Food and Agriculture Organization of the United Nations

World Health Organization

81st Joint FAO/WHO Expert Committee on Food Additives (JECFA) meeting, 2015

Teflubenzuron

Residue Monograph

This monograph was also published in: *Residue Evaluation of Certain Veterinary Drugs. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 81st meeting 2015. FAO JECFA Monographs 18*

7. Teflubenzuron

First draft prepared by

Susanne Rath, Campinas, SP, Brazil

Lynn G. Friedlander, Rockville, MD, USA

and

Rainer Reuss, Barton, Australia

Identity

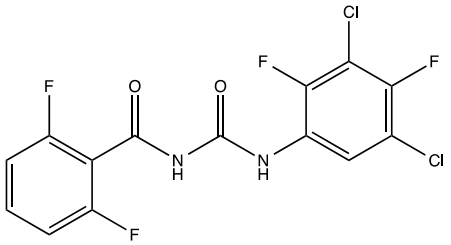
International Non-proprietary Name (INN): Teflubenzuron

Synonyms: AC 291898, CME 134, CME 134-01, CME 13406, Calicide, Ektobann, HOE 522, MK 139, Nomolt, Nomolt agro, OMS 3009, Tefluron

IUPAC Name: 1-(3,5-dichloro-2,4-difluorophenyl)-3-(2,6-difluorobenzoyl)urea.

Chemical Abstract Service Number: 83121-18-0

Structural formula:



Molecular formula: $C_{14}H_6Cl_2F_4N_2O_2$

Molecular weight: 381.1 g mol^{-1}

Other information on identity and properties

Pure active ingredient:	Teflubenzuron (purity ≥ 95%)
Appearance:	White to off-white crystalline solid
Melting point:	222.5 °C
Solubility in water:	0.6 g/L at 20 °C
Solubility in methanol:	1.8 g/L at 20 °C
Solubility in acetonitrile:	1.1 g/L at 20 °C
Solubility in dichloromethane:	1.8 g/L at 20 °C
Vapour pressure:	8 x 10^{-10} Pa at 20 °C
Log $K_{o/w}$:	4.56

Residues in food and their evaluation

Teflubenzuron is used for the treatment of sea lice in Atlantic salmon, as well as in agriculture to control codling moth, leaf miners, whiteflies and caterpillars on fruit trees, vines, vegetables, potatoes, soybean, tobacco and cotton.

Teflubenzuron (CAS No. 83121-18-0) is an acyl urea insecticide used as a veterinary drug in aquaculture for the treatment of sea lice (*Lepeophtheirus salmonis* Krøyer and *Caligus rogercresseyi* Boxshall & Bravo) infestations in Atlantic salmon (*Salmo salar* L.). It is also used for the control of a wide range of insect pests (larvae of Lepidoptera and Coleoptera being most sensitive) and some mites on fruits, vegetables, cereals, nuts and seeds. Teflubenzuron acts by inhibition of chitin synthesis and moulting, disrupting chitin deposition in the insect cuticle after ingestion.

An ADI of 0.01 mg/kg bw/day was established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR, 1995a), based on the dose-related effects in the liver and derived from an 18-month carcinogenicity study in mice described in the toxicological monograph prepared by the meeting (JMPR, 1995b). At the request of the manufacturer, the compound was removed from the review schedule for residues of the JMPR in 1994 and its residue aspects were reviewed for the first time by JMPR in 1996 (JMPR, 1996).

The U.S. Food and Drug Administration has established an import tolerance of 0.5 mg/kg teflubenzuron in muscle with adhering skin of Atlantic salmon (U.S.F.D.A., 2014).

In the European Union, teflubenzuron was included in Annex I of Directive 91/414/EEC by means of Commission Directive 2009/37/EC (EC, 2009) for use as an insecticide only in glasshouses (on artificial substrate or closed hydroponic systems) (EFSA, 2012).

The 22nd Session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) requested that JECFA conduct an evaluation of teflubenzuron, with establishment of an MRL in finfish (salmon) muscle and skin in natural proportions (FAO/WHO, 2015).

Conditions of use

Teflubenzuron is registered for use in aquaculture in Canada, Norway, UK and Ireland. Medicated feed is prepared by coating commercial fish feed pellets with teflubenzuron (at least 95% chemically pure) as a powder to a concentration of 2 g/kg feed. Spraying the diet with fish oil increases the adherence of the material to the feed pellet. The MRLs and withdrawal periods established in each country are summarized in Table 7.1.

Dosage

The intended oral dose is 10 mg teflubenzuron per kg of fish biomass once daily for seven consecutive days.

Table 7.1. Countries in which teflubenzuron is registered with the MRLs and adopted withdrawal periods.

Country	MRL (µg/kg)	Withdrawal period
United Kingdom	500 (muscle and skin in natural proportion)	7 days
Ireland	500 (muscle and skin in natural proportion)	45 degree days
Canada	300 in muscle and 320 in skin	11 days
Norway	500 (muscle and skin in natural proportion)	96 degree days

Pharmacokinetics and metabolism

Test material used in the radiolabelled pharmacokinetic and metabolism studies in salmon

Pharmacokinetic and metabolism studies were conducted with [^{14}C]-teflubenzuron uniformly labelled within the benzoyl ring (Figure 7.1A) and aniline ring (Figure 7.1B) of the compound.

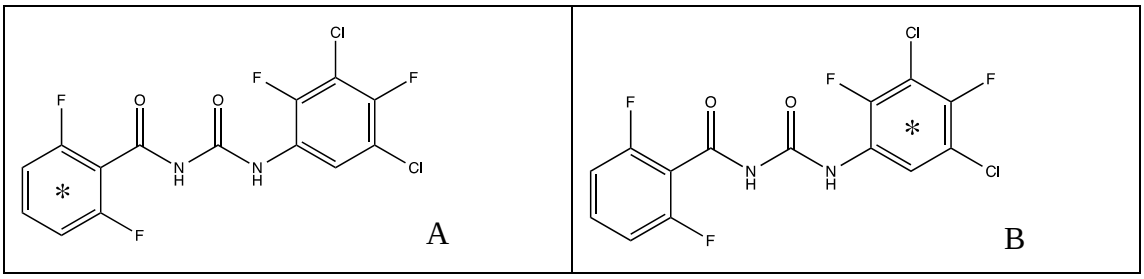


Figure 7.1. Structure of the radiolabelled compounds: A – [^{14}C]-teflubenzuron labelled at the benzoyl ring and B - [^{14}C]-teflubenzuron labelled at the aniline ring.

The purities of the radiolabelled compounds used throughout the studies were greater than 97%, as determined using high performance liquid chromatography (HPLC) coupled to a radiochemical detector.

The radiolabelled compounds were prepared in separate solutions in tetrahydrofuran. For the studies, the solutions were mixed in order to produce an equal mix of radioactivity. Tetrahydrofuran was removed and, the day before dosing, the test material was re-suspended in dimethyl sulphoxide (DMSO). The radiochemical dose was about 1850 kBq/kg (50 µCi/kg) {925 kBq/kg of the labelled compound on the aniline ring and 925 kBq/kg of the labelled compound on the benzoyl ring}.

The radiolabelled teflubenzuron dissolved in DMSO (40 mg/mL, corresponding to a radiochemical concentration of 200 µCi/mL) was added onto control diet at a rate of 100 µL dose formulation per 400 g fish bw. In order to facilitate the detection of regurgitation post-dosing, the formulation containing teflubenzuron was dispensed onto control diet that had been crushed and mixed with Barbour red food dye. The fortified diet was administered directly into the fish by intra-oesophageal intubation.

Pharmacokinetics in laboratory animals

Rats

Teflubenzuron is only partially absorbed from the gastrointestinal tract in rats (4% to 19%) and the absorption is dose-dependent and saturable (EMEA, 1999). Maximum concentrations in plasma are reached within 8 to 24 h after a single oral dose. The saturable kinetics of teflubenzuron in plasma is essentially constant after repeated administration of teflubenzuron in diet at concentrations greater than or equal to 1000 to 2000 mg/kg feed (77 to 158 mg/kg bw/day). Following repeated administration of radiolabelled [^{14}C]-teflubenzuron, the highest concentrations of the compound are present in fat, liver and kidneys. The distribution is rapid and the maximum concentrations for almost all tissues occur at 6 hours post-dosing. Residues in organs and tissues decline quickly and there is no evidence of accumulation of teflubenzuron. The compound 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 represents only a minor pathway (0.4% to 1.4% of the dose). The Committee noted that the values provided for the excretion and absorption will exceed 100%. There is no difference in excretion pattern between males and females after single or repeated administrations of teflubenzuron.

Pharmacokinetic in Food-producing Animals

Salmon

In a GLP-compliant study, Atlantic salmon, *Salmo salar* L., (100 fish, 173-395 g) were treated with a single dose of teflubenzuron by intravenous injection to give a nominal concentration of 2 mg/kg bw (Jenkins, 1996a). The fish were maintained in sea water at 13 -14 °C. Plasma samples were taken 15 min, 3 h, 6 h, 9 h, 12 h, 24 h, 48 h, 72 h, 120 h and 168 h post treatment. Teflubenzuron was quantified in the plasma samples (0.5 mL) by a validated HPLC-UV method with the following validation parameters: linear range: 10 to 5000 ng/mL, recovery: 90% and limit of quantification: 10 ng/mL; within-run precision: 1.0 – 5.9% and within-run accuracy: 97.5 to 110%.

In a related experiment, Atlantic salmon, *Salmo salar* L., (90 fish, 104 to 425 g) were treated with a single dose of medicated diet by oral gavage at a nominal concentration of 10 mg/kg bw (Jenkins 1996a). The fish were maintained in sea water at 13 - 14 °C and plasma samples were taken at 3 h, 6 h, 9 h, 12 h, 24 h, 48 h, 72 h, 120 h and 168 h post treatment. Teflubenzuron was quantified in the plasma samples (results shown in Table 2) by the same validated HPLC-UV method described previously with the following validation parameters reported for this study: linear range: 10 to 5000 ng/mL, recovery: 60% and limit of quantification: 25 ng/mL; within-run precision: 3.8 – 19.6% and within-run accuracy: 80.3 – 117%. The change in concentration of teflubenzuron in plasma with time is shown in Table 7.2.

Table 7.2. Mean concentration of teflubenzuron in plasma of Atlantic salmon (n=10 fish) following a single intravenous (IV) administration at a dose of 2 mg/kg bw {single oral (gavage) dose of 10 mg/kg bw}, water temperature: 13-14 °C (Jenkins, 1996a).

AR	Time post-dose (ng/mL)	15 min	3 h	6 h	9 h	12 h	24 h	48 h	72 h	120 h and 168 h
IV	Conc.	5192	883	559	497	518	157	86	29	<LOQ
	±SD	±4934	±297	±301	±217	±128	±103	±29	±14	
Gavage	Conc.	-	226	430	527	521	136	30	13	<LOQ
	±SD		±52	±123	±95	±122	±31	±8	±4	

AR: administration route of the compound; LOQ: 25 ng/mL; SD: standard deviation.

The calculated mean pharmacokinetic parameters for teflubenzuron in Atlantic salmon plasma (intravenous injection and gavage) are shown in Table 7.3.

Table 7.3. Mean pharmacokinetic parameters for teflubenzuron in Atlantic salmon plasma after dosing by intravenous injection or gavage, water temperature: 13-14 °C (Jenkins 1996b).

Route of administration	Dose (mg/kg per day)	T_{max} (h)	C_{max} (µg/mL)	$AUC_{(0-72)}$ (µg.h/mL)	$t_{1/2}$ (h)	CL (mL/kg per minute)
IV	2	0.25	5.2	23.4	15.3	1.4
Gavage	10	9.0	0.57	10.9	14.2	-

AR: administration route of the compound; T_{max}: time to peak plasma concentration; C_{max}: peak plasma concentration; AUC: area under the curve; $t_{1/2}$: elimination half-life and CL: body clearance.

In a multiple dose study (Jenkins, 1995), Atlantic salmon, *Salmo salar* L., weighing from 626 to 918 g, held in sea water at 7 – 8 °C, were treated with medicated feed (dose of 10 mg/kg bw/day) for seven consecutive days. Plasma samples were collected during the feeding period (6 h post-feeding on days 1-7) and 30 h and 48 h after administration of the last medicated feed. Residues of teflubenzuron were quantified with a validated HPLC-UV method. The concentrations of teflubenzuron in plasma found in this study are shown in Table 7.4.

Table 7.4. Mean concentration (n=5) of teflubenzuron in plasma following administration of medicated feed (dose of 10 mg/kg bw/day) to Atlantic salmon for seven consecutive days. Water temperature: 7 - 8 °C (Jenkins 1995).

Time post-dose	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	30 h*	48 h*
Conc.	79	144	139	118	177	250	196	100	56
±SD (ng/mL)	±41	±79	±50	±26	±48	±74	±65	±65	±11

* Time after last dose; SD: standard deviation.

The mean concentration of teflubenzuron in plasma was 144 ng/mL approximately 30 hours (Day 2) after first treatment and a maximum of 250 ng/mL was achieved after six days. The increased concentration of teflubenzuron in plasma on day 6 may be a result of the variability in the feeding levels of the fish observed during the test. Following the end of the treatment with medicated feed, mean concentrations of teflubenzuron in plasma fell rapidly and on day 9 (48 h after last treatment) at the end of the test, were 29% of the mean concentration

determined on day 7. The elimination phase rate constant was estimated to be 0.02949, giving a half-life after oral administration of 23.5 h.

Metabolism in Laboratory Animals

Rats

In an elimination and metabolism study, three test groups of rats, 5 males and 5 females per group, were treated with [^{14}C]-teflubenzuron (purity > 99%) by oral gavage (U.S.F.D.A., 2014). Group A received a single oral dose of 25 mg/kg bw [^{14}C]-teflubenzuron: Group B received a 14-day repeated oral dose of 25 mg/kg bw of non-radiolabelled teflubenzuron followed by a single oral dose of 25 mg/kg bw [^{14}C]-teflubenzuron; and Group C received a single oral dose of 750 mg/kg bw [^{14}C]-teflubenzuron. Urine and faeces were collected at 24, 48, 72, 120, 144, 168 and 192 h post-dosing with the radiolabelled drug.

In group A, more than 85% of the dose was eliminated in the faeces within 24 h after dosing; overall radioactivity recovered in faeces was higher than 90%. Similar results were obtained for rats of group B. For rats that received the higher dose of radiolabelled teflubenzuron (group C), greater than 90% of the administered dose was recovered in faeces within 48 h after dosing. After 8 days, total radioactivity recovered in urine, faeces and carcass was 0.15%, 94.2% and less than 0.1% of the administered dose, respectively. Unextractable and non-extracted radioactive residues in faeces accounted for between 0.7% and 4.5% of the administered dose for all three groups.

The greatest portion of radioactivity in faeces, determined by thin layer chromatography (TLC), was unchanged parent drug (82.2% to 91.4% of the administered dose). One metabolite identified in faeces was 3,5-dichloro-2,4-difluorophenyl-urea (maximum of 0.2% of the administered dose in Group C and 0.5% to 1.0% of the administered dose in Groups A and B).

In another study, the bile ducts of two groups of Wistar rats, 3 males and 3 females per group, were cannulated prior the administration of a single dose of 25 mg/kg bw [^{14}C]-teflubenzuron by oral gavage or a single dose of 750 mg/kg bw [^{14}C]-teflubenzuron by gastric intubation (U.S.F.D.A., 2014; Hawkins & Mayo, 1988). In each test group, bile was collected until 48 h post-dose. Urine and faeces were collected over the periods 0 to 24 h and 24 to 48 h post-dose. At 48 h, all animals were sacrificed and the gastro-intestinal tracts, livers and carcasses were collected. The radioactivity in bile and urine was measured by liquid scintillation counting. For the animals that received the single low dose (25 mg/kg bw), mean quantities of about 16% and 1.4% of the administered dose were excreted in the 0-48 h bile and urine, respectively. A mean of about 46% of the dose was excreted in the faeces collected in the first 48 h and 23% of the administered dose was measured in the gastrointestinal tract at 48 after dosing. About 0.4% and 1.6% of the administered dose were found in the liver and remaining carcass, respectively, at 48 h post-dose.

After the single high-level dose (750 mg/kg bw), means of about 1.9% and 0.4% of the administered dose were excreted in the 0-48 h bile and urine, respectively. A mean of about 65% of the dose was excreted in the 0-48 h faeces and 19% of the administered dose was measured in the gastrointestinal tract at 48 h after dosing. Approximately 0.06% and 1.2% dose

were measured in the liver and remaining carcass, respectively, at 48 h. The sum of the radioactivity excreted in urine and bile and the radioactivity in the liver indicated a total absorption of about 18% and 2% of the dose after administration at doses of 25 mg/kg bw and 750 mg/kg bw, respectively. This demonstrates that absorption of teflubenzuron is dose-dependent in rats, with greater absorption at the lower dose.

The majority of radioactivity in the faeces (approximately 43% of the low dose and 56% of the high dose), was identified by TLC as the unchanged parent drug. The concentrations of metabolites found were typically less than 1% of the administered dose. The metabolite, 3,5-dichloro- 2,4-difluorophenyl urea, was found in faeces. A substantial portion of the biotransformation products in bile was unidentified polar material (14.1-15.5% of the administered dose for the low dose rats).

In another study, the nature of the radioactive residues in urine and faeces of male and female rats treated with seven daily doses (25 mg/kg bw) of [^{14}C]-teflubenzuron, labelled in the aniline ring, was investigated (Schlüter, 1984). The radiolabelled compound (purity > 99%, specific radioactivity of 36.7 Bq/µg) was dissolved in dimethyl sulfoxide (0.5 mL) and administered by gavage. Faeces and urine were collected at daily intervals until day 8 post-dose. About 90% of the radioactive material was excreted with the faeces (89.9% male and 92.9% female on day 8 post-dose). Most of this amount (70-75%) passed the gastro-intestinal tract and was excreted as unchanged parent compound. The remainder (11-13%) was composed of various extractable trace compounds (at least 15), none of which exceeded 1% of radioactivity and of a portion of about 5%, which remained unextractable with organic solvents and additional acidic treatment. Only 2-3% of the radioactive material was excreted with the urine. Three metabolites, as hydroxylated compounds, showed that teflubenzuron was metabolized by substitution of a halogen atom and/or hydroxylation once it was resorbed. Differences in biokinetics were not observed between males and females.

In a similar study (JMPR, 1995b), the biotransformation of teflubenzuron was investigated in urine and faeces of Wistar rats treated with a single dose of 25 or 750 mg/kg bw of aniline ring-labelled [^{14}C]-teflubenzuron or single doses of 25 mg/kg bw of non-radiolabelled compound for 14 consecutive days followed by a single dose of 25 mg/kg bw of labelled compound. The main compound identified in faeces was teflubenzuron. Trace amounts of more polar compounds were noted in each treatment group in the faeces. One of these compounds was identified as 3,5-dichloro-2,4-difluorophenyl urea. Thin layer chromatography indicated that the low level of radiolabel found in urine consisted mainly of very polar compounds. A proposed biotransformation pathway is shown in Figure 7.2.

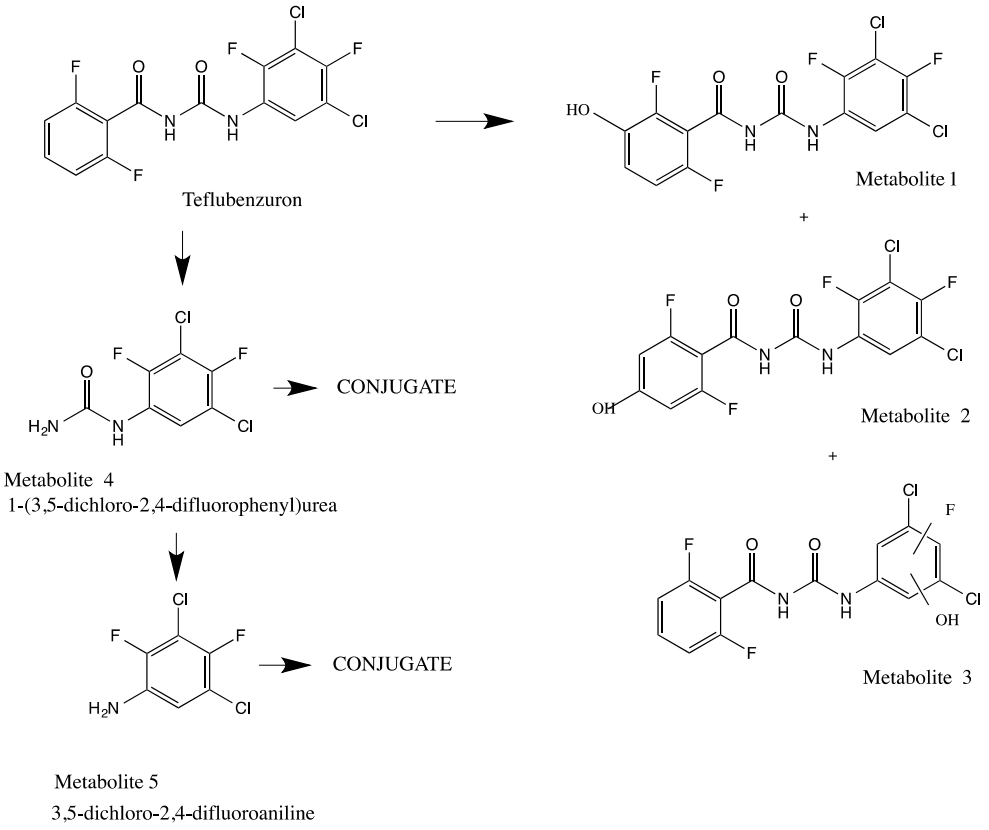


Figure 7.2. Biotransformation pathway of teflubenzuron in rats (JMPR, 1995b).

In an *in vivo* metabolism study (Koerts *et al.*, 1997), male Wistar rats (350 – 400 g) were exposed to 1 to 53 µmol teflubenzuron (in olive oil with 20% DMSO) by oral gavage. After dosing, 0-24 h, 24-48 h urine and 0-48 h faeces samples were collected. Identification of the metabolites was done by ^{19}F-NMR and, for the quantification, 4-fluorobenzoic acid was used as internal standard. Analysis of the faeces revealed the presence of mainly unmodified teflubenzuron. Within 48 h almost the total dose of teflubenzuron was recovered, partly as metabolites in the urine (4-6% of the dose administered) and mainly in unmodified form in the faeces (90% of the dose administered).

The 24-hour urinary metabolic profile of teflubenzuron is shown in Table 7.5.

Table 7.5. Urinary (24-h) metabolic profile of teflubenzuron (Koerts et al. 1997).

Metabolite	Total metabolite (%)
2,6-Difluorobenzoic acid	81.4 ± 3.8
2,6-Difluorobenzoylglycine	4.0 ± 0.5
2,6-difluorobenzamide	2.0 ± 0.3
2-Amino-3,5-difluoro-4,6-dichlorophenylsulfate	2.8 ± 0.5
2-Amino-3,5-difluoro-4,6-dichlorophenylglucoronide	1.2 ± 0.3
4-Amino-2,6-dichloro-3-fluorophenylsulfate	1.2 ± 0.4
4-Acetamido-2,6-dichloro-3-fluorophenylsulfate	0.6 ± 0.5
(3,5-Dichloro-2-fluoro-4-phenylsulfatephenyl)urea	0.3 ± 0.6
(3,5-Dichloro-2-fluoro-4-phenyglucoronidephenyl)urea	5.2 ± 2.4
Unidentified	1.3 ± 0.7

The metabolic profile shows that the benzoate part of teflubenzuron is mainly excreted as 2,6-difluorobenzoic acid and, to a minor extent, as 2,6-difluorobenzoylglycine and 2,6-difluorobenzamide. The aniline ring of teflubenzuron was excreted as the sulphated and glucuronidated conjugates of (4-hydroxy-3,5-dichloro-2-flurophenyl) urea and 2-amino-3,5-difluoro-4,6-dichlorophenol, the sulphated conjugate of 4-amino-2,6-dichloro-3-fluorophenol and the glucuronidated conjugate of 4-acetido-2,6-dichlorofluorophenol. The amount of benzoate-derived metabolites identified in urine was 87% of the total unidentified metabolic pattern whereas the aniline derivative was 13%. It was also shown that the excretion efficiency of the aniline-type compounds from teflubenzuron is at least eight-fold lower than that of benzoates.

Metabolism in Food Producing animals

Goats

In order to study the metabolic profile of teflubenzuron in goats, [^{14}C]-teflubenzuron, uniformly labelled in the aniline ring, was administered orally to two lactating goats, twice daily for 7.5 days, at a daily dose of 7 mg/kg bw (JMPR, 1996). Milk, plasma, urine, faeces, bile, organs and tissues were analysed for the identification and quantification of radioactive metabolites. The main route of elimination of radioactivity was in the faeces, accounting for 99% of the total administered dose, including intestinal contents at post-mortem. The major radioactive component (76.9%) in faeces was attributed to teflubenzuron parent compound using HPLC and TLC analyses. The radioactive residues in all organs, tissues and body fluids examined post-mortem were low in relation to the total dose. The highest mean concentrations of radiolabelled residues were in the liver and lung, with 486 µg eq/kg and 136 µg eq/kg, respectively, corresponding to 0.14 and 0.02% of the total administered dose in the whole organs. Relatively high concentrations were also detected in bile (mean concentrations of 1306 µg eq/L, 0.002% of the total administered dose). The concentrations of radioactivity in the liver and bile indicate biliary excretion as being important in the elimination of the absorbed fraction of an orally administered dose. The absence of similar concentrations in the plasma suggests that much of the absorbed radioactivity is removed by "first-pass metabolism" in the liver. The radioactivity in the bile was mainly in β-glucuronide (or possible sulphate) conjugates. The concentrations of radioactivity in all other organs, tissues and body fluids were generally less than 100 µg eq/kg. Teflubenzuron was, therefore, shown to be poorly absorbed after oral administration; the absorbed fraction appears to be metabolized in the liver and conjugated before elimination, mainly in the bile. Traces of material co-chromatographing with 1-(3,5-dichloro-2,4-difluorphenyl)-3-(2,6-difluoro-3- hydroxybenzoyl) urea (Metabolite 1, Figure 7.2) were detected. None of the extracts contained any radioactive components with similar characteristics to either 3,5-dichloro-2,4-difluoroaniline or 3,5-dichloro-2,4-difluorophenylurea.

Chickens

A total dose of 1.25 mg/kg bw per day was administered to laying hens orally twice daily for 7.5 days (JMPR, 1995b). Teflubenzuron was identified by mass spectrometry as the major component in excreta. Two components were observed in liver and kidney extracts by TLC

and HPLC, one of which was identified as teflubenzuron, while the other could not be identified. A third component found in kidney extracts was shown to be 3,5-dichloro-2,4-difluorophenylurea (Metabolite 4, Figure 7.2). In bile, 22% of the total radioactivity was found and a very polar compound was identified, which on treatment with β-glucuronidase yielded a compound with similar chromatographic characteristics to the meta-hydroxybenzoyl derivative of teflubenzuron (Metabolite 1, Figure 7.2).

Salmon

In a GLP-compliant metabolic profiling study, conducted at a water temperature of 9.9 ± 0.2 °C, a dose equivalent to 10 mg/kg bw (1.85 MBq/kg; 50 µCi/kg) [^{14}C]-teflubenzuron was administered to Atlantic salmon, *Salmo salar* L., (57 fish, 537 to 999 g) by intra-oesophageal intubation (Auger et al. 1995). Two time points after dosing were selected for sampling (days 1 and 8 post-dose). Approximately one quarter of the total liver sample for each fish (5 fish, per time point) was taken and pooled together for extraction. This procedure was also employed for kidney. For muscle, 10 g of sample from each fish per time point was taken and pooled together for extraction. For the skin, half of the total skin for each fish was taken and macerated with solid carbon dioxide and half of the macerated sample was used for extraction. The pooled samples were extracted with three successive portions of acetonitrile (7:3 v/w) at 80 ºC for 3 min. The acetonitrile was partitioned with hexane to remove fatty material, concentrated and analysed by HPLC. Chromatographic separation was achieved using a Lichrosorb RP18 (250 x 4.6 mm; 10 µm) column and a mobile phase of acetonitrile:water:trifluoroacetic acid under gradient elution. Detection was performed using a radiochemical detector and a UV detector (254 nm).

Chromatographic analysis of muscle, skin, liver and kidney tissues collected on days 1 and 8 provided determination of teflubenzuron and some metabolites (Table 7.6). The structures were confirmed by liquid chromatography coupled to mass spectrometry (LC-MS). The analysis of muscle and skin showed the presence of only the parent compound, teflubenzuron. In addition to teflubenzuron, the acetonitrile extracts of the liver showed the presence of three components (one unknown) on day 1. Only parent teflubenzuron and one unknown component were present on day 8. In addition to teflubenzuron, the acetonitrile extracts of the kidney showed the presence of five compounds on day 1 (three unknown); two of the unidentified compounds were more lipophilic in nature than the parent compound. Only parent teflubenzuron and one unknown were present in kidney extracts on day 8 (Table 7.6).

In a similar GLP study using repeated dosing, Atlantic salmon, *Salmo salar* L., (508-1297 g) were maintained at 10 °C in sea water (Auger *et al.*, 1996). Non-radiolabelled teflubenzuron was administered via medicated feed at a dose of 10 mg/kg bw for 6 consecutive days. On the seventh day, a dose of 10 mg/kg bw (1.85 MBq/kg; 50 µCi/kg) [^{14}C]-teflubenzuron was administered directly into the fish by intra-oesophageal intubation. Acetonitrile extracts of tissues samples taken on days 1 and 8 post-dose were analysed by HPLC and LC-MS to study the metabolism. The sample preparation and quantification of the compounds were the same as previously described.

Table 7.6. Compounds identified in the chromatograms (HPLC-radioactivity detector), with their respective concentrations and total radioactive residue in Atlantic salmon tissues sampled 1 and 8 days after single oral administration of [^{14}C]-teflubenzuron at a dose of 10 mg/kg bw (Auger *et al*., 1995)**.**

Tissue	Compound	Day 1		Day 8	
		Concentration (µk/kg)	TRR (%)	Concentration (µk/kg)	TRR (%)
Muscle	Teflubenzuron	398	97.1	74	78.7
Skin	Teflubenzuron	749	99.5	108	58.1
Liver	Teflubenzuron	888	46.0	212	14.2
	3,5-dichloro-2,4-difluoroaniline	59	3.1		
	3'-hydroxy-teflubenzuron	64	3.3		
	Unknown	124	6.4	181	12.1
Kidney	Teflubenzuron	498	66.3	190	
	2'-hydroxy-teflubenzuron	34	4.55	-	
	3'-hydroxy-teflubenzuron	12	1.6	-	40.2
	Unknown 1	84	11.2	46	9.7
	Unknown 2	16	2.1	-	
	Unknown 3	22	2.9	-	

TRR: total radioactive residue.

Analysis of the acetonitrile extracts of the liver, kidney, muscle and skin from day 1 post-dose revealed that the major component detected was the parent compound; structural confirmation was by LC-MS. In the liver from day 1, three minor components were detected: 3'-hydroxydiflubenzuron and 3,5-dichloro-2,4-difluorophenyl urea and one unknown substance. In samples from day 8, teflubenzuron was detected in muscle and skin. Two minor components were also detected in the muscle but the structures were not elucidated.

Tissue residue depletion studies

Salmon

Several GLP-compliant depletion studies at a water temperature of 6 °C or 10 °C were evaluated to assess total [^{14}C]-teflubenzuron residues (total radiolabelled residues, TRR) or residues of teflubenzuron parent compound (marker residue, MR) from studies using non-radiolabelled drug in Atlantic salmon (*Salmo salar* L.) tissues. These studies included single oral and repeated doses of radiolabelled and/or non-radiolabelled teflubenzuron. A summary of these studies is shown in Table 7.7 and described in more detail in the following paragraph.

Studies 2 and 5 are correlated and were carried out at the same time using the same experimental design with the difference being that, in Study 2, fish received a single oral dose

of [^{14}C]-teflubenzuron at Day 7 whereas, in Study 5, the same dose of non-radiolabelled teflubenzuron was administered. The same situation occurs for Studies 3 and 4.

Table 7.7. Summary of the residue depletion studies.

Study	Water temperature (°C)	Dose	Administration	Sampling Time post-dose
1*NTO/007	10 °C	Single oral dose of [^{14}C]-teflubenzuron (10 mg/kg bw; 1.85 MBq/kg).	Intra-oesophageal intubation	9 h, 1, 3, 4, 6, 8, 13 and 18 days
2*NTO/009	10 °C	Daily oral dose of teflubenzuron (10 mg/kg bw) for 6 days + single oral dose [^{14}C]-teflubenzuron (1.85 MBq/kg) on Day 7.	Medicated feed + intra-oesophageal intubation	1, 4, 8, 12 18, 24, 35, 50 and 120 days
3*NTO/013	6 °C	Daily oral dose of teflubenzuron (10 mg/kg bw) for 13 days + single oral dose [^{14}C]-teflubenzuron (1.80 MBq/kg) on Day 14.	Medicated feed + intra-oesophageal intubation	1, 8, 16, 24, 35, 50, 75 and 97 days
4*NTO/014	6 °C	Daily oral dose of teflubenzuron (10 mg/kg bw/day) for 13 days + single oral dose (10 mg/kg bw/day) on Day 14.	Medicated feed + intra-oesophageal intubation	1, 8, 16, 24, 35, 50[a], 75[a] and 97[a] days
5*NTO/010	10 °C	Daily oral dose of teflubenzuron (10 mg/kg bw) for 6 days + single oral dose of teflubenzuron (10 mg/kg bw/day) on Day 7.	Medicated feed + intra-oesophageal intubation	1, 4, 8, 12, 18, 24, 35, 50 and 120 days

*1- (Auger *et al*., 1995); 2 - (Auger *et al.*, 1996); 3 - (Auger & Bounds, 1996); 4 - (McGuire *et al.*, 1996a); 5 - (McGuire *et al.*, 1996b).[a]: samples not analysed.

Radiolabelled residue depletion studies

Salmon

All the following studies used the target species Atlantic salmon (*Salmo salar* L.) with a weight of approximately 1 kg and administration of teflubenzuron with purity higher than 97%. The compound was radiolabelled with carbon-14 to form radiolabelled test materials: [^{14}C]-benzoyl-CME 134 with purity >99% and [^{14}C]-aniline-CME 134 with purity >98%. The purity of both forms was confirmed by HPLC. The test material was prepared with equal amounts of the two radioactive forms of teflubenzuron.

In all studies, the total radioactive residue (TRR) was determined using liquid scintillation counting, either by (i) direct solubilization of tissues using Soluene-350, followed by decolourization using hydrogen peroxide and mixing with scintillation fluid before analysis; or (ii) solubilization of the skin samples using 2 mol/L potassium hydroxide in methanol:water 1:1 v/v; or (iii) combustion of the gastro-intestinal content and tank effluent and mixing with scintillation fluid.

Study 1 – NTO/007

In the first GLP-compliant depletion study (Auger *et al.*, 1995), conducted at a water temperature of 9.9 ± 0.2 °C, [^{14}C]-teflubenzuron at an intended dose equivalent to 10 mg/kg bw (1.85 MBq/kg; 50 µCi/kg) (actual dose 9.45 ± 0.15 mg/kg bw) was administered to Atlantic salmon (537 to 999 g) by intra-oesophageal intubation. In the study, 57 fish, approximately 24 months of age (33 females and 24 males), were housed in two tanks at a stocking density lower than 25 kg/m^3. Six fish were sampled at each of the following intervals: 9 h, and 1, 3, 4, 6, 8, 13 and 18 days post-dosing. Samples (mucus, liver, kidney, muscle, skin and gall bladder) were collected and the TRR was determined using liquid scintillation spectrometry.

Total recovered radioactivity on Day 1 post-dose in the acetonitrile extract was 98.6% (muscle), 103.8% (skin), 59.5% (liver) and 95.5% (kidney). The calculated marker to total residue ratios in the edible tissues are shown in Tables 7.8 and 7.9.

Table 7.8. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in muscle and skin of Atlantic salmon dosed with [^{14}C]-teflubenzuron at 10 mg/kg bw and held in sea water at 10 °C (Auger *et al.*, 1995).

Time	Muscle			Skin		
post-dose (days)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	410	404	98.6	753	782	103.8
8	93.6	79	84.0	185	143	77.1

Table 7.9. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in liver and kidney of Atlantic salmon dosed with [^{14}C]-teflubenzuron at 10 mg/kg bw and held in sea water at 10 °C (Auger *et al.*, 1995).

Time post-dose (days)	Liver TRR (µg/kg)	Liver MR (µg/kg)	Liver MR/TRR ratio (%)	Kidney TRR (µg/kg)	Kidney MR (µg/kg)	Kidney MR/TRR ratio (%)
1	1930	1149	59.5	752	718	95.5
8	1490	543	36.4	473	257	54.4

The residues remaining after acetonitrile extraction, for the liver at 1 and 8 days post treatment and the kidney at 8 days post treatment, were further treated by hydrolysis under acidic or basic conditions. Acidic hydrolysis released very little of the remaining radioactivity, whereas basic hydrolysis released 44% and 58% of the activity of the residues in the liver at 1 and 8 days post-dose, respectively, and 97% of the residues in the kidney at 8 days post-dose.

The changes in concentrations of radioactivity in tissues from Atlantic salmon with time are presented in Table 7.10. The highest concentration of radioactivity in muscle (410 ± 89.0 µg eq/kg) and skin (753 ± 224 µg eq/kg) was found 1 day after administration of the drug. The concentrations decreased with elimination half-lives of 4.7 and 6.5 days for muscle and skin, respectively. The highest concentrations of radioactive material were found in the tissues associated with metabolism and excretion. The maximum concentration was determined in gall bladder (119000 ± 31500 µg eq/kg) at 2 days and is assumed to be associated with the bile in this tissue. The concentration in the liver was at a maximum (2500 ± 538 µg eq/kg) at 9 hours after administration and decreased to 1060 ± 319 µg eq/kg at 18 days post-dose. The relatively slow rate of elimination from the liver and gall bladder (half-lives of 16.5 and 7.1 days, respectively) is an indicator of the process of enterohepatic recirculation.

Table 7.10. Mean concentration (six fish at each time) of radioactivity in tissues from Atlantic salmon with time after a single oral dose equivalent to 10 mg/kg bw (1.85 MBq/kg) of [^{14}C]-teflubenzuron (Auger *et al*., 1995).

Time	Mean concentration of radioactivity (µg eq/kg) ± SD

post-dose	Liver	Kidney	Muscle	Skin	Mucus	Gall bladder
9 hours	2500 ±538	656 ±103	266 ±61.2	395 ±82.5	93.2 ±85.2	7750 ±8190
1 day	1930 ±451	752 ±156	410 ±89.0	753 ±224	87.8 ±63.3	70100 ±28100
2 days	2100 ±505	670 ±150	215 ±29.0	439 ±75.7	61.6 ±61.6	119000 ±31500
3 days	1830 ±377	558 ±68.9	184 ±29.2	301 ±52.4	62.0 ±59.4	118000 ±20500
4 days	1720 ±524	477 ±125	149 ±35.3	260 ±65.2	40.2 ±29.8	107000 ±57400
6 days	1400 ±344	459 ±127	102 ±17.1	187 ±21.4	5.8 ±8.9	129000 ±67500
8 days	1490 ±230	473 ±93.3	93.6 ±17.3	185 ±31.6	31.6 ±14.6	104000 ±48400
13 days	1050 ±290	314 ±102	37.0 ±9.0	89.3 ±22.6	22.2 ±7.0	41700 ±38200
18 days	1060 ±319	310 ±65.9	20.9 ±4.2	73.0 ±5.7	22.9 ±12.7	28300 ±11500

SD: standard deviation.

Study 2- NTO/009

In a similar depletion study (GLP-compliant), salmon (508 to 1297 g) were maintained at 10 °C and were treated with non-radiolabelled teflubenzuron in medicated feed, at a dose of 10 mg/kg bw, for 6 consecutive days. On the seventh day, a dose of 10 mg/kg bw [14C]-teflubenzuron (1.85 MBq/kg; 50 µCi/kg) was administered directly into the fish by intra-oesophageal intubation (Auger *et al.*, 1996).

The depletion was evaluated (six fish per time) at the sampling days 1, 4, 8, 12 18, 24, 35, 50 and 120 post-dose. The gastro-intestinal contents and the tank environment were also examined to evaluate the excretion of the radiolabelled drug. Only a small amount of radioactive material was distributed into the tissues examined, *i.e.*, the majority of material was excreted from the fish. The highest quantity of radioactive material (1.5 ± 0.6%, corresponding to 310 ± 124 µg eq/kg) was detected in the muscle 1 day after administration, with an initial half-life of elimination of 2.6 days over the period of 1 to 18 days. The drug was slowly eliminated from the muscle with a terminal half-life of 38.5 days over a period of 35 to 120 days, to a level of 1.1 ± 1.3 µg eq/kg by Day 120. The maximum concentration of [^{14}C]-teflubenzuron in skin was determined on day 1 (554 ± 178 µg eq/kg). The initial half-life of elimination from the skin, over the period 1 to 18 days was calculated to be 3.6 days. The terminal half-life of elimination from skin, over the period of 18 to 120 days was calculated to be 49.5 days.

Consistent with the results in *Study 1* (Auger *et al.*, 1995), the highest concentrations of radioactive material were in the tissues associated with metabolism and excretion. The highest concentrations were in the liver, with a maximum of 1880 ± 153 µg eq/kg on day 1. The concentration in liver decreased to 793 ± 153 µg eq/kg on day 8 with an initial half-life of elimination of 5.7 days (over the period of 1 to 8 days). Results are shown in Table 7.11.

Table 7.11. Mean concentration (six fish at each time) of radioactivity in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.*, 1996).

Time post-dose (days)	Mean concentration of radioactivity (µg eq/kg) ± SD				
	Liver	Kidney	Muscle	Skin	Mucus
1	1880± 1110	651 ± 401	310 ± 124	55.4 ± 178	196 ± 165
4	1230 ± 686	239 ± 98.0	57.8± 18.6	122 ± 30.9	8.3 ± 9.3
8	793 ± 153	99.2± 28.2	14.6± 8.0	40.8 ± 13.1	ND
12	864 ± 268	135 ± 60.5	12.0± 5.1	33.7 ± 5.2	5.7 ± 9.4
18	1440 ± 434	217 ± 68.5	<LOD	16.3 ± 2.6	<LOD
24	765 ± 417	103 ± 51.3	<LOD	13.4 ± 3.2	ND
35	573 ± 95.6	64.1± 11.5	6.1± 2.3	8.4 ± 0.7	<LOD
50	522 ± 275	70.2± 36.7	<LOD	9.2 ± 1.5	<LOD
120	127 ± 16.3	25.7± 10.8	<LOD	<LOD	5.8 ± 5.3

SD: standard deviation. ND: not detected. Limit of detection: 5 µg eq/kg.

Total recovered radioactivity on day 1 post-dose in the acetonitrile extract was 95.6% (muscle), 83.4% (skin), 47.7% (liver) and 87.4% (kidney). The calculated marker to total residue ratios in the edible tissues are shown in Tables 7.12 and 7.13.

Table 7.12. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in muscle and skin of Atlantic salmon after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.*, 1996).

Time post-dose (days)	Muscle			Skin		
	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	313	300	95.6	568	474	83.4
8	14	18	128.6	41	34	81.9

Table 7.13. Concentrations (µg/kg) of total radioactive residues (TRR) and teflubenzuron residues (Marker Residue, MR) in liver and kidney of Atlantic salmon after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.,* 1996)**.**

Time post-dose (days)	Liver			Kidney		
	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)	TRR (µg/kg)	MR (µg/kg)	MR/TRR ratio (%)
1	1545	738	47.7	993	868	87.4
8	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

N.A.: not analyzed.

For the combined muscle and skin, the maximum concentration of radioactive material occurred on day 1 and decreased with an initial half-life of elimination of 3.8 days over the period 1 to 24 days. The terminal elimination half-life was 38.4 days over the period 35 to 120 days.

A summary of the pharmacokinetic parameters of radioactive material determined from the depletion data in this study is shown in Table 7.14.

Table 7.14. Pharmacokinetic parameters of radioactivity in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 7 via intra-oesophageal intubation. Water temperature of 10 ºC (Auger *et al.,* 1996)**.**

	Initial Elimination Phase			Terminal Elimination Phase		
Tissue	**Time, range (d)**	**Initial Half-life (d)**	**Rate constant (d^{-1})**	**Time, range (d)**	**Initial Half-life (d)**	**Rate constant (d^{-1})**
Liver	1-8	5.7	-0.12	24-120	38.5	-0.02
Kidney	1-8	2.6	-0.27	24-120	49.5	-0.01
Muscle	1-18	2.6	-0.26	35-120	38.5	-0.02
Skin	1-18	3.6	-0.19	18-120	49.5	-0.01

It is postulated that there is a biphasic elimination of radioactive material from the tissues. The initial elimination half-lives are between 2.6 and 5.7 days long. The terminal elimination half-lives are between 38.5 and 49.5 days long, which is attributable to the binding of the test material to the tissue matrix

This result is corroborated with the high level of radioactive material (> 95% in liver) that is unextractable from the tissues after 50 days post-dose.

Study 3 – NTO/013

A third depletion study was carried out using a similar experimental design and analytical methods as described in Study 2, using a water temperature of 6 °C instead of 10 °C (Auger & Bounds, 1996). In this study, Atlantic salmon (527 to 1403 g) were fed non-radiolabelled teflubenzuron in the diet for thirteen days at a dose of 10 mg/kg bw. On the fourteenth day the fish were not fed but received a dose equivalent to 10 mg/kg bw of [^{14}C]-teflubenzuron (50.3 ± 0.70 µCi/kg) by oral intubation. Tissues were collected after 1, 8, 16, 24, 35, 50, 75 and 97 days post-treatment. In this study, a background level of teflubenzuron was detected in the closed re-circulating water system, but it was concluded that this did not affect the validity of this study in terms of determining the metabolism and initial rate of depletion (McGuire *et al.*, 1996b). Very little systemic tissue absorption of radioactive material was observed following the final dose. The highest recovery of radioactivity was determined in muscle on Day 1 (0.7% ± 0.2% of the radiochemical dose). All other tissues analysed contained less than 0.1% of the radiochemical dose 8 days following administration. Liver contained the highest concentration of radioactive material, with a maximum of 1170 ± 336 µg eq/kg on day 1, which decreased with an initial elimination half-life of 16.9 days over the period of 1 to 24 days. It was not possible to determine the terminal elimination half-life for residues in liver due to variations in tissue concentrations after day 24. For muscle and skin, the maximum concentrations occurred at 1 day following the final dose (153 ± 40 µg eq/kg for the muscle and 218 ± 83 µg eq/kg for the skin). The initial elimination half-lives were 3.8 days for muscle and 5.7 days for (skin, respectively, over the period from 1-24 days. The terminal elimination half-life for the skin was 99 days over the period of 27 to 97 days.

The change in concentrations of radioactivity in tissues with time is shown in Table 7.15. The Committee noted that some values provided by the sponsor (marked with * at Table 7.15) are below the limit of detection of the method.

Table 7.15. Mean concentration ± SD (six fish at each time) of radioactivity in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 13 consecutive days followed by a single oral dose of [^{14}C]-teflubenzuron (1.85 MBq/kg) on day 14 via intra-oesophageal intubation at a water temperature of 6 ºC (Auger & Bounds, 1996).

Time post-dose (days)	Mean concentration of radioactivity (µg eq/kg) ± SD				
	Liver	Kidney	Muscle	Skin	Mucus
1	1170± 336	328 ± 74.5	153 ± 39.7	218 ± 82.6	8.6 ± 5.5
8	722 ± 181	114 ± 49.1	16.5 ± 6.0	28.5 ± 12.7	0.9* ± 2.3
16	512 ± 204	89.2 ± 31.1	7.5 ± 6.8	16.4 ± 6.9	2.2* ± 3.5
24	455 ± 192	61.3 ± 36.6	1.7* ± 4.2	10.0 ± 1.9	ND
35	655 ± 492	81.9 ± 57.5	ND	10.9 ± 4.9	ND
50	334 ± 122	53.4 ± 9.9	5.9* ± 3.2	8.7 ± 2.7	ND
75	328 ± 100	46.0 ± 22.4	ND	7.2 ± 4.3	ND
97	340 ± 161	31.6 ± 20.4	ND	6.1 ± 1.7	ND

SD: standard deviation. ND: not detected. * Limit of detection: 6 µg eq/kg.

The radiolabelled studies indicated that the main residue in muscle and skin is the parent compound and that the excretion of teflubenzuron is predominantly via faeces.

Residue depletion studies with non-radiolabelled drug

Salmon

Residues of teflubenzuron in salmon tissues in the following studies were quantified by a validated HPLC-UV method with a limit of quantification for the determination of teflubenzuron in muscle and skin of 20 µg/kg (McGuire, 1995). A full evaluation of the method is provided in "Methods of analysis for residues in tissues". Briefly, the samples (approx. 3 g) were extracted with hot acetonitrile and the solvent volume was reduced on a rotary evaporator. The remaining extract was diluted with dichloromethane, reduced by evaporation, then washed with water. The final organic extract was evaporated to dryness at 50 °C and the residue was then re-suspended in 5% diethyl ether in hexane before clean-up on silica and C8 solid phase extraction cartridges. The quantification of teflubenzuron was carried out by HPLC with a UV detector at 254 nm. The LOQ's for muscle and skin were 20 and 50 µg/kg, respectively.

Study 4 – NTO/014

In a GLP-compliant repeat dose study (McGuire *et al*., 1996a), Atlantic salmon (527 to 1403 g) kept at a water temperature of 6 °C were fed with a diet containing teflubenzuron for thirteen days at an intended dose of 10 mg/kg bw (actual dose of 9.76 mg/kg bw). On the fourteenth day the fish were not fed but were treated with the same dose of teflubenzuron by oral intubation. Tissues were collected 1, 8, 16, 24, 35, 50, 75 and 97 days post treatment, however the samples collected on days 50, 75 and 97 post-dose were not analysed. In this study, a background level of teflubenzuron in the closed re-circulating water system was detected but it was concluded that this did not affect the validity of this study to determine the initial rate of drug depletion.

The results of the analysis of skin and muscle with time are shown in Table 7.16. The recoveries of teflubenzuron in fortified blank skin and muscle samples were in the range of 70 to 129% and 73 to 104%, respectively.

Table 7.16. Mean concentration ± SD (ten fish at each time) of teflubenzuron in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 13 consecutive days followed by a single oral dose of teflubenzuron (10 mg/kg bw) on day 14 via intra-oesophageal intubation; water temperature 6 ºC (McGuire *et al.*, 1996a).

Time post-dose (days)	Mean concentration ± SD of Teflubenzuron (µg/kg)		
	Skin	Muscle	Muscle + Skin
1	443 ± 211	405 ± 176	407 ± 155
8	106 ± 32	63 ± 27	67 ± 26
16	54 ± 33	45 ± 7	46 ± 8
24	62 ± 17	41 ± 19	42 ± 17
35	44± 9	23 ± 4	25 ± 4

The initial half-life of elimination calculated from the residue data from days 1 to 16 in the combined tissues muscle and skin was 4.8 days (EMEA, 1999).

Study 5 – NTO/010

In a GLP-compliant repeated oral dose residue study (McGuire *et al.*, 1996b) conducted at a water temperature of 10 ± 1 °C, Atlantic salmon (508 to 1297 g; 25 male and 29 female) were treated with teflubenzuron medicated feed (5.7 g of medicated diet/kg bw, corresponding to 9.46 mg of teflubenzuron per kg bw; intended dose 10 mg/kg bw) over a seven day period. One fish per group received a single oral dose of 10 mg/kg bw teflubenzuron by intra-oesophageal intubation on feeding Day 7. Samples of muscle and skin (3 g) were collected on days 1, 4, 8, 12, 18, 24, 35, 50 and 120 post-dose. The concentrations of teflubenzuron in muscle and skin were determined using a validated HPLC-UV method (McGuire, 1995). Recoveries for muscle and skin ranged from 75-391% and 70-187% of the administered dose, respectively. The depletion results are presented in Table 7.17. Values lower than LOQ of 20 µg/kg in muscle

and skin were considered in the reported values as 20 µg/kg for calculations of the average values with their respective standard deviations.

Table 7.17. Mean concentration ± SD (ten fish at each time) of teflubenzuron in tissues from Atlantic salmon with time after oral daily dose (medicated feed) of teflubenzuron (10 mg/kg bw) for 6 consecutive days followed by a single oral dose of teflubenzuron (10 mg/kg bw) on day 7 via intra-oesophageal intubation; water temperature 10 ºC (McGuire *et al.,* 1996b)**.**

Time after treatment (days)	Mean concentration ± SD of Teflubenzuron (µg/kg)		
	Skin	Muscle	Muscle and skin*
1	1310 ± 436	894 ± 501	932 ± 475
4	353 ± 316	329 ± 206	331 ± 213
8	221 ± 229	103 ± 52	116 ± 64
12	86 ± 42	52 ± 23	56 ± 22
18	50 ± 12	26 ± 9	29 ± 8
24	39 ± 20	28 ± 16	29 ± 16
35	43 ± 13	37 ± 17	38 ± 16

* The combined concentrations of teflubenzuron in muscle and skin were obtained using the following equation:

$$\text{Muscle+skin}=\frac{\left(\text{Conc. Teflubenzuron }_{\text{muscle}} \text{ x weight}_{\text{muscle}}\right)+\left(\text{Conc. Teflubenzuron }_{\text{skin}} \text{ x weight}_{\text{skin}}\right)}{\text{Total weight }_{\text{muscle+skin}}}$$

The initial half-life of elimination calculated from the residue data from days 1 to 18 in the combined muscle and skin was 3.4 days. The tissue concentrations appeared to reach a plateau of 30 to 40 µg/kg at 24 to 35 days post treatment. This has been attributed to the background level of teflubenzuron which was found in the test system (EMEA, 1999).

A summary of the pharmacokinetic parameters obtained in the depletion studies (*Study 1, Study 2, Study 3, Study 4 and Study 5*) is presented in Table 7.18.

Table 7.18. Elimination rate constants and half-lives of elimination obtained from the total radioactive depletion and residue Studies 1 to 5 (O'Connor, 1996).

Study	Tissue	Initial Elimination Phase	Terminal Elimination Phase

		Time range (d)	Initial half-life(d)	Rate constant (d^{-1})	Time range (d)	Initial half-life(d)	Rate constant (d^{-1})
1	Liver	1-18	16.5	-0.04	--	--	--
	Kidney	3-18	17.4	-0.04	--	--	--
	Muscle	2-18	4.7	-0.15	--	--	--
	Skin	2-18	6.5	-0.11	--	--	--
	Muscle and skin	1-18	4.6	-0.15	--	--	--
	Mucus[a]	0.375-18	8.4	-0.08	--	-	--
	Gall bladder	2-18	7.1	-0.10	--	--	--
2	Liver	1-18	5.7	-0.12	24-120	38.5	-0.02
	Kidney	1-18	2.6	-0.27	24-120	49.5	-0.01
	Muscle	1-18	2.6	-0.26	35-120	38.5	-0.02
	Skin	1-18	3.6	-0.19	18-120	49.5	-0.01
	Muscle and skin	1-24	3.8	-0.18	35-120	38.4	-0.02
3	Liver	1-18	16.9	-0.04	*	*	*
	Kidney	1-24	10.0	-0.07	35-97	49.5	-0.01
	Muscle	1-24	3.8	-0.18	**	**	**
	Skin	1-24	5.5	-0.13	24-97	99.0	-0.01
**	Muscle and skin	1-35	4.9	-0.14	**	**	**
4	Muscle	1-16	4.8	-0.14	--	--	--
	Skin	1-16	5.0	-0.14	--	--	--
	Muscle and skin	1-16	4.8	-0.14	--	--	--
5	Muscle	1-18	3.4	-0.21	--	--	--
	Skin	1-18	3.8	-0.18	--	--	--
	Muscle and skin	1-18	3.4	-0.20	--	--	--

-- Insufficient data to determine terminal elimination phase.

[a] Day 6 was not included for mucus as data were considered as outliers.

* Data variation too high to determine the terminal elimination phase.

**Terminal elimination phase not observed.

Teflubenzuron residues depleted in muscle and skin with different half-lives depending on the water temperature. Peak residue concentrations were higher in the experiment performed at 10 ºC than in the experiment at 6 ºC, however the initial rates of depletion of tissue residues were similar. The slow terminal phase of elimination was attributed to background levels of teflubenzuron in the recirculated sea water in the tanks were the fish were maintained.

Methods of analysis for residues in tissues

Due to the physical and chemical properties of teflubenzuron (*i.e.*, its polarity and low volatility), liquid chromatography is the method of choice for the determination of drug residues in food, feed and biological matrices.

Most protocols use solvent extraction of teflubenzuron from the sample followed by clean-up steps, including solid phase extraction procedures and more recently the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach (Anastassiades *et al.*, 2003). Pressurized liquid extraction (PLE) has also been employed (Brutti *et al.*, 2010). Chromatographic separation is commonly performed using reverse-phase chromatography. For the quantification of teflubenzuron, UV and, more recently, tandem mass spectrometry detectors have been employed. In the latter case, electrospray ionization is usually employed in the positive ion mode using acquisition of ions in the selected reaction-monitoring mode (SRM). The Committee assessed the validation data against the analytical requirements as published in the Codex guidelines for analytical methods for residue control, CAC/GL 71-2009 (FAO/WHO, 2014).

Quantitative methods

Liquid chromatography

A validated single-residue method, using high performance liquid chromatography (HPLC) coupled to ultraviolet (UV) detection was used in depletion studies carried out twenty years ago for the determination of teflubenzuron in salmon tissues (McGuire, 1995). Teflubenzuron was extracted from the tissues (3 g) using acetonitrile (3 x 7 mL) at 80 °C. The combined acetonitrile extracts were reduced to approximately 2 mL on a rotary evaporator at 50 °C. The extract was diluted in dichloromethane (approximately 10 mL) and the volume was reduced again to 2 mL. The remaining dichloromethane extract was quantitatively diluted to 10 mL with dichloromethane. An aliquot of 5 mL of this extract was diluted with dichloromethane (20 mL) and the organic phase partitioned with 3 x 50 mL of water. The aqueous phase was back-partitioned with 25 mL dichloromethane. The organic extract was reduced to dryness and the residue re-dissolved in 5 mL of diethyl ether:hexane, 5:95 v/v. Finally, the extract was cleaned-up by solid phase extraction, first using a silica cartridge (500 mg), followed by a C8 cartridge (500 mg). The eluate (1 mL) was transferred to a vial and analysed by HPLC-UV at 250 nm or 254 nm. For the chromatographic separation a Supelcosil LC-ABZ column (25 cm x 4.6 mm) column at 28 °C and a mobile phase containing methanol:acetonitrile:water 60:20:20 v/v/v was employed. The external calibration curve covered the concentration range of 0.02 to 1.0 µg/mL (corresponding to 20 µg/kg to 1000 µg/kg) with a linearity of 0.9950. The recoveries of teflubenzuron were >70% and the limit of quantification was 20 µg/kg in salmon skin and muscle.

Confirmatory methods

Liquid chromatography – tandem mass spectrometry (LC-MS/MS)

Liquid chromatography coupled to tandem mass spectrometry, using electrospray ionization (ESI), has been widely used as technique for the determination of residues of teflubenzuron in food and biological matrices due to its selectivity. However, in order to overcome matrix suppression, matrix-matched standards are mandatory.

The FDA monitors teflubenzuron in salmon tissues using a multi-residue pesticide monitoring procedure, described in the FDA Laboratory Information Bulletin 4463 (Chamkasem *et al.*, 2010). The sample preparation is a modification of the QuEChERS approach developed by Anastassiades and co-workers (Anastassiades *et al.*, 2003) without using the dispersive sample clean-up step. Briefly, salmon tissue (10 g) is shaken with a mixture of 5 mL water and 15 mL acetonitrile in the presence of 1.5 g NaCl and 6 g $MgSO_4$. The mixture is centrifuged, and a volume of 600 µL of the extract is added of the same volume of a solution of 4 mmol/L ammonium formate and 0.1% formic acid. The solution is mixed and filtered through a 0.2 µm PVDF syringe filter. The filtrate is injected into the LC-MS/MS system, using the electrospray ionization source in the negative mode. The chromatographic separation is carried out on a reversed phase C18 column (Restek Ultra Aqueous, 100 x 2.1 mm; 3 µm), at 50 °C, and a mobile phase of ammonium formate and formic acid under gradient elution. Teflubenzuron has a precursor ion at 379 *m/z* and two product ions at 339.1 *m/z* and 358.9 *m/z*. The two product ions were used for quantification and confirmation purposes. Concentrations in salmon muscle and skin were determined by external calibration curves (standards in acetonitrile); i.e., without using an internal standard. The linear range of 0.1 to 100 ng/mL corresponds to 0.3 to 300 µg/kg of teflubenzuron in tissues. The limit of quantification was estimated at a signal to noise ratio ≥ 10.

The method validation parameters are presented in Table 7.19. Analysis of incurred residues in salmon was also carried out. Salmon were fed by oral gavage with a single dose of teflubenzuron at 20 mg/kg bw. Four fish were sacrificed at 24 h and 48 h after feeding and average concentrations of teflubenzuron in muscle tissue with skin of 4.4 µg/kg and 16.4 µg/kg were determined, respectively.

Table 7.19. Validation parameters of the LC-MS/MS method for the determination of teflubenzuron in salmon tissues (Chamkasem *et al.,* 2010)**.**

Parameter Assessed	Muscle with skin Fortified samples	 Incurred samples
Intraday accuracy (% bias)		
Intraday precision (% CV)	6.02% (1 µg /kg) 1.64% (10 µg /kg) 3.31% (100 µg /kg)	 3.2% (4.4 µg/kg)[a] 2.2% (16.4 µg/kg)[b]
Accuracy	65.9% (1 µg /kg) 88.4% (10 µg /kg) 101.4% (100 µg /kg)	
Estimated LOQ (µg/kg)	0.3	
Analytical range (ng/mL)	0.1 -100	
Linearity (r)	0.9995	
Selectivity	No interference observed	
Extraction recovery (n=6)	65.9 (1 µg/kg) 88.5 (10 µg/kg) 101.37 (100 µg/kg)	

a: 24 h post feeding (n=4); b: 48 post feeding (n=4)

A method using pressurized liquid extraction (PLE) for the extraction of benzoylureas, including teflubenzuron, in animal products (milk, eggs and meat) has been developed and validated (Brutti *et al.,* 2010). Quantification was carried out by LC-MS/MS using an ion trap (IT) mass analyser and an APCI source. Sample test portions (5 g) were homogenized with diatomaceous earth (4-5 g) and extracted with 22 mL ethyl acetate at 80 °C and 1500 psi. The extract was concentrated to 1 mL at 40 °C, transferred quantitatively to another flask using 2 x 2.5 mL methanol and evaporated to dryness. The residue was reconstituted in methanol. The method presented linearity (r) higher than 0.99. Recoveries at a fortification level of 10 µg/kg were 78% (RSD, 16%), 84% (RSD, 8%) and 90% (RSD 10%) for milk, eggs and beef meat, respectively. The limit of quantification was in the range of 2 to 10 µg/kg.

A simple and fast method for the determination and monitoring of occurrence of eight pesticides, including teflubenzuron, in fish and shellfish by matrix solid-phase dispersion (MSPD) with anhydrous sodium sulphate and C18 as dispersants, silica as an adsorbent and LC-MS/MS quantification has been reported (Carro *et al.*, 2012). Sample test portions (0.2 g) were blended with 0.5 g sodium sulphate anhydrous and 2 g of C18. The mixture was transferred to a 6 mL SPE empty cartridge and 2 g of silica was added at the top. Acetic acid:acetonitrile, 5:95 v/v, was used to elute the analytes. The quantification was performed by LC-MS/MS with an ESI interface, which was operated simultaneously in the positive and negative mode. For the chromatographic separation, a Hypersil ODS (100 mm x 3.2 mm, 3 µm particle size) column at 30 °C and a mobile phase 5 mM ammonium acetate in acetonitrile were used. Linearity was evaluated using matrix-matched standards in the range of 5 to 500 µg/kg. Linearity was demonstrated, with a correlation coefficient > 0.996 and intra-day precision

(n=6) was 5.8%. A matrix effect of 18.1% was verified. The limits of detection and quantification for teflubenzuron were 1.5 µg/kg and 4.7 µg/kg, respectively.

Appraisal

Teflubenzuron has not been previously reviewed by the Committee, but was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), in 1994, when an ADI of 0-0.01 mg/kg bw per day was established based on the dose-related effects in the liver and derived from the 18-month carcinogenicity study in mice.

Teflubenzuron is an acyl urea insecticide registered for aquaculture use in the treatment of Atlantic salmon at a maximum dose of 10 mg per kg fish for seven days, administered through feed (pelleted diet at a level of 2 g/kg), for control of infestation of sea lice. It is also used in agriculture to control a wide range of insect pests.

Teflubenzuron residues depleted in muscle and skin with different half-lives, depending on the water temperature. Peak residue concentrations were higher in the experiment performed at 10 ºC than in the experiment at 6 ºC, however the initial rates of depletion of tissue residues were similar.

Metabolism data are available for a variety of animal species, including rats, goats, chicken and salmon. Teflubenzuron is predominantly unmetabolized and biliary excretion is the main path for elimination. The metabolic profiles are similar throughout animal species.

Metabolic profiling in salmon was available. Two studies were carried out following single or repeated dose administration of radiolabelled teflubenzuron to salmon. Unchanged teflubenzuron was the major component in liver and kidney. Minor metabolites (less than 5%) were identified in salmon liver and kidney: 3,5-dichloro-2,4-difluoroaniline, 2'-hydroxy-teflubenzuron, 3'-hydroxy-teflubenzuron and 3,5-dichloro-2,4-difluorophenyl urea. Some metabolites remained unknown. In salmon muscle and skin only teflubenzuron was identified.

Radiolabelled data are available for the depletion of teflubenzuron residues in salmon at a water temperature of 10 ºC, following single or repeated dose. Teflubenzuron was identified as the marker residue in salmon muscle and skin. Based on the results of these two studies, the Committee identified teflubenzuron as the marker residue in salmon muscle and skin and determined that a value of 0.8 was appropriate for the MR:TRR. This value was the mean value of the MR:TRR of muscle and skin determined 8 days post last dose of teflubenzuron at a water temperature of 10 ºC, excluding the value of 1.28 that was considered an outlier.

The highest concentration (less than 1000 µg/kg) of teflubenzuron in salmon muscle and skin occurs 1 day after administration of the drug.

The residue depletion studies in salmon were conducted in the mid 90's using HPLC-UV methods, which required complex sample preparation procedures for extraction and clean-up. The limit of quantification (LOQ) was 20 µg/kg in salmon muscle and skin. The state-of-the-art methods (LC-MS/MS) use simpler sample preparation procedures, based on the QuEChERS approach, and have a LOQ about of 0.3 µg/kg.

The recommended MRL of 400 µg/kg of teflubenzuron in fillet (muscle with skin in natural proportions) and salmon muscle was based on a withdrawal period of 96 degree days and calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile (UTL 95/95) of residue concentrations in salmon muscle and skin derived from the pivotal residue depletion study, conducted at a water temperature of 10 ºC, from 1 to 18 days after treatment. The tolerance limits for teflubenzuron residues in salmon muscle and skin are shown in Figure 7.3.

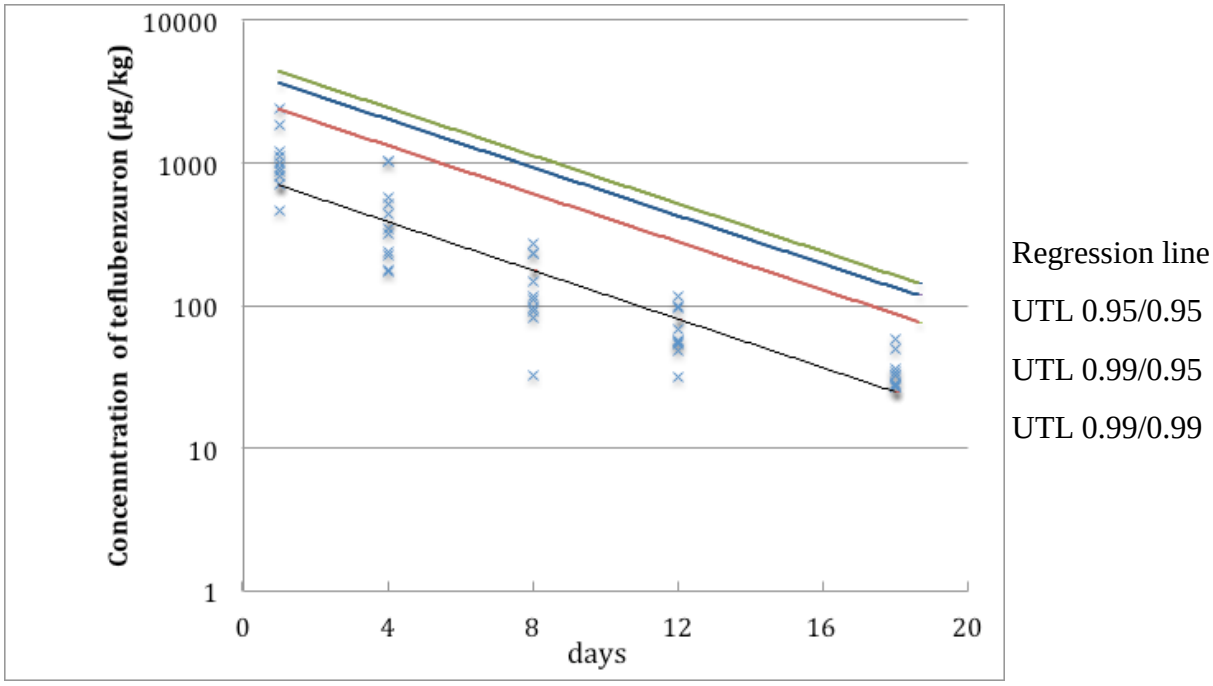


Figure 7.3. Tolerance limit considerations for teflubenzuron in salmon muscle with skin.

Dietary exposure assessment

The ADI of 0-5 µg/kg bw for teflubenzuron established by the Committee was based on a chronic effect, so a GEADE was not determined. In this dietary exposure assessment, fish was the only contributor to dietary exposure.

The EDI for teflubenzuron was calculated based on median residues found in salmon muscle and skin (water temperature 10 ºC) at 8 days post-dosing, with an associated ratio of the concentration of marker residue to the concentration of total residue of 80%. The median (n = 10) calculated 8 days after treatment was used as input into the chronic dietary exposure estimates (Table 7.20).

Table 7.20. Median concentrations of teflubenzuron residues in salmon skin and muscle at a water temperature of 10 ºC.

Time post dosing (days)	Concentration of marker residue in salmon muscle and skin µg/kg										
	animal 1	animal 2	animal 3	animal 4	animal 5	animal 6	animal 7	animal 8	animal 9	animal 10	Median
1	813	882	465	1123	1021	712	946	1862	1226	2448	984
4	352	177	180	577	450	321	243	519	226	1045	337
8	237	83	32	92	99	236	116	148	112	273	114
12	101	55	117	57	56	49	69	57	32	98	57
18	29	58	50	35	37	33	27	28	27	33	33

The estimated dietary exposure expressed as the EDI was 42.9 µg/person/day, which represents 14% of the upper bound of the ADI of 0-5 µg/kg bw/day (or 300 µg/person/day) (Table 7.21).

Table 7.21. The Estimated Dietary Intake of teflubenzuron residues from salmon muscle.

Tissue	Median concentration*(µg/kg)	Standard Food Basket (kg)	MR:TR ratio[1]	Daily intake (µg/person/day)
Muscle	114	0.3	0.8	42.9
TOTAL				42.9

Using the median residue in salmon muscle and skin and fish consumption as inputs, the GECDE for the general population was 1.6 µg/kg bw/day, which is equivalent to 31% of the upper bound of the ADI. The higher exposure estimate compared to the EDI was due to the higher consumption of fish used in the GECDE, 655 g/person compared 300 g of muscle (fish) used in the EDI model diet (Table 7.22).

In children, the GECDE was 2.1 µg/kg bw/day which represents 43% of the upper bound of the ADI. This estimate was higher than the whole population estimate. While the fish consumption amount is lower than for adults and the model diet, the lower bodyweight of children leads to comparatively higher exposure on a bodyweight basis. Exposure of infants was estimated to be lower at 0.9 µg/kg bw/day (18% of the upper bound of the ADI) because fish consumption of infants is only 10% of the consumption amount used in the model diet.

Table 7.22. The Global Estimate of Chronic Dietary Exposure (GECDE) to teflubenzuron residues in salmon muscle for the general population, children and infants.

Category	Type	Mean consumption[1] whole population, g/d	97.5th consumption[2] consumers only, g/d	Exposure µg/kg bw/day		GECDE[3] µg/kg bw/day	ADI %
				Mean	97.5th		
General Population							
Fish and seafood	Fish	27	655	0.06	1.56	1.56	31.1
TOTAL				0.0	1.6	1.6	31
Children							
Fish and seafood	Fish	24	226	0.22	2.15	2.15	42.93
TOTAL				0.0	2.0	2.0	43
Infants							
Fish and seafood	Fish	1	33	0.04	0.92	0.92	8.8
TOTAL				0.0	0.9	0.9	18

[1]highest mean consumption figures based on whole population considered from the available dataset
[2]highest 97.5th food consumption figures based on consumers only considered from the available dataset
[3]GECDE is the sum of the highest exposure at the 97.5th percentile of consumption for a food and the mean dietary exposures of the other foods.

Maximum Residue Limits

In recommending MRLs for teflubenzuron in salmon, the Committee considered the following factors:

- Teflubenzuron is authorized for use in salmon in several countries. The maximum recommended dose is 10 mg/kg fish per day for 7 consecutive days, administered through medicated feed. The withdrawal periods range from 7 to 11 days and from 45 to 96 degree-days.
- An ADI of 0-5 µg/kg bw for teflubenzuron was established by the Committee.
- Teflubenzuron is the marker residue in tissues.
- The ratio of the concentration of marker residue to the concentration of total residue of 0.8 was calculated in muscle and skin in natural proportion of salmon. Residue data were provided using a validated analytical method to quantify teflubenzuron in salmon tissues.
- A validated analytical method for the determination of teflubenzuron in edible salmon tissues is available in the literature and may be used for monitoring purposes.

The MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of total residue concentrations (95/95 UTL) in salmon muscle and skin derived from the pivotal study used for this assessment, conducted at a water temperature of 10 °C and a withdrawal period of 96 degree-days (10 days).

The Committee recommended MRLs for teflubenzuron in salmon of 400 µg/kg in fillet (muscle plus skin in natural proportion) and in muscle.

The EDI is 42.9 µg/person per day, on the basis of a 60 kg individual, which represents approximately 14% of the upper bound of the ADI.

The GECDE for the general population is 1.6 µg/kg bw per day, which represents 31% of the upper bound of the ADI; for children, 2.1 µg/kg bw per day, which represents 43% of the upper bound of the ADI; and for infants, 0.9 µg/kg bw per day, which represents 18% of the upper bound of the ADI.

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