ASSURING THE SAFETY, QUALITY AND EFFICACY OF VETERINARY MEDICINES

United Kingdom
Veterinary Medicines Directorate
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(Reference Member State)

MUTUAL RECOGNITION PROCEDURE

PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY MEDICINAL PRODUCT

Pyceze 500 mg/ml concentrate for solution for fish treatment

PuAR correct as of 01/05/2018 when RMS was transferred to FR.
Please contact the RMS for future updates.

MODULE 1

PRODUCT SUMMARY

EU Procedure number	UK/V/0311/001/MR
Name, strength and pharmaceutical form	Pyceze 500mg/ml concentrate for solution for fish treatment
Applicant	Elanco Europe Ltd Lilly House Priestley Road Basingstoke Hampshire RG24 9NL
Active substance(s)	Bronopol
ATC Vetcode	QD01AE91
Target species	Farmed Atlantic salmon and rainbow trout eggs, Atlantic salmon and rainbow trout
Indication for use	Prevention of growth of fungal infections(Saprolegnia spp) in the face of suspected or known challenge in farmed Atlantic salmon and rainbow trout kept in fresh water. The product is most effective if used at the first signs of fungal infection Prevention of growth of fungal infections (Saprolegnia spp) in the face of suspected or known challenge in farmed Atlantic salmon eggs and rainbow trout eggs. The product is most effective if used at the first signs of fungal infection.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the Heads of Medicines Agencies (veterinary) (HMA(v)) website (www.hma.eu).

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	Full Mutual Recognition application in accordance with Article 12(3) of Directive 2001/82/EC as amended.
Date of completion of the original mutual recognition procedure	2/7/08
Date product first authorised in the Reference Member State (MRP only)	November 2003
Concerned Member States for original procedure	Denmark France Germany Greece Iceland Ireland Italy Norway Poland Spain

I. SCIENTIFIC OVERVIEW

Pyceze 500 mg/ml concentrate for solution for fish treatment is produced and controlled using validated methods and tests which ensure the consistency of the product released on the market.
It has been shown that the product can be safely used in the target species; the slight reactions observed are indicated in the SPC.
The product is safe for the user and for the environment, when used as recommended. Suitable warnings and precautions are indicated in the SPC.
The efficacy of the product was demonstrated according to the claims made in the SPC. The product is indicated for farmed atlantic salmon and rainbow trout eggs, atlantic salmon and rainbow trout for prevention of growth of fungal infections (*Saprolegnia* spp) in the face of suspected or known challenge in farmed atlantic salmon eggs and rainbow trout eggs, prevention or reduction of fungal infections (*Saprolegnia* spp) in farmed atlantic salmon and rainbow trout kept in fresh water. The product is most effective if used at the first signs of fungal infection. The product is presented in one litre and five litre polyamide/HDPE bottles.
The overall risk/benefit analysis is in favour of granting a marketing authorisation.

II. QUALITY ASPECTS

Composition

The product contains the active substances Bronopol 50% w/v. The product also contains the following excipients; dipropylene glycol monomethyl ether (dowanol DPM) and water purified.

The container/closure system is a one litre and five litre polyamide/HDPE bottle containing polypropylene screw cap with an internal low density polyethylene/aluminium/low density polyethylene/paper/polyethylene foam seal (1L bottle) and high density polyethylene screw cap with an internal low density polyethylene/polyethylene terephthalate/aluminium/paper/wax/paper/polyethylene foam seal (5L bottle). The particulars of the containers and controls performed were provided and conform to the regulations.

The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice from a licensed manufacturing site.

Process validation data on the product have been presented in accordance with the relevant European guidelines.

C. Control of Starting Materials

The active substance, bronopol, is not included in the European Pharmacopoeia, but is the subject of a monograph in the British Pharmacopoeia. The active substance is manufactured in accordance with the principles of good manufacturing practice.

The supporting data for bronopol are presented in the form of a European Active Substance Master File (ASMF). The open part of the ASMF is included in the dossier submitted by the applicant. The active substance manufacturer applies tighter limits in respect of related substances than is required in the monograph. The two named impurities are each limited to 0.2 %, other impurities are limited individually to 0.1 % and in total to 0.2 %. The active substance manufacturer applies a validated HPLC method for assay, which has been shown to correlate with the titrimetric method of the pharmacopoeia. The compliance with the pharmacopoeial monograph is therefore assured.

The specification applied by the manufacturer of the finished product to the solvent dipropylene glycol monomethyl ether, Dowanol DPM, includes limits for appearance, water content, density and assay. A summary of the supporting validation data has been presented for the supplier's method. Three certificates of analysis are presented which demonstrate compliance with the proposed specification.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

A declaration and format 3 statements are presented together with supporting evidence which indicate that there are no ingredients of animal origin used in the manufacture of Pyceze.

E. Control on intermediate products

Not applicable. There are no intermediate products.

F. Control Tests on the Finished Product

The finished product specification controls the relevant parameters for the pharmaceutical form. The tests in the specification, and their limits, have been justified and are considered appropriate to adequately control the quality of the product.
Satisfactory validation data for the analytical methods have been provided.
Batch analytical data from the proposed production site have been provided demonstrating compliance with the specification.

Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substances when stored under the approved conditions.

H. Genetically Modified Organisms

Not applicable.

J. Other Information

Special precautions for storage
Store in the original container.
Keep the container tightly closed.
Do not store above 25ºC.

Shelf life
Shelf life of the veterinary medicinal product as packaged for sale: 3 years.
Shelf-life after first opening the immediate packaging 30 days.
Once diluted, the product must be used immediately.

III. SAFETY AND RESIDUES ASSESSMENT (PHARMACO-TOXICOLOGICAL)

III.A Safety Testing

Pharmacological Studies

Pharmacodynamics:
No new data pertaining to the pharmacodynamics of bronopol were presented in this application. The UK Licensing Authority and the CVMP as part of the MRL procedure for bronopol have already assessed the data submitted. The CVMP concluded on pharmacodynamics as follow:
Bronopol was reported to be active against a wide range of bacteria but to be less active against moulds and yeasts. The precise antimicrobial mode of action of bronopol is not known. It is believed that bronopol causes inhibition of thiol-containing dehydrogenase enzymes that are membrane bound, resulting in cell leakage and eventual collapse. There were no data presented on the secondary pharmacological effects of bronopol in laboratory animals.

Pharmacokinetics:
No new data pertaining to the pharmacodynamics of bronopol were presented in this application. The UK Licensing Authority and also the CVMP as part of the MRL procedure for bronopol had already assessed the data submitted. The CVMP concluded on pharmacokinetics as follows:

After administration of a single oral dose of 1 mg/kg body weight C-bronopol to rats and beagle dogs, peak plasma concentrations in the range of 0.5 to 0.61 and of 0.92 to 1.4 µg-equivalents/ml were observed in both rats and dogs respectively, 0.5 to 2 hours after dosing. The bioavailability in rats after a single oral dose of 10 mg/kg body weight or 50 mg/kg bw was estimated to be at least 75% in both cases. After single or repeated oral dosing to rats, bronopol-related residues were widely distributed to the tissues; there was no evidence of accumulation and excretion was rapid. Following a single oral dose of 10 mg/kg body weight C-bronopol to rats, over 60% of the administered dose was recovered from the urine during the first 12 hours after dosing and 1.3 to 7.4% from the faeces. Total faecal excretion for the first 168 hours after dosing accounted for approximately 10% of the administered dose. In the first 48 hours after dosing 4.1% and 1.8% of the dose was recovered in males and females respectively, from expired carbon dioxide.

Unmetabolised bronopol was not detected in the plasma or urine of treated rats at any time point. The main metabolised component in rat urine collected up to 24 hours after oral dosing with 10 or 50 mg/kg body weight C-bronopol was 2-itropropane-1,3-diol which accounted for around 50% of the radioactivity present. Other metabolites could not be identified due to technical problems. In an *in vitro* experiment, samples of ^{14}C-bronopol added to samples of rat, dog and rabbit plasma were completely degraded within 5 minutes yielding 2- nitropropane-1,3-diol. In a special study designed to investigate the fate of the bromine moiety, rats were given a single oral dose of 1 or 50 mg/kg body weight of bronopol and the resulting urine was analysed for bromide ions. It was claimed that the 1 mg/kg body weight dose resulted in bromide ion concentrations which were not above these levels and that around 17% of the 50 mg/kg bw dose was excreted as bromide over the period 0 to 120 hours.

At pH 7 and 9, the rate of hydrolysis of bronopol was rapid but showed a distinct concentration dependence with the rate of hydrolysis increasing as concentrations were reduced. At bronopol concentrations in the range 0.01 to 0.1%, the hydrolysis half-life was less than one day at 25ºC. At pH 4, bronopol was intrinsically more stable but the results indicated a steady hydrolysis with time and a similar though less marked concentration effect. Four degradation pathways were identified; three of these proceeded via 2-bromo-2-nitroethanol, which was formed by the reversible loss of formaldehyde. The fourth pathway involved the irreversible conversion of bronopol into tris(hydroxymethyl)nitromethane

Toxicological Studies

- Single Dose Toxicity

No new data pertaining to the acute toxicity of bronopol were submitted in this application. The UK Licensing Authority and also the CVMP as part of the MRL procedure for bronopol had already assessed the data submitted. The CVMP concluded on acute toxicity follows:

Bronopol was of moderate acute toxicity. The acute oral LD_{50} values ranged from 180 to 400 mg/kg body weight in rats and 250 to 500 mg/kg body weight in mice. In rats, signs of toxicity were usually observed within 30 minutes of oral dosing and included sedation, salivation, wheezing, gasping or laboured breathing, nasal exudates, cyanosis (blue tinged mucous membranes) and ataxia (unsteadiness). Gross pathological examination of rats, which died revealed gastrointestinal irritation, small spleens and enlarged, dark-coloured adrenal glands. The acute LD_{50} after percutaneous administration to rats was reported to be greater than 1600 mg/kg body weight. In rats, the LC_{50} value following a 6-hour inhalation exposure was 5 mg/l of air.

- Repeated Dose Toxicity

No new data pertaining to the acute toxicity of bronopol were submitted in this application. The UK Licensing Authority and also the CVMP as part of the MRL procedure for bronopol had already assessed the data submitted. The CVMP concluded on acute toxicity follows:

The groups of 20 rats were given daily oral doses of 0 (distilled water), 20, 80 or 160 mg/kg body weight per day of bronopol by stomach tube for 13 weeks. All the rats given 160 mg/kg body weight died or were euthanased by day 9 of the study. Seven males and nine females in the 80 mg/kg body weight group also died. Overt signs of toxicity were observed at 80 and 160 mg/kg body weight and in one rat given 20 mg/kg body weight and included respiratory distress and abdominal swelling. Body weight gain and food consumption were adversely affected at 80 and 160 mg/kg body weight. The pathological changes in the rats, which died included gaseous or fluid swelling in the gastrointestinal tract, swelling of the lung lobes and regressive changes in the thyroid. The terminal histopathology revealed renal changes (dilated tubules containing eosinophilic material) in 2 rats given 20 mg/kg body weight and 2 rats given 80 mg/kg body weight. A “No Observable Effect Level” was not established.

Groups of 3 beagle dogs were given daily oral doses of 0 (distilled water), 4, 8 or 20 mg/kg body weight per day of bronopol for 13 weeks. For the first 6 weeks, the dogs given 20 mg/kg body weight vomited around 30 minutes after dosing. Thereafter the dogs were fed 2 hours before dosing and vomiting was observed in only one dog on one occasion. After 12 weeks of dosing, white blood cell counts were significantly reduced in the groups given 8 and 20 mg/kg bodyweight. At termination, mean absolute and relative spleen weights and mean relative liver weights were significantly increased in the 20 mg/kg body weight group but there were no corresponding pathological changes.

Observations in Humans

The study was carried out using a typical cream base (*Soltan 3*), containing 0.1% C-bronopol, of specific activity 52 kBq/mg. Approximately 222 kBq of C-bronopol was applied to 200 cm^2 of skin on the abdominal wall of 2 volunteers.
The 1.8 % and 5.0 % of the dose was recovered from the urine of the 2 volunteers, all of this during the period 2 - 48 hours after application. No radio-labelled material was detected in the faeces of either volunteer. Eight hours after the application, the sites of application were swabbed; 65.7 % and 92 % of the dose were recovered from the 2 volunteers. However significant amounts of radioactivity remained at the application site and proved resistant to removal by vigorous washing and depilation. It was concluded that a significant amount had penetrated the skin and/or hair follicles and remained bound in those areas.
In another experiment, 5g of *Soltan* cream containing 0.1% bronopol was applied to the skin (abdominal wall) of 5 male and 5 female human volunteers. After 8 hours, the application sites were swabbed. In males, around 63 % was recovered from the skin and in females 80 %. The difference was attributed to the larger number of hair follicles in the males.

Microbiological Studies

When considering the MRL application for bronopol, the CVMP concluded as follows:

In vitro MIC data were provided for six strains (3 genera) of Gram-positive bacteria and 15 strains (7 genera) of Gram-negative bacteria. The species tested included several which were representative of the normal human gut flora. The MIC values were generally in the range 12.5 to 25 µg/ml indicating that the bacteria were relatively insensitive to bronopol. No studies were provided on effects of bronopol on the food industrial processes were carried out; it was considered that such data were not necessary bearing in mind the proposed use of the substance.

User Safety

The applicant has included the following user warnings in the SPC and product literature:

IRRITANT (alongside the St Andrews Cross symbol)
Irritating to eyes, lungs and skin.

Wear protective clothing, impervious gloves (0.3mm nitrile rubber) and use either a disposable half mask respirator conforming to European Standard EN149 together with suitable eye protection or a non-disposable respirator conforming to European Standard EN 140 with a filter to EN 143 when mixing and handling the product. After contact with skin, wash immediately and – in case of contact with the eyes – rinse immediately with plenty of water and seek medical advice. Do not smoke, drink or eat when using the product and wash hands carefully after use.

The user safety warnings are considered appropriate for a product of this type.

Pyceze is not expected to present an undue hazard to the operator under normal conditions of use and subject to use of appropriate personal protective equipment.

Ecotoxicity

Pyceze contains the active substance bronopol (50 % w/v) an aliphatic halogenitro compound active against bacteria and fungi. It is indicated for the treatment and control of fungal infections in farmed salmonid eggs and the reduction and control of fungal infections in farmed atlantic salmon and rainbow trout.

Eggs are treated in the incubator (beginning 24 hours after fertilisation) with bronopol at a concentration of 50 mg/l for 30 minutes. This treatment is repeated daily as necessary. Fish are treated with bronopol at a concentration of 20 mg/l for 30 minutes. This treatment is repeated daily for up to 14 consecutive days.

The following information relevant to environmental safety is presented in the SPC:

> Do not contaminate surface waters or ditches with product, diluted product or used container.
>
> Any unused veterinary medicinal product or waste materials derived from such veterinary medicinal products should be disposed of in accordance with local requirements.

III.B Residues documentation

Residue Studies

The reports of pharmacokinetic and metabolism studies in rats and dogs were provided. The substance was well absorbed in these species after oral administration with a bioavailability of over 75 %. It was rapidly metabolised and excreted so that bronopol itself was undetectable in plasma and urine at all time points. Bronopol was quickly hydrolysed in aqueous solution, at concentrations between 0.01 and 0.1% (w/v), the hydrolysis half-life was less than one day at 25˚C. The main metabolite found in rat urine was 2-nitropropane-1,3-diol accounting for 50 % of the total residue. This substance was also found in the plasma of rats, dogs and rabbits in an *in vitro* study.

In the pivotal residue depletion study, attempts were made to identify the metabolites present in samples of muscle and skin. In this study, unmetabolised bronopol was not found in edible tissues. When bronopol was assessed by the CVMP for extension for the existing Annex II entry for fish eggs to include whole fish they concluded as follows:

Atlantic salmon were exposed by immersion in a tank containing a solution of (C)-bronopol (21.91 mg/l water) at 14º C, for a period of 30 minutes. At the end of treatment, the fish were transferred to a clean water tank. Groups of 10 fish were collected at the end of the exposure period (0 hours) and at 6, 12, 24, 72 and 168 hours after treatment. Concentrations of radioactivity in samples of muscle with skin were determined by combustion followed by liquid scintillation counting. The mean total residues depleted from 259 µg equivalents/kg at 0 hours, to 266 µg equivalents/kg at 6 hours, to 255 µg equivalents/kg at 12 hours, to 194 µg equivalents/kg at 24 hours, to 102 µg equivalents/kg at 3 days and to 39 µg equivalents/kg at 7 days.

The attempts were made to identify the components of the residues in pooled tissue samples, by comparison with authentic samples of known mammalian metabolites and degradation products of bronopol. Radioactivity was extracted from the samples with extraction efficiencies of 79 to 93%. The HPLC analysis indicated one major metabolite, which could not be identified. LC-MS indicated 2 possible structures for the unknown metabolite.

Based on the results of the study in which salmon were given a single treatment of (C)-bronopol, the estimated mean consumer intakes of total residues were 80 µg, 77 µg and 58 µg at 6 hours, 12 hours and 24 hours respectively, after the end of the exposure period. These values represented approximately 7%, 6% and 5% of the ADI for bronopol. Because up to 14 consecutive daily treatments were possible, a risk assessment of the likely consumer intake of total residues in muscle plus skin from fish treated with bronopol for 14 days was conducted. A worst-case estimate of consumer intake of total residues was determined assuming that residues are depleted only on the day of dose administration and that fish are eaten 6 hours after the 14th and final treatment. This scenario would result in the accumulation of 13 times 200 µg/kg (the average residue concentration a day after a single treatment) plus 300 µg/kg (the average intake of total residues after 6 hours) equalling a cumulative concentration of 2900 µg/kg resulting in a consumer intake of 870 µg, representing around 73% of the ADI.

Withdrawal Periods

Do not use in salmonid eggs intended for human consumption.
Meat : zero degree days

IV CLINICAL ASSESSMENT (EFFICACY)

IV.A Pre-Clinical Studies

Pharmacology

EGGS

Pharmacodynamics

The expert stated that Pyceze contains bronopol (2-bromo-2 nitropropane-1, 3-diol) a member of the aliphatic halogenitro compounds. Its mode of action was considered to result from blocking thiol containing enzymes such as membrane bound dehydrogenase, causing alterations to the cell membrane, leading to cell leakage and destruction. It is more active against bacteria than fungi, and more active against metabolically active than resting cells. Bronopol is currently included in Annex II of the EC Council Regulation No. 2377/90 for salmonid eggs. There is also a CVMP recommendation that the existing entry for bronopol be extended to cover all stages of fin-fish.

An *in vitro* study tested PHA01- bronopol against *Saprolegnia parasitica* using 2 standardised anti fungal protocols. It was determined that PHA01 had marked effects on zoosporulation after 5 minutes exposure at 10 ppm or less. Despite the mycelia penetrating the agar and gaining some protection from the test substance, it was determined that a 20 minute exposure to 1000 ppm prevented all subsequent growth. An exposure of 100 ppm for 80 minutes also had inhibitory effects. When naked fungal hyphae were exposed 50 ppm for 5 minutes was found to have an inhibitory effect on subsequent growth, increasing to 100 ppm for 10 minutes. It was concluded that although less active than malachite green, PHA01 clearly possessed anti fungal potential.

In an *in vitro* MIC study bronopol was demonstrated to inhibit *Saprolegnia parasitica* at concentrations in the range 2 - 30 mg/litre for 30 minutes. The efficacy was dose dependant: 20 % at the lower dose and 80 % at the higher dose. No absolute kill of zoospores was observed.

Another *in vitro* MIC study, similar to the above, used concentrations of bronopol of 5, 30, 50 & 100 mg/litre for 30 minutes. Again, the efficacy was dose dependant with *Saprolegnia parasitica* zoospore density being reduced by 20 %, 56 %, 96 % & 100 % respectively. In all cases, however, new growth was observed after 48 hours. Both daily and second day treatments using 50 mg/litre prevented confluent growth of *S. parasitica* in flasks containing hemp seeds. Although both treatments killed the fungus on the surface of the seeds, the

fungus survived in the centre of the seeds and was capable of growth after up to 9 days.
From the study, the expert concluded that the MIC for *S. parasitica* zoospore viability was between 50 & 100 mg/litre, although no treatment appeared to kill all the vegetative mycelia.

A study was undertaken to assess the homogeneity of bronopol in water when administered 3 ways. This was to establish the best method of administration. A water trough containing 400 litres was visually divided into 5 sections of equal size, identified by the letters A to E. These were not physical divisions and so water could move freely between sections. Three methods of administering 50 mg/litre bronopol were undertaken by adding pre-diluted concentrations:
- at either positions B or D
- by pouring evenly along the length of the trough
- by administering at position E and continuously re-circulating the water by using a water pump.

Water samples were taken from the mid-point of each section, just below the surface at intervals of 5, 10, 20, 30, 40, 60, 90 & 120 minutes after administration. One sample was also taken from the base of the trough at position E, 120 minutes after administration. The results demonstrated that bronopol concentrations were only homogeneous when water was re-circulated. As re-circulation or flow-through systems are used in the field, this should adequately ensure homogeneity of the treatment

Pharmacokinetics
No data have been presented in eggs, but reference is made to the residues depletion data submitted for fish.

Tolerance in the Target Species of Animals

A tolerance study was conducted on sensitivity of rainbow trout eggs exposed to bronopol for 1 hour at 2XRTD (100 mg/l) or 4XRTD (200 mg/l) for 31 consecutive days until hatching. The results of egg counts for survival to hatchability demonstrated that daily treatment with bronopol caused no adverse or toxic effects and produced better hatching figures than similar groups of eggs treated with malachite green or untreated controls. It was concluded that Pyceze is well tolerated by incubating fertilised rainbow trout eggs during extended exposure from start of incubation to the first fry stage.

Resistance

A discussion of the antibacterial bacteriostatic activity of bronopol against common human pathogens, was provided, as bronopol is well established in pharmaceutical, personal care, household and industrial products, as a preservative and for prevention of bio fouling. It was noted that the activity of bronopol against fungi is more variable, though no data are reported for *Saprolegnia spp*. It was noted that resistance or a high level of tolerance in micro-organisms has not been reported with use of bronopol, after 30 years of worldwide use.

Pharmacology

FISH

Pharmacodynamics

The expert stated that bronopol was a member of the aliphatic halogenitro compounds. Its mode of action was considered to result from blocking thiol containing enzymes such as membrane bound dehydrogenase, causing alterations to the cell membrane, leading to cell leakage and destruction. It is more active against bacteria than fungi, and more active against metabolically active than resting cells.

In an *in vitro* MIC study, bronopol was demonstrated to inhibit *Saprolegnia parasitica* at concentrations in the range 2 - 30 mg/litre for 30 minutes. The efficacy was dose dependant: 20% at the lower dose and 80 % at the higher dose. No absolute kill of zoospores was observed.

Another *in vitro* MIC study used the concentrations of bronopol of 5, 30, 50 & 100 mg/litre for 30 minutes. Again, the efficacy was dose dependant with *Saprolegnia parasitica* zoospore density being reduced by 20 %, 56 %, 96 % & 100 % respectively. In all cases, however, new growth was observed after 48 hours. Both daily and alternate day treatments using 50mg/litre prevented confluent growth of *S. parasitica* in flasks containing hemp seeds. Although both treatments killed the fungus on the surface of the seeds, the fungus survived in the centre of the seeds and was capable of growth after up to 9 days under both treatment regimes tested.

The expert concluded from the above that the MIC for *S. parasitica* zoospore viability was between 50 & 100 mg/litre, although no treatment appeared to kill all the vegetative mycelia.

A study was undertaken to assess the homogeneity of bronopol in water when administered 3 ways. This was to establish the best method of administration. A water trough containing 400 litres was visually divided into 5 sections of equal size, identified by the letters A to E. These were not physical divisions and so water could move freely between sections. Three methods of administering 50mg/litre bronopol were undertaken by adding pre-diluted concentrations:

- at either positions B or D
- by pouring evenly along the length of the trough
- by administering at position E and continuously re-circulating the water by using a water pump.

Water samples were taken from the mid-point of each section, just below the surface at intervals of 5, 10, 20, 30, 40, 60, 90 & 120 minutes after administration. One sample was also taken from the base of the trough at position E, 120 minutes after administration. The results demonstrated that bronopol concentrations were only homogeneous when water was re-circulated. As re-circulation or flow-through systems are used in the field, this should ensure homogeneity.

Pharmacokinetics

(C14)-Bronopol residue depletion study was conducted for determination of a MRL. The 2-Bromo-2-nitro[2-^{14}C]propane-1,3-diol (^{14}C-bronopol) with a specific activity of 925 kBq/mg and a radiochemical purity of greater than 98% was used. A fish tank containing tap water was fortified with ^{14}C-bronopol to 21.91 mg/l (i.e. 20.27 MBq/l).

Atlantic salmon (*Salmo salar*), mean body weight 32.4 g, were obtained. The fish were exposed by immersion in a tank of water fortified with ^{14}C-bronopol for a specific period of time. The fish were immediately transferred to a clean water tank. The groups of 10 fish were removed at the end of the exposure period (0 hours), at 6, 12 and 24 hours and at 3 and 7 days. They were quickly rinsed in clean water and killed. The head, tail, fins and viscera were removed and the muscle with skin was filleted from the bone. The concentrations of radioactivity in the samples of muscle with skin were determined by combustion followed by liquid scintillation counting. The confirmation of the metabolites present in skin + muscle samples was attempted using LC-MS.

The attempts were made to identify the components of the residues in pooled tissue samples by co-chromatography with metabolite standards. The incurred residues were extracted from pooled skin + fat from 10 fish per time point in acetonitrile and methanol. The extraction recovery of radioactivity homogenates was around 79 – 93 %. HPLC analysis (with UV 225 nm and in-line radio detection) showed the existence of only one major component with a retention time of around 5 minutes. The retention time of ^{14}C-bronopol was around 10 minutes under the same chromatographic conditions. Bronopol was not detected. The retention time of 2-bromo-nitroethanol was around 12.6 minutes indicative of its being too hydrophobic to be the unknown metabolite.

Tris hydroxymethyl-nitromethane eluted at a similar retention time (*ca* 5 minutes) and produced an LC-MS fragmentation pattern (i.e. *m/z* 120 to *m/z* 72, *m/z* 120

to *m/z* 46 and *m/z* 150 to *m/z* 120) similar to the unknown metabolite. However, as the molecular ion *m/z* 150 was not detected with *tris* hydroxymethyl nitromethane. The observed fragmentation could also be attributed to the metabolite 2-nitropropane-1,3-diol.

Co-chromatography of tissue extracts with *tris* hydroxymethyl-nitromethane and 2-nitropropane-1,3-diol followed by LC-MS analysis did not confirm the identity of the unknown metabolite as both substances eluted at the same retention time. Consequently, it was not possible to assign a structure to the unknown metabolite

Tolerance in the Target Species of Animals

This was a pilot study. The objective was to assess the tolerance of recently hatched Rainbow trout alevins to bronopol at dose rates of 20, 60 & 100 mg/litre for 90 minutes. The study was conducted in the UK in 1998 and was not GLP compliant.

Three hundred alevins, hatched from the eggs treated in the egg dose titration study of approx. 1 week of age, were allocated to three troughs (100/trough)
The troughs were randomly allocated to three treatment groups:

- 20 mg/litre for 90 minutes (X1 the proposed dosage for X3 the proposed duration)
- 60 mg/litre for 90 minutes (X3 the proposed dosage for X3 the proposed duration)
- 100 mg/litre for 90 minutes (X5 the proposed dosage for X3 the proposed duration)

After the water had been turned off for 2 minutes, treatments were administered. The water was then turned on again and the treatment flushed out of the tank
The observations were made for behaviour and general demeanour pre-treatment, at 30, 60 & 90 minutes during treatment and, at 120, 180, 240 & 300 minutes post-treatment.
At the end of the study all alevins were sacrificed and disposed of. No histological examination of tissues and no statistical analysis of the results was carried out.

- In the 20 mg/litre group, no signs of distress, reaction to treatment, deviations from normal or mortalities were reported
- In the 60 mg/litre group, no abnormalities were observed during treatment but there were signs of toxicity approx. 2½ hours after the treatment period when dead alevins were observed. Mortality was approx. 10 %.
- In the 100 mg/litre group, no abnormalities were observed during treatment but at approx. 1 - 1½ hours after treatment the activity level appeared to be depressed. By 2½ hours after the treatment period mortality was approx. 25 %.

From the study, it was concluded that there were no adverse effects in Rainbow trout alevins treated with 20 mg/litre bronopol for 90 minutes (X1 the proposed dosage for X3 the proposed duration) but that 60 and 100 mg/litre bronopol for 90 minutes resulted in both clinical signs of intolerance and mortality.

Another study was conducted to assess the tolerance of rainbow trout and atlantic salmon to bronopol at a dose rate of 200 mg/litre for 90 minutes.
Five trout and 5 salmon were used, with each species being kept in a separate tank. The trout were approx. 12 months of age and salmon approx. 9 months of age. The dosage used represents X10 that proposed and the treatment duration X3 that proposed. At the end of treatment, the fish were placed in a different tank with clean untreated water. The observations were made for behaviour and general demeanour pre-treatment, at 5, 15, 30, 60 & 90 minutes during treatment and, at 24 & 48 hours after the start of the treatment period. At 48 hours post-treatment all the fish were sacrificed. Samples of kidney, liver, heart, gills and skin/muscle were sampled for histopathology. No statistical analysis of the results was carried out. All fish appeared healthy and normal before treatment.
Salmon - no abnormalities were observed during treatment but when the fish were transferred post-treatment to clean water, a slightly reduced response to stimulus was evident. By 24 hours post-treatment, 1 fish was dead and the remaining 4 were dull and lethargic.
Trout - all were normal for the first 60 minutes of the treatment period. Mild signs of distress and behavioural changes were observed after 80 minutes exposure. On transfer post-treatment to clean water, a reduced response to stimulus was evident in all fish. At 24 hours post-treatment, 1 fish was dead and the remaining 4 exhibited a lack of response to stimulus. By 24 hours post-treatment, the 4 surviving fish showed abnormal swimming movements and activity and were sacrificed on welfare grounds. The mild pathological changes were evident in the tissue samples from both species. Kidney - 1 trout, liver - 2 salmon, gill - 2 salmon & 3 trout, skin - 4 salmon & 4 trout and muscle - 3 salmon & 1 trout.
From the study, it was concluded that clinical signs of intolerance and histological changes, mainly in the skin and muscle, in Rainbow trout and Atlantic salmon, were produced by bronopol when administered once at 200 mg/litre for 90 minutes.

The another study was conducted to determine the tolerance of rainbow trout to bronopol at concentrations of 0, 20, 60 and 100 mg/litre administered for 60 minutes daily for 28 consecutive days.
The regime used represents X0, X1, X3 & X5 the proposed dose, X2 the proposed daily treatment duration and X2 the proposed treatment frequency.
At the start of the study, the mean weight of the fish was 13.0 grams. All were female. Two hundred and forty fish were randomly allocated to 8 tanks (30/tank). Fish were held for 3 weeks before treatment was started. Dead or moribund fish were replaced from stock to maintain numbers. On Day -1, all fish were removed, anaesthetised, weighed and replaced. Statistical analysis of the weights revealed differences between tanks and so fish were exchanged so that the tanks contained fish of a similar mean weight at the start of the study.
Duplicate tanks were either untreated controls or treated with 20, 60 or 100 mg/litre bronopol. After the 60 minutes bronopol exposure, dilution was performed rapidly to avoid extended exposure.
Water samples were taken for analysis of bronopol concentration on Days 1, 7, 14 & 21; before treatment and at 60 & 120 minutes after the start of exposure for

the treated tanks, and, at 30 minutes after the start of exposure for the untreated, control tanks.
Fish behaviour and appearance were observed on Day 0 before treatment started and on each day during treatment. The trial was terminated on Day 29, the day after the last treatment, when all fish were sacrificed, weighed and necropsied. The observer and pathologist were blinded to the treatment. From each tank 8 fish were selected for histological examination of limited tissues (gill, liver, spleen and posterior kidney) and a further 2 were selected for more detailed histological examination (the above tissues + eye, anterior kidney, brain, heart, muscle & attached skin, spleen, stomach, small intestine and colon). At the request of the pathologist, samples were collected for histology from one further fish.
The mean concentrations of bronopol, measured 1 hour after treatment was completed, were all ≤5.5 % of the initial targets, indicating satisfactory flush out.
The maximum daily dosage, calculated using the minimum water replacement rate, was estimated, by integration of the area under the exponential curve over the 23 hours post-treatment period, to be X1.45 nominal. The actual daily dosage was calculated as X1.34 nominal.
There were no mortalities which were considered to result from bronopol exposure. There were no consistent changes in behaviour or appearance at any time. At final sampling, there were no gross pathological or histopathological abnormalities which were considered to result from bronopol exposure. There were significant differences in fish weights between the controls and the treated groups. In all cases, the mean weight of the control group was less than that of the bronopol treated group. This suggested some beneficial effect of bronopol. At the end of the study a sub-clinical infestation with *Ichthyophthirius multifilis* (‘white spot’) was identified. Bronopol may have had a beneficial effect on this.
It was concluded that Rainbow trout treated with bronopol at 20, 60 and 100 mg/litre for 60 minutes daily, for 28 consecutive days, had no adverse effects. The safety margin was, therefore, > X5 dose, X2 exposure time and X2 treatment duration.

Resistance

A discussion of the antibacterial bacteriostatic activity of bronopol against common human pathogens, was provided, as bronopol is well established in pharmaceutical, personal care, household and industrial products, as a preservative and for prevention of bio fouling. It was noted that the activity of bronopol against fungi is more variable, though no data are reported for *Saprolegnia spp*. It was noted that resistance or a high level of tolerance in micro-organisms has not been reported with use of bronopol, after 30 years of worldwide use.

IV.B Clinical Studies

Laboratory Trials

The study was conducted to assess the efficacy of bronopol against a natural infection of *Saprolegnia parasitica* in brown trout. The study was negatively controlled and was not conducted according to GCP guidelines. Approx. 1,270 commercially farmed brown trout of 5-7 years of age and of mixed sex, weighing approx. 1.2 kg were included in the trial. The fish were in 6 tanks. These were randomly allocated to either treatment with bronopol at 20mg/litre, for 30 minutes daily for 14 consecutive days (3 tanks), or, to untreated controls (3 tanks). The numbers of fish in each tank were not the same. The study concluded that the administration of bronopol at a dose of 20mg/litre litre for 30 minutes daily, for 14 consecutive days, substantially reduced fungal infections and mortality rates caused by *Saprolegnia parasitica* in brown trout.

Field Trials

The objective of this study was to assess the efficacy of bronopol against a natural infection of *Saprolegnia parasitica* in atlantic salmon, rainbow trout and brown trout. The study was conducted according to EU GCP guidelines. A total of 64 tanks and 25,878 fish were used. The tanks and ponds, each containing 13-5,000 fish, were randomly allocated on each site so that equal numbers were either treated with bronopol or placebo. On each site 1-6 pairs of tanks were used. The fish were 1-6 years of age, of mixed sex and of various commercial strains. The results for each site showed that the placebo treated fish had a greater severity of infection than the bronopol treated ones at the end of the trial.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the risk benefit profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)

The post-authorisation assessment (PAA) contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

The PAA for this product is available on the Product Information Database of the Veterinary Medicines Directorate website.

(www.gov.uk/check-animal-medicine-licensed)