



Aquaflor™
Aquaferen®
Florocol™
(florfenicol)



Technical
Monograph



Schering-Plough Animal Health

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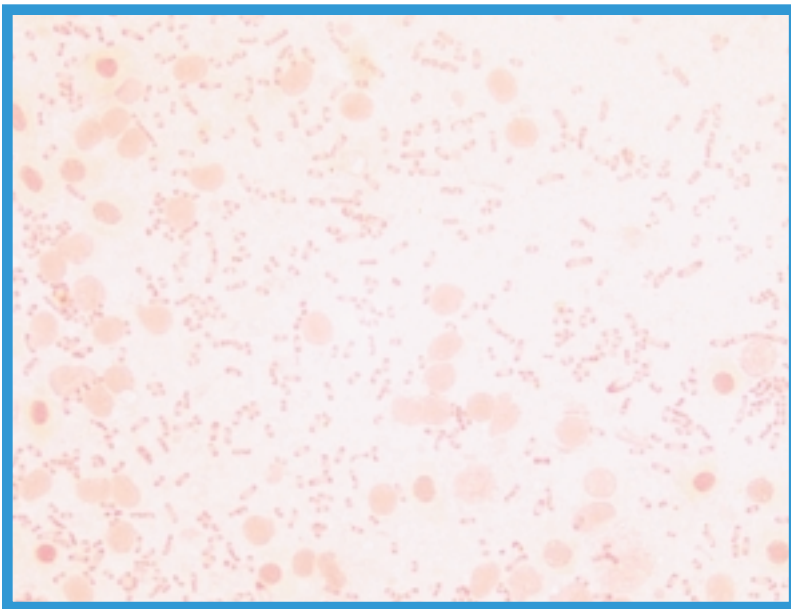
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1 Introduction

Bacterial diseases represent some of the most significant challenges facing fish farming worldwide. Furunculosis, vibriosis, cold-water vibriosis, rickettsiosis, rainbow trout fry syndrome and enteric redmouth disease are just a few of the more significant diseases that have plagued aquaculture in recent years. *Aeromonas salmonicida* is the causal agent of furunculosis and a disease outbreak can result in the rapid onset of high mortality with little clinical pathology, or a more common chronic form of the disease can occur with lower mortality rates and external boils or “furuncles”. Furunculosis occurs in freshwater

and seawater fish and the most significant losses are usually associated with a change in husbandry e.g. movement to sea, lice treatment or environmental change such as a rise in water temperature. *Vibrio* spp. infections are predominantly marine disease problems, although there have been occasional reports of disease caused by *Vibrio* spp. in freshwater. *V. anguillarum* and *V. salmonicida* infections give rise to the septicemic diseases known as vibriosis and cold-water vibriosis respectively, and as with furunculosis can present as either acute disease with high mortality rates or as a more chronic condition with lower mortality rates but clinical signs more typical of a septicemia e.g. internal and external hemorrhages. In a similar situation



Spleen smear from sea bream infected with *Photobacterium damsela* (pasteurellosis) (Gram stain).

to furunculosis, husbandry and environmental stress play significant roles in determining whether or not infection results in clinical disease, however, these bacteria are considered primary pathogens and successful therapy relies on the availability of effective antibacterial agents.

An increase of antibacterial resistance by some fish bacterial pathogens, most notably with *A. salmonicida*¹⁻³, has been reported and the need for antibiotics that will combat these pathogens is of increasing importance.

Aquaflor[®] is a feed premix containing the broad spectrum antibiotic, florfenicol, in a 50% formulation. Florfenicol has activity against a wide range of fish pathogens *in vitro* and *in vivo*, including *Aeromonas salmonicida* and *Vibrio salmonicida* in salmon. Florfenicol added to fish feed (either incorporated prior to pelleting or coated onto pellets), and administered at a dose rate of 10mg/kg body weight for 10 consecutive days, results in a rapid decline in mortality rates caused by susceptible bacterial diseases such as furunculosis and vibriosis.

Key characteristics

- **Administered in feed**
- **Well tolerated by fish**
- **Effective against a range of bacterial diseases**
- **Effective in a range of water temperatures**
- **Effective in fresh and salt water**
- **Different mode of action compared to other antibiotics**
- **Minimal environmental effect**

Florfenicol is a synthetically produced antibacterial that has been specifically developed for veterinary use. It is a fluorinated analogue of thiamphenicol, a chloramphenicol analogue, and these structural modifications confer advantages in activity, particularly against bacteria resistant to thiamphenicol and chloramphenicol⁴. Florfenicol is chemically different from chloramphenicol and lacks the functional group responsible for chloramphenicol's human toxicity concerns (bone marrow suppression and aplastic anemia).

Studies with florfenicol indicated potent activity against a number of bacterial fish pathogens *in vitro* and in a variety of fish species *in vivo*. Experimental efficacy has been demonstrated against *Pasteurella piscicida* (now *Photobacterium damsela* subsp. *piscicida*) in yellowtail, *Edwardsiella tarda* in eels, *V. anguillarum* in goldfish⁵ and *A. salmonicida* in Atlantic salmon⁶, and indicated that florfenicol was a potential candidate for the control of furunculosis and vibriosis in salmon.

There are a limited number of products approved for treatment of bacterial disease in fish and antibacterial resistance to some of these products has already become widespread, in particular with *A. salmonicida*.^{2,3} Oral administration of antimicrobials is the preferred route of chemotherapy in finfish aquaculture due to the ease of use and lack of any additional stress to the fish during treatment. Any reduction in appetite due to clinical disease can be addressed by higher feed inclusion rates of the medication.

Florfenicol has been approved in Japan since 1990 for treatment of susceptible bacterial diseases, including pasteurellosis and streptococcosis in yellowtail, red sea bream, coho salmon, horse mackerel, rainbow trout, sweetfish, tilapia and eel. In 1991 florfenicol was approved in South Korea for treatment of bacterial diseases in yellowtail and in 1993 florfenicol was introduced into Norway for treatment of furunculosis in salmon. Florfenicol was approved in Chile (Aquafer[®]) in 1995 and in Canada (Aquaflor[®]) in 1997 for treatment of furunculosis in salmon. The UK authorities approved florfenicol feed premix (Florocol[®]) for the treatment of furunculosis in farmed salmon in 1999. A 30% injectable

form of florfenicol (Nuflor™) has been approved for respiratory disease in cattle in the European Community (1994) and respiratory disease and foot rot in cattle in the US (1996). Florfenicol has been approved for the treatment and control of pleuropneumonia in pigs (1992) and colibacillosis in poultry (1993) in Japan and in major European markets for swine respiratory disease in 2000.

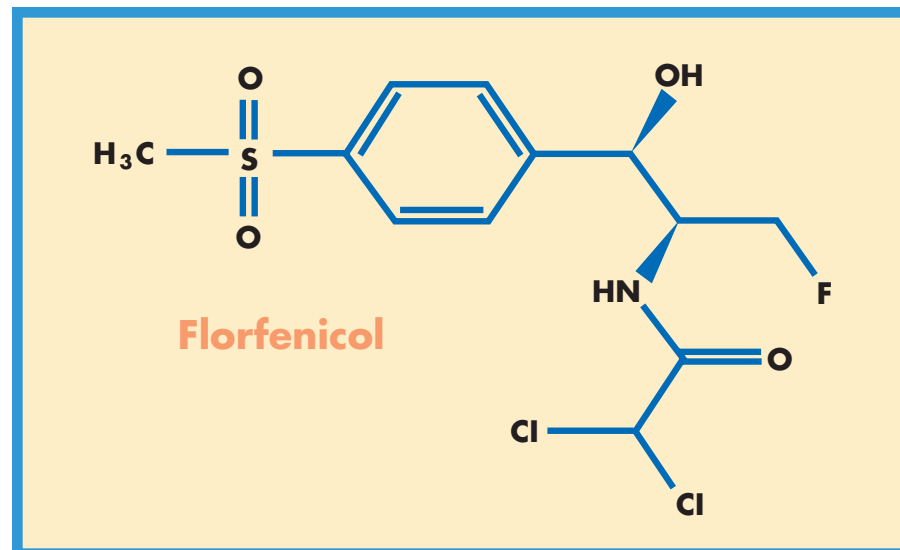
Aquaflor®/Florocol® was developed by the Research Division of Schering-Plough Animal Health specifically to provide fish producers with a product that combines highly effective control of susceptible bacterial diseases with safety for fish, ease of administration and a different mode of action from other antibiotics. Because florfenicol is in a novel class, it is likely to be effective against pathogens that are resistant to existing drugs.

CHEMISTRY

The following data describe the active ingredient:

Florfenicol is the active ingredient in Aquaflor®/Florocol®. Florfenicol is a monofluorinated derivative of thiamphenicol, a chloramphenicol analogue in which the *p*-nitro group on the aromatic ring is substituted with a sulphonylmethyl group.

Chemical structure



Scientific Name ([R-(R*,S*)]-2,2-Dichloro-N-[(1R)-1-hydroxy-2-(2-fluoroethyl)ethyl]acetamide)

Generic Name florfenicol

Molecular Formula C₁₂H₁₄Cl₂FNO₄S

Molecular Weight 358.21

DOSAGE FORM

Premix

Aquaflor[®] is a 50% (w/w) medicated premix for inclusion into fish feed.

The composition is 50% florfenicol and 50% inert carriers with the following characteristics:

- Supplied in 2 kg foil packages, with eight 2 kg packages in a fibre drum.
- Aquaflor[®] has a shelf life of three years.

Medicated Feed Production (see label for explanation)

- The product can be mixed in unmedicated fish feed prior to pelleting or surface coated on pellets.
- It should be added to feed to deliver 10 mg florfenicol per kg body weight daily. See table for recommended Aquaflor feed inclusion rate.

Recommended AquaflorTM Inclusion Rates for Preparation of Medicated Feed

Feeding Rate (% of body wt.)	Aquaflor [®] per ton of Feed		Fish medicated for 10 days per ton of feed	
	kg/metric ton	lb/US ton	kg fish/metric ton feed	lbs fish/ US ton feed
0.5%	4.0	8.0	20,000	40,000
1.0%	2.0	4.0	10,000	20,000
2.0%	1.0	2.0	5,000	10,000
3.0%	0.66	1.32	3,333	6,666
5.0%	0.40	0.8	2,000	4,000

Dose rate

- Recommended dose rate is 10 mg of florfenicol/kg body weight/day for 10 consecutive days



Offshore salmon farm.

MODE OF ACTION

Florfenicol is a synthetic, broad spectrum antibiotic active against many Gram-negative and Gram-positive bacteria. Florfenicol acts by binding to the 50S ribosomal subunit thereby preventing bacterial protein synthesis.⁴

In vitro activity has been demonstrated against commonly isolated bacterial fish pathogens including *Aeromonas salmonicida*, *Vibrio anguillarum*, *V. salmonicida*, *Vibrio* spp., *Edwardsiella tarda*, *Yersinia ruckeri*, *Photobacterium damsela* subsp. *piscicida* and *Flavobacterium psychrophilum*.^{2, 3, 5, 7, 8, 9, 10, 11} (see table below)

Bacteria resistant to chloramphenicol, through chloramphenicol acetyltransferase production, are sensitive to florfenicol.⁴ *In vitro* tests with fish pathogens and a range of chemotherapeutants, including chloramphenicol, streptomycin, sulfamonomethoxine, tetracycline, ampicillin, trimethoprim, furazolidone, kanamycin, naladixic acid, amoxycillin, oxolinic acid and florfenicol, demonstrated that florfenicol showed the most effective antibacterial activity of all the chemicals tested.^{2, 3, 8, 9}

***In vitro* activity (Minimum Inhibitory Concentrations) of florfenicol to some fish pathogens (µg/ml)**

Organism	No. of isolates	MIC range (µg/ml)
<i>Aeromonas salmonicida</i>	34	0.3 - 1.25 ⁷
<i>A. salmonicida</i>	2	0.4 ⁵
<i>A. salmonicida</i>	49	0.4 - 1.6 ¹⁰
<i>A. salmonicida</i>	11	0.3 - 1.25 ³
<i>A. salmonicida</i>	2	0.25 - 1.0 ²
<i>Edwardsiella tarda</i>	52	0.4 - 1.6 ⁵
<i>Flavobacterium psychrophilum</i>	48	0.00098 - 16 ¹¹
<i>Pasteurella piscicida</i> *	50	0.2 - 0.4 ⁵
<i>Pasteurella piscicida</i> *	175	0.004 - 0.6 ⁹
<i>Vibrio anguillarum</i>	114	0.2 - 0.8 ⁸
<i>Vibrio anguillarum</i>	37	0.4 - 0.8 ⁵
<i>Vibrio salmonicida</i>	10	0.8 ¹²
<i>Vibrio</i> spp.	3	1.25 ⁷
<i>Yersinia ruckeri</i>	5	0.6 - 10 ⁷

*now *Photobacterium damsela*



Land-based fish farm.



Atlantic salmon post-smolt affected by acute furunculosis (note pale gills, some bloody ascites and small hemorrhages on the liver).



Gross pathology of Atlantic salmon affected by chronic furunculosis. Note hemorrhagic flank swelling and petechiae on swim bladder.

Pharmacokinetics

The absorption, distribution, metabolism and excretion of florfenicol has been studied in cattle, pigs, Muscovy ducks, broiler chickens, horses and rainbow trout, as well as Atlantic salmon in freshwater and seawater.¹³⁻²¹ Conclusions from these studies were consistent for all species in that florfenicol was well absorbed, and excreted in bile, feces and urine.

Studies in salmon

Atlantic salmon (*Salmo salar*) were used in a number of studies to determine the fate of florfenicol in the fish; one study being a whole body autoradiography study with salmon in seawater at 8 to 11°C¹⁹ and others being radiolabeled residue depletion studies conducted with salmon in seawater at 5°C and 10°C. Results from the whole body autoradiography study indicated that florfenicol had the following properties:

- rapidly absorbed from the intestinal tract and transferred to other tissues
- widely distributed to salmon tissues with maximum levels found at 12 hours after the end of treatment with similar concentrations in blood and muscle, but lower levels in fat and brain
- maximal levels of florfenicol achieved in the muscle exceeded the Minimum Inhibitory Concentration (MIC) values reported for most fish pathogenic bacteria.¹⁹

In radiolabeled residue depletion studies, salmon received either a single dose of feed containing radiolabeled florfenicol, or 9 days of florfenicol medicated feed and 1 day of feed containing radiolabeled florfenicol at 10 mg/kg body weight. Results showed that:

- maximum radioactivity concentrations in all tissues occurred around 6 to 24 hours after final dose delivery and the highest levels were observed in the kidney and liver,
- tissue radioactivity declined faster at 10°C than at 5°C indicating that florfenicol is cleared from tissues faster at higher temperatures,
- residue concentrations were lower in muscle than in skin and depleted somewhat faster from muscle than from skin.



Japanese fish farmer feeding cages of red sea bream near Nagasaki.

Toxicology

A complete toxicological evaluation has been conducted with florfenicol. These include extensive published and unpublished studies in the mouse, rat, cattle, dog, swine, poultry, rainbow trout, salmon and bluegill sunfish.^{22, 23} From studies with florfenicol on reproduction in rats a No Observable Effect Level (NOEL) of 1.0 mg/kg/day has been established. With a safety factor of 100, an acceptable daily intake (ADI) has been calculated at 10 µg/kg/day. The metabolism of florfenicol in salmon is qualitatively similar to the metabolism in the rat.

Maximum Residue Limit or Safe Concentration

An HPLC assay based on the quantitative conversion of florfenicol and its metabolites to florfenicol amine was validated in muscle, skin and intact muscle/skin tissues of Atlantic salmon.²⁴ Since the assay involves conversion of metabolites to florfenicol amine, the marker residue represents 100% of the residues.

The Maximum Residue Limit (MRL) or equivalent Safe Concentration varies from country to country and is based on the marker residue, florfenicol amine. The following are the established or proposed limits or concentrations:

European Community (EC) – MRL is 1.0 mg/kg for muscle with attached skin.

USA – proposed safe concentration is 1.0 mg/kg for muscle with attached skin.

Canada – safe concentration is 0.8 mg/kg for muscle, 8.0 mg/kg for skin.

Norway – safe concentration is 1.0 mg/kg for muscle with attached skin.

Withdrawal period

The withdrawal period varies from country to country but the following are established or proposed:

<i>Country</i>	<i>Withdrawal period</i>
Japan	5 – 14 days
South Korea	7 days
Norway	30 days
Chile	28 days
Canada	12 days
UK	15 days @ 10°C or 150 degree-days
USA	pending

Safety studies: salmon

Target animal safety and tolerance studies were performed with florfenicol in Atlantic salmon (*Salmo salar*) in both freshwater and seawater. A summary of the results for these studies is presented below.

One study tested the effect on Atlantic salmon post-smolts in seawater of multiples of the recommended dose and treatment period on growth and internal organ pathology. The second study tested the effects of multiples of the recommended dose and treatment period in freshwater on growth and internal organ pathology of Atlantic salmon parr and further investigated any effects on tissues by histology.⁶ Weights of fish at the start and end of the trial were also recorded.

Details of tolerance trials with orally administered florfenicol^(a)

Life stage	Parr	Post-smolts
No. fish per treatment group	11.5	98
Mean weight (g)	10	35
No. of days treatment at:		
1x ^b	60	61 ^c
5x	60	61 ^d
10x	10	11 ^e

^a all were 10 or 11 day treatments with 10 day intervals between each medication regimen

^b multiples of recommended dose of 10 mg/kg/day

^c actual dose received was 6.4 mg/kg/day

^d actual dose received was 31.4 mg/kg/day

^e actual dose received was 85.5 mg/kg/day

Salmon leaping in a sea cage.





Clinical signs and feed consumption were recorded daily and fish were weighed at the beginning and end of the trials. Any fish that died during the course of the studies had post-mortem examinations conducted and bacteriology performed. In the seawater trial 380 fish were separated into 10 tanks, examined macroscopically and randomly selected for histology on

day 11 and day 61 of the trial. A total of 100 fish were examined histologically.

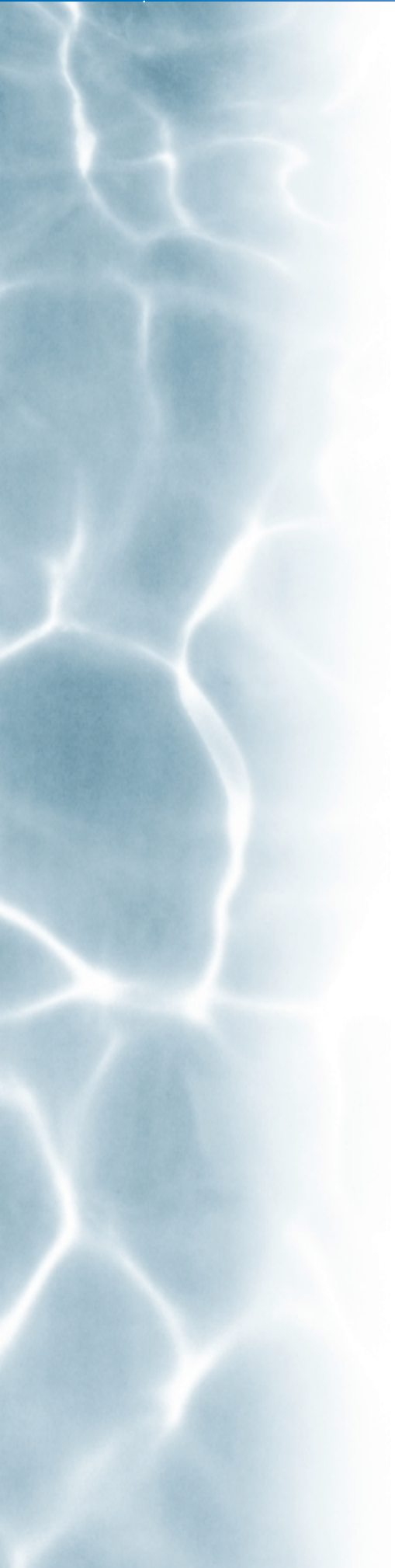
In the freshwater trial 100 fish were allocated to five tanks. Ten (10) fish from the high dose treatment group were sacrificed for histology after 10 days and following 60 days, 10 fish from each of the remaining treated groups and the controls were also sampled for histology. A total of 40 fish was examined histologically.

Results

No palatability problems, adverse reactions or abnormal clinical behavior were observed in any of the trials even with 10x the recommended dose of 10 mg/kg body weight fed for ten days. In the freshwater trial, doses of 10, 5 and 1x the recommended dose were fed for 10 days. The 5x and 1x doses were repeated three times following intervals of 10 days on normal diets and no adverse clinical signs were observed.

The objective of the seawater trial was to administer 10, 5 and 1x the recommended dose, however, due to the growth of the fish they received lower than intended levels with 85.5, 31.4 and 6.4 mg/kg body weight being the actual doses fed respectively. The highest dose was fed for 11 days and the remaining two dose groups repeated three times for 11 days each with normal feed fed for 10 days in-between. No palatability problems, adverse reactions or abnormal clinical behavior were observed that were associated with the medication. No treatment-related mortality was observed. In all freshwater and seawater trial groups, no tissue abnormalities were observed by histopathology that were associated with medication and all groups gained weight.

From these results it is clear that up to 10-fold the recommended dose produced no adverse clinical or pathological reactions in freshwater salmon. Similarly, up to 8.5-fold the recommended dose fed to seawater salmon produced no adverse clinical or pathological reactions.



4 Environment

The levels of florfenicol and its metabolites in the receiving environment will be dependent on the quantities administered and consumed and their fate in the receiving environment. Florfenicol will reach the sediment as uneaten, medicated feed or in feces. Metabolites will enter the sediment in excreta. Both florfenicol and its metabolites will enter the water column by leaching from feed and feces and by excretion in the aqueous phase of the excreta.

Environmental risk assessment included evaluation of all available data for florfenicol, and its metabolites, and generation of data specific for the use of florfenicol in the freshwater and marine environments.

Details

Florfenicol has a molecular weight of 358.21 daltons and a solubility in water of 1.32g/L at pH7 and a log K_{OW} value (partition coefficient) of 0.37, the latter indicating little potential for bioaccumulation. As the physiochemical characteristics of florfenicol and its major metabolites show, it is unlikely that florfenicol or its degradation products will concentrate in sediment. Experimental studies of the persistence of antibacterial agents in marine sediments have indicated that the concentration of florfenicol decreased rapidly in the sediment with a calculated half-life of 4.5 days.^{25, 26} The metabolite, florfenicol amine, was detected and it appears that florfenicol is rapidly degraded in sediment. This was in stark contrast to the persistence of the other antibacterials examined in marine sediments where the half-life was shown to be in excess of 50 days at the sediment surface.²⁵

From these studies and others undertaken by Schering-Plough Animal Health, a comprehensive data set has been developed on the toxicity of florfenicol to invertebrates, fish, birds and mammals.²³ These data enabled predictive risk assessments to be undertaken in which 10-100 fold assessment (safety) factors have been applied to the toxicity data for the most sensitive of the species appropriate for the environments considered.

To establish the potential for an adverse effect in the environment, the Minimum Inhibitory Concentrations (MICs), LC₅₀, or EC₅₀ values and NOELs were compared to the Predicted Environmental Concentration (PEC) in water and sediment in both marine and freshwater environments. The PEC values shown on the following page were refined based on degradation and dissipation.

PEC values of florfenicol

Compartment	PEC Value
Marine sediment	224 µg/kg
Seawater	0.0035 µg/l
Freshwater	<0.0625 µg/l

The PEC to Predicted No Effect Concentration (PNEC) ratio values were established for a range of micro-organisms, plants, invertebrates and fish, with the PNEC values derived by applying a one hundred fold safety factor to the highest concentration tested. Details of the environmental risk characterisation data for selected species are shown below:

Environmental risk characterisation data for florfenicol

Organism	MIC EC ₅₀ /LC ₅₀ (mg/l)	NOEC* (mg/l)	PNEC (µg/l)	PEC:MIC PEC:PNEC
<i>Bacillus subtilis</i>	0.4			0.560
<i>Nostoc</i>	4.0	2.0		0.00003
<i>Selenastrum capricornutum</i>	1.5	0.75	75	0.0008
<i>Daphnia magna</i>	>330	<100	3,300	<0.00002
<i>Oncorhynchus mykiss</i>	>780	780	7,800	<0.000008
<i>Lepomis macrochirus</i>	>830	830	8,300	<0.000008
<i>Skeletonema costatum</i>	0.0128	0.0042	0.42	0.008

* No Observed Effect Concentration

The very low PEC/PNEC ratios reflect the lack of toxicity of florfenicol to aquatic organisms and the rapid degradation and dissipation of the compound. With regard to fish metabolites present in excreta, the soil degradation studies indicate that florfenicol and its degradates are ultimately mineralized.

Although significant amounts of florfenicol can enter the aquatic environment, its rapid degradation and dissipation decreases its environmental concentration. The existing toxicity data indicates that florfenicol is, in general, more active against prokaryotic than eukaryotic organisms. However, the likelihood of environmental effects are limited, given the MIC:PNEC and PEC:PNEC ratios calculated based on the intended use patterns, its fate in the receiving environments, and its toxicity. The calculated PEC values are below the MIC or PNEC values determined. The mobility and rapid loss of activity of florfenicol in soils and sediments indicates that any exposure will be of limited duration. These data indicate that marine organisms are unlikely to be adversely affected by exposure to florfenicol following its use in fish in the marine environment. Likewise, the use of florfenicol administered in feed to fish reared in freshwater in commercial hatchery and production facilities would not affect the aquatic environment.

Efficacy

EFFICACY: RESULTS OF CLINICAL STUDIES

Overview

Experimental models have been developed for some bacterial fish pathogens however there is still a paucity of knowledge on the routes of infection and pathogenesis of many fish pathogens. Natural bacterial disease outbreaks on farms represent an alternative testing arena although these are often a complicated and expensive means of testing efficacy. Further trials on farms with untreated controls may not be acceptable either ethically or economically and the usual requirement is that the test compound must be tested against products already established as effective against the disease.

Dose rate determination and dose rate confirmation studies were conducted at several locations in Scotland and one in Norway. In the confirmation studies in salmon, the challenge bacteria were *Aeromonas salmonicida* and *Vibrio salmonicida* and florfenicol medicated feed was administered to supply the recommended dose rate of 10 mg/kg for 10 consecutive days.

Commercial field trials were conducted in Norway and Canada against furunculosis. In Norway the efficacy of florfenicol was compared to that of other antibacterials administered in feed, specifically oxolinic acid, potentiated sulphonamide (trimethoprim & sulphadiazine) and flumequine. In the Canadian trials the efficacy of florfenicol was compared to erythromycin or unmedicated feed. Results from the five study sites in Norway and the three study sites in Canada showed that florfenicol medicated feed provided better sustained efficacy against furunculosis when compared to positive and negative controls.

Characteristics of Aquaflor[®]/Florocol[®]

- High level efficacy: Aquaflor[®]/Florocol[®] rapidly reduced mortalities due to furunculosis or cold-water vibriosis
- Excellent efficacy in comparative response to therapy: Aquaflor[®] reduced mortalities more rapidly and to a lower level in the field trials when compared to fish treated with oxolinic acid, potentiated sulphonamide, flumequine and erythromycin.
- Well tolerated: Aquaflor[®]/Florocol[®] was well tolerated with no mortality or reduction in feeding associated with treatment.

Efficacy evaluation details

The efficacy evaluation for the control of bacterial diseases using florfenicol was conducted as follows:

- 1 Seawater and freshwater dose titration studies against induced infections of *A. salmonicida* in Scotland and Norway plus a seawater dose titration study in a natural furunculosis outbreak on a farm in Scotland
- 2 Seawater dose confirmation studies, in cases of furunculosis or coldwater vibriosis in comparison to oxolinic acid, oxytetracycline, potentiated sulphonamide and flumequine
- 3 Seawater field trials in Norway against furunculosis in comparison to oxolinic acid, potentiated sulphonamide and flumequine.
- 4 Seawater field trials in Canada against furunculosis in comparison to erythromycin or unmedicated feed.

Dose titration and dose confirmation in salmon

Six trials were conducted in Scotland and Norway to determine and confirm the optimum dose rate of florfenicol. Atlantic salmon (*Salmo salar*) ranging in size from 11.5 g parr to 650 g post-smolts were given a pelleted feed coated with florfenicol in fish oil.

During these trials, treatment groups were fed florfenicol at various dose rates for 10 consecutive days. Control groups were either positive controls fed oxolinic acid, oxytetracycline, potentiated sulphonamide or flumequine, or were negative controls fed unmedicated feed at the same rate. Efficacy was assessed by total mortality at the end of the treatment period.

A total of 6,406 Atlantic salmon were utilized in the dose titration and dose confirmation studies, with 3,211 fish in treatment groups that received florfenicol medicated feed and 1,520 in the positive control groups and 1,675 in the negative or unmedicated feed groups.

Results and significant findings

Results of trials 1 to 4 (see table) indicated that a florfenicol medicated diet given to fish for 10 consecutive days (at dose rates between 5 and 23 mg/kg/day) resulted in effective control of furunculosis. In two of these trials (1 and 3) there was significant evidence that the dose rate of 10 mg/kg/day was more efficacious than 5 mg/kg/day in reducing mortalities due to furunculosis.⁷ Results of trials 5 and 6 (see table) validated the recommended dosage for florfenicol of 10 mg/kg/day for 10 consecutive days.²⁷

Dose titration studies with florfenicol: cumulative mortalities (%) due to challenge with *A. salmonicida*

Trial No.	Descriptive/Objective	Dose rate (mg/kg/day)	Cumulative mortalities (%)	Summary results
1 ⁶	Dose range finding studies: 20 parr (freshwater) per tank and 13 tanks bath challenged with <i>A. salmonicida</i>	0 5 10 20	75 17 13 5	Excellent palatability at all doses. Effective in reducing furunculosis mortalities between 5 and 20 mg/kg/day
2	Dose titration study: 108 post-smolts per tank, and eight tanks challenged by intramuscular injection with <i>A. salmonicida</i>	0 11.6 17.4 23.2	88 25 25 22	This was a severe challenge trial and there was a poor feeding response at the start of medication, with the possibility that many fish did not receive the targeted dose rates, however, there was clear evidence that florfenicol controlled an acute infection of <i>A. salmonicida</i> .
3	Dose titration study: 100 post-smolts in each of six tanks, to which were added 15 salmon injected with <i>A. salmonicida</i>	0 5 10	50 14.8 8.1	Excellent palatability of medicated feed. Florfenicol was efficacious in controlling mortalities due to <i>A. salmonicida</i> and 10 mg/kg was statistically more effective than 5 mg/kg ($P = 0.01$)
4	Dose titration study: post-smolts in eight sea cages, with 176 fish per cage. These fish were naturally infected with <i>A. salmonicida</i> .	0 5 10 15	24.7 3.7 3.2 4.6	Excellent palatability and significant reduction in mortality due to furunculosis with florfenicol.

Dose confirmation studies with florfenicol: cumulative mortalities (%) due to challenge with *A. salmonicida* and *Vibrio salmonicida* and comparison with other medicines²⁷

Trial no.	Description/Objective	Treatment and dosage (mg/kg/day)	Cumulative mortalities (%)	Summary results
5	Dose confirmation study where 18 tanks (100 smolts/tank) had 15 <i>A. salmonicida</i> experimentally infected fish introduced per tank.	Unmedicated	48	Mortality rates significantly lower in florfenicol treated groups when compared to untreated and were better than or equal to other medicines.
		Florfenicol (10)	14	
		Oxolinic acid (25)	21	
		Oxytetracycline (100)	16	
		Trimethoprim/sulphadiazine(30)	21	
		Flumequine (25)	17	
6	Dose confirmation study where six tanks each holding 100 smolts were exposed to <i>Vibrio salmonicida</i> by injection.	Unmedicated	97	Good efficacy and excellent feeding response with florfenicol.
		Florfenicol (10)	24	
		Oxolinic acid	31	

Efficacy - Commercial Field Trials: Norway

The Norwegian farm trials were based on the results from five marine farms affected by furunculosis.

Trial details

Environment: These trials were conducted in the summer months of June to September when sea water temperatures varied from 12.0 to 16.8° C.

Treatment design: The test unit on each farm was comprised of four cages, divided into two blocks of two pens of identical fish, with treatments randomly allocated within blocks. Florfenicol was fed to two cages in each trial at 10 mg/kg/day for 10 days and the other two cages were fed a standard medicine, the details of which are shown in the table. Fish were 1991 and 1992 generation Atlantic salmon (*Salmo salar*).

Evaluation of efficacy: Efficacy was based on mortality figures with dead fish collected daily and the percentage mortality calculated for the first 10 days after introduction of medication. During the first period samples from each dead fish were cultured for the presence of *A. salmonicida*.²⁸

Commercial field trials in Norway with florfenicol and standard medicines versus furunculosis²⁸

Trial no.	No. cages	Florfenicol			Positive Control		
		No. fish	Mortality*		No. fish	Mortality*	
			No.	Percent		No.	Percent
7	4	13,881	146	1.05	13,656	116	0.85
		13,963	263	1.88	13,862	353	2.55
8	4	8,708	129	1.48	6,950	196	2.82
		8,925	140	1.57	8,188	112	1.37
9	4	24,691	21	0.09	25,837	42	0.16
		28,657	51	0.18	24,641	17	0.07
10	4	2,233	50	2.24	2,219	110	4.96
		2,309	15	0.65	2,294	13	0.57
11	4	6,264	271	4.33	6,736	429	6.37
		5,614	27	0.48	5,514	40	0.73
Overall		115,245	1,113	0.97	109,897	1,428	1.30

Positive Controls:

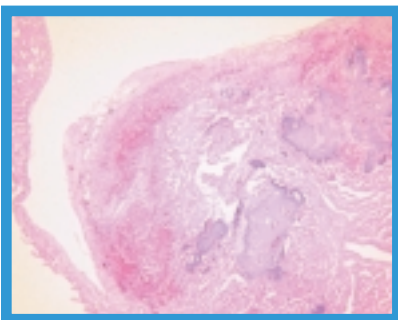
Trial nos. 7 & 8 - oxolinic acid at 25mg/kg/day

Trial nos. 9 & 10 - flumequine at 25mg/kg/day

Trial no. 11 - trimethoprim/sulfadiazine at 30 mg/kg/day

* mortality figures are those specifically due to furunculosis as confirmed by bacteriology

Results



Histological section of cardiac ventricle taken from a salmon affected by furunculosis. Note large bacterial colonies which stain up as purple areas and associated tissue damage (H&E stain).

- All medicated feeds were well accepted by the fish.
- The differences between florfenicol and each standard product were significantly in favor of florfenicol. Overall specific mortality was 0.97% (range 0.09 to 4.33%) for florfenicol and 1.30% (range 0.07 to 6.64%) for other antibacterial agents.
- The majority of deaths due to *A. salmonicida* infection occurred during the first three of four days of medication.
- At a daily dose rate of 10 mg/kg bodyweight/day for 10 consecutive days, florfenicol was shown to be highly effective and well tolerated for the treatment of furunculosis in Atlantic salmon under field conditions.

Efficacy – Commercial Field Trials: Canada

Three Canadian field trials were conducted at marine sites where salmon were affected by furunculosis.

Trial details

Environment: These trials were conducted in the months of August and October when sea water temperatures varied between 14 to 18°C and 9 to 10°C, respectively.

Treatment design: The test unit on each farm was comprised of randomly selected sea cages within pontoons. A number of cages in each pontoon were fed florfenicol medicated feed and other cages acted as either positive controls, where they were medicated with erythromycin, or as negative controls where they were fed unmedicated feed. All fish treated in the trials were Atlantic salmon (*Salmo salar*).

Evaluation of efficacy: Efficacy was based on mortality with dead fish collected every two to three days by divers. The percentage mortality was calculated for the 10 days after the start of medication.

Commercial field trials in Canada with florfenicol and standard medicines, or unmedicated feed, versus furunculosis

Trial no.	No. cages	Florfenicol			Control*		
		No. fish (no. cages)	Mortality**		No. fish (no. cages)	Mortality**	
			No.	Percent		No.	Percent
12	13	129, 629 (9)	129	0.10	91, 876 (4)	171	0.19
13	12	56, 428 (4)	135	0.24	90, 441 (8)	194	0.22
14 ²⁹	20	189, 904 (12)	1, 547	0.82	129, 464 (8)	2, 116	1.63
Overall		375, 961	1, 811	0.48	311, 781	2, 481	0.80

*The control cages in trials 12 and 13 were medicated with erythromycin, which was fed at 100 mg/kg/day. Three of the eight control cages in Trial 13 originally were on unmedicated feed but then switched to erythromycin.

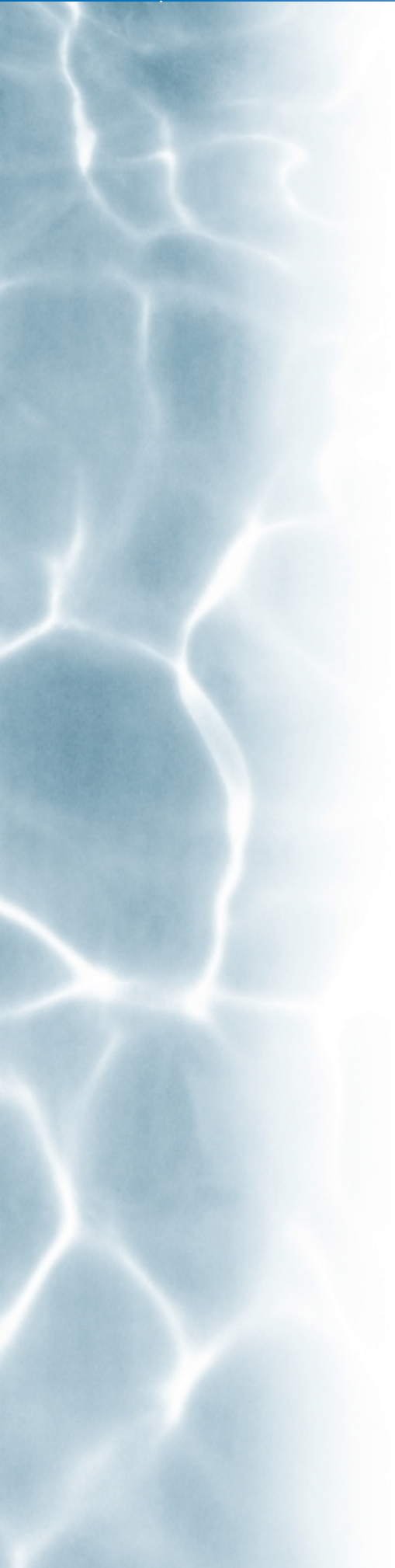
** The mortalities are the total mortality during the medication period i.e. during and including Day 1 to Day 10 of each trial.

Results

- All medicated feeds were well accepted by the fish
- Overall mortality for the medication period demonstrated that florfenicol compared favorably to the control cages fed either erythromycin or unmedicated feed; overall results were 0.48% (range 0.10 to 0.82) for florfenicol and 0.80 (range 0.19 to 1.63) for the controls.
- At a daily dose rate of 10 mg/kg bodyweight/day for 10 consecutive days, florfenicol was shown to be highly effective and well tolerated for the treatment of furunculosis in Atlantic salmon under field conditions

Conclusions

Aquaflor® has been shown to provide highly effective control of bacterial diseases of fish with excellent palatability of medicated feed and a wide margin of safety for fish.



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