The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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May 1997

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TEFLUBENZURON

SUMMARY REPORT (1)

1. Teflubenzuron [1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea] is an acyl urea derivate classified as an insecticide for use in treatment of infestation with sea lice in salmon. Teflubenzuron is admixed with pelleted diet at a level of 2 g/kg. The intended dosage level of teflubenzuron is 10 mg/kg bw administered once daily for 7 consecutive days. The substance is also used as a pesticide on crops. Very few substances are available for treatment of sea lice in salmon.

2. Teflubenzuron acts by interference with the synthesis of chitin. Demand for chitin is greatest at the moult between growth stages and hence insects are killed due to disruption of the moulting process. No specific studies on pharmacodynamic properties of teflubenzuron were submitted.

3. In rats, teflubenzuron is only partially absorbed from the gastrointestinal tract (4 to 19%) and the absorption is dose-dependent and saturable. Maximal plasma concentrations are reached within 8-24 hours after a single oral dose. The saturable kinetics of teflubenzuron results in plasma levels that are essentially constant after repeated administration of teflubenzuron in the diet at concentrations of greater than or equal to 1000 to 2000 mg/kg feed (77 to 158 mg/kg bw/day). Following repeated administrations of ^{14}C-teflubenzuron the highest concentrations of teflubenzuron are present in fat, liver and kidneys. The distribution is rapid and the maximum concentration for almost all tissues occurs at 6 hours post dosing. Residues in organs and tissues decline quickly and there is no evidence of accumulation of teflubenzuron. Teflubenzuron is rapidly and completely excreted, mainly via the faeces (more than 90% of the dose). Absorbed teflubenzuron is largely excreted in the bile (2 to 16% of the dose), while the urinary excretion only represents a minor pathway (0.4 to 1.4% of the dose). There is no difference in excretion pattern between males and females neither after single nor after repeated administrations of teflubenzuron, mainly due to saturable kinetics. No pharmacokinetic studies in other laboratory species were provided.

4. Teflubenzuron is eliminated largely unchanged in faeces of rats. A minor faecal metabolite, less than 1% of the dose, characterised as 3,5-dichloro-2,4-difluorophenylurea, indicates that cleavage of the benzoylurea moiety occurs. This metabolite and 3,5-dichloro-2,4-difluoroaniline were found in urine at low levels (less than 0.1%). The presence of conjugates of these metabolites including unchanged 3,5-dichloro-2,4-difluorophenylurea was detected in bile. In urine three additional minor (less than 1%) hydroxylated metabolites of teflubenzuron were identified, two hydroxylated benzoyl derivatives and one hydroxylated aniline derivative.

5. Teflubenzuron has a low acute oral toxicity in rats and mice (LD_{50} = greater than 5000 mg/kg bw) and a low acute dermal and inhalation toxicity in rats (LD_{50} = greater than 2000 mg/kg bw, LC_{50} = greater than 5038 mg/m^3). No deaths occurred in these studies. Teflubenzuron was more acutely toxic after intraperitoneal administration in rats (LD_{50} = greater than 2000 mg/kg bw), including a few deaths, peritonitis and symptoms such as sedation, dyspnea, ataxia, ruffled hair, body weight loss and diarrhoea.

7 Westferry Circus, Canary Wharf, London E14 4HB, UK
Tel: (+44-171) 418 84 00 Fax: (+44-171) 418 84 16

6. Short term repeat dose toxicity tests in rats, mice and dogs revealed that the major target organ for the toxic effect of teflubenzuron is the liver. The main findings attributed to hepatotoxicity were increased liver weights and liver lesions, such as hepatocellular swelling, collapsed stroma, fatty changes, necrosis and cell infiltration.

   In a 90-day toxicity study in rats, teflubenzuron was administered via the diet at concentrations of 0, 100, 1000 or 10 000 mg/kg feed, equal to 0, 8, 82 or 809 mg/kg bw/day in males and 0, 9, 94 or 942 mg/kg bw/day in females. Increased activity of serum enzymes and increased liver weights were noted in the two highest dose groups. The NOEL was 100 mg/kg feed, equal to 8 mg/kg bw/day.

   In a 90-day toxicity study carried out in mice, teflubenzuron was administered via the diet at concentrations of 0, 100, 1000 or 10 000 mg/kg feed, equal to 0, 12, 115 or 1210 mg/kg bw/day in males and 0, 14, 143 or 1450 mg/kg bw/day in females. Animals in the two highest dose groups showed increased activity of serum enzymes, enlargement of livers and hepatocellular swelling. In addition, centrilobular fatty change in the mid-dose group and discoloration of the liver in the high-dose group were noted. The NOEL was 100 mg/kg feed, equal to 12 mg/kg bw/day.

   Dogs were administered teflubenzuron for 90 days in the feed at concentrations of 0, 100, 1000 or 10 000 mg/kg feed, equal to 0, 3.2, 30.4 or 323 mg/kg bw/day for both sexes. Effects observed were increased activity of serum enzymes in the low- and high-dose group and increased liver weights in the high dose group. Animals from the high-dose group showed slight to moderate hepatotoxicity, characterised mainly by single cell necrosis, cell infiltration and collapsed stroma. In the low-dose group a slight hepatotoxicity was found in one male and moderate necrosis of the liver was noted in another male dog. Macroscopic and microscopic findings in the stomach revealed red foci, nodular foci, focal gastritis and hyperplasia in the two highest dose groups. No statistical analysis of the histopathological effects was performed. In view of the possible treatment related hepatotoxicity in the livers of two of eight dogs at 100 mg/kg feed, equal to 3.2 mg/kg bw/day, no NOEL could be established in this study. In a supplementary 90-day toxicity study in dogs, designed to establish a definitive NOEL, concentrations of teflubenzuron in feed were 0, 30 or 100 mg/kg feed, equal to 0, 1.2 or 4.1 mg/kg bw/day for both sexes. No treatment related effects were noted in the study and the NOEL was 100 mg/kg feed, equal to 4.1 mg/kg bw/day.

7. Long term repeat dose toxicity was studied in dogs. In a one-year (52 weeks) chronic toxicity study, dogs were fed diets containing 0, 30, 100 or 500 mg/kg feed, equal to 0, 1, 3.2 or 17.3 mg/kg bw/day in males and 0, 1.2, 4 or 18 mg/kg bw/day in females. Based on increased liver weights in the high dose group the NOEL was 100 mg/kg feed, equal to 3.2 mg/kg bw/day.

8. Tolerance studies have been performed in Atlantic salmon. Teflubenzuron was well tolerated in single doses of 0, 500, 1000 or 1500 mg/kg bw, apart from that all fish including controls swam slowly and the majority of the fish were pale in colour. After repeated doses of five times the recommended dose and three times the recommended treatment period the only effect seen was a slight discoloration in the majority of the fish, although a small number of fish were distinctly darker. After administration of about 130 mg/kg bw/day (thirteen times the recommended dosage) for 10 days the swimming activity of the fish was decreased and they had problems maintaining a fixed position in the water. However, these symptoms disappeared after cessation of the treatment.

9. A two-generation reproduction study (one litter/generation) was performed in rats, with dietary concentrations of 0, 20, 100 or 500 mg/kg feed, which corresponds to 0, 1.5 to 1.9, 7.4 to 9.6 or 36.9 to 48.2 mg/kg bw/day in males and 0, 1.6 to 3.6, 7.9 to 18.5 or 39.5 to 89.3 mg/kg bw/day in females. There was no evidence of toxicity or effects on reproductive performance in either of the two generations. The NOEL for reproductive effects was 500 mg/kg feed, equal to 36.9 mg/kg bw/day.

10. In a teratogenicity study in rats teflubenzuron was administered orally by gavage at doses of 0, 10, 50 or 250 mg/kg bw/day. A slightly reduced number of foetuses in the highest dose group was noted. The NOEL for foetotoxicity was 50 mg/kg bw/day and for maternal toxicity 250 mg/kg bw/day. In a teratogenicity study in rabbits teflubenzuron was administered orally by gavage at doses of 0 or 1000 mg/kg bw/day. A NOEL for maternal toxicity could not be established conclusively in view of a possible effect on the liver. No evidence of teratogenicity or embryotoxicity/foetotoxicity was found and the NOEL was 1000 mg/kg bw/day. In another teratogenicity study in rabbits no effects were observed after being given oral doses of 0, 10, 50 or 250 mg/kg bw/day and the NOEL for maternal toxicity, foetotoxicity and teratogenicity was 250 mg/kg bw/day.

11. The mutagenic potential of teflubenzuron was explored in a battery of properly conducted *in vitro* and *in vivo* tests. Tests performed were the Ames test, HGPRT test, chromosome aberrations, unscheduled DNA synthesis and micronucleus assay. Negative results were obtained in all studies and enables the conclusion that teflubenzuron is not mutagenic.

    The potential for covalent binding of teflubenzuron to mouse liver DNA was investigated after a single oral administration of ^{14}C-teflubenzuron (40 mg/kg bw) to mice. The covalent binding index (CBI) for teflubenzuron was 0.1, which indicates that it is unlikely that DNA binding represents a potential mechanism for liver adenoma induction by teflubenzuron.

12. In a 18-month carcinogenicity study in mice, teflubenzuron was given via the diet at concentrations of 0, 15, 75 or 375 mg/kg feed, equal to 0, 2.1, 10.5 or 53.6 mg/kg bw/day in males and 0, 3.1, 15.4 or 71.7 mg/kg bw/day in females. Toxicological findings observed were increased liver weights at the two highest doses and enhanced activity of serum enzymes in the highest dose group. Non-neoplastic dose-dependent hepatic changes were observed in both sexes of all treated groups, such as hypertrophy, hyperplasia, single cell necrosis, phagocytic cell foci, lipofuscin accumulation and glycogen storage. The lowest dose 15 mg/kg feed, equal to 2.1 mg/kg bw/day, was the LOEL (lowest observed effect level), with only an increased incidence of hypertrophy, single cell necrosis and phagocytic cell foci (only in males). No statistical analysis of the non-neoplastic lesions was performed. However, a larger percentage of animals revealed hepatic changes at 15 mg/kg feed (hypertrophy 29/60 and necrosis 26/60) compared with control animals (hypertrophy 12/60 and necrosis 13/60). The incidence of hepatocellular adenomas at 18-month (terminal kill) was significantly increased in males at the two highest dose groups: 22% in the mid-dose group and 32% in the high-dose group, versus 12% in the concurrent control group. There were no changes in the incidence of hepatocellular carcinomas. Since the increased incidence of hepatocellular adenomas is likely to be secondary to the hepatotoxicity of teflubenzuron and was of a type generally not considered to be of concern for human health if not accompanied by other evidence for carcinogenicity, the mouse study was considered not to provide evidence for carcinogenicity of teflubenzuron.

    In a 120-week chronic toxicity/carcinogenicity study, rats were fed diets containing 0, 20, 100 or 500 mg/kg feed, equal to 0, 1, 4.8 or 24.8 mg/kg bw/day in males and 0, 1.2, 5.9 and 29.9 mg/kg bw/day in females. The mean plasma concentrations of teflubenzuron were found to be dose-related. Based on increased activities of serum enzymes and increased liver weights in males the NOEL was 100 mg/kg feed, equal to 4.8 mg/kg bw/day. Histopathological examination indicated an increased incidence of mesenteric lymph node hemangiomas in males in the high-dose group (17%) in comparison with rats in concurrent controls (2%), but not when compared to the incidence in historical controls. A significantly increased incidence of pancreatic exocrine carcinomas in male rats in the high-dose group (4.3%), was based on a low number of affected rats (2/47) and was therefore not considered to be treatment related.

In a supplementary study, rats were fed diets containing 0, 2500 or 10 000 mg/kg feed, equal to 0, 122.5 or 487.3 mg/kg bw/day in males and 0, 154 or 615.2 mg/kg bw/day in females, for 111 weeks. The mean plasma concentrations of teflubenzuron increased with time but not with dose, and were lower in females than in males throughout the study. No NOEL could be established because increased activity of serum enzymes and also non-neoplastic liver changes were noted at both doses. Liver lesions found were cell foci, fatty change, hyperthrophy and hyperplasia. The incidence of adenocarcinomas in the uterus was 10% in females at the high-dose group, versus no such tumours in concurrent controls. In comparison with controls in the previous 120-week toxicity/carcinogenicity study the incidence of adenocarcinomas in uterus was not increased and therefore not considered to be treatment related. The increase of mesenteric lymph node haemangiomas found in the 120 week study was not reproduced in the 111 week study and therefore considered not to be treatment related. The overall conclusion from the three mouse and rat studies, taking into consideration the negative results of the mutagenicity studies, there was no evidence for carcinogenicity of teflubenzuron. The lowest dose (2.1 mg/kg bw/day) in the mouse study showed minor hepatotoxic effects.

13. Test for delayed hypersensitivity with teflubenzuron (dissolved in polypropylene glycol/saline 1:1) was carried out in a Magnusson and Kligman tests. The results of two topical challenges after intradermal or topical induction indicated that teflubenzuron did not show any sensitizing properties.

    The potential of teflubenzuron to produce skin or eye irritation was investigated in rabbits in two separate studies. There was no evidence of either skin or eye irritation.

14. Studies on the microbiological properties of teflubenzuron were not submitted and are not considered to be necessary in view of the nature of the compound.

15. No studies were submitted on observations in humans.

16. The Joint FAO/WHO Meeting on Pesticides Residues (JMPR) evaluated teflubenzuron in 1994 and established an ADI of 0.01 mg/kg bw/day, based on the dose-related effects in the target tissue liver in the mouse carcinogenicity study submitted.

17. The proposed ADI by JMPR may be accepted provisionally. The LOEL of 2.1 mg/kg bw/day was based on the dose-related effects in the target tissue liver and derived from the 18-month carcinogenicity study in mice, which can be considered as the most sensitive species, and applying a safety factor of 200 since no NOEL was identified, a toxicological ADI of 0.01 mg/kg bw (equivalent to 600 µg/day for a 60 kg person) can be provisionally established for teflubenzuron.

18. After a single oral administration of 10 mg teflubenzuron/kg bw to Atlantic salmon held at 7 to 8ºC, 9ºC or 13 to 14ºC, the highest mean plasma concentrations, 0.311, 0.150 and 0.572 µg/ml respectively, were obtained 9 to 24 hours post dose. The plasma levels fell progressively with half-lives of 15 to 20 hours. After a single intravenous dose of 2 mg teflubenzuron/kg bw at 13 to 14ºC the mean peak plasma level was 5.192 µg/ml at 15 minutes post injection. The bioavailability of teflubenzuron in salmon was very low, approximately 4 % at 9ºC and 9 % at 13 to 14ºC indicating a temperature-dependent absorption.

    In repeated administration of medicated feed at a level of 10 mg teflubenzuron/kg bw/day for 7 days to Atlantic salmon at 7-8ºC (actual dose 7.8 mg/kg) and 9ºC steady-state levels of 0.170 µg/ml and 0.450 µg/ml in plasma were achieved after approximately 3 days. The elimination half-life was estimated to be 23 hours.

    No specific study on excretion has been performed in the target species.

19. Three radiometric depletion studies were conducted in Atlantic Salmon. In two of these studies, performed at a water temperature of 10ºC, the metabolism was investigated. The metabolites which were identified were also found in the rat.

    Following a single oral treatment of 10 mg ^{14}C-teflubenzuron/kg bw in salmon kept at 10ºC, the highest amounts of radioactivity were present in the gall bladder, liver and kidney. For muscle and skin the mean maximum concentrations occurred at 24 hours post dosing, 410 µg equivalents/kg and 753 µg equivalents/kg respectively. The levels decreased with half-lives of elimination of 4.7 and 6.5 days for muscle and skin respectively.

    After 24 hours about 99% and 104% of the radioactivity could be extracted from muscle and skin. After 8 days 84% and 77% of the radioactivity was extracted from muscle and skin, respectively. Only unchanged teflubenzuron was detected at both day 1 and day 8 in both muscle and skin. The ratio of parent compound to total residues was 97% and 79% at day 1 and day 8 respectively for muscle, and 99% and 58% at the same time-points for skin. In the liver and kidney unchanged teflubenzuron was the major component; 77% and 69% respectively at day 1. Minor metabolites were also detected, of which 2-hydroxy and 3-hydroxyteflubenzuron (4.6% and 1.6% of the radioactivity in the kidney) and 3,5-dichloro-2,4-difluoroaniline (3.1% of the radioactivity in the liver) were identified.

20. In a repeated dose study at 10ºC salmon were administered teflubenzuron mixed with feed to give a dose of 10 mg/kg bw/day for 6 days followed by a single administration of 10 mg/kg bw of ^{14}C-teflubenzuron. The highest concentration of radioactivity was detected in liver at day 1. For muscle and skin the mean maximum concentration of radioactivity occurred at day 1; 310 µg equivalents/kg for muscle and 554 µg equivalents/kg for skin. The levels decreased with initial half-lives of 2.6 and 3.6 days for muscle and skin respectively (over a period of 18 days). The mean levels of radioactivity were very low in muscle and skin at day 8; 15 and 41 µg equivalents/kg respectively. The concentration levels given were those resulting from the radioactive administration only. Any material resulting from the previous six cold doses was not detected by this experimental procedure.

    Extract analysis for the presence of metabolites produced essentially similar results to those obtained in the single dose study. The major component present in all the tissues examined was unchanged teflubenzuron at day 1. In muscle and skin respectively about 96% and 88% of the radioactivity could be extracted at 24 hours. The ratio of parent compound to total residues for muscle and skin calculated from the last dose was 77% and 82% at this time-point. Eight days after the administration these ratios were 19% for both muscle and skin, but the values of radioactivity measured were low and no level of quantification was given for the radio HPLC method used. Three minor metabolites were detected in liver. Two of them were identified as 3-hydroxy-teflubenzuron (7.9 % of the radioactivity) and 3,5-dichloro-2,4-difluorophenyl urea (2.8 % of the radioactivity).

    No data were provided which show that the ratios calculated from the last dose of the repeated metabolism study are the same for residues resulting from the whole recommended repeated treatment schedule.

21. In a similar study in salmon maintained at 6ºC and receiving 10 mg teflubenzuron/kg bw/day for 13 days followed by a single dose of 10 mg/kg bw of ^{14}C-teflubenzuron the highest amounts of radioactivity were present in liver. The total residues, resulting from the last dose, in muscle and skin one day after dosing were equivalent to 153 µg/kg and 218 µg/kg, respectively and the levels decreased with initial half-lives of 3.8 and 5.5 days for the two tissues, respectively. The metabolism was not investigated in this study.

22. According to the studies performed unchanged teflubenzuron can be considered as the marker residue and accounts for approximately 80% of total residues at the time-point (day 1) when total residues has decreased to below the ADI in the standard food package. This ratio can be used for calculating a provisional MRL for teflubenzuron.

23. Two non-radiometric residue depletion studies that meet the requirements in Volume VI of the Rules Governing Medicinal Products in the European Community were conducted in Atlantic salmon. The concentrations in muscle and skin were determined by the HPLC-UV method which is proposed as the routine analytical method.

    In the first study, fish kept at 10ºC received a dose of 9.46 mg teflubenzuron/kg bw/day for 7 days in the feed. Highest mean levels of teflubenzuron in both skin and muscle samples were measured one day after the last treatment, 1310 (813 to 1863) µg/kg in skin and 894 (373 to 2000) µg/kg in muscle. The calculated value for combined muscle and skin was 931 (377 to 1983) µg/kg after one day. After 4 days the mean concentrations had declined to 353 (66 to 1126) µg/kg in skin, 329 (136 to 818) µg/kg in muscle and 331(144 to 847) µg/kg in the combined skin and muscle. After 8 days the levels were 221 (48 to 784), 103 (20 to 189) and 116 (26 to 221) µg/kg for skin, muscle and the combined skin and muscle, respectively. The initial half-life of elimination calculated from the residue data from days 1 to 18 in the combined skin and muscle was 3.4 days. The tissue levels seemed to reach a plateau of 30 to 40 µg/kg 24 to 35 days post treatment. This was probably a result of the background level of teflubenzuron which was found in the test system.

    In the second study, fish kept at 6ºC received a dose of 9.76 mg/kg bw/day for 14 days in the feed. Highest mean levels of teflubenzuron in both skin and muscle were measured one day after the last treatment, 443 (226 to 913) µg/kg in skin and 405 (213 to 772) µg/kg in muscle. The calculated value for combined muscle and skin was 407 (245 to 747) µg/kg after 1 day. After 8 days the mean concentrations in skin, muscle and combined skin and muscle were 106 (66 to 161), 63 (40 to 130) and 67 (43 to 128) µg/kg respectively. The initial half-life of elimination calculated from the residue data from days 1 to 16 in the combined skin and muscle was 4.8 days. In this study the tissue levels reached a plateau of 25 to 50 µg/kg after 24 to 35 days.

    No residue depletion study at a higher water temperature, e.g. 14 to 16-ºC, was performed.

24. The routine analytical method for the determination of residues in salmon muscle and skin separately is based on HPLC with UV detection. The limit of quantification can be set to 50 at µg/kg for both muscle and skin, provisionally. A limit of detection was not estimated, but can be set at the lowest level of quantification of 20 µg/kg used in the validation of the analytical method. There was no information concerning specificity and possible interference from residues of other substances which might be present in fish tissues.

Conclusion and recommendation

Having considered that :

- an ADI of 0.01 mg/kg (600 µg/person) is provisionally set for teflubenzuron and only limited information is necessary to establish a final ADI;
- teflubenzuron is the marker residue and a ratio of marker residue to total residues of 0.80 can be assumed from the studies performed;
- the residue profile in muscle and skin is sufficiently similar to allow establishment of an MRL for muscle and skin in natural proportions;
- consumer intake arising from pesticidal uses is estimated to account for 60% of the ADI according to information from one European country;
- a validated analytical method is available to detect residues in muscle and skin independently but not for muscle plus skin in natural proportion as recommended in Volume VI.

The Committee recommends the inclusion of teflubenzuron in Annex III of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Teflubenzuron	Teflubenzuron	Salmonidae	500 µg/kg	Muscle and skin in natural proportions	Provisional MRL expires on 1.7.1999

Based on this MRL value, the daily consumer intake of teflubenzuron from veterinary use in fish would account for about 30% of the ADI.

LIST OF QUESTIONS

Safety file

1. The degree of purity of teflubenzuron is stated to be equal to or greater than 95%, but should probably be less than or equal to 95%. Furthermore, no information about the chemical names of the impurities, characterised as E13, E72, E37 and E16, has been given.-Information on the purity of teflubenzuron used is missing in several studies. The applicant should comment on this.

2. The applicant should provide more information on the pharmacodynamic action of teflubenzuron.

3. In the 13-week subchronic toxicity study in rats (RCC. Project 018843), the applicant has investigated clinical biochemical parameters. In the results the statistically significant changes noted (page 31) are not in agreement with the significant changes in the tables. The applicant should clarify this discrepancy.

4. The whole report of the 13-week toxicity study in mice (Report T-15) should be translated from Japanese into English and submitted.

5. In the carcinogenicity studies information about tumour incidence in historical controls is not included in the documentation and should be submitted.

6. The applicant should perform a statistical evaluation of the non-neoplastic lesions of the liver found in the 18-month carcinogenicity study in mice.

Residue file

7. The applicant should confirm or provide new information on the estimated consumer intake arising from the pesticidal uses of teflubenzuron.

8. The routine analytical method should be simplified, if possible, to be more applicable for surveillance purposes. The applicant should provide a fully validated analytical method for muscle and skin in natural proportions in accordance with Volume VI. The method should be presented in an internationally recognised format (e.g. ISO 78/2).