Food and Agriculture Organization of the United Nations

World Health Organization

Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 85th Meeting 2017

Lufenuron

This monograph was also published in: Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 85th meeting 2017. FAO JECFA Monographs 21

Lufenuron

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Identity

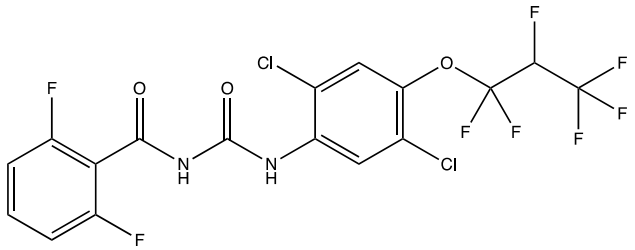
International Non-proprietary Name (INN): Lufenuron

Synonyms: IMVIXA^{TM}, Match EC, Match 5 EC, Curyom 550 EC

IUPAC Name: (RS)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoro-propoxy)-phenyl]-3-(2,6-difluorobenzoyl)-urea

Chemical abstract service Nº: 103055-07-8

Structural formula:



Molecular formula: $C_{17}H_8Cl_2F_8N_2O_3$

Molecular weight: 511.15 g mol^{-1}

Other information on identity and properties

Pure active ingredient: Lufenuron (purity ≥ 98 %)

Appearance: White to pale yellow powder

Melting point: 174.1 °C

Solubility in water: 46 µg/L at 25 °C

Solubility in methanol: 45 g/L at 20 °C

Solubility in acetonitrile: 50 g/L at 20 °C

Solubility in dichloromethane: 70 g/L at 20 °C

Vapor pressure: <1.3 x 10^{-7} Pa at 20 °C

Log $K_{o/w}$: 5.12

Chirality: Equimolar racemic mixture of R- and S-isomers

Polimorphism: Seven crystalline forms

Impurities: Less than 2 % (total) and less than 0.5 % for each compound. 2,6-difluoro benzamide, N-(2,5-dichloro-4-hydroxyphenyl-aminocarbonyl)-2,6-difluoro-benzamide, N-[5-chloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl-aminocarbonyl]-2,6-difluoro-benzamide, N-[2-chloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl-aminocarbonyl]-2,6-difluoro-benzamide, N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl-aminocarbonyl]-2-chloro-6-fluoro-benzamide, N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl]-2-fluoro-benzamide, N,N'-bis[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl]-urea and carbonic acid 2,5-dichloro-4-[3-(2,6-difluoro-benzoyl)-ureido]-phenylester phenyl ester

Background

Lufenuron (CAS No. 103055-07-8) is a benzoyl phenylurea insecticide used as veterinary drug for the treatment of sea lice infestations (*Caligus rogercresseyi*) in farmed Atlantic salmon (*Salmo salar* L.) and as an insecticide for the control of flea infestation in companion animals (cats and dogs). It is also used in crop protection as an insecticide for various fruits and vegetables, being active against larvae of Lepidoptera and Coleoptera. Lufenuron consists of an equimolar racemic mixture of R- and S- enantiomers.

The toxicology and residues of lufenuron were evaluated by the FAO/WHO JMPR in 2015. At that meeting, JMPR established an ADI of 0-0.02 mg/kg bw for lufenuron based on the no-observed-adverse-effect level (NOAEL) of 1.93 mg/kg bw per day for tonic-clonic seizures and findings in lungs, gastrointestinal tract, liver and urinary tract in the 2-year dietary study in rats. The Committee on this Meeting also concluded that it was not necessary to establish an acute reference dose (ARfD) for lufenuron in view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be occasioned by a single dose (JMPR, 2015a).

The U.S. Food and Drug Administration established an import tolerance of 1.35 ppm lufenuron in salmonids muscle with adhering skin (U.S.FDA, 2016).

The European Commission adopted a MRL of 1350 µg/kg lufenuron in fin fish (Regulation EU 967/2014), based on a toxicological ADI of 0.015 mg/kg bw based on the NOEL of 1.5 mg/kg bw/day for the observed signs of hepatotoxicity in a 1-year dog toxicity study (EMA, 2015).

The European Food Safety Authority reviewed the MRL for lufenuron in 2016 (EFSA, 2017).

At the Twentieth Third Session, the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) requested evaluation of lufenuron with establishment of MRL in finfish (salmon/trout) muscle and skin in natural proportions.

Residues in food and their evaluation

Conditions of use

Lufenuron is a benzoyl phenyl-urea insecticide used in aquaculture for the treatment of sea lice on farmed salmon. It is also used as an insecticide on a wide range of vegetable crops, oilseeds, root crops maize, sugarcane and coffee for the control of the larvae of many insect pest.

Lufenuron acts by inhibition of chitin synthesis, disrupting the formation of chitin and prevents larvae from moulting.

The treatment is based on administration of a high dose of the active ingredient to Atlantic salmon when they still are in the life stage of smolts and maintained in fresh water. The drug protect fish whilst they are in seawater, facing continual sea-lice pressure.

Dosage

Lufenuron is registered for use in aquaculture in Chile since 2016 with a withdrawal period of 2050 degree-days for salmon. The MRL adopted is 1350 µg/kg of lufenuron in muscle and skin in natural proportions.

The pre-mix containing lufenuron 10 % (w/w), corn starch 88 % (w/w) and colloidal silicon dioxide 2 % (w/w) is incorporated by top-coating or vacuum coating onto fish feed. Considering a feeding rate of 1 % biomass/day the premix incorporation rate would be 5 kg to 1000 kg feed, which represents a final concentration of 0.05 % w/w of lufenuron in the feed.

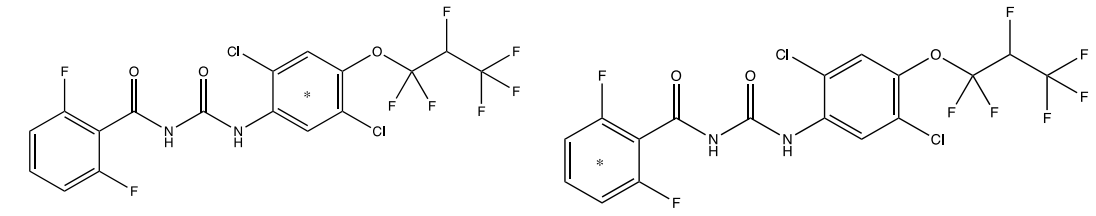
The recommended dose is 5 mg lufenuron per kg of fish biomass orally once daily for seven consecutive days. The treatment starts at the life stage of smolts, when the fish are maintained in fresh water and before they are transferred to sea. When the expected feeding rate is disrupted, the feeding period may need to be extended from 7 days to a maximum of 14 days to ensure the fish receive the full therapeutic dose of 35 mg/kg bw per day.

Pharmacokinetics and metabolism

Test material used in the radiolabelled pharmacokinetic and metabolism studies

Pharmacokinetic and metabolism studies were conducted with [^{14}C]-lufenuron labelled within the dichlorophenyl ring (Figure 1A) or the difluorophenyl ring (Figure 1B). The purities of these radiolabelled compounds, determined by high performance liquid chromatography (HPLC), used throughout the studies, were greater than 96 %.

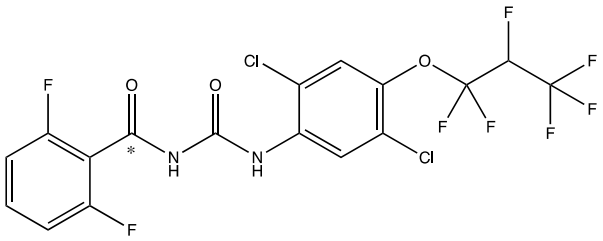
Figure 1. Structure of the radiolabelled compounds: A – [^{14}C]-lufenuron labelled at the dichlorophenyl ring and B - [^{14}C]-lufenuron labelled at the difluorophenyl ring



A B

For the ADME studies on salmon [^{14}C]-lufenuron labelled at the carbon on the benzamide group was used (Figure 2). The purity of the radiolabelled compound, determined by HPLC, was greater than 99 %.

Figure 2. Structure of the [^{14}C]-lufenuron labelled at the carbon of the benazamide group



Pharmacokinetics in laboratory animals

Rats

In a GLP-compliant ADME study (Bissig, 1990), three male and three female rats (Tif: RAIf, SPF), 7 to 11 weeks old and with a body weight of 200-260 g, separated in two groups (E1 and E2), were treated with radiolabelled [^{14}C]-lufenuron (purity higher than 99 %, specific activity 51.4 µCi/mg, labelled at the dichlorophenyl ring), at two dose levels: 0.5 mg/kg bw and 100 mg/kg bw. Blood was collected at different time points:1, 2, 4, 8, 12, 24, 32, 48 and 56 h post-dose for Group E1 and 1, 2, 4, 8, 12, 24, 32, 48, 56 h and 72 h post-dose for Group E2. The blood kinetics indicated moderate absorption rates from the intestinal tract into the systemic circulation. The maximum blood levels were reached about 8 h after administration and declined to one half of the maximum value about 45 and 60 h following treatment for the low and high dose level, respectively.

The depletion of residues in tissues followed biphasic first order kinetics with half-life times of 2 to 6 days for Phase 1. For the slower Phase 2, different half-life times were observed for the two dose levels: 5 to 9 days at the low dose level and 10 to 37 days at the high dose level, respectively (Bissig, 1990).

In another study, non-GLP compliant (Okada, 1997), female (3) and male (3) rats (Sprague-Dawley [Crj:CD(SD)] were treated by oral gavage with radiolabelled [^{14}C]-lufenuron (purity higher than 97.8 %, specific activity 1.89 MBq/mg, labelled at the dichlorophenyl ring) at the

dose of 0.5 mg/kg bw for 14 consecutive days. Blood samples were collected at 8 and 24 h after each daily administration for 13 consecutive days and then 8, 24, 48 and 168 h after the final administration. After the first dose blood levels reached 0.040 µg eq/mL and 0.025 µg eq/mL at 8 h and 24 h, respectively. Lufenuron blood concentrations increased during the dosing period, reaching 0.184 µg eq/mL and 0.178 µg eq/mL in males and females 8 h after the last dose (Day 14). After dosing ceased, lufenuron blood concentrations decreased gradually, reaching 0.095 µg eq/mL and 0.101 µg eq/mL in males and females, respectively, with estimate elimination half-lives of 208 and 323 h in males and females, respectively.

Dogs

Pharmacokinetics and bioavailability of lufenuron in dogs were investigated in two GLP-compliant studies (Maurer and Hotz, 1999). In the first study, two Beagle dogs (Animal 511, female, 15.6 kg and Animal 534, male, 12.3 kg) were treated intravenously with a single dose of 2.6 mg/kg bw [^{14}C]-lufenuron (purity higher than 99 %, specific activity 51.4 µCi/mg, labelled at the dichlorophenyl ring). The drug was dissolved in Tween 80/Solketal and administered before feeding. For the second study, two Beagle dogs (Animal 508, female, 12.3 kg and Animal 313, male 12.6 kg) were administered a single oral dose of 10 mg/kg bw [^{14}C]-lufenuron (purity higher than 98.9 %, specific activity 0.104 MBq/mg, labelled at the dichlorophenyl ring). Blood samples were taken at regular intervals for up to 21 days and lufenuron quantified by HPLC. The blood concentration profiles for both studies are shown in Figure 3.

Figure 3. Blood concentration profile of [^{14}C]-lufenuron following intravenous (IV) and oral administration to Beagle dogs (Maurer and Hotz, 1999). The pharmacokinetic parameters were estimated from both studies and data are summarized in Table 1

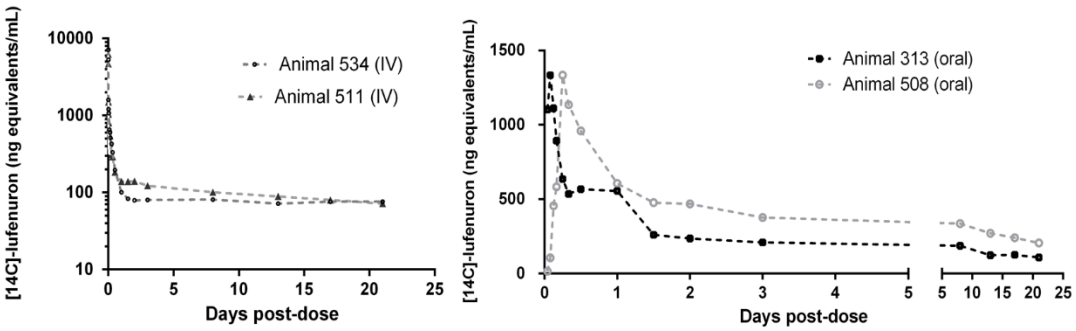


Table 1. Pharmacokinetic parameters for lufenuron in Beagle dog plasma after dosing by intravenous injection or oral administration (Maurer and Hotz, 1999)

Administration route and dose	Dog n.	t_{max} (days)	C_{max} (ng/mL)	$t_{1/2}$ (days)	k_e $days^{-1}$	$AUC_{(0-t)}$ (ng.days/mL)	$AUC_{(0-inf)}$ (ng.days/mL)	MRT (days)
Oral	508	0.25	1334	20.2	0.034	6920	12890	27.8
10 mg/kg bw	313	0.083	1334	18.0	0.039	3833	6735	24.1
Intravenous	511	0	6223	23.8	0.029	2272	4747	32.9
2.5 mg/kg bw	534	0	145	198.4	0.0035	1905	23682	283.5

t_{max}: time to peak plasma concentration; C_{max}: peak plasma concentration; AUC: area under the curve; $t_{1/2}$: elimination half-life and MRT: mean residence time.

Animal 534 showed an extremely slow elimination and was considered as an outlier. The bioavailability after oral administration was estimated using $AUC_{(0-t)}$, instead of $AUC_{(0-inf)}$ once to the prolonged distribution phase of Animal 534 that overlapped the elimination and thus led to an overestimated elimination half-life time. The bioavailability is estimated to be about 70 %. Due to generally slow elimination of lufenuron a significant amount of the administered dose remained in the bodies of the dogs 21 days after oral administration.

Pharmacokinetic in Food-producing Animals

Salmon

In six GLP-compliant studies, carried out in Canada (3 studies), Chile (one study) and Norway (2 studies), Atlantic salmon smolts (mixed sex) held in a fresh water hatchery were treated with lufenuron at a nominal dose of 5 mg/kg bw for seven consecutive days (Table 2). Fish were transferred to sea cages after the end of medication and remained at sea until the end of the study. Blood samples were collected up to 12 months after the last dose and lufenuron quantified by a validated liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method, using $^{13}C_6$-lufenuron as an internal standard. The limit of quantitation (LOQ) of the method was 10 ng/mL. The lufenuron blood depletion curves are shown in Figure 4. Values lower than the LOQ were not used for the calculations of the average values.

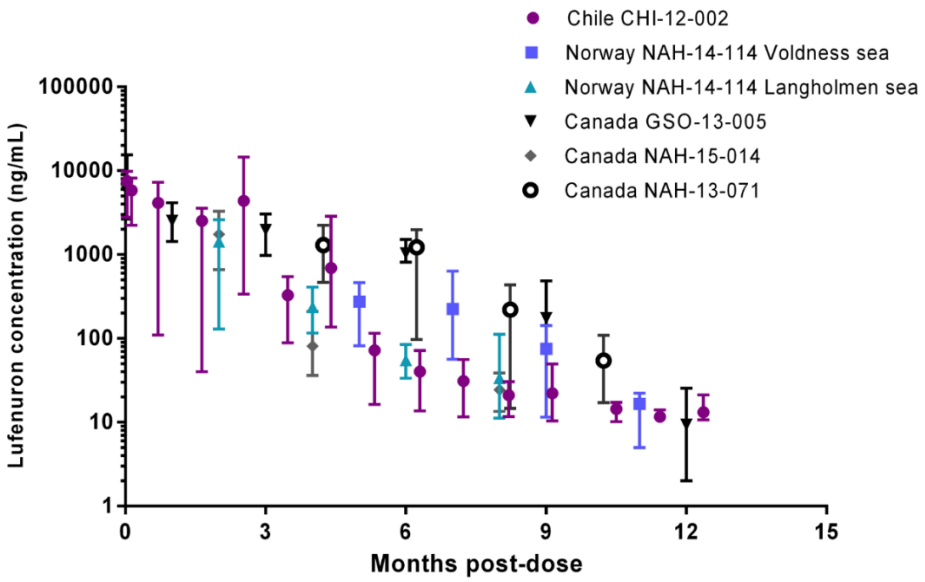
The half-life of lufenuron in blood determined in the study carried out in Chile was 53.7 days or 635.1 degree-days. Two additional similar studies were carried out using a lower dose (0.5 mg/kg bw) and a higher dose (10 mg/kg bw) as in the first study. The half-lives were 344 degree-days (23.4 days) and 373 degree-days (25.2 days) for the lower and higher doses, respectively. Some residue data were excluded.

Table 2. Experimental designs used for the determination of lufenuron in blood of Atlantic salmon treated with a nominal dose of 5 mg/kg bw for seven consecutive days

Local of the study	Study number/ Study report	Number of fish	Average weight	Transfer to sea cages after medication	Temperature (ºC)		Reference
					Hatchery	Sea	
Canada[a]	GSO-13-005/ ATL-14-1123	36	115 g	9 – 10 days	10.3	1.7-13.7	Hervy, 2015b
Canada[b]	NAH-13-071/ ATL-15-1351	48	88 g	7 days	10	0.7-13.2	Hervy, 2016b
Canada[c]	NAH-15-014/ ATL-15-1430	196	62 g	14 days	13	4-16	Hervy, 2016c
Chile[d]	CHI-12-002	124	125 g	6 days	11	9-18	Lewis, 2015
Norway[e] **Voldness sea**	NAH-14-114/ ATL-15-1352	48	102 g	7 days	10-12	5.8-15.6	Hervy, 2015d
Norway[f] **Langholmen sea**	NAH-14-114/ ATL-15-1363	48	98 g	1-3 weeks	3.5	3.7-13.3	Hervy, 2016a

[a]Blood samples were collected at 1 day, 1, 3, 6, 9 and 12 months after the end of the treatment. Six fish were sampled for each time point. [b]Blood samples were collected at 127, 183, 246 and 308 days after the end of the treatment. Twelve fish were sampled for each time point. [c]Blood samples were collected at at 2, 4, 6 and 8 months after the end of the treatment. Eighteen fish were sampled for each time point. [d]Blood samples were collected at 1 (n= 6), 4 (n= 6), 21 (n=12), 49 (n=11), 76 (n=10), 104 (n=11), 132 (n=10), 160 (n=11), 189 (n=6), 217 (n= 9), 246 (n=9), 274 (n=6), 315 (n=4), 343 (n=7) and 371(n= 6) days post last dose. [e]Blood samples were collected at 3, 5, 7, 9 and 11 months after the end of the treatment. Twelve fish were sampled for each time point. [f]Blood samples were collected at 2, 4, 6 and 8 months after the end of the treatment. Twelve fish were sampled for each time point.

Figure 4. Blood concentration profile of lufenuron following oral administration of 5 mg/kg bw for 7 consecutive days

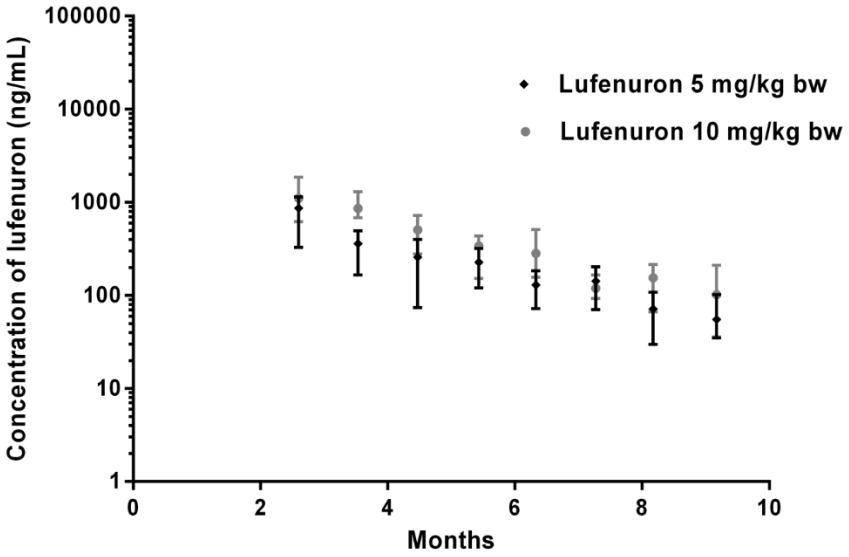


Rainbow trout

Pharmacokinetics of lufenuron in rainbow trout (*Oncorhynchus mykiss*) were evaluated in a GLP-compliant study conducted in Chile (Lewis and Jacob, 2017). Two groups of rainbow trout smolts (mixed sex and an average weight of 212 g at the beginning of the treatment) held in a fresh water hatchery (11.2 ºC) were treated with lufenuron incorporated onto feed at a nominal dose of 5 mg/kg bw and 10 mg/kg bw for seven consecutive days. Fish were transferred to sea cages after the end of medication and remained at sea until the end of the study. The temperature of the sea varied from 9.2 to 17.1 ºC. Blood samples (1 mL) drawn from the caudal vein were collected at 1, 6, 21, 49, 78, 106,134, 163, 190, 218, 245 and 275 days post last dose. Six fish per time point were sampled. In order to provide a reliable depletion curve for blood residues, the data obtained for days 1, 6, 21 and 49 were excluded. The lufenuron blood depletion curves are shown in Figure 5.

The half-time of lufenuron in blood was 591.1 degree-days or 54.1 days for the first treatment group (dose of 5 mg/ kg bw for seven consecutive days) and 584.1 degree-days or 53.4 days for the fish that were treated with 10 mg/kg bw for seven consecutive days.

Figure 5. Blood concentration profile of lufenuron in rainbow trout following oral administration of 5 mg/kg bw and 10 mg/kg bw for 7 consecutive days. Studies carried out in Chile



Metabolism in Laboratory Animals

Rats

In a GLP-compliant ADME study, 66 male and 15 female rats (Tif: RAIf, SPF), separated into eight groups (Table 3), were treated with radiolabelled [^{14}C]-lufenuron (purity higher than 99 %, specific activity 51.4 µCi/mg, labelled at the dichlorophenyl ring), at two dose levels: 0.5 mg/kg and 100 mg/kg (Bissig, 1990). The test substance was dissolved in ethanol:polyethylene glycol 200 (3:7, v/v). Each animal received the dose in 0.5 mL by stomach tube, except the animals of Group C that received the dose in 0.6 mL. The control animals (12 rats) received a single dose of the administration vehicle without the test substance. The following tissues and organs were collected from the animals of the Groups B, C, D, F1, F2, F3 and F4: bone, brain, abdominal fat, testes, ovaries, uterus, heart, kidneys, liver, lungs, whole blood, plasma, skeletal muscle, spleen, thymus, thyroid and residual carcass.

Table 3. Experimental design for ADME studies of lufenuron in rats (Bissig, 1990)

Group	Animals	Body weight/age	Dose (mg/kg bw)	Sample collection
B	5 males 5 females	200 g/ 7 weeks 200 g/9 weeks	0.50 – 0.51 0.51 – 0.52	Urine[a], faeces[a] and various tissues after 7 days post-dose
C	5 males 5 females	210–260 g/8-weeks 210–260 g/11 weeks	0.49 – 0.53 * 0.54 – 0.58 *	Urine,[a] faeces[a] and various tissues after 7 days post-dose
D	5 males 5 females	200 g/ 7 weeks 200 g/9 weeks	97.7 – 108.1 100.3 – 109.3	Urine[a], faeces[a], expired air[b] and various tissues after 7 days
E1	3 males	200 g/ 7 weeks	0.47 - 0.51	Blood at different time points (1, 2, 4, 8, 12, 24, 32, 48 and 56 h post-dose)
E2	3 females	200 g/9 weeks	102.9 – 103.5	Blood at different time points (1, 2, 4, 8, 12, 24, 32, 48 56 and 72 h post-dose)
F1, F2	20 males	200 g/ 7 weeks	0.47 – 0.53	Various tissues after 8 h, 45 h, 5 days and 12 days post-dose

F3, F4	20 males	200 g/ 7 weeks	91.6 – 101.0	Various tissues after 8 h, 60 h, 5 days and 12 days post-dose
G	5 males (bile fistulated)	260 g/8 weeks	0.48 – 0.49	Bile[c], urine[d] and faeces[d]

***** Preceded by 14 consecutive daily low doses of non-labelled lufenuron. a: 0-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h post-dose; b: 0-8 and 8-24 h post-dose; c: 0-0.5, 0.5-1, 1-2, 2-4, 4-8, 8-18, 18-24, 24-42 and 42 – 48 h post-dose; d: 0-24 and 24-24 h post-dose.

The extent of absorption of the orally administered lufenuron from the gastrointestinal tract into the systemic circulation was about 45 % and 10 % of the orally administered lufenuron at the low dose (0.5 mg/kg bw, Groups B and C) and the high dose level (100 mg/kg bw, Group D), respectively. Within the first 24 h an average of 25 % and 70 % of the administered dose were eliminated by the animals of Group B and Group D, respectively. Pre-treatment of the animal by 14 consecutive daily oral doses (0.5 mg/kg bw), lightly accelerated the faecal excretion in male rats (Group C). Within 48 h about 2 %, < 1 % and 52 % of the administered dose of the bile cannulated rats (Group G) were excreted with the bile, urine and faeces, respectively. Within 7 days post-dose the male and female rats of Group B and Group C excreted about 45 % and 56 % of the dose. The high dosed animals (Group D) eliminated more than 80 % of the administered dose, almost exclusively with the faeces. Seven days after the single oral dose of [^{14}C]-lufenuron 0.5 mg/kg bw, significant residues (40 % of the dose in animals of Group B and 10 % of the dose in animals of Group D) were present in most of the tissues collected, especially in fat (1.91 mg eq/kg in male and 2.40 mg eq/kg in female). Less than 0.01 % of the administered radioactivity was detected in exhaled volatile and carbon dioxide traps. The extent of absorption and faecal excretion was dose dependent. The greatest portion of radioactivity in faeces, determined by thin layer chromatography (TLC), was unchanged parent drug (77 % to 79 % and 37 % to 48 % of the administered high and low doses, respectively). The total radioactivity in faeces varied in the range of 78 % to 80 % and 43 % to 53 % of the high and low doses, respectively. The faecal metabolite pattern of the bile-duct cannulated rats (Group G) was similar to that of animals of Group B, but with a higher percentage (48 %) of the unchanged parent drug. One metabolite was identified as 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). The urinary metabolite pattern was not determined because the low radioactivity recovered in the samples (less than 1 % of the administered dose). Almost 80 % of the radioactive compounds were extracted from tissues and 95 % from faeces. Profiling of fat, liver, kidneys, lung and carcass indicated only a single component, the parent compound. Moreover, lufenuron was the major component in faeces, 5 minor metabolites were noted, each of 7 % or less of the TRR. Most of the seven biliary metabolite fractions were more polar than the faecal metabolites. Two metabolites were identified: 1-(2,5-dichloro-4-(1,1,2,3.3.3-hexafluoropropoxy)-phenyl)-urea (0.1 %) and (2,5-dichloro-4-(1,1,2,3.3.3-hexafluoropropoxy)-aniline (< 0.1 %).

In another study, non-GLP compliant, six weeks old rats of both sexes (Sprague-Dawley [Crj:CD(SD)] were treated by oral gavage with radiolabelled [^{14}C]-lufenuron (purity higher than 97.8 %, specific activity 1.89 MBq/mg, labelled at the dichlorophenyl ring) at the dose of

0.5 mg/kg bw for 14 consecutive days (Okada, 1997). Whole blood, plasma, cerebrum, cerebellum, pituitary, thyroid, heart, lung, liver, kidney, adrenal glands, spleen, fat, skin, muscle, femur, testis, seminal vesicle, ovary, and carcass were collected and the radioactivity determined by a liquid scintillation counter. Almost 58 to 64 % of the parent compound was excreted until 168 h after the last dose on Day 14. Faecal excretion was the major route of elimination: 57.1 % and 62.1 % of TRR was determined in male and female faeces, respectively. Only 0.9 % and 1.4 % were eliminated into urine from male and female rats, respectively. Concentration in the cerebrum and cerebellum increased with dosing, and were 2-3 times higher than that in blood concentrations by 8 h after the last dose. However, these concentrations were significantly lower than those determined in other tissues (Table 4). At 168 h post-dose, in both sexes, about 1 % of TTR remained in the liver and only 0.1 % or less remained in other tissues. The residual carcasses contained 34.6 % ± 4.1 % (n=3) and 29.5 % ± 1.9 % (n=3) of the TTR in males and females, respectively, indicating retention of the non-excreted radiolabelled compound. In plasma, more than 96 % of the radioactivity was extracted with methanol and four radioactive compounds were identified by thin layer chromatography: parent compound (major component, 70-80 %), 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl)urea, 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)aniline and an unknown metabolite. In the cerebrum, more than 94 % of the radioactivity was extracted almost in the methanol fraction and a small portion in the n-hexane fraction. Only the parent compound (about 92 %) and 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)aniline (less than 1.1 %) were identified.

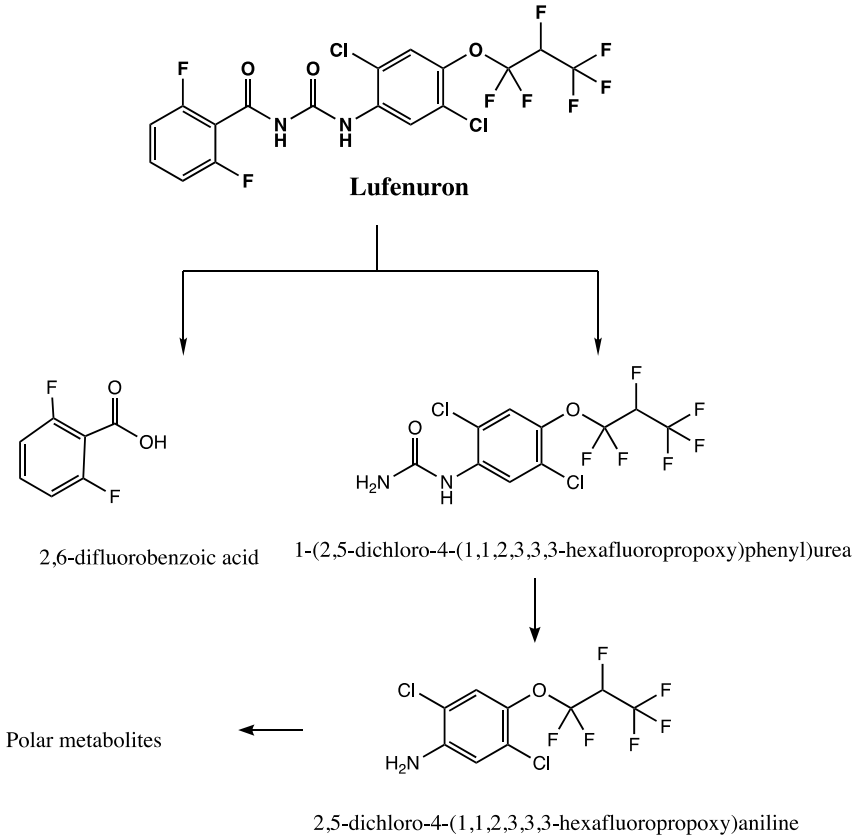
Table 4. Average concentration (± standard deviation, n=3) of radioactivity in rat tissues and organs after 8 h and 168 h post-dose. [^{14}C]-lufenuron was administrated orally (gavage) at a dose of 5 mg/kg bw of for 14 consecutive days (Okada, 1997)

Tissue	Concentration of radioactivity (µg eq/g or µg eq/mL)			
	Female (8 h)	Female (168 h)	Male (8 h)	Male (168 h)
Blood	0.15 ± 0.01	0.06 ± 0.01	0.15 ± 0.01	0.06 ± 0.01
Plasma	0.18 ± 0.02	0.07 ± 0.00	0.18 ± 0.02	0.07 ± 0.01
Cerebrum	0.38 ± 0.16	0.10 ± 0.02	0.38 ± 0.16	0.10 ± 0.05
Cerebelum	0.35 ± 0.15	0.10 ± 0.103	0.35 ± 0.15	0.11 ± 0.06
Pituitary	1.67 ± 0.27	0.46 ± 0.02	1.67 ± 0.27	0.40 ± 0.01
Thyroid	2.30 ± 0.37	0.53 ± 0.03	2.30 ± 0.37	0.70 ± 0.14
Lung	2.00 ± 0.10	0.73 ± 0.12	2.00 ± 0.10	0.62 ± 0.08
Heart	1.89 ± 0.18	0.63 ± 0.03	1.89 ± 0.18	0.58 ± 0.04
Liver	4.09 ± 0.10	1.26 ± 0.05	4.09 ± 0.10	1.20 ± 0.07
Kidney	2.11 ± 0.12	0.72 ± 0.03	2.11 ± 0.12	0.68 ± 0.04
Adrenal	4.87 ± 0.64	1.62 ± 0.03	4.87 ± 0.64	1.52 ± 0.05
Spleen	1.08 ± 0.04	0.37 ± 0.01	1.08 ± 0.04	0.36 ± 0.05
Muscle	1.02 ± 0.14	0.33 ± 0.02	1.02 ± 0.14	0.35 ± 0.043
Bone	0.41 ± 0.07	0.15 ± 0.02	0.41 ± 0.07	0.15 ± 0.04
Fat	34.68 ± 3.12	18.93± 0.53	34.68 ± 3.12	16.59 ± 1.28
Skin	3.74 ± 2.34	2.00 ± 1.2	3.28 ± 0.83	2.14± 1.06
Ovary/Testis	2.89 ± 0.27	1.06 ± 0.14	3.74 ± 2.34	0.17 ± 0.05
Seminal vesicle			2.89 ± 0.127	0.722 ± 0.06

In another GLP-compliant study, the nature of the radioactive residues in urine and faeces of male and female rats Tif: RAI f (SPF) treated with seven daily doses of [^{14}C]-dichlorophenyl ring-labelled lufenuron (100 mg/kg bw) was investigated (Thanei, 1990). The radiochemical purity was about 99 % with a specific radioactivity of 51.4 µCi/mg. Faeces were collected at the sampling time 8-24 h and 24-48 h and fat at 168 h post-dose. Almost 100 % of the radioactivity in faeces was extractable with methanol and 97 % with methanol /chloroform in fat. Two non-polar metabolite fractions representing less than 0.5 % of TRR was observed. In this fractions two metabolites were identified by TLC and confirmed by spectroscopic analysis: 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl)urea and 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)aniline. Moreover, a cluster of polar metabolites was observed, representing 0.5 % of TRR, however, the compounds remained unidentified.

In summary, lufenuron is mainly excreted in faeces and predominantly retained in fat tissue and therefore poorly metabolized in rats. The minor degradation pathway is the cleavage of the benzamide moiety yielding 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl)urea and 2,6-difluorobenzoic acid. Further cleavage of the ureido moiety leads to the aniline derivative 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)aniline (Figure 6).

Figure 6. Proposed biotransformation pathway of lufenuron in rats (Thainey, 1990)



Dogs

In an ADME study two Beagle dogs (one female and one male, 12 – 13 kg body weight) received a single oral dose of 10 mg/kg bw [^{14}C]-lufenuron (purity higher than 98.9 %, specific activity 0.104 MBq/mg, labelled at the dichlorophenyl ring) (Maurer & Hotz, 1999). At the end of the study (21 days post-dose), the animals were sacrificed and the following tissues were collected: skin, brain, liver, subcutaneous fat, kidney fat, omental fat, skeletal muscles, heart, and lungs. The total radioactivity in faeces (days 1 to day 21) was 52 % to 53 % and in urine 0.84 % to 2.18 % of the total administered dose. Fat tissues contributed with 7.2 % to 8.4 % of the TRR. The distribution of radioactivity in tissues and organs are shown in Table 5.

Table 5. Total radioactive residues in tissues and organs after 21 days post-dose. [^{14}C]-lufenuron was administrated orally at a single dose of 10 mg/kg bw (Maurer and Hotz, 1999)

Tissue	TRR (%)	
	Dog n^0. 1	Dog n^0. 2
Skin	15.8	4.72
Brain	0.01	0.01
Liver	0.65	0.38
Bile	0.29	0.11
Spleen	0.03	0.01
Fat*	7.21	8.44
Muscle	11.33	6.36
Heart	0.07	0.06
Lungs	0.08	0.04
Kidney	0.05	0.02
Blood	0.3	0.13
Total	35.78	20.28

*Sum of total fat.

The metabolic profiling, using high performance thin layer chromatography (HPTLC), was limited to faecal extracts, once only 0.8 to 2.2 % of the total administered dose was found in urine. At the first day post-dose only the unchanged [^{14}C]-lufenuron was identified in the faeces collected during the period of 0 – 24 h from the two animals. Extracts of the faeces collected between 24 – 48 h post-dose yielded one major peak with an R_f value identical with the reference compound (97.9 %, animal 1 and 64.7 %, animal 2), one minor peak with an R_f higher than the reference compound (less polar compound, 2.1 %, animal 1 and 20.9 %, animal 2) and three other minor metabolites (sum of 14.4 %, animal 2) with very low R_f values (more polar compounds). The identities of the metabolites were not confirmed. In the third fraction of faeces collected over the period of 48 -72 h post-dose, unchanged [^{14}C]-lufenuron was determined at a concentration of 89.9 % (animal 1) and 45.5 % (animal 2). In this fraction, also a peak with a higher R_f value as the parent compound and three minor unidentified peaks (4.8 %, animal 1 and 19.4 %, animal 2) were verified.

Metabolism in Food Producing animals

Goats

In order to study the metabolic profile of lufenuron in goats, [^{14}C]-lufenuron was administered orally (by gavage) for ten consecutive days to two lactating British Sannen goats (Cameron, *et.al*., 1992a). One goat (40 kg bw) received [^{14}C]-lufenuron labelled in the difluorophenyl ring (specific activity 34.93 µCi/mg, purity higher than 97 %) at a dose of 8.3-8.5 mg/day and the second goat (38 kg bw) received [^{14}C]-lufenuron labelled in the dichlorophenyl ring (specific activity 51.90 µCi/mg, purity higher than 98 %) at a dose of 8.5-8.7 mg/day. Following administration of the first dose, urine and faeces were collected in the morning prior to dose administration at 24 h intervals. The sample collection periods were: 0-24 h, 24-48 h, 48-72 h,

72-96 h, 96-120 h, 120-144 h, 144-168 h, 168-192 h, 192-216 h and 216-240 h after the first dose. Blood samples were collected at 0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h and 216 h after the first dose. During the study period the goats were milked in the morning prior to dosing and in the afternoon, ca 7 h after dose administration. The animals were slaughtered 24 h after the last dose and the following tissues were collected: tenderloin muscle, hind quarter muscle, forequarter muscle, omental fat, renal fat, subcutaneous fat, liver, kidneys, rumen and intestinal contents. Radioactivity was measured by combustion and liquid scintillation counting. The main route of elimination of radioactivity was in the faeces, accounting for 73 %-74 % of the total administered dose. Only 1 %-2 % of the total dose was eliminated via urine. The rate of excretion was slow resulting in incomplete excretion of radioactivity over the first 24 h and over the period of the study (240 h). The radioactivity in milk was about 6 %-7 % of the total administered dose over 24 h. The highest level of radioactivity, about 4.2 %-5.4 % of TRR, was determined in fat 24 h post-dose of the tenth day. In muscle, concentration in the range of 0.038 – 0.080 mg eq/kg was detected. Approximately 5 %-7 % of the total administered dose was estimated to remain in tissues at sacrifice and 5 %-10 % remained in the rumen and intestinal contents (Table 6). There were no significant differences in the excretion and distribution pattern of radioactivity observed between the two labelled forms of [^{14}C]-lufenuron.

Table 6. Radioactive tissue residues 24 h following the tenth consecutive daily dose (8.3-8.7 mg) of ([^{14}C]-lufenuron labelled in the difluorophenyl ring or dichlorophenyl ring to a lactating goat (Cameron *et.al.*, 1992a)

	Goat treated with ([^{14}C]-lufenuron labelled in the difluorophenyl ring		**Goat treated with ([^{14}C]-lufenuron labelled in the dichlorophenyl ring**	
Tissue	**Concentration (mg eq/kg)**	**% Total dose**	**Concentration (mg eq/kg)**	**% Total dose**
Hindquarter muscle	0.066	1.555*	0.039	0.774*
Forequarter muscle	0.080		0.038	
Tender loin muscle	0.071		0.040	
Omental fat	2.288	5.356*	2.411	4.216*
Subcutaneous fat	0.883		0.821	
Renal fat	2.434		1.640	
Liver	0.417	0.297	0.367	0.281
Kidney	0.114	0.017	0.118	0.014
Blood cells	0.017	0.018	0.015	0.012
Plasma	0.014	0.032	0.017	0.038
Total	-	7.275	-	5.335
Rumen and intestinal contents	0.350	5.043	0.752	10.098

* Calculated on the basis that the total weight of liver, kidney, rumen and intestinal contents is known and that the total weight of muscle and fat represents 45 % of the body weight.

In a separate study, using the samples of the aforementioned study, metabolic profiling was carried out (Schulze-Aurich, 1992). The radioactive residues were almost completely extractable from all samples (fat, muscle, kidneys, liver, milk, and faeces). In fat, muscle, kidney, and milk unchanged lufenuron was the only detectable residue and accounted for more than 92 % of the extracted radioactivity. In liver besides unchanged lufenuron (>80 %) minor amounts of metabolites were detected. In faeces, besides unchanged lufenuron, 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea, 2,6-difluorbenzoic acid and 2,6-difluorobenzamide were detected as metabolites. In urine, only 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea was identified as a minor metabolite.

Chickens

In a GLP-compliant study radiolabelled lufenuron at a dose of 0.59 mg was administered in capsules via pharynx to six laying Leghorn hens daily for 14 consecutive days (Cameron *et.al*., 1992b). Three animals (1.30 – 1.74 kg body weight) received [^{14}C]-lufenuron labelled in the difluorophenyl ring (specific activity 34.93 µCi/mg, purity higher than 97 %) and the other three hens (1.35 – 1.59 kg body weight) received [^{14}C]-lufenuron labelled in the dichlorophenyl ring (specific activity 51.90 µCi/mg, purity higher than 98 %). Radioactivity was measured by combustion and liquid scintillation counting.

Excreta were collected in the morning prior to dose administration at 24 h intervals until 336 h after the first dose. Only a single blood sample was taken at the end of the study prior to sacrifice (336 h after the first dose). Eggs were collected throughout the day and in the morning prior to dose administration over 24 h intervals. At the end of the experiment, 24 h following the fourteenth dose, the animals were slaughtered and the following tissues collected: lean meat, skin, peritoneal fat, liver, kidneys and intestinal contents. Radioactivity was determined using a liquid scintillation counter and the results are presented in Table 7.

Table 7. Tissue concentrations of radioactivity 24 h following the fourteenth consecutive daily dose (0.59 mg) of ([^{14}C]-lufenuron labelled in the difluorophenyl ring or dichlorophenyl ring to laying hens (Cameron *et.al*., 1992b)

	Hens treated with ([^{14}C]-lufenuron labelled in the difluorophenyl ring		**Hens treated with ([^{14}C]-lufenuron labelled in the dichlorophenyl ring**	
Tissue	Concentration (mg eq/kg)	% Total dose	Concentration (mg eq/kg)	% Total dose
Lean meat	0.24 ± 0.16	1.15 ± 0.61	0.10 ± 0.01	0.55 ± 0.05
Skin (including fat)	2.56 ± 0.51	-	1.30 ± 0.33	-
Peritoneal fat	13.04 ± 0.69	8.83 ± 0.92	7.19 ± 1.32	5.09 ± 1.06
Liver	1.45 ± 0.11	0.64 ± 0.13	0.83 ± 0.14	0.40 ± 0.09
Kidney	0.74 ± 0.03	0.09 ± 0.02	0.52 ± 0.11	0.07 ± 0.03
Whole blood	0.29 ± 0.09	0.14 ± 0.03	0.19 ± 0.31	0.10 ± 0.02
Intestinal contents	0.37 ± 0.06	0.21 ± 0.05	0.41 ± 0.37	0.15 ± 0.03

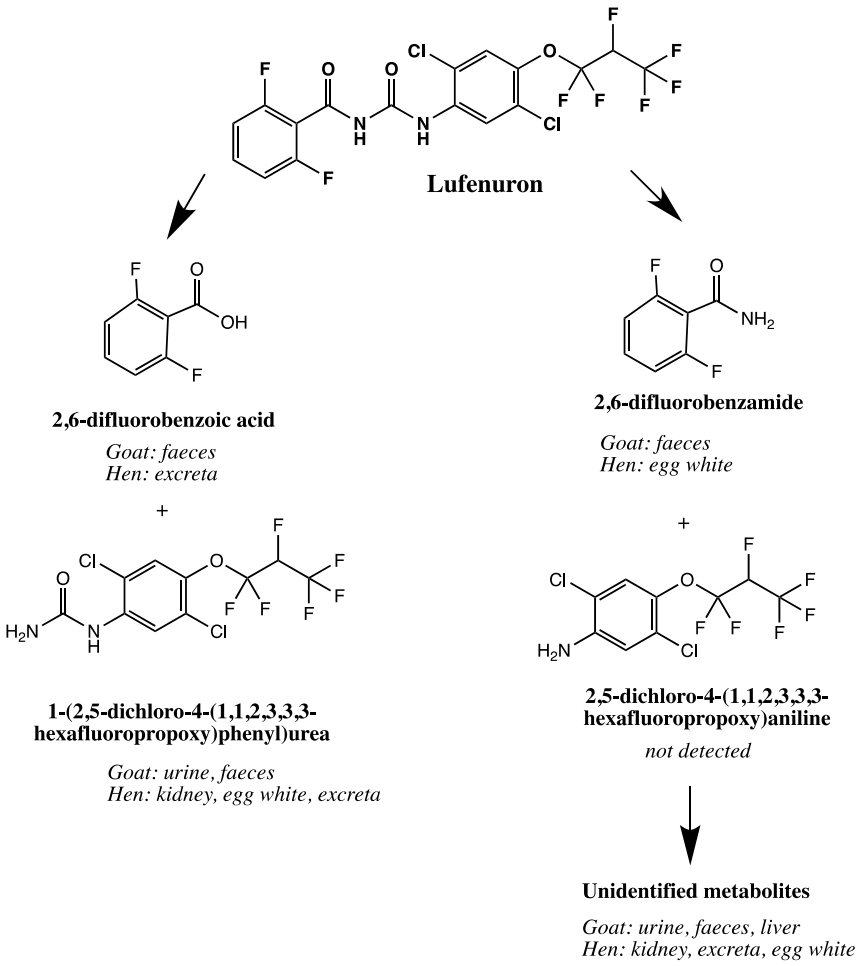
Recovery of radioactivity was 75 %-79 % of the total administered dose. There were no significant differences in the excretion and tissue distribution pattern dependent on the site of labelling of the test substance. About 10 % of the total dose was eliminated via eggs, mainly in the egg yolk. The majority of the radioactivity (55 %-64 %) was measured in the accumulated excreta. The concentration of radioactivity in peritoneal fat was 38-45 times higher than that in whole blood at the time of sacrifice, indicating selective uptake of the radiolabelled compound and or its metabolites in fat.

The results indicated that the radiolabelled compound is slowly eliminated, mainly by excreta, and tend to accumulate. Highest tissues residues were found in fat.

Metabolic profiling was carried out in another study (Schulze-Aurich, 1992) using the samples of the previous study. Metabolites were characterized by TLC with co-chromatography with standards. The radioactive residues were almost completely extractable with solvents (89 %-98 %) from fat including skin, liver, lean meat, egg white, and egg yolk. For both labels, unchanged lufenuron was the major residue in all tissues and egg yolk (79.3 % – 93.7 % TRR). In egg white, lufenuron summed 37.6 % to 44.1 % of the TRR and 2,6-difluorobenzamide was the only metabolite identified for the difluorophenyl-label (0.001 mg eq/kg, 17.3 % TRR). For the dichlorophenyl-label 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea was identified in kidney (0.028 mg eq/kg, 5.3 %) and egg white (< 0.001 mg eq/kg, 7% TRR). Unknown metabolites summed 3.1 % to 42.1 % TRR for the difluorophenyl-label and 3.0 % to 37.4 % TRR for the dichlorophenyl label. The highest unidentified metabolites were measured in egg white. In hen excreta > 90 % TRR was extracted and lufenuron was the major compound identified (>82 %). The two minor metabolites detected in kidney and egg white were also identified in excreta, 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea (3 % TRR) and 2,6-difluorobenzamide (<4.3 % TRR).

The fate of lufenuron in hens and goats after multiple oral administration is assumed to be similar and resembles closely that observed in rats. The same metabolites were identified by co-chromatography in tissues, eggs, milk and excreta and a metabolic pathway of lufenuron in goats and hens was proposed (Figure 7). Lufenuron is metabolized to a limited extent by cleave of the benzoyl ureido bridge leading to 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea and 2,6-difluorobenzoic acid and by cleavage of the urea bridge leading to the 2,6-difluorobenzamide and very likely to the 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)aniline (not detected). In goats, the extent of metabolism is assumed to be slightly higher than in hens, as higher amounts of metabolites are eliminated with excreta.

Figure 7. Proposed biotransformation pathway of lufenuron in goats and hens (Schulze-Aurich, 1992)



Salmon

In a GLP-compliant study 64 post-smolt salmons (*Salmo salar* L.) with a body weight of 107-185 g, housed in tanks with seawater, were treated with [^{14}C]-lufenuron (specific activity 95.5 µCi/mg, purity higher than 99.8 %, labelled at the carbon of the benzamide moiety, Figure 2) at an intended dose of 5 mg/kg bw (calculated dose of 5.34 mg/kg bw) for 7 consecutive days (Hobbs, 2014). The water temperature ranged from 4 to 14 ºC. Ten fish were sacrificed at 1 day, 30 days, 90 days and 178 days after the end of treatment and muscle plus skin, liver, hindgut and residual carcasses sampled for analysis (Table 8). Faeces were collected during the treatment period and throughout the live phase. TRR was almost completely extracted from fillet and faeces (>91.2 %).

Table 8. Concentration of total radioactivity in Atlantic salmon tissues with time. Oral administration of [^{14}C]-lufenuron at a dose of 5.34 mg/kg bw for seven consecutive days (Hobbs, 2014)

Concentration of total radioactivity (mg eq/kg) ± SD				
Time (days)	**Fillet**	**Liver**	**Residual carcass**	**Hindgut***
1	24.8 ± 7.7	33.9 ± 9.9	34.8 ± 9.5	46.9
30	14.3 ± 8.2	15.8 ± 8.1	20.1 ± 14.4	11.1
90	10.5 ± 5.1	13.2 ± 5.7	18.1 ± 8.1	7.3
178	2.7 ± 1.4	3.5 ± 1.7	5.3 ± 2.7	2.2

* Single measurement of combined samples; SD: standard deviation (n= 10 fish).

TRR declined progressively from 1 day after treatment to 178 days post-dose in all tissues. Lufenuron was the only residue detected in all samples analysed accounting for 79.2 % to 95.3 % TRR in the salmon fillet. The concentration of TRR was also determined in blood, organs, and tissues of salmon (mean of 3 fish) at Day 35, Day 90 and Day 178 after the end of the treatment (Table 9).

Table 9. Mean concentration of total radioactivity in Atlantic salmon tissues with time. Oral administration of [^{14}C]-lufenuron at a dose of 5.34 mg/kg bw for seven consecutive days (Hobbs, 2014)

Tissue	**Concentration of total radioactivity (mg eq/kg) ± SD**		
	Day 35	Day 90	Day 178
Bladder	4.8±2.9	3.8±0.8	0.8±0.6
Blood	5.7±1.9	2.8±0.4	0.6±0.2
Brain	5.6±2.9	3.4±0.6	0.5±0.3
Brown fat	16.6±6.2	11.9±4.2	1.9±0.8
Muscle	3.4±1.3	1.8±0.3	0.3±0.2
Gill-arch	53.8±20.6	44.4±4.6	9.5±4.2
Gill-rakes	5.0±2.2	3.9±0.2	0.7±0.4
Gill-whole	12.1±5.4	14.1±3.2	2.0±1.0
Heart muscle	7.6±2.6	6.0±1.5	0.9±0.2
Intestine wall	19.4±9.6	11.2±1.7	1.3±0.4
Kidney	12.3±2.3	11.1±1.5	3.4±0.4
Liver	14.4±5.9	11.5±2.2	2.8±1.2
Skeleton	12.8±8.5	11.8±3.4	2.4±1.8
Spinal cord	8.4±4.3	11.2±6.1	0.8±0.2
Spleen	7.9±4.0	9.2±1.5	4.7±3.4
Stomach wall	7.7±4.2	6.1±1.3	1.0±0.3
Swim bladder wall	9.2±10.2	5.8±2.0	1.2±0.5
White fat	67.6±35.9	61.0±5.3	15.2±10.0

SD: standard deviation (n= 3 fish).

The highest concentration of TRR was determined in white fat with a mean concentration of 67.6 mg eq/kg at 35 days post-dose. Lufenuron is excreted as unchanged compound in the faeces. The TRR data indicate a slow depletion of radioactivity.

Quantitative whole body autoradiography (QWBA) analysis of longitudinal fish sections indicated that lufenuron is located primarily in the fat and viscera. No metabolism of lufenuron in Atlantic salmon was detected over the six-month period.

Fillet and pooled faecal samples were extracted with acetonitrile (2 x 10 mL) for metabolite profiling using HPLC with LSC detection. Unextracted residues (TRR) were in fillet: 1.1 % (day 1 post-dose), 1.4 % (day 30 post-dose), 8.8 % (day 90 post-dose) and 3.4 % (day 178 post-dose), and in faeces 3.3 %.

No metabolites were detected over the 6-month period after medication. In the chromatograms, only one peak was observed and identified as the parent compound. This result was also confirmed by TLC. No polar metabolites were detected.

In vivo metabolism in fish

Bluegill

In a GLP-compliant study (Forbis, 1987) one hundred and twenty bluegill (*Lepomis macrochirus*), with an initial mean weight of 5.4 g (±1.5 g), were exposed to [^{14}C]-lufenuron (specific activity 31.7 µCi/mg, purity higher than 98 %, labelled at the difluorophenyl ring) in water at a concentration of 13 µg/L over 49 days at 22 ºC (equivalent to 1078 degree-days). The water (100 L in the aquaria) was replenished in a continuous flow system and the test substance was added to the water with the aid of a small quantity of acetone (0.1 mL). After the 49-day period fish were transferred to clean water for 28 days to evaluate the depuration. Fillet, viscera and whole fish were sampled at days 0, 0.17, 1, 3,7,14, 21, 28, 35, 42 and 49 for the uptake phase and at days 1, 3, 7, 10, 14, 21 and 28 for the depuration phase. Water was sampled at the same days for analysis. Radioactivity was measured by combustion and liquid scintillation counting. Daily bioconcentration factors ranged from 11 to 2500X, 29 to 4000X and 45 to 6800X for fillet, whole fish and viscera, respectively. Uptake concentration ranged from 0.19 to 25 mg eq/kg for fillet, 0.52 to 40 mg eq/kg for whole fish and 0.81 to 68 mg eq/kg for viscera. Radioanalysis throughout the depuration phase indicated a decrease of 50 %, 42 % and 46 % of lufenuron from fillet, whole fish and viscera, respectively.

For metabolic profiling, fish tissues were sampled from day 49 of the uptake period (Leak, 1987). Characterization was performed by TLC in two different solvent systems. The TRR was completely extracted with dichloromethane and only lufenuron was detected.

Fathead minnow

In a similar GLP-compliant study (Maynard *et.al.*, 2004), four hundred and sixteen fathead minnow (*Phimephales promelas*), with a weight in the range of 2.1 to 6.3 g, were exposed to [^{14}C]-lufenuron (specific activity 1.8 MBq/ mg, purity of 95.7 %, labelled at the dichlorophenyl ring) in water at nominal [^{14}C]-lufenuron concentrations of 1 and 10 µg/L over 60 days at 25 ºC

(equivalent to 1500 degree-days). After this period, fish were transferred to clean water for the depuration phase, that comprised 60 days at 25 ºC or 1500 degree-days. The water (120 L in the aquaria) was replenished in a continuous flow system and the test substance was dissolved in triethylene glycol. Viscera, flesh, carcass and whole fish were sampled at days 7, 14, 21, 28, 35, 42, 49, 52, 54, 58 and 60 for the uptake phase and at days 1, 3, 8, 15, 22, 29, 36, 43, 57 and 60 for the depuration phase. Total radioactive residues in the viscera, flesh, carcass and whole fish were analysed during the uptake and depuration phases. The uptake rate constants were calculated to be 936 day^{-1} and 644 day^{-1} for the 1 and 10 µg/L of [^{14}C]-lufenuron treatments, respectively. During the depuration phase the concentration of [^{14}C]-lufenuron in the whole fish decreased steadily. Within 29 days the concentration dropped from the mean plateau phase concentrations by 49 % and 25 % in the nominal 1 and 10 µg/L treatments, respectively. After 60 days, 83 % and 79 % depuration had been achieved for the lower and higher [^{14}C]-lufenuron concentrations. The depuration constants were calculated to be 0.0335 day^{-1} and 0.0239 day^{-1} for the 1 and 10 µg/L of [^{14}C]-lufenuron treatments, respectively. The kinetic bioconcentration factor, calculated as the ratio of the uptake rate constant to the depuration rate constant, were 28000 and 27000, for the lower and higher [^{14}C]-lufenuron concentrations, respectively.

Fish analysis by TLC confirmed that the residues in fish tissues were predominantly lufenuron (91 %-96 %) and, therefore, no metabolite characterisation was performed. The mean recovery of radioactivity in the acetonitrile extracts analysed by TLC was in the range of 82 to 106 %.

Comparative metabolism

The metabolic pathway of lufenuron is similar in rodents (rats), ruminants (goat) and poultry (chicken). Lufenuron is metabolized to a very limited extent by cleavage of the benzoyl ureido bridge leading to 1-(2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl)urea and 2,6-difluorobenzoic acid and by cleavage of the urea bridge leading to the 2,6-difluorobenzamide. The same minor metabolites were identified in tissues, eggs, milk and excreta. Some minor uncharacterized metabolites were detected in goat excreta and liver and in hen excreta, kidney and egg white. Important routes of elimination were milk in lactating goats and egg yolk in laying hens.

Lufenuron is not metabolized in bluegill sunfish and fathead minnow, independent of the label of the molecule; the only residue was the parent compound. In Atlantic salmon, also no metabolites were detected and lufenuron is only slowly eliminated. The main route of elimination in fish is via feaces and lufenuron is mainly distributed in fat tissues.

Tissue residue depletion studies

Radiolabelled residue depletion studies

Salmon

In a GLP-compliant study (Hobbs, 2014), conducted at a water temperature of 4 to 14 °C, [^{14}C]-lufenuron (specific activity 95.5 µCi/mg, purity higher than 99.8 %, labelled at the acyl carbon adjacent to the difluorophenyl ring) at an intended dose equivalent to 5 mg/kg bw (actual dose 5.34 mg/kg bw) was administered in feed to 64 post-smolt salmons (*Salmo salar* L.) (107-185 g) held in tanks supplied with seawater and aerated via air stones.

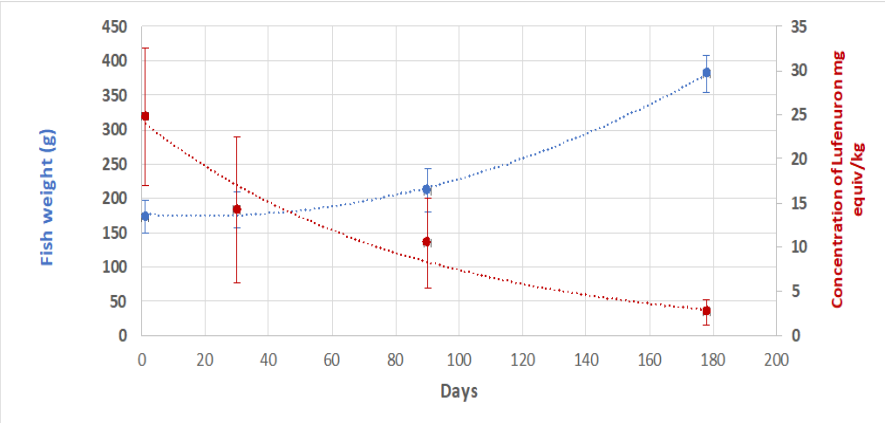
Ten fish were sacrificed at 1 day, 30 days, 90 days and 178 days after the end of treatment and muscle plus skin (fillet) sampled for analysis. TRR was determined by LSC using Premafluor®E scintillation fluid. Acetonitrile extracts of salmon fillets were analysed by HPLC with a radiochemical detector. Combustion efficiencies were higher than 97 %. The limit of detection for TRR and chromatography were assessed to be < 0.01 mg/kg. TRR was almost completely extracted from fillet and the parent compound was the only residue detected in salmon fillet (Table 10).

The highest concentration of radioactivity in fillet was determined 1 day after end of the treatment phase.

Lufenuron was the only residue detected in fish faeces. Extraction of the combined faecal samples released 96.8 % of the TRR (5.36 mg eq/kg). Radioactive residues in the faeces declined from about 7 mg eq/kg to 2 mg eq/kg at the end of the in-life phase of the study.

Smolting and migration of Atlantic salmon from freshwater to saltwater is energy demanding and affects the growth rate. Atlantic salmon have a period of low growth rate followed by a period of excessive growth (compensatory growth). In general, smolts transferred to seawater cages reach harvest size of 4 kg in 10 – 15 months. However, in this study the growth of fish was impaired, they only doubled their body weight in 6 months, which is characteristic of fish held in confined tanks. Therefore, the data of this study are not adequate to be considered for a depletion study. However, the data indicate that the concentration of lufenuron decreases with time in the same manner as the fish body weight increases (Figure 8). These results suggest that lufenuron is distributed in the fatty fish tissues rather than eliminated.

Figure 8. Fish body weight and lufernuron depletion in Atlantic salmon fillet following a total dose of 5.34 mg/kg bw



Residue depletion studies with unlabelled drug

Salmon

Six GLP-compliant studies were conducted with unlabelled lufenuron in different geographic locations: Canada (three studies), Chile (one study) and Norway (two studies) (Table 10).

Atlantic salmon smolts (mixed sex), held in a freshwater hatchery, were treated with lufenuron via feed at a nominal dose of 5 mg/kg bw for seven consecutive days. Fish were transferred to sea cages after the end of medication and remained at sea until the end of the study. Water temperatures varied according to the geographic site where the studies were conducted. Following treatment, fish were transferred to sea cages and kept at sea until the end of the studies. Fish were harvested at various time points after treatment.

Table 10. Summary of the residue depletion studies carried out in different geographic locations

Country of the study	Study number/ Study report	Season (Sea transfer)	Temperature (°C)		Reference
			Hatchery	Sea	
Canada	GSO-13-005/ ATL-14-1123	Autumn	10.3	1.7-13.7	Hervy, 2015b
Canada	NAH-13-071/ ATL-15-1351	Autumn	10	0.7-13.2	Hervy, 2016b
Canada	NAH-15-014/ ATL-15-1430	Spring	13	4-16	Hervy, 2016c
Chile	CHI-12-002	Spring	11	9-18	Lewis, 2015
Norway Voldness sea	NAH-14-114/ ATL-15-1352	Autumn	10-12	5.8-15.6	Hervy, 2015d
Norway Langholmen sea	NAH-14-114/ ATL-15-1363	Spring	3.5	3.7-13.3	Hervy, 2016a

Residue depletion data were obtained using the entire fillet from one side of the fish including skin, scales and belly flap. Lufenuron was quantified in fillet using a validated UHPLC-MS/MS method. Fluazuron (N-((4-chloro-3-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)carbamoyl)-2,6-difluorobenzamide) was used as internal standard. The sample preparation procedure consisted of extraction of lufenuron from minced and homogenized fillet by solid-liquid extraction with acetonitrile. The clean-up of the extracts was performed by solid phase extraction on C18 sorbents. Recoveries were above 90 % and the limit of quantitation of the method was 50 µg/kg.

Study GSO-13-005

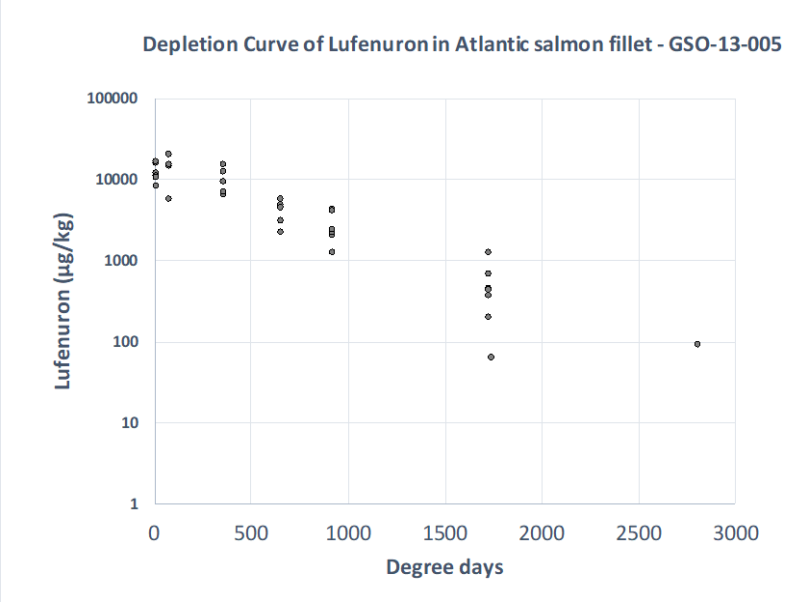
In a GLP-compliant study carried out in Canada (Hervy, 2015b), Atlantic salmon smolts (average weight of 115 g, nine days prior the start of treatment) were fed lufenuron in the diet for seven days at an intended dose of 5 mg/kg bw (actual total dose of 33.9 mg/kg bw). The water temperature at sea varied in the range of 1.7 ºC to 13.7 ºC. Six fish were sampled at each time point (1, 8, 37, 96, 192, 283 and 369 days post-dose) and fillet was collected, with exception of the last time point (369 days) where only two fish were sampled. Lufenuron was quantified by a validated UHPLC-MS/MS method. The mean concentrations of lufenuron in fillet are shown in Table 11.

Table 11. Mean concentration of lufenuron in fillet from Atlantic salmon with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Hervy, 2015b)

Time post-dose (d)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of lufenuron (µg/kg)			Standard deviation (µg/kg)
				Mean	Minimum	Maximum	
1	20.7	10.3	6	12315	8090	16600	3342
8	85.3	5.7	6	14223	5640	20400	4766
37	364.4	8.4	6	10428	6300	15400	3594
96	658.6	3.1	6	4088	2170	5590	1261
192	925.7	4.8	6	2693	1270	4240	1187
283	1730	13.2	6	564	201	1270	378
369	2814	12.3	2	77	64	91	19

Figure 9 shows the concentration of lufenuron in Atlantic salmon versus days post-dose from the depletion study carried out in Canada.

Figure 9. Concentration of lufenuron in Atlantic salmon fillet versus days post-dose



The initial half-life of elimination calculated from the residue data from days 1 to 369 in the fillet was 359 degree-days (EMEA 1999).

Study NAH-13-071

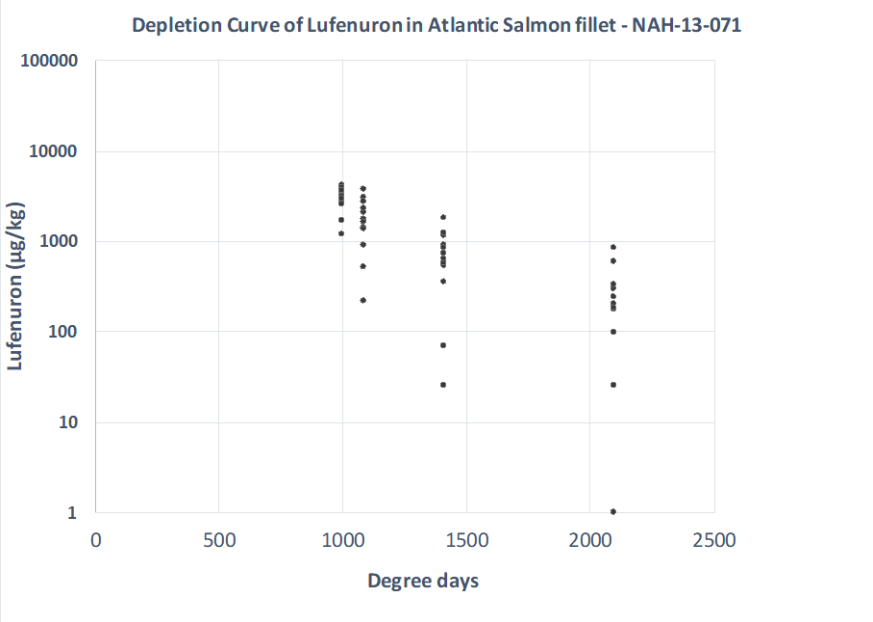
In another GLP-compliant study conducted in Canada (Hervy, 2016b), Atlantic salmon smolts (average weight at the start of treatment, 88 g) were fed lufenuron in the diet for seven days at an intended dose of 5 mg/kg bw (actual total dose of 34.2 mg/kg bw). The water temperature at sea varied in the range of 0.7 ºC to 13.2 ºC. Tissues were collected after 127, 187, 247 and 307 days post-treatment. Twelve fish were sampled for each time point. Lufenuron was quantified by a validated UHPLC-MS/MS method. The mean concentrations of lufenuron in fillet are shown in Table 12 and the depletion curve in Figure 10.

Table 12. Mean concentration of lufenuron in fillet from Atlantic salmon with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Hervy, 2016b)

Time post-dose (d)	Time post-dose (DD)	Number of fish	Concentration of lufenuron (µg/kg)			Standard deviation (µg/kg)
			Mean	Minimum	Maximum	
127	999	12	3023	1190	4200	858
183	1088	12	1964	217	3680	958
246	1407	12	776	25	1820	464
308	2099	12	324	25	851	283

Two samples presented a lufenuron concentration lower than the LOQ. These values were reported as half of LOQ (25 µg/kg).

Figure 10. Depletion curve of lufenuron in salmon fillet



Study NAH-15-014

In another GLP-compliant study conducted in Canada (Hervy, 2016c), Atlantic salmon smolts (average weight three days prior to the start of treatment, 62 g) were fed with lufenuron diet for seven days at an intended dose of 5 mg/kg bw (actual total dose of 33.8 mg/kg bw). The seawater temperature ranged from 4 ºC to 16 ºC. The fish growth in the hatchery had been delayed due to the unusually low temperature of the fresh water. Due to logistical considerations, the smolts were divided into two batches (Endris *et.al*., 2017). Fillet samples were collected after 2, 4, 6 and 8 months post-treatment and lufenuron was quantified by a validated UHPLC-MS/MS method. The mean concentrations of lufenuron in fillet are shown in Table 13. Unlike the previous study results, it was not possible to establish the depletion curve for this study due to the difficulty in assigning correct degree-days due to the sampling times being reported in months.

Table 13. Mean concentration of lufenuron in fillet from Atlantic salmon with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Hervy, 2016c)

Time post-dose (months)	Number of fish	Concentration of lufenuron (µg/kg)			Standard deviation (µg/kg)
		Mean	Minimum	Maximum	
2	18	3659	<LOQ	8330	2295
4	18	492	<LOQ	965	342
6	18	96	<LOQ	149	78
8	17	110	<LOQ	177	56

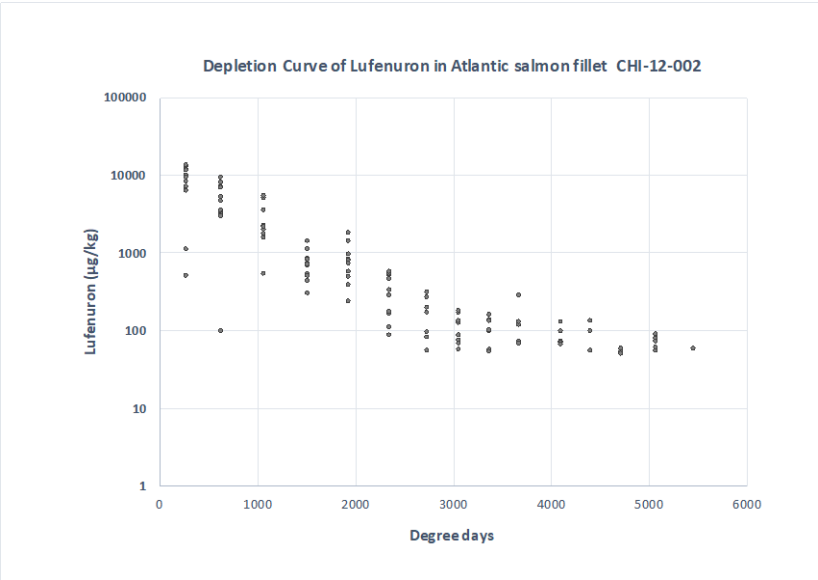
Study CHI-12-002

In a GLP-compliant study carried out in Chile (Lewis, 2015), Atlantic salmon smolts (average weight prior the start of treatment, 125 g, mixed sex), held in freshwater hatchery tanks (water temperature of 11 ºC), were fed with lufenuron diet for seven days at an intended dose of 5 mg/kg bw (actual total dose of 33.9 mg/kg bw). Six days post end of the treatment fish were transferred to sea cages. The seawater temperature ranged from 4 ºC to 16 ºC. Tissues were collected after 1, 4, 21, 49, 76, 104, 132, 160, 189, 217, 246, 274, 315, 343, 371, 401 and 430 days post-treatment. Fish were sampled for each time point and lufenuron quantified by a validated UHPLC-MS/MS method. The mean concentrations of lufenuron in fillet are shown in Table 14 and the depletion curve is shown in Figure 11.

Table 14. Mean concentration of lufenuron in fillet from Atlantic salmon with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Lewis, 2015)

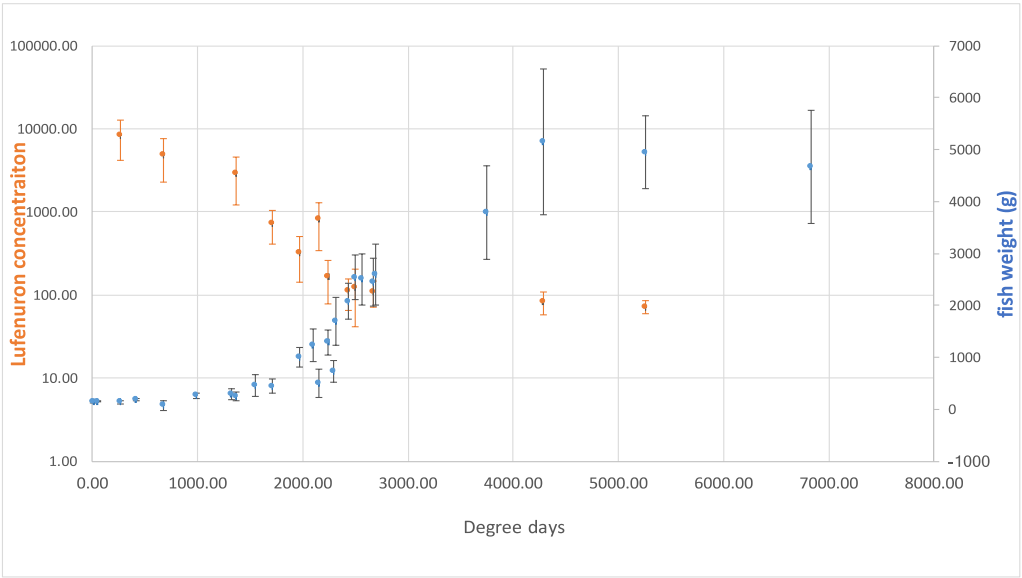
Time post-dose (d)	Time post-dose (DD)	T (ºC)	Number of fish	Concentration of lufenuron (µg/kg)			Standard deviation (µg/kg)
				Mean	Minimum	Maximum	
21	265.5	12.6	12	8349	510	13300	4178
49	620.6	12.7	11	4908	98	9210	2651
76	1063	14.0	10	2908	538	5310	1700
104	1516	14.6	11	734	298	1430	320
132	1932	14.6	10	813	235	1790	473
160	2351	14.7	11	322	87	570	179
189	2728	14.4	8	167	55	309	90
217	3052	14.1	8	111	56	177	47
246	3373	13.7	8	109	54	159	38
274	3667	13.4	6	123	68	281	81
315	4099	13.0	6	83	65	128	25
343	4405	12.8	3	94	55	131	38
371	4720	12.7	3	54	50	58	4
401	5070	12.6	6	72	55	90	13
430	5457	12.7	1	59	59	59	

Figure 11. Depletion curve of lufenuron with time in degree-days



It is possible to verify that the concentration of lufenuron decreases as the fish weight increases (Figure 12). The Atlantic salmon were medicated when they still are in freshwater (smolts, with a body weight about 100 g). When they are transferred to sea cages the growth rate is affected and very limited in the first period (until 1000 degree-days). In the second phase, an excessive growth rate is observed at the same time that the concentration of lufenuron decreases. Even some depletion occurs because lufenuron is detected in faeces, the concentration in fillet remains high. As fish increases in size, the percentage of fat in the fillet also increases which may explain the low elimination rate of lufenuron. About 55 % of the fillet is fat and lufenuron is a very lipophilic compound.

Figure 12. Concentration of lufenuron and fish body weight in function of time post-dose of lufenuron to Atlantic salmon



Study NAH-14-114 **(***Voldnes Sea)*

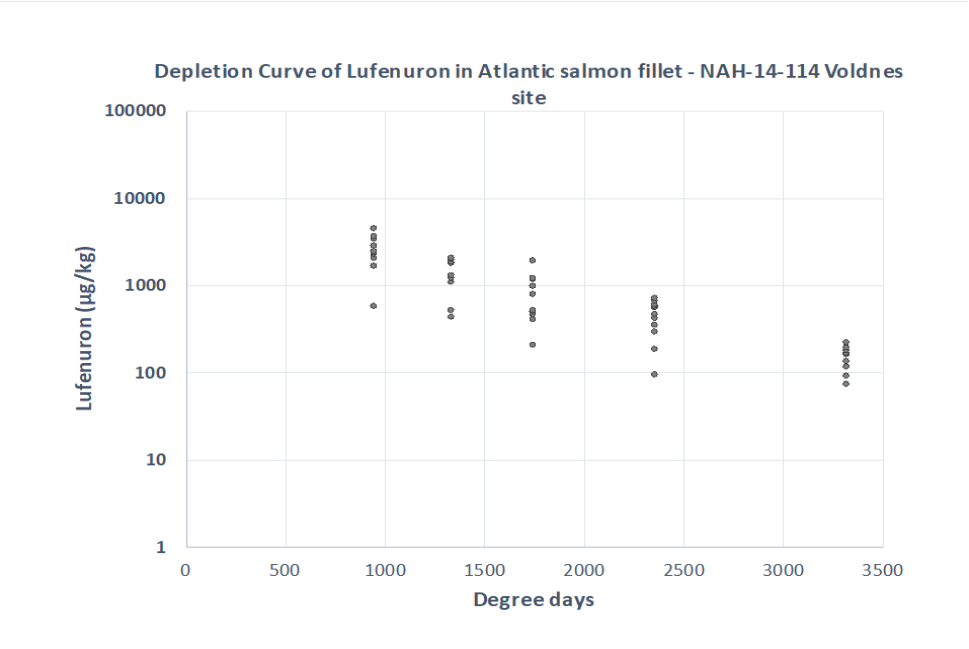
In a GLP-compliant study carried out in a commercial fish farm (Voldnes) in southern Norway (Hervy, 2015d), Atlantic salmon smolts (average weight at the start of treatment, 102 g, mixed sex), held in freshwater hatchery tanks (water temperature of 10-12 ºC), were fed with lufenuron diet for seven days at an intended dose of 5 mg/kg bw (actual total dose of 36.2 mg/kg bw). Six days post end of the treatment fish were transferred to sea cages and maintained in seawater until end of the study. The seawater temperature ranged from 5.8 ºC to 15.6 ºC. Fillet samples were collected after 104, 167, 222, 246, 279 and 347 days post-treatment. Twelve fish were sampled for each time point and lufenuron quantified by a validated UHPLC-MS/MS method. Lufenuron was quantified in fish fillet and the mean concentrations are shown on Table 15.

Table 15. Mean concentration of lufenuron in fillet from Atlantic salmon with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Hervy, 2015d)

Time post-dose (d)	**Time post-dose (DD)**	**Number of fish**	**Concentration of lufenuron (µg/kg)**			**Standard deviation (µg/kg)**
			Mean	Minimum	Maximum	
104	944	12	2498	1980	4300	843
167	1335	12	1255	423	1920	526
222	1743	12	862	393	1860	547
279	2358	12	446	284	688	154
347	3318	12	158	72	214	56

The maximum lufenuron concentration was determined in the first-time point (4300 µg/kg). The fish weights were not provided in this study; only the weight and length of the fillets were given for each sample. The data fit well to the log-e transformed regression model and the ln-linearity, normal distribution of errors and homogeneity of variances assumptions were satisfied. The depletion curve is shown in Figure 13.

Figure 13. Depletion curve of lufenuron in salmon fillet



Study NAH-14-114 (Langolmen Sea)

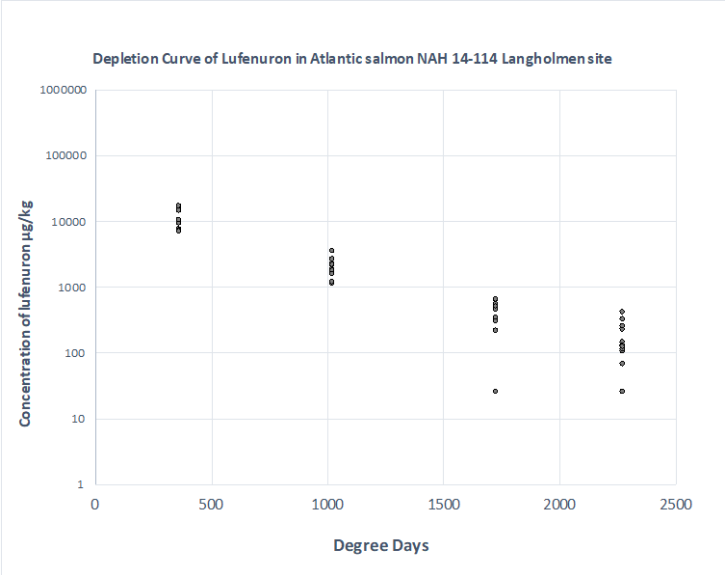
In a GLP-compliant study carried out in a commercial fish farm (Langholmen) in northern Norway (Hervy, 2016a), Atlantic salmon smolts (average weight at the start of treatment, 98 g, mixed sex), held in freshwater hatchery tanks (water temperature of 3.5 ºC), were fed with lufenuron diet for seven days at an intended dose of 5 mg/kg bw (actual total dose of 35.0 mg/kg bw). One to three weeks post end of the treatment fish were transferred to seawater cages and maintained in seawater until the end of the study. The seawater temperature ranged from 3.7 ºC to 13.3 ºC. Tissues were collected after 62, 127, 184 and 246 days post treatment. Twelve fish were sampled for the first two time points, eleven for the third time point and ten for the last time point. Lufenuron was quantified in the collected fillet samples by a validated UHPLC-MS/MS method. The mean concentrations of lufenuron determined are shown on Table 16.

Table 16. Mean concentration of lufenuron in fillet from Atlantic salmon with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Hervy, 2016a)

Time post-dose (d)	Time post-dose (DD)	Number of fish	Concentration of lufenuron (µg/kg)			Standard deviation (µg/kg)
			Mean	Minimum	Maximum	
62	362	12	10891	7240	15900	3858
127	1024	12	2010	1760	2670	358
184	1725	12	438	321	553	88
246	2273	12	178	25	412	149

The concentration of lufenuron as a function of time in degree-days is shown in Figure 14. Using all data points, it was verified that the residues are not normally distributed and are not constant over time. After excluding three results (below of LOQ) the data fitted the method of least square.

Figure 14. Depletion curve of lufenuron in salmon fillet



The depletion curves of the studies carried out in Canada, Norway and Chile are summarized in Figure 15 and the log-e transformed curves in Figure 16.

Figure 15. Depletion curves of all studies (except study NAH 15-014)

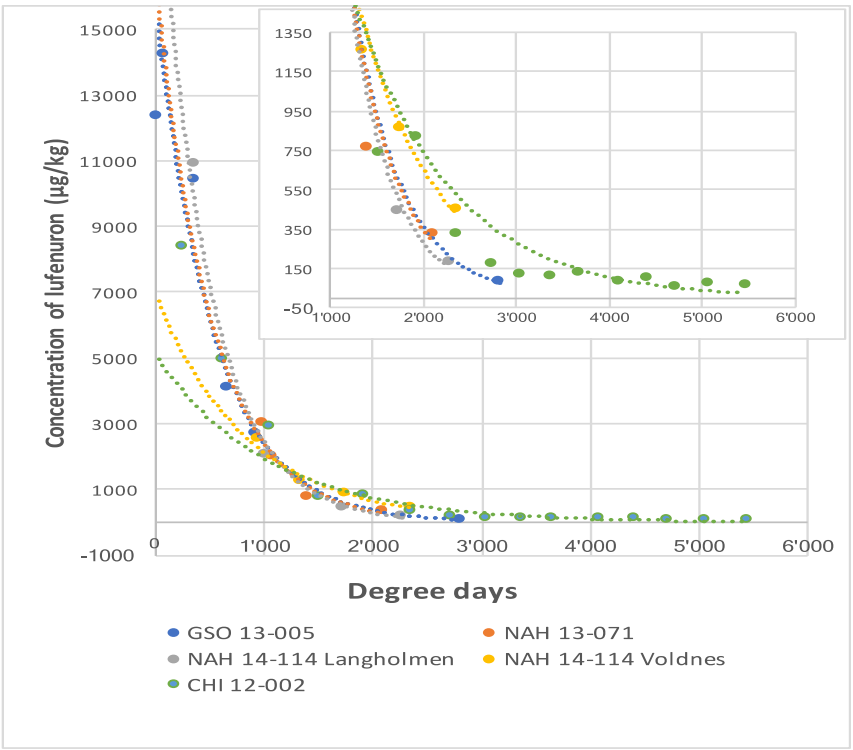
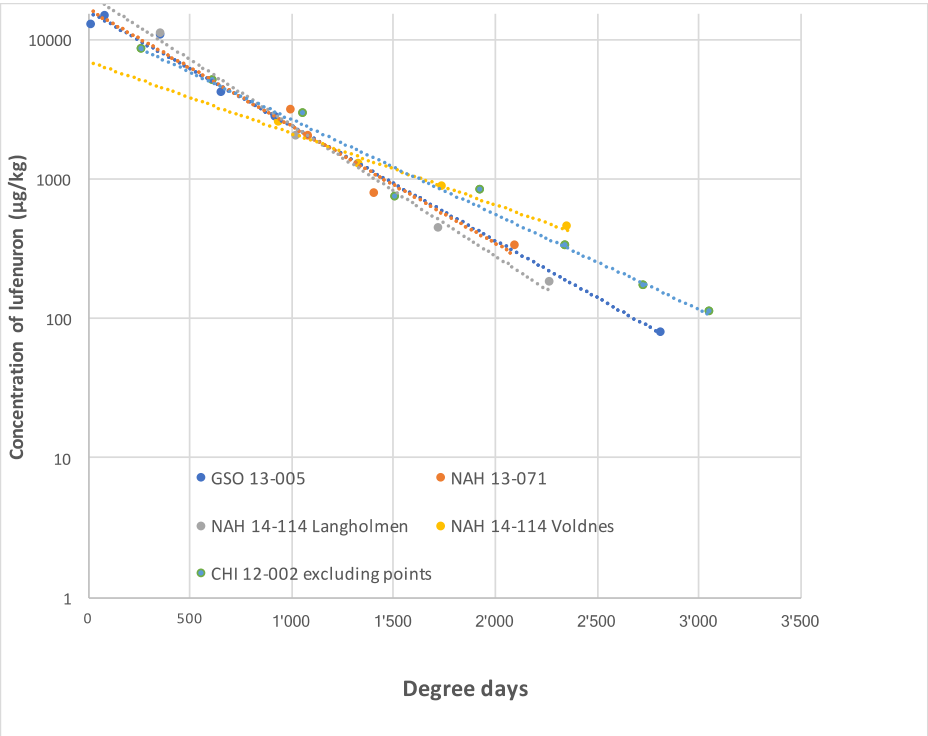


Figure 16. Depletion curves (log-e transformed) of all studies (except study NAH 15-014)



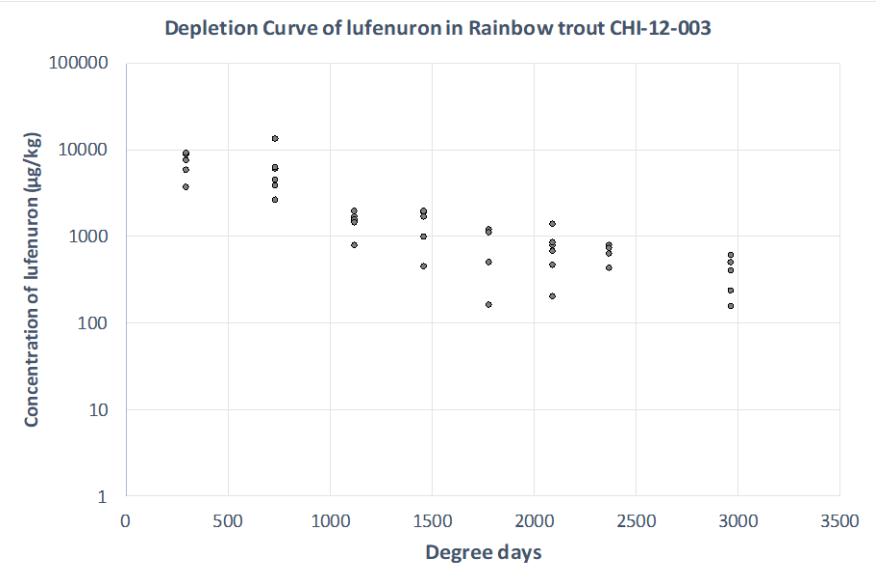
Trout

In a GLP-compliant study carried out in Chile (Lewis *et.al.*, 2017), rainbow trout smolts (*Oncorhynchus mykiss*) (average weight at the start of treatment, 212 g, mixed sex), held in freshwater hatchery tanks (water temperature of 11.2 ºC), were fed with lufenuron diet for seven days at a dose of 5 mg/kg bw (actual total dose of 35.0 mg/kg bw). Eight days post end of the treatment fish were transferred to sea cages and maintained in seawater until end of the study. The seawater temperature ranged from 9.2 ºC tp 17.1 ºC. Whole fillet samples were collected after 21, 49, 78, 106, 134, 163, 190 and 244 days post-treatment. Four to six fish were sampled for each time point and lufenuron was quantified in the collected fillet samples by a validated UHPLC-MS/MS method. The mean, maximum and minimum concentrations of lufenuron determined are shown on Table 17 and the depletion curve in Figure 17.

Table 17. Mean concentration of lufenuron in fillet from rainbow trout with time after oral daily dose (medicated feed) of lufenuron (5 mg/kg bw) for 7 days (Hervy, 2016a)

Time post-dose (d)	Time post-dose (DD)	Number of fish	Concentration of lufenuron (µg/kg)			Standard deviation (µg/kg)
			Mean	Minimum	Maximum	
21	300	6	7142	3600	9000	2148
49	736	6	5892	2540	12800	3635
78	1126	6	1451	763	1910	381
106	1467	5	1359	444	1913	633
134	1780	4	728	160	1180	485
163	2094	6	711	196	1370	396
190	2375	5	586	415	766	164
245	2967	5	372	153	592	181

Figure 17. Depletion of lufenuron in Rainbow trout in function of time (degree-days)



Method of analysis for residues in tissues

Due to their low volatility and thermal instability benzoylurea insecticides, including lufenuron, are usually determined in food, feed and biological matrices by liquid chromatography. There are no methods reported in the scientific literature about the determination of lufenuron in fish. Some methods are reported for the determination of residues of lufenuron in agricultural commodities and environmental matrices.

Liquid chromatography

Analytical methods based on the quantitation of lufenuron in plants and food of animal origin by high performance liquid chromatography with an ultraviolet detector were described in the

JMPR evaluation (2015) of pesticide residues in food. In general, lufenuron was extracted by solvents and the extracts cleaned-up by solid phase extraction using silica gel, cyano or C18 sorbents. For samples rich in proteins, such as milk, a protein precipitation step is introduced in the procedure. The quantitation of lufenuron is performed at the wavelength of 255 nm. The limit of quantitation is about 0.01 mg/kg for plant and 0.02 mg/kg for animal commodities.

Confirmatory methods

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

An ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) method was validated in compliance with GLP to determine residues of lufenuron in fish fillet (muscle and skin in natural proportions) from salmon and rainbow trout in the studies submitted to the current Meeting of the Committee (Adams, 2013). This original method was revalidated in another laboratory for the determination of lufenuron in salmon fillet (Hervy, 2013c) and trout fillet (Hervy, 2013d). The Committee assessed the validation data against the requirements for analytical methods as published in Codex Guideline CAC/GL 71-2009 (FAO/WHO, 2014).

In summary, the sample preparation consists of addition of fluazuron (N-((4-chloro-3-((3-chloro-5-(trifluoromethyl)pyridin-2-yl)oxy)phenyl)carbamoyl)-2,6-difluorobenz- amide) as internal standard to the mechanically homogenized tissues (0.5 g) followed by solid-liquid extraction with acetonitrile (5 mL). After centrifugation (15 min), the supernatant is diluted with 10 mL water and percolated on a solid phase extraction cartridge containing a C18 sorbent. The cartridge is washed with 2 mL acetonitrile:water 30:70 v/v, dried under vacuum and the retained analytes eluted with 10 mL acetonitrile:water 70:30 v/v. The eluate is filtered and 10 µL injected into the UHPLC-MS/MS. The separation of lufenuron and the internal standard is performed on a Waters Acquity CSH C18 column (2.1 x 50 mm, 1.7 µm), at 40 ºC, with a mobile phase containing 0.05 % v/v formic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 0.4 mL/min, under gradient elution (0 to 0.3 min, 80:20 v/v A:B to 55:45 v/v A:B; 1.6 min 50:50 v/v A:B; 3.1 min 0:100 v/v A:B; 6.8 min 0:100 v/v A:B and 7 min 80:20 v/v A:B). The mass spectrometer conditions were: electrospray source operating in the negative mode, capillary voltage of 3.25 kV, source temperature of 120 ºC, dessolvation temperature of 280 ºC, cone gas flow of 100 L/h and desolvation gas flow of 700 L/h. Quantitation is performed using acquisition of ions in the selected reaction-monitoring mode, using the transitions of *m/z* 508.9→326.0 for lufenuron and *m/z* 504.1→305.1 for fluazuron. For identification and identity confirmation two additional transitions for lufenuron were monitored: *m/z* 508.9→174.0 and *m/z* 508.9→201.9. A plot of the area response ratio of lufenuron to fluazuron versus concentration of lufenuron was fitted to a quadratic regression model with 1/x weighting. Linearity was observed in the range of 2.5 to 200 ng/mL equivalent to 50 to 4000 µg/kg.

The LOD and LOQ were estimated as the signal to noise ratio of 3 and 10, respectively, at the retention time of lufenuron. The matrix effect and extraction recovery were evaluated at the LOQ, using at least six different batches of control matrix. The method validation parameters, using fortified samples, are presented in Table 18.

Table 18. Validation parameters of the UHPLC-MS/MS method for the determination of lufenuron in salmon tissues (Adams, 2013)

Parameter	Salmon fillet	Trout fillet
Interday precision	8.6 % (100 µg /kg)	5.7 % (1500 µg /kg)
	9.4 % (600 µg /kg)	
(CV, n=18)	7.2 % (1500 µg /kg)	
	6.4 % (3000 µg /kg)	
Accuracy (n=18)	102 % (100 µg /kg)	97.7 % (1500 µg /kg)
	101 % (600 µg /kg)	
	97.6 % (1500 µg /kg)	
	102 % (3000 µg /kg)	
LOQ	18 µg/kg (100 µg/kg)[a]	21 µg/kg (100 µg/kg)[a]
LOD	5 µg/kg	6 µg/kg
Analytical range (µg/kg)	50 - 4000	50 - 4000
Linearity (r)	>0.99	>0.99
Specificity/selectivity	No interference observed	No interference observed
Extraction recovery	93.4 %	94.8 %
Matrix effect	+19.4 %	+5.6 %

a: For practical reasons, regarding a proposed MRL of 1350 µg/kg, the LOQ of the method was set as 100 µg/kg for both fish species.

For the selectivity test, possible interference of other veterinary drugs was assessed: emamectin benzoate, oxytetracycline, teflubenzuron, amoxicillin, deltamethrin, diflubenzuron, florfenicol, azamethiphos and cypermethrin. No interference was observed on the determination of lufenuron in the presence of these drugs.

For matrix co-extractives, 20 and 18 control blank samples of salmon and trout were analysed, respectively. No endogenous interferents with concentrations higher than the LOD were verified.

Minor modifications were introduced in the original analytical method described by Adams (2013) regarding to the sample homogenization (using a Turrax® instead of a food processor) and sample weight (2.5 g instead of 0.5 g) and the method was revalidated (Hervy, 2015a) for the determination of lufenuron in salmon fillet (Hervy, 2013c) and trout fillet (Hervy, 2013d). Also, incurred samples were included. The method validation parameters, using fortified samples, are presented in Table 19.

Table 19. Validation parameters of the UHPLC-MS/MS method for the determination of lufenuron in salmon and trout tissues (Hervy, 2015a)

Parameter	Salmon fillet	Trout fillet
Precision, within run (CV)	14.1 % (50 µg /kg) 2.6 % (600 µg /kg) 4.0 % (3500 µg /kg)	4.0 % (50 µg /kg) 3.0 % (600 µg /kg) 3.1 % (3500 µg /kg)
Precision, between run (CV)	0 % (50 µg /kg) 6.6 % (600 µg /kg) 0 % (3500 µg /kg)	8.9 % (50 µg /kg) 2.5 % (600 µg /kg) 3.2 % (3500 µg /kg)
Accuracy	105.4 % (50 µg /kg) 102.7 % (600 µg /kg) 98.9 % (3500 µg /kg)	101.0 % (50 µg /kg) 106.7 % (600 µg /kg) 101.4 % (3500 µg /kg)
Limit of quantitation	50 µg/kg	50 µg/kg
Limit of detection	5 µg/kg	6 µg/kg
Analytical range (µg/kg)	50 - 4000	50 - 4000
Linearity (r)	>0.99	>0.99
Specificity/selectivity	No interference observed	No interference observed

The estimated limit of quantitation was 18 µg/kg and 21 µg/kg for salmon and trout fillet, respectively. However, for this method, the lowest calibration point was 50 µg/kg and this was set as the LOQ.

Fifteen incurred salmon fillet samples, with lufenuron residues ranging from about 600 to 2400 µg/kg, were chosen from two depletion studies and analyzed in triplicate in the same analytical run. Sixty percent of the samples analyzed have a coefficient of variation <10 %. The highest value, from one sample, was 22 %.

The FDA monitors lufenuron in salmon tissues using the same multi-residue pesticide monitoring procedure as for teflubenzuron, described in the FDA Laboratory Information Bulletin 4463 (U.S.FDA, 2016).

The method is fully validated and suitable for the depletion studies summarized above. However, the Committee noted that fluazuron, an approved veterinary drug, could affect the analytical result, and therefore may not be an appropriate internal standard for the method used for monitoring purposes.

Stability of residues

The stock solutions of lufenuron (100 µg/mL) and internal standard (100 µg/mL) prepared in acetonitrile are stable for 12 months at 5 ºC. At the same conditions, working standard solutions of lufenuron prepared in acetonitrile:water 30:70 v/v are stable for 4 months. The final extracts diluted in mobile phase was proved to be stable for 4 days at ambient temperature.

Lufenuron is stable in salmon fillet and rainbow trout stored at -20 ºC at least for 10 months.

Fortified blank salmon fillets with lufenuron (200 µg/kg) were stable over at least three cycles of freeze (20 h)/thaw (4 h).

Appraisal

Lufenuron has not been previously reviewed by the Committee, but was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), in 2015, when an ADI of 0-0.02 mg/kg bw per day was established based on the basis of a NOAEL of 1.93 mg/kg bw for tonic-clonic seizures and findings in lungs, gastrointestinal tract, liver and urinary tract in the 2-year dietary study in rats, using a safety factor of 100.

Lufenuron is a benzoyl phenylurea insecticide registered for aquaculture use in the treatment of Atlantic salmon at a dose of 5 mg per kg fish for seven days (full therapeutic dose of 35 mg/kg bw), administered through feed (pelleted diet at a level of 5 g/kg), for control of infestation of sea lice. It is also used for the control of flea infestation in companion animals (cats and dogs) and in agriculture to control a wide range of insect pests.

Metabolism data are available for a variety of animal species, including rats, dogs, goats and chicken. In mammals, lufenuron is only metabolized to a limited extent by cleave of the benzoyl ureido bridge and the biliary excretion is the main path for elimination.

Metabolic profiling in salmon was available. In fish lufenuron is not metabolized and the parent drug is distributed in the fatty fish tissues; no metabolites were detected over a 6-month period after medication.

Radiolabelled data are available for the depletion of lufenuron in salmon at a water temperature of 4 to 14 ºC, following repeated dose. Lufenuron was identified as the marker residue in salmon fillet. Lufenuron is almost quantitatively extracted from fish fillet, indicating there are no bound residues. Based on the results of this study, the Committee identified lufenuron as the marker residue in salmon fillet and determined that a value of 1.0 was appropriate for the MR:TRR.

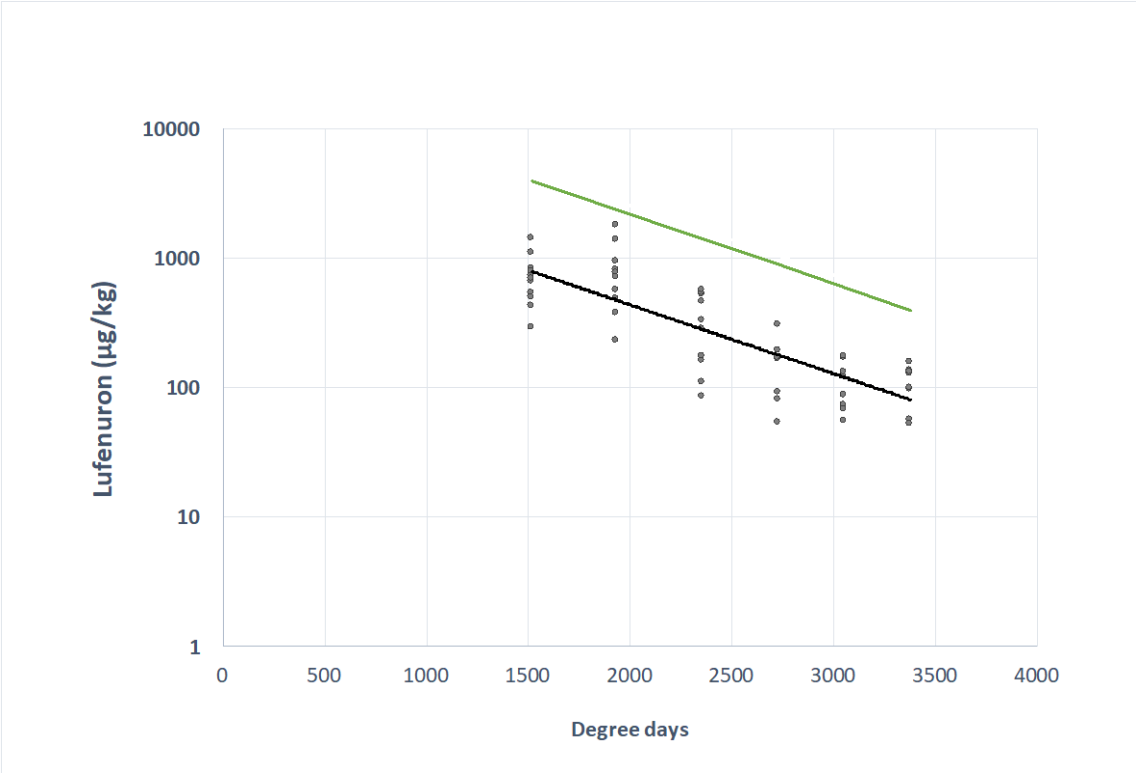
The highest concentration (about 20400 µg/kg) of lufenuron in salmon fillet was registered in one study carried out in Canada 8 days after administration of the drug.

The residue depletion studies in salmon and trout fillets were conducted with a validated method using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). The limit of quantification (LOQ) was 50 µg/kg in salmon and trout fillet.

The recommended MRL of 1350 µg/kg of lufenuron in salmon fillet muscle with skin in natural proportions was 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 fillet derived from the data provided, in accordance with Good Veterinary Practice and a withdrawal period of 2050 degree-days.

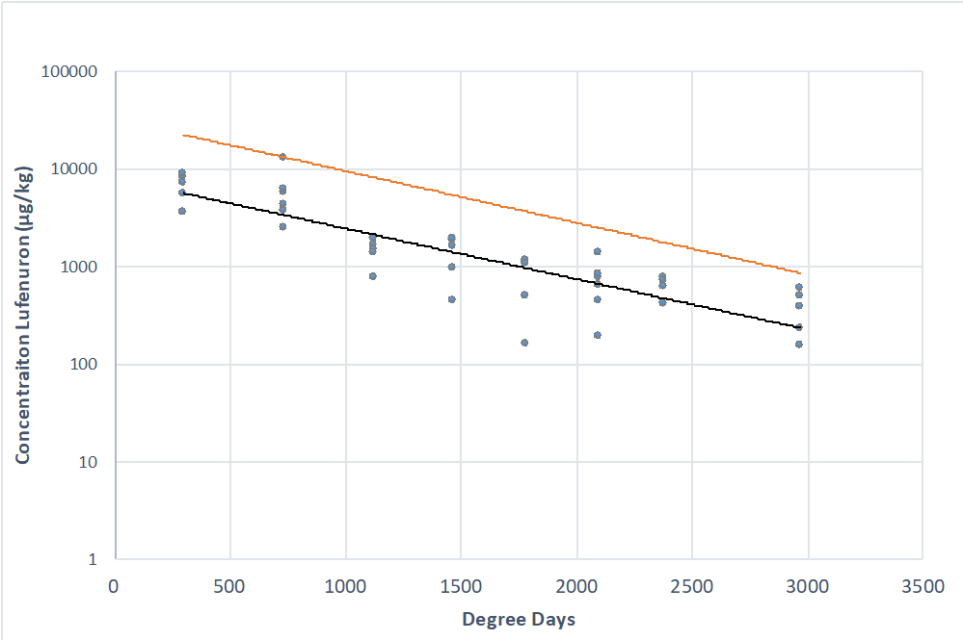
The tolerance limits for lufenuron residues in salmon fillet are shown in Figure 18. The first three and six last time points were excluded.

Figure 18. Tolerance limit considerations for lufenuron in salmon fillet. Regression line (black) and UTL 0.95/0.95 regression line (green)



For rainbow trout, the tolerance limits are shown in Figure 19. The upper limit of the one-sided 95 % confidence interval over the 95th percentile (UTL 95/95) lead to the MRL of 1350 µg/kg at 2643 DD.

Figure 19. Tolerance limit considerations for lufenuron in trout fillet. Regression line (black) and UTL 0.95/0.95 regression line (orange)



Dietary Exposure Assessment

Dietary exposure from pesticide residues (IEDI)

Exposure to lufenuron residues may occur through its use as a pesticide as well as a veterinary drug.

When used as a pesticide, the exposure of lufenuron was 0–4 % of the upper bound of the ADI.

Dietary exposure from veterinary drug residues (GECDE)

When used as a veterinary drug, chronic dietary exposure in the general population was estimated. Based on the toxicological profile of the compound, dietary exposure estimates for children, shorter than lifetime dietary exposure or acute dietary exposure were not required.

Dietary exposure was estimated based on the potential occurrence of residues in salmon and trout muscle and skin in natural proportion. Other finfish were not included in the exposure estimate.

The GECDE for the general population is 1.1 µg/kg bw per day, which represents 5.5 % of the upper bound of the ADI of 0.02 mg/kg bw per day (Table 20). Salmon was the major contributor to chronic dietary exposure. It should be noted that no reliable high percentile consumption value was available for trout.

In addition to the accepted GECDE methodology, further calculations were carried out. Instead of using the highest mean and the highest 97.5th percentile consumption across surveys, the calculations were carried out using the mean and the highest reliable percentile for each individual national survey from available data sets (CIFOCOss). The mean and ranges across surveys were reported (Table 21). The mean of 25 estimates for adults or the general population was 0.35 µg/kg bw per day (2 % of the upper bound of the ADI), with a range of 0.0001-1.1 (<0.01-5.5 % of the upper bound of the ADI).

Combined chronic dietary exposure from pesticide and veterinary drug residues (Extended GECDE)

Modified methods based on the GECDE were used to estimate combined chronic dietary exposure (Table 22). The usual GECDE approach was extended to include additional commodities that were assessed for the compound by JMPR (“Extended GECDE”). It should be noted that this new exposure assessment methodology is still being piloted. It should further be noted that the median residues used as inputs were extracted from JMPR publications and have not been validated for this assessment.

Combined chronic dietary exposure from veterinary drug and pesticide residues was considered for the general population based on the potential occurrence of residues in salmon, trout, mammalian meat, mammalian fats, edible mammalian offal, milks, poultry meat, poultry fats, edible poultry offal, eggs and plant products (cucumber, melons, except watermelon, sweet pepper, potato, tomato, tomato juice and tomato products).

The Extended GECDE for the general population is 1.8 µg/kg bw per day, which represents 9 % of the upper bound of the ADI of 0.02 mg/kg bw per day. Milks (cow and sheep milk) are the major contributors to combined lufenuron chronic dietary exposure.

Table 20. Estimated chronic dietary exposure to lufenuron (GECDE) occurring in salmon and trout muscle and skin in natural proportion

Category	Type	Median concentration[1] (µg/kg)	Mean consumption[2] (whole population, g/kg bw/d)	Highest reliable percentile consumption[3] (consumers only, g/kg bw) /[percentile used]	MR:TR ratio	Exposure (µg/kg bw/day)		GECDE[4]	
						mean	97.5th	µg/kg bw/day	ADI%
General Population									
Fish and seafood	Salmon[5]	425	0.18	2.6 [97.5]	1	0.077	*1.1*		
Fish and seafood	Trout[5]	348	0.0003	n/a	1	*0.0001*			
TOTAL						0.0001	1.1	1.1	5.5

[1]Median concentration at the end of treatment;
[2]Highest mean consumption figures based on whole population considered from the available dataset;
[3]Highest food consumption figures based on consumers only considered from the available dataset;
[4]GECDE is the sum of the highest exposure at the highest reliable percentile of consumption for a food and the mean dietary exposures of the other foods;
[5]Muscle and skin in natural proportions.

Table 21. National estimates of chronic dietary exposure to lufenuron median residues in adults or the general population, based on GECDE methodology, occurring in salmon and trout muscle and skin in natural proportion

Country	Population group	Study	Estimate of chronic dietary exposure (µg/kg bw per day)	% ADI	Main contributing food
Belgium	Adults	Diet_National_2004	0.64	3.2	Pacific salmon
China	General population	2002 China Nutrition and Health Survey	<0.01	<0.05	Pacific salmon
Czech Republic	Adults	SISP04	<0.01	<0.05	Pacific salmon
Denmark	Adults	Danish_Dietary_Survey	0.20	1.0	Pacific salmon
Finland	Adults	FINDIET_2007	1.09	5.4	Pacific salmon
France	Adults	INCA2	0.29	1.5	Pacific salmon
Germany	Adults	National_Nutrition_Survey_II	0.82	4.1	Pacific salmon
Hungary	Adults	National_Repr_Surv	<0.01	<0.05	Pacific salmon
Ireland	Adults	NSIFCS	0.48	2.4	Pacific salmon
Italy	Adults	INRAN_SCAI_2005_06	0.50	2.5	Pacific salmon
Latvia	Adults	EFSA_TEST	0.33	1.7	Pacific salmon
Netherlands	Adults	DNFCS_2003	0.28	1.4	Pacific salmon
Spain	Adults	AESAN	0.02	0.09	Pacific salmon
Spain	Adults	AESAN_FIAB	0.43	2.1	Pacific salmon
Sweden	Adults	Riksmaten_1997_98	0.11	0.6	Pacific salmon
United Kingdom	Adults	NDNS	0.45	2.3	Pacific salmon
		Mean	0.35	1.8	
		Minimum	<0.01	<0.05	
		Maximum	0.109	5.4	

Table 22. Estimated chronic dietary exposure to lufenuron (GECDE) occurring in salmon and trout muscle and skin in natural proportion

Category	Type	Median residue[1] µg/kg	Mean consumption[2] g/kg bw/d	Percentile consumption[3] g/kg bw/d (percentile used)	Exposure µg/kg bw/day		GECDE[4]
					Mean	HRP	µg/kg bw/d
Meat from mammals other than marine mammals	Beef and other bovines meat	12	0.96	4.4 (97.5)	*0.012*	0.053	0.012
	Goat and other caprines	12	0.006	4.8 (97.5)	*0.0001*	0.058	0.0001
	Horse and other equines	12	0.019	4.7 (95)	*0.0002*	0.056	0.0002
	Pork and other porcines	12	0.99	6.3 (97.5)	*0.012*	0.076	0.012
	Rabbit meat	12	0.067	3.5 (97.5)	*0.001*	0.042	0.001
	Sheep and other ovines	12	0.16	4.3 (97.5)	*0.002*	0.052	0.002
Mammalian fats	Cattle fat	300	0.13	0.61 (97.5)	*0.038*	0.18	0.038
	Goat fat	300	0.00002	0.047 (90)	*0.00001*	0.014	0.00001
	Pig fat	300	0.082	1.5 (95)	*0.025*	0.46	0.025
	Sheep fat	300	0.002	0.11 (97.5)	*0.0005*	0.032	0.0005
Edible offal (Mammalian)	Cattle, liver	25	0.031	3.3 (97.5)	*0.001*	0.083	0.001
	Pig liver	25	0.041	2.3 (97.5)	*0.001*	0.057	0.001
	Sheep liver	25	0.009	2.1 (95)	*0.0002*	0.053	0.0002
	Cattle, kidney	25	0.007	No HRP	*0.0002*	-	0.0002
	Pig kidney	25	0.004	1.8 (97.5)	*0.0001*	0.046	0.0001
	Sheep kidney	25	0.002	0.11 (90)	*0.0001*	0.003	0.0001
	Cattle, offal of, nes	25	0.037	4.0 (97.5)	*0.001*	0.099	0.001
	Goat, offal of	25	0.001	2.8 (90)	*0.00003*	0.070	0.00003
	Horse,offal of	25	0.000	No HRP	*0.00001*	-	0.00001
	Pig, offal of, nes	25	0.061	2.8 (97.5)	*0.002*	0.071	0.002
	Sheep, offal of, nes	25	0.003	3.0 (97.5)	*0.000*	0.075	0.0001
Milks	Cow milk	66	4.5	16.9 (97.5)	*0.29*	1.1	0.29
	Goat milk	66	0.017	No HRP	*0.001*	-	0.001
	Sheep milk	66	0.012	18.0 (95)	0.001	*1.2*	1.2

Poultry meat	Chicken meat	0.6	0.62	6.7 (97.5)	*0.0004*	0.004	0.0004
	Duck meat	0.6	0.055	4.3 (97.5)	*0.00003*	0.003	0.00003
	Goose meat	0.6	0.012	4.0 (95)	*0.00001*	0.002	0.00001
	Poultry meat, nes	0.6	0.037	2.4 (97.5)	*0.00002*	0.001	0.00002
	Chicken fat	27	0.0003	No HRP	*0.00001*	-	0.00001
Poultry fats	Poultry fats	27	0.001	1.1 (90)	*0.00003*	0.029	0.00003
Poultry, edible offal of	Chicken, offal of	4	0.10	2.7 (97.5)	*0.0004*	0.011	0.0004
	Duck, offal of	4	0.004	2.7 (97.5)	*0.00002*	0.011	0.00002
	Goose, offal of	4	0.008	1.0 (90)	*0.00003*	0.004	0.00003
	Poultry offals, unprocessed	4	0.017	2.0 (97.5)	*0.0001*	0.008	0.0001
Eggs	Chicken eggs	10	0.46	3.1 (97.5)	*0.005*	0.031	0.005
	Duck eggs	10	0.016	2.7 (97.5)	*0.0002*	0.027	0.0002
Plant products	Cucumber	20	0.33	4.6 (97.5)	*0.007*	0.092	0.007
	Melons, except watermelon	20	0.21	15.8 (97.5)	*0.004*	0.32	0.004
	Peppers, sweet	150	0.088	0.68 (97.5)	*0.013*	0.10	0.013
	Potato	10	1.7	6.7 (97.5)	*0.017*	0.067	0.017
	Tomato	80	1.2	10.6 (97.5)	*0.093*	0.85	0.093
	Tomato juice	14	0.12	6.9 (97.5)	*0.002*	0.097	0.002
	Tomato sauce	78	0.022	2.1 (97.5)	*0.002*	0.17	0.002
Fish and seafood	Salmon, Pacific	425	0.18	2.6 (97.5)	*0.076*	1.1	0.076
	Trout	348	0.0003	No HRP	*0.0001*	-	0.0001
						TOTAL	1.8
						% ADI	9.0

[1]For non-fish food types, median residue concentrations were taken from JMPR (2016)

[2]highest mean consumption figures based on whole population considered from the available dataset

[3]highest reliable percentile food consumption figures based on consumers only considered from the available dataset

[4]GECDE is the sum of the highest exposure at the highest reliable percentile of consumption for a food and the mean dietary exposures of the other foods

No HRP: No highest reliable percentile could be defined due to low numbers of consumers

Maximum Residue Limits

In recommending MRLs for lufenuron in salmon and trout fillet the Committee considered the following factors:

- An ADI for lufenuron of 0-0.02 mg/kg bw was established by the Committee.
- An ARfD was considered unnecessary.
- The Committee considered that for lufenuron there are no specific concerns for less-than-lifetime exposure.
- Lufenuron is used as both a pesticide and a veterinary drug.
- Lufenuron is authorised for use in salmon in one Member State. The maximum recommended dose is 5 mg/kg per day for 7 consecutive days, administered through medicated feed. The withdrawal period is 2050 degree-days.
- Lufenuron is the marker residue in fillet.
- The ratio of the concentration of marker residue to the concentration of total residue of 1.0 was calculated.
    - Residue data for salmon and trout were provided using a validated analytical method to quantify lufenuron in fillet.
    - A validated analytical method (UHPLC-MS/MS) for the determination of lufenuron in salmon and trout fillet is available and may be used for monitoring purposes, with the reservation noted above.

An MRL was 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 fillet derived from the data provided, in accordance with Good Veterinary Practice and a withdrawal period of 2050 degree-days.

The Committee recommended an MRL of 1350 µg/kg for lufenuron in salmon fillet.

For trout, using the same approach, the Committee could recommend an identical MRL should lufenuron be approved for use in trout. This MRL is compatible with a withdrawal period of 2643 degree-days based on the residue data provided.

The Committee could not extrapolate the MRL to other fish species or to fin fish in general considering that:

(i) lufenuron is a lipophilic drug and its concentration is higher in fatty tissues, the fat content in fish depends on the species and growing conditions,

(ii) the decrease in concentration of lufenuron in studies in Atlantic salmon and in rainbow trout is dependent on the time after the last drug administration as well as the increase in body weight, both of which are dependent on the water temperature,

(iii) no depletion data were provided for species other than salmonids.

The GECDE for the general population is 1.1 µg//kg bw per day, which represents 5.5 % of the upper bound of the ADI of 0.02 mg/kg bw per day. Salmon was the major contributor to chronic dietary exposure. It should be noted that no reliable high percentile consumption value was available for trout.

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