



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee for Medicinal Products for Veterinary Use (CVMP)

European public MRL assessment report (EPMAR)

Lufenuron (fin fish)

On 12 September 2014 the European Commission adopted a Regulation¹ establishing a maximum residue limit for lufenuron in fin fish, valid throughout the European Union. This maximum residue limit was based on the favourable opinion and the assessment report adopted by the CVMP.

Lufenuron is intended for use in fin fish for the control of sea lice infestations as a premix formulation.

Novartis Animal Health Inc (Switzerland) submitted the application for the establishment of maximum residue limits to the European Medicines Agency, on 6 March 2013.

Based on the original and complementary data in the dossier, the CVMP recommended on 12 December 2013 the establishment of maximum residue limits for lufenuron in fin fish.

Subsequently the Commission recommended on 19 March 2014 that a maximum residue limit in fin fish is established. This recommendation was confirmed on 9 April 2014 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 12 September 2014.

¹ Commission Implementing Regulation (EU) No 967/2014, O.J. L 272, of 13.09.2014



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Lufenuron
Therapeutic class:	Antiparasitic agents / Agents acting against ectoparasites
Procedure number:	EMEA/V/MRL/003749/FULL/0001
Applicant:	Novartis Animal Health Inc (Switzerland)
Target species:	Atlantic salmon and Rainbow trout
Intended therapeutic indication:	Control of sea lice infestations
Route(s) of administration:	In-feed use

1. Introduction

Lufenuron is a benzoyl phenylurea characterised as an insect development inhibitor.

Lufenuron is intended for use in salmonidae for the control of sea lice infestations as a premix formulation. The proposed treatment is by feeding over a 7-day period to juvenile fish prior to sea transfer at a maximum dose of 10 mg/kg bodyweight (bw).

In veterinary medicine lufenuron has been approved as an insecticide for the control of flea infestations in dogs and cats. It is given orally at a dose of 10 mg/kg bw and 30 mg/kg bw once a month in dogs and cats respectively. Additionally a suspension for subcutaneous administration is available for the treatment of cats.

Lufenuron is used as a pesticide in crop protection. It is registered in France, Portugal, Spain, Italy and Greece for use in grapes, tomato, artichoke, apple, aubergine, peppers, brassica heads, citrus fruits, cucumbers, peach, pear, potato and strawberry.

2. Scientific risk assessment

2.1. Safety assessment

The structure of lufenuron is similar to teflubenzuron and diflubenzuron, which are used for the treatment of sea lice infestation in salmon. Teflubenzuron and diflubenzuron were assessed by the CVMP in 1999 and MRLs for muscle and skin have been established (EMEA/MRL/547/99-FINAL; EMEA/MRL/621/99-FINAL).

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties

A literature review on the mode of action of acyl ureas shows this substance class acting as chitin synthesis inhibitors. Diflubenzuron as a member of the group has been shown to inhibit the process of incorporation of N-acetylglucosamine into insect chitin by depolarisation of the transport vesicle membranes. A comparable mode of action is assumed for lufenuron. The use of lufenuron for the control of fleas in dogs and cats shows, that female fleas feeding on treated animals will produce unviable eggs. The same inhibitory mechanism was found in salmon lice in a field study.

The general pharmacology of lufenuron in rabbits, rats and mice was briefly summarised in a published Japanese paper. Lufenuron did not induce significant pharmacological effects in rabbits after intravenous application. In mice the substance caused persistent behavioural changes and reduced mobility at doses of 500 mg/kg bw and higher. Slight alterations in the rotor rod in the high dose groups (1250 mg/kg bw) were seen.

In male rats occult blood was observed in urine at subcutaneous doses of 500 mg/kg bw and higher. In both sexes inconsistent changes in urine composition regarding sodium and potassium ion discharge were seen.

Pharmacokinetic properties

Several pharmacokinetic studies were conducted in mammals (rat, dog, goat), poultry (hen) and fish (bluegill, fathead minnow). Most of these studies used radiolabelled lufenuron and were designed to investigate absorption, distribution, metabolism and excretion (ADME) of lufenuron in laboratory animals as well as uptake, depuration, metabolism and biodegradation of lufenuron in fish.

Pharmacokinetic properties of lufenuron were comparable in all species. [¹⁴C]-lufenuron was only moderately absorbed following oral uptake and was retained mostly in the body fat and to a lower extent in liver, skin and muscle. Maximum blood levels were reached after 8 hours. The decrease in blood levels was slow with long half-lives (323 hours and 20 days in female rats and beagle dogs, respectively). Main radioactivity was found in liver, kidney, heart and lungs and to a lesser extent in the brain, primarily in the pituitary and pineal glands.

The major metabolic pathway of lufenuron is almost identical in rodents, ruminants and poultry. [¹⁴C]-lufenuron was only minimally metabolised by partial degradation of the benzoyl-ureido bridge, leading to the urea derivative and difluorobenzoic acid and by cleavage of the urea bridge leading to difluorobenzamide and probably the aniline derivative, which was, however, not detected in goats and hens. Some minor uncharacterised metabolites were detected in goats (in urine, faeces and liver), in hens (in excreta, kidney and egg white), in the faeces of dogs and in rat plasma. In bluegill sunfish the only residue present was the parent compound. In fathead minnow 91 to 96% of the residues were characterised as lufenuron. The remaining substances were not further characterised.

The major route of elimination was *via* faeces (50 to 80%) and to a far lesser extent urine (1 to 2%). Additional important routes of elimination were milk in lactating goats and egg yolk in laying hens.

No specific pharmacokinetic and metabolism studies have been conducted in the target species (salmon and trout). However, the behaviour of lufenuron has been found to be comparable across mammals and birds, and also the studies conducted in bluegill sunfish and fathead minnow did not reveal significant differences in the kinetics and metabolism of the substance compared to mammals and birds.

2.1.2. Calculation of pharmacological ADI, if relevant

Based on the evidence provided it can be concluded that establishment of a pharmacological ADI is not required since lufenuron is considered not pharmacologically active in humans.

2.1.3. Overview of toxicology

Single-dose toxicity

Single oral dose toxicity studies have been performed in mice and rats showing LD₅₀ values of more than 2000 mg/kg for males and females in both species. The main signs of toxicity observed were ruffled fur, dyspnoea, curved body position, and exophthalmos. Signs of toxicity were all transient and cleared by day 11 at the latest.

Repeat-dose toxicity

Subchronic toxicity

Several oral repeated dose subchronic toxicity studies in rats, dogs and mice have been provided. All studies were performed according to GLP and in compliance with the relevant guidelines.

Rat: In an oral repeated dose toxicity study a total of 50 albino rats, five males and five females per dose group, were treated with lufenuron in the diet for 29 days at five dose levels (0, 50, 400, 3000 and 20000 mg/kg feed). The mean daily intake of lufenuron was 4.10, 30.8, 254 and 1690 mg/kg bw in males, and 4.07, 32.6, 254 and 1740 mg/kg bw in females, respectively. No deaths occurred during the study. For males treated with the high dose a slightly reduced mean body weight gain was related to decreased mean food consumption from experimental week 2 onwards. Absolute and relative thymus weights were reduced in animals treated at 3000 and 20000 mg/kg feed. The no observable effect level (NOEL) for lufenuron in this study was 30.8 mg/kg bw in males and 32.6 mg/kg bw in females.

In a 3-month repeated toxicity study rats (20 animals/sex/dose in control and high dose; 10 animals/sex/dose in remaining groups) were dosed daily in feed with lufenuron at doses of 0, 25, 150, 1500 and 15000 mg/kg feed. The mean daily intake of lufenuron was 1.60, 9.68, 101 and 998 mg/kg bw in males, and 1.70, 10.2, 103 and 1050 mg/kg bw in females, respectively. Subgroups (10 animals/sex/ dose) of the control and high dose animals were kept for an additional treatment-free recovery period of one month. Clinical signs consisted of tonic-clonic seizures and were observed from week 10 onwards at both highest feed levels. One high dose female was found dead on day 98. In animals of the two highest dose levels minor variations in mean body weight gain, blood parameters and haematology were noted. Absolute and relative liver and adrenal weights were increased in the high dose groups, but the organs showed no macroscopic or histopathologic morphological alterations. Based on clinical signs and organ weight alterations the NOEL for lufenuron in this study is 150 mg/kg feed corresponding to a mean daily intake of 9.68 mg/kg bw in males and 10.2 mg/kg bw in females.

Mice: In two 3-month repeated dose toxicity studies lufenuron was administered daily *via* feed to mice (10 animals/sex/ dose) for 3 months at doses of 0, 1000, 3000 and 9000 mg/kg feed in the first and 0, 4, 8, 20, 100 and 1000 mg/kg feed in the second study (only female mice). Due to mortality, the first study was terminated after 48 days for the mid and high doses and after 65 days for the low dose and control. The second study was terminated after 70 days in the 1000 mg/kg feed dose and after 91 days in the doses 0, 4, 8, 20 and 100 mg/kg feed respectively. Clinical signs consisted of tonic-clonic seizures, which were observed in all dose groups. There was a high mortality at 1000 mg/kg feed in the second study between days 57 and 71. Four of the animals showed tonic-clonic seizures prior to death, from week 7 onwards. A reduced mean body weight gain was noted for males of the mid dose group at week 7 and for males of the low dose group from week 8 onwards. These effects were correlated to reduced

food consumption. Macroscopical and microscopical examination did not reveal any treatment-related changes. A no-observed adverse effect level (NOAEL) could not be established in the first study. From the second study a NOEL of 100 mg/kg feed (14.5 mg/kg bw/day) was derived based on neurological disorders and mortality at 143 mg/kg bw/day.

Dog: In a repeated dose subchronic toxicity study four dogs (two animals/sex/dose) received lufenuron at doses of 200 and 50000 mg/kg feed (corresponding to approximately 10 mg/kg bw/day up to approximately 2850 mg/kg bw/day, respectively) in feed over a period of 28 days. There were no mortalities and no clinical signs observed for any of the animals. The no observed effect level in this study was the highest dose level of 50000 mg/kg feed/day (approximately 2850 mg/kg bw/day).

In a second repeated dose toxicity study beagle dogs received lufenuron at constant concentrations of 200, 3000 or 50000 mg/kg feed/day for 13 weeks in the diet. Control animals remained untreated. Each group consisted of four males and four females (low and mid dose groups) or six males and six females (control and high dose groups). The mean daily intakes of lufenuron were 7.8, 121.6 and 2023 mg/kg bw for the males and 7.9, 122.5 and 1933 mg/kg bw for the females, respectively. The only findings consisted of effects on blood chemistry and liver weights. Changes in blood chemistry at the two highest doses were: slight decreases of potassium and phosphorus concentrations, increased alkaline phosphatase activity and a moderate to marked increase of cholesterol concentration. Liver weights were increased at mid and high dose levels. There were no macroscopic or histopathological treatment-related changes. Based on the presence of elevated liver weights and the effect on blood chemical parameters, the feeding level of 200 mg/kg feed (corresponding to 7.8 and 7.9 mg/kg bw/day in males and females respectively) was considered the NOEL.

In two repeated dose toxicity studies lufenuron was administered to groups of four male and four female dogs daily in the diet for one year. Concentrations in the first study: 0, 100, 2000 and 50000 mg/kg feed corresponding to mean daily intakes of 0, 3.8, 72 and 1930 mg/kg bw/day, respectively.

Concentrations in the second study: 0, 10, 50, 250 and 1000 mg/kg feed corresponding to daily intakes of 0.31, 1.42, 7.02 and 29.8 mg/kg bw/day in males and 0.33, 1.55, 7.72 and 31.8 mg/kg bw/day in females, respectively.

In the first study three animals of the 2000 mg/kg feed dose group died (week 33) or were sacrificed moribund (week 37). In the second study one female of the 1000 mg/kg feed dose group was found dead at week 31 and one female and two males were sacrificed at weeks 28, 43 and 49, respectively. Prior to death, all affected animals showed marked clinical signs, i.e. convulsions, tremor, atactic gait, reduced locomotor activity, aggressiveness, nervousness, impeded respiration, vomiting and salivation.

Generalised convulsions occurred after at least 20 weeks of treatment. A reduction in mean body weight gain was noted in the two highest doses. Haematological changes consisted of increases in platelets from 1000 mg/kg feed onwards. Dose related increases in cholesterol, phospholipids, alkaline phosphatase and alanine aminotransferase were noted in both high dose groups. Increased liver, adrenal and thyroid weights were noted in all treatment groups. Treatment-related lesions were noted in the liver, thyroid, adrenals and lungs. Hepatocytic alterations were present from 250 mg/kg feed onwards. Minimal to slight follicular dilatation with increased eosinophilic staining of the colloid in the thyroid was noted in animals from all treatment groups in the first study, these findings were not reported in the second study. The adrenals of animals from the two highest dose groups showed cortical hyperplasia. Furthermore intra-alveolar histiocytosis was observed in the lungs of the animals of the two highest dose groups. In the dose group of 1000 mg/kg feed mean thymus weight was slightly decreased, a minimal to moderate hypocellularity (depletion of lymphocytes) of Peyer's patches was present in the small intestine and phagocytic cells as well as hypocellularity of lymphatic tissue were seen in the mesenteric lymph node. In this group also moderate to marked thymic atrophy and foamy histocytes (alveolar foam cells) were present with increased incidence and severity. A clear NOEL could not be derived from the first study.

From the second study a NOEL of 50 mg/kg feed, equivalent to daily doses of 1.42 and 1.55 mg/kg bw for males and females, respectively, can be derived due to histopathological changes in the liver at 250 mg/kg feed.

Chronic toxicity

One combined 24-month carcinogenicity and chronic toxicity study in the rat was provided.

A total of 800 albino rats (80 animals/sex/dose) were administered lufenuron *via* the diet at doses of 0, 5, 50, 500 and 1500 mg/kg feed corresponding to 0, 0.19, 1.93, 20.4 and 108 mg/kg bw in males and 0, 0.23, 2.34, 24.8 and 114 mg/kg bw in females. No treatment-related mortalities were observed. Whole body tonic-clonic convulsions occurred with earliest onset at week 6 in the majority of males and females at the two highest dose groups, rats of the highest dose group were sacrificed in week 14. Additional clinical signs consisted of vaginal discharge in females, higher incidence of reddened/swollen eyelids, often associated with eye exudate, body weight changes and minor changes in plasma protein, albumin, plasma potassium and inorganic phosphorus levels in females. These changes were observed mainly in the two highest dose groups and usually resolved after several weeks. No treatment-related organ weight changes were observed. Higher incidences of mottled lungs were seen in males and females at 500 mg/kg feed at macroscopy. Histopathology revealed a higher incidence of aggregations of pulmonary alveolar foam cells in both sexes of the two highest dose groups, in association with a dilatation of the right heart ventricle in some females. Minor changes like ulcerative and inflammatory lesions in the non-glandular stomach (both sexes), increased incidence of fatty change of the perilobular region of the liver (females) and inflammation of the female urinary tract were observed at 500 mg/kg feed. Additionally, focal haemorrhagic, necrotic, ulcerative, and inflammatory lesions were seen in caecum and colon in the two highest dose groups in both sexes. A NOEL of 50 mg/kg feed, corresponding to an average daily intake of approximately 2 mg/kg bw was established.

Reproductive toxicity, including developmental toxicity

One oral two generation reproductive toxicity study was performed in the rat. In two successive generations (P and F1), young adult male and female rats were continuously exposed to lufenuron in the diet at concentrations of 0, 5, 25, 100 or 250 mg/kg feed. Mean daily intake in males was initially approximately 0, 1, 3, 10 and 30 mg/kg bw. Intake in females was similar until the lactation period, when, due to increased food intake, the intake nearly doubled (0, 1, 5, 20 and 50 mg/kg bw).

P Generation and F1 offspring

The only effect noted in the F1 generation was a minimal delay of the surface righting reflex (by about 0.2 days) in the high dose group relative to controls.

F1 Generation and F2 offspring

There were no mortalities in F1 adults. The incidence of skin wounds/crust on head/trunk was increased in the male high dose group. Body weights during the premating period in high dose animals were significantly higher than in controls. Male and female mating and fertility indices, maternal gestation and parturition indices and duration of gestation were unaffected by treatment. In F2 offspring the only treatment related effect noted was a delay of the surface righting reflex in the high dose group by about 0.4 days relative to the controls. There were no treatment-related observations at gross necropsy in F1 adults or F2 offspring. Organ weights were higher in high dose males and females, consistent with the increased body weights in this group. There were no treatment-related histopathological changes in the reproductive system or in any other organs examined in the F1 adult animals.

The reproduction and parental NOAEL are 250 mg/kg feed (20 mg/kg bw), the highest dose tested.

The offspring NOEL is 100 mg/kg feed (5 mg/kg bw) based on a minimal delay in the emergence of the surface righting reflex in both generations at the highest dose of 250 mg/kg feed (20 mg/kg bw).

Developmental studies were performed in two species, rats and rabbits.

Rat: Lufenuron was given by oral gavage to groups of 25 mated female rats at dosages of 0, 100, 500, or 1000 mg/kg bw/day from day 6 through day 15 of gestation (period of 10 consecutive days). Minimal maternal toxicity was observed at 1000 mg/kg bw/day indicated by a short period (gravid days 6 to 9) of slightly reduced body weight gain and food consumption. No embryo foetal toxicity was seen at any dose level. The mean number of malformed live foetuses after treatment with lufenuron was similar to controls. Thus, the NOEL was 500 mg/kg bw/day for maternal toxicity and 1000 mg/kg bw/day for developmental toxicity.

Rabbit: Lufenuron was given by oral gavage to groups of 16 mated female rabbits at dosages of 0, 100, 500, or 1000 mg/kg bw/day from day 7 through day 19 of gestation (period of 13 consecutive days). No signs of maternal toxicity, embryo-foetal toxicity or teratogenicity were observed at any dose level. Thus, the NOEL for maternal and embryo-foetal toxicity, and teratogenicity was 1000 mg/kg bw/day in this study.

Genotoxicity

Lufenuron was tested for its mutagenic potential in a comprehensive set of *in vitro* and *in vivo* studies according to GLP and current OECD guidelines: A bacterial reverse mutation test (Ames test), a chromosome aberration test (Chinese hamster ovary cells), an *in vivo* micronucleus test and five unscheduled DNA synthesis tests (three *in vitro* and two *in vitro/in vivo*). All tests were negative and lufenuron can be considered as non-mutagenic.

Carcinogenicity

One carcinogenicity study was performed in albino mice for 18 months in accordance with OECD Guideline 451. A second study on carcinogenicity was conducted in albino rats according to OECD Guideline 453.

A total of 600 Albino mice (60 animals / sex/ group) were administered lufenuron *via* the diet at doses of 0, 2, 20, 200 and 400 mg/kg feed. The mean daily intake of lufenuron was 0, 0.222, 2.25, 22.6 and 62.9 mg/kg bw in males and 0, 0.217, 2.12, 22 and 61.2 mg/kg bw in females, respectively. Increased mortality was seen in both sexes at 200 mg/kg feed: 36% in males and 52% in females, respectively. At 400 mg/kg feed, five males and 29 females died during the first nine weeks of the study. Therefore, all survivors of this group were sacrificed in week 9 and 10.

Tonic-clonic convulsions (spontaneous/stimulated) up to 30 seconds were observed in a number of males and females of the two highest dose groups, followed by a period of inactivity before normal behaviour resumed. No substance-related effects were recorded for food consumption. After week 54, females at 200 mg/kg feed showed slightly lower mean body weights until the end of the study. Treatment had no effects on haematological data. Mean adrenal weights and ratio of females at 200 mg/kg feed were higher than controls, but not associated with microscopic changes. In male mice, masses and nodules were found in the lungs, but without a clear dose response relation at 2 and 200 mg/kg feed. At 200 mg/kg feed in male mice, a higher incidence of single and multiple lung adenomas was observed.

In mice of both highest dose groups, hepatic lipidosis was observed in both sexes, which was partly accompanied by necrotic changes in female mice. Males treated with 200 mg/kg feed had a higher incidence of inflammatory changes in the prostate. Furthermore, cystic dilatation of the prostatic glands was increased. The elevated incidence of lung adenomas was considered to be of no relevance regarding carcinogenicity as bronchiole-alveolar adenomas are the most common type of benign lung tumour in mice, no progression to malignant growth has been observed, there was no dose dependency and the

incidence in the male controls of the present study (20%) was already higher than the upper range of the historical control data. A NOEL of 20 mg/kg feed (2.25 or 2.12 mg/kg bw for males and females, respectively) was established.

A total of 800 albino rats (80 animals/sex/dose) were administered lufenuron *via* the diet for 24 months at doses of 0, 5, 50, 500 and 1500 mg/kg feed. The mean daily intake of lufenuron was 0, 0.19, 1.93, 20.4 and 108 mg/kg bw in males and 0, 0.23, 2.34, 24.8 and 114 mg/kg bw in females, respectively. A scheduled interim sacrifice of 10 animals per sex and dose group was performed at 12 months. No treatment-related mortality was observed. Whole body tonic-clonic convulsions occurred with earliest onset at week 6 in the majority of males and females at the two highest dose groups and due to these symptoms rats of the highest dose group were sacrificed in week 14. Additional clinical signs consisted of bite marks on the tail, vaginal discharge in females, reddened/ swollen eyelides, often associated with eye exudate. These changes usually resolved after several weeks. In the two highest dose groups mean bodyweights were reduced until day 23. At 50 and 5 mg/kg feed no treatment-related changes were observed. Regarding haematology no treatment-related changes were observed. Slightly lower values for plasma protein and albumin and higher levels of plasma potassium and inorganic phosphorus were recorded for females of the high dose group. At 500 mg/kg feed, gamma-glutamyltransferase was increased in females compared to values of control animals. No treatment-related changes were observed in organ weights. Higher incidences of mottled lungs were seen in males and females at 500 mg/kg feed. Higher incidences of aggregations of pulmonary alveolar foam cells increasing in severity dependant on treatment duration were seen in both sexes in both high dose groups. These findings were associated with a dilatation of the right heart ventricle developing due to the increased pulmonary pressure in some females. Ulcerative and inflammatory lesions in the non-glandular stomach (both sexes), increased incidence of fatty change of the perilobular region of the liver (females) and inflammation of the female urinary tract were observed at 500 mg/kg feed. Additionally, focal haemorrhagic, necrotic, ulcerative, and inflammatory lesions were seen in cecum and colon at 500 and 1500 mg/kg feed in both sexes. No increases of hyperplastic or neoplastic lesions were related to the treatment. Statistically significant increases of benign interstitial cell tumours of the testes (6.25%) and of granular cell tumours of the meninges (3.75%) of males at 500 mg/kg feed were within the range of historical control data and were therefore considered not treatment-related. A NOEL of 50 mg/kg feed, corresponding to an average daily intake of approximately 2 mg/kg bw was established.

Based on the available information, it can be concluded that lufenuron has no carcinogenic potential.

Studies of other effects including immunotoxicity and neurotoxicity

Skin and eye irritation, skin sensitisation

Lufenuron was classified as non-irritating to skin and eye in albino rabbits according to the classification of Commission Directive 83/467/EEC.

A skin sensitisation test challenge with lufenuron at sites pretreated with lufenuron during the induction phase did lead to slight erythema in 4 and 9 animals 24 and 48 hours after the removal of the dressing respectively. One female showed a well defined erythema in combination with a slight oedema 48 hours after the removal of the dressing. Lufenuron is therefore classified as a moderate sensitizer in albino guinea pigs according to the maximisation grading of Magnusson and Klingman.

Neurotoxicity

In a subchronic neurotoxicity study following an OECD draft guideline on Neurotoxicity screening battery (now OECD guideline 424) lufenuron was applied orally in the diet for four months to male rats. Each dose group at concentrations of 0, 5, 25, 100 or 500 mg/kg feed (corresponding to mean daily intakes of 0.25, 1.2, 5.4 and 27 mg/kg bw) comprised 10 animals. Additionally 10 animals in the control and high dose

were investigated for reversibility of effects over a 2-month period subsequent to the treatment period. Pentylenetetrazol potentiation tests were performed at the end of the treatment and recovery period. None of the animals died of treatment with lufenuron and body weights and food consumption were not affected by treatment. Two animals of the high dose group showed clinical signs of neurotoxicity (fasciculation and tonic-clonic convulsions respectively). No treatment-related effects were noted on any of the examined neurological parameters. Motor activity was not affected. Startle habituation and mean startle amplitude were comparable for all groups. Pentylenetetrazol treatment at the end of the treatment period induced clonic-tonic seizures in all animals in all dose groups. At the end of the recovery period the mean convulsion scores at the high dose were still higher than in control animals and 6 out of 10 animals showed convulsion scores of 5 (compared with 7 out of 10 at the end of the treatment period).

In the maze learning test, mean error scores, percentage of blind alley visits, and time to complete 12 arm choices did not differ between the control and any treated group. No treatment-related macroscopic or microscopic changes were seen in the central and peripheral nervous system or in muscles.

The NOEL was 100 mg/kg feed (5.4 mg/kgbw/day) observed as functional changes in neurons based on an increased excitability of nerve cells.

Effects on the endocrine tissue in rats

To assess the impact of lufenuron on the endocrine tissue 55 female and 50 male rats were treated for three weeks with 500 and 1500 mg/kg feed corresponding to 39.4 and 30.5 mg/kg bw/day (500 mg/kg feed) and to 120.1 and 92.5 mg/kg (1500 mg/kg feed) bw/day, in males and females respectively. No deaths occurred during the study. Food consumption was unchanged, a slight reduction of body weight gain in females given 1500 mg/kg feed was noted. No influence of lufenuron on mean oestrus cycle length or the relative density of cornified and nucleated epithelial cells was found. No significant changes in estradiol, progesterone, corticosterone, aldosterone, prolactin, luteinizing hormone, follicle stimulating hormone (FSH), adrenocorticotrophic hormone (ACTH) and testosterone were noted in relation to the control group in all females and in males from the low dose group. However, a significant increase in values for prolactin, FSH and ACTH were noted in high dose males. No treatment-related macro- or microscopic findings and no organ weight changes were evident. No influence on cholinesterase activity in the plasma, erythrocytes or brain samples was observed.

Tissue levels in blood, fat and brain

Tissue levels of lufenuron were assessed in rats and dogs during the repeated dose toxicity studies and the subchronic neurotoxicity study (rats).

Rat: In the 3-month repeated dose study the concentration of lufenuron in fat increased proportionally to the dose at the three lower dose levels. At the two higher doses the concentrations were similar, indicating a saturation process at about 3000 mg/kg lufenuron/fat or 1500 mg/kg feed (100 mg/kg bw/day) respectively. The data from the neurotoxicity study (4-month) show a dose dependant increase of lufenuron levels in blood and fat. After the 2-month recovery period 61% and 25% depletion of lufenuron was demonstrated in fat and blood, respectively (comparison lufenuron concentration directly after last dose and after recovery period).

Dog: Two 1-year studies were performed. In both studies lufenuron levels in blood reached a plateau after approximately 26 weeks in all those groups. The feed concentrations of 2000 and 50 000 mg/kg feed (72 and 1930 mg/kg bw, respectively) resulted in similar tissue levels, however in the second study significantly higher tissue concentrations were seen at 1000 mg/kg feed. The blood to fat concentration ratio was approximately 1:120 and remained constant over all doses in both studies, whereas in the brains the concentrations increased dose dependently. After 52 weeks brain concentrations were similar

to those in the blood at the low dose levels (10, 50 and 100 mg/kg feed) and were up to eight times higher at the higher dose levels (250, 1000, 2000 and 50000 mg/kg feed).

2.1.4. Calculation of the toxicological ADI or alternative limit

The lowest NOEL was 1.42 mg/kg bw/day observed in male dogs in the one year repeated toxicity study and is based on signs of hepatotoxicity. This NOEL is considered the most relevant for the establishment of the toxicological ADI. The NOEL is rounded up to 1.5 mg /kg bw/day.

This conclusion is in line with the EFSA evaluation of lufenuron in 2008 (EFSA Scientific Report (2008) 189, 1-130).

Using the standard uncertainty factor of 100, accounting for inter- and intra-species variability a toxicological ADI of 0.015 mg/kg bw/day or 0.9 mg/person/day is established.

2.1.5. Overview of microbiological properties of residues

No microbiological data were provided which is acceptable as no microbiological effects are expected from a substance of this nature.

2.1.6. Calculation of microbiological ADI

As no microbiological effects are expected a microbiological ADI is not necessary.

2.1.7. Observations in humans

Lufenuron is not used in humans. Observations in humans are therefore not available.

2.1.8. Findings of EU or international scientific bodies

Lufenuron has been evaluated by EFSA in 2008 and 2011 with regard to its use as pesticide. The agreed acceptable daily intake (ADI) was 0.015 mg/kg bw/day based on the NOEL of 1.5 mg /kg bw/day for the observed signs of hepatotoxicity in a 1-year dog toxicity study and applying an uncertainty factor of 100.

2.1.9. Overall conclusions on the ADI

The overall ADI is the toxicological ADI of 0.015 mg/kg bw/day or 0.9 mg/person/day.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

No specific radiolabelled studies have been conducted in the target species (salmon and trout). Pharmacokinetic studies in bluegill sunfish and fathead minnow were available. In bluegill sunfish the only residue present was the parent compound. And in fathead minnow 91-96% of the residues were characterised as lufenuron. The remaining substances were not further characterised. However, the evidence from all studies in mammals, poultry and different fish species, suggests that lufenuron shows comparable pharmacokinetic and metabolic properties over all species tested. The studies conducted in

bluegill sunfish and fathead minnow did not reveal significant differences in the kinetics and metabolism of the substance compared to mammals and birds.

2.2.2. Residue depletion studies

A combined (non GLP) field study to investigate the efficacy and safety and residues of lufenuron as a treatment for the prevention and control of sea lice, *L. salmonis*, infestations on Atlantic salmon was provided. The description of the study is limited to the residue findings.

Young Atlantic salmon were fed with lufenuron while at sea. The active ingredient was formulated as an in-feed formulation and dosed at 3, 5 and 10 mg/kg bw/day delivered over 7 days. Fish were sampled for residues 1 and 8 days after the end of medicated feeding and at approximately monthly intervals thereafter, up to 11 months after treatment.

Lufenuron was extracted from fillets, faeces, mucus, blood, kidney, pyloric caecae and liver and tissue concentrations were determined with a validated HPLC-MS/MS method. Significant amounts were found in all sample types from treated fish and these amounts appeared to be proportional to the initial dose rates. The distribution/partition of lufenuron over the different sample types was relatively consistent, irrespective of dose rate (blood:caudal fillet:dorsal fillet:liver:kidney: 1:3:10:3:4). The lufenuron content in fillet samples is likely to arise from fat depots (which retain lufenuron) and the higher residues in dorsal fillet are presumably due to higher fat content. Lufenuron levels depleted with time in all sample types. The highest levels at the target dose of 10 mg/kg bw were 27915 µg/kg in caudal fillet (dorsal fillet not measured at day 1 after treatment and these depleted to 614 µg/kg at day 324 (last time point measured)). In dorsal filet the residue was 3177µg/kg at day 205 after treatment (first time point with measurements) and the residue depleted to 1385 µg/kg at day 324.

Selection of marker residue and ratio of marker to total residues

Based on the general metabolic behaviour of the substance in various species and metabolic data in non-target fish species, lufenuron (parent compound) is considered suitable as the marker residue.

As the metabolic data suggest that lufenuron is not significantly metabolised in any species (including fish) the ratio of marker to total residues is considered to be 1.

2.2.3. Monitoring or exposure data

Lufenuron is monitored in several EU member states in relation to its use as pesticide. No findings of non-compliance have been reported.

2.2.4. Analytical method for monitoring of residues

An HPLC/MS/MS method for determination of lufenuron, in salmon (muscle and skin in natural proportions) and trout (muscle and skin in natural proportions) is available and has been validated according to the requirements of Volume 8 of The Rules Governing Medicinal Products in the European Union. The limits of detection in salmon and trout were 5.0 µg/kg and 5.8 µg/kg respectively. The limits of quantification were 100 µg/kg for muscle and skin in natural proportions in either species (salmon and trout). The method is expected to be applicable to other fin fish species.

The relevant European Reference laboratory has reviewed the proposed analytical method and is in agreement with the above evaluation.

2.2.5. Findings of EU or international scientific bodies

MRLs for animal commodities arising from crop protection use (indirect exposure through contaminated feed) are set in the EU as default levels (based limit of detection of the analytical method) as 20 µg/kg for all animal tissues, milk, eggs and honey. No MRL for fish commodities is established.

EFSA has estimated that the theoretical maximum daily intake (TMDI) from current crop protection use accounts for 3.4% of ADI but can be as high as 20.2% for the Portuguese diet. These estimations are based on non-vegetarian diets.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

The substance is not intended for use in dairy cattle and therefore potential effects in dairy products were not investigated.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

No such considerations were identified.

3.3. Elaboration of MRLs

According to the EFSA review report for lufenuron the theoretical maximum daily intake (TMDI) (excluding water and products of animal origin) for a 60 kg adult is 3.4% and 20.2% of the Acceptable Daily Intake (ADI) for the FAO/WHO European Diet and national Portuguese diet, respectively.

EFSA considered that additional intake from water and products of animal origin is not expected to give rise to intake problems (SANCO/165/08 – final rev 1, 21 November 2011).

For dual use substances consideration of the use as pesticide should be addressed.

ADI (µg per person)	Statement of the value of the relevant ADI
% Total used for veterinary products	45
% ADI used for pesticide products	3.42 WHO/FAO diet (20.2 Portuguese Diet)*
% ADI used for biocide products (products of animal origin)	3.9
% Total (veterinary + pesticide use)	52.32 (69.1 Portuguese diet)

*in brackets the figures on the basis of the Portuguese diet

Assuming an ADI of 15 µg/kg bw i.e. 900 µg/person/day and the standard 45% allocation for veterinary use of dual use compounds (according to Volume 8, see table above), the maximum allowable intake is 405 µg/person day and this amount may be maximally contained in 300 g fish fillet (muscle and skin in natural proportions).

The MRL resulting from this calculation is 1350 µg/kg for the RS-isomers of lufenuron (ratio 1:1).

The intake from fish based on the MRL (405 µg) plus intake from plant derived commodities (30.6 µg) plus the plus the TMDI for products of animal origin as milk (33 µg/day) eggs (2.2 µg) and honey (0.2 µg) would lead to a total of 471 µg or approximately 50% of the ADI. When calculated with the worst case scenario (Portuguese diet) the intake would lead to a total of 622.2 µg of lufenuron residues or approximately 69.1% of the ADI which is still well below the ADI.

An MRL of 1350 mg/kg leaves reasonable room for potential future uses of the substance (in the veterinary or pesticide/biocide area) and is considered conservative enough to even accommodate for the risk to consumers with specific consumption habits (e.g. high vegetable/fish diet, pescetarians). This approach is in line with MRLs for comparable substances.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EC) No 470/2009 the CVMP considered the possibility of extrapolating its recommendation on maximum residue limits for lufenuron on the basis of residue data in fish to other food producing species and commodities. Taking into account the current scientific knowledge the recommendations on extrapolation are justified as follows:

Animal species / food commodities	Extrapolation possible (Yes/No)	Justification
Fin fish	Yes	Pharmacokinetic data in two different fish species were available indicating that lufenuron is not significantly metabolised and has similar distribution in fish species. Therefore the MRL recommended for Atlantic salmon and Rainbow trout can be extrapolated to other fin fish species.
All food producing species (except fin fish)	No	The metabolism in fish is known to be more limited than in mammalian and avian species and therefore an extrapolation to non-fish species is not considered possible.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- a toxicological ADI of 0.015 mg/kg bw/day or 0.9 mg/person/day has been established and considered the overall ADI for lufenuron;
- lufenuron (RS isomers) can be considered as the marker residue;
- a ratio of marker to total residues can be set to 1;
- a validated routine analytical method for monitoring of residues in Atlantic salmon and Rainbow trout (muscle and skin in natural proportions) is available and should be applicable to other fin fish species;
- the MRLs for dual use substances should in principle not use more than 45% of the ADI;

the Committee recommends establishment of maximum residue limits for lufenuron in accordance with the following table:

Pharmaco-ologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Lufenuron (RS-isomers)	Lufenuron (RS-isomers)	Fin fish	1350 µg/kg	Muscle and skin in natural proportions	NO ENTRY	Antiparasitic agents / Agents (acting) against ectoparasites

4. Background information on the procedure

Submission of the dossier	6 March 2013
Steps taken for assessment of the substance	
Application validated:	20 March 2013
Clock started:	21 March 2013
List of questions adopted:	18 July 2013
Consolidated response to list of questions submitted:	13 September 2013
Clock re-started:	14 September 2013
CVMP opinion adopted:	12 December 2013