# LUFENURON

*First draft prepared by*
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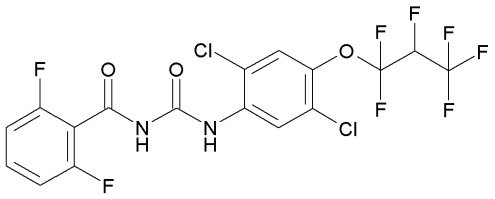
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## Explanation

Lufenuron (Fig. 1) is the International Organization for Standardization (ISO)–approved common name for (*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 103055-07-8.

***Fig. 1. Structure of lufenuron***



Lufenuron is an insecticide initially registered for use on a wide range of crops for the control of the larvae of many insect pests. Lufenuron inhibits chitin synthesis, probably through enzymatic interference, and prevents the larvae from moulting.

Lufenuron has not been evaluated previously 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).

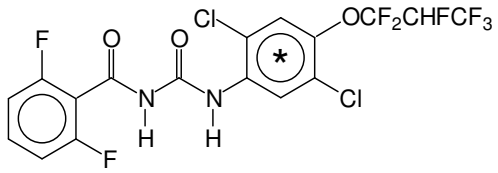
## Evaluation for acceptable intake

### 1. Biochemical aspects

#### *1.1 Absorption, distribution and excretion*

To investigate the absorption, distribution and excretion of lufenuron, a single oral dose of [$^{14}$C]lufenuron (batch no. ILA-178 7A; > 98% radiochemical purity; see Fig. 2 for position of the radiolabel) was administered by gavage to four groups of four male rats (Alpk:AP$_f$SD, Wistar-derived) at a dose of 0.1, 1.0, 10 or 100 mg/kg body weight (bw), or a single intravenous dose of [$^{14}$C]lufenuron was administered to two groups of four male rats at a dose of 0.1 or 10 mg/kg bw. The dose vehicle was 7:3 (volume per volume [v/v]) polyethylene glycol (PEG) 400/ethanol. Urine and faeces were collected daily for 7 days following dosing. Subsequently, faeces were collected daily on days 8–21 following dosing, and urine was collected on days 10–11, 14–15 and 19–21. Serial blood samples were collected at 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 288 and 384 hours after dosing and by cardiac puncture at termination. Twenty-one days after dosing, each rat was terminated.

***Fig. 2. Position of radiolabel***




* = $^{14}$C position

The study was not performed according to GLP, but it met all criteria specified in the study guidelines (Organisation for Economic Co-operation and Development [OECD] Test Guideline 417).

Over the 21-day duration of the study, the mean total proportion of administered radioactivity excreted in urine following oral dosing at 0.1, 1.0, 10 or 100 mg/kg bw was 0.6%, 0.5%, 0.6% and 0.2%, respectively, whereas faecal excretion accounted for 80.6%, 72.9%, 80.4% and 91.9%, respectively. The mean total percentage of dose excreted in urine following an intravenous dose of 0.1 or 10 mg/kg bw was 0.6% and 0.8%, respectively, whereas faecal excretion accounted for 66.5% and 62.2%, respectively. A characteristic feature of faecal excretion was the slow, sustained period of elimination. Faecal excretion was still measurable 21 days following administration of a single oral or intravenous dose, irrespective of dose level (Table 1).

The area under the blood concentration–time curves over 120 hours ($AUC_{0-120\ h}$) after dosing, for both dose routes, clearly show that for dose levels between 0.1 and 10 mg/kg bw, systemic exposure is directly proportional to the administered dose. The systemic bioavailability of lufenuron can be directly determined by the classical method of calculating the ratio of AUC between an oral and an intravenous dose. This gives a value of approximately 70% for the 0.1 and 10 mg/kg bw dose levels. The amount of radioactivity in urine following oral and intravenous dosing can also be treated

*Table 1. Comparison of kinetics following single oral or intravenous administration of lufenuron*

| Route | Dose (mg/kg bw) | Radioactivity (% of dose at 21 days after dosing) | | | | $AUC_{0–120\ h}$ (h·µg eq/g) |
|---|---|---|---|---|---|---|
| | | Urine | Faeces | Tissue and residual carcass | Total recovery | |
| Oral | 0.1 | 0.64 | 80.59 | 21.00 | 102.93 | 0.40 |
| | 1.0 | 0.51 | 72.93 | 23.55 | 97.23 | 4.37 |
| | 10 | 0.57 | 80.37 | 14.83 | 96.05 | 41.25 |
| | 100 | 0.17 | 91.87 | 5.17 | 97.43 | 83.88 |
| Intravenous | 0.1 | 0.64 | 66.50 | 32.03 | 99.77 | 0.56 |
| | 10 | 0.82 | 62.23 | 34.73 | 98.11 | 60.69 |

$AUC_{0–120\ h}$: area under the blood concentration–time curve over 120 hours; bw: body weight; eq: equivalents
*Source:* Booth (2004)

similarly to obtain an estimate of absorption. For the 10 mg/kg bw dose level, the urinary data indicate an absorption value of 70%; for the 0.1 mg/kg bw dose level, the absorption is estimated at 100%.

The blood $AUC_{0–120\ h}$ increased with dose over the range 0.1–100 mg/kg bw. At the highest oral dose level, 100 mg/kg bw, evidence of saturation was observed, and the AUC was no longer proportional to the administered dose. Saturation was more evident in the relationship between blood $AUC_{0–\infty}$ and dose. The time to reach the maximum concentration ($T_{max}$) was 8 hours after oral administration and 2 hours after intravenous administration (Table 2).

The mean terminal half-life of faecal excretion following oral dosing was 256 hours, and following intravenous dosing, 232 hours. At termination of the study, the mean total proportion of administered radioactivity present in tissues and carcass following oral dosing at 0.1, 1.0, 10 or 100 mg/kg bw was 21.0%, 23.6%, 14.8% and 5.2%, respectively. The mean total proportion of dose present in tissues following an intravenous dose of 0.1 or 10 mg/kg bw was 32.0% and 34.7%, respectively. Radioactivity was located mainly in the fat and, to a much lesser extent, in residual carcass, followed by liver and kidneys. The total mean percentage recoveries of administered radioactivity, including excreta, tissues and residual carcasses, following oral dosing at 0.1, 1.0, 10 or 100 mg/kg bw were 102.9%, 97.2%, 96.0% and 97.4%, respectively. The total mean percentage recoveries of administered radioactivity, including excreta, tissues and residual carcasses, following an intravenous dose of 0.1 or 10 mg/kg bw were 99.8% and 98.1%, respectively.

*Table 2. Blood kinetics data following a single oral or intravenous dose of [$^{14}$C]lufenuron*

| Nominal dose, route | Mean achieved dose (mg/kg bw) | $AUC_{0–120\ h}$ (h·µg/g) | $AUC_{0–\infty}$ (h·µg/g) | $T_{max}$ (h) | $C_{max}$ (µg/g) |
|---|---|---|---|---|---|
| 0.1 mg/kg bw, oral gavage | 0.10 | 0.40 | 0.49 | 8 | 0.008 |
| 1.0 mg/kg bw, oral gavage | 1.08 | 4.37 | 10.73 | 8 | 0.097 |
| 10 mg/kg bw, oral gavage | 10.52 | 41.25 | 90.44 | 8 | 0.89 |
| 100 mg/kg bw, oral gavage | 101.85 | 83.88 | 216.36 | 8 | 1.341 |
| 0.1 mg/kg bw, intravenous | 0.11 | 0.56 | 0.94 | 2 | 0.017 |
| 10 mg/kg bw, intravenous | 10.35 | 60.69 | 153.76 | 2 | 1.907 |

$AUC_{0–120\ h}$: area under the blood concentration–time curve over 120 hours; $AUC_{0–\infty}$: area under the blood concentration–time curve from time 0 to infinity; bw: body weight; $C_{max}$; maximum concentration; $T_{max}$: time to reach maximum concentration
*Source:* Booth (2004)

From the AUC values determined for blood, the dose–response curve does not appear to be linear at the high dose level following oral gavage dosing (Booth, 2004).

To investigate the absorption, distribution and excretion of lufenuron, [$^{14}$C]lufenuron (batch no. Le-78.3A; approximately 99% radiochemical purity) was administered by gavage to groups of male and female rats (Tif: RAIf (SPF)) at a single dose of either 0.5 or 100 mg/kg bw following the study design indicated in Table 3.

***Table 3. Terminal toxicokinetic studies in the rat – experimental scheme***

| Group | Number and sex | Route and dose level of [$^{14}$C]lufenuron | Sample collection times |
|---|---|---|---|
| B | 5 males, 5 females | Single oral dose of 0.5 mg/kg bw<br>199 kBq/animal | Urine: 0–8, 8–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h<br>Faeces: 0–8, 8–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h<br>Volatiles: 0–8 and 8–24 h<br>Expired air: 0–8 and 8–24 h<br>Tissues: 7 days after administration of the $^{14}$C-labelled material, tissues and organs were taken for analysis |
| C | 5 males, 5 females | Single oral dose of 0.5 mg/kg bw [$^{14}$C]lufenuron preceded by 14 consecutive daily oral doses of 0.5 mg unlabelled lufenuron/kg bw<br>239 kBq/animal | Collection of urine, faeces, volatiles, expired air and tissues as in Group B |
| D | 5 males, 5 females | Single oral dose of 100 mg/kg bw<br>7 785 kBq/animal<br>(diluted with batch no. P1 to a specific activity of 385 kBq/mg) | Collection of urine, faeces, volatiles, expired air and tissues as in Group B |
| E1 | 3 males | Single oral dose of 0.5 mg/kg bw<br>196 kBq/animal | Blood: 1, 2, 4, 8, 12, 24, 32, 48 and 56 h after administration (determination of $T_{max}$ and $T_{max/2}$ of this dose) |
| E2 | 3 males | Single oral dose of 100 mg/kg bw<br>1 423 kBq/animal (diluted with batch no. AMS 266/101 to a specific activity of 72 kBq/mg) | Blood: 1, 2, 4, 8, 12, 24, 32, 48, 56 and 72 h after administration (determination of $T_{max}$ and $T_{max/2}$ of this dose) |
| F1 and F2 | 10 males | Single oral dose of 0.5 mg/kg bw<br>191 kBq/animal | Tissues: 8 h ($T_{max}$), 45 h ($T_{max/2}$), 5 days and 12 days after administration, selected tissues and organs were taken for analysis |
| F3 and F4 | 10 males | Single oral dose of 100 mg/kg bw<br>1 378 kBq/animal (diluted with batch no. AMS 266/101 to a specific activity of 70 kBq/mg) | Tissues: 8 h ($T_{max}$), 60 h ($T_{max/2}$), 5 days and 12 days after administration, selected tissues and organs were taken for analysis |
| G | 5 males, bile duct cannulated under anaesthesia | Single oral dose of 0.5 mg/kg bw<br>237 kBq/animal | Urine: 0–24 and 24–48 h<br>Faeces: 0–24 and 24–48 h<br>Bile: 0–0.5, 0.5–1, 1–2, 2–4, 4–8, 8–18, 18–24, 24–42 and 42–48 h |

bw: body weight; $T_{max}$: time to reach maximum concentration; $T_{max/2}$: time to decline to half the maximum concentration
*Source*: Bissig (1990)

Irrespective of dose level or sex, less than 1% of an oral dose of [$^{14}$C]lufenuron was excreted in urine, and less than 0.01% of the administered radioactivity was detected in exhaled volatile and carbon dioxide traps. The extent of absorption of a 0.5 mg/kg bw dose was 44%, based upon radioactivity present in urine and residual carcass and tissues at 7 days after administration. However, the extent of absorption and faecal excretion were dose dependent. Despite the similarities in faecal excretion of a 0.5 mg/kg bw dose by bile duct–cannulated and non-cannulated male rats and the low biliary elimination over 48 hours after dosing, the slow rate of faecal excretion by non-cannulated rats suggests that some faecal radioactivity was of biliary origin. The extent of absorption of a 100 mg/kg bw dose was much lower, accounting for at least 9% of the dose, based mainly on radioactivity present in tissues at the termination of the experiment. Even though some of the systemic dose was likely to have been eliminated in bile, the much lower proportion of dose retained in tissue and the very low urinary excretion indicate that at the high dose level, the capacity to absorb the test material was reduced (Table 4).

***Table 4. Excretion of lufenuron by rats following an oral [$^{14}$C]lufenuron dose of 0.5 or 100 mg/kg bw***

| | | **Excretion (% of administered radioactivity)** | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **B (single dose 0.5 mg/kg bw)** | | **C (repeated dose 0.5 mg/kg bw per day)** | | **D (single dose 100 mg/kg bw)** | | **G (bile duct cannulated, single dose 0.5 mg/kg bw)** |
| | | **Males** | **Females** | **Males** | **Females** | **Males** | **Females** | **Males** |
| Bile | 0–8 h | – | – | – | – | – | – | 0.33 |
| | 8–24 h | – | – | – | – | – | – | 0.54 |
| | 24–48 h | – | – | – | – | – | – | 0.82 |
| | Subtotal | | | | | | | 1.70 |
| Urine | 0–24 h | 0.27 | 0.29 | 0.19 | 0.31 | 0.10 | 0.14 | 0.09 |
| | 24–48 h | – | – | – | – | – | – | 0.08 |
| | 24–96 h | 0.35 | 0.27 | 0.25 | 0.28 | 0.10 | 0.10 | – |
| | 96–168 h | 0.20 | 0.16 | 0.16 | 0.17 | 0.05 | 0.04 | – |
| | Subtotal | 0.82 | 0.72 | 0.60 | 0.75 | 0.26 | 0.28 | 0.17 |
| Faeces | 0–24 h | 25.97 | 23.71 | 38.83 | 23.27 | 66.88 | 73.20 | 8.57 |
| | 24–48 h | – | – | – | – | – | – | 43.03 |
| | 24–96 h | 17.71 | 15.12 | 11.17 | 12.93 | 13.44 | 8.75 | – |
| | 96–168 h | 8.26 | 8.92 | 5.31 | 7.84 | 2.10 | 1.32 | – |
| | Subtotal | 51.95 | 47.74 | 55.31 | 44.04 | 82.42 | 83.26 | 51.60 |
| Expired air 0–24 h | | Not measured | Not measured | Not measured | Not measured | < 0.01 | < 0.01 | Not measured |
| Total excretion | | 52.77 | 48.46 | 55.91 | 44.79 | 82.68 | 83.54 | 53.47 |
| Cage wash | | 0.07 | 0.07 | 0.05 | 0.06 | 0.08 | 0.14 | – |
| Tissue residues | | 5.41 | 4.95 | 5.87 | 8.36 | 1.96 | 1.50 | – |
| Carcass | | 38.09 | 43.80 | 37.09 | 44.42 | 9.65 | 7.42 | – |
| Total recovery 0–168 h | | 96.34 | 97.27 | 98.92 | 97.64 | 94.37 | 92.59 | 53.47 |

bw: body weight
*Source:* Bissig (1990)

The urinary excretion and tissue residue data showed that at least 44% and 9% of the orally administered lufenuron were absorbed from the intestinal tract into the systemic circulation by the low-dose rats (groups B and C) and the high-dose rats (group D), respectively.

Seven days after a single oral dose of 0.5 mg/kg bw, significant residues were present in most tissues in both sexes, predominantly in fat (1.91 parts per million [ppm] in males and 2.40 ppm in females). Residues above 0.1 ppm were also measured in ovaries (0.44 ppm), uterus (0.23 ppm), thyroid (0.22 ppm in males and 0.16 ppm in females), liver (0.13 ppm in males and 0.15 ppm in females), lungs (0.11 ppm in females) and kidneys (0.11 ppm in females). As a consequence of the slightly faster excretion by males, the residues in virtually all tissues in males in groups B and C were slightly lower than those in females, with the single exception of the thyroid in group B. As approximately 43–49% of a single 0.5 mg/kg bw dose of [$^{14}$C]lufenuron was present in tissues, predominantly fat, after 7 days and as similar proportions of a 0.5 mg/kg bw dose of radiolabelled lufeuron were present in tissues of rats pretreated with 14 daily doses of unlabelled lufenuron, substantial proportions of each unlabelled dose are also likely to be present in tissues. In contrast, 9–12% of a 100 mg/kg bw dose remained in tissues 7 days after dosing, reflecting the lower extent of absorption. Accordingly, at this dose level, tissue residues were only 40–60 times and 30–40 times higher in males and females, respectively. The only exception to this observation was thyroid in females, which contained approximately 100 times higher residue levels. As for the low dose level, the highest residue levels were present in the fat.

The blood kinetics of rats in groups E1 and E2 (single oral doses of 0.5 and 100 mg/kg bw, respectively) showed fairly rapid absorption of lufenuron from the intestinal tract into the systemic circulation. At both dose levels, the maximum blood levels ($C_{max}$) were reached about 8 hours after dosing. The blood concentrations declined to half of the maximum value ($C_{max/2}$) about 45 and 60 hours ($T_{max/2}$) after dosing at the low and high dose levels, respectively.

At the low dose level, the rate of tissue depletion followed first-order kinetics (45 hours – 12 days; with a coefficient of correlation $r^2 > 0.97$ for most tissues). Tissue concentrations for both low and high doses demonstrated similar depletion kinetics for all tissues. Half-lives of elimination, expressed over two time intervals, showed an initial faster rate followed by a slower terminal rate. Irrespective of dose level or tissue concentration, the initial rates of tissue elimination were similar across the range of tissues analysed, ranging from 2 to 6 days. At the low dose level, the terminal half-lives ranged between 5 and 13 days; the corresponding values at the high dose level ranged from 10 to 37 days (Bissig, 1990).

To investigate the absorption and depletion kinetics of lufenuron, a single oral dose of [$^{14}$C]lufenuron (batch no. Le-78.3A-1; 97% radiochemical purity) was administered by gavage to two groups of three male and three female rats (Tif: RAIf (SPF)) at a dose of 0.5 or 100 mg/kg bw.

In both sexes, the peak blood concentration of radioactivity was reached 8 and 12 hours after administration of the low and high dose levels, respectively. The $^{14}$C concentration in blood declined to half of these values after 56 and 42 hours ($T_{max/2}$) at the low dose level and after 86 and 68 hours at the high dose level in males and females, respectively. Assuming first-order kinetics, a monophasic decline of radioactivity in blood was observed at both the low and high dose levels. When applying linear regression, the calculated half-lives of depuration were 84 and 97 hours at the low dose level and 119 and 111 hours at the high dose level for male and female rats, respectively (Table 5).

The shapes of the areas under the concentration–time curves ($AUC_{0–168\ h}$) were essentially identical for both sexes at both dose levels, accounting at the low dose level for 2.5 h·µg/g. At the high dose level, the respective values were 116 and 106 h·µg/g for male and female rats, respectively (Table 5). When comparing the AUCs at the low and high dose levels, a 46-fold increase in males and a 42-fold increase in females were observed, indicating that a smaller proportion of the dose was bioavailable at the higher dose level. The AUC values were virtually the same for both sexes, demonstrating similar absorption of lufenuron by both males and females (Müller, 1995).

***Table 5. Blood kinetic data following a single oral [$^{14}$C]lufenuron dose of 0.5 or 100 mg/kg bw***

| | 0.5 mg/kg bw | | 100 mg/kg bw | |
|---|---|---|---|---|
| | **Males** | **Females** | **Males** | **Females** |
| $C_{max}$ (ppm) | 0.033 | 0.035 | 1.331 | 1.176 |
| $T_{max}$ (h) | 8 | 8 | 12 | 12 |
| Calculated $t_{½}$ (h) | 84 | 97 | 119 | 111 |
| $AUC_{0–168\ h}$ (h·µg/g) | 2.5 | 2.5 | 116.3 | 105.8 |

$AUC_{0–168\ h}$: area under the concentration–time curve over 168 hours; bw: body weight; $C_{max}$; maximum concentration; ppm: parts per million; $t_{½}$: half-life; $T_{max}$: time to reach maximum concentration
*Source*: Müller (1995)

To investigate the absorption, distribution and excretion of lufenuron after multiple oral administrations, [$^{14}$C]lufenuron (batch no. ILA-178.3; 97.0% radiochemical purity) was administered by gavage to four groups of four male rats (HanBrl: WIST (SPF)). The dosing was performed by 14 consecutive daily doses at a dose of 0.5 mg/kg bw. One group of four males was sacrificed after 1 day of dosing, another group of four males after 7 days of dosing, and the remaining two groups after 14 days of dosing.

Approximately 1% of the dose was excreted in urine, and 58% in faeces. Approximately 38% of the cumulative dose remained in tissues and the residual carcass at the conclusion of this study. The total recovery of administered radioactivity was greater than 97%. The unexcreted dose was accumulated in tissues, and the rate of faecal excretion suggested that such tissue residues would be eliminated slowly and predominantly in faeces. About 80% of the administered dose was found in tissues and residual carcass 1 day after a single oral dose, which indicates that absorption of a 0.5 mg/kg bw single oral dose was approximately 80%.

With the single exception of testes, all tissue concentrations of radioactivity increased to a maximum 1 day after the final dose. The results suggest that most tissue concentrations would plateau within 2–3 weeks of similar repeated dosing. The highest residues were present in fat (29 ppm lufenuron equivalents). Much lower residue levels were present in adrenals (4.2 ppm), pancreas (3.2 ppm), thyroid (3.0 ppm), liver (2.1 ppm), kidneys (1.3 ppm), heart (1.2 ppm), lungs (1.1 ppm) and thymus (1.1 ppm). All other tissues attained maximum levels below 1 ppm lufenuron equivalent. The calculated half-life ($t_{½}$) for the depuration of tissue $^{14}$C residues, assuming a monophasic first-order kinetics, typically ranged from 7 to 12 days. A faster depletion was observed for thyroid ($t_{½}$ = 4 days). Elimination half-lives of residues from testes, lungs and fat ranged from 14 to 16 days, although testes showed lower residues after 14 days than after 7 days during the accumulation phase (Table 6).

Seven days after the last of 14 consecutive daily doses, tissue residues represented approximately 38% of the total administered radioactivity. There was a similar distribution pattern between these profiles and those seen 7 days after a similar single oral dose, and there was an approximate 10-fold difference in the comparative tissue concentrations (Table 6).

To conclude, the rates and routes of excretion and the tissue distribution did not change upon multiple dosing compared with single dosing. The tissue residues increased with ongoing dosing, not reaching a plateau within the dosing period. Indication is given that residue levels in most of the tissues will reach a plateau within a dosing period of 3 weeks (Hassler, 2003).

To investigate the absorption, excretion, tissue distribution and pharmacokinetics of lufenuron after multiple oral administrations, [$^{14}$C]lufenuron (batch no. ISL-158.1; 97.8% radiochemical purity) was administered by gavage to groups of male and female rats (Sprague Dawley Crj:CD(SD)) for 1, 7 or 14 days, respectively. The dosing was performed by 14 consecutive daily doses at a dose of 0.5 mg/kg bw. This study was not compliant with GLP, but it was conducted in Japan to standards apparently equivalent to GLP.

***Table 6. Tissue residues of radioactivity during and following 14 daily oral [$^{14}$C]lufenuron doses of 0.5 mg/kg bw***

| | Tissue residues (µg eq/g tissue) | | | | | |
|---|---|---|---|---|---|---|
| | Single dose[a] | Multiple doses | | | | $t_{½}$ (days) |
| | Day 7[b] | Day 1[b] | Day 7[b] | Day 14[b] | Day 20[b,c] | |
| Adrenals | n.a. | 0.741 8 | 2.379 3 | 4.187 9 | 2.391 5 | 7 |
| Blood | 0.008 4 | 0.031 2 | 0.110 5 | 0.165 6 | 0.099 1 | 8 |
| Bone | 0.038 9 | 0.095 7 | 0.302 1 | 0.329 7 | 0.235 1 | 12 |
| Brain | 0.013 1 | 0.031 3 | 0.111 3 | 0.139 6 | 0.081 6 | 8 |
| Fat | 1.914 2 | 3.480 9 | 21.150 2 | 29.245 2 | 22.660 2 | 16 |
| Heart | 0.080 2 | 0.261 4 | 0.926 1 | 1.247 2 | 0.774 6 | 9 |
| Kidneys | 0.087 9 | 0.292 3 | 1.067 7 | 1.348 1 | 0.884 5 | 10 |
| Liver | 0.129 2 | 0.462 0 | 1.600 5 | 2.119 2 | 1.346 0 | 9 |
| Lungs | 0.094 2 | 0.299 4 | 0.826 6 | 1.096 3 | 0.833 8 | 15 |
| Muscle | 0.040 4 | 0.156 4 | 0.576 4 | 0.636 7 | 0.395 6 | 9 |
| Pancreas | n.a. | 0.595 7 | 2.164 5 | 3.169 6 | 2.165 1 | 11 |
| Plasma | 0.010 4 | 0.039 5 | 0.138 9 | 0.231 7 | 0.130 7 | 7 |
| Spleen | 0.046 5 | 0.160 0 | 0.547 7 | 0.724 2 | 0.512 6 | 12 |
| Testes | 0.026 0 | 0.082 2 | 0.367 3 | 0.279 0 | 0.207 6 | 14 |
| Thymus | 0.056 0 | 0.173 4 | 0.728 4 | 1.087 0 | 0.618 5 | 7 |
| Thyroids | 0.220 0 | 0.413 1 | 2.441 2 | 3.023 6 | 1.097 5 | 4 |

eq: equivalents; n.a.: not available; ppm: parts per million
[a] From Bissig (1990).
[b] Days after start of dosing.
[c] Seven days after last dose.
*Source*: Hassler (2003)

Concentrations of radioactivity in blood measured 8 hours after each dose showed a progressive increase during and following the 14-day repeated-dose experiment, corresponding to the peak blood concentration following a single similar dose. After an initial rapid increase, blood concentrations rose gradually to maximum values of 0.184 and 0.178 µg equivalents of lufenuron per millilitre after the final dose. Concentrations then declined gradually, with estimated elimination half-lives of 208 and 323 hours in males and females, respectively. There was considered to be no marked sex difference in accumulation or depletion profiles.

Excretion by 168 hours after the final dose in male and female rats administered 14 daily oral [$^{14}$C]lufenuron doses of 0.5 mg/kg bw was 58% of the cumulative dose in males and 64% in females. There was no pronounced sex difference in excretion. Urinary excretion accounted for only about 1% of the cumulative dose in both males and females, whereas faecal excretion accounted for the remainder. The unexcreted dose was accumulated in tissues, and the rate of faecal excretion suggested that such tissue residues would be eliminated slowly and predominantly in faeces.

Concentrations of radioactivity during and following 14 daily oral [$^{14}$C]lufenuron doses of 0.5 mg/kg bw showed an increase in all tissues until 8 hours after the 14th dose and then a decline. All tissue concentrations exceeded the plasma concentration at all time points, although concentrations in the cerebrum and cerebellum were only slightly higher than those in plasma. The greatest accumulation was observed in fat (28.1 µg eq/g in males and 34.7 µg eq/g in females), with markedly lower peak concentrations in other tissues. Progressively lower peak levels were present in adrenal

glands, liver, skin, thyroid, lungs, heart and pituitary. All other tissue concentrations attained peak values of less than 1 µg eq/g. Over the 7 days after the cessation of dosing, all tissue concentrations declined steadily, including fat residues. By this time, the residual carcasses contained 34.6% and 29.5% of the total administered radioactivity in males and females, respectively. There was no marked sex difference in either accumulation or tissue depletion.

Radioluminograms showed very little radioactivity in the brain 8 hours after the first dose. For both sexes, the concentration in the pituitary increased only by a factor of 2 between the first and 14th daily doses, followed by a decline, as observed for other tissues. Although the pineal body was not detected in sections for females, the concentrations in this tissue matched those in the pituitary in both sexes. The peak concentrations in these tissues were much lower than those observed in major organs, such as the liver, kidney, heart and lung (Okada, 1997).

In conclusion, lufenuron accumulates in fat, from which it is slowly released into faeces, with terminal half-lives in fat of 5–13 days at 0.5 mg/kg bw and 10–37 days at 100 mg/kg bw. This accumulation has to be taken into account when determining the oral absorption, as part of the faeces measured at 7–21 days after dosing will consist of the initially absorbed amount from tissues/carcass.

The oral absorption after a single low dose (0.1–0.5 mg/kg bw) is at least 70%, based on the ratio of AUC between oral and intravenous doses (Booth, 2004). This is confirmed by Hassler (2003), who found an oral absorption of around 80%, based on the concentration in tissues/carcass 1 day after a single dose of 0.5 mg/kg bw.

There are no studies with tissue/carcass measurements at 1 day to determine the oral absorption after a single high dose. Taking an average half-life of 14 days in carcass and tissues, the tissue/carcass concentrations at 21 days (Booth, 2004) or 7 days (Bissig, 1990) after a single high dose of 100 mg/kg bw would indicate an initial tissue/carcass concentration of around 15–20% of the administered dose. The faecal excretion in these studies at 1 day after dosing was around 70–80%. This figure also indicates that the oral absorption after a single high dose of 100 mg/kg bw would be around 20%.

### *1.2 Biotransformation*

In a study described in section 1.1, a single major component was identified in faecal extracts after single oral doses of 0.1, 1.0, 10 or 100 mg/kg bw, and this was identified as parent lufenuron. The levels of administered radioactivity in urine were very low (< 1% of the dose), and metabolite identification was not performed on these samples (Booth, 2004).

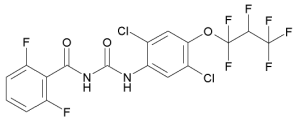
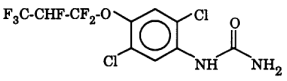
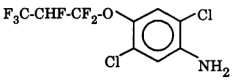
In Hassler (2003), described in section 1.1, chromatographic analysis of urine revealed a metabolite profile comprising at least 10 metabolite fractions. Although the total amount of radiolabelled metabolites was very low, based on very low urinary excretion, the patterns were qualitatively and quantitatively similar after single and repeated dosing.

Thin-layer chromatographic (TLC) analysis of the faecal extracts revealed at least four metabolite fractions. The extractability and the metabolite pattern of the faeces were essentially identical for all three selected sampling intervals, with slight quantitative variations mainly between the first time interval (0–1 day) and the later selected time intervals. The major metabolite fraction (3) corresponded to unchanged lufenuron, accounting for approximately 30% of the daily dose. Apart from unchanged parent, only minor metabolites were present in the faecal extracts (Hassler, 2003).

In Okada (1997), described in section 1.1, chromatographic analysis of metabolite profiles in plasma and cerebrum revealed the following. In plasma, 96% of the radioactivity was extracted into methanol and was resolved into four metabolites. The major component was unchanged lufenuron, and the other major metabolite was CGA 238277; one of the minor metabolites corresponded to

CGA 224443, and the other was unidentified (Table 7). The proportion of lufenuron in all samples was approximately 70–80% and appeared to be unaffected by the number of doses given or the sampling time. More than 94% of radioactivity in the cerebrum was extracted and comprised only unchanged lufenuron, representing more than 92%, and CGA 238277, accounting for 1.1% or less of the radioactivity (Okada, 1997).

***Table 7. Summary of lufenuron-derived metabolites in the rat***

| Metabolite code | Chemical name | Structure |
|---|---|---|
| Lufenuron, (CGA 184699) | (*RS*)-1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-3-(2,6-difluorobenzoyl)urea |  |
| CGA 238277 | 1-[2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]-urea |  |
| CGA 224443 | 2,5-Dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)aniline |  |

*Source*: Thanei (1990)

In another study described in section 1.1 (Bissig, 1990), the urinary metabolite pattern after a single dose of either 0.5 or 100 mg/kg bw was not determined because of the low recovery of radioactivity in urine (< 1% of the dose). Analysis of faeces by one-dimensional TLC revealed no significant differences between the sexes and showed no significant influence of pretreatment with unlabelled lufenuron, but showed slightly dose-dependent differences. The metabolite pattern consisted of one dominant fraction (F3), which was characterized as unchanged lufenuron. As a consequence of the higher amount excreted in faeces at the high dose level, metabolite fraction F3 represented a higher percentage of the dose (77–79%) than at the low dose level (37–48%). In contrast, the most polar fractions (F1 and F2) were more pronounced at the low dose than at the high dose.

The faecal metabolite pattern of the bile duct–cannulated rats (group G) was similar to that of the animals of group B, but with a higher percentage of unchanged lufenuron. Radioactivity accounted for only 1.5% of the administered dose. Metabolite fraction G5, which represented 0.1% of the administered dose, co-chromatographed with unchanged lufenuron. The metabolite fraction G2 (corresponding to 0.1% of the dose) behaved as the authentic reference compound CGA 238277 (i.e. the free urea conjugate of the dichlorophenyl moiety). The least polar metabolite fraction G6 co-chromatographed with CGA 224443, the free amine derivative of the dichlorophenyl moiety (< 0.1% of the dose).

Samples of fat, liver, kidneys, lungs and carcass were pooled by sex and group for the analysis of $^{14}C$ residues. TLC analysis of organic solvent and aqueous extracts of tissues revealed a single metabolite fraction in all extracts, co-chromatographing with unchanged lufenuron (Bissig, 1990).

To further investigate the metabolism of lufenuron after a single oral administration, samples used in this study originated from a study described above (Bissig, 1990). Faeces and fat collected from male and female rats administered a single oral dose of [$^{14}C$]lufenuron at 100 mg/kg bw were used in the present study.

The 8- to 48-hour faecal pools (representing 76% of the dose) for male and female rats were extracted with methanol. Almost 100% of the radioactivity in faeces was extractable. The TLC analysis revealed one dominant metabolite fraction, accounting for 75% of the dose. This major metabolite fraction was identified by spectroscopic means to be unchanged lufenuron. The minor metabolite fractions accounted for less than 1% of the dose each. Lufenuron was metabolized to a very small extent. In addition, two metabolites were characterized by chromatographic comparison with authentic reference compounds (Table 7).

Organic solvent extraction of the single pool of fat from male and female rats extracted approximately 93% of the radioactivity present. Purification by preparative TLC yielded two fractions. The fraction containing almost all the radioactivity was further purified by high-performance liquid chromatography. It was shown by mass spectroscopy and by co-chromatography to be identical to the parent compound, lufenuron. By chromatographic comparison, the second fraction corresponded to metabolite F2.

Mostly unchanged lufenuron was excreted in faeces and was also retained in tissues, predominantly in fat, demonstrating that lufenuron was poorly metabolized. It is assumed that a minor route of degradation is cleavage of the benzamide moiety, yielding the urea CGA 238277 and 2,6-difluorobenzoic acid. Further cleavage of the ureido moiety of CGA 238277 leads to the aniline CGA 224443. It is established that rats excrete 2,6-difluorobenzoic acid largely unchanged. Based on the faecal and fat metabolites identified or characterized, which represented approximately 85% of the dose, the proposed metabolic pathway in the rat is summarized in Fig. 3 (Thanei, 1990).

## 2. Toxicological studies

### *2.1 Acute toxicity*

*(a) Lethal doses*

The results of acute toxicity studies with lufenuron administered orally, dermally or by inhalation are summarized in Table 8. All the studies were certified to comply with GLP and performed according to United States Environmental Protection Agency (USEPA) and OECD guidelines.

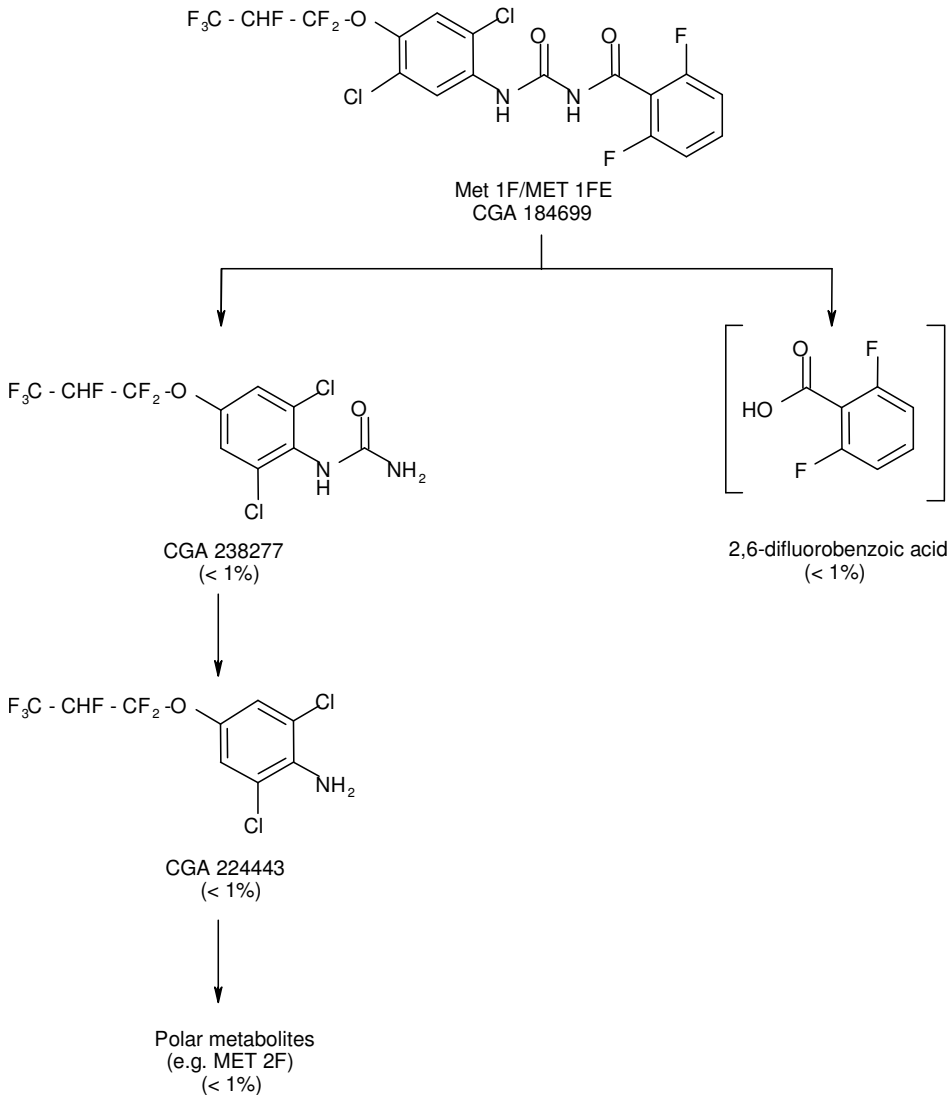
Lufenuron has low acute toxicity when administered orally, dermally and via inhalation to rats.

In an acute oral toxicity study, groups of five Tif:MAG f (SPF) mice of each sex received an oral dose of lufenuron (purity 95.2%) at 2000 mg/kg bw. The test material was suspended in Oleum arachidis Ph.H.VI. There were no deaths, and clinical signs were limited to piloerection, hunched posture and dyspnoea. Clinical signs were slight, and animals recovered within 6 days. Body weight gains were normal. There were no gross necropsy findings. The median lethal dose ($LD_{50}$) following oral exposure was greater than 2000 mg/kg bw (Hartmann, 1989).

In an acute oral toxicity study, groups of five Tif: RAIf (SPF) rats of each sex received an oral lufenuron (purity 95.2%) dose of 2000 mg/kg bw. The test was suspended in Oleum arachidis Ph.H.VI. There were no deaths, and clinical signs were limited to ruffled fur, dyspnoea, hunched posture and exophthalmos, ranging from moderate directly after treatment to slight up to 10 days after treatment. Body weight gains were normal. There were no gross necropsy findings. The $LD_{50}$ following oral exposure was greater than 2000 mg/kg bw (Hartmann, 1988a).

In an acute dermal toxicity study, groups of five Tif: RAIf (SPF) rats of each sex were treated with lufenuron (purity 95.2%) via dermal exposure at a limit dose of 2000 mg/kg bw. The test material was suspended in Oleum arachidis Ph.H.VI and administered in 0.4 mL/kg bw to approximately 10% of the body surface area under an occlusive dressing for 24 hours. There were no deaths, and the animals showed ruffled fur, dyspnoea, abnormal body positions and reduced spontaneous activity. Clinical signs were slight, and animals recovered within 5 days. No skin

***Fig. 3. Proposed metabolic pathway for lufenuron in the rat***




*Source*: Thanei (1990)

***Table 8. Acute toxicity of lufenuron***

| Species | Strain | Sex | Route | Purity (%) | Result | Reference |
|---|---|---|---|---|---|---|
| Mouse | Tif:MAG f (SPF) | Male and female | Oral | 95.2 | $LD_{50}$ > 2 000 mg/kg bw | Hartmann (1989) |
| Rat | Tif: RAIf (SPF) | Male and female | Oral | 95.2 | $LD_{50}$ > 2 000 mg/kg bw | Hartmann (1988a) |
| Rat | Tif: RAIf (SPF) | Male and female | Dermal | 95.2 | $LD_{50}$ > 2 000 mg/kg bw | Hartmann (1988b) |
| Rat | Tif: RAIf (SPF) | Male and female | Inhalation | 95.2 | $LC_{50}$ > 2.35 mg/L (maximal attainable concentration) | Hartmann (1988c) |

bw: body weight; $LC_{50}$: median lethal concentration; $LD_{50}$: median lethal dose

irritation was noted. Body weight gains were normal. There were no gross necropsy findings. The $LD_{50}$ following dermal exposure was greater than 2000 mg/kg bw (Hartmann, 1988b).

In an acute inhalation toxicity study, groups of five Tif: RAIf (SPF) rats of each sex were exposed to lufenuron (purity 95.2%) for 4 hours via nose-only inhalation exposure at a test atmosphere concentration of 2.35 mg/L (nominal 3.0 mg/L, mass median aerodynamic diameter 1.9–2.8 μm, geometric standard deviation 2.2–2.5 μm). There were no deaths, and clinical signs recorded during exposure were moderate piloerection, slight hunched posture and moderate dyspnoea. All animals recovered within 2 days. The males exposed to the test article exhibited a significantly higher body weight increase during the second week after exposure. There were no notable necropsy findings at termination. The acute inhalation median lethal concentration ($LC_{50}$) was greater than 2.35 mg/L (Hartmann, 1988c).

*(b) Dermal irritation*

In a skin irritation study, three female New Zealand White (KFM-NZW) rabbits were treated with lufenuron (purity 95.2%) via dermal exposure at a dose of 0.5 g applied to the flank under an occlusive dressing for 4 hours. The (solid) test material was applied to the skin using a gauze patch that was moistened before application with distilled water containing 0.5% carboxymethyl cellulose and 0.1% polysorbate 80. Very slight erythema was observed in two animals at 1 hour after removal of the test substance. No other skin reactions were observed in any animal during the study. Body weight gains were normal (Schneider, 1988a).

*(c) Ocular irritation*

In an eye irritation study, three male New Zealand White (KFM-NZW) rabbits were treated with lufenuron (purity 95.2%) via ocular exposure at a dose of 0.1 mL (70 mg). Slight irritation of the cornea, iris and conjunctiva was observed at 1 hour after instillation in all three animals. Slight conjunctival redness was also found in one male 24 hours after instillation. No signs of eye irritation were present at the 48- and 72-hour examinations (Schneider, 1988b).

*(d) Dermal sensitization*

In a Magnusson and Kligman maximization test, lufenuron (purity 95.2%) was tested using 10 male and 10 female Pirbright White Strain (Tif: DHP) guinea-pigs in the test substance group and five male and five female guinea-pigs in the control group. No results of the intradermal injection/ topical induction are presented. Following challenge with lufenuron in petrolatum at 10% weight per weight (w/w), dermal responses were observed in 4/20 test animals at 24 hours after challenge and in 9/20 test animals at 48 hours after challenge. No skin reactions were observed in control animals. Up to 45% of the animals were sensitized by lufenuron under the experimental conditions employed. Therefore, lufenuron is considered to be a skin sensitizer in the guinea-pig. Sensitization of this strain of animals was positively tested with dinitrochlorobenzene (positive control), which gave very severe allergic reactions in all areas treated (Schneider, 1988c).

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

*(a) Oral administration*

*Mice*

In a dose range–finding study, lufenuron (purity 95.2%) was fed to groups of 10 male and 10 female mice (Tif: MAGf (SPF), hybrids of NIH × MAG) at a concentration of 0, 1000, 3000 or 9000 ppm (mean substance intakes: 0, 151, 449 and 1470 mg/kg bw per day for males and 0, 189, 517 and 1440 mg/kg bw per day for females, respectively) for up to 65 days. No haematological examinations were carried out, and no organ weights were measured.

As a result of high mortality and neurotoxic effects of the test substance (tonic-clonic seizures), the surviving animals at 3000 and 9000 ppm were sacrificed on day 49. Eleven animals (four males and seven females) treated at 3000 ppm and 19 animals (nine males and 10 females) treated at 9000 ppm were found dead between days 17 and 48. At 1000 ppm, there was a slight increase in mortality (one male and three females died between days 54 and 65). Tonic-clonic seizures were also observed in two animals in the 1000 ppm dose group. A small reduction in body weight gain and decreased feed consumption were observed in all treated males. It was concluded that the maximum tolerated dose was exceeded even at 1000 ppm (equal to 151 and 189 mg/kg bw per day for males and females, respectively) (Fankhauser, 1989a).

In a second dose range–finding study intended for test substance residue and blood level determination, lufenuron (purity 95.2%) was fed to groups of 30 female mice (Tif: MAGf (SPF), hybrids of NIH × MAG) at a concentration of 0, 4/8, 20, 100 or 1000 ppm (mean substance intakes: 0, 0.47/1.1, 2.94, 14.5 and 143 mg/kg bw per day, respectively) for up to 91 days (only 71 days for the high dose). From day 57 onwards, the diet of the low-dose group inadvertently contained 8 ppm instead of 4 ppm. Subgroups of nine mice were killed at weeks 9, 11 and 14; the remaining three animals per dose group were intended for eventual replacement or pharmacological investigations. Fat and blood samples were collected at 9 and 11 weeks, and brain samples were collected at terminal sacrifice at week 14. No haematological or clinical chemistry examinations were carried out, and no organ weights were measured.

There was a significant increase in mortality (eight animals) and signs of neurotoxicity (tonic-clonic seizures in four animals) at 1000 ppm. Therefore, the remaining animals at 1000 ppm (six from the last sacrifice group and the replacement group) were sacrificed on day 71. No effects on body weight or feed consumption were observed, and there were no macroscopic findings. Concentrations of lufenuron in blood, fat and brain were dose dependent, with equilibrium being reached by week 9 of the study. Lufenuron concentrations in fat were approximately 100-fold those in blood and brain (Table 9).

The no-observed-adverse-effect level (NOAEL) after 90 days in this dose range–finding study in female mice was 100 ppm (equal to 14.5 mg/kg bw per day), based on mortality and neurotoxicity at 1000 ppm (equal to 143 mg/kg bw per day) (Fankhauser, 1990).

***Table 9. Blood, fat and brain concentrations of lufenuron observed in a 3-month range-finding study in female mice given lufenuron in the diet***

| Dietary concentration (ppm) | Blood (mg/L) | | | Fat (mg/kg) | | | Brain (mg/kg) |
|---|---|---|---|---|---|---|---|
| | Week 9 | Week 11 | Week 14 | Week 9 | Week 11 | Week 14 | Week 14 |
| 0 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.08 |
| 4/8 | 0.13 | 0.36 | 0.52 | 10.8 | 29.9 | 49.9 | 0.41 |
| 20 | 0.87 | 0.9 | 1.45 | 81.7 | 76.5 | 81.6 | 0.65 |
| 100 | 5.02 | 5.24 | 5.05 | 488.7 | 499.5 | 487.8 | 5.19 |
| 1 000 | 48.5 | 43.4 | n.a. | 4 537.7 | 5 400 (all survivors) | n.a. | 187 (week 11) |

n.a.: not applicable, as this dose group was terminated in week 11; ppm: parts per million
*Source*: Fankhauser (1990)

*Rats*

In a 28-day range-finding study, groups of five male and five female Tif: RAIf (SPF) rats were administered lufenuron (purity 95.2%) in the diet at a concentration of 0, 50, 400, 3000 or 20 000 ppm (mean substance intakes: 0, 4.10, 30.8, 254 and 1692 mg/kg bw per day for males and 0, 4.07, 32.6, 254 and 1741 mg/kg bw per day for females, respectively). Investigations included clinical signs, body weights, feed consumption, haematology and clinical chemistry at termination, urine analysis, organ weights, gross necropsy and histopathological examination.

No mortality and no treatment-related clinical findings were observed. At weeks 3 and 4, the mean body weight gain and feed consumption of males fed 20 000 ppm lufenuron were reduced, not statistically significantly, by about 13% and 12–16%, respectively. Feed utilization values were not affected in any group. There were no statistically significant differences in water consumption between the groups.

There were no treatment-related differences between the groups in haematological or blood clinical chemistry parameters; some minor differences did achieve statistical significance, but these were representative of the normal variation of the respective parameters. No treatment-related differences were observed in urinary clinical chemistry parameters.

There were no treatment-related differences in macroscopic findings postmortem. After exsanguination, the mean body weight of males fed 20 000 ppm lufenuron was about 15% lower than that of the control group. The increased mean testis to body weight ratio and decreased liver weights seen in these males are therefore considered unrelated to treatment. Absolute and relative thymus weights of animals fed 3000 or 20 000 ppm lufenuron were reduced by about 13–30% compared with controls. The thymus weight of 400 ppm males was only slightly reduced (9%), and the statistical significance was due to a rather low standard deviation at 400 ppm compared with the one for controls. Moreover, the thymus weight at 400 ppm in males was within the range of controls. The reduced thymus weight at 400 ppm was therefore not considered to be adverse. In females fed 20 000 ppm lufenuron, slightly increased ovary weights were noted (Table 10). There were no treatment-related changes seen during histopathological examination.

The NOAEL for lufenuron when fed to rats continuously for a 28-day period was 400 ppm (equal to 30.8 and 32.6 mg/kg bw per day for males and females, respectively), based on decreased thymus weight at 3000 ppm (equal to 254 mg/kg bw per day for both males and females) (Fankhauser, 1988).

In a 90-day toxicity study, groups of 20 male and 20 female Tif: RAIf (SPF) rats were fed diets containing 0 or 15 000 ppm lufenuron (purity 95.2%), and 10 male and 10 female rats were fed diets containing 25, 150 or 1500 ppm lufenuron (purity 95.2%), for 92 or 93 consecutive days. Mean substance intakes were 0, 1.6, 9.68, 101 and 998 mg/kg bw per day for males and 0, 1.7, 10.2, 103 and 1050 mg/kg bw per day for females at 0, 25, 150, 1500 and 15 000 ppm, respectively. In each dose group, 10 animals of each sex per group were killed at the end of the treatment period. In the control and highest-dose groups, 10 animals of each sex were kept for a 4-week recovery period before sacrifice. Clinical observations, body weights, and feed and water consumption were measured throughout the study. Ophthalmological examinations were made on control and highest-dose animals pre-study and towards the end of the treatment period and recovery period. Haematology and blood chemistry analyses were carried out on all animals towards the end of the treatment period. At the end of the scheduled period, all animals were killed and subjected to a postmortem examination, selected organs were weighed and specified tissues were taken for subsequent histopathology.

One female fed 15 000 ppm lufenuron was found dead on day 98, after showing signs of emaciated condition and motor disorder. There were no other treatment-related mortalities. Tonic-clonic seizures were observed in 9/20 males and 8/20 females fed 15 000 ppm lufenuron and in 1/10 females fed 1500 ppm lufenuron. Two males still exhibited tonic-clonic seizures in the last week of the recovery period. There was no evidence of any effect of treatment on the eyes. The mean body weight gain of males from groups fed 1500 or 15 000 ppm lufenuron was decreased from week 2 until

*Table 10. Intergroup comparison of selected organ weights*

| | Absolute/relative (to body weight) organ weights | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | | | | Females | | | | |
| Organ | 0 ppm | 50 ppm | 400 ppm | 3 000 ppm | 20 000 ppm | 0 ppm | 50 ppm | 400 ppm | 3 000 ppm | 20 000 ppm |
| Thymus | | | | | | | | | | |
| Absolute (mg) | | | | | | | | | | |
| Mean | 792.9 | 749.5 | 725.0* | 597.5 | 556.1 | 524.2 | 434.8 | 455.1 | 405.0 | 393.9 |
| SD | 239.9 | 103.3 | 89.22 | 116.7 | 110.6 | 214.1 | 106.7 | 67.25 | 69.45 | 59.65 |
| Min | 530.5 | 639.8 | 637.9 | 471.2 | 447.5 | 338.7 | 313.8 | 354.9 | 316.4 | 332.4 |
| Max | 1 086 | 872.4 | 836.2 | 752.7 | 733.6 | 799.4 | 601.6 | 536.5 | 492.7 | 492.1 |
| Relative (%) | | | | | | | | | | |
| Mean | 2.530 | 2.451 | 2.337* | 2.008 | 2.091 | 2.665 | 2.311 | 2.309 | 2.332* | 2.109 |
| SD | 0.595 | 0.409 | 0.257 | 0.343 | 0.287 | 0.884 | 0.544 | 0.311 | 0.266 | 0.326 |
| Min | 1.882 | 1.919 | 1.993 | 1.660 | 1.792 | 1.933 | 1.616 | 1.767 | 2.105 | 1.781 |
| Max | 3.104 | 2.848 | 2.607 | 2.514 | 2.568 | 3.863 | 3.096 | 2.535 | 2.678 | 2.622 |
| Ovary/testis | | | | | | | | | | |
| Absolute (mg) | 3.305 | 3.573 | 3.430 | 3.382 | 3.517 | 171.3 | 161.0 | 166.7 | 158.5 | 192.8 |
| Relative (%) | 10.68 | 11.63 | 11.08* | 11.41 | 13.29* | 0.886 | 0.856 | 0.844 | 0.912 | 1.031 |
| Liver | | | | | | | | | | |
| Absolute (g) | 14.95 | 15.00 | 15.69 | 15.44 | 13.44 | 8.309 | 7.941 | 9.454 | 8.182 | 8.408 |
| Relative (%) | 48.30 | 48.61 | 50.32 | 51.96 | 50.74 | 43.13 | 42.16 | 48.02 | 47.25 | 44.92 |

Max: maximum; Min: minimum; ppm: parts per million; SD: standard deviation; *: $P < 0.05$
*Source*: Fankhauser (1988)

the end of treatment. At 15 000 ppm lufenuron, mean body weights comparable with control values were reached at the end of the recovery period. A similar tendency was seen in females at these dose levels. During the treatment period, slightly, but not statistically significantly, reduced feed consumption was seen in animals fed 1500 or 15 000 ppm lufenuron. A compensatory increase was seen in the animals retained for the recovery period. Water consumption was not affected in any group.

Haematocrit values and prothrombin time were increased slightly in females fed 1500 and 15 000 ppm lufenuron at the end of treatment, but were reversible within the recovery period. White blood cell counts in females fed 15 000 ppm lufenuron were at the upper end of the normal range at week 14 and at the end of the recovery period. Some changes in plasma phosphate and sodium concentrations and in alkaline phosphatase activity were seen in male and/or female animals fed 1500 or 15 000 ppm lufenuron; these changes were not reversible over the recovery period. Other minor changes in blood chemistry, which were reversible, were seen in cholesterol, albumin, protein and chloride levels and decreased albumin to globulin ratios. Alanine aminotransferase (ALAT) and alkaline phosphatase activities were slightly higher in females fed 15 000 ppm lufenuron. Other haematological and clinical chemistry parameters that achieved a statistically significant difference from control values were not biologically significant.

There were no treatment-related changes in macroscopic findings postmortem. Absolute and relative liver weights of females fed 15 000 ppm lufenuron were increased by 13–16% and 20–32% at the end of the treatment period and recovery period, respectively. In males at this dose, the ratio was increased by 13% at the end of the treatment period, with values comparable with control values after the recovery period. Adrenal weights in both sexes at this dose level were increased by 20–36%; in females at 1500 ppm lufenuron, they were increased by 11–16%. Adrenal weights were comparable with control values at all doses at the end of the recovery period. Other findings in organ weights that achieved a statistically significant difference from control values were not biologically significant. There were no treatment-related changes in microscopic findings postmortem.

In order to support the selection of dose levels for a 2-year toxicity and oncogenicity study and to study the accumulation of lufenuron in fat tissues, fat samples were collected at terminal sacrifice at week 14. The content of lufenuron in fat increased dose-dependently in the 25, 150 and 1500 ppm dose groups. The concentrations were similar at 1500 and 15 000 ppm, indicating that a solubility limit in fat had been reached (Table 11). Fat tissue concentrations were similar in males and females.

***Table 11. Fat concentrations of lufenuron in rats after a 3-month treatment***

| | **Concentrations of lufenuron (mg/kg fat)** | | | | |
|---|---|---|---|---|---|
| | **0 ppm** | **25 ppm** | **150 ppm** | **1 500 ppm** | **15 000 ppm** |
| Males | 3.2[a] | 47.2 | 378 | 3 025 | 2 710 |
| Females | 4.4[a] | 44 | 447 | 3 875 | 3 176 |

ppm: parts per million
[a] Most probably due to contamination of samples.
*Source*: Fankhauser (1989b)

Some of the control samples contained traces of the test article. Reconstruction of the sampling procedure and a confirmational analysis of leftover samples indicated that this was most probably due to contamination of the samples rather than a feeding error. The interpretation of the analytical results was not considered to be compromised by this finding.

The NOAEL for lufenuron in rats when fed continuously in the diet for a period of up to 3 months was 150 ppm (equal to 9.68 and 10.2 mg/kg bw per day for male and female animals, respectively), based on clinical signs (tonic-clonic seizures), decreased body weight gain and feed consumption, slight changes in haematology and clinical chemistry parameters, and increased adrenal weights at 1500 ppm (equal to 101 and 103 mg/kg bw per day for male and female animals, respectively) (Fankhauser, 1989b).

*Dogs*

In a 4-week range-finding study, groups of two male and two female Beagle dogs received lufenuron (purity 95.2%) in their diet at a concentration of 200 or 50 000 ppm (equal to 8.43 and 2200 mg/kg bw per day for males and 10.1 and 2648 mg/kg bw per day for females, respectively). Blood samples were taken pretest and at intervals during the study and were analysed for proof of absorption. All the dogs were examined daily for clinical condition and mortality. Detailed clinical examination, body weights and feed consumption were measured or recorded at intervals during the study. Haematological and clinical chemistry examinations of blood were performed pretest and after 2 and 4 weeks. At the end of the study, all the animals were killed humanely and given a macroscopic examination postmortem. Final body weights and selected organ weights were recorded. A comprehensive range of tissues was examined histopathologically.

There were no mortalities, and no clinical signs of toxicity were seen during the study. No abnormalities were detected during the detailed clinical and physical examinations. There was no

effect on body weight or feed consumption. There were no toxicologically significant changes in any of the haematological or clinical chemistry parameters examined. No macroscopic or microscopic abnormalities were detected, and there was no effect on organ weights. The mean levels of lufenuron found in the blood were 4 and 16 ppm, and those in the fat were 615 and 2750 ppm, at the low and high doses, respectively.

The NOAEL was 50 000 ppm (equal to 2200 and 2648 mg/kg bw per day for males and females, respectively), the highest dose tested (Briffaux, 1989a).

In another 4-week range-finding study, groups of two male and two female Beagle dogs received an oral dose of 50 mg/kg bw per day of a water-based veterinary preparation containing 15% lufenuron. According to the report, this was 5 times the anticipated therapeutic dose. No further information on the test formulation was provided in the report; hence, the exact composition remains unclear. The following information is provided in the European Union Draft Assessment Report (Portugal, 2006):

> The Notifier informed that the test formulation used was a pilot formulation for a commercial veterinary product whose recipe is proprietary information now belonging to another company. The Notifier assumed that the main component is water, with the addition of glycerine and avicel (a mixture of microcrystalline cellulose and carboxymethylcellulose sodium, which acts as a water insoluble dispersible colloidal excipient) and on this basis found it reasonable to believe that the toxicity seen in this study is due to the 15% lufenuron in the formulation, and not to any of the formulation additives.

All the dogs were examined daily for clinical condition and mortality. Detailed clinical examination, ophthalmology, body weights and feed consumption were measured or recorded at intervals during the study. Haematological and clinical chemistry examinations of blood were performed pretest and after 2 and 4 weeks. Blood samples were taken at intervals and analysed for lufenuron concentration. At the end of the study, all the animals were killed humanely and given a macroscopic examination postmortem. Final body weights and selected organ weights were recorded. Tissues were not examined histopathologically.

The levels of lufenuron in blood showed a steady increase during the 4-week study. After 1 week, they ranged from 0.57 to 7.51 µg/mL, after 2 weeks, from 1.63 to 9.80 µg/mL, and after 4 weeks, from 2.46 to 16.0 µg/mL. There were no mortalities, and no clinical signs of toxicity were seen during the study. No abnormalities were detected during the detailed clinical and physical examinations, including ophthalmology. There was no effect on body weight or feed consumption. Haematological examination showed that platelet counts were slightly elevated in the males at 2 and 4 weeks compared with the pretest values. No other haematological or blood chemistry changes were detected. No significant macroscopic abnormalities were seen, and there was no effect on organ weights.

In this 4-week range-finding study in dogs, the NOAEL of a veterinary lufenuron formulation with 15% active substance was 50 mg/kg bw per day (corresponding to a lufenuron dose of 7.5 mg/kg bw per day), the only dose tested (Pavkov & Macaskill, 1989).

In a 90-day toxicity study, groups of four male and four female Beagle dogs received lufenuron (purity 95.2%) in their diet at a concentration of 0, 200, 3000 or 50 000 ppm (equal to 0, 7.8, 121.6 and 2023 mg/kg bw per day for males and 0, 7.9, 122.5 and 1933 mg/kg bw per day for females, respectively). An additional two animals of each sex in the control and high-dose groups were given a 4-week recovery period after the 90-day dosing period. Clinical observations (including ophthalmoscopy), body weights, feed consumption, haematology and clinical biochemistry were measured throughout the study. At the end of the scheduled periods, the animals were killed and subjected to a postmortem examination. Bone marrow smears were taken, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no mortalities. No treatment-related clinical or ophthalmological findings were recorded. Feed consumption and body weight were not affected by treatment. No changes in haematology parameters attributable to a toxic effect of the compound were observed. A slight decrease in blood phosphorus level was observed in the 3000 and 50 000 ppm groups in both sexes at week 6 and in females at week 13. At week 17 (end of the recovery period), mean blood phosphorus levels were similar for control and 50 000 ppm animals. A slight decrease in blood potassium level was observed at week 13 in the 3000 and 50 000 ppm females, without a dose–response relationship. At week 17, mean blood potassium levels were similar for control and 50 000 ppm animals. A moderate to marked increase in total blood cholesterol level was observed at weeks 6 and 13 for both sexes at 3000 and 50 000 ppm. A moderate increase was still present at week 17 in the 50 000 ppm males, although this was not statistically significant. A moderate increase in alkaline phosphatase activity was seen at weeks 6 and 13 for females in the 200 or 3000 ppm groups and at week 13 for males in the 3000 ppm group, with no evidence of a clear dose–response relationship. At week 17, all individual alkaline phosphatase values were considered normal (Table 12). Statistically significant differences from controls were also seen in some glucose, albumin and transaminase values recorded, but these differences were considered not to be of toxicological significance and were not treatment related.

An apparent increase in the volume of urine associated with a decrease in specific gravity was seen at weeks 6, 13 and 17 in the 50 000 ppm males. When the values at pretest and in weeks 6, 13 and 17 were compared for individual dogs, no trends were evident. The semiquantitative estimations and the microscopic examination of the spun deposits in the urine did not reveal any treatment-related changes.

Absolute liver weights were increased at terminal sacrifice at week 13 in both sexes in the 3000 and 50 000 ppm groups (132% and 131% for males and 125% and 132% for females, respectively), reaching statistical significance only in 50 000 ppm males. Liver to terminal body weight ratios were increased statistically significantly at terminal sacrifice at week 13 in both sexes in the 3000 and 50 000 ppm groups (142% and 142% for males and 131% and 131% for females, respectively). Liver to brain weight ratios were statistically significantly increased in males at 50 000 ppm (135%). At the end of the recovery period, there was some evidence of reversibility, with none of the increases being statistically significant (Table 12).

No gross or microscopic changes were observed that correlated with the increased liver weights observed in the 3000 or 50 000 ppm dogs. There were no macroscopic or microscopic findings postmortem that could be attributed to treatment with lufenuron.

The NOAEL was 200 ppm (equal to 7.8 and 7.9 mg/kg bw per day for males and females, respectively), based on increased blood cholesterol levels and absolute and relative liver weights, reductions in blood potassium and phosphorus levels, and an increase in serum alkaline phosphatase activity for some animals at 3000 ppm (equal to 121.6 and 122.5 mg/kg bw per day for males and females, respectively) (Briffaux, 1989b).

In a 1-year toxicity study, groups of four male and four female Beagle dogs received lufenuron (purity 95.3%) in their diet at a concentration of 0, 100, 2000 or 50 000 ppm (equal to 0, 3.97, 65.4 and 1879 mg/kg bw per day for males and 0, 3.64, 78.3 and 1977 mg/kg bw per day for females, respectively). Clinical observations and feed consumption were recorded daily. Body weight was recorded weekly. A detailed veterinary examination and ophthalmoscopy were carried out at intervals during the study, and a range of haematological and clinical biochemistry (blood and urine) parameters was measured throughout the study. Lufenuron levels in the blood were analysed from all surviving dogs in weeks 34, 37 and 52. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Terminal blood and bone marrow samples were taken, selected organs were weighed and specified tissues were taken for subsequent histopathology examination. In order to support the interpretation of the results obtained in this study and to demonstrate saturation, the concentrations of the test article were determined in blood, fat and brain

***Table 12. Intergroup comparison of blood biochemistry (selected parameters) and liver weights in a 90-day dog study[a]***

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **Week** | **0 ppm** | **200 ppm** | **3 000 ppm** | **50 000 ppm** | **0 ppm** | **200 ppm** | **3 000 ppm** | **50 000 ppm** |
| Phosphorus (mg/L) | 0 | 75 | 77 | 72 | 71 | 70 | 71 | 72 | 70 |
| | 6 | 67 | 67 | 58** | 56*** | 59 | 60 | 52*** | 49*** |
| | 13 | 59 | 56 | 48 | 50 | 54 | 52 | 41* | 44** |
| | 17 | 50 | – | – | 53 | 47 | – | – | 46 |
| Potassium (meq/L) | 0 | 4.7 | 4.9 | 5.0 | 4.9 | 4.8 | 5.1 | 4.7 | 4.8 |
| | 6 | 4.6 | 4.9 | 4.6 | 4.7 | 4.8 | 5.1 | 4.7 | 4.8 |
| | 13 | 5.0 | 5.2 | 4.8 | 4.7 | 4.9 | 4.9 | 4.2* | 4.3* |
| | 17 | 4.3 | – | – | 4.7 | 4.7 | – | – | 4.5 |
| Cholesterol (g/L) | 0 | 1.37 | 1.28 | 1.36 | 1.39 | 1.23 | 1.29 | 1.43 | 1.30 |
| | 6 | 1.20 | 1.45 | 1.95* | 2.22*** | 1.35 | 1.48 | 2.17* | 2.13* |
| | 13 | 1.20 | 1.42 | 1.86* | 1.98** | 1.40 | 1.43 | 2.42* | 2.09* |
| | 17 | 1.12 | – | – | 1.93 | 1.43 | – | – | 1.64 |
| Alkaline phosphatase (mU/mL) | 0 | 284 | 244 | 253 | 248 | 244 | 263 | 294 | 241 |
| | 6 | 218 | 216 | 262 | 241 | 202 | 308* | 395** | 269 |
| | 13 | 168 | 168 | 260* | 247 | 174 | 279* | 674* | 291 |
| | 17 | 99 | – | – | 187 | 140 | – | – | 200 |
| Liver weight | | | | | | | | | |
| Absolute (g) | 13 | 257 | 299 | 338 | 337* | 258 | 247 | 323 | 340 |
| Relative (%) | | | | | | | | | |
| - To body | 13 | 2.6 | 3.0 | 3.7** | 3.7** | 2.9 | 2.9 | 3.8* | 3.8** |
| - To brain | 13 | 3.4 | 4.1 | 4.2 | 4.6** | 3.4 | 3.4 | 4.3 | 4.4 |
| Absolute (g) | 17 | 241 | – | – | 341 | 254 | – | – | 319 |
| Relative (%) | | | | | | | | | |
| - To body | 17 | 2.6 | – | – | 3.2 | 2.8 | – | – | 3.3 |
| - To brain | 17 | 3.4 | – | – | 4.4 | 3.5 | – | – | 4.5 |

eq: equivalents; ppm: parts per million; U: units; week 0: pretest value; –: no measurements; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

[a] Because of the number of animals killed at week 17, no statistical analysis was performed for the recovery period

*Source*: Briffaux (1989b)

samples. Blood samples were taken at 34, 37 and 52 weeks of feeding. At final sacrifice after 24 months, fat and brain tissues were sampled from all surviving animals.

Mortality was observed in the mid-dose group of 2000 ppm: one male died during week 33, and one male and one female were killed moribund during week 37. Neuromuscular signs were noted for these three animals. This generally consisted of tremors, salivation, dyspnoea, unsteady gait and convulsions. There were no other mortalities. Neuromuscular clinical signs were noted in 3/4 animals of each group fed with 2000 and 50 000 ppm after at least 20 weeks of treatment. These signs generally included stiffness, convulsions (tonic or clonic) and salivation, followed by tremors, decreased activity and unsteady gait. The incidence of these signs was biologically similar in the 2000

and 50 000 ppm dose groups. At 2000 ppm, three animals died, but in dogs fed 50 000 ppm, neuromuscular signs were transient and reversible. A very high incidence of vomiting was noted throughout the treatment period for one female dosed at 50 000 ppm. The ophthalmological examination did not reveal any treatment-related changes. A reduction in mean body weight gain was seen in the 50 000 ppm males and females. At 2000 ppm, a slight decrease in body weight and a reduced body weight gain were seen, particularly in the males. Feed consumption was not affected.

An increase in platelet count was noted in groups fed 2000 and 50 000 ppm. In comparison with pretest and control values, blood chemistry analyses showed that there was a dose-related increase in cholesterol at several time points in the 2000 and 50 000 ppm groups. Phospholipids were elevated in males dosed at 2000 and 50 000 ppm and in females dosed at 50 000 ppm. A dose–response relationship was evident within the males. Alkaline phosphatase activity was higher in the 2000 ppm dose group (males and females) and in the 50 000 ppm males. No statistically significant changes in ALAT activity were noted. However, in comparison with control animals, elevated ALAT values were noted in males and females of the 2000 ppm dose group and in males dosed at 50 000 ppm. For one female at 2000 ppm, the elevated value noted at week 13 was associated with an increase in gamma-glutamyltranspeptidase (GGT) activity. These elevated ALAT and GGT values were considered to be treatment related (Table 13).

Increases in mean adrenal weights (absolute and relative to body and brain weights) were noted in males and females at 2000 and 50 000 ppm. Increases in mean thyroid weights (absolute and relative to body and brain weights) were noted in males of all dose groups and in females at 2000 and 50 000 ppm. Increases in liver weights (absolute and relative to body and brain weights) were noted in males from all treated groups (not dose related) and in females at 2000 and 50 000 ppm (not dose related). This increase was statistically significant in males and females at 2000 and 50 000 ppm for the relative values and in females at 2000 ppm for the absolute values (Table 13).

At the end of the treatment period, an enlargement of the adrenals was noted in one female at 2000 ppm, and pale area(s) in the lungs were observed for two animals at 2000 ppm and for 50 000 ppm animals. Histopathological lesions that could be attributed to treatment were seen in the liver, thyroid, adrenals and lungs. Hepatocytic hypertrophy was present in dogs fed 2000 and 50 000 ppm, associated with an increased intracellular dark pigmentation of the hepatocytes. Cell necrosis was noted in one female fed 2000 ppm. In the thyroid, follicular dilatation with increased eosinophilic staining of the colloid was seen in one dog at 100 ppm (grading: minimal), three dogs at 2000 ppm (grading: minimal [two animals] to slight [one animal]) and five dogs at 50 000 ppm (grading: minimal [one animal] to slight [four animals]). Cortical hyperplasia of the adrenal was present in dogs fed 2000 and 50 000 ppm, the highest severity being noted at 2000 ppm. In the lungs, intra-alveolar histiocytosis was seen generally associated with cholesterol clefts at 2000 and 50 000 ppm.

The blood, fat and brain tissue concentrations of lufenuron were similar at 2000 and 50 000 ppm. Thus, the blood and tissues seemed to be saturated to a similar extent at both of these feeding levels. At the low feeding level of 100 ppm, the blood and tissue concentrations were lower by a factor of approximately 10. The similar blood and tissue levels in the 2000 and 50 000 ppm groups were in good agreement with the absence of a dose–response relationship for the majority of treatment-related findings. Major clinical signs (neuromuscular signs, convulsions) were seen after at least 20 weeks of treatment in three animals at 2000 ppm and in three animals at 50 000 ppm. Although the incidence was similar in both groups, the animals at 2000 ppm were more severely affected. At 50 000 ppm, neuromuscular signs were transient and reversible. In view of the small number of animals and the similar blood and tissue levels, mortality could have occurred in the 2000 ppm group and not in the 50 000 ppm group only by chance (Table 14).

At 100 ppm, a slight increase in liver weight without any associated histopathological changes was observed, and one dog showed a slight increase in thyroid weight and histopathological evidence of minimal dilatation of the follicles. Considering the nature and low severity of these changes and that the histopathological changes in the thyroid were seen in only one animal, the NOAEL was 100 ppm (equal to 3.97 mg/kg bw per day for males and 3.64 mg/kg bw per day for females), based on mortality, neuromuscular signs, including convulsions, reduced body weight gains

*Table 13. Haematology, clinical biochemistry and organ weights in a 52-week oral study in dogs*

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 ppm | 100 ppm | 2 000 ppm | 50 000 ppm | 0 ppm | 100 ppm | 2 000 ppm | 50 000 ppm |
| Platelet count | | | | | | | | |
| 13 | 353 | 360 | 397 | 598** | 325 | 356 | 383 | 421 |
| 26 | 317 | 343 | 448 | 629*** | 371 | 351 | 420 | 524 |
| 39 | 279 | 352 | 451* | 622*** | 317 | 354 | 417 | 532** |
| 52 | 289 | 363 | 532** | 643*** | 346 | 345 | 469 | 572** |
| Cholesterol (g/L) | | | | | | | | |
| 13 | 1.27 | 1.53 | 1.71 | 2.21* | 1.22 | 1.16 | 1.72 | 1.83 |
| 26 | 1.24 | 1.55 | 1.60 | 2.09* | 1.32 | 1.49 | 1.59 | 1.92 |
| 39 | 1.34 | 1.61 | 1.85 | 1.91 | 1.27 | 1.21 | 1.45 | 1.96 |
| 52 | 1.23 | 1.49 | 1.73 | 2.24* | 1.53 | 1.34 | 1.68 | 2.17* |
| Phospholipids (g/L) | | | | | | | | |
| 13 | 2.59 | 2.85 | 2.91 | 3.68* | 2.42 | 2.36 | 3.00 | 3.26 |
| 26 | 2.66 | 2.99 | 3.04 | 3.45 | 2.71 | 3.08 | 2.90 | 3.48 |
| 39 | 2.74 | 2.78 | 3.16 | 3.36 | 2.33 | 2.48 | 2.64 | 3.45* |
| 52 | 2.97 | 3.15 | 3.53 | 3.94 | 3.34 | 3.11 | 3.50 | 4.13 |
| Alkaline phosphatase (IU/L) | | | | | | | | |
| 13 | 197 | 242 | 178 | 247 | 240 | 170 | 272 | 251 |
| 26 | 131 | 206 | 150 | 227 | 154 | 130 | 478 | 208 |
| 39 | 116 | 170 | 207 | 235 | 153 | 124 | 347 | 222 |
| 52 | 110 | 169 | 173 | 281* | 154 | 151 | 323 | 254 |
| ALAT (IU/L) | | | | | | | | |
| 13 | 40 | 42 | 36 | 48 | 48 | 38 | 67 | 34 |
| 26 | 44 | 40 | 35 | 38 | 43 | 33 | 54 | 35 |
| 39 | 40 | 38 | 35 | 35 | 41 | 35 | 44 | 33 |
| 52 | 43 | 42 | 40 | 33 | 37 | 37 | 63 | 33 |
| Adrenal weight | | | | | | | | |
| Absolute (g) | 1.349 | 1.270 | 1.606 | 1.844* | 1.462 | 1.412 | 2.686** | 1.562 |
| Relative (%) | | | | | | | | |
| - To body | 0.017 6 | 0.016 8 | 0.022 1 | 0.023 7* | 0.019 7 | 0.020 4 | 0.036 7*** | 0.022 2 |
| - To brain | 0.012 7 | 0.013 0 | 0.016 9 | 0.019 6* | 0.016 6 | 0.015 8 | 0.031 9*** | 0.020 1 |
| Thyroid weight | | | | | | | | |
| Absolute (g) | 0.652 | 0.970 | 0.834 | 0.944 | 0.549 | 0.618 | 0.761 | 0.791 |
| Relative (%) | | | | | | | | |
| - To body | 0.008 4 | 0.012 7 | 0.011 3 | 0.012 2 | 0.007 4 | 0.008 9 | 0.010 5 | 0.011 2 |

| | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 ppm | 100 ppm | 2 000 ppm | 50 000 ppm | 0 ppm | 100 ppm | 2 000 ppm | 50 000 ppm |
| - To brain | 0.006 0 | 0.010 0 | 0.008 7 | 0.009 7 | 0.006 3 | 0.007 0 | 0.009 1 | 0.010 1 |
| Liver weight | | | | | | | | |
| Absolute (g) | 261.7 | 331.8 | 432.7 | 387.2 | 262.5 | 281.0 | 419.3** | 339.6 |
| Relative (%) | | | | | | | | |
| - To body | 3.41 | 4.34 | 5.94** | 4.96* | 3.57 | 4.05 | 5.75*** | 4.81** |
| - To brain | 2.42 | 3.42 | 4.54** | 4.03** | 3.01 | 3.13 | 4.96*** | 4.33*** |

ALAT: alanine aminotransferase; IU: International Units; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
*Source*: Briffaux (1992)

***Table 14. Blood, fat and brain concentrations in a 52-week oral toxicity study in dogs***

| Tissue | Week | Sex | 0 ppm | 100 ppm | 2 000 ppm | 50 000 ppm |
|---|---|---|---|---|---|---|
| Blood (µg/mL blood) | 34 | Males | < 0.1 | 5.8 | 31.7 | 29.2 |
| | | Females | < 0.1 | 4.3 | 29.7 | 26.7 |
| | 37 | Males | < 0.1 | 5.6 | 33.3 | 33.7 |
| | | Females | < 0.1 | 4.7 | 29.2 | 30.7 |
| | 52 | Males | < 0.1 | 5.1 | 42.5 | 55.5 |
| | | Females | < 0.1 | 6.3 | 34.7 | 41.7 |
| Fat (mg/kg tissue) | 52 | Males | 10.5[a] | 670 | 6 000 | 5 025 |
| | | Females | 9.2[a] | 797 | 4 433 | 4 925 |
| Brain (mg/kg tissue) | 52 | Males | < 2 | 15.5 | 250 | 242.5 |
| | | Females | < 2 | 9 | 282.5 | 197.5 |

ppm: parts per million
[a] Most probably due to contamination of samples.
*Source*: Briffaux (1992)

and changes in clinical pathology parameters, organ weights and histopathological lesions in adrenals, liver, thyroid and lungs observed at 2000 ppm (equal to 65.4 mg/kg bw per day for males and 78.3 mg/kg bw per day for females) (Briffaux, 1992).

In another 1-year toxicity study, groups of four male and four female Beagle dogs received lufenuron (purity 97.1%) in their diet at a concentration of 0, 10, 50, 250 or 1000 ppm (equal to 0, 0.31, 1.42, 7.02 and 29.8 mg/kg bw per day for males and 0, 0.33, 1.55, 7.72 and 31.8 mg/kg bw per day for females, respectively). Clinical observations and feed consumption were recorded daily. Body weight was recorded weekly. A detailed neurological examination and ophthalmoscopy were carried out at intervals during the study, and a range of haematological and clinical biochemistry (blood and urine) parameters was measured throughout the study. At the end of the scheduled period, the animals were killed and subjected to a postmortem examination. Terminal blood and bone marrow samples were taken, selected organs were weighed and specified tissues were taken for subsequent histopathological examination. In order to support the interpretation of the results obtained in this study and to demonstrate saturation, the concentrations of the test article were determined in blood, fat and brain samples. Blood samples were taken at 26 and 52 weeks of feeding. At final sacrifice after 24 months, fat and brain tissues were sampled from all surviving animals.

One female in the 1000 ppm group was found dead in week 31. Prior to death, this animal had shown marked clinical signs and deterioration of general state of health. A further one female and two males from this dose group were killed in weeks 28, 43 and 49 following clinical signs of convulsions. Treatment-related clinical signs were restricted to 1000 ppm animals that died or were killed prior to scheduled termination. These signs included convulsions, tremor, atactic gait, reduced locomotor activity, aggressiveness, nervousness, impeded respiration, vomiting and salivation. During the first third of the treatment period, body weight gain of treated animals was comparable with that of controls. After this time, loss in body weight was recorded for all 1000 ppm animals, resulting in an overall weight loss or depressed body weight gain. At the end of the treatment period, one male in the 250 ppm group lost weight from week 47 onwards (~5%: 10.4 kg in week 52 compared with ~11.0 kg during weeks 14–46), whereas the mean body weight of the 250 ppm group males in week 52 (12.00 kg) was comparable with the control value (12.08 kg). The results of the ophthalmoscopic examinations and measurement of neurological parameters showed no treatment-related effects.

Mean feed consumption for the treated groups was comparable with that of controls except for single periods of depressed feed intake in the 1000 ppm group caused by animals with clinical signs prior to early sacrifice. There were no statistically significant differences. Males and females in the 1000 ppm group had minimally but consistently higher platelet counts throughout the treatment period. Alterations of blood chemistry parameters were confined to the 1000 ppm animals. Higher activities of alkaline phosphatase were recorded in both sexes at weeks 26 and 52, and males also had higher GGT activities when measured at week 52. Plasma globulin levels were slightly increased in both sexes at the end of treatment and in males at week 26. Higher plasma cholesterol levels were observed in males at weeks 13, 26 and 52 and in females at week 52. Also, males had minimally lower plasma phosphate and thyroxine levels, whereas females showed higher plasma glucose levels at each assessment period during treatment (Table 15).

***Table 15. Haematological and blood chemistry findings in a 52-week oral toxicity study in dogs***

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter / week** | **0 ppm** | **10 ppm** | **50 ppm** | **250 ppm** | **1 000 ppm** | **0 ppm** | **10 ppm** | **50 ppm** | **250 ppm** | **1 000 ppm** |
| Platelets (g/L) | | | | | | | | | | |
| –1 | 264.8 | 287.8 | 295.0 | 268.5 | 249.8 | 254.3 | 267.8 | 284.0 | 247.0 | 298.8 |
| 13 | 248.5 | 294.0 | 299.0 | 259.0 | 314.0 | 272.0 | 292.8 | 283.0 | 301.3 | 364.8* |
| 26 | 243.0 | 354.8* | 327.0* | 337.8* | 350.6* | 294.8 | 322.3 | 308.0 | 299.8 | 454.5 |
| 52 | 274.5 | 381.3 | 311.5 | 386.3 | 405.0 | 305.3 | 307.8 | 356.0 | 383.0 | 654.0+ |
| Alkaline phosphatase (U/L) | | | | | | | | | | |
| –1 | 122.3 | 85.45 | 88.28 | 112.5 | 105.6 | 84.05 | 72.80 | 93.53 | 96.63 | 74.68 |
| 13 | 97.48 | 68.40 | 73.08 | 83.70 | 92.65 | 68.60 | 57.53 | 81.15 | 76.16 | 74.48 |
| 26 | 67.55 | 47.88 | 54.45 | 58.70 | 135.6* | 48.15 | 46.50 | 64.33 | 70.18 | 113.9*+ |
| 52 | 53.30 | 40.53 | 46.50 | 59.23 | 144.1 | 48.03 | 47.90 | 65.78 | 67.68 | 173.9 |
| GGT (U/L) | | | | | | | | | | |
| –1 | 1.375 | 2.000 | 1.375 | 3.375* | 0.725 | 1.375 | 2.200 | 0.550 | 2.950 | 2.925 |
| 13 | 4.450 | 4.325 | 4.625 | 3.500 | 4.800 | 4.175 | 3.800 | 4.300 | 4.175 | 4.625 |
| 26 | 4.700 | 4.150 | 4.050 | 3.625 | 4.550 | 4.150 | 3.225 | 3.625 | 4.313 | 5.338 |

| | Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter / week** | **0 ppm** | **10 ppm** | **50 ppm** | **250 ppm** | **1 000 ppm** | **0 ppm** | **10 ppm** | **50 ppm** | **250 ppm** | **1 000 ppm** |
| 52 | 2.850 | 2.700 | 3.325 | 3.025 | 5.100 | 1.750 | 3.350 | 3.350 | 3.888 | 3.650 |
| Globulin (g/L) | | | | | | | | | | |
| –1 | 21.01 | 22.72 | 21.34 | 21.28 | 22.71 | 21.18 | 20.76 | 21.60 | 19.45 | 20.50 |
| 13 | 24.02 | 25.10 | 23.05 | 23.86 | 24.47 | 22.70 | 23.34 | 22.21 | 21.87 | 22.17 |
| 26 | 24.44 | 25.23 | 23.99 | 24.22 | 27.02 | 22.38 | 23.57 | 22.62 | 21.83 | 22.36 |
| 52 | 25.89 | 28.09 | 25.14 | 26.95 | 30.61 | 23.88 | 25.95 | 24.99 | 24.25 | 27.40 |
| Cholesterol (mmol/L) | | | | | | | | | | |
| –1 | 3.475 | 3.408 | 3.630 | 3.593 | 4.061* | 3.305 | 3.170 | 3.135 | 3.203 | 3.245 |
| 13 | 3.868 | 3.720 | 3.873 | 3.885 | 4.985* | 3.513 | 3.708 | 3.588 | 3.515 | 3.805 |
| 26 | 3.840 | 3.113* | 3.855 | 3.998 | 5.063*+ | 3.320 | 3.735 | 3.558 | 3.413 | 3.863 |
| 52 | 3.610 | 3.193 | 3.688 | 3.875 | 4.735 | 3.458 | 3.450 | 3.465 | 3.460 | 4.465 |
| Phosphate (mmol/L) | | | | | | | | | | |
| –1 | 2.138 | 2.010 | 2.225 | 2.035 | 2.015 | 1.813 | 1.945 | 1.890 | 1.973 | 1.920 |
| 13 | 1.628 | 1.533 | 1.675 | 1.588 | 1.350* | 1.385 | 1.340 | 1.345 | 1.398 | 1.350 |
| 26 | 1.468 | 1.378 | 1.458 | 1.230* | 1.114*– | 1.306 | 1.298 | 1.298 | 1.124 | 1.045 |
| 52 | 1.185 | 1.275 | 1.175 | 1.083 | 0.810 | 1.063 | 1.115 | 0.858 | 0.985 | 0.828 |
| Thyroxine (nmol/L) | | | | | | | | | | |
| –1 | 37.84 | 36.73 | 31.25 | 33.32 | 35.00 | 33.48 | 35.06 | 36.10 | 32.35 | 41.65 |
| 13 | 35.20 | 32.26 | 28.40 | 32.40 | 27.03 | 33.50 | 35.15 | 35.27 | 32.54 | 31.02 |
| 26 | 33.75 | 34.37 | 27.00 | 25.17*– | 25.42– | 25.02 | 28.73 | 33.50 | 27.38 | 30.21 |
| 52 | 43.71 | 40.39 | 37.19 | 34.99 | 29.43 | 43.02 | 40.88 | 45.34 | 35.93 | 33.57 |
| Glucose (mmol/L) | | | | | | | | | | |
| –1 | 5.715 | 6.110 | 5.840 | 5.818 | 6.108 | 5.900 | 6.023 | 5.723 | 5.770 | 6.270 |
| 13 | 5.143 | 5.420 | 5.430 | 5.385 | 6.008*– | 5.065 | 5.455 | 5.213 | 5.365 | 6.050* |
| 26 | 5.420 | 5.323 | 5.495 | 5.350 | 6.185 | 5.275 | 5.953 | 5.560 | 5.665 | 8.820* |
| 52 | 5.120 | 5.035 | 5.145 | 5.203 | 6.200 | 5.235 | 5.558 | 5.490 | 5.278 | 6.480 |

GGT: gamma-glutamyltranspeptidase; ppm: parts per million; U: units; *: $P < 0.05$ (Wilcoxon); +/–: $P < 0.01$ (Jonckheere trend test)
*Source*: Altmann (1995)

There was a dose-related increase in both absolute and relative (to body weight) liver weights for both sexes in the 250 ppm group (absolute: 126% of control values for both sexes; relative: 129% and 135% of control values for males and females, respectively) and in the surviving animals in the 1000 ppm group (absolute: 147% and 141% of control values for males and females, respectively; relative: 164% and 172% of control values for males and females, respectively). Mean absolute and relative adrenal weights were increased in the surviving animals in the 1000 ppm group. In addition, a

tendency to an increase in adrenal weights was recorded in one male and one female treated with 250 ppm, but without pathological correlate. Mean thymus weight was slightly decreased in the surviving animals in both 1000 ppm groups.

At necropsy, the liver was described as enlarged in one dog of each sex in the 1000 ppm group, and enlarged adrenal glands were found in most animals in this group. Microscopically, a minimal to moderate hypertrophy of hepatocytes was found in males and females in the 250 and 1000 ppm groups. Pigmentation of liver Kupffer cells was increased in incidence and severity in the 1000 ppm males. A minimal to marked diffuse adrenal cortical hyperplasia was seen in all animals in the 1000 ppm group. In a few females in this group, cortical haemorrhage was also present. A minimal to moderate depletion of lymphocytes of the Peyer's patches showed an increased incidence in the small intestine of the 1000 ppm animals. Depletion of lymphocytes in the mesenteric lymph node showed an increased incidence and severity in both 1000 ppm groups. Moderate to marked thymic atrophy showed an increased incidence and severity in 1000 ppm females. Aggregation of alveolar foam cells was found in the lungs of both sexes at 1000 ppm. Other findings noted at microscopic examination were considered to be unrelated to treatment. No effects were observed on thyroid weight or thyroid histopathology (Table 16).

The blood, fat and brain concentrations were similar in males and females. The blood levels at week 52 were similar to or only slightly higher than those at week 26, indicating that a plateau was reached. The blood to fat ratio was approximately 1:100 or slightly higher. In the brains, the concentrations were similar to those in the blood at the two lowest feeding levels and up to about 5 times higher than those in the blood at the two highest dose levels (Table 17).

The effects observed at a dose level of 250 ppm were confined to body weight loss in one male, a tendency to increased adrenal weight in one male and one female, without pathological correlates, and increased absolute and relative liver weights in males and females with hypertrophy of hepatocytes. The liver effect should be seen as an adaptive response, in the absence of further histopathological damage and relevant clinical chemistry changes. The effects on body weight and adrenals at this dose had no effect on the group means.

The NOAEL was 250 ppm (equal to 7.02 mg/kg bw per day for males and 7.72 mg/kg bw per day for females), based on treatment-related mortality and clinical findings, effects on body weight and effects on the liver and adrenals, with associated histopathology and/or clinical chemistry changes, at 1000 ppm (equal to 29.8 mg/kg bw per day for males and 31.8 mg/kg bw per day for females) (Altmann, 1995).

*(b) Dermal application*

*Rats*

In a 28-day dermal toxicity study, groups of five male and five female albino rats (Tif: RAIf (SPF), hybrids of RII/1 × RII/2) were treated dermally with lufenuron (purity 95.2%) moistened with 0.5% carboxymethyl cellulose in 0.1% aqueous polysorbate 80 (wetting agent) at a dose level of 0, 100, 300 or 1000 mg/kg bw per day, 5 days/week, for 4 weeks. The exposure period was 6 hours/day under an occlusive dressing. Mortality and signs of systemic toxicity were determined daily, and signs of local skin irritation were determined approximately 17 hours after removing the gauze patches. Body weights and feed consumption were recorded weekly. Laboratory investigations (haematology and blood chemistry) were carried out on all surviving animals of each dose group at the end of the treatment period. At the end of the test period, all control and treated rats were subjected to detailed necropsy. Besides the weight of the exsanguinated body, the following organs were weighed: brain, heart, liver, kidneys, adrenals, thymus, ovaries/testes and spleen. Selected organs and tissues were preserved; the following samples were subjected to microscopic examination: skin application site, skin remote site, brain, liver, kidneys, adrenal glands, thymus and spleen (histopathological evaluation of paired organs was performed on both of them).

There were no systemic or local effects at any dose level. The NOAEL of the study was 1000 mg/kg bw per day, the highest dose tested (Schneider, 1990).

*Table 16. Pathology findings in a 52-week oral toxicity study in dogs*

| | Males ($n$ = 4) | | | | | Females ($n$ = 4) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Parameter** | **0 ppm** | **10 ppm** | **50 ppm** | **250 ppm** | **1 000 ppm** | **0 ppm** | **10 ppm** | **50 ppm** | **250 ppm** | **1 000 ppm** |
| Mortality (weeks) | 0 | 0 | 0 | 0 | 2 (43/49) | 0 | 0 | 0 | 0 | 2 (28/31) |
| Body weight (kg) | 11.56 | 10.88 | 11.10 | 11.44 | 10.53 | 10.64 | 10.14 | 10.42 | 9.905 | 8.740 |
| Liver | | | | | | | | | | |
| Liver weight | | | | | | | | | | |
| - Absolute (g) | 356.3 | 359.6 | 354.6 | 451.8 | 523.0* | 326.1 | 299.4 | 334.3 | 410.6* | 462.1 |
| - Relative (%) | 30.72 | 33.08 | 32.01 | 39.84 | 50.45 | 30.77 | 29.67 | 32.07 | 41.43* | 52.95+ |
| Enlarged liver | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| HH | 0 | 0 | 0 | 4 | 3 | 0 | 0 | 0 | 3 | 1 |
| KCP | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 |
| Adrenals (both) | | | | | | | | | | |
| Adrenal weight | | | | | | | | | | |
| - Absolute (g) | 1.470 | 1.461 | 1.583 | 1.732 | 2.485+ | 1.606 | 1.728 | 1.613 | 1.819 | 3.078 |
| - Relative (%) | 0.127 | 0.134 | 0.143 | 0.154 | 0.236+ | 0.151 | 0.173 | 0.155 | 0.184 | 0.356 |
| Enlarged adrenals | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 4 |
| ACH | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
| Thymus | | | | | | | | | | |
| Thymus weight | | | | | | | | | | |
| - Absolute (g) | 6.714 | 4.899 | 7.549 | 7.410 | 4.673 | 8.429 | 7.611 | 6.101 | 6.051 | 3.055 |
| Atrophy | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 3 | 3 | 4 |
| Thyroid | | | | | | | | | | |
| Thyroid weight | | | | | | | | | | |
| - Absolute (g) | 1.162 | 1.088 | 1.034 | 1.077 | 1.079 | 0.820 | 1.040 | 0.960 | 0.981 | 1.020 |
| - Relative (%) | 0.010 1 | 0.010 0 | 0.009 3 | 0.009 4 | 0.010 7 | 0.007 7 | 0.010 7 | 0.009 2 | 0.009 9 | 0.011 8 |
| Small intestine | | | | | | | | | | |
| HPP | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 3 |
| Mesenteric lymph node | | | | | | | | | | |
| PC | 4 | 3 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 |
| HLT | 0 | 1 | 0 | 0 | 3 | 1 | 1 | 0 | 1 | 3 |
| Lungs | | | | | | | | | | |
| AFC | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 1 |

ACH: adrenocortical hyperplasia; AFC: alveolar foam cells; HH: hypertrophy of hepatocytes; HLT: hypocellularity of lymphatic tissue; HPP: hypocellularity of Peyer's patch; KCP: Kupffer cell pigmentation; PC: phagocytic cells; ppm: parts per million; *: $P < 0.05$; +: $P < 0.01$

*Source*: Altmann (1995)

***Table 17. Blood, fat and brain concentrations in a 52-week oral toxicity study in dogs***

| Tissue | Week | 0 ppm | 10 ppm | 50 ppm | 250 ppm | 1 000 ppm |
|---|---|---|---|---|---|---|
| Blood (µg/mL blood) | 26 | < 0.1 | 0.19 | 1.7 | 10 | 41 |
| | 52 | < 0.1 | 0.18 | 1.9 | 24 | 66 |
| Fat (mg/kg tissue) | 52 | < 0.5 | 28 | 260 | 2 500 | 8 700 |
| Brain (mg/kg tissue) | 52 | < 0.1 | 0.2 | 1.9 | 62 | 340 |

ppm: parts per million
*Source*: Altmann (1995)

*(c) Exposure by inhalation*

No study was submitted.

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

*Mice*

In a chronic toxicity and carcinogenicity study, groups of 60 male and 60 female mice (Tif:MAGf (SPF), hybrids of NIH × MAG) received lufenuron (purity 96.2%) in their diets at a concentration of 0, 2, 20, 200 or 400 ppm (equal to 0, 0.222, 2.25, 22.6 and 62.9 mg/kg bw per day for males and 0, 0.217, 2.12, 22.0 and 61.2 mg/kg bw per day for females, respectively) for 18 consecutive months. As a result of high mortality in the high-dose group, surviving animals in this dose group were terminated in weeks 9 and 10. Samples were taken from selected animals at weeks 53 and 78 for haematology. Clinical chemistry parameters were not assessed. At termination, all surviving animals were subjected to a gross necropsy, and selected organs were weighed. Gross necropsies were performed for all animals that died prior to scheduled termination (found dead or euthanized moribund). Samples of selected tissues were processed for histopathological evaluation from all surviving test animals and from any unscheduled deaths that occurred during the study.

During the first 9 weeks of the study, five males and 29 females in the 400 ppm group were found dead. All survivors in this group were sacrificed early in weeks 9 and 10. The mortality recorded for the 2 and 20 ppm groups was similar to control values, but there was a higher mortality rate than for controls in the 200 ppm group. The percentage of animals surviving to termination at 18 months was 76%, 74%, 84% and 64% for the males and 82%, 82%, 88% and 48% for the females in the 0 (controls), 2, 20 and 200 ppm groups, respectively. Tonic-clonic convulsions, occurring spontaneously or in response to external stimuli such as handling, were exhibited by a number of animals of both sexes in the 200 and 400 ppm groups. The convulsions were of short duration (up to 30 seconds), during which time forelimb paddling, uncoordinated movement, jumping and straub tail occurred. Convulsions recorded in the other dose groups, including controls, were of a shorter duration and less intense (tonic phase only) and were regarded as spontaneous events, unrelated to treatment. No other clinical signs of reaction to treatment were observed.

No effects on body weight were recorded for either sex in the 2 and 20 ppm groups or for males in the 200 ppm group. Females in the 200 ppm group showed a small reduction in mean body weights (which did not attain statistical significance) compared with controls during the last 6 months of the study. Feed consumption for both sexes in the 2, 20 and 200 ppm groups was not adversely affected by treatment. No treatment-related haematological changes were noted in either sex at either 12 months or study termination (18 months). Slight increases in absolute and relative (to body weight) adrenal weights were observed in the 200 ppm females compared with controls (absolute weight: 113%; relative weight: 122%). However, all values were within the range recorded among controls, and microscopy revealed no morphological changes in this organ. Macroscopic postmortem examination revealed a higher incidence of single nodules of the lung in male mice of the 2 ppm group and of single and multiple nodules of the lung in male mice of the 200 ppm group compared

with controls. The incidence at 20 ppm was similar to that of controls, indicating no dose–response relationship.

Microscopic examination revealed higher incidences of fatty liver compared with controls, accompanied by necrotic changes in females of the 200 ppm group. Similar findings were seen in the 400 ppm group. Males in the 200 ppm group had a higher incidence of inflammatory changes in the prostate. Males in this group also showed an increased incidence, compared with controls, of both single and multiple lung adenomas, relating to the increased numbers of lung nodules seen macroscopically. The incidences observed across the treated groups in this study were not dose related, suggesting that the variation in incidence was spontaneous rather than as a result of treatment. In addition, the incidence of lung adenomas is known to be very variable in aged mice, and thus these isolated higher incidences are not considered to indicate an effect of treatment (Table 18).

***Table 18. Pathology findings in an 18-month oral toxicity and carcinogenicity study in mice***

| | **Incidence of finding** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Males** | | | | | **Females** | | | | |
| **Observation** | **0 ppm** | **2 ppm** | **20 ppm** | **200 ppm** | **400 ppm** | **0 ppm** | **2 ppm** | **20 ppm** | **200 ppm** | **400 ppm** |
| *Number examined* | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 |
| Lung | | | | | | | | | | |
| Adenoma | 12 | 19 | 4 | 19 | 0 | 8 | 5 | 8 | 7 | 0 |
| Liver | | | | | | | | | | |
| Fatty change | 42 | 41 | 38 | 53 | 37 | 46 | 39 | 46 | 56 | 52 |
| Recent necrosis | 4 | 3 | 1 | 0 | 0 | 8 | 6 | 8 | 13 | 20 |
| Hepatocellular necrosis | 4 | 10 | 7 | 7 | 1 | 5 | 2 | 5 | 12 | 6 |
| Prostate | | | | | | | | | | |
| Total inflammatory lesions | 3 | 6 | 4 | 10 | 1 | – | – | – | – | – |

ppm: parts per million
*Source*: Bachmann (1993a)

The NOAEL for systemic toxicity was 20 ppm (equal to 2.25 mg/kg bw per day for males and 2.12 mg/kg bw per day for females), based on increased mortality, clinical signs (tonic-clonic convulsive episodes), increased incidences of fatty liver (in females accompanied by necrotic changes) and a higher incidence of inflammatory changes in the prostate at 200 ppm (equal to 22.6 mg/kg bw per day for males and 22.0 mg/kg bw per day for females).

No treatment-related tumours were observed in male or female mice (Bachmann, 1993a).

*Rats*

In a combined chronic toxicity and carcinogenicity study in rats, groups of 80 male and 80 female Tif: RAIf (SPF) rats (70 of each sex in main groups, 10 of each sex in satellite groups) received lufenuron (purity 96.2%) in their diets at a concentration of 0, 5, 50, 500 or 1500 ppm (equal to 0, 0.19, 1.93, 20.4 and 108 mg/kg bw per day for males and 0, 0.23, 2.34, 24.8 and 114 mg/kg bw per day for females, respectively) for 104 weeks. As a result of overt toxicity at 1500 ppm, all animals in this group were terminated in week 14. Clinical observations (including ophthalmology), body weights, and feed and water consumption were measured throughout the study. Haematology, blood chemistry and urine analysis were carried out on selected animals periodically throughout the study

and at the end of the treatment period. At the end of the scheduled period, all animals were killed and subjected to a postmortem examination, selected organs were weighed and specified tissues were taken for subsequent histopathology. Samples of fat and blood were taken from all 1-year sacrifice animals and from selected surviving animals at study termination for analytical investigation, the results of which were reported as a separate study that was not submitted.

As a result of overt toxicity recorded in the 1500 ppm group, all animals in this group were terminated in week 14. The mortality rate in the remaining treated groups was similar to that of the controls. The percentage of animals surviving to termination at 18 months was 50%, 46%, 57% and 59% for the males and 51%, 57%, 59% and 61% for the females in the 0 (controls), 5, 50 and 500 ppm groups, respectively. A total of 46 males and 57 females in the 1500 ppm group and 47 males and 58 females in the 500 ppm group exhibited whole-body tonic-clonic convulsions from weeks 6 to 7. The convulsive episodes, lasting approximately 30–90 seconds, were observed mainly during and after handling and occurred repeatedly over several weeks in the majority of affected animals. As the study progressed, the incidence of observed convulsions in the 500 ppm animals diminished. A low frequency of spontaneous convulsive episodes was seen in the control and low-dose groups. Several females in the 500 ppm group had vaginal discharge during the latter part of the treatment period.

Males and females in the 1500 ppm group had body weights approximately 10% lower than those of controls in week 12 prior to early sacrifice. Slightly lower mean body weights were recorded for both sexes in the 500 ppm group up to week 27. Thereafter, an increased body weight gain in the females resulted in mean body weights approximately 20% higher than control values by the end of the study. The males in the 500 ppm group continued to have body weights slightly lower than control values to the end of the study (92% of controls). Higher feed intake, compared with controls, was recorded in week 1 for both sexes in the 500 and 1500 ppm groups (111–114% of controls). Thereafter, the feed intake of the 1500 ppm group tended to be lower than that of controls up to the early termination of this group. Lower feed intake, compared with controls, was recorded in the 500 ppm males for the next 26 weeks (95% of controls), after which time the feed intakes were similar to control values. Females in the 500 ppm group showed an 18% increase in feed intake from week 23 to termination.

The week 13 examination of the blood, 1 week prior to the termination of the 1500 ppm animals, showed 8/20 males in this group with platelet counts above the concurrent control range and 3/20 females with white blood cell counts above the control range. Other parameters in this group were not affected by treatment, and subsequent examinations revealed no evidence of a treatment-related influence on the haematological profile of treated rats. Slightly lower levels of plasma protein and albumin and higher levels of plasma potassium and inorganic phosphorus were recorded for females in the 1500 ppm group prior to termination of this group in week 14. No clear effect of treatment on the blood chemistry profile of males or females was seen in the 5, 50 or 500 ppm groups throughout the study. A number of differences between the mean values attained a level of statistical significance, but the differences were small and considered to be of no biological relevance. No effects on the urinary parameters were observed.

No treatment-related effects on organ weights were recorded in this study. Compared with the controls, levels of statistical significance were achieved for absolute and relative adrenal gland weights for females in the 50 and 500 ppm groups sacrificed at week 53. However, no changes were detected in the adrenals by microscopy. Therefore, the difference at week 53 was considered to have occurred by chance.

At macroscopy, higher incidences of mottled lungs in males and females of the 500 ppm group were considered to be treatment related. Microscopic examination revealed an increased incidence of pulmonary alveolar foam cells in both sexes of the 500 and 1500 ppm groups, with a higher severity level in the 500 ppm animals. This lesion was considered to be associated with a dilatation of the right heart ventricle developing due to increased pulmonary pressure caused by massive aggregations of foam cells in some females in the 500 ppm group.

Large adrenal glands were reported in 2/80, 5/80, 5/80 and 8/80 males and 3/80, 4/80, 2/80 and 11/80 females at 0, 5, 50 and 500 ppm, respectively. On microscopic examination, various

lesions, such as cysts, sinusoidal cystic dilatations, and hyperplasias or tumours of cortex or medulla, were found in these adrenals. The incidences of these lesions in the different groups did not indicate any treatment-related effect. The microscopic verification of some other macroscopic findings reported in higher numbers of treated animals revealed no treatment-related gross pathological effects.

An increased incidence of ulcerative and inflammatory lesions was seen in the non-glandular stomachs of males and females in the 500 ppm group. Focal haemorrhagic, necrotic, ulcerative and inflammatory lesions were found in the caecum and/or colon of both males and females in the 500 and 1500 ppm groups. An increased incidence of fatty change was seen in the perilobular region of the liver in females in the 500 ppm group. In addition, inflammation of the female urinary tract was markedly increased in 500 ppm animals (Table 19).

***Table 19. Selected microscopic findings in a 2-year oral toxicity and carcinogenicity study in rats***

| | **Incidence of finding** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Males (*n* = 80)** | | | | | **Females (*n* = 80)** | | | | |
| **Observation** | **0 ppm** | **5 ppm** | **50 ppm** | **500 ppm** | **1 500 ppm** | **0 ppm** | **5 ppm** | **50 ppm** | **500 ppm** | **1 500 ppm** |
| **Non-neoplastic findings** | | | | | | | | | | |
| Lung: foam cells | | | | | | | | | | |
| Minimal | 20 | 24 | 19 | 24 | 61 | 21 | 25 | 21 | 22 | 69 |
| Moderate | 12 | 12 | 10 | 19 | 6 | 14 | 15 | 16 | 24 | 5 |
| Marked | 5 | 4 | 5 | 11 | 0 | 8 | 3 | 6 | 19 | 0 |
| Heart: dilatation of right ventricle | 6 | 5 | 3 | 4 | 0 | 4 | 1 | 4 | 12 | 0 |
| Non-glandular stomach: ulceration | 0 | 0 | 1 | 4 | 0 | 1 | 0 | 1 | 2 | 0 |
| Stomach: chronic inflammation | 2 | 0 | 1 | 4 | 0 | 0 | 1 | 4 | 1 | 11 |
| Caecum/colon: lesions total count | 0 | 0 | 0 | 5 | 10 | 1 | 1 | 1 | 5 | 6 |
| Liver (centrilobular): fatty change | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 36 | 0 |
| Urinary bladder: inflammation | 10 | 5 | 3 | 1 | 0 | 1 | 2 | 2 | 22 | 0 |
| Kidney: inflammation of pelvis | 11 | 5 | 2 | 2 | 0 | 2 | 4 | 2 | 20 | 0 |
| **Neoplastic findings** | | | | | | | | | | |
| Testis: benign interstitial cell tumour | 2 | 2 | 1 | 5 | – | – | – | – | – | – |
| Cerebral meninges: benign granular cell tumour | 1 | 0 | 1 | 3 | – | 0 | 0 | 0 | 1 | – |

ppm: parts per million
*Source*: Bachmann (1993b)

No treatment-related increase in the incidence of hyperplastic or neoplastic lesions was found. The incidence of animals (*n* = 80) bearing primary tumours was 49, 52, 55, 42 and 0 for males and 57,

67, 54, 41 and 0 for females in the 0, 5, 50, 500 and 1500 ppm groups, respectively. Evidence of an apparently statistically significant dose-related effect at 500 ppm was shown for the benign interstitial cell tumour of testis found at incidences of 2/80 at 0 ppm, 2/80 at 5 ppm, 1/80 at 50 ppm and 5/80 (6.25%) at 500 ppm, as well as the benign granular cell tumour of the cerebral meninges found in male animals at incidences of 1/80 at 0 ppm, 0/80 at 5 ppm, 1/80 at 50 ppm and 3/80 (3.75%) at 500 ppm. Both tumour incidences were in line with the historical control data presented in the study report, even though the historical control data were not completely within JMPR standards (i.e. rat strain not specified and presented studies were from 1978 to 1989, which is not within 2 years of the in-life phase of the lufenuron study). The historical control data from the laboratory and from the Registry of Industrial Toxicology Animal-data (RITA) database show a variable incidence, with no obvious change in incidence over the period 1978–1989. On this basis, it can be considered that all these data are applicable historical control data for the lufenuron 2-year rat study, which started in January 1990 (see Appendix 1).

Treatment with lufenuron for 24 months resulted in whole-body tonic-clonic convulsions at 500 and 1500 ppm. The extent of the reaction showed that the maximum tolerated dose (MTD) was exceeded at the high dose level of 1500 ppm, and this group was terminated in week 14. At 500 ppm, an initially reduced body weight gain was observed, which in females reversed to a marked increase in body weights from week 27 onwards, associated with a marked increase in feed intake. Histopathological changes at 500 and/or 1500 ppm included an increase in the incidence of pulmonary alveolar foam cells, ulcerative and inflammatory lesions in the non-glandular stomach and focal lesions in the caecum and/or colon. Additionally, increased incidences of fatty change in the liver and inflammation of the urinary tract were detected in females. There was no evidence of a tumorigenic response.

The NOAEL for systemic toxicity was 50 ppm (equal to 1.93 mg/kg bw per day for males and 2.34 mg/kg bw per day for females), based on tonic-clonic convulsions, decreased body weight and (histo)pathological effects on lungs, liver, non-glandular stomach, intestines and urinary tract at 500 ppm (equal to 20.4 mg/kg bw per day for males and 24.8 mg/kg bw per day for females). There was no evidence of a tumorigenic response (Bachmann, 1993b).

### *2.4 Genotoxicity*

The results of studies of genotoxicity with lufenuron are summarized in Table 20. All the studies were certified to comply with GLP and performed according to internationally accepted guidelines. No evidence of genotoxicity was observed.

### *2.5 Reproductive and developmental toxicity*

#### *(a) Multigeneration studies*

In a two-generation study on reproductive toxicity, groups of 30 male and 30 female Sprague-Dawley-derived Tif: RAIf (SPF) rats received lufenuron (purity 96.2%) in their diets at a concentration of 0, 5, 25, 100 or 250 ppm over the course of two generations (P and $F_1$). Mean lufenuron intakes for combined P and $F_1$ generations during the premating period were 0, 0.41, 2.1, 8.3 and 20.9 mg/kg bw per day for males and 0, 0.44, 2.2, 8.9 and 22.2 mg/kg bw per day for females, respectively.

After 10 weeks, the animals were mated (1:1) within each dose group and allowed to rear the ensuing $F_1$ litters to weaning. Litters were culled to four male and four female pups, where possible, on day 4 postpartum. The breeding programme was repeated with the $F_1$ parents selected from the $F_1$ offspring. Test diets were fed continuously throughout the study. Parental feed consumption and body weights were measured throughout the study. Reproductive performance, pup survival and developmental parameters were measured. Gross necropsy findings and histopathological observations in target organs of parental animals and pups not selected for mating were recorded.

*Table 20. Results of studies of genotoxicity with lufenuron*

| End-point | Test object | Concentration | Purity (%) | Result | Reference |
|---|---|---|---|---|---|
| In vitro | | | | | |
| Reverse mutation | *Salmonella typhimurium* TA98, TA100, TA1535, T1537 | 20–5 000 µg/0.1 mL (±S9) | 95.2 | Negative | Deparade (1988) |
| Mammalian cell gene mutation | V79 Chinese hamster cells, HPRT test | 37.5–900 µg/mL (+S9) and up to 25–500 µg/mL (−S9) | 95.2 | Negative | Dollenmeier (1988) |
| Chromosomal aberration | CCL 61 CHO cells | 200–1 600 µg/mL (+S9) and up to 25–200 µg/mL (−S9) | 95.2 | Negative | Strasser (1989) |
| Unscheduled DNA synthesis | Male Tif: RAIf (SPF) rat hepatocytes | 2–2 000 µg/mL | 95.2 | Negative | Hertner (1988) |
| Unscheduled DNA synthesis | Human fibroblasts | 28.40–6 900 µg/mL | 95.2 | Negative | Meyer (1988) |
| Unscheduled DNA synthesis | Cultured human MRC-9 lung cells | 0.15–5 µg/mL (±S9) | 97.1 | Negative | Tanaka (1997) |
| In vivo | | | | | |
| Mouse micronucleus | Tif: MAGF, SPF mice | 0, 1 250, 2 500 or 5 000 mg/kg bw (gavage) | 95.2 | Negative | Meyer (1989a) |
| Unscheduled DNA synthesis | Male Tif: RAIf (SPF) rats | 0, 1 250, 2 500 or 5 000 mg/kg bw (gavage) | 97.1 | Negative | Hertner (1994) |
| Unscheduled DNA synthesis | Male HanIbM: WIST (SPF) rats | 0, 1 000 or 2 000 mg/kg bw (gavage) | 97.1 | Negative | Ogorek (2000) |

bw: body weight; CHO: Chinese hamster ovary; DNA: deoxyribonucleic acid; HPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* fraction from Aroclor 1254 or phenobarbital/5,6-benzoflavone–induced pretreated rat liver homogenate

There were no mortalities or treatment-related clinical observations in either generation of parents. There was an increased incidence of skin wounds/crust on the head/trunk in the $F_1$ male 250 ppm group. Body weights and feed consumption were unaffected by treatment for the P parents. Body weights of the $F_1$ 250 ppm animals of both sexes were significantly higher than control values during the premating period. Feed consumption was also slightly higher than control values during this period in the same groups, indicating that the increased body weight was a direct result of increased feed consumption.

For both generations, male and female mating and fertility indices, maternal gestation and parturition indices, and duration of gestation were unaffected by treatment. There were no treatment-related observations at gross necropsy in either generation of adults. No effects on organ weights were seen in the P parents. Organ weights were higher in the 250 ppm male and female $F_1$ parents, consistent with the higher body weights in this group, but there were no statistically significant differences from control values in the organ to body weight ratios in this group. There were no treatment-related histopathological changes in the reproductive system or in any organs examined in both the P and $F_1$ parental generations.

In both the $F_1$ and $F_2$ offspring, live births, postnatal survival indices and litter weights were unaffected by treatment. The mean time of appearance of the surface righting reflex was slightly

delayed by about 0.2–0.4 day in the 250 ppm group compared with controls for both generations. There were no treatment-related observations at postmortem examination in the $F_1$ or $F_2$ offspring.

In this study, the highest dose did not cause any toxicity, which is required according to OECD Test Guideline 416. However, from the 2-year rat study, it is clear that 1500 ppm was above the MTD (animals euthanized in week 14 because of the extent of tonic-clonic convulsions) and 500 ppm was the lowest-observed-adverse-effect level (LOAEL), based on, for example, tonic-clonic convulsions. A highest dose level of 250 ppm is therefore considered acceptable for this two-generation reproductive toxicity study.

The NOAEL for parental and reproductive effects was 250 ppm (equal to 20.9 mg/kg bw per day for males and 22.2 mg/kg bw per day for females), the highest dose tested.

The NOAEL for offspring toxicity was 100 ppm (equal to 8.3 mg/kg bw per day for males and 8.9 mg/kg bw per day for females), based on the slight delay of 0.2–0.4 day in righting reflex in pups at 250 ppm (equal to 20.9 mg/kg bw per day for males and 22.2 mg/kg bw per day for females) (Fitzgerald & Khalil, 1992).

*(b) Developmental toxicity*

*Rats*

In a study of developmental toxicity, groups of 25 mated female Sprague-Dawley (Crl:CD® (SD) BRVAF/Plus™) rats were administered lufenuron (purity 94.7%) at a dose of 0, 100, 500 or 1000 mg/kg bw per day in 3% cornstarch and 0.5% Tween 80 via oral gavage on gestation day (GD) 6 to GD 15 (inclusive). On day 21 of gestation, the rats were killed and examined for macroscopic changes in the oral cavity and all organs of the thoracic and abdominal cavities. The ovaries and uterus with cervix were removed, trimmed, weighed intact and examined. Corpora lutea were counted. The uterus was opened and examined for the number and distribution of live fetuses and intrauterine deaths. All fetuses were weighed, sexed and examined for external variations and malformations. Live fetuses were killed, and half of the fetuses from each litter were eviscerated and subsequently processed and examined for skeletal variations and malformations. The remaining fetuses from each litter had the heads removed and fixed in Bouin's fixative for examination of the structure by the serial cross-section technique. The trunks of these fetuses were given an internal examination of the soft tissue, then eviscerated and processed for skeletal examination.

Twenty-five sperm-positive female rats were assigned to the 0, 100, 500 or 1000 mg/kg bw per day dose levels, of which 22, 23, 19 and 25 animals, respectively, were pregnant. No animals died prior to scheduled sacrifice, and no significant treatment-related signs of toxicity were seen in the individual clinical observations recorded. Minimal maternal toxicity occurred at 1000 mg/kg bw per day, with slight, but statistically significant, reductions in body weight gain on GDs 7–9 and in feed consumption on GDs 6–9.

All pregnant females had live fetuses at termination, and no animals delivered prior to scheduled sacrifice. No embryo/fetal toxicity was apparent at any of the dose levels tested. Statistically significant reductions in the mean number of implantations per dam at 100 mg/kg bw per day and in the number of live fetuses per dam at 100 and 1000 mg/kg bw per day were considered not to be adverse effects of treatment. The reduction at 100 mg/kg bw per day reflected a slight reduction in the number of corpora lutea (developed before treatment), and the less than 4% reduction in mean number of live fetuses at 1000 mg/kg bw per day, compared with concurrent controls, exceeded the mean values for controls from the previous five studies.

The historical control data were presented in the study report as data from control CD rats. No further details were provided; the data were apparently from the performing laboratory, but the exact rat strain is not stated.

There were no significant increases in the test groups, compared with controls, in the occurrences of external, soft tissue or skeletal observations either singularly or collectively when classified as variations or malformations. The mean number of malformed live fetuses in treated

groups was similar to control values. Malformations were observed in six, six, three and four fetuses from five, four, two and four litters in the 0, 100, 500 and 1000 mg/kg bw per day groups, respectively. All of these malformations were considered to have occurred spontaneously.

The NOAEL for maternal toxicity was 500 mg/kg bw per day, based on a transient reduction in body weight gain on GDs 7–9 and in feed consumption on GDs 6–9 at 1000 mg/kg bw per day.

The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested. Lufenuron did not show teratogenic potential in rats (Gilles, 1989).

*Rabbits*

In a study of developmental toxicity, groups of 16 mated Hra: New Zealand White SPF female rabbits were dosed by gavage at 0, 100, 500 or 1000 mg/kg bw per day with lufenuron (purity 94.7%) in aqueous 3% cornstarch and 0.5% Tween 80 on GD 7 to GD 19 (inclusive). The females were monitored daily for clinical signs. Body weights and feed consumption were recorded at selected intervals during gestation. On day 29 of gestation, the rabbits were killed and examined for macroscopic changes in all organs of the thoracic and abdominal cavities. The ovaries and uterus with cervix were removed, trimmed, weighed intact and examined. Corpora lutea were counted. The uterus was opened and examined for the number and distribution of live fetuses and intrauterine deaths. All fetuses were weighed and examined for external variations and malformations. Each fetus was examined for both soft tissue and skeletal malformations and variations. A transverse section of the brain was made, and organ structure was examined. The trunks of the fetuses were given an internal examination of the soft tissue, sexed, and then eviscerated and processed for skeletal examination.

One female in the control group died on day 18, and one female in the 100 mg/kg bw per day group was found dead on day 11. Both of these deaths were a result of trauma induced by gavage dosing. Pregnancy was observed at scheduled sacrifice in 13, 15, 12 and 15 females in the 0 (controls), 100, 500 and 1000 mg/kg bw per day dose groups, respectively. There was no treatment-related effect on fertility. The fertility index was 88%, 100%, 75% and 94% in the 0 (controls), 100, 500 and 1000 mg/kg bw per day dose groups, respectively. No significant treatment-related signs of toxicity were seen in the individual clinical observations recorded, and there were no treatment-related effects on body weight, body weight gain or feed consumption. At necropsy of the dams, there were no findings that appeared to be treatment related, and no effects of treatment on mean absolute or relative organ weights were observed.

All surviving pregnant females had live fetuses on GD 29. There were no statistically significant changes in fetal weights or other intrauterine parameters. There was no significant increase, relative to controls, in the occurrence of any external, soft tissue or skeletal malformation or variation in fetuses from does treated with lufenuron.

The NOAEL for maternal toxicity and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Meyer, 1989b).

### *2.6 Special studies*

*(a) Neurotoxicity*

In a repeated-dose neurotoxicity study, groups of 10 male Tif: RAIf (SPF) rats received lufenuron (purity 96.2%) in their diet at 0, 5, 25, 100 or 500 ppm (equal to 0, 0.26, 1.22, 5.43 and 27.0 mg/kg bw per day, respectively) for 4 months. An additional 10 males in the control and high-dose groups served as recovery animals. The investigation was limited to males, as previous studies conducted in rats showed that males were as sensitive as or slightly more sensitive than females to the effects of lufenuron. Animals were observed daily except for weekends throughout the study. Detailed clinical observations were not made “blind” (trained observers unaware of the actual treatment group at reading). No data were presented to provide evidence of the ability to detect and quantify, as appropriate, changes in the different end-points recommended for observation, such as autonomic signs, sensory reactivity, limb grip strength and motor activity (use of positive controls).

Ophthalmological examination was not carried out in this study, but data are already available from other studies of similar duration and at similar dose levels. Any observable gross changes were apparently not recorded. The deviations are considered not to compromise the scientific validity of the study. In five animals per dose group, the lufenuron concentrations in fat and blood were determined.

There were no treatment-related mortalities during the study. In the 500 ppm group, single episodes of hyper-reactivity to handling, fasciculations and tonic-clonic convulsions were observed in one animal each between weeks 13 and 18. There were no other compound-related clinical signs in the treated animals. Mean body weight and feed consumption were comparable for all groups over the whole study period. No compound-related effects were noted in any group throughout the study period in any of the neurology parameters measured. Motor activity parameters were not affected in any group, and mean startle habituation reactions were comparable for all groups throughout the study. The maze learning error scores did not differ between controls and treated groups at the end of the treatment period or at the end of the recovery period.

Following intraperitoneal injection with 25 mg/kg bw of pentylenetetrazol at the end of the treatment period, generalized tonic-clonic convulsions involving the hindlimbs were induced in top-dose animals only. Comparable motor seizures limited to the head, neck and forelimb areas were induced in all other groups. After a generalized tonic-clonic convulsion, subsequent seizure activity was inhibited, whereas motor seizures were shown frequently throughout the 30-minute observation period. At the end of the recovery period, the incidence rate for generalized convulsions in top-dose animals was comparable with that seen at the end of the treatment period. Median convulsion scores in the top-dose animals were lower than at the end of the treatment period, but still higher than in control animals, thus indicating partial recovery from the proconvulsive effect induced by the test article.

At the end of the treatment period, the concentrations of lufenuron in fatty tissue were 16, 150, 660 and 2600 mg/kg tissue for the 5, 25, 100 and 500 ppm groups, respectively. Corresponding concentrations in whole blood were 0.1, 0.6, 2.6 and 17 mg/L, indicating a preferential accumulation in fat. In top-dose animals, concentrations of 1600 mg/kg in fat and 4.3 mg/L in blood were measured at the end of the 2-month recovery period.

No treatment-related macroscopic or microscopic changes were seen in the central or peripheral nervous system or in muscles.

The NOAEL for systemic toxicity was 500 ppm (equal to 27.0 mg/kg bw per day), the highest dose tested. The NOAEL for neurotoxicity was 100 ppm (equal to 5.43 mg/kg bw per day), based on spontaneous tonic-clonic convulsions or fasciculations observed in weeks 13–18 and facilitated pentylenetetrazol-induced generalized convulsions at 500 ppm (equal to 27.0 mg/kg bw per day), (Classen, 1992).

*(b) Mechanistic studies*

A study was performed to determine the effects of treatment of lufenuron in the diet for 3 weeks on the estrous cycle in female rats and various plasma hormone levels in male and female rats. Forty-five rats (Crj: Sprague-Dawley (SD)) of each sex were randomly allocated to three groups: 0, 500 or 1500 ppm (equal to 0, 30.5 and 92.5 mg/kg bw per day for males and 0, 39.4 and 120.1 mg/kg bw per day for females, respectively). All animals were checked twice a day for mortality and clinical signs. Body weights were recorded at the start of the study and weekly thereafter. Two-day feed consumption per cage was measured weekly, and mean daily feed consumption values per rat were calculated. Vaginal smears were taken each morning from 10 females per group. Proestrous, estrous, metestrous and diestrous stages were determined, and vaginal cytology was examined. Each smear was scored for the relative density of cornified and nucleated cells. At necropsy, 10 mL blood samples were collected under ether anaesthesia from the abdominal aorta of 10 animals per group for analysis of plasma hormone levels (estradiol, progesterone, corticosterone, aldosterone, prolactin, luteinizing hormone [LH], follicle stimulating hormone [FSH], adrenocorticotropic hormone [ACTH] and testosterone). The left ovaries, adrenals and testes from 10 animals per group were taken for the

analysis of hormone levels in tissue. Gross pathology of these animals consisted of macroscopic examination of the organs and tissues in the thoracic and abdominal cavities and removal of uterus, ovaries, vagina, testes, adrenals and pituitary for weighing and/or processing for microscopic examination. After 3 weeks of administration of test diet, cholinesterase activity in plasma erythrocyte and brain samples from five rats per group was determined.

No deaths or changes of general condition related to lufenuron administration were observed. With the exception of body weight gain retardation in the 1500 ppm females at week 3 (96% of controls), there were no differences in mean body weight of either sex between the groups given lufenuron and the control groups. There was no difference between the feed consumption of test and control animals of both sexes.

No statistically significant differences in mean estrous cycle length were found between the treated groups and controls, although there were two cases in the 500 ppm group of 7 and 10 days' length, respectively, and one case in the 1500 ppm group of 4.6 days' length, demonstrating prolongation. There was no statistically significant difference in the relative density of cornified and nucleated epithelial cells between the treated and control animals. Statistically significant elevation of mean prolactin, FSH and ACTH levels was noted for the 1500 ppm male group. For prolactin, 8/10 animals in the 1500 ppm group had plasma levels that were within the control range; therefore, the higher mean value for this group was not considered to be biologically significant. The slightly higher FSH levels in the 1500 ppm group were also not considered large enough to be biologically significant. The increase in ACTH levels is relatively small and may indicate a stress response in these animals that is unrelated to treatment. No other statistically significant differences in hormone levels were found between any of the groups given lufenuron and controls (Table 21).

***Table 21. Hormone level data: selected parameters in a 3-week mechanistic study in rats***

| | **Hormone levels** | | | | | |
|---|---|---|---|---|---|---|
| | **Males** | | | **Females** | | |
| **Parameter** | **0 ppm** | **500 ppm** | **1 500 ppm** | **0 ppm** | **500 ppm** | **1 500 ppm** |
| Prolactin (ng/mL) | 20.200 | 22.635 | 33.420* | 85.610 | < 80.460 | 95.870 |
| FSH (MIU/mL) | 19.4 | 21.64 | 22.47** | 18.26 | 16.72 | 21.50 |
| ACTH (pg/mL) | 41.35 | 50.95 | 67.88** | 115.22 | 130.57 | 118.29 |
| Corticosterone (ng/mL) | 295.30 | 325.15 | 336.46 | 950.10 | 977.20 | 953.30 |

ACTH: adrenocorticotropic hormone; FSH: follicle stimulating hormone; ppm: parts per million; IU: international units; *: $P < 0.05$; **: $P < 0.01$
*Source*: Tamano (1997)

No gross pathological abnormalities were observed in any of the groups, other than inner dilatation of the uterus in one rat in the 1500 ppm group. Significantly lower relative uterus weights were noted in the 500 ppm group compared with controls. This was unlikely to be treatment related, as the effect was slight, and there was no dose dependence. There were no statistically significant differences between test and control animals in the weights of the ovaries, testes, adrenals or pituitary.

Angiectasis in the adrenals was observed in one to three female rats in each group and one male rat fed 1500 ppm lufenuron. Fatty metamorphosis in the adrenals was also noted in one or two male rats in each group. Inner dilatation of the uterus was observed in one rat in each of the control and 1500 ppm groups. No abnormal histopathological findings were noted for the pituitary, ovaries, vagina or testes in any of the groups.

No statistically significant differences in any of the cholinesterase activity samples were noted between the groups of rats given lufenuron and the controls.

The results of this investigation, which focused on the pituitary, adrenal and genital organs, suggest that there is no effect of lufenuron on the endocrine system in rats of either sex. This conclusion is supported by the reproductive toxicity study in rats, which showed no effect of lufenuron on any reproductive end-point (Tamano, 1997).

## 3. Observations in humans

The sponsor submitted the following information:

Manufacturing employees in Switzerland are medically examined by a company physician at the beginning of their employment and then routinely once a year according to the criteria of the Swiss Accident Insurance Institution (SUVA). Routine medical examinations include:

- Anamnesis
- Physical examination including blood pressure
- Blood analysis: hemoglobin, erythrocytes, leukocytes, thrombocytes, complete blood count, blood sedimentation rate, blood sugar, blood pressure, cholesterol, triglycerides, ALAT, ASAT, alkaline phosphatase, bilirubin, creatinine, uric acid
- Urine analysis

The Occupational Health group of Syngenta (formerly ICI/Zeneca Agrochemical) has maintained a database of incidents involving chemical exposure of workers since 1983. Since 1994, data has been collected on the occurrence of occupationally related illnesses from all our manufacturing, formulation and packaging sites around the world. Analysis of the Syngenta internal database confirmed that no cases of adverse reactions that related to the handling of lufenuron have been reported up to July 2015.

In conclusion, lufenuron has been handled in large quantities over the last 22 years at a number of sites. With the use of appropriate control strategies, no adverse health effects associated with the material have been reported in the workforce.

In companion animals lufenuron is used for flea control. From 1995-1997, 57 incidents of human exposure involving accidental ingestion and/or therapeutic mishaps ("<1/4 tablet" to 409mg (1.0–26.4mg/kg)) were reported to 2 poison centres in the US. At the time of the call a 15 month old male vomited within 2 hours, without further symptoms and a 13 year old female experienced diarrhoea that resolved within 8 hours. The other patients remained asymptomatic for up to 2 hours post ingestion. Lufenuron has shown no significant toxicity in humans from the limited amounts ingested to date. (Ref: J. of Toxicol Clin Toxicol, Vol.36, No.5, pp. 491)

A breast feeding mother accidentally ingested an anti-flea preparation of lufenuron. The infant was exposed to an average dose of 0.032 mg/kg bw per day, which is only 3% of the reported acute overdose. No adverse effects were reported during 7 months of follow-up. (Ref: Bar-Oz (2000), J. of Human Lactation, 16 (3), 229-30)

## Comments

### Biochemical aspects

Lufenuron is only partially absorbed following a single oral dose, with the extent of absorption being dose related; approximately 20% of a single 100 mg/kg bw dose appears to be absorbed, compared with about 70% of a single 0.1 or 0.5 mg/kg bw dose. A large proportion of the absorbed dose partitions into fat, with very much lower uptake by other tissues, including the brain. All tissue concentrations of radioactivity increased to a maximum 1 day after the last of 14 repeated low doses. The results suggest that most tissue concentrations would plateau within 2–3 weeks of similar repeated dosing. The fat depot is slowly released, with a terminal half-life of up to 5–13 days at 0.5 mg/kg bw and 10–37 days at 100 mg/kg bw, leading to an increase in concentrations of lufenuron in the brain over long periods (see below). Excretion of the absorbed dose is predominantly via faeces, with only about 1% of the dose being excreted in urine, independent of the dose.

Metabolism of lufenuron is minimal, with only about 1% of an oral dose being metabolized by deacylation followed by cleavage of the ureido group. There is no marked sex difference in absorption, tissue distribution, metabolism or excretion. The pattern of excretion and metabolism is not affected by repeated dosing (Bissig, 1990; Thanei, 1990; Müller, 1995; Okada, 1997; Hassler, 2003; Booth, 2004).

**Toxicological data**

Lufenuron has low acute toxicity when administered orally or dermally ($LD_{50}$ > 2000 mg/kg bw) or via inhalation ($LC_{50}$ > 2.35 mg/L, maximal attainable concentration) to rats (Hartmann, 1988a,b,c, 1989). Lufenuron produced very slight skin and eye irritation in rabbits and is considered to be a skin sensitizer in the guinea-pig (Schneider, 1988a,b,c).

The most significant toxicological end-point for lufenuron is convulsions, observed after prolonged treatment at high dose levels. Convulsions are observable in all species treated with lufenuron at daily doses of more than 20 mg/kg bw for extended periods (2–3 months in rodents, > 3 months in dogs). As lufenuron is a very lipophilic compound (log octanol–water partition coefficient [$K_{ow}$] = 5.12), it has the potential to accumulate in fatty tissues. It has been shown in toxicity studies with rats, mice and dogs that following prolonged exposure to high doses of 20 mg/kg bw per day or more, fat compartments may become saturated. If exposure is continued after saturation occurs, concentrations in the brain increase, leading to tonic-clonic convulsions.

In a dose range–finding study, lufenuron was fed to mice at a concentration of 0, 1000, 3000 or 9000 ppm (equal to 0, 151, 449 and 1470 mg/kg bw per day for males and 0, 189, 517 and 1440 mg/kg bw per day for females, respectively) for up to 65 days. As a result of mortality and neurotoxic effects of the test substance (tonic-clonic seizures) at all dose levels, it was concluded that the MTD was exceeded even at 1000 ppm (equal to 151 mg/kg bw per day) (Fankhauser, 1989a).

In a second dose range–finding study, intended for test substance residue and blood level determination, lufenuron was fed to female mice at a concentration of 0, 4/8, 20, 100 or 1000 ppm (equal to 0, 0.47/1.1, 2.94, 14.5 and 143 mg/kg bw per day, respectively) for up to 91 days (only 71 days for the high dose). From day 57 onwards, the diet of the low-dose group inadvertently contained 8 ppm instead of 4 ppm. The NOAEL was 100 ppm (equal to 14.5 mg/kg bw per day), based on mortality and neurotoxicity (tonic-clonic seizures) at 1000 ppm (equal to 143 mg/kg bw per day) (Fankhauser, 1990).

In a 28-day range-finding study, rats were administered lufenuron in the diet at a concentration of 0, 50, 400, 3000 or 20 000 ppm (equal to 0, 4.10, 30.8, 254 and 1692 mg/kg bw per day for males and 0, 4.07, 32.6, 254 and 1741 mg/kg bw per day for females, respectively). The NOAEL was 400 ppm (equal to 30.8 mg/kg bw per day), based on decreased thymus weight at 3000 ppm (equal to 254 mg/kg bw per day) (Fankhauser, 1988).

In a 90-day toxicity study, rats were fed diets containing 0, 25, 150, 1500 or 15 000 ppm lufenuron (equal to 0, 1.6, 9.68, 101 and 998 mg/kg bw per day for males and 0, 1.7, 10.2, 103 and 1050 mg/kg bw per day for females, respectively). The NOAEL was 150 ppm (equal to 9.68 mg/kg bw per day), based on clinical signs (tonic-clonic seizures), decreased body weight gain and feed consumption, slight changes in haematology and clinical chemistry parameters and increased adrenal weights at 1500 ppm (equal to 101 mg/kg bw per day) (Fankhauser, 1989b).

In a 4-week range-finding study, dogs received lufenuron in their diet at a concentration of 200 or 50 000 ppm (equal to 8.43 and 2200 mg/kg bw per day for males and 10.1 and 2648 mg/kg bw per day for females, respectively). The NOAEL was 50 000 ppm (equal to 2200 mg/kg bw per day), the highest dose tested (Briffaux, 1989a).

In a 90-day toxicity study, dogs received lufenuron in their diet at a concentration of 0, 200, 3000 or 50 000 ppm (equal to 0, 7.8, 121.6 and 2023 mg/kg bw per day for males and 0, 7.9, 122.5 and 1933 mg/kg bw per day for females, respectively). The NOAEL was 200 ppm (equal to 7.8 mg/kg bw per day), based on increases in blood cholesterol levels and absolute and relative liver weights,

reductions in blood potassium and phosphorus levels, and an increase in serum alkaline phosphatase activity for some animals at 3000 ppm (equal to 121.6 mg/kg bw per day) (Briffaux, 1989b).

In a 1-year toxicity study, dogs received lufenuron in their diet at a concentration of 0, 100, 2000 or 50 000 ppm (equal to 0, 3.97, 65.4 and 1879 mg/kg bw per day for males and 0, 3.64, 78.3 and 1977 mg/kg bw per day for females, respectively). The main target organs were the brain, adrenals, liver, thyroid and lungs. The NOAEL was 100 ppm (equal to 3.64 mg/kg bw per day), based on mortality, neuromuscular signs, including convulsions, reduced body weight gains, changes in clinical pathology parameters and histopathological lesions in adrenals, liver, thyroid and lungs observed at 2000 ppm (equal to 65.4 mg/kg bw per day) (Briffaux, 1992).

In another 1-year toxicity study, dogs received lufenuron in their diet at a concentration of 0, 10, 50, 250 or 1000 ppm (equal to 0, 0.31, 1.42, 7.02 and 29.8 mg/kg bw per day for males and 0, 0.33, 1.55, 7.72 and 31.8 mg/kg bw per day for females, respectively). The NOAEL was 250 ppm (equal to 7.02 mg/kg bw per day), based on treatment-related mortality and clinical findings, including convulsions, effects on body weight and effects on the liver and adrenals, with associated histopathology and/or clinical chemistry changes, at 1000 ppm (equal to 29.8 mg/kg bw per day) (Altmann, 1995).

An overall NOAEL of 250 ppm (equal to 7.02 mg/kg bw per day) can be identified on the basis of the two 1-year dog studies. The 90-day dog study should not be included in the overall NOAEL, as the observed effects (blood parameters and liver weights) are far less severe than the effects in the 1-year dog studies (e.g. mortality) at similar dose levels. This can be explained by the fat accumulation, which is not yet saturated in the 90-day study; this leads to higher concentrations of the parent compound in the brain in the longer-term studies.

In an 18-month dietary toxicity and carcinogenicity study, mice received lufenuron at a concentration of 0, 2, 20, 200 or 400 ppm (equal to 0, 0.222, 2.25, 22.6 and 62.9 mg/kg bw per day for males and 0, 0.217, 2.12, 22.0 and 61.2 mg/kg bw per day for females, respectively). As a result of high mortality in the high-dose group, surviving animals in this dose group were terminated in weeks 9 and 10. The NOAEL was 20 ppm (equal to 2.12 mg/kg bw per day), based on increased mortality, clinical signs (tonic-clonic convulsive episodes), increased incidences of fatty liver (in females accompanied by necrotic changes) and a higher incidence of inflammatory changes in the prostate at 200 ppm (equal to 22.0 mg/kg bw per day). No treatment-related tumours were observed (Bachmann, 1993a).

In a 2-year dietary toxicity and carcinogenicity study, rats received lufenuron at a concentration of 0, 5, 50, 500 or 1500 ppm (equal to 0, 0.19, 1.93, 20.4 and 108 mg/kg bw per day for males and 0, 0.23, 2.34, 24.8 and 114 mg/kg bw per day for females, respectively). As a result of overt toxicity at 1500 ppm, all animals in this group were terminated in week 14. The NOAEL was 50 ppm (equal to 1.93 mg/kg bw per day), based on clinical signs (tonic-clonic convulsions), decreased body weight and (histo)pathological effects on lungs, liver, non-glandular stomach, intestines and urinary tract at 500 ppm (equal to 20.4 mg/kg bw per day). No treatment-related tumours were observed (Bachmann, 1993b).

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

Lufenuron was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that lufenuron is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that lufenuron is unlikely to pose a carcinogenic risk to humans.

In a two-generation study on reproductive toxicity, rats received lufenuron in their diet at a concentration of 0, 5, 25, 100 or 250 ppm (equal to 0, 0.41, 2.1, 8.3 and 20.9 mg/kg bw per day for males and 0, 0.44, 2.2, 8.9 and 22.2 mg/kg bw per day for females, respectively, based on mean intakes for combined P and $F_1$ generations during the premating period). The NOAEL for parental and reproductive effects was 250 ppm (equal to 20.9 mg/kg bw per day), the highest dose tested. The

NOAEL for offspring toxicity was 100 ppm (equal to 8.3 mg/kg bw per day), based on the slight delay in righting reflex in pups at 250 ppm (equal to 20.9 mg/kg bw per day) (Fitzgerald & Khalil, 1992).

In a study of developmental toxicity, rats were administered lufenuron via gavage at a dose of 0, 100, 500 or 1000 mg/kg bw per day. The NOAEL for maternal toxicity was 500 mg/kg bw per day, based on a transient reduction in body weight gain on GDs 7–9 and feed consumption on GDs 6–9 at 1000 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Gilles, 1989).

In a study of developmental toxicity, rabbits were dosed at 0, 100, 500 or 1000 mg/kg bw per day with lufenuron via gavage. The NOAEL for maternal toxicity and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Meyer, 1989b).

The Meeting concluded that lufenuron is not teratogenic.

In a repeated-dose neurotoxicity study, male rats received lufenuron in their diet at 0, 5, 25, 100 or 500 ppm (equal to 0, 0.26, 1.22, 5.43 and 27.0 mg/kg bw per day, respectively) for 4 months. No systemic toxicity was observed. The NOAEL for neurotoxicity was 100 ppm (equal to 5.43 mg/kg bw per day), based on spontaneous tonic-clonic convulsions or fasciculations observed in weeks 13–18 and facilitated pentylenetetrazol-induced generalized convulsions at 500 ppm (equal to 27.0 mg/kg bw per day) (Classen, 1992).

Convulsions are observed in all species after prolonged treatment with lufenuron, owing to saturation of the accumulation in fatty tissues, with subsequent increased lufenuron levels in the brain. The neurotoxic effects are not expected to occur after a single dose. The Meeting concluded that lufenuron is not acutely neurotoxic, but is neurotoxic after prolonged treatment.

A study was performed to determine the effects of treatment of lufenuron for 3 weeks on the estrous cycle in female rats and various plasma hormone levels (estradiol, progesterone, corticosterone, aldosterone, prolactin, LH, FSH, ACTH and testosterone) in male and female rats administered a dietary concentration of 0, 500 or 1500 ppm (equal to 0, 30.5 and 92.5 mg/kg bw per day for males and 0, 39.4 and 120.1 mg/kg bw per day for females, respectively). The results of this investigation, focused on the pituitary, adrenal and genital organs, suggest that there is no effect of lufenuron on the endocrine system in rats of either sex (Tamano, 1997). This conclusion is supported by the reproductive toxicity study in rats, which showed no effect of lufenuron on any reproductive end-point (Fitzgerald & Khalil, 1992).

No specific studies on immunotoxicity were submitted. The available repeated-dose studies do not indicate an immunotoxic potential for lufenuron following exposure by the oral route.

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

No metabolites of concern were identified.

**Human data**

In reports on manufacturing plant personnel, no adverse health effects were noted. Several incident reports indicate no significant toxicity in humans.

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

## Toxicological evaluation

An acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established on the basis of the NOAEL of 1.93 mg/kg bw per day for tonic-clonic seizures and findings in lungs, gastrointestinal tract, liver and urinary tract in the 2-year dietary study in rats, using a safety factor of 100.

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

***Levels relevant to risk assessment of lufenuron***

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| Mouse | Eighteen-month study of toxicity and carcinogenicity[a] | Toxicity | 20 ppm, equal to 2.12 mg/kg bw per day | 200 ppm, equal to 22.0 mg/kg bw per day |
| | | Carcinogenicity | 400 ppm, equal to 61.2 mg/kg bw per day[b] | – |
| Rat | Two-year study of toxicity and carcinogenicity[a] | Toxicity | 50 ppm, equal to 1.93 mg/kg bw per day | 500 ppm, equal to 20.4 mg/kg bw per day |
| | | Carcinogenicity | 1 500 ppm, equal to 108 mg/kg bw per day[b] | – |
| | Two-generation study of reproductive toxicity[a] | Reproductive toxicity | 250 ppm, equal to 20.9 mg/kg bw per day[b] | – |
| | | Parental toxicity | 250 ppm, equal to 20.9 mg/kg bw per day[b] | – |
| | | Offspring toxicity | 100 ppm, equal to 8.3 mg/kg bw per day | 250 ppm, equal to 20.9 mg/kg bw per day |
| | Developmental toxicity study[c] | Maternal toxicity | 500 mg/kg bw per day | 1 000 mg/kg bw per day |
| | | Embryo and fetal toxicity | 1 000 mg/kg bw per day[b] | – |
| | Four-month neurotoxicity study[a] | Neurotoxicity | 100 ppm, equal to 5.43 mg/kg bw per day | 500 ppm, equal to 27.0 mg/kg bw per day |
| Rabbit | Developmental toxicity study[c] | Maternal toxicity | 1 000 mg/kg bw per day[b] | – |
| | | Embryo and fetal toxicity | 1 000 mg/kg bw per day[b] | – |
| Dog | One-year studies of toxicity[a,d] | Toxicity | 250 ppm, equal to 7.02 mg/kg bw per day | 1 000 ppm, equal to 29.8 mg/kg bw per day |

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

*Estimate of acceptable daily intake (ADI)*

0–0.02 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 lufenuron***

| | |
|---|---|
| *Absorption, distribution, excretion and metabolism in mammals* | |
| Rate and extent of oral absorption | ~70% within 24 h at 0.1 or 0.5 mg/kg bw; ~20% within 24 h at 100 mg/kg bw |
| Dermal absorption | No data |
| Distribution | Widely distributed; highest concentrations in fat; a plateau within 2–3 weeks of dosing is suggested for all tissues. Brain levels are initially low, but rise after prolonged exposure due to saturation of fat storage. |
| Potential for accumulation | High fat accumulation, with slow release (terminal half-life 5–13 days at 0.5 mg/kg bw and 10–37 days at 100 mg/kg bw) |
| Rate and extent of excretion | Predominantly in faeces, with < 1% in urine, independent of dose |
| Metabolism in animals | Minimal |
| Toxicologically significant compounds in animals and plants | Parent compound |
| *Acute toxicity* | |
| Rat, $LD_{50}$, oral | > 2 000 mg/kg bw |
| Rat, $LD_{50}$, dermal | > 2 000 mg/kg bw |
| Rat, $LC_{50}$, inhalation | > 2.35 mg/L, maximal attainable concentration |
| Rabbit, dermal irritation | Mildly irritating |
| Rabbit, ocular irritation | Mildly irritating |
| Guinea-pig, dermal sensitization | Sensitizing (Magnusson and Kligman maximization test) |
| *Short-term studies of toxicity* | |
| Target/critical effect | Mortality and neurotoxicity (tonic-clonic convulsions) |
| Lowest relevant oral NOAEL | 7.02 mg/kg bw per day (1 year; dog) |
| Lowest relevant dermal NOAEL | 1 000 mg/kg bw per day (28 d; highest dose tested; rat) |
| Lowest relevant inhalation NOAEC | No data |
| *Long-term studies of toxicity and carcinogenicity* | |
| Target/critical effect | Neurotoxicity (tonic-clonic convulsions), (histo)pathological findings in lungs, liver, non-glandular stomach, intestines and urinary tract |
| Lowest relevant NOAEL | 1.93 mg/kg bw per day (2 years; rat) |

| | |
|---|---|
| Carcinogenicity | Not carcinogenic in mice or rats[a] |
| *Genotoxicity* | |
| | No evidence of genotoxicity[a] |
| *Reproductive toxicity* | |
| Target/critical effect | Slight delay in righting reflex in pups |
| Lowest relevant parental NOAEL | 20.9 mg/kg bw per day (highest dose tested; rat) |
| Lowest relevant offspring NOAEL | 8.3 mg/kg bw per day (rat) |
| Lowest relevant reproductive NOAEL | 20.9 mg/kg bw per day (highest dose tested; rat) |
| *Developmental toxicity* | |
| Target/critical effect | Transient reduction in maternal body weight gain and feed consumption (rat) |
| Lowest relevant maternal NOAEL | 500 mg/kg bw per day (rat) |
| Lowest relevant embryo/fetal NOAEL | 1 000 mg/kg bw per day (rat, rabbit; highest dose tested) |
| *Neurotoxicity* | |
| Acute neurotoxicity NOAEL | No evidence of acute neurotoxicity |
| Subchronic neurotoxicity NOAEL | 5.43 mg/kg bw per day (4 months; rat) |
| Developmental neurotoxicity NOAEL | No data |
| *Other toxicological studies* | |
| Immunotoxicity | No data |
| Studies on toxicologically relevant metabolites | No metabolites of concern were identified |
| *Medical data* | |
| | No evidence of adverse effects in personnel exposed to lufenuron; several incident reports indicate no significant toxicity in humans |

[a] Unlikely to pose a carcinogenic risk to humans from the diet.

***Summary***

| | **Value** | **Study** | **Safety factor** |
|---|---|---|---|
| ADI | 0–0.02 mg/kg bw | Two-year toxicity and carcinogenicity study (rat) | 100 |
| ARfD | Unnecessary | – | – |

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## Appendix 1. Historical control data for 2-year rat study

***Table A-1. Historical control data for benign interstitial cell tumour of testis in the 2-year rat study***

| Laboratory database | | | | RITA database for SD rats, 1985–1995, 2-year oral studies | | | |
|---|---|---|---|---|---|---|---|
| **Study number** | **Date of first dose** | **Incidence of benign interstitial cell tumours** | **Number of testes examined** | **Study number** | **Date of first dose** | **Incidence of benign interstitial cell tumours** | **Number of testes examined** |
| 785271 | Oct 78 | 2 | 90 | 1 | Mar 85 | 4 | 68 |
| 801440 | Mar 81 | 6 | 80 | 40 | Sep 88 | 2 | 50 |
| 800218 | Jun 82 | 1 | 58 | 45 | Jul 89 | 2 | 55 |
| 820872 | Aug 82 | 1 | 79 | 46 | Jul 86 | 2 | 49 |
| 820650 | Apr 83 | 2 | 80 | 47 | Feb 89 | 4 | 71 |
| 82648 | Oct 83 | 0 | 79 | 50 | Sep 89 | 1 | 55 |
| 821482 | Oct 83 | 2 | 79 | 52 | Jul 86 | 1 | 50 |
| 820559 | Feb 84 | 1 | 80 | 58 | Nov 87 | 0 | 50 |
| 821638 | Apr 84 | 3 | 70 | 62 | Aug 87 | 6 | 60 |
| 830732 | Nov 84 | 2 | 79 | 70 | Jul 86 | 2 | 48 |
| 840171 | Mar 85 | 4 | 78 | 96 | Nov 93 | 4 | 60 |
| 850335 | Feb 86 | 6 | 80 | 101 | Oct 92 | 0 | 55 |
| 850853 | May 86 | 1 | 80 | 102 | Oct 92 | 1 | 55 |
| 874076 | May 87 | 1 | 50 | 106 | Nov 93 | 1 | 60 |
| 860087 | Sep 87 | 3 | 80 | 111 | Jul 95 | 5 | 59 |
| 850703 | Jan 88 | 1 | 79 | 140 | Oct 94 | 1 | 60 |
| 876047 | May 88 | 2 | 70 | 141 | Oct 94 | 2 | 60 |
| 861139 | Feb 89 | 4 | 80 | | | | |
| 861154 | Mar 89 | 0 | 80 | | | | |
| 886178 | Aug 89 | 2 | 70 | | | | |

| Laboratory database | | | | RITA database for SD rats, 1985–1995, 2-year oral studies | | | |
|---|---|---|---|---|---|---|---|
| **Study number** | **Date of first dose** | **Incidence of benign interstitial cell tumours** | **Number of testes examined** | **Study number** | **Date of first dose** | **Incidence of benign interstitial cell tumours** | **Number of testes examined** |
| 901463 | Dec 90 | 4 | 80 | | | | |
| 891326 | Feb 91 | 1 | 60 | | | | |
| 901483 | May 92 | 2 | 60 | | | | |
| 911123 | Aug 92 | 0 | 60 | | | | |

RITA: Registry of Industrial Toxicology Animal-data; SD: Sprague-Dawley

***Table A-2. Historical control data (laboratory database) for benign granular cell tumour of the cerebral meninges in the 2-year rat study***

| Study number | Date of first dose | Incidence of benign granular cell tumours in the meninges | Number of organs examined |
|---|---|---|---|
| 785271 | Oct 78 | 0 | 88 |
| 800218 | Jun 82 | 0 | 60 |
| 801440 | Mar 81 | 0 | 79 |
| 820559 | Feb 84 | 0 | 80 |
| 820648 | Oct 83 | 1 | 78 |
| 820650 | Apr 83 | 0 | 80 |
| 820872 | Aug 82 | 0 | 80 |
| 821482 | Oct 83 | 1 | 79 |
| 821638 | Apr 84 | 1 | 68 |
| 830732 | Nov 84 | 1 | 77 |
| 840171 | Mar 85 | 1 | 78 |
| 850703 | Jan 88 | 3 | 80 |
| 860087 | Sep 87 | 3 | 80 |
| 861139 | Feb 89 | 4 | 80 |
| 861154 | Mar 89 | 1 | 80 |
| 874076 | May 87 | 2 | 50 |
| 876047 | May 88 | 2 | 70 |
| 886178 | Aug 89 | 0 | 70 |