LUFENURON FOR SALMONIDS

ENVIRONMENTAL ASSESSMENT
IN SUPPORT OF AN
IMPORT TOLERANCE REQUEST

Final: Original signed and on file

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1.0 General information

Sponsor: Elanco Animal Health U.S.
2500 Innovation Way
Greenfield, Indiana 46140

Established Name: Lufenuron

2.0 Purpose and need for the proposed action

Elanco Animal Health is requesting establishment of an import tolerance for lufenuron so that meat from salmonids treated with lufenuron may be imported into the United States (U.S.) for human consumption. No drug products containing lufenuron are currently approved for use in fish in the U.S.; therefore, an import tolerance needs to be established. The environmental impact on the U.S. environment from lufenuron residues in salmonid flesh, and from lufenuron residues that may be transported by water into the U.S. environment, will be evaluated herein based on the expected exposure pathways and appropriate physical-chemical properties and fate data for the drug.

3.0 Identification of the substance

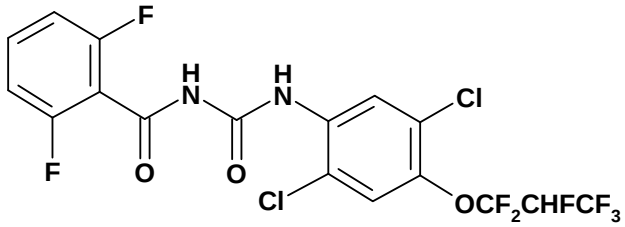
Lufenuron is a member of the benzoyl-phenyl-ureas and acts as a chitin synthesis inhibitor; it is classified as a growth regulator for animals with a chitin exoskeleton.

Lufenuron does not currently have a marketing authorization for use in food animals in the U.S. However, lufenuron has a marketing authorization in Chile as IMVIXA (lufenuron 10% oral powder) for use in salmonids (Appendix A below). Lufenuron has also been approved for investigational use in clinical studies with medicated feed administered to fish in foreign countries including Canada and Norway. In addition, lufenuron is authorized or registered for use on companion animals, as termite bait, and for use on food crops in a number of foreign countries.

In Chile, IMVIXA is administered to the fish in feed. This occurs while the fish are being reared in freshwater facilities. Following treatment, the fish are held for approximately 7 additional days to allow excretion of unabsorbed active pharmaceutical ingredient before they are transferred to marine (seawater) grow out sites. IMVIXA is indicated for prevention and control of infestations caused by sea lice, *Caligus rogercresseyi*.

Lufenuron has an octanol/water partition coefficient (log P_{ow}) value of 5.12, Table 3.0.1. The volatility and vapor pressure values indicate that lufenuron is unlikely to enter or exist as a vapor in the atmosphere. Additional structural and physicochemical characteristic details of lufenuron are presented in Table 3.0.1.

Table 3.0.1: Identity and physicochemical properties

International Nonproprietary Name (INN)	Lufenuron
International Union of Pure and Applied Chemistry (IUPAC) Name	N-[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)phenyl]carbamoyl]-2,6-difluorobenzamide and/or (*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl]-3-(2,6-difluorobenzoyl)-urea
Chemical Abstracts (CA) Name	N-[[[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoro-propoxy)-phenyl]amino]carbonyl]-2,6-difluoro-benzamide
Chemical Abstracts Service (CAS) Number	103055-07-8
Structure	
Empirical formula	$C_{17}H_8Cl_2F_8N_2O_3$
Molecular weight	511.15 g/mol
Vapor pressure (Pa at 25°C) [a]	$<4 \times 10^{-6}$
Volatility [b]	3.7×10^{6}
Octanol/water partition coefficient (log P_{ow} at 25°C) [c]	5.12 (±0.14)

[a] Geoffroy, 1992; [b] Reischmann, 1995; [c] Rodler, 1992
* Summaries of proprietary studies referenced in this table are provided in Appendix B below.

4.0 Sites of introduction and exposure pathways

There are two general types of exposure pathways for lufenuron to the U.S. environment that could potentially exist due to the establishment of an import tolerance for this drug in salmonid tissues: 1) pathways arising from the release of drug residues, if present, from imported food derived from treated fish, or 2) pathways arising from use of the drug on salmonids in countries where it is legally authorized.

With respect to the first of the two general types of exposure pathways, potential points of introduction into the U.S. environment arising from the import of salmonid flesh from fish previously treated with lufenuron include:

- disposal of seized fish and waste from processing of treated fish to landfills;
- effluent from wastewater treatment;
- application of biosolids from wastewater treatment as fertilizer to soil.

With respect to the second of the two general types of exposure pathways, a potential point of introduction to the U.S. environment could consist of water flow from treatment of fish in countries adjoining the U.S., specifically Canada where aquaculture of salmonids is common.

The environmental exposure and likelihood of lufenuron to cause impacts on U.S. ecosystems at the sites of introduction are evaluated in Section 5.0.

5.0 Analysis of exposure and risk

The potential exposures due to the pathways listed in Section 4.0 are evaluated based on metabolism and environmental fate data for lufenuron.

5.1 Metabolism in fish

Lufenuron was the only molecule detected in the tissues or excreta in salmon fed [^{14}C]-lufenuron, other than non-extractable residues (NER) (Hobbs, 2014; Appendix B below). Less than 9% of the total radioactive residues (TRR) were present as NER in the fillet and only 3.3% in the feces. The highest TRR levels were found in white fat deposits associated with the pyloric caecae though high concentrations were also present in the skin throughout the study to 178 days after treatment (DAT). As fish are grown at sea for up to 22 months following treatment it is considered unlikely that the residues in imported fillets will be above the maximum residue level of 1,350 µg.kg^{-1} set by the European authorities (CVMP, 2013). However, if fish were condemned for exceeding a set import tolerance then it must be considered possible that this residue level might be exceeded in fish flesh sent for disposal.

5.2 Environmental fate

5.2.1 Fate in water sediment systems

In a sediment adsorption/desorption study conducted following the Organization for Economic Cooperation and Development (OECD) Guideline 106, adsorption to sediments was rapid with equilibrium being reached within 30 minutes (Commander, 2014; Appendix B below). The log Freundlich adsorption constants (K_F^{ads}) were determined to be 2.12 and 3.07 for the high organic carbon (HOC) and low organic carbon (LOC) systems, respectively. The corresponding log Freundlich organic carbon normalized adsorption constants (K_{Foc}) were 3.37 and 5.38, respectively. The results indicate that lufenuron would be of low mobility in the HOC sediment and immobile in the LOC sediment.

When [^{14}C]-lufenuron was sprayed as an emulsifiable concentrate onto microcosms containing water, sediments, animals and plants, a median dissipation half-life (DT-50) value of 18 hours for total radioactivity (lufenuron and any metabolites) in water was determined (Volz, 2003; Appendix B below). The study was conducted according to guidance documents from the Society of Environmental Toxicology and Chemistry-Europe (SETAC-Europe), OECD, and European Commission (EC) Workshop and the U.S. Environmental Protection Agency (EPA). A biota accumulation factor (BAF) of 327 for lufenuron was also determined from this study.

In microcosms prepared with Pond and Rhine sediments and water, degradation half-lives of 27.4 and 52.5 days in the pond, and 159 and 187 days in the Rhine system were determined for lufenuron, depending on the location of the ^{14}C (Gonzalez-Valero, 1994; Appendix B below). The Pond and Rhine systems were identical and only the sources of sediment and water were different for the two systems. While metabolites were transiently detected, a large proportion of the radiolabel was found as NER at the end of the 360 day incubation. In the pond system, 30.2% were present as NERs with 49.4% of the radiolabelled carbon being mineralized to CO_2. The corresponding values for NER and mineralization to CO_2 for the Rhine system were 20.7% and 40.0%, respectively.

5.2.2 Fate in soil

Adsorption of lufenuron to soils was assessed in a study that complied with several guidelines, including OECD 106, EPA Pesticide Assessment, and Canadian Pesticide Registration (Ellgehausen, 1992; Appendix B below). [^{14}C]-lufenuron, dissolved in acetone, reached equilibrium in the system within 30 minutes in most soils and by 85 minutes in all five soil types. Lufenuron concentrations were determined by combustion, Liquid Scintillation Counting (LSC) and Thin Layer Chromatography (TLC). A 2-hour equilibration period was adopted and K_{ads} values of 166-2,350 were determined. The mean K_{ads} was 1,056 and mean K_{oc} was calculated as 41,182 (log K_{oc} of 4.615). Desorption data from the same study indicated that the adsorption was partially irreversible with desorption constants, K_{des}, of 219-4,017 $\mu g.g^{-1}$ derived from the first desorption step. The adsorption and desorption values indicate that lufenuron should be of low mobility.

The low mobility of lufenuron in soil was also established in four soil types when [^{14}C]-labelled lufenuron was applied in irrigated soil columns with a depth of 30 cm in accordance with Dutch Registration Guidelines (Ellgehausen, 1990; Appendix B below). Lufenuron penetrated to depths of 2 to 8 cm. Concentrations in leachate from the 30 cm columns were negligible (0.04-0.58% of the applied dose) following 200 mm of irrigation water within 48 hours. The same soil types were studied again with radiolabelled lufenuron in accordance with EPA Pesticide Assessment Guidelines (Ellgehausen, 1991a; Appendix B below). For this study, lufenuron was aged in soil in metabolic chambers for 30 days, then applied to columns and irrigated for 45 days with a total 508 mm artificial rain. Lufenuron and its metabolites were largely restricted to the top 2 cm with only negligible amounts in the 2-4 cm layer. Analysis of the leachates again revealed negligible amounts of radioactivity, 0.6%-1.2%.

Biodegradation of radiolabelled lufenuron was determined in a study conducted in compliance with the EPA Pesticide Assessment Guidelines and those of the German and Dutch Authorities (Ellgehausen, 1991b; Appendix B below). [^{14}C]-lufenuron, labelled in either the dichlorophenyl or difluorophenyl rings, was incubated under aerobic, aerobic-anaerobic, or aerobic sterile conditions at 20°C in loamy sand or loam soils. Under aerobic conditions, degradation half-life values of 13 to 23.7 days were determined for parent lufenuron (NERs were considered as dissipated or degraded chemical when calculating the lufenuron degradation half-lives). Limited degradation occurred under anaerobic conditions and no degradation occurred under sterile conditions. Mineralization was found with both ring labels with 58.6% of the label being found as

^{14}C-CO_2 after 1 year with the difluorophenyl and 9.9 to 15.1% from the dichlorophenyl. In the course of the incubations a number of metabolites were transiently detected up to 26.9% of the applied dose and NERs were found. NERs were highest after 240 and 60 days with 70.7-78.6% and 36.1% for the dichlorophenyl- and difluorophenyl-labelled compound, respectively.

5.2.3 Summary of environmental fate

The available environmental fate data reviewed herein indicate that the great majority of lufenuron is expected to partition to the soil and sediment phase and remain in the country of use. In addition, any lufenuron entering the U.S. environment will dissipate from the aqueous environment, become bound to particulate and soil/sediment matrices, and be slowly degraded. The bound material will be of markedly reduced bioavailability. The specific routes of introduction into the U.S. environment are considered below.

5.3 Analysis of environmental risk from potential pathways arising from the release of drug residues, if present, from imported food derived from treated fish

5.3.1 Disposal of seized fish and waste from processing of treated fish at U.S. processing plants to landfills

Any treated fish that are processed for food in the U.S. or that are seized by U.S. authorities for non-compliance with Federal, or local, regulations might be disposed of in a landfill or incinerated. Disposal to landfill could create a potential route for lufenuron to enter groundwater or be washed out as surface run-off. However, the lack of mobility of lufenuron in soils and lack of leaching into deeper layers or elution as detailed above makes transfer to groundwater improbable. In addition, such disposal events are sporadic and rare, and therefore, only a negligible amount of lufenuron is expected to be available to potentially leach from salmon products disposed of in landfills.

Furthermore, landfills in the U.S. are highly regulated by local, state and Federal authorities to prevent environmental contamination. For example, most landfills are required to have caps and liners of clay or an impermeable membrane to prevent leaching of water or fluids therein (and any contaminants they may contain) to groundwater and/or local surface waters (e.g., rivers and lakes). As a result of these controls, there is expected to be minimal or no movement of lufenuron out of U.S. landfills and into the adjacent U.S. environment (groundwater or surface water). In addition, because lufenuron has a low vapor pressure ($<4 \times 10^{-6}$ Pa at 25°C; see Table 3.0.1 above) it is not expected to volatilize from landfills or enter air to any significant extent. Therefore, based on lack of exposure, significant environmental impacts on the terrestrial and aquatic environments are not expected from residues of lufenuron in imported food derived from treated salmon that are disposed of in U.S. landfills.

5.3.2 Effluent from wastewater treatment

Wastewater treatment plants may receive inputs of excreta arising from human consumers of lufenuron-treated fish, or potentially of industrial effluent from factories processing fish previously treated with lufenuron.

When the input is from human consumption of treated fish then excretion will occur slowly over a prolonged period following consumption. Within the treatment plant it can be expected that there will be negligible degradation of lufenuron within the transit time through the plant. However, the low concentrations expected in human feces following consumption of treated fish will be further reduced (diluted) by the excreta from other consumers who had not eaten salmon previously treated with lufenuron. Additionally, consumption rates of salmon in the U.S. are low compared to those for most other types of meats, and the distribution of the excreted residues, if any, in the U.S. environment will likely be spatially and temporally variable. For both effluent from factories processing fish and human consumption, the partitioning characteristics are such that lufenuron will remain largely adsorbed to the organic matter and inorganic filtration matrices of the treatment plant and be disposed of as biosolids to land, landfill, or incineration.

On the basis of these behaviors it is considered unlikely that the produced water will contain unbound lufenuron at active levels and as such pose no risk in the receiving waters.

5.3.3 Application of residues from wastewater treatment as fertilizer to soil

In the event that biosolids from wastewater treatment plants were applied to soil as fertilizer there could be potential for the introduction of low levels of lufenuron into the soil. However, the exposure to lufenuron from this pathway is expected to be *de minimis* for the reasons described in Section 5.3.2 above (e.g., low concentrations in biosolids), as well as considerable dilution in the soil. Furthermore, the binding characteristics of lufenuron make it unlikely that the applied residues will migrate far into the soil or enter groundwater. It has been concluded that lufenuron will not be taken up by plants as the high log K_{ow} will restrict the potential for uptake in crops (EFSA, 2008).

5.4 Water flow from treatment of fish in foreign countries (e.g., Canada) adjoining the U.S.

Although lufenuron is not currently approved in Canada, it is reasonably foreseeable that it could be approved there because salmon farming is a major industry in Canada. Therefore, for this reason and because of Canada's close proximity to the U.S., the potential impact to the environment of the U.S. from the potential use of lufenuron in Canada is evaluated below.

The distribution of lufenuron in fish, and its physicochemical characteristics, indicates that the main route of entry into the environment will be in excreta from fish over an extended period with the released lufenuron present in feces. There could also be some introduction of lufenuron to the environment from uneaten feed. The highest concentrations are expected to arise in the effluents from freshwater hatcheries during treatment of the fish due to uneaten feed and excretion of lufenuron that was not absorbed by the fish. The concentrations entering the

environment are expected to decrease post treatment and further still when fish are transferred to sea, where the particulate material will settle from the water column.

The operation of fish farms in Canada is regulated to prevent adverse impacts on the environment around the farms. Given this, it is unlikely that an animal drug being used on a Canadian farm would enter U.S. waters at concentrations that could have adverse environmental impacts to the U.S. environment. In addition, the physicochemical properties of lufenuron indicate that the great majority of residues entering the freshwater aquatic environment via uneaten feed or fish feces will be removed by filtration and/or settling at the aquaculture facility prior to discharge. If lufenuron enters receiving waters, it will dissipate to the sediment phase and remain primarily within the country of use.

When treated fish are moved to marine sites, it is expected that the aqueous lufenuron concentrations in Canadian marine water will be reduced compared to that in freshwater due to larger dilution in the marine environment as well as decreasing excretion from fish on a per-day basis. Lufenuron in the marine environment would also undergo sorption to solids and partition to the sediment, remaining primarily in the country of use. On this basis it is considered that waters (fresh or marine) arising from Canadian farms using lufenuron should pose no unacceptable risk to U.S. waters.

6.0 Description of any alternatives to the proposed use

Elanco is proposing to establish a tolerance for lufenuron in salmonids imported into the U.S. for human consumption. The only alternative to the proposed action is the ‘no action’ alternative, which would be the failure to establish a tolerance for lufenuron in salmonids. However, based on our analysis in this EA, we do not believe that significant environmental impacts will occur from this action; therefore, the preferred alternative is the establishment of a tolerance for lufenuron in salmonids imported into the U.S. and the no action alternative was eliminated from consideration.

7.0 Conclusions

Based on the available information on the metabolism, environmental fate, and exposure of lufenuron, and the potential exposure pathways presented in the EA, there is expected to be little or no exposure to lufenuron residues in the U.S. Therefore, it is concluded that the proposed action of establishing an import tolerance for lufenuron residues in salmonids will not result in significant impacts to the U.S. environment.

8.0 Agencies and persons consulted

This EA was prepared with input and assistance from members of the Environmental Safety Team in the Office of New Animal Drug Evaluation in the United States Food and Drug Administration’s Center for Veterinary Medicine.

9.0 Author

John G McHenery

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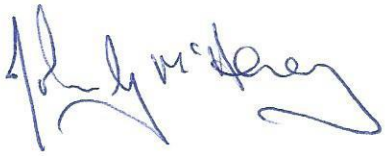
Elanco Animal Health

4002-Basel, Switzerland

10.0 Signature

The undersigned certifies that the information presented in this Environmental Assessment is to the best of their knowledge true, accurate, and complete.



Date: 5 September 2016.

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Appendix A

Drug information
Translation of Chilean registered label

IMVIXA™ 10%, Lufenuron 10% Oral powder

LABEL TEXT PROPOSAL PRIMARY PACKAGING

VETERINARY USE

IMVIXA™
LUFENURON 10%, Oral powder
Content: 2.5 kilograms

COMPOSITION:
Each kilogram of product contains:

Lufenuron	100 grams
Excipients q.s	1000 grams

INDICATION OF USE:
For the prevention and control of infestations caused by sea lice, *Caligus rogercresseyi* on farmed salmonids. To be administered orally through the feed prior transfer to sea sites. The product can be administered only in freshwater facilities with an active effluent system treatment that allow the retention of suspended solids, according to valid regulatory requirements.

SPECIES:
Salmonids.

ROUTE OF ADMINISTRATION:
Oral through medicated feed

DOSE AND FREQUENCY
Lufenuron is incorporated into a 10% premix formulation. Medicated feed is prepared through the addition of the premix to commercial fish feeds by top-coating or vacuum-coating. IMVIXA™ medicated feed is to be prepared only at authorized facilities to produce medicated feed, not at fish farming sites.
The concentration of IMVIXA™ in feed must be adjusted proportionally to the feeding rate required to achieve a lufenuron dose of 5 mg/kg/day for a total dose of 35 mg/kg in the treated fish. For example, at a feeding rate of 1% biomass/day the incorporation rate would be 5 kg premix to 1000 kg pre-transfer feed to deliver a concentration of 0.05% lufenuron in the feed. In instances when the feeding rate is lower than expected, 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.
To warrant the efficacy in preventing and controlling sea lice infestations, it is recommended to use IMVIXA™ according to the following considerations:

- Use the product in the absence of any concurrent disease or environmental condition affecting appetite.
- Prepare an appropriate amount of medicated feed to ensure complete and homogeneous consumption.
- Ensure administration of correct dose over a minimum 7 day period.
- Monitor feeding behaviour of the fish during administration.
- Transfer to sea no sooner than 7 days post-treatment.

SPECIAL WARNINGS AND PRECAUTIONS:
- For animal treatment only.
- Do not administer the product on sea water sites or lakes.
- Ensure adequate administration to all fish in the group through good animal feeding practices and do not exceed recommended treatment dose.

SPECIAL PRECAUTIONS FOR THE OPERATOR:
- Wear protective gloves, glasses and masks while handling the veterinary medicinal product and medicated feed.
- In case of accidental contact to skin or eyes, wash immediately with water.
- In case of accidental ingestion seek medical advice immediately.
- Do not eat, drink or smoke whilst handling the veterinary medicinal product or medicated feed.
- Wash hands and exposed skin after handling the veterinary medicinal product or medicated feed.

CONTRAINDICATIONS:
- Do not use when the fish show clinical signs of disease or when it is unlikely that the necessary quantity of medicated feed will be consumed to ensure the 35 mg/kg therapeutic dose.
- Do not use for treatment of salmonids in facilities that do not have an effective method of retaining suspended solids according to local and national requirements.
- Do not use in broodstock.
- Do not use in a concomitant manner with other pharmaceutical products.

WITHDRAWAL PERIOD:
Treated fish must not be slaughtered for human consumption for at least 2050 degree days after the end of the treatment with this veterinary medicine.

SHELF LIFE:
Shelf life of product as packed for sale: 24 months.
Use immediately once open and dispose remaining product.

STORAGE CONDITIONS:
Store in the original container. Keep the container tightly closed. Store between 15-30°C in a dry place.

SPECIAL PRECAUTIONS FOR UNUSED PRODUCTS OR WASTE MATERIAL DISPOSAL:
Do not discharge empty containers or with product remainings into the soil, water sources or regular waste containers. All unused product or waste material should be eliminated by authorised companies to ensure the service is performed in a secure manner.

ENVIRONMENTAL PRECAUTIONS:
Do not administer this product in fish farms located in the sea or lakes or sites located within rivers. Product use is restricted to freshwater facilities with an effluent treatment system that allows the retention of suspended solids, according to valid regulatory requirements. IMVIXA™ is environmentally safe provided that it is administered according to the authorized dose, time schedule and directions for use authorized.

Manufacturer:
Novartis Sante Animale S.A.S.
Usine de Huningue
26, Rue de la Chapelle
F-68330 Huningue
France

Imported and Distributed by:
Novartis Chile S.A.
Ruta 5 Sur Km. 1012, Puerto Varas
Telephone: 65-2231400, Fax: 65-2231411

Under License of:
Novartis Animal Health Inc.,
Basel, Switzerland.

Manufacturing date:

Expiry date:

Batch number:

Register SAG No: 2325

Sales Under withheld Veterinary Prescription.

Keep out of the sight and reach of children.

Appendix B

Executive study summaries

Commander RF. 2014. Lufenuron: Adsorption to two marine sediments. Unpublished Novartis Animal Health Report BR0853, p.57. GLP compliant.

Sponsor	Novartis (Elanco) Animal Health Inc., Park View, Riverside Way, Watchmoor Park, Camberley, Surrey, GU15 3YL, UK
Test facility, location of raw data and final report	Brixham Environmental Laboratory, AstraZeneca UK Limited, Freshwater Quarry, Brixham, Devon, TQ5 8BA, UK
Test substance common name	[^{14}C]-lufenuron
Test	Adsorption to two marine sediments
Guideline	OECD Guideline for the Testing of Chemicals. Test Guideline 106, Adsorption – Desorption using a batch equilibrium method. Adopted 21 January 2000
Nominal test temperature	12 ± 2°C

Results

Sediment	Organic carbon in sediment (%)	pH (in 0.01M $CaCl_2$)	Derived adsorption parameters (0.5 h equilibration period, 1:25 sediment water ratio)			
			Log K_F^{ads}	1/n (ads)	Log K_{Foc}	K_{Foc}
HOC	5.6	7.7	2.12	0.742	3.37	2,335
LOC	0.5	9.0	3.07	1.058	5.38	237,427

HOC:	High organic carbon
LOC:	Low organic carbon
Log K_F^{ads}	Freundlich adsorption coefficient $\mu g^{1-1/n}$ $(mL)^{1/n}/g$
1/n (ads)	The regression constant, 1/n, generally ranges between 0.7–1.0, indicating that sorption data are frequently slightly nonlinear
K_{Foc}	The organic carbon Freundlich linearized adsorption coefficient

Discussion

Adsorption of [^{14}C]-lufenuron to sediments occurred very quickly, with equilibrium being reached within 30 minutes in all test systems.

The adsorption characteristics of [^{14}C]-lufenuron differed among the two sediment types investigated. After a 30 minute equilibration period with 1:25 sediment:water ratio, the Freundlich adsorption coefficients (K_F^{ads}) were 131 and 1,187 for the HOC and LOC sediment, respectively. The derived K_{Foc}^{ads} values were 2,335 and 237,427 for the HOC and LOC sediment, respectively. These K_{Foc}^{ads} values indicated that [^{14}C]-lufenuron can be considered slightly mobile in the HOC sediment and immobile in the LOC sediment.

Ellgehausen H. 1990. Leaching model study with CGA 184699 in four soils. Unpublished Ciba-Geigy, Basel Report, PR 25/90, p.34. GLP compliant.

The leaching behaviour of the experimental insecticide lufenuron (CGA 184699) was studied in a soil column model experiment and compared to a reference substance, monuron, which is known to be a moderate “leacher” in the field.

The mobility of the test substance was investigated in four different soils, i.e. in two sandy and two silt loam soils. For this purpose, the ^{14}C-labelled test substance was applied onto the top of 30 cm soil columns and thereafter submitted to artificial rain of 200 mm within 48 hours. Results were compared with those of monuron tested on the same columns simultaneously.

The penetration depth of lufenuron into the soil profile ranged between 2 and 8 cm. The mobility of the test substance was found to be one fourth of the reference substance (relative mobility of lufenuron related to monuron: <0.28).

Only negligible amounts of lufenuron (0.04-0.58% of the dose applied) were found in the leachates. The recovery of lufenuron and monuron in the leaching experiments was, on average, 103.5 and 89.3%, respectively.

Based on these findings, lufenuron can be considered as having little mobility in the soil.

Ellgehausen H. 1991a. Leaching characteristics of aged soil; residues of CGA 184699 in two soils after percolation of 508 mm artificial rain. Unpublished Ciba-Geigy, Basel Report, PR 11/91, p.54. GLP compliant.

The present experiments were designed to obtain information on the leaching behaviour of lufenuron, i.e. N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylaminocarbonyl]-2,6-difluorobenzamide, and its soil metabolites.

For this purpose a loamy sand and a loam were treated separately with lufenuron ^{14}C-labelled in the dichlorophenylring (lufenuron A) or in the difluorophenylring (lufenuron B). The treatment corresponded to the maximum recommended field rate for pesticide use of 100 g a.i./ha. Thereafter, treated soil samples were aged for 30 days in a metabolism apparatus and then an aliquot applied onto the top of untreated soil columns filled with the same soil types. Artificial rainfall of 11.3 mm was daily applied onto the soil columns over a period of 45 days giving a total amount of 508 mm. Daily eluates were collected. Amounts and nature of radioactivity in daily eluates and in soil layers after percolation were determined.

Besides non-extractable radioactivity and small amounts of ^{14}C-CO_2, the primary degradation products of lufenuron were CGA 238277, i.e. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylurea, and CGA 224443, i.e. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-aniline, after aerobic incubation of lufenuron A. Lufenuron B was primarily broken down into non-extractable radioactivity and ^{14}C-CO_2 showing practically no other degradates.

Leaching of aged soil residues clearly showed the immobility of the parent compound and of CGA 238277 in both soils, i.e. both compounds were only found in the top 2 cm of the soils. CGA 224443 was also found to be practically immobile since it was mainly found in the top layer of the soils and to a small extent in the adjacent soil layer. These results are confirmed by analysis of total leachates, which only contained minor amounts of radioactivity (0.6-1.2%).

Ellgehausen H. 1991b. Degradation of CGA 184699 in soil under aerobic, aerobic/ anaerobic and sterile/aerobic conditions. Unpublished Ciba-Geigy, Basel, Report, PR 37/90, p.77. GLP compliant.

[^{14}C]-lufenuron (CGA 184699) labelled in the dichlorophenylring or in the difluorophenylring was incubated in separate studies in a loamy sand and loam soil under aerobic, aerobic-anaerobic and aerobic sterile conditions in the laboratory under controlled conditions. The objectives of the studies were to elucidate the rate of degradation of the molecule and to determine its metabolic pathway.

The study showed that lufenuron was rapidly broken down in soil under aerobic conditions with half-lives of 13 to 23.7 days. Furthermore, it was clearly demonstrated that both ring moieties were mineralized to carbon dioxide representing the most prominent metabolite of the difluorophenylring-labelled molecule (with 58.6% after one year). For the dichlorophenylring-labelled compound the corresponding figure ranged from 9.9 to 15.1%. Non-extractables (both labels), CGA 238277, i.e. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylurea, and CGA 224443, i.e. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-aniline, the latter compounds representing degradation products of the dichlorophenylring-labelled parent compound, are considered to be transient, aerobic degradation products of the microbial breakdown of lufenuron. CGA 238277 and CGA 224443 amounted at highest to 23.1-24.3% after 14 days and 21.6-26.9% after 59 days, respectively. At the end of the study their corresponding amounts were 1.6-2.7% and 4.1-5.2%. Non-extractables were highest after 240 and 60 days with 70.7-78.6% and 36.1% for the dichlorophenyl- and difluorophenyl-labelled compound, respectively. After one year their corresponding amounts were 66.8-74.9% and 28.3%. Therefore, the molecule was shown to degrade or become non-extractable in soil with time.

Ellgehausen, H. 1992. Adsorption/desorption CGA 184699 in various soil types. Unpublished Ciba-Geigy, Basel, Report, PR 6/92, p.47. GLP compliant.

The adsorption and desorption of lufenuron (CGA 184699) was studied in various soil types and compared to other pesticides.

The Freundlich adsorption constants K_F^{ads} varied between 166 µg/g of soil in soil Les Evouettes and 2,350 µg/g of soil in soil Illarsaz (humic soil). The mean adsorption constant normalized to organic matter (Q) was 22,482 µg/g OM and the mean adsorption constant corrected for the organic carbon content (OC), $K_{oc,}$ was stated in the report as 38,756 but recalculated herein as 41,182 µg/g OC. These values characterize lufenuron as a very strongly adsorbing compound like fluorodifen and parathion.

The desorption data of lufenuron showed that adsorption was partially irreversible. Desorption constants determined in the study ranged from 219 to 4,017 µg/g of soil after the first desorption step and from 157 to 7,026 µg/g of soil after the second desorption step.

Recoveries ranged from 86.30 to 102.96% of the dose applied. Analysis of soil extracts and aqueous phases after adsorption of the highest test concentration showed, beside the known impurities, no degradation products.

Geoffroy A. 1992 (Amended 1994). CGA 189633 Report on vapour pressure curve. Unpublished Ciba-Geigy, Basel, Report, Test Number AG 91/16P.VPC, p.9. GLP compliant.

The vapour pressure was determined by the gas saturation method in accordance with OECD guideline 104. The vapour pressure at 25°C was determined as $<4x10^{-6}$ Pa by comparison of the values obtained with those for hexachlorobenzene and those given in the guideline. The flow rate of the carrier gas , nitrogen, was varied to show that equilibrium was obtained and that trapping of the substance in the -26°C

condenser was complete. The lufenuron trapped in the condenser was quantitatively dissolved in organic solvent and analyzed by HPLC with UV detection which could discriminate between lufenuron and potential degradation products.

Gonzalez-Valero J. 1994. Metabolism of CGA 184699 under aerobic conditions in aquatic systems. Unpublished Ciba-Geigy, Basel, Report, PR 8/94, p.87. GLP compliant.

Two series of two different natural sediment/water systems were either treated with [^{14}C]-dichlorophenylring-labelled lufenuron or [^{14}C]-difluorophenylring-labelled lufenuron at a treatment rate of about 0.14 mg/L of water and incubated under aerobic conditions in aquatic model systems in the laboratory. The objectives of these experiments were to elucidate the rate of degradation of lufenuron, to determine the rates of dissipation for 50 and 90% of the test substance under these conditions, and to determine its fate in the aquatic environment.

The study has shown that lufenuron very rapidly disappeared from the treated water with a DT-50 of less than 1 day and a time to reach 90% dissipation (DT-90) of less than 3 days. Degradation half-lives were rapid for the Pond aquatic system and slower for the Rhine aquatic system. The degradation results for lufenuron in the aquatic system can be summarized as follows:

Aquatic system	Dichlorophenylring label		Difluorophenylring label		Average of both labels	
	Degradation half-life (days)	Time to reach 90% degradation (days)	Degradation half-life (days)	Time to reach 90% degradation (days)	Degradation half-life (days)	Time to reach 90% degradation (days)
Pond	27.4	805	52.5	448	37.9	550
Rhine	159	529	187	622	172	574

The recovery of radioactivity was on average 96.8 ± 2.6 and 99.1 ± 3.12% for the Pond and Rhine aquatic systems, respectively, treated with dichlorophenylring-labelled lufenuron. The corresponding figures for the systems treated with difluorophenylring-labelled lufenuron were 97.8 ± 2.43 and 96.7 ± 3.13%, respectively.

Degradation of dichlorophenylring-labelled lufenuron was microbially degraded into CGA 238277, i.e. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenylurea, and subsequently into CGA 224443, i.e. 2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-aniline. Both metabolites were transient by nature reaching in the biologically more active Pond system their highest concentration with 47.5 and 26% after 59 and 120 days, respectively. At the end of the study (360 days) their corresponding amounts were only 6.3 and 17.4% of the radioactivity applied. Calculated dissipation half-lives were for the corresponding metabolites in the range of 9-11 and 24-36 days for CGA 238277 and CGA 224443, respectively. In addition to these major extractable metabolites only one minor metabolite, M5, i.e. N-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy)-phenyl]-N-methylurea (only in Pond system with 6.7% at highest), and non-extractable radioactivity was formed in large amounts reaching at the end of the study 43.9%.

In the Rhine aquatic systems the amounts of the corresponding metabolites were found to be lower reaching their highest concentrations with 19.8% (CGA 238277) and 12.8% (CGA 224443) after 120 and 182 days, respectively. Here again, large amounts of NER were found amounting after 360 days to 40.3%. Finally, small amounts of ^{14}C-CO_2, 1.5-4%, were formed indicating a low but significant mineralization of the dichlorophenylring moiety.

For the difluorophenylring-labelled test compound mainly ^{14}C-CO_2 and bound residues were observed besides negligible amounts of extractable metabolites. At the end of the study, carbon dioxide amounted to 49.4 and 40.0% in the Pond and Rhine aquatic systems, respectively. The bound residues amounted to 30.2 and 20.7% in the Pond and Rhine aquatic systems, respectively. These figures demonstrate the pronounced mineralization of the difluorophenylring moiety in aquatic systems. Harsh extraction of sediments from incubation day 360 liberated 9.7 and 9.3% of the dose applied for Pond and Rhine sediment, respectively. However HPLC and TLC of these extracts demonstrate that only matrix radioactivity was extracted by this procedure, showing no discrete peaks on the chromatograms.

In conclusion, lufenuron was rapidly broken down at the beginning of the study in the Pond aquatic system with high microbial biomass. In the Rhine aquatic system with lower microbial biomass a slower degradation was observed. Thereafter, lower degradation rates were observed for both systems. Finally, the formation of ^{14}C-CO_2 from both radiolabelled moieties demonstrates the mineralization of lufenuron with time.

Hobbs G. 2014. The metabolism, excretion and residue depletion of [^{14}C]-Lufenuron in the Atlantic salmon (*Salmo salar* L.). Charles River: 34776, p.148. GLP Compliant. Unpublished Elanco Animal Health Report.

The aim of the study was to determine the distribution and extent of metabolism, excretion and residue depletion of lufenuron in the edible tissue of the Atlantic salmon (*Salmo salar L*.), following a 7-day feeding period to simulate potential consumer and environmental exposure.

Fish diet fortified with [^{14}C]-lufenuron was administered to a single group of fish at a target dose of 5 mg lufenuron/kg body weight/day over a 7 day period. The achieved dose rate, taking into account uneaten feed during the administration period, was calculated to be 5.34 mg lufenuron/ kg estimated body weight per day.

Ten whole fish were taken at 1 day, 30 days, 90 days and 178 days after the end of treatment and were euthanized with a blow to the head. Samples of skin plus muscle in natural proportions (known as fillet) were taken as well as hindgut contents, liver and the residual carcass. Feces were collected during the treatment period and throughout the live phase. At each sampling point three fish were taken, euthanized as above, flash frozen at the time of sampling to reduce damage from ice crystals and retained whole. These whole fish from 35 days, 90 days and 178 days after the end of treatment were imaged by quantitative whole-body autoradiography (QWBA).

Sub-samples of the fish samples were taken for initial total radioactive residue (TRR) determination employing sample oxidation with LSC analysis. Representative combined samples of fillet and feces were extracted with acetonitrile and the extractable residues analyzed by HPLC and TLC to determine the nature of the residues.

Tissue distribution of TRR was determined by QWBA of the whole fish samples. Sagittal sections were taken to ensure analysis of all major organs and tissues.

A summary of the TRR in selected organs and tissues following oral administration of [^{14}C]-lufenuron to Atlantic salmon is presented below:

Sample Point	Fillet (mg/kg)	Liver (mg/kg)	Residual carcass (mg/kg)	Hindgut content (mg/kg)
1DAT	24.828	33.946	34.832	46.936
30DAT	14.274	15.841	26.071	11.072
90DAT	10.545	13.200	18.067	7.264
178DAT	2.658	3.531	5.321	2.152

TRR were readily extracted from both fillet and fecal samples (≥91.2% TRR). No metabolism of lufenuron in Atlantic salmon (*Salmo salar* L.) was detected over the 6-month period. Residues in the fish decrease over time and are excreted as lufenuron in the feces.

QWBA analysis of the salmon indicates the concentration of residues in the fat of the fish.

Reischman F-J.1995. Volatilization of CGA 184699 from water (Calculation). Unpublished Ciba-Geigy, Basel, Report, 95RF08, p.9. GLP compliant.

The calculation was undertaken according to Prufung des Verhaltens von Pflanzenbehandlungsmitteln im Wasser, Merkblatt Nr. 55. Tiel I und II of the BBA 1980. From the relationships detailed in the guidance the volatility which is described by the Henry's Law Constant was determined from the data:

	Dimension	Value
Molecular weight (M)	g/Mol	511.16
Solubility (S)	Mg/L = g/m^3	0.1
Vapor pressure (p)	Pa	1.30E-07
Gas constant (R)	$(Pa*m^3)/(Mol*K)$	8.3143
Temperature (T)	K	293.15

And the relationships:

	Formula	Dimension
Molar solubility (S)	S = L/M	Mol/m^3
Henry's constant (H)	H = p/S	$Pa*m^3/Mol$
C–water/C-air	C–water/C-air = (R*T)/H	$(mg/L)/(\mu g/cm^3)$

Such that:

M	S	p at 20°C	H	C–water/C-air
511.2	1.00E-01	1.30E-07	6.65E-04	3.67E+06

Comparison of the results for lufenuron with those for other chemicals indicate that it is in the low volatility range.

Rodler M. 1992. (Amended 1994). Report on octanol/water partition coefficient. Unpublished Ciba-Geigy, Basel, Report, EA 165165, p.13. GLP compliant.

The test was conducted according to the OECD 117 HPLC method with a Nucleosil C_{18} column at 25°C, an acetonitrile:water/60:40 mobile phase and a diode array detector. A reference solution containing seven standards and a test solution of lufenuron were prepared. The reference solution was injected followed by two injections of the test solution with the procedure repeated twice. From the three injections

of the reference solution a plot of log k versus log P was prepared using least squares linear regression and the log k values from the six runs of lufenuron were used to determine the corresponding log P_{OW} values with an average for the six runs of:

log P_{OW} = 5.12 (±0.14, standard deviation)

Volz E. 2003. Fish bioaccumulation and fate of [Difluoro-phenyl- U-^{14}C] CGA 184699 EC050 (A7814A) in outdoor microcosms. Unpublished Syngenta Crop Protection Report 2012657, p.203. GLP compliant.

Lufenuron (CGA184699) bioaccumulates in fish under maintained exposure conditions in the laboratory (bioconcentration factor = 5,300). However, its lipophilic nature also means that it is lost rapidly from the water phase in water-sediment systems, and adsorbs to the sediment, most likely thereby reducing its bioavailabilty. Consequently under field conditions where exposure will be rapidly reduced, it is likely that bioaccumulation potential indicated from laboratory studies may significantly overestimate bioaccumulation in the field. To investigate this hypothesis, the amount and distribution of residues of lufenuron and its metabolites in water, sediment, plants and fish were measured under simulated field conditions.

Bluegill sunfish (*Lepomis macrochirus*) were added to 759 L aquatic microcosms (water depth 30 cm, sediment depth 10 cm), which contained established communities of phytoplankton, zooplankton, macroinvertebrates and plants. Thus the potential for food chain accumulation via water, sediment and food routes could be evaluated.

Three microcosms were established. Two were used to evaluate bioaccumulation at two treatment levels (erroneously labelled 'bioconcentration tanks' as the exposure pathways included water sediment and food) and a third was used to investigate the fate of lufenuron in water, sediment and plants ('fate tank').

Difluoro-phenyl-U-^{14}C labelled lufenuron was added to an emulsifiable concentrate blank formulation (A7814A) in order to facilitate spray application.

On 16 July 2002, the two bioconcentration tanks were sprayed once at rates equivalent to 1.5 and 15 g lufenuron/ha. These two treatments resulted in nominal water-phase concentrations of 0.5 and 5 µg lufenuron/L, respectively. The fate tank was also treated once at the 5 µg/L rate. Fish from the bioconcentration tanks were sampled up to 105 days after treatment (DAT). Water and plant samples from the fate tanks were taken at intervals following the application up to 105 DAT. Sediment samples were removed from the fate tank at intervals until 190 DAT.

The distribution of lufenuron was determined initially by measuring the total radioactive residues (TRR) in various matrices. The TRR was then characterized in order to identify how much of the measured radioactivity was attributed to lufenuron or its primary (CGA238277, CGA149772) or secondary (CGA224443) metabolites. These analyses showed that lufenuron was rapidly and extensively degraded in the test system.

The TRR in the water phase (expressed as lufenuron in µg/L) decreased from 5.14 µg/L (103% of nominal) one hour after treatment to 0.09 µg/L (1.8% of nominal) at 105 DAT. Immediately after application, total radioactivity dissipated rapidly and already at 1 DAT only half of the nominal initial concentration was found. Thereafter, the dissipation of TRR slowed, indicating two-phase mechanism. The median dissipation (DT-50), calculated by non-linear regression, was 18 hours. Approximately 80% of TRR in the water were dissipated by 11 DAT and about 90% by 20 DAT (DT-90 by non-linear regression).

Characterization of the TRR demonstrated that degradation of lufenuron in the water was extremely rapid. One hour after treatment, the concentration of lufenuron was 0.48 µg/L. The concentration of lufenuron continued to decrease rapidly and by 7 DAT, lufenuron was no longer detectable.

The extensive degradation of the parent resulted in the rapid formation of the two primary metabolites CGA238277 and CGA149772. At one hour after treatment, the concentrations reached a maximum of 3.83 µg CGA238277/L (106% of nominal concentration corrected for the relative molecular weights of the parent and metabolite) and 0.13 µg CGA149772/L (8.2% of nominal concentration corrected for the relative molecular weights of the parent and metabolite) in the water phase. The concentrations of the metabolites declined rapidly from this point onwards. CGA238277 was only measured until 28 DAT, whereafter it was no longer detected. Its degradation product CGA224443 was measured at low concentrations (0.08 µg/L) between 7 and 28 DAT, whereafter it was no longer detected. The DT-50 values of CGA184699 and CGA238277, determined by non-linear regression were 15 minutes and 21 hours, respectively. The DT-90 values of CGA184699 and CGA238277, determined by non-linear regression were 1 hour and 32 days, respectively. The other primary metabolite CGA149772 was degraded extremely rapidly and was no longer detected at 2 DAT.

The TRR in sediment (expressed as lufenuron equivalents in µg/kg) increased from 0.35 µg/kg (3.5% of nominal calculated using the mass of applied radioactivity and the mass of the sediment) at three hours after treatment to a maximum value of 3.85 µg/kg (38%of nominal) at 64 DAT. After this peak, total radioactivity dissipated with a DT-50 and DT-90 of 19 and 163 days, respectively. By 190 DAT, the TRR in the sediment had declined to 0.11 µg/kg (1.1% of nominal).

Characterization of the measured radioactivity revealed that the concentration of lufenuron in sediment reached a maximum value of 1.687 µg/kg (17% of nominal) at 28 DAT and declined thereafter to <LOQ at 190 DAT with DT-50 and DT-90 values of 63 and 208 days, respectively. The metabolites CGA238277 and CGA224443 appeared in the sediment between 7 and 105 DAT at concentrations of up to 0.430 µg/kg (5.9% of nominal correcting for molecular mass) and 1.260 µg/kg (20% of nominal), respectively. By the end of the study, no residues of lufenuron or its metabolites were detected in sediment.

A summary of the results for water and sediment analysis is presented below:

	Max. Measured Values [% Nom.]				
	TRR	Lufenuron (CGA184699)	CGA238277	CGA224443	CGA149772
Water (max. measured value- % nominal)	103	10	1	2.6	8.2
DT-50	18 h	15 min	21 h	nd	<2 d
DT-90	20 d	1 h	32 d	nd	nd
Sediment (max. measured value- % nominal)	38	16.8	5.9	19.5	-
DT-50	49 d	63 d	nd	nd	nd
DT-90	163 d	208 d	nd	nd	nd

nd = not determined due to insufficient data

The TRR (expressed as lufenuron equivalents in mg/kg) in plants increased from 3.74 mg/kg at the start of the exposure (3 hours after application) to a maximum of 8.12 mg/kg at 3 DAT and decreased to 3.05 mg equivalent/kg by 105 DAT. The concentration of lufenuron in plants reached a maximum value of 10.6 mg/kg at 1 DAT and declined thereafter again to 0.064 mg/kg at 105 DAT. The metabolite CGA238277 appeared in plant extracts between day 1 and 64 of exposure. CGA224443 was detected between day 3 and day 28. CGA149772 was only found in plant extracts at 28 DAT.

Radioactivity was taken up rapidly by the fish. The TRR reached a maximum concentration (expressed as lufenuron equivalents in mg/kg) of 0.147 mg/kg (whole fish) and 1.8 mg/kg at 7 DAT in the 0.5 µg/L and 5.0 µg/L treatments, respectively. TRR were then continuously eliminated from the fish tissues with DT-50 values of 45 and 42 days for treatment concentrations of 0.5 µg/L and 5.0 µg/L, respectively. The DT-90 values, determined by non-linear regression, were 130 and 121 days for treatment concentrations of 0.5 µg/L and 5.0 µg/L, respectively. More than 80% of the bioconcentrated radioactivity was eliminated by 56 and 65 days after the peak tissue concentration in the 0.5 µg/L and 5.0 µg/L treatment concentrations, respectively.

The amount of TRR that could be extracted by solvents from fish tissues was on average 90% at 14 DAT and 51% at 105 DAT. All the extracted radioactivity was identified as lufenuron. None of the metabolites described above were detected, with the exception at day 14, where 0.028 mg/kg CGA238277 (2.2% of total parent equivalents) and 0.015 mg/kg of CGA224443 (1.3% of total parent equivalents) were measured in fish tissues of bioconcentration tank dosed at 5.0 µg/L. Total radioactivity was used to calculate biota accumulation factors (BAF). This means that BAF estimates made at later time points in the study are conservative because they include non-extractable radioactivity, which is most likely ^{14}C which has been incorporated into the fish tissue rather than lufenuron or its metabolites.
One hour after application, the average BAF in whole fish was 8. The BAF increased to reach a maximum value of 327 at 7 DAT. From 7 DAT onwards, the BAF fell continuously to an average value of 66 at 105 DAT.