8 September 2015
EMA/CVMP/347671/2014
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Hexaflumuron (Fin fish)

On 3 July 2015 the European Commission adopted a Regulation[1] establishing maximum residue limits for hexaflumuron in fin fish, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Hexaflumuron is intended for use in fin fish for the control of sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) infestations in farmed Atlantic salmon *(Salmo salar),* rainbow trout (Onchorhyncus mykiss), and other fin fish.

PHARMAQ AS submitted the application for the establishment of maximum residue limits to the European Medicines Agency, on 29 May 2013.

Based on the original and complementary data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 10 July 2014 the establishment of maximum residue limits for hexaflumuron in fin fish.

Subsequently the Commission recommended on 23 May 2015 that maximum residue limits in fin fish are established. This recommendation was confirmed on 13 June 2015 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 3 July 2015.

[1] Commission Implementing Regulation (EU) No 2015/1079, O.J. L 175, of 03 July 2015

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Hexaflumuron
Therapeutic class:	Antiparasitic agents / Agents (acting) against ectoparasites
Procedure number:	EMEA/V/MRL/003802/FULL/0001
Applicant:	PHARMAQ AS
Target species:	Fin fish
Intended therapeutic indication:	Ectoparasiticide for the control of sea lice
Route(s) of administration:	Bath (immersion)

1. Introduction

Hexaflumuron is an acyl urea (benzoylphenylurea) chitin synthesis inhibitor. It is intended for use in fin fish for the control of sea lice (*Lepeophtheirus salmonis* and *Caligus elongatus*) infestations in farmed Atlantic salmon *(Salmo salar),* rainbow trout (Onchorhyncus mykiss), and other fin fish.

The substance is intended for use in bath (immersion) treatment, with an intended dose of 2 mg hexaflumuron/litre of sea water. Fish will be immersed for 60 to 120 minutes.

Hexaflumuron was previously authorised at a national level in some Member States for use in plant protection products but it is currently classified as 'Not approved' under Regulation (EC) No 1107/2009.

Hexaflumuron is however registered in plant protection products outside the EU, for instance in the USA and Australia.

2. Scientific risk assessment

2.1. Safety assessment

Hexaflumuron is an acyl urea derivate and is similar in structure to teflobenzuron, diflubenzuron and lufenuron. Teflubenzuron and diflubenzuron have been assessed by the CVMP and maximum residue limits for muscle and skin have been established for both (see published Summary reports EMEA/MRL/547/99-FINAL and EMEA/MRL/621/99-FINAL, respectively). Lufenuron has also been assessed by the CVMP and a maximum residue limit for muscle and skin has been recommended. The recommendation has not yet been adopted by the European Commission.

2.1.1. Overview of pharmacological properties

Pharmacodynamic properties

No original pharmacodynamic studies or studies from public literature on the mode of action of hexaflumuron are available.

Generally chitin synthesis inhibitors prevent the synthesis of chitin at moulting and hence prevent growth and development in insects. Chitin synthesis inhibitors do not inhibit hexosamine transferases, which are

responsible for connective tissue glycosaminoglycan formation in mammals, and hence their mode of action is specific to insects and they are of low toxicity to mammals.

Pharmacokinetic properties

Two pharmacokinetic studies in laboratory species have been conducted: one in rats and one in dogs. Both studies used hexaflumuron labelled at two positions (benzoyl and phenylamino labelled) and two doses of each radiolabelled compound. In rats hexaflumuron was rapidly absorbed and eliminated within approximately 24 hours. A saturation effect on absorption was reported. The tissue concentrations were generally low with the highest concentrations seen in fat, kidney and liver. The major metabolite in urine was 2,6-difluorobenzoic acid. The remaining metabolites were not identified.

In dogs, absorption of hexaflumuron was rapid but incomplete. A number of metabolites were formed but most remain unidentified. Extensive biotransformation of absorbed compound occurred and, as with the rat, absorption appears to have been saturated at higher doses. However only one animal was used per group, and one high dose group was eliminated from the study due to low feed intake.

2.1.2. Calculation of pharmacological ADI, if relevant

The pharmacological activity of hexaflumuron is considered to be insect specific and not of relevance for mammals. Consequently a pharmacological ADI is not considered necessary.

2.1.3. Overview of toxicology

Single-dose toxicity

Hexaflumuron was of low acute oral toxicity in the rat where the LD_{50} value was in excess of 5000 mg/kg bw.

Repeated dose toxicity

Mice: Hexaflumuron was studied in 4 and 13-week oral studies in mice. In the 4-week study using doses of up to 1500 mg/kg bw/day there was no compound related mortality and no major signs of toxicity. The main effects noted in this study were slight increases in hepatic enzymes at 750 and 1500 mg/kg bw/day, slight increases in methaemoglobinaemia in males and females at 125 mg/kg bw/day and above, and an increase in haemosiderin deposition in erythrocytes at 750 and 1500 mg/kg bw/day. The NOEL in this study was 25 mg/kg bw/day.

In the 13-week study, methaemoglobinaemia and elevated hepatic enzymes occurred at the highest dose used, 250 mg/kg bw/day, in females. There was also an increased frequency of hepatic enlargement at 250 and 25 mg/kg/bw/day. The NOEL in this study was 5 mg/kg bw/day.

Rats: Hexaflumuron was studied in 13 and 52-week oral studies and in a 3-week dermal study.

In the 13-week study, rats were administered doses from 25 to 1500 mg/kg bw/day hexaflumuron. There was no compound related mortality or signs of overt toxicity. Methaemoglobinaemia occurred in males administered 125 mg/kg bw/day and above, and in females given 750 and 1500 mg/kg bw/day. Increased haemosiderin deposits occurred in the spleens of females in a dose-related manner at 125 mg/kg bw/day and above. There were increases in absolute liver weights in females at 25 and 750 mg/kg bw/day, with increases in relative liver weights in low and high dose females. A NOEL could not be determined.

In the 52-week study, rats were given doses of 0, 5, 75 and 500 mg/kg bw/day. There were no treatment-related mortalities or signs of overt toxicity and methaemoglobinaemia was not observed. High dose females had elevated serum cholesterol levels at weeks 26 and 51, with elevated alkaline phosphatase levels in high dose males at week 51. Low dose females showed an increase in the incidence of absent *corpora lutea* but this was not seen in rats given 75 or 500 mg/kg bw/day and so the effect is unlikely to be due to compound administration. The NOEL in this study was 75 mg/kg bw/day.

Dogs: The major effects noted in a 28-day oral study in dogs were related to the induction of methaemoglobinaemia and included extramedullary erythropoiesis, Heinz bodies and increases in splenic weights. These effects occurred at all doses used, 25, 125 and 500 mg/kg bw/day but were minimal at the lowest dose used. A NOEL could not be determined in this study.

In a 52-week oral study in dogs, animals were given 0, 0.5, 2.0, 2.5 and 25 mg/kg bw/day. There were slight increases in methaemoglobinaemia noted in dogs given 2 mg/kg bw/day hexaflumuron and above, with increases in Heinz bodies and haemosiderin in the liver. The NOEL in this study was 0.5 mg/kg bw/day.

Reproductive toxicity, including developmental toxicity

A two-generation study was conducted in the rat using doses of 0, 5, 25 and 125 mg/kg bw/day.

The main effects noted were in parental animals administered 25 mg/kg bw/day and above, and these were haematological effects, mainly methaemoglobinaemia. There was a slight increase in pup mortality but no effects on pup development or on general indices of reproductive performance.

In this study the NOEL for parental toxicity was 5 mg/kg bw/day, while the NOEL for pup survival was 25 mg/kg bw/day. The NOEL for pup development was 125 mg/kg bw/day.

In an oral study in rats, animals were given doses of up to 1000 mg/kg bw/day from days 6 to 15 of gestation. There were no effects on food consumption, maternal body weights, mortality or clinical signs in maternal animals, and no effects on any of the indices of gestation. The NOEL in this study was 1000 mg/kg bw/day.

Genotoxicity

Hexaflumuron was tested in two assays for mutagenic activity (bacterial and mammalian cells) and in *in vitro* and *in vivo* tests for clastogenic activity (chromosomal aberrations in rat lymphocytes and in the mouse bone marrow micronucleus test.) Hexaflumuron gave negative results in these studies and is therefore not considered to be genotoxic.

Carcinogenicity

Hexaflumuron was tested in an 80-week mouse carcinogenicity study and in a 104-week combined chronic toxicity and carcinogenicity study in the rat.

In the mouse study, groups of male and female mice were administered doses of 0, 2, 5 and 25 mg/kg bw/day in the diet. Hexaflumuron had no adverse effects on survival and there were no signs of overt toxicity. The major finding in this study was an increase in pulmonary adenomatosis and a slight increase in the incidence of lung tumours (adenoma and adenocarcinomas) in male mice given the highest dose. However there was no statistical difference between treated mice and untreated controls. Furthermore, the incidence of these lesions was within historical control limits. Hexaflumuron was considered not to be carcinogenic in this study.

In the rat combined chronic toxicity and carcinogenicity study, animals were administered doses of 0, 5, 75 and 500 mg/kg bw/day hexaflumuron for 104 weeks. There were no major adverse effects of

hexaflumuron in any dose group. The only finding was an increase in unilateral adrenal enlargement but this was mainly seen in low dose animals and is therefore unlikely to be due to hexaflumuron. There was also an increase in the incidence and severity of hepatic pale cell foci in both males and females given the highest dose. There was no increase in the incidence of any tumour type. Hexaflumuron was not carcinogenic in the rat and the NOEL in this study was 75 mg/kg bw/day.

Overall, in view of the negative results from genotoxicity studies, the negative results from bioassays in two rodent species, and as hexaflumuron bears no structural alerts for carcinogenicity and is not related structurally to any known carcinogens, it is concluded that the substance is not carcinogenic.

Studies of other effects including immunotoxicity and neurotoxicity

Although no signs of neurotoxicity were noted in any of the toxicity studies the drug was investigated for the possible induction of delayed neurotoxicity in the domestic hen.

Groups of 10 birds were used to determine the oral LD_{50} of hexaflumuron. In the neurotoxicity segment, groups of 10 birds were administered 0 or 5000 mg/kg bw hexaflumuron. A further group of 10 birds was administered 500 mg/kg bw triorthocresyl phosphate, the positive control and a known inducer of delayed neurotoxicity.

Acute toxicity was very low in the hen, and no significant toxicity was noted at the highest dose used, 5000 mg/kg bw. The acute oral LD_{50} value is therefore in excess of 5000 mg/kg bw hexaflumuron.

No evidence of delayed neurotoxicity was seen in vehicle control birds or in birds treated with 5000 mg/kg bw hexaflumuron. Histopathological examination of brain, spinal cord and peripheral nerves revealed no differences between birds given the vehicle only and those given hexaflumuron. Triorthocresyl phosphate produced evidence of delayed neurotoxicity with significant histopathological lesions in the nervous system.

2.1.4. Calculation of the toxicological ADI or alternative limit

A range of toxicological studies has been conducted with hexaflumuron. The major effects noted in animal models were the induction of methaemoglobinaemia, along with accompanying effects such as the induction of haemosiderosis and Heinz bodies. The ability to produce methaemoglobinaemia has been noted with other benzoylurea compounds including diflubenzuron and lufenuron, whereas other compounds in the series do not lead to this effect, possibly as a result of poor absorption after oral administration.

Summary of NOELs from all relevant studies:

Endpoint	**Route**	**Species**	**NOEL** **mg/kg bw/day**
28 day	Oral	Mice	25
13 weeks	Oral	Mice	5
13 weeks	Oral	Rat	No NOEL determined
52 weeks	Oral	Rat	75
28 day	Oral	Dog	No NOEL

			determined
52 weeks	Oral	Dog	0.5
2 generation reproduction study	Oral	Rat	5
Developmental toxicity	Oral	Rat	1000
Developmental toxicity	Oral	Rabbit	1000
80 weeks	Oral	Mouse	25
104 weeks	Oral	Rat	75

The lowest NOEL was 0.5 mg/kg bw/day, seen in the 52 week study in dogs. An uncertainty factor of 100 was selected to account for inter- and intra-species variability. A toxicological ADI of 0.005 mg/kg bw, equivalent to 0.3 mg/person is therefore 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 type.

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

Hexaflumuron has been reported to have caused facial contact dermatitis in contractors who were present in an area where hexaflumuron was being sprayed onto cellulose material. This is not considered relevant to the current consumer safety evaluation.

2.1.8. Findings of EU or international scientific bodies

No evaluations by other EU or international committees were available.

2.1.9. Overall conclusions on the ADI

As pharmacological and microbiological ADIs are not considered relevant for hexaflumuron, the toxicological ADI of 0.005 mg/kg bw, or 0.3 mg/person for a 60 kg person, is established as the overall ADI.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

In a radiolabelled study in Atlantic salmon low systemic exposure was observed after 30 minutes bath immersion at 15ºC. Hexaflumuron was the major residue in fillet and liver. No data on hexaflumuron concentration in fat are available. Concentrations declined from 6 hours to 14 days after exposure, with a calculated half-life in fish of 16 to 27 days at 15ºC.

2.2.2. Residue depletion studies

A residue study in Atlantic salmon (*Salmo salar*) was conducted using unlabelled hexaflumuron. This study was conducted at two temperatures, 6 and 15°C, representative of cold and warm regions where salmon are farmed commercially. Groups of fish were either sham exposed (0 mg/l hexaflumuron) or they were exposed to 2 mg/l hexaflumuron by bath treatment for 2 hours, in a manner comparable to the proposed commercial use of the product. After treatment, fish were returned to the holding tanks maintained at 6 or 15°C, and aliquots of control and treated fish were removed at intervals for sacrifice and residues analysis in muscle and skin. A validated analytical method was used for the determination of residues in tissues.

The data demonstrate the almost linear depletion of residues in skin and muscle at both temperatures. At 6ºC hexaflumuron concentration was 144 µg/kg two weeks after treatment and declined to 74 µg/kg at 20 weeks after treatment. At 15ºC hexaflumuron concentration declined from 240 µg/kg at 1 week after treatment to 133 µg/kg 10 weeks after treatment. At the higher temperature, residues were higher than at 6°C while the rate of depletion was faster. This is likely to be due to the higher metabolic capacity of the fish at the higher temperature.

Hexaflumuron could be used in other fin fish species infested with moulting ectoparasites, including freshwater fish. It is accepted that water salinity is unlikely to significantly impact on uptake and depletion of hexaflumuron and that fat content of the fish and water temperature will be more important determinants of residue depletion for this lipophilic molecule. It is agreed that residue depletion in Atlantic salmon represents the worst case scenario in relation to consumer exposure to residues as it is a fatty fish farmed at low temperatures. The data for this species, farmed at low temperatures, can therefore be considered sufficiently conservative to ensure that exposure to residues from other fin fish, reared at higher temperatures, will not represent a threat to consumer safety.

Selection of marker residue and ratio of marker to total residues

The results from the metabolism study in salmon, and from the residues study confirm that the only substance detected as a significant residue was hexaflumuron, the parent substance. Hence, the marker residue is hexaflumuron and the ratio of marker to total residues is considered to be 1.

2.2.3. Monitoring or exposure data

No monitoring or exposure data other than that described elsewhere in this report are available.

2.2.4. Analytical method for monitoring of residues

An HPLC/MS/MS method for determination of the marker residue, hexaflumuron, in salmon muscle and skin in natural proportions has been provided and described in an internationally recognised format. The limits of detection and quantification for the method are 1.2 and 10 µg/kg, respectively. The method fulfils the requirements of Volume 8 of The Rules Governing Medicinal Products in the European Union and is considered to have been validated. Furthermore, the method has been demonstrated to be basically applicable in rainbow trout and sea-bass.

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

2.2.5. Findings of EU or international scientific bodies

No evaluations by other international committees were available with regards to fin-fish species.

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

The results of the residue study in Atlantic Salmon show that at both temperatures employed, 6 and 15ºC, residues of hexaflumuron were, at all time points, at levels that result in a theoretical maximum daily intake (TMDI) below the acceptable daily intake (ADI) of 300 µg/kg/person (5 µg/kg bw).

The highest residue concentration found at 6ºC (week 4) results in a residues intake equivalent to 29% of the ADI, while the highest residue concentration found at 15ºC (week 1) results in a residues intake equivalent to 79% of the ADI.

Based on the available residue data and considering the established ADI, an MRL 500 µg/kg in muscle and skin is recommended for Atlantic Salmon and, taking into account the CVMP guideline on the establishment of MRLs for Salmonidae and other fin fish (EMEA/CVMP/153b/97), can be extended to fin fish in general.

Calculation of theoretical daily intake of residues

Edible Tissue	Daily Consumption (kg)	MRL Proposed (µg/kg)	Ratio of marker to total residues	Total Residue (µg)	% ADI

Skin and muscle in natural proportions	0.30	500	1	150	50

For dual use substances consideration should be given to consumer exposure resulting from the pesticide use of the substance. In such cases the CVMP generally considers that consumer exposure resulting from residues in crop products may account for up to 55% of the ADI while consumer exposure resulting from residues in animal products may account for up to 45% of the ADI.

Hexaflumuron was previously authorised at a national level in Some Member states for use in plant protection products. However, no complete dossier was submitted to the competent authorities following introduction of Directive 91/414/EEC concerning the placing of plant protection products on the market, as a result of which the substance is currently classified as 'Not approved' under Regulation (EC) No 1107/2009 concerning the placing on the market of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Directive 2004/129/EEC required Member States to ensure that authorisations for plant protection products containing hexaflumuron were withdrawn. The default MRL of 0.01 mg/kg applies for residues of hexaflumuron in products of vegetable and animal origin but does not apply to residues in fish, in line with Regulation (EC) No. 396/2005.

As the use of hexaflumuron in pesticides is no longer allowed, it is considered acceptable that the consumer exposure to residues from use of the substance in veterinary medicinal products should account for 50% of the ADI.

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 hexaflumuron in fin fish to other food producing species and commodities. However, 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:

- the toxicological ADI of 5 µg/kg bw (i.e. 300 µg/person) was established as the overall ADI for hexaflumuron,
- hexaflumuron was retained as the marker residue,
- the ratio of marker to total residues can be set to 1,
- A validated analytical method for the monitoring of residues of hexaflumuron in Atlantic salmon is available and has been demonstrated to be applicable for monitoring of residues in and other fin fish;

the Committee recommends the establishment of maximum residue limits in fin fish in accordance with the following table:

Pharmaco-logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Hexaflumuron	Hexaflumuron	Fin fish	500 µg/kg	Muscle and skin in natural proportions	NO ENTRY	Antiparasitic agents / Agents (acting) against ectoparasites

Based on the MRLs for fin fish, the theoretical maximum daily intake (TMDI) from muscle and skin in natural proportions is 150 µg which accounts for 50% of the ADI.

4. Background information on the procedure

Submission of the dossier	29 May 2013
Steps taken for assessment of the substance	
Application validated:	12 June 2013
Clock started:	13 June 2013
List of questions adopted:	10 October 2013
Consolidated response to list of questions submitted:	4 April 2014
Clock re-started:	12 April 2014
CVMP opinion adopted:	10 July 2014