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TEFLUBENZURON page 99-139

Toxicological evaluation of certain veterinary drug residues in food

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Teflubenzuron

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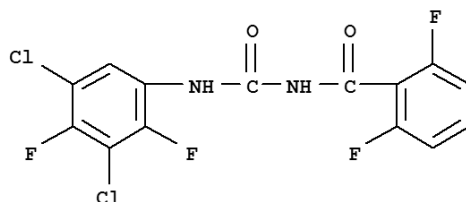
1. Explanation	100
2. Biological data	101
2.1 Biochemical aspects	101
2.1.1 Absorption, distribution and excretion	101
(a) Rats	101
2.1.2 Biotransformation	104
2.2 Toxicological studies	107
2.2.1 Acute toxicity	107
(a) Lethal doses	107
(b) Dermal irritation	109
(c) Ocular irritation	109
(d) Dermal sensitization	109
2.2.2 Short-term studies of toxicity	110
(a) Mice	110
(b) Rats	110
(c) Dogs	111
2.2.3 Long-term studies of toxicity and carcinogenicity	113
(a) Mice	113
(b) Rats	116
2.2.4 Genotoxicity	118
2.2.5 Reproductive and developmental toxicity	120
(a) Multigeneration reproductive toxicity	120
(b) Developmental toxicity	121
2.2.6 Special studies	124
(a) Haematological toxicity	124
(b) Effects on mammalian cells in vitro	125
(c) Microbiological effects	125
2.2.7 Dose–response analysis	125
2.3 Observations in humans	126
3. Comments	126
3.1 Biochemical data	126
3.2 Toxicological data	128
3.3 Microbiological data	135
4. Evaluation	135
5. References	136

1. Explanation

Teflubenzuron (International Union of Pure and Applied Chemistry name: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea; Chemical Abstracts Service no. 83121-18-0; Fig. 1) is an insecticide belonging to the benzoylurea group of compounds. Its mode of action is through inhibition of the synthesis of chitin, and hence it is most effective on the developmental stages of insects. Insects are killed as a result of the disruption of their moulting. Teflubenzuron is approved in many countries as an insecticide for use in plant production as well as for control of sea lice (*Lepeophtheirus salmonis* and *Caligus rogercresceyi*) in aquaculture. Teflubenzuron is used as a premix coated onto non-medicated fish feed pellets to achieve an intended dose of 10 mg/kg body weight (bw) per day for 7 consecutive days. The withdrawal periods range from 7 to 11 days and from 45 to 96 degree-days.

Fig. 1

Chemical structure of teflubenzuron



Teflubenzuron has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), although it was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) as a pesticide in 1994 and 1996 (FAO/WHO, 1995, 1997) and is scheduled for periodic re-evaluation at JMPR's September 2016 meeting. JMPR established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw on the basis of a lowest-observed-adverse-effect level (LOAEL) of 2.1 mg/kg bw per day in a mouse carcinogenicity study with the application of an uncertainty factor of 200, including an additional factor of 2 to account for the use of a LOAEL instead of a no-observed-adverse-effect level (NOAEL).

The Committee evaluated teflubenzuron at the present meeting at the request of the Twenty-second Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2015), with a view to establishing an ADI and recommending maximum residue limits (MRLs) in finfish tissues.

For this assessment, a data package was submitted by a sponsor. Additionally, the following databases of published literature were searched using the search term “teflubenzuron”: Agricola (1970 – September 2015), CAB

Abstracts (1973 – 2015 week 38), Embase (1974 – September 2015), Food Science and Technology Abstracts (1969 – 2015 September week 3), Global Health (1973 – 2015 week 38), International Pharmaceutical Abstracts (1970 – September 2015) and Medline (1946 – September 2015). In total, 876 articles were retrieved, of which 271 were removed as duplicates; the titles and abstracts of the remaining 605 articles were screened to determine their relevance. Articles that investigated the pharmacodynamics, pharmacokinetics, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, or carcinogenicity of teflubenzuron in laboratory animals or humans were considered relevant for the assessment. Identified relevant articles and those submitted by the sponsor were reviewed. Most of the original studies provided by the sponsor were performed in the 1980s and conform to the good laboratory practice (GLP) guidelines, although the GLP-compliant status of a few studies could not be verified. Overall, available studies were considered sufficient for the evaluation of teflubenzuron.

2. Biological data

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Rats

In a GLP-compliant study, groups of Wistar (Chbb:THOM) rats (five of each sex per group) were administered uniformly ^{14}C aniline ring-labelled teflubenzuron (radiochemical purity >97%) in aqueous solutions of 1% Tylose and 1% Tween 80 by gavage at a single dose of 25 or 750 mg/kg bw. Serial blood samples were collected for up to 168 hours after treatment. Concentrations of radioactivity in plasma were determined by liquid scintillation counting. In the low-dose group, nearly constant concentrations of 0.38–0.46 μg teflubenzuron equivalents per millilitre in males and 0.22–0.25 $\mu\text{g/mL}$ in females were maintained between 1 and 8 hours, which declined thereafter to below 0.01 $\mu\text{g/mL}$ by 168 hours post-treatment. In the high-dose group, nearly constant concentrations of 0.98–1.43 $\mu\text{g/mL}$ were detected between 20 minutes and 24 hours in female rats. In the males, with the exception of a distinct peak (3.27 $\mu\text{g/mL}$) at 24 hours, nearly constant concentrations of 1.17–1.72 $\mu\text{g/mL}$ were maintained between 40 minutes and 24 hours. In both dose groups, the concentration of radioactivity in whole blood was slightly lower than that in plasma, but the time course was comparable. A 30-fold dose increase led to only a 4- to 6-fold increase in the concentration of the drug in plasma in animals of both sexes, suggesting a difference in absorption rates at different doses (Schlüter, 1986).

In another GLP-compliant study, concentrations of teflubenzuron in plasma in groups of five male and five female Wistar (KFM-Han) rats were determined after feeding the rats a diet medicated with teflubenzuron (purity 92.4%) at a nominal concentration of 0, 500, 1000, 2000, 4000, 8000, 16 000 or 32 000 mg/kg feed (equal to 0, 39, 77, 154, 305, 624, 1259 and 2520 mg/kg bw per day for males and 0, 38, 78, 158, 320, 607, 1313 and 2544 mg/kg bw per day for females, respectively) for a period of 28 days. Blood samples were collected on day 28, and plasma was analysed by high-performance liquid chromatography (HPLC). The concentrations reached a plateau in male rats (0.37 µg/mL) at teflubenzuron concentrations of 1000 mg/kg feed and higher (equivalent to ≥ 77 mg/kg bw per day) and in female rats (0.43 µg/mL) at teflubenzuron concentrations of 2000 mg/kg feed and higher (equivalent to ≥ 158 mg/kg bw per day) (Ellgehausen et al., 1986).

In a GLP-compliant study intended to address carcinogenicity and chronic toxicity, Wistar (KFM-Han) rats were administered teflubenzuron (purity 92.4%) at a nominal concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.1, 5.4 and 27.6 mg/kg bw per day for males and 0, 1.3, 6.5 and 32.0 mg/kg bw per day for females, respectively). Plasma concentrations, determined on surviving animals in the chronic toxicity study (10 of each sex per group) at week 107, reflected the dietary dose: 0, 0.02, 0.06 and 0.27 µg/mL in males and 0, 0.2, 0.04 and 0.15 µg/mL in females, respectively (Suter et al., 1987a).

In a second GLP-compliant chronic toxicity and carcinogenicity study, Wistar (KFM-Han) rats were administered teflubenzuron (purity 92.4%) at a nominal concentration of 0, 2500 or 10 000 mg/kg feed (equal to 0, 123 and 487 mg/kg bw per day for males and 0, 154 and 615 mg/kg bw per day for females, respectively), and plasma concentrations were determined at weeks 4, 26, 52 and 104. In males, plasma concentrations of teflubenzuron increased from 0.18 µg/mL at week 4 to 0.79 µg/mL at week 104 of treatment at the low dose and from 0.23 µg/mL at week 4 to 0.70 µg/mL at week 104 at the high dose. In females, plasma concentrations of teflubenzuron increased from 0.18 µg/mL at week 4 to 0.60 µg/mL at week 104 at the low dose and from 0.14 µg/mL at week 4 to 0.43 µg/mL at week 104 at the high dose (Tennekes et al., 1989).

In a study described previously (Schlüter, 1986), additional groups of five male and five female Wistar (Chbb:THOM) rats were administered uniformly ^{14}C -aniline ring-labelled teflubenzuron (aqueous solution in 1% Tylose and 1% Tween 80) by gavage at either a single dose of 25 or 750 mg/kg bw or 14 daily doses of unlabelled teflubenzuron at 25 mg/kg bw per day followed by the same dose of radiolabelled compound 24 hours after the last dose. Urine and faeces were collected for 8 days post-treatment, after which the animals were killed and radioactivity in the carcass was determined. Irrespective of sex, dose or duration of treatment, most radioactivity (91–95% of the total dose) was excreted

in the faeces, predominantly (>85%) within the first 24 hours, and only small quantities (0.6–0.9% of the total dose for the low-dose group and 0.15–0.16% for the high-dose group) were excreted in urine. Radioactivity remaining in the carcass was negligible (low-dose group, 0.04–0.08% of the total dose; high-dose group, <0.01% of the total dose). Total recoveries of radioactive residues were 91–96%. Additionally, the radioactivity was measured in the expired air collected over a 24-hour period in one male and one female rat treated with ^{14}C -labelled teflubenzuron by gavage at 25 mg/kg bw. No radioactivity was detected in the expired air (Schlüter, 1986).

In a preliminary non-GLP-compliant study, nine male and nine female Wistar (Chbb:THOM) rats were administered uniformly ^{14}C -aniline ring-labelled teflubenzuron (radiochemical purity >99%) via gavage in dimethyl sulfoxide (DMSO) at a dose of 25 mg/kg bw per day for 7 consecutive days. Radioactivity in excreta was assessed in four rats of each sex during the dosing period and for 8 days following the last dose, after which the rats were killed to determine the remaining radioactivity in the carcass. Of the remaining animals, one rat of each sex was killed at 1, 6, 24, 48 and 120 hours after the last of the seven doses, and radioactivity in organs and tissues was determined. Excretion of radioactivity was comparable between sexes. Total recovery of radioactivity was 90–93% of the total dose in faeces, 2–3% in urine and less than 0.1% in the carcass (Schlüter, 1984). The excretion pattern of teflubenzuron was comparable after a single or a multiple oral administration (Schlüter, 1984, 1986).

In a GLP-compliant study, groups of bile duct-cannulated Wistar rats (three of each sex) were dosed by gavage with ^{14}C -aniline ring-labelled teflubenzuron (radiochemical purity >98%) in an aqueous solution containing 1% Tylose and 1% Tween 80 at 25 or 750 mg/kg bw. In the low-dose group, between 0 and 48 hours after dosing, 15.3–17% of the total dose was excreted in bile, 1.3–1.5% in urine and 45.4–46.6% in faeces. At 48 hours, 19.3–26.6% of the total dose was measured in the gastrointestinal tract, 0.4% in liver and 1.3–1.9% in the rest of the carcass. In contrast, in the high-dose group, only a small proportion of the total dose (1.6–2.1%) was excreted in bile, 0.3–0.4% in urine and 57.9–71.8% in the faeces between 0 and 48 hours post-treatment. The remaining radioactivity at 48 hours accounted for 12.2–25.0%, 0.06% and 1.0–1.4% of the total dose in the gastrointestinal tract, liver and the rest of the carcass, respectively. If the sum of the radioactivity in urine, bile, liver and the rest of the carcass is considered as an indication of absorption, 18.3–20.8% of the total dose was absorbed at the low dose compared with 3.0–4.0% at the high dose, suggesting a dose-dependent absorption of this drug from the gastrointestinal tract (Hawkins & Mayo, 1988).

2.1.2 Biotransformation

The nature of the radioactive residues in urine and faeces of male and female rats treated orally for 7 consecutive days with ^{14}C -aniline ring-labelled teflubenzuron at a dose of 25 mg/kg bw in DMSO was investigated in a study described above (Schlüter, 1984). No difference was noted in drug metabolism between males and females. Metabolites in faeces were identified by thin-layer chromatography (TLC). The parent compound represented the majority of radioactivity in faeces (73.3% of the administered dose, 80.2% of faecal radioactivity). The remainder was represented by at least 15 unidentified metabolites, each representing less than 1% of the administered dose. Metabolites in urine were identified by TLC and mass spectroscopy (MS). Three metabolites were characterized in urine. Two were identified as structural isomers of hydroxylated (benzoyl ring positions 3 and 4) teflubenzuron (metabolites I and II, see Fig. 2 below). The third metabolite was formed by substitution of a fluoride atom by a hydroxide group (metabolite III, see Fig. 2). Each of these metabolites represented less than 1% of the dose (Schlüter, 1985).

The biotransformation of teflubenzuron in Wistar rats was also investigated in a study described above (Schlüter, 1986) by analysing the urine and faeces after a single oral dose of 25 (low) or 750 (high) mg/kg bw ^{14}C -labelled compound or daily oral doses of 25 mg/kg bw of unlabelled compound for 14 consecutive days followed by a single dose of labelled compound. Samples were analysed by TLC, HPLC, ultraviolet spectroscopy and MS. No difference in metabolism was noted between the sexes or between animals administered a single or multiple doses of the drug. Most radioactivity excreted in faeces (>90% in single-dose groups and >95% in repeated-dose groups) co-chromatographed with the parent compound. Trace amounts of diverse, mostly polar, compounds were detected in faeces of all treatment groups. One of these metabolites was identified as (3,5-dichloro-2,4-difluorophenyl)urea (metabolite IV, see Fig. 2). This metabolite in faeces represented less than 0.2% of the dose in the high-dose group and less than 1% of the dose in the low-dose group. Given the low amounts present, no other metabolites in faeces were characterized. In urine, the radioactivity was due mainly to very polar compounds, although traces of parent compound were also reported. However, the possibility that the parent compound present in urine was due to cross-contamination by faecal particles was not ruled out. Urine from the high-dose group also contained a trace amount of the same metabolite, (3,5-dichloro-2,4-difluorophenyl)urea (metabolite IV), identified in faeces. Some polar compounds in urine were identified to be conjugated metabolites, although exact identification was not made (Schlüter, 1986).

In another study using the bile duct-cannulated rats described above (Hawkins & Mayo, 1988), metabolites in faeces, urine and bile were analysed by TLC. In 0- to 48-hour pooled faecal samples, most radioactivity (>97% at 25 mg/kg bw and 87% at 750 mg/kg bw) was identified as the parent teflubenzuron. In 0- to 48-hour urine, most radioactivity (>81%) was associated with unidentified polar material. Hydrolysis of urine indicated the presence of conjugates of metabolites that co-chromatographed with (3,5-dichloro-2,4-difluorophenyl)-urea (metabolite IV), its corresponding substituted aniline metabolite (metabolite VII) and the meta-hydroxybenzoyl derivative (metabolite I) of teflubenzuron (see Fig. 2). In 0- to 48-hour bile, most radioactivity (>85%) was represented by unidentified polar material, and only <1–5% of radioactivity co-chromatographed with (3,5-dichloro-2,4-difluorophenyl)urea (metabolite IV) and 1% with teflubenzuron (see Fig. 2). Hydrolysis of bile extracts indicated the presence of conjugates of (3,5-dichloro-2,4-difluorophenyl)urea (metabolite IV), the corresponding substituted aniline (metabolite VII) and the *meta*-hydroxybenzoyl derivative of teflubenzuron (metabolite I, see Fig. 2). As the biliary excretion contained only a low proportion of teflubenzuron, the unchanged teflubenzuron in faeces likely represented the non-absorbed drug (Hawkins & Mayo, 1988).

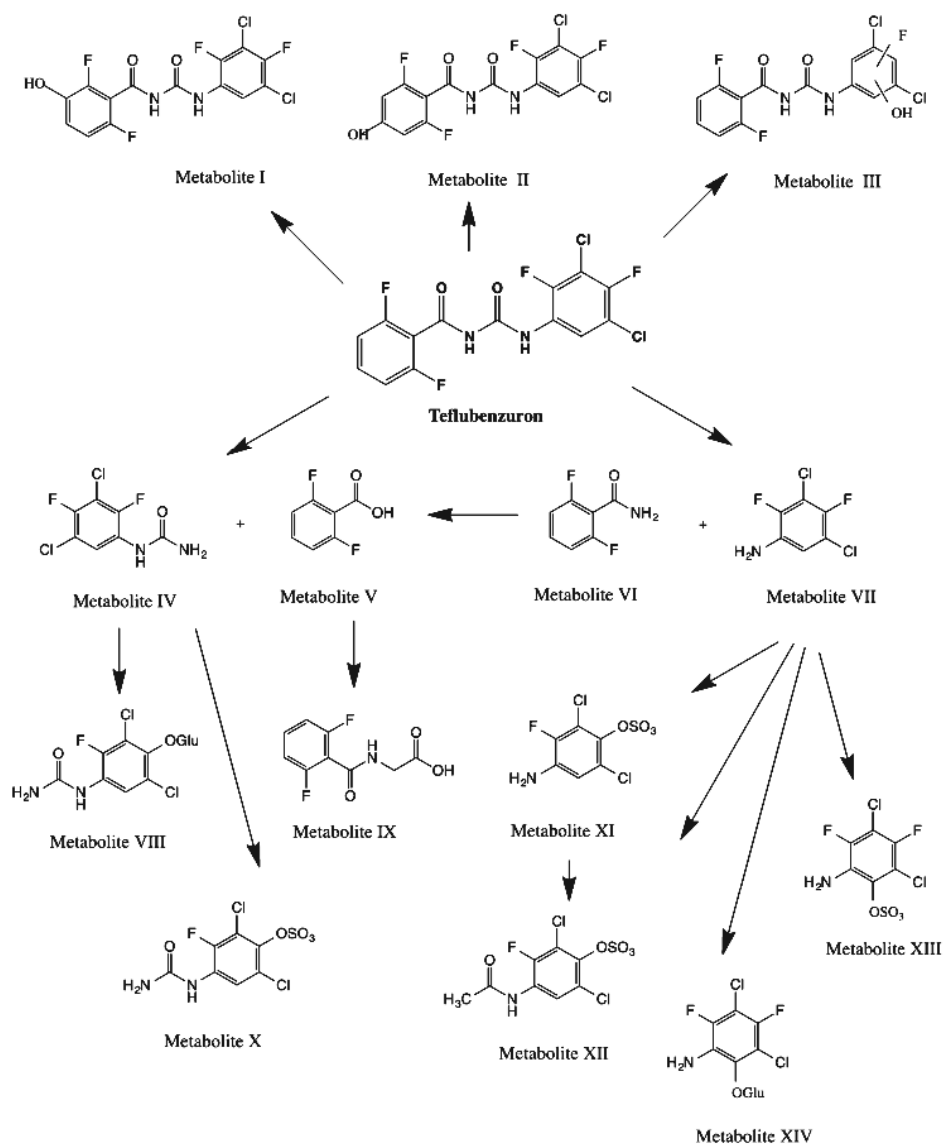
In a non-GLP-compliant study, the metabolites of teflubenzuron in rats were analysed using ^{19}F nuclear magnetic resonance (NMR) spectral analysis. Adult male Wistar rats weighing 350–400 g (number not specified) were administered 20 mg (approximately 55 mg/kg bw) teflubenzuron by gavage in olive oil with 20% DMSO, and urine (0–24 and 24–48 hours) and faeces (0–48 hours) were collected and analysed by the NMR technique after an appropriate extraction procedure. The identity of metabolite conjugates was analysed after treating the extracted samples with acid, β -glucuronidase or arylsulfatase. Teflubenzuron metabolism was supplemented with additional metabolism studies in rats separately administered four scission products (metabolites IV–VII, see Fig. 2) formed by hydrolytic cleavage of the phenylurea bridge of teflubenzuron – namely, (a) (3,5-dichloro-2,4-difluorophenyl)urea (metabolite IV), (b) 2,6-difluorobenzoic acid (metabolite V), (c) 2,6-difluorobenzamide (metabolite VI) and (d) 3,5-dichloro-2,4-difluoroaniline (metabolite VII). When rats were administered the scission products of teflubenzuron, most of the administered chemicals were absorbed and excreted via urine. In contrast, in rats administered teflubenzuron, more than 90% of the dose was excreted in faeces, primarily as the parent compound (91%). NMR spectral analysis could not identify any metabolites in faeces irrespective of whether the teflubenzuron or its scission products were administered. Analyses of urine samples identified a number of metabolites: scission products, other metabolites and/or their conjugates (see Fig. 2). Most of the urinary metabolites were characterized. Only a small portion (12% of the urinary metabolites, approximately 1% of the total

dose) could not be identified, but these were postulated to be compounds with an intact teflubenzuron structure (Koerts et al., 1997).

The proposed biotransformation pathway of teflubenzuron in rats is presented in Fig. 2.

Fig. 2

Proposed biotransformation pathway of teflubenzuron in rats



Two pathways for the hydrolytic cleavage of the phenylurea bridge of teflubenzuron are proposed: pathway 1 yields (3,5-dichloro-2,4-difluorophenyl)-urea (metabolite IV) and 2,6-difluorobenzoic acid (metabolite V), and pathway 2 yields 2,6-difluorobenzamide (metabolite VI) and 3,5-dichloro-2,4-difluoroaniline (metabolite VII). The benzoate part of teflubenzuron was mainly excreted as 2,6-difluorobenzoic acid and, to a minor extent, as 2,6-difluorobenzoylglycine (metabolite IX) and 2,6-difluorobenzamide. The aniline part of teflubenzuron was excreted as the glucuronidated (metabolite VIII) or sulfated (metabolite X) conjugates of (4-hydroxy-3,5-dichloro-2-fluorophenyl)urea, the sulfated conjugate of 4-amino-2,6-dichloro-3-fluorophenol (metabolite XI), the sulfated conjugate of 4-acetamido-2,6-dichloro-3-fluorophenol (metabolite XII) and the sulfated (metabolite XIII) or glucuronidated (metabolite XIV) conjugates of 2-amino-3,5-difluoro-4,6-dichlorophenol (Fig. 2). The relative distribution of various metabolites in the urine of rats orally dosed with teflubenzuron is presented in Table 1.

Table 1

Relative distribution of metabolites in male rat urine (0–24 hours post-dosing) administered teflubenzuron by gavage

Metabolite	% of total metabolites	Metabolite designation ^a
2,6-Difluorobenzoic acid	81.4 ± 3.8	V
2,6-Difluorobenzamide	2.0 ± 0.3	VI
(3,5-Dichloro-2-fluoro-4-phenylglucuronidephenyl)urea	5.2 ± 2.4	VIII
2,6-Difluorobenzoylglycine	4.0 ± 0.5	IX
(3,5-Dichloro-2-fluoro-4-phenylsulfatephenyl)urea	0.3 ± 0.6	X
4-Amino-2,6-dichloro-3-fluorophenylsulfate	1.2 ± 0.4	XI
4-Acetamido-2,6-dichloro-3-fluorophenylsulfate	0.6 ± 0.5	XII
2-Amino-3,5-difluoro-4,6-dichlorophenylsulfate	2.8 ± 0.5	XIII
2-Amino-3,5-difluoro-4,6-dichlorophenylglucuronide	1.2 ± 0.3	XIV
Unidentified	1.3 ± 0.7	—

^a See Fig. 2.

Source: After Koerts et al. (1997)

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity studies with teflubenzuron conducted according to Organisation for Economic Co-operation and Development (OECD) test guidelines (GLP compliant or quality assured) are summarized in Table 2.

Table 2

Single-dose toxicity studies with teflubenzuron in laboratory animals

Species	No. of animals per sex per group	Route	Dose (mg/kg bw) / concentration (mg/m ³)	LD ₅₀ (mg/kg bw) / LC ₅₀ (mg/m ³)	Reference
Mouse	5	Oral	1 000, 5 000	> 5 000	Ullmann (1983d)
Rat	5	Oral	1 000, 5 000	> 5 000	Ullmann (1983e)
Rat	5	Oral	5 000	> 5 000	Ullmann, Sacher & Vogel (1988)
Rat	5	Dermal	2 000	> 2 000	Ullmann (1983b)
Rat	5	Inhalation	5 038 mg/m ³	> 5 038 mg/m ³	Ullmann (1983a)
Rat	5	Intraperitoneal	300, 500, 800, 1 200, 2 000	> 2 000	Ullmann (1983c)

bw: body weight; LC₅₀: median lethal concentration; LD₅₀: median lethal dose

NMRI mice or Wistar (KFM-Han) rats (five of each sex per group) were gavaged once with teflubenzuron (purity 96.5–97.5%) in either 2% carboxymethyl cellulose (one rat study) or polyethylene glycol (PEG) 400 (one rat and one mouse study) at 1000 or 5000 mg/kg bw and were observed for 14 days. At 1000 mg/kg bw, no symptoms were observed in mice, but ruffled fur was observed in a few rats. Ruffled fur, dyspnoea (in both rats and mice), sedation and hunched posture (only in rats) were observed in animals dosed at 5000 mg/kg bw, which resolved by 2–4 days post-treatment. At necropsy, the only abnormality noted was mottled (dark red) lungs in three male rats of the low-dose group. No mortality was observed in either species, suggesting a median lethal dose (LD₅₀) of greater than 5000 mg/kg bw in mice and rats (Ullmann, 1983d,e; Ullmann, Sacher & Vogel, 1988).

Teflubenzuron (purity 96.5%) in PEG 400 at a dose of 2000 mg/kg bw was applied once on the clipped skin surface of Wistar (KFM-Han) rats (five of each sex), and the area was covered with a dressing pad. Skin reaction was assessed 24 hours after the treatment, and animals were observed for a period of 14 days, followed by a full necropsy examination. Clinical examination revealed no adverse skin reactions, and no abnormality was detected in necropsy, suggesting an LD₅₀ for dermal exposure of greater than 2000 mg/kg bw (Ullmann, 1983b).

Wistar (KFM-Han) rats (five of each sex) were exposed via inhalation to teflubenzuron (purity not specified) at a measured concentration of 5038 (4508–5244) mg/m³ air for 4 hours and observed for 14 days, followed by a full necropsy. Dyspnoea and ruffled fur were observed in all rats starting within 2 hours of exposure and lasting until 24 hours post-treatment. No mortality or macroscopic changes were noted in treated animals, suggesting a median lethal concentration (LC₅₀) of greater than 5000 mg/m³ air (Ullmann, 1983a).

Wistar (KFM-Han) rats (five of each sex per group) were injected intraperitoneally (300, 500, 800, 1200 or 2000 mg/kg bw) with teflubenzuron (purity 96.5%) in PEG 400 and observed for 14–22 days, followed by a necropsy of surviving animals. During the first week after dosing, slight to moderate clinical signs occurred in all dose groups, which consisted of ataxia, dyspnoea, sedation, hunched appearance and ruffled fur. Mortality at different doses ranged from 0% to 30% and was not dose dependent. Postmortem examination revealed extensive peritonitis in all dose groups. The LD₅₀ was greater than 2000 mg/kg bw per day (Ullmann, 1983c).

(b) Dermal irritation

The skin irritating potential of teflubenzuron (purity 96.5%) was investigated in three adult New Zealand White rabbits. The drug was applied to intact clipped skin on the dorsum of each rabbit. Half a gram of test article, moistened with tap water, was applied to the skin under a patch of surgical gauze and was held in place for 4 hours. The skin reaction at the application site was observed at 1, 24, 48 and 72 hours after removal of the patch. Under the conditions of the test, there was no evidence of skin irritation by teflubenzuron (Ullmann, 1983g).

(c) Ocular irritation

The eye irritating potential of teflubenzuron (purity 96.5%) was investigated in three adult New Zealand White rabbits. The drug (0.1 g) was deposited into the conjunctival sac of the left eye, and the eyelid was held closed briefly to prevent the loss of the drug. The right eye served as control. Eye irritation was recorded at 1, 24, 48 and 72 hours post-treatment. Slight reddening of the ventral conjunctivae and sclera was observed at 1 hour, but there was no sign of eye irritation on subsequent observations (Ullmann, 1983f), suggesting that teflubenzuron was not an eye irritant under the conditions of the test.

(d) Dermal sensitization

The delayed hypersensitivity potential of teflubenzuron was investigated using the guinea-pig (Dunkin-Hartley) maximization test. Ten animals of each sex were used as test animals, and five animals of each sex served as controls. After initial induction by intradermal injection and topical patch application, animals received a percutaneous application of either 25% teflubenzuron (purity 93.5%) in a 1:1 mixture of propylene glycol and normal saline or the vehicle alone. At 24 hours after removal of the medicated pad, 25% (5/20) of the treated animals had a positive mild skin sensitizing reaction, compared with 30% (3/10) of the controls. A second reading performed 48 hours after removal of the medicated pad showed a positive response in 5% (1/20) of the treated animals compared

with none (0/10) of the controls. In a second challenge study conducted 1 week after the first study, none of the treated or control animals had a positive skin reaction at 24 and 48 hours after removal of the medicated pad (Ullmann, 1984), suggesting that teflubenzuron did not sensitize skin under the test conditions.

2.2.2 Short-term studies of toxicity

(a) Mice

In a study conducted according to Japan Ministry of Agriculture, Forestry and Fisheries guidelines (GLP status unknown, study protocol unavailable), groups of CD-1 mice (12 of each sex per group) were administered teflubenzuron (purity not reported) at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 12, 115 and 1213 mg/kg bw per day for males and 0, 14, 142 and 1450 mg/kg bw per day for females, respectively) for 13 weeks.

No differences in feed consumption, body weight gain, mortality, clinical observations, or ophthalmoscopic, urine analysis and haematological parameters were noted between the groups. Biochemical changes noted included significantly increased alkaline phosphatase activity in males and alanine transaminase activity in females, increased total cholesterol level in females, decreased blood glucose level in males and increased blood glucose level in females at 10 000 mg/kg feed. Observations at necropsy were enlargement (both sexes) and discoloration (males only) of the liver at the middle and high doses. Absolute and relative liver weights were increased at the middle and high doses in both sexes. Significant histopathological changes noted were centrilobular hepatocellular swelling (both sexes) and fatty change (males only) at the middle and high doses.

The NOAEL was 100 mg/kg feed (equal to 12 mg/kg bw per day), based on liver changes in groups treated at 1000 mg/kg feed (equal to 115 mg/kg bw per day) (Takahashi et al., 1987).

(b) Rats

In a GLP-compliant study, groups of Wistar (KFM-Han) rats (10 of each sex per group) were administered teflubenzuron (purity 96.5%) at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 8, 82 and 809 mg/kg bw per day for males and 0, 9, 94 and 942 mg/kg bw per day for females, respectively) for 13 weeks. Additional satellite groups of five rats of each sex were included in the control and the high-dose groups, which, after 13 weeks of treatment, were maintained for an additional 4 weeks on unmedicated diet to determine the reversibility of effects.

There were no deaths or treatment-related signs of toxicity. Body weights, feed consumption, urine analysis, haematology and ophthalmoscopy did not reveal any meaningful treatment-related changes. Clinical biochemistry revealed

increased activities of ornithine transcarbamylase (in males at the high dose at week 6 and in both sexes at the high dose at week 13), aspartate transaminase (in males at the middle and high doses at week 6 and in both sexes at the same doses at week 13), alanine transaminase (in males at the middle and high doses at week 6 and at the high dose at week 13) and alkaline phosphatase (in all treated males at week 6 and in males at the low and high doses at week 13). Increases were more pronounced in males than in females. Additionally, lactate dehydrogenase activity was significantly elevated in males of the high-dose group at week 13. After 4 weeks of drug withdrawal, clinical biochemistry parameters returned to control levels. The only abnormal gross pathological and histopathological findings were the increased absolute and relative weights of liver in females and of testes in males at the high dose. In the satellite group, 4 weeks after cessation of treatment, absolute and relative thymus weights were higher in rats treated for 13 weeks at a teflubenzuron concentration of 10 000 mg/kg feed.

Being less than 1.5-fold above the control value and within the normal physiological range, the change in alkaline phosphatase activity in male rats at the low dose was considered not to be biologically relevant. Based on effects on liver enzyme activities at 1000 mg/kg feed (equal to 82 mg/kg bw per day), a NOAEL of 100 mg/kg feed (equal to 8 mg/kg bw per day) was identified (Suter et al., 1987c).

(c) Dogs

The short-term toxicity of teflubenzuron in dogs was assessed in two 13-week feeding studies and a 52-week feeding study.

In the first GLP-compliant study, groups of Beagle dogs (four of each sex per group) were fed a diet containing teflubenzuron (purity 96.5%) at a nominal concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 3.5, 33.7 and 318.2 mg/kg bw per day for males and 0, 4.0, 42.8 and 417.1 mg/kg bw per day for females, respectively) for 13 weeks.

No mortality or signs of toxicity were observed during the study. There were no changes in feed consumption or body weight gain, and a hearing test, ophthalmoscopic examination, haematology analysis and urine analysis did not reveal any changes of toxicological significance. Clinical biochemistry revealed that in a male and a female dog of the high-dose group, activities of alanine transaminase (male: weeks 4, 8 and 13; female: week 4), aspartate transaminase (male and female: week 4), alkaline phosphatase (male: weeks 4 and 8; female: week 4) and ornithine transcarbamylase (male: weeks 4 and 8; female: week 4) were elevated. Additionally, serum ornithine transcarbamylase activity was increased in another male of the same dose at week 4. Absolute and relative liver

weights were elevated in animals of both sexes at the high dose. Gross pathology revealed an increased incidence of nodular foci in the pyloric or fundic region of dogs in the high-dose group. Isolated dark red foci were also noted in the pyloric region of the stomach of two mid-dose and two high-dose dogs. A male dog at the high dose had a firm liver with irregular contour of the capsule. Microscopic changes identified were limited to hepatitis and gastritis in some dogs. One male at the low dose (100 mg/kg feed) had mild hepatitis, and another had moderate centrilobular necrosis of the liver. Slight round cell infiltration was noted in one male at each of the middle and high doses. A moderate chronic active hepatitis was diagnosed in one animal of each sex at the high dose (10 000 mg/kg feed), which was characterized by single-cell necrosis, cell infiltration and collapsed stroma. Focal gastritis was observed in females receiving teflubenzuron at a concentration of 1000 (one animal) or 10 000 (two animals) mg/kg feed. Follicular hyperplasia of the pyloric mucosa was noted in most animals of both sexes at a concentration of 10 000 mg/kg feed.

Although mild microscopic changes in liver were noted in two dogs at the lowest dose (100 mg/kg feed), given the lack of a clear dose–response relationship, 100 mg/kg feed (equal to 3.5 mg/kg bw per day) was identified as the NOAEL, based on stomach lesions at 1000 mg/kg feed (equal to 33.7 mg/kg bw per day) (Bathe et al., 1985).

A supplementary GLP-compliant study was conducted to clarify the effects on liver in groups of Beagle dogs (four of each sex per group) that were fed a diet containing teflubenzuron (purity 92.4%) at a nominal concentration of 0, 30 or 100 mg/kg feed (equal to 0, 1.2 and 4.4 mg/kg bw per day for males and 0, 1.5 and 5.1 mg/kg bw per day for females, respectively) for 13 weeks.

There was no mortality, and there were no treatment-related signs of toxicity. Feed consumption and body weight gain were not affected by treatment, and no treatment-related toxicological changes were noted in the hearing test, ophthalmoscopic examination, haematology, clinical biochemistry, urine analysis, organ weights, and macroscopic or microscopic examination of various organs.

The highest dose of 100 mg/kg feed (equal to 4.4 mg/kg bw per day) was identified as the NOAEL (Bathe et al., 1987).

Groups of Beagle dogs (four of each sex per group) were fed diets containing teflubenzuron (purity 92.4%) at a concentration of 0, 30, 100 or 500 mg/kg feed (equal to 0, 1.0, 3.2 and 17.3 mg/kg bw per day for males and 0, 1.2, 4.0 and 18.0 mg/kg bw per day for females, respectively) for 52 weeks under GLP-compliant study conditions.

There were no deaths or treatment-related signs of toxicity. No treatment-related differences were noted in feed consumption, body weight gain, auditory test (pretest and week 52), ophthalmic examinations (pretest and week 52), haematology (pretest and weeks 13, 26 and 52), clinical biochemistry (pretest and weeks 13, 26 and 52) or urine analysis (pretest and weeks 13, 26 and 52). At necropsy, the only notable change observed was an increase in absolute liver weight in males at the highest dose (500 mg/kg feed). No treatment-related changes were noted in gross pathological or histopathological examinations.

The NOAEL from this study was identified as 100 mg/kg feed (equal to 3.2 mg/kg bw day), based on the liver weight change in males at 500 mg/kg feed (equal to 17.3 mg/kg bw per day) (Sachsse et al., 1986).

The Committee identified an overall NOAEL of 100 mg/kg feed (equal to 4.4 mg/kg bw per day) from the three short-term studies in dogs, based on the findings of adverse effects in liver at a dose of 500 mg/kg feed (equal to 17.3 mg/kg bw per day).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

In a GLP-compliant carcinogenicity study, teflubenzuron (purity 92.4%) was administered in the diet to groups of NMRI mice (60 of each sex per group) at a concentration of 0, 15, 75 or 375 mg/kg feed (equal to 0, 2.1, 10.5 and 53.6 mg/kg bw per day for males and 0, 3.1, 15.4 and 71.7 mg/kg bw per day for females, respectively) for 78 weeks (18 months). Ten animals of each sex in each group were killed at 12 months (interim kill), and the remainder were killed at 18 months (terminal kill). Animals were observed for clinical signs of toxicity, and feed consumption and body weights were recorded regularly. Ophthalmoscopic examinations, haematology and clinical biochemistry were evaluated at prespecified intervals. Animals that died prior to or on their scheduled killing dates were subjected to gross pathological and histopathological examinations.

There were no treatment-related signs of toxicity or changes in ophthalmoscopic or haematological parameters. Mortality in high-dose males (10%) was lower than that in the control (33%) and the low-dose (27%) males. The mortality was considered to be within biological variation and not treatment related. Body weight gain was reduced (9% for interim sacrifice group and 5% for terminal kill group) in males at the high dose. Compared with controls, in male mice treated at the high dose, activities of aspartate transaminase, alanine transaminase and ornithine transcarbamylase were elevated at both weeks 52 and 78, whereas lactate dehydrogenase and alkaline phosphatase activities were elevated only at week 52. In females treated at this dose, only the alanine

transaminase activity was elevated significantly. Slight, but non-significant, increases in the activities of alanine transaminase and ornithine transcarbamylase were noted in males treated at the middle dose at week 78 and in females treated at the high dose in both weeks 52 and 78, respectively.

At interim and terminal kill necropsy, the absolute and relative liver weights were higher in both sexes of the high-dose group. At interim kill, relative liver weight was slightly increased for males at the middle dose. Macroscopic examinations revealed an increased incidence of hepatic nodules (30% versus 16.7% in controls) in males at the high dose. Histopathological investigations in terminal kill animals indicated an increased incidence of hepatocellular adenomas in males at the middle and high doses. The incidence of hepatocellular adenoma was 12% in controls, 10% at the low dose, 22% at the middle dose and 32% at the high dose. The incidence of hepatocellular adenoma recorded among controls in this study (12%) was similar to the mean incidence of 10.9% (range 4.5–16.4%) recorded in male NMRI mice used in four long-term studies (concurrent historical controls) in the same laboratory. Therefore, the incidence of hepatocellular adenoma in both the mid- and high-dose groups was higher than the highest range observed in the historical controls. No hepatocellular carcinoma was identified in females. Among the males, the incidence of hepatocellular carcinoma was not affected by the treatment, being 3/50, 5/50, 4/50 and 5/50 in the control, low-dose, mid-dose and high-dose groups, respectively.

Several treatment-related, dose-dependent non-neoplastic hepatic changes were reported in animals of both sexes at both the interim and terminal kills. They included hepatocellular hypertrophy in combination with single-cell necrosis, diffuse Kupffer cell proliferation, phagocytic cell foci, lipofuscin accumulation and patchy glycogen storage. These changes were more pronounced in males than in females; significant changes were detected in females only in the high-dose group. The proportions of male mice with dose-dependent non-neoplastic hepatic lesions in the control, low-, mid- and high-dose groups, respectively, were as follows: hepatocellular hypertrophy – 12/60, 29/60, 46/60 and 56/60; single-cell necrosis – 13/60, 26/60, 42/60 and 56/60; phagocytic cell foci – 17/60, 21/60, 43/60 and 54/60; and lipofuscin accumulation – 8/60, 11/60, 20/60 and 27/60. In addition, in the mid- and high-dose males, an increased incidence of nodular hepatic hyperplasia was observed at terminal kill. A slight bile duct proliferation was observed in males at the middle (3/50) and high (10/50) doses, with a concomitant decrease in normal centrilobular fat storage. In the high-dose females, patchy fatty change was noted in the liver.

In the low-dose males, although the incidence of non-neoplastic hepatic lesions was significantly increased, the severity of these lesions was comparable with that observed in the controls. For example, the severity grades in low-dose and control males were, respectively, 2.3 and 2.1 for hepatocellular hypertrophy

and 1.9 and 1.9 for single-cell necrosis. The sponsor considered non-neoplastic hepatic changes observed in the low-dose group to be common background findings in mice of this age and strain and proposed the lowest dose (15 mg/kg feed, equal to 2.1 mg/kg bw per day) as the NOAEL (Suter et al., 1987b).

The non-neoplastic lesions (based on the re-evaluation of the liver sections) from male mice in the terminal kill group from the carcinogenicity study reported above were analysed using a linear extrapolation approach, and a point of departure of 1 mg teflubenzuron per kilogram feed was proposed (Tennekes, 1989). However, given that better dose–response modelling approaches are currently available, the Committee did not agree with the modelling approach.

Histopathological sections of liver from male mice in the above study were subjected to re-evaluation by an independent pathologist. In total, seven slides (one routine and six additional slides) from each liver were examined for nodular liver lesions: hepatocellular hyperplastic nodules, adenomas and carcinomas, based on predefined and more detailed diagnostic criteria. The pathologist identified hepatocellular hyperplastic nodules in 2/60, 0/60, 6/60 and 12/60 animals, hepatocellular adenomas in 8/60, 5/60, 13/60 and 13/60 animals, and hepatocellular carcinoma in 2/60, 6/60, 3/60 and 3/60 animals in the control, low-, mid- and high-dose groups, respectively. Based on these data, a dose-related increase in the incidence of hepatocellular hyperplastic nodules and a slight but statistically non-significant increase in the hepatocellular adenomas were proposed. The pathologist's conclusion was that whereas teflubenzuron increased the incidence of non-neoplastic hepatocellular nodules at the high dose (375 mg/kg feed), it did not manifest either the tumorigenic effect (enhancement of hepatocellular adenomas) or carcinogenic effect (enhancement of hepatocellular carcinomas) (Vesselinovitch, 1988).

Considering all available information, the Committee concluded that teflubenzuron induced hyperplastic proliferation in liver of mice by an unknown mechanism and considered the non-neoplastic lesions observed in the low-dose group as adverse. The lowest dietary concentration of 15 mg/kg feed (equal to 2.1 mg/kg bw per day) was identified as the LOAEL.

In the absence of a NOAEL, to better characterize the point of departure, the Committee conducted a dose–response analysis of these data using the benchmark dose (BMD) approach (see [section 2.2.7](#) below). The Committee considered the lower confidence limit on the benchmark dose for a 10% response over the controls ($BMDL_{10}$) of 0.54 mg/kg bw per day for the BMD of 0.73 mg/kg bw per day, estimated by the multistage model for hepatocellular hypertrophy, the most toxicologically relevant effect, as the most appropriate point of departure for this study.

(b) Rats

In a GLP-compliant chronic toxicity and carcinogenicity study, teflubenzuron (purity 92.4%) was administered to groups of Wistar (KFM-Han) rats (70 of each sex per group) for a period of 120 weeks at a dietary concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.0, 4.8 and 24.8 mg/kg bw per day for males and 0, 1.2, 5.9 and 29.9 mg/kg bw per day for females, respectively). Ten rats of each sex were killed at week 53 (interim kill 1) and 107 (interim kill 2), and the remainder were killed at week 120 (terminal kill). Animals were observed for clinical signs of toxicity, and feed consumption and body weight were recorded regularly. Ophthalmoscopic examinations, a hearing test, haematology, urine analysis and clinical biochemistry were evaluated at prespecified intervals. Animals that died prior to or on their scheduled killing dates were subjected to gross pathological and histopathological examinations.

There was no effect of treatment on mortality; it ranged in different groups from 5% to 20% among the animals killed at weeks 53 and 107 and from 40% to 50% among the animals killed at week 120. There were no signs of toxicity or treatment-related effects on feed consumption or on ophthalmoscopic, hearing, haematological or urine analysis parameters. The body weight gain in males at the middle and high doses was slightly, but non-significantly, reduced.

Clinical biochemistry revealed increased activities (1.5- to 3-fold) of aspartate and alanine transaminases at weeks 14, 26, 53 and 78, of alanine transaminase at week 120 and of ornithine transcarbamylase at weeks 53 and 78 in males treated at the high dose.

At terminal kill, absolute and relative liver weights were increased in males at the high dose. No treatment-related changes were noted in gross pathology. Several non-neoplastic microscopic changes were noted in different organs, mainly the endocrine and large parenchymatous organs. However, they were not treatment related. The only statistically significant increases noted in the trend analysis of the incidence of neoplastic lesions were haemangiomas in mesenteric lymph nodes in males treated at the high dose (8/47 versus 1/48 controls) and pancreatic exocrine carcinoma in males at the same dose (2/47 versus 0/50 in controls). However, when compared with the historical controls, the incidence of this neoplasm in the high-dose males was not significantly different. Also, the number of animals with pancreatic exocrine carcinoma was too small to allow a meaningful conclusion to be drawn (Suter et al., 1987a). It was noted that the study used a rat strain prone to a high incidence of spontaneous neoplasms in a number of organs. For example, among the controls, 40% of females had mammary gland fibroadenoma, 35% of male rats and 78% of female rats had pituitary adenoma, and 18% of male rats and 12% of female rats had various skin neoplasms. The

Committee considered that high rates of spontaneous neoplasms in control rats significantly reduced the sensitivity of the study to identify carcinogenic effects.

Based on the effect on liver enzymes and liver weight at 500 mg/kg feed (equal to 24.8 mg/kg bw per day), 100 mg/kg feed (equal to 4.8 mg/kg bw per day) was identified as the NOAEL from this study (Suter et al., 1987a).

In a supplemental GLP-compliant carcinogenicity study, groups of Wistar (KFM-Han) rats (60 of each sex per group) were administered teflubenzuron (purity 92.4%) at a concentration of 0, 2500 or 10 000 mg/kg feed (equal to 0, 123 and 487 mg/kg bw per day for males and 0, 154 and 615 mg/kg bw per day for females, respectively) for a period of 111 weeks. Ten rats of each sex were killed at week 104 (interim kill), and the remainder were killed at the end of the study. Animals were observed for clinical signs of toxicity, and feed consumption and body weight were recorded regularly. Haematology, urine analysis and clinical biochemistry were evaluated at prespecified intervals. Animals were subjected to gross pathological and histopathological examinations.

There were no signs of toxicity or treatment-related changes in feed consumption. Mortality was significantly reduced in the high-dose males (28%) compared with the controls. There was no treatment-related difference in mortality (39–44%) among females or between the low-dose and control males. However, a non-significant reduction in body weight gain was noted in females at both the low (6%) and high (7.5%) doses. No treatment-related changes were observed in haematology or urine analysis parameters. Clinical biochemistry revealed increased activities of alanine transaminase and aspartate transaminase in males at both treatment doses. Aspartate transaminase activity was also elevated in females at the high dose.

At interim kill, compared with controls, the absolute and relative liver and kidney weights were increased in males treated at the high dose. Females in the same group also had a slight, but statistically non-significant, increase in absolute and relative liver weights. At terminal kill, absolute and relative liver weights were increased in the high-dose males. On gross pathology, dose-dependent increases in the incidence of diffuse, clay-coloured discoloration and focal and multifocal discoloration of the liver were observed in treated males, the incidences being higher at the high dose than at the low dose. Treatment-related non-neoplastic microscopic changes were noted in the liver of both sexes at both doses tested. These lesions were more frequent and more severe in males than in females. They included increased incidence of fatty changes and centrilobular hepatocellular hypertrophy in both sexes at both dose levels, increased incidence of mixed cell and basophilic cell foci in males at both dose levels, increased incidence of focal hepatocellular hyperplasia and spongiosis hepatitis in males at the high dose and increased incidence of basophilic cell foci in females at the high dose (Tennekes et

al., 1989). The incidences of mesenteric lymph node haemangioma and pancreatic exocrine carcinoma in males, which were identified in the previous study, were not different between the treated rats and untreated controls in this study. As in the previous study, some of the experimental animals (including controls) had a high incidence of spontaneous neoplasms in various organs, which were not treatment related, but likely reduced the sensitivity of the test.

A NOAEL could not be identified from this study because of elevated activities of liver enzymes, macroscopic liver findings and non-neoplastic microscopic hepatic changes in treated rats at both dose levels.

2.2.4 Genotoxicity

To examine the genotoxic potential of teflubenzuron, four in vitro and two in vivo studies were conducted. The results of these studies are summarized in Table 3. All studies were conducted under GLP-compliant conditions, except the reverse mutation study in *Salmonella typhimurium*. The latter, however, was audited for quality assurance.

Table 3
Results of genotoxicity assays on teflubenzuron

Test system	Test object	Concentration/dose	Results	Reference
Bacterial reverse mutation (Ames test)	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	125, 250, 500, 1 250, 2 500 and 5 000 µg/plate	Negative ^a	Kramer (1982)
Point mutation (<i>hprt</i> locus) assay	Chinese hamster V79 cells	5, 10, 25 and 50 µg/mL	Negative ^a	Heidemann (1986)
Chromosomal aberration assay	Chinese hamster V79 cells	4, 25 and 50 µg/mL	Negative ^a	Heidemann (1985)
Unscheduled DNA synthesis	Wistar CF HB male rat hepatocytes	1, 3.3, 10, 33.3 and 100 µg/mL	Negative	Müller (1986)
In vivo mouse micronucleus assay	NMRI mice (both sexes)	5 000 mg/kg bw	Negative	Guenard (1984)
In vivo DNA covalent binding assay	NMRI male mice	40 mg/kg bw	Negative	Kugler-Steigmeier, Lutz & Schlatter (1988)

bw: body weight; DNA: deoxyribonucleic acid; *hprt*: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

^a In the presence and absence of metabolic activation (S9).

The potential of teflubenzuron (purity not reported) to induce reverse mutation was tested in vitro in five histidine-dependent strains of *Salmonella typhimurium* – namely, TA100, TA1535 (base mutation–sensitive strains), TA98, TA1537 and TA1538 (frameshift mutation–sensitive strains). A direct plate incorporation method was used to test six concentrations (125, 250, 500, 1250, 2500 and 5000 µg/plate) of the test substance in four replicate plates with or without a metabolic activation system (S9) and with appropriate negative and positive controls. The test substance was dissolved in DMSO. Higher concentrations

(1250–5000 µg/plate) of teflubenzuron precipitated in the top agar layer. Under the conditions of the test, there was no evidence that teflubenzuron induced reverse mutations in the test strains of *S. typhimurium* in both the presence and absence of S9 (Kramer, 1982).

The potential of teflubenzuron to induce a point mutation (*hprt* locus) in Chinese hamster V79 cells was investigated in vitro in two independent experiments. The cells were exposed to the test substance (purity 92.4%) dissolved in DMSO for 4 hours at a final concentration of 5, 10, 25 or 50 µg/mL in either the presence or absence of S9. Appropriate negative and positive controls were used. The positive control used for testing without metabolic activation was ethylmethanesulfonate, and that for testing with metabolic activation was 9,10-dimethyl-1,2-benzanthracene. Concentrations of teflubenzuron higher than 50 µg/mL precipitated in the medium. At all concentrations of the test substance, there was no increase in mutation rate in teflubenzuron-treated cells when compared with negative controls in either the presence or absence of S9. The sensitivity of the test system was demonstrated by the enhanced mutation rates found in cells treated with the positive controls. Under the test conditions used, there was no evidence that teflubenzuron induces point mutations in the *hprt* locus in Chinese hamster V79 cells (Heidemann, 1986).

Chromosomal aberration rates were determined in Chinese hamster V79 cells treated with teflubenzuron (purity 92.4%) solubilized in DMSO at a final concentration of 4, 25 or 50 µg/mL of medium for 4 hours. Experiments were conducted with or without S9. Appropriate negative and positive controls were used. The positive control used for testing without metabolic activation was ethylmethanesulfonate, and that for testing with metabolic activation was cyclophosphamide. Structural aberrations recorded included gaps, breaks, exchanges and partial or total disintegrations of chromosomes. At all concentrations of the test substance, there was no increase in chromosomal aberration rate in teflubenzuron-treated cells when compared with negative controls in either the presence or absence of S9. The sensitivity of the test system was demonstrated by the enhanced chromosomal aberration rates in cells treated with positive controls. Under the test conditions used, there was no evidence that teflubenzuron induces chromosomal aberrations (Heidemann, 1985).

The ability of teflubenzuron (purity 92.4%) to induce unscheduled DNA synthesis in primary rat hepatocytes (male Wistar rats) in vitro was evaluated. Five concentrations of teflubenzuron (1, 3.3, 10, 33.3 or 100 µg/mL) dissolved in acetone were tested. The test system included both negative and positive (7,12-dimethylbenz(a)anthracene) controls. Rat hepatocytes were incubated at 37 °C in the culture medium for 1 hour followed by treatment with the test or control substance for 3 hours in the presence of [³H]thymidine and incubated further for an additional 3 hours. The extracted DNA was analysed for [³H]-

thymidine incorporation by liquid scintillation counting. There was no increased incorporation of radioactivity in test groups when compared with negative controls, although a 2.1-fold increase was noted in the positive control. Under the conditions of the assay, there was no evidence that teflubenzuron induced unscheduled DNA synthesis in rat hepatocytes (Müller, 1986).

The mutagenic potential of teflubenzuron (purity 96.5%) was tested in an *in vivo* mouse micronucleus assay. Groups of NMRI mice (18 of each sex per treatment) were administered, by gavage, teflubenzuron in 2% carboxymethyl cellulose at 5000 mg/kg bw, the vehicle (negative control) or cyclophosphamide in 0.9% saline solution at 50 mg/kg bw (positive control). Six mice of each sex per group were killed at 24, 48 and 72 hours post-treatment, and bone marrow was collected and analysed. At all time points examined, no significant treatment-related increase in micronucleated polychromatic erythrocytes was observed in mice of either sex treated with teflubenzuron. In contrast, increased micronucleated cells were observed in mice treated with the positive control. Thus, teflubenzuron was negative in the *in vivo* mouse micronucleus assay (Guenard, 1984).

To investigate whether the tumorigenic activity of teflubenzuron in liver was associated with its potential to covalently bind to DNA, an *in vivo* study was conducted in male NMRI mice. Six mice (two groups of three each) were dosed by gavage with either aniline ring-labelled [^{14}C]teflubenzuron (radiochemical purity >98%) or benzoyl ring-labelled [^{14}C]teflubenzuron (radiochemical purity >99%) at a nominal dose of 40 mg/kg bw. Three mice were used as the negative controls. Mice were killed 24 hours post-treatment, and DNA was purified from the liver. Radioactivity in purified liver DNA from the treated animals was below the limit of detection, with a calculated upper limit of covalent binding index of 0.1, suggesting that teflubenzuron did not form DNA adducts in this *in vivo* test (Kugler-Steigmeier, Lutz & Schlatter, 1988).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration reproductive toxicity

Groups of Sprague-Dawley rats (25 of each sex per group) were fed a diet containing teflubenzuron (purity 92.4%) at a concentration of 0, 20, 100 or 500 mg/kg feed (for males, equal to 0, 1.5, 7.4 and 36.9 mg/kg bw per day in the parental generation and 0, 1.9, 9.6 and 48.2 mg/kg bw per day in the F_1 generation; for females, equal to 0, 1.6–3.6, 7.9–18.5 and 39.5–89.3 mg/kg bw per day [the range represents mean drug intakes for the parental and F_1 generations prior to mating, during gestation and during lactation], respectively) starting at the age of 6–7 weeks. At 10 weeks of age, a male and a female (parental generation) of the same dose group were housed together until a successful mating or for a maximum

of 14 days. Females were allowed to litter and rear pups to weaning (21 days), when the parents were killed for necropsy. From each litter, one or two pups (F_1 generation) of each sex were selected (minimum 25 of each sex per group) for further breeding (F_1 parent), and the rest of the pups were necropsied at weaning. Selected F_1 parents continued to receive the same teflubenzuron-medicated diet as their dams and were individually mated at the age of 12 weeks. As the parental generation, F_1 parents also littered and reared their pups until weaning, when both the parents and their pups were necropsied. All breeding rats and each litter born to both generations were observed daily for signs of toxicity. Other reproductive and general health parameters recorded were body weight, feed consumption, mating performance, fertility rate, duration of gestation, pinna unfolding, generalized hair growth, tooth eruption, eye opening, litter size, pup weight, pup mortality and sex ratio. Additionally, F_1 pups underwent functional tests, such as pupillary reflex, startle response and ability to learn the use of water maze. Histopathological examinations were limited to sex organs of parents of both generations receiving teflubenzuron at concentrations of 0 and 500 mg/kg feed.

The sponsor's study report suggested that there was no evidence of toxicity and no effects of teflubenzuron on reproductive performance (Osterburg, 1989). Closer examination of the necropsy findings in F_1 generation pups revealed a dose-related increase in the incidence of unilateral and bilateral dilatation of the renal pelvis; the incidences were 0.9% (2/214) in controls, 1.9% (5/262) at the low dose, 3.3% (7/215) at the middle dose and 6.8% (15/221) at the high dose, with the incidence at the high dose being statistically different from the control incidence. No such effect was seen in the F_2 generation. No other signs of maternal or offspring toxicity were observed.

The NOAEL for offspring toxicity was 100 mg/kg feed (equal to 7.4 mg/kg bw per day), based on the increased incidence of renal pelvic dilatation seen at 500 mg/kg feed (equal to 36.9 mg/kg bw per day). The NOAEL for parental and reproductive toxicity was 500 mg/kg feed (equal to 36.9 mg/kg bw per day), the highest dose tested.

(b) Developmental toxicity

(i) Rats

In a GLP-compliant study, teflubenzuron (purity 92.4%) in 0.5% aqueous carboxymethyl cellulose was administered by gavage at a dose of 0, 10, 50 or 250 mg/kg bw per day to groups of pregnant Wistar rats (25 per group) from day 6 to day 15 of gestation following natural mating. Treated rats were killed on day 20 of gestation, and fetuses were removed by caesarean section and examined for developmental abnormalities.

There were no deaths or adverse clinical findings in dams, and feed consumption and body weight were not affected by treatment. The number of corpora lutea and implantation rate were similar between the groups. At necropsy, there were no dead fetuses and no treatment-related organ or skeletal malformations. The proportion of dams having spontaneous abortion (compared with total bred or pregnant), implantation rates, number of resorptions and number of congenital runts were not different between the treatment groups, although the absolute number of rats ($n = 4$) with complete abortion was higher in the high-dose group. There was a dose-related decrease in the absolute number of live pups: 240, 236, 223 and 208 in the 0, 10, 50 and 250 mg/kg bw per day dose groups, respectively. Consequently, the number of live pups per dam was significantly reduced in the high-dose group when compared with the controls.

Under the test conditions described, teflubenzuron had weak embryo/fetal toxicity. Therefore, from this study, a NOAEL of 50 mg/kg bw per day was identified for embryo/fetal toxicity, based on a reduction in the number of live pups per dam at 250 mg/kg bw per day. The NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested (Gleich, Weisse & Unkelbach, 1986).

Teflubenzuron (purity 92.1%) in 0.5% aqueous carboxymethyl cellulose was administered by gavage to groups of 25 pregnant Wistar rats at 0, 100, 300 or 1000 mg/kg bw per day during days 7–17 of gestation, and the effect on dams and fetuses was observed. The highest dose tested was the upper “limit” recommended according to the Japanese standard guidelines followed in the study. Treated animals were killed on day 20 of gestation.

In dams, no treatment-related changes were noted in general condition, body weight, feed consumption or necropsy. No treatment-related changes were observed in conditions of pregnancy, including number of corpora lutea, number of implantations, proportions of dead or resorbed fetuses, number of live fetuses and placental weight. No difference was noted in sex ratio or body weight of live fetuses, and no external abnormalities were observed. Additionally, no visceral or skeletal abnormalities were observed in fetuses.

The NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Ishida et al., 1987).

(ii) Rabbits

Groups of naturally mated and considered pregnant Himalayan rabbits (15 per group) were gavaged with teflubenzuron (purity 92.4%) in 0.5% carboxymethyl cellulose at 0, 10, 50 or 250 mg/kg bw per day from day 6 to day 18 of gestation under GLP-compliant conditions. Clinical observations, body weight and feed consumption were recorded. On day 29 of gestation, animals were

killed and examined macroscopically, and the ovaries and uterus were removed. The numbers of corpora lutea, implantations, live and dead fetuses, resorptions and congenital runts, fetal weights and fetal sex were recorded. All live fetuses were observed for a 24-hour period in an incubator to determine early viability. Subsequently, all fetuses were examined for malformations by X-ray and given a macroscopic as well as microscopic examination.

There were no signs of maternal toxicity at any dose, and none of the rabbits died during the study. The frequency distribution of corpora lutea, implantations and live fetuses per dam as well as the early and late resorption rates did not differ between the groups. There were no treatment-related effects on abortions, fetal deaths, fetal weight, sex ratio, number of congenital runts, and visceral or skeletal malformations. The only significant effect noted was decreased survival of offspring during the first 24 hours in the low-dose (94%) and high-dose (88.5%) groups compared with the controls (100%). However, the survival of offspring in the low-dose group within the first 24 hours was not different from survival in historical controls (94.9%).

Whereas the study author concluded that the compound was not embryotoxic, maternally toxic or teratogenic at the highest dose tested (Gleich et al., 1985), the Committee considered it to exhibit slight embryo/fetal toxicity at the highest dose tested (based on decreased survival of offspring in the first 24 hours after birth) and identified the middle dose of 50 mg/kg bw per day as the NOAEL for embryo/fetal toxicity. The NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested.

To further elucidate whether teflubenzuron has an embryotoxic effect in rabbits, a non-GLP-compliant supplementary study was conducted. Groups of female Himalayan rabbits (five per group) considered to be pregnant after natural mating were gavaged with teflubenzuron (purity not reported) in 0.5% carboxymethyl cellulose at 0, 250 or 500 mg/kg bw per day (two groups at the high dose) on days 6–18 of gestation. All animals, except the second group at the high dose, were killed on day 19, and their livers were removed, weighed, homogenized and assayed for cytochrome P450 and *O*- and *N*-demethylase activities. Additionally, the numbers of corpora lutea, live embryos and resorptions were recorded. The remaining animals of the high-dose group were killed on day 29 of gestation, and their livers were examined as described above. Fetuses were examined for skeletal and visceral malformations as described in the previous study.

The only changes noted were a few early resorptions and a reduced total number of living embryos in treated animals. The study author considered teflubenzuron not to be embryotoxic or teratogenic in rabbits, as the observed

effects could be a chance finding due to the small number of animals used (Gleich, 1985). A closer analysis of the data, however, suggested a treatment-related increase in the number of early resorptions (0 in control versus 3 and 4 in the 250 and 500 mg/kg bw per day groups, respectively) and a decrease in the number of live fetuses (32 in controls versus 20 in each treatment group), showing a potential embryo/fetal toxic effect of teflubenzuron. The Committee, however, considered the findings of this study to be of limited significance for risk assessment.

In a GLP-compliant study, 22 pregnant New Zealand White rabbits were dosed by gavage with teflubenzuron (purity 92.4%) in 0.5% carboxymethyl cellulose at 1000 mg/kg bw per day during days 6–18 of pregnancy, and the effects of teflubenzuron on maternal and fetal health were compared with the effects in 16 controls. Rabbits were killed on gestation day 28 and examined for gross pathology and reproductive performance, including effects on developing fetuses.

No treatment-related changes in the behaviour or clinical conditions of the dams were noted. Treated rabbits had a higher incidence of macroscopic liver lesions – grossly granulated cut surface (8/22 in treated rabbits versus 1/16 in controls). Body weight, feed consumption and number, weight or sex of fetuses as well as preimplantation or postimplantation losses were not affected by treatment. There were no treatment-related developmental abnormalities.

In view of the observed liver lesions, no NOAEL could be identified for maternal toxicity, but the only dose tested (1000 mg/kg bw per day) was identified as a NOAEL for embryo/fetal toxicity in rabbits (Osterburg, 1987). However, this study did not evaluate the offspring survival during the first 24 hours, and hence the Committee cannot discount the effects seen in the previous study.

2.2.6 Special studies

(a) Haematological toxicity

The influence on haematological parameters, including the potential to form methaemoglobin, of five benzoyl urea insecticides, including teflubenzuron, was investigated in groups of 10 adult Wistar rats (sex and GLP compliance not specified). Ten rats served as controls. Animals received aqueous solutions of the active ingredient by gavage at 100 mg/kg bw per day for 28 days, and blood samples were collected at the end of the study.

No overt clinical signs of toxicity were observed during treatment. Compared with controls, teflubenzuron did not cause any changes in haemoglobin, haematocrit, red blood cell count, mean corpuscular volume or mean corpuscular haemoglobin concentration. Reticulocyte count was increased in all treated rats. Methaemoglobin levels were elevated in rats treated with diflubenzuron

and triflumuron, but not in those treated with flufenoxuron, hexaflumuron or teflubenzuron. Red blood cells and the haemoglobin concentrations in blood were not affected in rats treated with teflubenzuron when compared with controls (Tasheva & Hristeva, 1993).

(b) Effects on mammalian cells in vitro

The effects of teflubenzuron and other pesticides on the human intestinal Caco-2 cell line were investigated in vitro in a cell culture model. Among others, the effects of pesticides on cell viability, morphological changes, cell monolayer integrity, alkaline phosphatase activity, pesticide-induced oxidative stress and pesticide-induced cell death mechanism were investigated. The results indicated that various adverse effects could be observed on the in vitro cell culture at teflubenzuron concentrations of 5 µmol/L and above (Ilboudo et al., 2014). However, the Committee considered that the findings of this study were of limited relevance to the risk assessment of teflubenzuron as a veterinary drug.

(c) Microbiological effects

Considering the chemical structure and mode of action of teflubenzuron, the Committee did not anticipate any adverse effects of teflubenzuron residues on human gastrointestinal microbiota.

2.2.7 Dose–response analysis

The Committee considered that of the non-neoplastic changes observed in the liver of male mice in the carcinogenicity study (Suter et al., 1987b), hepatocellular hypertrophy was the most relevant end-point for the dose–response analysis. To better characterize the point of departure, the Committee conducted a dose–response analysis of these data using the United States Environmental Protection Agency’s Benchmark Dose (BMD) Software. The BMD and a lower confidence limit on the BMD for a 10% response over the controls (BMD₁₀ and BMDL₁₀, respectively) for hepatocellular hypertrophy were determined using nine different dichotomous models. A summary of these analyses is presented in [Table 4](#).

The LogLogistic, LogProbit and Multistage models provided acceptable fits based on statistical considerations. However, the BMD₁₀ and BMDL₁₀ estimated by the LogLogistic and LogProbit models were much lower than the lowest dose used in the study. Furthermore, the Multistage model provided a better fit of the BMDL value for the benchmark response at the low end of the observed range of the data ([Fig. 3](#)). Therefore, the Committee considered the BMDL₁₀ of 0.54 mg/kg bw per day for the BMD₁₀ of 0.73 mg/kg bw per day estimated by the Multistage model as the most appropriate point of departure for this study.

Table 4

BMD₁₀ and BMDL₁₀ for hepatocellular hypertrophy^a in male mice in the carcinogenicity study

Model	BMD ₁₀ (mg/kg bw per day)	BMDL ₁₀ (mg/kg bw per day)	P-value	AIC	Scaled residual for	
					Treatment	Control
Gamma	1.66	1.22	0.000 8	254.8	1.362	−1.861
Logistic	3.4	2.6	0	263.8	0.798	−3.089
LogLogistic	0.37	0.082	0.997	243.7	0	0
LogProbit	0.38	0.086	0.850	243.8	0.012	0.012
Multistage	0.73	0.54	0.248	245.1	−0.45	−0.45
Multistage-Cancer	1.7	1.2	0.000 8	254.8	1.362	−1.861
Probit	4.0	3.2	0	265.9	0.714	−3.258
Weibull	1.7	1.2	0.000 8	254.8	1.362	−1.861
Quantal-Linear	1.7	1.2	0.000 8	254.8	1.362	−1.861

AIC: Akaike information criterion; BMD₁₀: benchmark dose for a 10% response; BMDL₁₀: lower 95% confidence limit on the benchmark dose for a 10% response^a Proportion affected: 12/60, 29/60, 46/60 and 56/60 at control, low, middle and high doses, respectively.

2.3 Observations in humans

No information was available on the toxicity of teflubenzuron in humans.

3. Comments

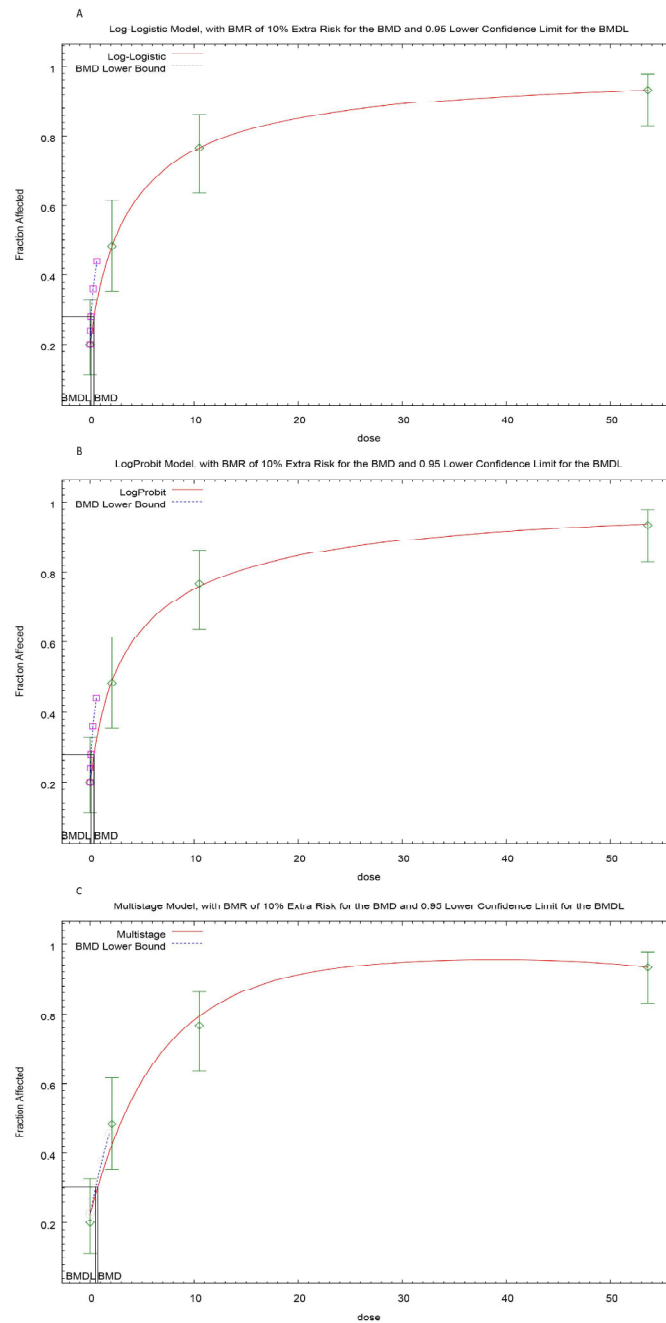
The Committee considered data on the pharmacokinetics, short- and long-term toxicity, reproductive and developmental toxicity, genotoxicity and carcinogenicity of teflubenzuron. In addition to a sponsor's submission, relevant studies retrieved from the published literature were evaluated. Most studies submitted by the sponsor were conducted under GLP-compliant conditions. Those that were not conducted under GLP-compliant conditions are identified below.

3.1 Biochemical data

Orally administered teflubenzuron was only partially, but relatively quickly, absorbed in rats. Following administration of a single oral dose of radiolabelled teflubenzuron at 25 mg/kg bw, approximately 20% of the radioactivity was absorbed; only 4% was absorbed when rats were dosed at 750 mg/kg bw, suggesting a dose-dependent absorption (Schlüter, 1986; Hawkins & Mayo, 1988). Peak plasma concentrations were reached within 1–2 hours post-dosing and were maintained at similar levels for up to 8 hours (low dose) or 24 hours (high dose) (Schlüter, 1986). In repeatedly dosed animals, there was some evidence of a dose-dependent plateau in plasma concentration (Ellgehausen et al., 1986).

Fig. 3

Benchmark dose–response curve for hepatocellular hypertrophy in mice for 10% extra risk for the BMD and BMDL: A. LogLogistic model, B. LogProbit model, and C. Multistage model



Most (90–95%) radiolabelled teflubenzuron administered by gavage to rats (single dose at 25 or 750 mg/kg bw or 14 daily doses of 25 mg/kg bw of unlabelled drug followed by a single dose of 25 mg/kg bw radiolabelled drug) was excreted in faeces, primarily as the parent compound. More than 85% of the drug was excreted within the first 24 hours of the dosing. Only a small fraction (0.15–3%) of the total oral dose of teflubenzuron was excreted in the urine. There was no difference in excretion pattern between sexes or between animals dosed with a single or multiple doses of the drug (Schlüter, 1984, 1986; Hawkins & Mayo, 1988). Absorbed teflubenzuron was mostly excreted through bile, predominantly as polar materials. Only negligible residues of teflubenzuron were detected in tissues and organs (<2% of the dose), with no evidence of accumulation (Schlüter, 1984, 1986; Hawkins & Mayo, 1988).

Metabolites identified in bile and urine were benzoyl or aniline ring hydroxylated teflubenzuron and conjugates of (3,5-dichloro-2,4-difluorophenyl)-urea and 3,5-dichloro-2,4-difluoroaniline (Schlüter, 1985; Hawkins & Mayo, 1988). Several polar metabolites were detected in faeces, but the only metabolite characterized was (3,5-dichloro-2,4-difluorophenyl)urea (Schlüter, 1986). Hydrolytic cleavage of the phenylurea bridge was identified as the predominant pathway of teflubenzuron metabolism in a non-GLP-compliant study in which rats were gavaged once with approximately 55 mg/kg bw of the drug. The scission products thus produced were either excreted unmodified or further metabolized and excreted (Koerts et al., 1997).

3.2 Toxicological data

Critical studies relevant to the risk assessment of teflubenzuron are summarized in Table 5. Most of the toxicity studies were conducted under GLP-compliant conditions, and the purity of the substance used, where specified, was greater than 90%.

Teflubenzuron was shown to have low acute toxicity in laboratory animals. The oral LD₅₀ in mice and rats was greater than 5000 mg/kg bw (Ullmann, 1983d,e; Ullmann, Sacher & Vogel, 1988). The dermal LD₅₀ in rats was greater than 2000 mg/kg bw (Ullmann, 1983b), and the inhalation LC₅₀ in rats was greater than 5000 mg/m³ air (Ullmann, 1983a). Teflubenzuron was not irritating to the skin (Ullmann, 1983g) or eyes (Ullmann, 1983f) of rabbits, and it did not cause skin sensitization in the guinea-pig maximization test (Ullmann, 1984).

Short-term toxicity studies of teflubenzuron in which the drug was administered in diet were conducted in mice (one 13-week study), rats (one 13-week study) and dogs (two 13-week studies and one 52-week study). In all three

Table 5
Studies relevant to risk assessment

Species / study type (route of administration)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Mouse				
Eighteen-month carcinogenicity study (diet)	0, 2.1, 10.5, 53.6	Hepatocellular adenoma	2.1	10.5
		Hepatocellular hypertrophy	–	2.1 ^a
			BMDL ₁₀ : 0.54*	–
Rat				
Two-year toxicity and carcinogenicity study (diet)	0, 1.0, 4.8, 24.8	Increased activity of liver enzymes, and increased liver weights in males	4.8 ^b	24.8 ^c
Two-generation reproductive toxicity study (diet)	0, 1.5, 7.4, 36.9	Unilateral and bilateral dilatation of the renal pelvis in F ₁ pups	Offspring toxicity: 7.4	36.9
			Parental toxicity: 36.9 ^d	–
			Reproductive toxicity: 36.9 ^d	–
Developmental toxicity studies ^e (gavage)	Study 1: 0, 10, 50, 250	Decreased number of live pups per dam	Embryo/fetal toxicity: 50 (study 1)	250 (study 1)
	Study 2: 0, 100, 300, 1 000		Maternal toxicity: 1 000 ^{b,d}	–
Rabbit				
Developmental toxicity studies ^e (gavage)	Study 1: 0, 10, 50, 250	Decreased survival of offspring within 24 h of birth	Embryo/fetal toxicity: 50 ^b	250 ^c
	Study 2: 0, 250, 500		Maternal toxicity: 500 ^{b,d}	–
	Study 3: 0, 1 000			
Dog				
Thirteen-week studies of toxicity ^e (diet)	Study 1: 0, 3.5, 33.7, 318.2	Increased liver weight	4.4 ^b	17.3 ^c
	Study 2: 0, 1.2, 4.4			
One-year study of toxicity (diet)	0, 1.0, 3.2, 17.3			

* Pivotal study value for the derivation of an ADI (Suter et al., 1987b)

^a Lowest dose tested.

^b Overall NOAEL.

^c Overall LOAEL.

^d Highest dose tested.

^e Two or more studies combined.

species, liver was identified as the target organ for toxic effects, as evidenced by elevated enzyme activities and/or adaptive cellular changes.

In a study whose GLP-compliant status could not be verified, mice were administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg diet (equal to 0, 12, 115 and 1213 mg/kg bw per day for males and 0, 14, 142

and 1450 mg/kg bw per day for females, respectively) for 13 weeks. In the high-dose group, activities of liver enzymes (alkaline phosphatase in males, alanine transaminase in females) and total cholesterol levels (females) were elevated, and blood glucose levels (both sexes) were altered. In the mid- and high-dose groups, absolute and relative liver weights were increased, with centrilobular hepatocellular swelling (both sexes) and microscopic fatty changes (in males). The NOAEL was identified as 100 mg/kg feed (equal to 12 mg/kg bw per day), based on liver changes at 1000 mg/kg feed (equal to 115 mg/kg bw per day) (Takahashi et al., 1987).

Rats were administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 8, 82 and 809 mg/kg bw per day for males and 0, 9, 94 and 942 mg/kg bw per day for females, respectively) for 13 weeks. Some animals from the control and high-dose groups were observed for an additional 4 weeks without treatment. Increased activities of several enzymes, notably aspartate transaminase (middle and high doses, both sexes), ornithine transcarbamylase (high dose, both sexes), alanine transaminase (mid- and high-dose males), lactate dehydrogenase (high-dose males) and alkaline phosphatase (all treated males), were observed, which returned to normal levels after 4 weeks of drug withdrawal. At necropsy, increased absolute and relative weights of liver in females and of testes in males were observed at the high dose. The statistically significant change in alkaline phosphatase activity in male rats of the low-dose group was not considered to be biologically relevant, being less than 1.5-fold above the control value and within the normal physiological range. Based on effects on liver enzymes at 1000 mg/kg feed (equal to 82 mg/kg bw per day), a NOAEL of 100 mg/kg feed (equal to 8 mg/kg bw per day) was identified (Suter et al., 1987c).

In a non-GLP-compliant study, the potential of teflubenzuron to form methaemoglobin and exert other effects on haematological parameters in rats was investigated in a comparative study that investigated five benzoylurea insecticides. Although reticulocyte count was increased in rats treated with teflubenzuron (100 mg/kg bw per day for 28 days), it was not associated with anaemia or the formation of methaemoglobin (Tasheva & Hristeva, 1993).

In dogs administered teflubenzuron at a concentration of 0, 100, 1000 or 10 000 mg/kg feed (equal to 0, 3.5, 33.7 and 318.2 mg/kg bw per day for males and 0, 4.0, 42.8 and 417.1 mg/kg bw per day for females, respectively) for 13 weeks, activities of alanine transaminase, aspartate transaminase, alkaline phosphatase and ornithine transcarbamylase were increased in both sexes at the high dose. At necropsy, absolute and relative liver weights were elevated and the incidence of nodular foci in the pyloric or fundic region of the stomach was increased at the high dose. Isolated dark red foci were noted in the pyloric region of the stomach at the middle and high doses. Focal gastritis was observed in females at the middle

and high doses, and follicular hyperplasia of the pyloric mucosa was noted in most animals at the high dose. Mild hepatitis in one male and centrilobular hepatic necrosis in another male were observed at the low dose. Also, moderate chronic active hepatitis was diagnosed in one animal of each sex at the high dose. Given the lack of a clear dose–response relationship for hepatitis, the mild microscopic hepatic changes noted in two dogs in the low-dose group were not considered to be a treatment-related adverse effect. The NOAEL was 100 mg/kg feed (equal to 3.5 mg/kg bw per day), based on stomach lesions at 1000 mg/kg feed (equal to 33.7 mg/kg bw per day) (Bathe et al., 1985).

In a supplementary study to clarify the effects on liver, dogs were administered teflubenzuron at a concentration of 0, 30 or 100 mg/kg feed (equal to 0, 1.2 and 4.4 mg/kg bw per day for males and 0, 1.5 and 5.1 mg/kg bw per day for females, respectively) for 13 weeks. There were no treatment-related adverse effects identified in clinical examination, laboratory testing, and macroscopic or microscopic examination of organs. The NOAEL was 100 mg/kg feed (equal to 4.4 mg/kg bw per day), the highest dose tested (Bathe et al., 1987).

In a 52-week study, dogs were administered teflubenzuron at a concentration of 0, 30, 100 or 500 mg/kg feed (equal to 0, 1.0, 3.2 and 17.3 mg/kg bw per day for males and 0, 1.2, 4.0 and 18.0 mg/kg bw per day for females, respectively). At necropsy, the absolute liver weight in males was increased at the high dose, but no treatment-related changes were noted in gross pathological or histopathological examinations. The NOAEL was 100 mg/kg feed (equal to 3.2 mg/kg bw per day), based on the liver weight change at 500 mg/kg feed (equal to 17.3 mg/kg bw per day) (Sachs et al., 1986).

The Committee identified an overall NOAEL of 100 mg/kg feed (equal to 4.4 mg/kg bw per day) from the three short-term studies in dogs, based on the findings of adverse effects in liver at a dose of 500 mg/kg feed (equal to 17.3 mg/kg bw per day).

In a carcinogenicity study in mice, teflubenzuron was administered at a concentration of 0, 15, 75 or 375 mg/kg feed (equal to 0, 2.1, 10.5 and 53.6 mg/kg bw per day for males and 0, 3.1, 15.4 and 71.7 mg/kg bw per day for females, respectively) for 78 weeks, with an interim kill at week 52. Aspartate transaminase, alanine transaminase, ornithine transcarbamylase, lactate dehydrogenase and alkaline phosphatase activities were elevated in high-dose males, but only alanine transaminase activity was elevated in high-dose females. Absolute and relative liver weights were higher in both sexes at the high dose, and relative liver weight was slightly increased in the mid-dose males. The incidence of macroscopic hepatic nodules was increased in the high-dose males. Histopathology indicated an increased incidence of hepatocellular adenomas and nodular hepatic hyperplasia in males treated at the middle and high doses compared with both concurrent and historical controls, but there was no difference in the incidence

of hepatic carcinoma. Several treatment-related, dose-dependent non-neoplastic hepatic changes were also observed, which were more pronounced in males than in females. In particular, males in the control, low-dose, mid-dose and high-dose groups, respectively, had dose-dependent incidences of hepatocellular hypertrophy (12/60, 29/60, 46/60 and 56/60), single-cell necrosis (13/60, 26/60, 42/60 and 56/60), phagocytic cell foci (17/60, 21/60, 43/60 and 54/60) and lipofuscin accumulation (8/60, 11/60, 20/60 and 27/60). In the low-dose group, the incidence, but not the severity, of these non-neoplastic hepatic changes was significantly higher when compared with the controls (Suter et al., 1987b).

Histopathological sections of liver from male mice in this study were re-evaluated by an independent pathologist, with a focus on nodular liver lesions. The pathologist concluded that there was a dose-related increase in the incidence of hepatocellular hyperplastic nodules and a slight, but statistically non-significant, increase in hepatocellular adenoma (Vesselinovitch, 1988).

Given that only hepatic adenomas were observed and that the genotoxicity test results were negative (see below), the Committee considered that teflubenzuron was not carcinogenic in mice. However, the Committee concluded that teflubenzuron induced hyperplastic proliferation in liver of mice by an unknown mechanism. Based on the increased incidence of non-neoplastic hepatic changes observed in liver (e.g. hepatocellular hypertrophy, single-cell necrosis, phagocytic cell foci, lipofuscin accumulation) at all doses, no NOAEL could be identified. The lowest dietary concentration, 15 mg/kg feed (equal to 2.1 mg/kg bw per day), was identified as the LOAEL.

In the absence of a NOAEL, to better characterize the point of departure, the Committee conducted a dose-response analysis of these data using the BMD approach. Of several non-neoplastic hepatic changes identified, hepatocellular hypertrophy was considered to be the most toxicologically relevant effect for dose-response modelling. The BMD_{10} and $BMDL_{10}$ were determined using nine different dichotomous models. Three models (LogLogistic, LogProbit and Multistage) provided acceptable fits based on statistical considerations. However, the BMD_{10} and $BMDL_{10}$ estimated by the LogLogistic and LogProbit models were much lower than the lowest dose used in the study. Furthermore, the Multistage model provided a better fit of the BMDL value for the benchmark response at the low end of the observed range of the data. Therefore, the Committee considered the $BMDL_{10}$ of 0.54 mg/kg bw per day for the BMD_{10} of 0.73 mg/kg bw per day estimated by the Multistage model as the most appropriate point of departure for this study.

In a carcinogenicity study, rats were administered teflubenzuron at a concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.0, 4.8 and 24.8 mg/kg bw per day for males and 0, 1.2, 5.9 and 29.9 mg/kg bw per day for females, respectively) for 120 weeks, with an interim kill at weeks 53 and 107. Mortality

ranged from 40% to 50% at week 120, which was not influenced by treatment. Increased (approximately 1.5- to 3-fold) activities of alanine transaminase, aspartate transaminase and ornithine transcarbamylase were noted in males in the high-dose group. Absolute and relative liver weights were increased in high-dose males. Several non-neoplastic microscopic changes were noted in different organs, but were not treatment related. Trend analysis identified increased incidences of haemangiomas in mesenteric lymph nodes and pancreatic exocrine carcinoma in the high-dose males. However, they were not significantly different when compared with historical controls. Also, the occurrence of pancreatic exocrine carcinoma was too infrequent (2/47 versus 0/50) to allow a meaningful comparison to be drawn. Based on the effect on liver enzymes and liver weight at 500 mg/kg feed (equal to 24.8 mg/kg bw per day), the NOAEL was 100 mg/kg feed (equal to 4.8 mg/kg bw per day) (Suter et al., 1987a).

In a supplemental carcinogenicity study, rats were administered teflubenzuron at a concentration of 0, 2500 or 10 000 mg/kg feed (equal to 0, 123 and 487 mg/kg bw per day for males and 0, 154 and 615 mg/kg bw per day for females, respectively) for 111 weeks, with an interim kill at week 104. Clinical biochemistry revealed increased activities of alanine transaminase and aspartate transaminase in males at both doses and of aspartate transaminase in females at the high dose. The absolute and relative liver (interim and terminal kill) and kidney (interim kill) weights were increased in males at the high dose compared with controls. Dose-dependent increases in the incidence of diffuse, clay-coloured discoloration and focal and multifocal discoloration of livers were observed in treated males (both doses). Also, treatment-related non-neoplastic microscopic changes (e.g. fatty changes, mixed cell and basophilic cell foci, focal hepatocellular hyperplasia, spongiosis hepatis) were noted in the liver of both sexes at both doses tested, lesions being more severe in males than in females. There was no compound-related increase in the incidence of any tumours observed in this study, including mesenteric lymph node haemangioma and pancreatic exocrine carcinoma in male rats, thus confirming the lack of association between substance administration and the occurrence of these tumours suggested from the previous study (Tennekes et al., 1989).

Although no NOAEL could be identified in the second study owing to non-neoplastic microscopic hepatic changes and elevated liver enzyme activities in both treatment groups, the Committee was able to identify an overall NOAEL of 100 mg/kg feed (equal to 4.8 mg/kg bw per day) from the two chronic toxicity and carcinogenicity studies in rats.

The Committee concluded that teflubenzuron is not carcinogenic in mice or rats.

The genotoxic potential of teflubenzuron was investigated in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was detected, and teflubenzuron was considered unlikely to be genotoxic.

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

In a multigeneration reproductive toxicity study, teflubenzuron was administered to rats at a concentration of 0, 20, 100 or 500 mg/kg feed (equal to 0, 1.5, 7.4 and 36.9 mg/kg bw per day for males and 0, 1.6, 7.9 and 39.5 mg/kg bw per day for females, respectively). The only treatment-related adverse effect noted was a significant increase in the incidence of unilateral and bilateral dilatation of the renal pelvis in F₁ pups in the high-dose group (6.8%) when compared with the controls (0.9%). No such effect was seen in the F₂ generation. The NOAEL for offspring toxicity was 100 mg/kg feed (equal to 7.4 mg/kg bw per day), and the NOAEL for both parental and reproductive toxicity was 500 mg/kg feed (equal to 36.9 mg/kg bw per day), the highest dose tested (Osterburg, 1989).

The developmental toxicity of teflubenzuron was investigated in pregnant rats by gavage administration at 0, 10, 50 or 250 mg/kg bw per day from days 6 to 15 of gestation. The number of live pups per dam was significantly reduced at the high dose when compared with the controls. The NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested, and the NOAEL for embryo/fetal toxicity was 50 mg/kg bw per day, based on a reduction in the number of live pups per dam at 250 mg/kg bw per day (Gleich, Weisse & Unkelbach, 1986).

In a second developmental toxicity study, teflubenzuron was administered by gavage to pregnant rats at 0, 100, 300 or 1000 mg/kg bw per day during days 7–17 of gestation. No treatment-related toxicity was observed in dams, for both general health and reproductive parameters. No external, visceral or skeletal abnormalities were observed in pups. The NOAEL for both maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Ishida et al., 1987).

An overall NOAEL of 1000 mg/kg bw per day for maternal toxicity was identified. No overall NOAEL for embryo/fetal toxicity could be identified, as the reason for the difference in NOAELs for embryo/fetal toxicity in these two developmental toxicity studies in rats was unknown.

Pregnant rabbits were dosed with teflubenzuron by gavage at 0, 10, 50 or 250 mg/kg bw per day from days 6 to 18 of gestation and killed on day 29 of gestation. No maternal or reproductive toxicity was observed, and there were no developmental abnormalities. The only significant effect noted in the offspring was decreased survival during the first 24 hours in the high-dose group (88.5%) compared with the controls (100%). The NOAEL for embryo/fetal toxicity was 50 mg/kg bw per day, based on decreased survival at 250 mg/kg bw per day, and the

NOAEL for maternal toxicity was 250 mg/kg bw per day, the highest dose tested (Gleich et al., 1985).

To further elucidate the embryotoxic effect in rabbits, a small supplementary study (five per group) was conducted by administering teflubenzuron by gavage at 0, 250, 500 (killed on day 19) or 500 mg/kg bw per day (killed at day 29) on days 6–18 of gestation. There was evidence of embryotoxicity in all treated animals, although no maternal toxicity was identified (Gleich, 1985).

In another developmental toxicity study in rabbits, pregnant animals were dosed with teflubenzuron by gavage at 0 or 1000 mg/kg bw per day during days 6–18 of pregnancy and killed on gestation day 28. Treated rabbits had a higher incidence of liver lesions compared with controls, but there were no treatment-related reproductive or developmental abnormalities. No NOAEL was identified for maternal toxicity, as effects were noted at the only dose tested. The NOAEL for embryo/fetal toxicity was 1000 mg/kg bw per day, the only dose tested. However, this study did not evaluate offspring survival during the first 24 hours, and hence the Committee cannot discount the effects seen in the previous study (Osterburg, 1987).

An overall NOAEL of 500 mg/kg bw per day was identified for maternal toxicity, and an overall NOAEL of 50 mg/kg bw per day was identified for embryo/fetal toxicity.

3.3 Microbiological data

Considering the chemical structure and mode of action of teflubenzuron, the Committee did not anticipate any adverse effects of teflubenzuron residues on human gastrointestinal microbiota.

4. Evaluation

An ADI of 0–5 µg/kg bw was established on the basis of a BMDL₁₀ of 0.54 mg/kg bw per day for hepatocellular hypertrophy in male mice observed in the carcinogenicity study, with application of an uncertainty factor of 100 to account for interspecies and intraspecies variability, and rounded to one significant figure.

The use profile of teflubenzuron as a veterinary drug is such that dietary exposure to teflubenzuron from a large portion is unlikely to be markedly greater than that from chronic consumption. The toxicological profile of teflubenzuron is such that it is unlikely to present an acute hazard. The Committee therefore concluded that it was not necessary to assess the acute risk from exposure to teflubenzuron when used as a veterinary drug.

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