ENVIRONMENTAL ASSESSMENT

FOR

AQUAFLOR® 50% TYPE A MEDICATED ARTICLE FOR CATFISH

SECTION 1. DATE: January 15, 2004

SECTION 2. NAME OF APPLICANT/PETITIONER: Schering-Plough Animal Health Corp.

SECTION 3. ADDRESS: 1095 Morris Ave.
Union, NJ 07083-1982

SECTION 4. DESCRIPTION OF THE PROPOSED ACTION

A new animal drug approval has been requested for the use of Aquaflor® 50% Type A Medicated Article in catfish (*Ictalurus punctatus*). Aquaflor® 50% Type A Medicated Article contains the active ingredient, florfenicol. Florfenicol is a synthetic, broad-spectrum antibiotic, with activity against a wide range of fish pathogens. In catfish aquaculture, the target fish pathogen for florfenicol is *Edwardsiella ictaluri,* which causes enteric septicemia in catfish (ESC).

The Aquaflor® 50% Type A Medicated Article is added to fish feed (incorporated prior to pelleting). The rate of administration of the premix to the feed will be dependent on the food consumption rate. The recommended dosage regimen in catfish is 10 mg a.i./kg body weight for 10 consecutive days. Therefore, at a feeding rate of 1% body weight per day a total of 2.0 g of the medicated article (1.0 g florfenicol) would be applied per kilogram of feed. The quantities of florfenicol being administered will be dependent on the quantities and weight of fish requiring treatment. The product is intended for use in farmed catfish in the freshwater environment.

Aquaflor® is currently being used in Japan, South Korea, Norway, Chile, Canada and the UK.

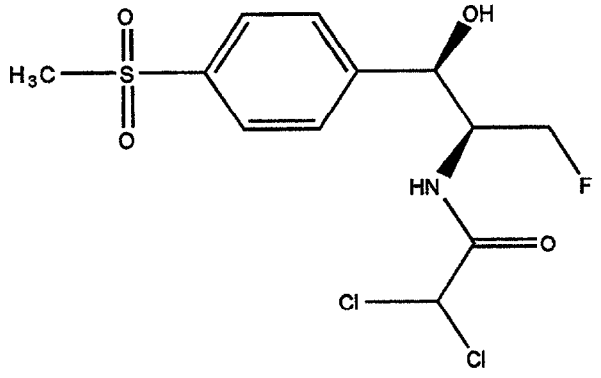
SECTION 5 IDENTIFICATION OF CHEMICAL SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

Florfenicol is the 3-fluoro derivative of thiamphenicol, which is a chloramphenicol analogue in which the p-nitro group on the aromatic ring is substituted with a sulfonylmethyl group. The structure and nomenclature are presented below. There are three primary metabolites: the amine, the alcohol, and the oxamic acid.

Chemical characteristics for florfenicol:

Chemical Name: ([R-(R*,S*)]-2,2-Dichloro-N-[α-(fluoromethyl)-β-hydroxy-β-[4-(methylsulfonyl)phenyl]ethyl]-acetamide

Structural Formula:



Florfenicol (SCH 25298)

The physico-chemical characteristics of florfenicol and its major metabolites have been determined, Table 1 (Appendix 1). Florfenicol has a molecular weight of 358.21 with solubility in water of 1.32 g/l at pH 7 and a log K_{ow} value of 0.37, the latter indicating little potential for bioaccumulation. In addition, florfenicol has a melting point of 153.5 - 154.5°C. Florfenicol is a nonvolatile solid and has a UV maximum at 224 nm. In view of these physico-chemical characteristics, and those listed for the metabolites in Table 1, it is unlikely that florfenicol, or its metabolites, will pose potential risks to the environment. This is particularly true when considering locally elevated concentrations are expected to be intermittent and of short duration. Environmental concerns are generally associated with materials of low solubility that readily adsorb or accumulate, but compounds such as florfenicol that have substantial solubility with an extremely

low Log K_{ow} would not be expected to accumulate in biota or other environmental matrices.

Florfenicol and its metabolites have molecular weights, which range from 69 to 89% of parent. The solubility and K_{ow} differ. The metabolites are markedly more soluble with reported solubilities ranging from 49.7 to >500 g/L and as would be expected the metabolites are markedly less lipophilic (i.e., have lower K_{ow}). These factors make the metabolites even more likely than florfenicol to enter and remain in water and not partition to sediment particles or accumulate in biota.

The studies pertaining to environmental toxicity have been conducted with the active ingredient, florfenicol, as well as metabolites. The aquaculture product contains 50% (w/w) florfenicol. Inert ingredients include 47% (w/w) lactose monohydrate as diluent and 3% (w/w) povidone (polyvinylpyrrolidone) as binder. Lactose will be degraded readily in the fish and if any reaches the environment, it will also be degraded readily there. Povidone (polyvinylpyrrolidone, CASRN 9003-39-8) is a high molecular weight, highly soluble (110g/L @ 20°C[1]) polymer that acts as a dispersing agent. It is a common ingredient in pharmaceuticals. Degradation would ultimately be to low molecular weight subunits that could undergo further degradation. Therefore, these excipients will not be the subject of this environmental assessment.

1 ChemIndex.cambridgesoft.com

TABLE 1 Physico-chemical characteristics of florfenicol and major metabolites

	Florfenicol	Principle Metabolite		
		Amine	Alcohol	Oxamic Acid
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
CAS Number	73231-34-2	76639-93-5	NA	NA
Empirical Formula	$C_{12}H_{14}Cl_2FNO_4S$	$C_{10}H_{14}FNO_3S$	$C_{12}H_{16}FNO_5S$	$C_{12}H_{14}FNO_6S$
Molecular Weight	358.21	247.28	305.32	319.30
Comparative Molecular Weight Ratio	1.000	0.690	0.852	0.891
Solubility, pH 7(g/l)	1.32	>500	49.7	>500
Dissociation Constant (pK_a)	NA	7.5	NA	1.99 2.03*
Partition Coefficient (K_{ow}) (Log K_{ow}), pH 7	2.36 (0.37)	0.100 (-0.965)	0.070 (-1.20)	0.001 (-3.0)
Density (g/cm^3)	1.68	1.32	1.42	1.45

NA = Not applicable/available
* = With ionic strength correction

SECTION 6 INTRODUCTION OF THE SUBSTANCES INTO THE ENVIRONMENT

The levels of florfenicol and its metabolites entering the receiving environment will be dependent on the use-pattern of Aquaflor, the pharmacokinetics, metabolism (in fish) and the environmental fate characteristics of the active ingredient and its metabolites. The amount of Aquaflor applied including the magnitude, timing, frequency and duration of application will be determined by

use-pattern, as specified on the label, combined with the biology of *Edwardsiella ictaluri,* the target bacterial species.

6.1 The role of use-pattern in determining environmental release

The frequency and timing of application of Aquaflor are limited by regulations and biology. Aquaflor will be regulated under the Veterinary Feeds Directive (VFD), which requires the presence of the disease in a population to be confirmed by an aquaculture veterinarian. The use is prescribed and the medicated feed must be formulated in an FDA certified feed mill. The VFD does not allow for prophylactic use. Aquaflor is the first aquaculture drug to be regulated under the VFD, which will result in minimized and more controlled use of this and similar products.

In addition, the use of Aquaflor is limited temporally by the biological characteristics of the target pathogen (*E. ictaluri*). Since the presence of the organism and pathological effects are required to get a prescription, the application is limited to two narrow windows of time in the fall (September / October) and spring (May / June). Enteric septicemia in catfish caused by *E. ictaluri* only occurs when the waters are between 20°C and 30°C. In catfish ponds this happens only twice per year lasting approximately 30-40 days each season (Appendix 2).

Florfenicol and its metabolites will enter the sediment in excreta. Both florfenicol and its metabolites will move into the water column through leaching from feces

and by mixing of the aqueous phase of excreta into the water column. Nearly all catfish feed is formulated as floating, extruded pellets. Unlike sinking feed used in the culture of other aquatic animals, floating catfish feed has high water stability and does not sink into sediments where it may become unavailable to the fish. Also, fish-feeding activity is readily observed when fish feed on these floating pellets. These characteristics, taken as a whole, mean that very little, if any, feed is not consumed by the fish and little is expected to reach the sediments. For the purposes of this assessment it is assumed that feed is 100% consumed by the catfish.

Channel catfish (*Ictalurus punctatus*) are raised commercially in the southeastern portion of the United States, mainly in Mississippi, Alabama, Arkansas, and Louisiana. Approximately 97% of domestic catfish production occurs in these states (Appendix 3). California and Missouri account for the other 3%. The fish to be harvested for food are grown in large ponds approximately 3.2 - 6 hectares in area and approximately 1 m in depth (Appendix 3). The ponds are dug out of soil such that the water level of the ponds is mainly below the soil surface and the levees of the pond are made from the soil removed from the pond area. The ponds are filled and maintained through pumping of surface or well water. To reduce water loss via overflow and the release of effluent from the ponds, the water level in the ponds is managed by maintaining a water level below the overflow structure of the pond. A 20 cm storage capacity below the overflow level is recommended. In this way, rainfall will not normally cause the ponds to overflow (Appendix 3). Generally the ponds are built in areas where clay is a predominant feature of

the soil and therefore leakage from the ponds is minimal (Appendix 3). All of these factors serve to limit the release of pond water and any florfenicol-related residues into the environment.

Susceptibility of catfish to enteric septicemia (ESC) caused by *E. ictaluri* is dependent on production methods and previous exposure of the fish to this pathogen. The culturing of catfish starts in brood fishponds with the production of eggs. To maximize egg production, the brood fish are kept at a low density and a low stress level. This minimizes the potential for infections. Also, since the brood fish are older fish, they most likely have been exposed previously to the disease and are therefore possibly less susceptible to subsequent infection under the conditions in the brood ponds. Brood ponds constitute about 1.2% of the total area of catfish ponds (Appendix 3). It is unlikely that florfenicol would be used in brood ponds.

Fingerlings are raised from eggs during about a 12-month period from the spring of one year to the spring of the next year. Fingerlings being both naive to the disease and under production conditions of high density are the stage most susceptible to infection by *E. ictaluri*. The most likely time for this infection to occur is in the autumn of the first year (mid-September to mid-October) and to some extent in the second spring (mid-April to late May when the water temperatures become conducive for ESC (Appendix 2). The fingerlings will therefore be the fish most likely to be treated with florfenicol. The fingerling (nursery) ponds are drained each year to facilitate harvesting of the fish. The harvesting usually occurs in the spring before the potential for infection with

enteric septicemia and the fingerlings are purchased for the stocking of production ponds. The fingerling ponds take up approximately 13% of the total area used for catfish production and are mainly located in the lower Mississippi River Valley where water temperatures and water supplies are less subject to variation and therefore better suited to the fingerling production (Appendix 3). Limited fingerling production also occurs in areas of Mississippi and Alabama where the water source is surface water from a watershed. Since runoff from a watershed is greater than the volume of rainwater falling directly into ponds, the overflow volume from watershed-type ponds is greater than for levee-type ponds supplied with well water. Thus watershed-type ponds provide greater potential for dilution and dispersion and; therefore, levee ponds are used as worst-case scenarios in this assessment.

In production ponds, the goal is to raise the fish to market size weight in the shortest period of time. This usually takes about 6-12 months of additional growth after the fingerling stage but is variable depending on weather conditions, water quality, growth rate characteristics of individual fish within the fish population and the preferred market weight of fish. Most production ponds contain fish of various ages and therefore sizes. Fish are harvested by seining on a "continual" basis, 1-3 times a year. Fingerlings are added each year, usually in the spring, to make up for the amount of fish harvested the previous year and any losses. Larger fish in production ponds could also become infected, but the likelihood is much less than in the nursery ponds since the recently added fingerlings, and these older fish in the ponds, would likely have had some previous exposure and hence acquired some immunity (Appendix 4)

to *E. ictaluri*. This would serve to reduce the possibility of infection and to dampen the spread of any infection. Catfish ponds are managed so that waste inputs (fish waste products and organic matter produced by algae) do not exceed the capacity of the pond microbial community to assimilate those wastes. This allows the pond to be used for years at a time without accumulation of nutrients or organic matter in either the water column or in the sediments. From the standpoint of the environmental effects of Aquaflor, the most important aspect of this "balanced ecosystem" is that organic matter derived from fish feces does not accumulate in sediments because organic matter decomposition is continuous, even in winter. On average, production ponds are drained every 6.5 years when the pond bottom and/or levees require renovation. At those times the pond bottom is leveled and the soil used to rebuild eroded levees.

Therefore, in the catfish growth cycle, fingerlings during their first autumn in a pond are the most susceptible to ESC and as a result, would be most likely to be treated at that time and to some extent in the following spring. After exposure the catfish are less likely to be infected again. Therefore, the major use of florfenicol would be in the nursery ponds rather than the brood, stocker or food fish production ponds. Although much smaller pond acreage is dedicated to fingerling production (13 %), the use of Aquaflor in fingerling ponds represents the "worst case scenario". The incidence of bacterial diseases is highest at this stage of production and fingerling ponds are drained annually. Fingerling ponds will be emphasized throughout this environmental assessment.

To be conservative the worst-case release scenario will involve draining of a fingerling pond 14 days (the minimum pre-harvest interval) after the last of 10 daily applications. This allows a 14-day observation period to detect any reoccurrence of infection in the population (see subsequent discussions). Harvest requires repeated seining over a 1-3 month harvest period. This scenario will result in the maximum amount of water potentially released after the minimum timeframe following application. This is the key exposure scenario used in this assessment, but other scenarios involving production ponds will also be incorporated into this assessment.

The target dose to the fish is 10 mg/kg for 10 days for a total dose of 100 mg/kg of fish. The concentration of florfenicol in the feed is adjusted based on the feeding rate of the fish. The total amount dosed to a pond is determined by the biomass of fish in the pond. Feeding rate is only used to determine the FFC concentration in the feed. The dose rate to fish will be 10 mg/kg/day. The biomass of catfish in a pond depends on the number and size of the fish in the pond. The biomass is usually estimated from the stocking density, food consumption in the pond over a period of time and the temperature of the water.

In fingerling ponds, the biomass in late fall would be around 3000 - 6000 lb of fish/acre or 1364 - 2727 kg/acre or 3370 - 6738 kg/hectare at 2.21 lb/kg and 2.47 acres/hectare (Appendix 3). At a total dose of 100 mg/kg of fish, 3.4×10^5 to 6.7×10^5 mg of florfenicol would be dosed per hectare of pond. The subsequent discussion regarding pharmacokinetics (Section 6.2, below) shows that florfenicol and related residues are rapidly and completely excreted. The

environmental fate discussion (below) shows that the parent florfenicol and the related residues will partition to and remain in water.

In production ponds, the biomass will also be dependent upon when the larger fish had been harvested. Just prior to harvesting in the fall, the biomass level of the fish could be as high as 10,000 lb/acre in a given pond (Appendix 3). Although on a farm, the average biomass in the pond would be more in the range of 6000 - 7000 lb/acre. If 10,000 lb/acre were used, the biomass of fish would be 11,200 kg/hectare, which if treated with florfenicol, would result in 11.2 x 10^5 mg of florfenicol being administered per hectare. The predicted concentration in water and sediment would range from 111 - 112 ppb in water and 11.2 - 30 ppb in sediment based on the K_d values of 0.1 and 0.27, respectively.

In all cases, the concentrations given above in sediment and water are assuming that florfenicol is excreted unchanged. As discussed later, extensive metabolism occurs in fish species treated with florfenicol. Also, it must be remembered that the partitioning of florfenicol between sediment and water is an equilibrium situation, so if the concentration in one compartment decreases, florfenicol will equilibrate from the other compartment. Based on metabolism and fate data, the concentration of florfenicol and its metabolites in the environment will subsequently be discussed.

As an enclosed aquaculture system, the catfish ponds do not represent the ambient environment and are not of environmental concern. However, any

overflow or release of water (draining) from the ponds into the general environment is of concern and will be the focus of this assessment.

6.2 Pharmacokinetics of florfenicol

Florfenicol is readily absorbed, distributed, metabolized and excreted by vertebrate organisms specifically fish. There is a body of scientific evidence characterizing the pharmacokinetics of florfenicol in fresh and saltwater salmonids. The results of this work are similar to results of studies with mammalian species e.g., cattle (NADA #141-063), swine (INAD #2729) and Poultry (INAD #2609). The results of this work are considered directly relevant to other fish species such as catfish (Appendix 12).

Using various routes of administration (intravenous, gavage, and dietary exposure) and a range of study designs, the following results demonstrate a consistent pattern of pharmacokinetics in trout and salmon (Appendices 5-10). The florfenicol-related residues observed included the parent florfenicol and three major metabolites (florfenicol amine, the alcohol, and the oxamic acid) and conjugates (e.g., glucuronides) of parent and metabolites (Appendices 5-10).

Radio-labeled florfenicol administered in feed to salmon has been shown to have a bioavailability of 96.5% and 99% (Appendices 5-9). The uptake into tissues is rapid with radioactivity being detected after three hours in tissue and urine, the latter indicating rapid elimination (Appendix 7). The main routes of excretion are via bile and urine, the levels in bile and kidney peaking at day

three. The metabolism and depuration of florfenicol are such that half-lives of 30 to 35 hours have been found for all organs, except kidney, following administration of a single dose (Appendix 7). The total residue levels were below the levels of detection in muscle, blood, brain and fat 28 days after administration but remained at low levels for up to 56 days in the kidney. In multiple dose studies half-lives of 25, 34 and 21 hours for florfenicol and 64, 92 and 198 hours for the amine metabolite were determined for muscle, liver and kidney respectively (Appendix 8). In these studies the amine metabolite was detected and was generally found at lower levels than the parent compound in the plasma. When administered intravenously the half-life was determined as 12.2 hours (Appendix 11).

Gavage studies with salmon show that while the parent compound and amine metabolite represent 90% and 7%, respectively, of the residues in muscle at 6 hours post dosing the relative respective proportions were 20% and 70% after 3 days, indicating rapid metabolism (Appendix 7). The results indicated that the florfenicol was well absorbed, excreted rapidly in bile, feces and urine and rapidly metabolized to florfenicol amine with florfenicol alcohol and oxamic acid being present as minor metabolites.

In residue depletion studies with salmon dosed orally with ^{14}C-labelled florfenicol and held at 5°C and 10°C extensive metabolism was recorded (Appendices 5 and 6). Florfenicol and its alcohol, oxamic acid, monochloro- and amine derivatives were identified together with a number of unidentified moieties, some of which were chromatographically similar to the glucuronides of the

alcohol, amine and parent compound. Concentrations of the metabolites, florfenicol amine and florfenicol alcohol, increased relative to florfenicol and accounted for the majority of the residue at later time points. However, analysis of a composite sample starting at three hours and stopping at 15 days and encompassing the major period of residue excretion, indicated that florfenicol was the major residue present. Including its glucuronide, it would constitute about 40% of the residue. All other components were less than 20% of total residues (Appendix 5).

These florfenicol-related residues were found to be present in the tissues, bile and excreta and it was concluded that the metabolism of florfenicol in fish was similar to that in cattle and other vertebrate species (NADA #141-063, INAD #2729, INAD #2609) with florfenicol being metabolized through the identified intermediates to florfenicol amine. These fish were dosed using the standard treatment pattern, but with radio labeled florfenicol included on the last treatment day. The highest concentrations detected in feces were equivalent to 56.9 μg, and 53.8 μg florfenicol equivalents per g of feces at 5°C and 10°C, respectively (Appendices 5 and 6). These peak concentrations were found at 24 hours and 12 hours at 5°C and 10°C, respectively, following the end of treatment, decreasing to 0.023 and 0.01 μg/g by day 60 (Appendices 5 and 6) indicating that florfenicol-related residues were rapidly excreted (Appendices 5 and 6).

In rainbow trout an elimination half-life of 8.8 hours was determined following intravenous injection at 10°C (Appendix 10). Following oral intubation at 10°C

and oral administration of medicated feed at 16°C bioavailabilities of 73.9% and 66.3%, respectively, were determined for rainbow trout (Appendix 10). The residue levels in the plasma of trout fed medicated feed treated with florfenicol at 10°C were found to be reduced by more than ten-fold relative to the peak recorded 12 hours after the final dose of a 10-day treatment (Appendix 13). After 8 days following the last dosing, the residues in the muscle and skin taken from the same fish were reduced more than ten-fold relative to the 12-hour peak concentration (Appendix 13).

Catfish are expected to have similar pharmacokinetics to salmonids, and other vertebrate species. The available data indicate that the pharmacokinetic distribution patterns for florfenicol in rainbow trout and salmon are similar, although the trout had lower rates of absorption and shorter elimination times at 10°C. That the metabolism should be similar in salmon and trout would be expected as it has been found that the metabolic degradation of florfenicol in cattle, rats and salmon are similar with the florfenicol being degraded to either the alcohol, oxamic acid or monochloro-florfenicol residues before being further metabolized to florfenicol amine (Appendix 5). The same or similar metabolism and kinetics are expected in catfish as well. The similarity in metabolism among fish species is further supported by the similar withdrawal times, i.e., 12 days for catfish and 15 days for salmonids.

The pharmacokinetics in the target species (fish) is the key route of release of florfenicol-related residues to the environment. The principle route of release of these residues is as excreted material including parent florfenicol, metabolites

and conjugates. Consumption is assumed to be complete and due to the high bioavailability, absorption is high with only a small amount of florfenicol being excreted unabsorbed. Further, clearance of florfenicol-related residues is rapid following single or multiple exposures limiting the magnitude of the potential environmental release of florfenicol and florfenicol-related residues to pond water occurs. Finally, these residues are released only from treated fish into the water of culture ponds and release to the ambient aquatic environment occurs only intermittently through overflow or release of pond water upon draining.

SECTION 7. FATE OF THE EMITTED SUBSTANCES IN THE ENVIRONMENT

The fate of florfenicol in the environment has been discussed in detail in support of its use in cattle (NADA 141-063) and to support its use in salmonids. The results of those studies and additional studies relating specifically to this proposed action will be discussed below. The effect of these fate characteristics on the concentration of florfenicol-related residues in the catfish pond environment and their PEC values in the pond effluents will be discussed.

7.1 Fate of florfenicol in water

The fate of florfenicol in water is determined primarily by the physicochemical and environmental fate characteristics. As discussed above (Table 1) florfenicol has a substantial solubility in water (1.32 g/L at pH 7), and a very small log K_{ow} (0.373) (Appendix 1), indicating that it will not partition to organic material (i.e., soils, sediment, suspended solids, or biota) but as a result of equilibration or

direct input (i.e., excretion from catfish) will enter and remain in the water column.

Table 2 Photolytic half-lives of florfenicol and major metabolites

	Florfenicol	Amine Metabolite	Alcohol Metabolite	Oxamic acid Metabolite
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
pH 5	NA	NSR	22.1 d	24.5 d
pH 7	NA	41.2 d	21.0 d	47.9 d
pH 9	94.8 d	51.4 d	22.8 d	23.9 d
Synthetic humic water	196 d	NA	NA	NA
Pure water	171 d	NA	NA	NA

NA = Not applicable/available
NSR = No significant regression

Florfenicol is hydrolytically stable. Based upon dark controls in a photolysis study the hydrolytic half-life is 350 days (Appendices 14-18). Hydrolysis is not an important fate process. This compound does not undergo rapid photolysis in water (Appendices 14-15). However, its metabolites do photodegrade in water at pH 7. The half-lives range from 21 to 48 days (Table 2). Photodegradation could occur in a catfish pond because the residence time of florfenicol in water would be long and under strong sunlight. However, photolysis is not generally a significant route of degradation in a system such as a catfish pond with low light penetration.

Florfenicol has a density of 1.68 gm/cm^3 (Appendix 1). Its K_{oc} ranges from 10-27. This is a very low value for K_{oc} and indicates that it will preferentially be in the water rather than in sediment or associated with suspended particles and would be considered highly mobile in soil or sediment. (Appendices 19-22).

The properties of florfenicol and its metabolites are given in Tables 5 and 6. The metabolites are more water-soluble than florfenicol and have a lower log K_{ow} (Appendix 1). However, the two ionizable metabolites, the amine and the oxamic acid metabolites, have higher K_{oc} values of 202 and 130, respectively, indicating a somewhat higher potential for partitioning to sediment compared to florfenicol. The alcohol is similar to florfenicol (Appendix 21).

Table 3 Sorption/desorption characteristics of florfenicol and major metabolites determined in three soil types with $CaCl_2$

	Florfenicol	Amine Metabolite	Alcohol Metabolite	Oxamic acid Metabolite
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
% Sorbed	2-10	23.9-39.9	1.3-8.2	7.5-43
% Desorbed	79-93	86.3-99.8	85.6-161	65-172
K_d	0.07-0.59	1.56-3.35	0.07-0.45	0.41-3.78
K_{oc} range (geom. mean)	10-27 (18.38)	162-241 (202.28)	7-76.5 (20.16)	36.4-642 (130.40)
Mobility	Highly	Partially	Highly	Moderately

The data summarized in Table 3 shows that parent florfenicol is highly mobile in soil and is not likely to sorb to soil or to remain sorbed to soils or other matrices such as feces. For the three metabolites, the K_{oc} values may be somewhat higher, but all of the florfenicol-related residues appear to have a very low potential for associating with organic matter. All florfenicol-related residues show an even stronger tendency to migrate away from organic matter as indicated by desorption data (Table 3).

Aerobic biodegradation[2] of florfenicol in water has not been established by a guideline study, due to flaws in the designs of numerous existing studies (Appendices 23-25). Test concentrations exceeded known inhibitory concentrations; therefore the results cannot be interpreted. There is, however, a body of evidence, which shows that florfenicol is rapidly biodegraded in soils in sediments (Appendices 26-28), and in a synthetic seawater system (Appendix 29). These studies provide the basis for a weight-of-evidence argument that biodegradation of florfenicol-related residues occurs and is a significant process in aquatic systems.

A study has been conducted on the degradation of florfenicol in synthetic seawater containing sand, at an initial concentration of 3 mg/L (Appendix 29). Bacterial growth inhibition was employed as a bioassay. Within one day, there was already a 31 - 38% loss of activity. There was an 82 - 93% loss over 31 days. Antimicrobial activity was reduced by 50% in approximately four days. The absence of sediments in some incubation media resulted in a reduced rate of loss of activity, particularly when the incubation vessel was shaded from direct sunlight. Based on the results of this study, florfenicol is predicted to degrade rapidly in natural water systems to products lacking antimicrobial activity. These results are further supported by the results of degradation studies in soils supplemented with excreta (Appendix 26) and the loss of florfenicol from marine sediment (Appendices 27 and 28), (see Section 7.2, below).

[2] FDA 3.11/OECD Ready Biodegradation, CO_2 Evolution Test.

This work is supported by the observation of the amine metabolite of florfenicol in sediments spiked with florfenicol and placed in a natural marine system (Appendices 27 and 28). The susceptibility of florfenicol and related residues to microbial biodegradation is confirmed in a GLP soil degradation study (Appendix 26, see subsequent discussion). Finally, soils from earthworm and plant toxicity studies show that degradation occurred during the tests. Taken as a whole these studies indicate that relatively rapid degradation in water should be expected. Finally, dispersion and dilution in receiving waters are important processes in reducing the environmental risk potentially posed by florfenicol-related residues in waters outside the catfish ponds.

7.2 Fate of florfenicol in soils, sediments and excreta

Studies on the adsorption and desorption of florfenicol and metabolites in three different soil types determined that florfenicol was generally classified as highly mobile, while the metabolites were less so and classified as moderately to highly mobile. These results are summarized in Table 3 (Appendices 19-22). K_d and K_{oc} values were determined to be 0.07-0.57 and 10-27, respectively, consistent with the low sorption characteristics.

In a study on the decomposition of florfenicol in chicken excreta suspended in water it was found that, at 37°C, 80% of the florfenicol had degraded by day 14 (Appendix 30) (Table 4). From the reported values an estimate of the half-life of *ca.* 10 days can be made in the presence of chicken excreta.

Degradation and mineralization studies of florfenicol, added at an initial concentration of 0.05 mg/kg, to three soil types amended with manure demonstrated that mineralization was extensive with mineralization half-lives ranging from 86 to 270 days (Appendix 26) and a mean value of 158 days at 22°C. Primary degradation, or transformation, of the florfenicol was considerably quicker and only 2.6% to 9% of the florfenicol could be recovered at the end of the 92-day study. Half-lives of 3.6 to 27.2 days were reported in this study. Conservatively, based on this data, a half-life of 27.2 days is taken for florfenicol in soils in calculations of environmental concentrations (Appendix 26). While degradation products appeared in the course of the study they did not accumulate. On this basis the same half-life has been adopted for the degradation metabolites. While no study has been undertaken on the degradation of florfenicol in manure alone it is considered unlikely that the degradation rate would be lower in manure than in soils in view of the microbial biomass associated with excreta. This should ensure that the half-life of florfenicol in excreta is no longer than that in manure amended soils, unless the levels of florfenicol in excreta initially inhibit the microbial activity.

In studies on the toxicity of florfenicol to worms and plants the concentrations of florfenicol in the soils were determined at the beginning and end of the respective 14 and 21 day exposure periods (Appendices 31-33). The analytical results indicate that the levels of florfenicol in the soils at the end of the studies represented 16%, *ca.* 34.6% and 67.2% of those initially detected in the plant studies at initial nominal concentrations of 1, 10 and 100 mg/kg respectively (Appendices 32 and 33). In the worm study the final concentration represented

87.5% of that detected at an initial nominal concentration of 1,000 mg/kg (Appendix 31). Based on degradation following first order kinetics then the half-lives at initial concentrations of 1, 10, 100 and 1,000 mg/kg, would be *ca.* 8, 14, 37 and 73 days, respectively.

In studies on the persistence of florfenicol in marine sediment systems the rate of loss of florfenicol, and the amine metabolite, indicated their potential to undergo transformation and elution from the sediments. A dissipation half-life of 5 days was determined based on elution and degradation. The amine metabolite measured in deeper segments was found to persist for longer than the florfenicol. This is most likely due to it being a degradation product with a higher K_d/K_{oc} (i.e., absorption potential), and somewhat lower mobility than the parent compound (Appendices 19-22). The detection of the metabolite at the first sampling point indicates that the microbial flora of the sediment was able to degrade the florfenicol at concentrations between 1 and 50 mg/kg. In a subsequent publication where the persistence and impact of a number of antibacterial agents were examined half-lives of 1.7 and 7.3 days were determined for florfenicol at the two depths studied, 0-1 cm and 5-7 cm respectively (Appendices 27 and 28). While it is possible that the more rapid reduction in concentrations in surface sediments was contributed to by greater wash out of the florfenicol at the surface, the appearance of the amine metabolite demonstrates that degradation was occurring. The degradation and/or washout indicate that florfenicol and its metabolites are unlikely to accumulate in sediments. This finding concurs with the results from soil systems and predictions based on physicochemical properties. It is reasonable

to include dissipation data in this assessment. It is particularly relevant, with episodic and intermittent uses where dilution and degradation are both expected to occur.

Existing biodegradation data from a range of studies and study designs are summarized qualitatively in Table 4. As discussed above the reliability of these studies ranges from GLP soil degradation studies (Appendix 26) to unique experiments with a lower level of documentation (Appendix 29). Due to the low reliability of many of these studies and the lack of delineation between dissipation and degradation the half-life of florfenicol aerobic biodegradation are difficult to quantify. However, in all these studies from manure amended-soils to marine sediments rapid degradation/dissipation/loss of activity is consistently observed. Conservatively an estimate of biodegradation half-life in aquatic systems (water) is estimated to be 30 days based primarily on the 27.2-day half-life in manure-amended soil and supported qualitatively by the remaining studies discussed above and summarized in Table 4. This 30-day half-life will be used in refining the water PECs and will stress the conservative nature of this half-life.

Table 4 Summary of florfenicol degradation/dissipation studies in water, soil, and sediments

Study	Reference	Matrix/System	Environmental Half-Lives
Aerobic Biodegradation in manure-amended soil	Appendix 26	Manure amended soil	Half-lives ranged from 3.6 to 27.2 days
Persistence of anti bacterial agents in marine sediments	Appendix 28	Marine sediments	Dissipation half-lives of 1.7 and 7.43 days at 0-1 and 5-7 cm, respectively
The decomposition characteristics in seawater	Appendix 29	A sediment water system	50% loss of anti-biotic activity in four days
Acute toxicity to the earthworm	Appendix 33	Bioassay soil (14 day earthworm study)	Half-lives for initial concentrations of 1,10, 100 and 1,000 mg/kg are 8, 14, 37 and 73 days, respectively.
Florfenicol: Terrestrial plants, growth test	Appendices 31 and 32	Bioassay soil (21 day plant study)	After 21 days 16%, ca 34.6% and 67.2% remained in soil treated with 1, 10 and 100 mg/kg, respectively
Diluted chicken excreta	30	Diluted chicken excreta	80% degradation in 14 days @37°C

7.3 Predicted environmental concentrations

7.3.1 Water

The persistence and levels of florfenicol, and its metabolites, in the receiving environment (i.e., outside of the catfish ponds) will be dependent on the quantities administered, the proportion consumed, the proportion excreted as parent compound and as metabolites, and the partitioning within the pond environment and the volume, frequency and timing of water released to the receiving environment. While there is extensive metabolic degradation of

florfenicol in the treated fish, the residence half-life in the fish is short, see section 6. For the purpose of this assessment an extreme worst-case scenario will be presented in that the initial degradation in the fish is discounted by considering all florfenicol-related materials reaching the environment as unmetabolized florfenicol (parent) although the metabolism in fish is extensive and the metabolites generally exhibit higher NOEC values in toxicity studies.

Florfenicol, which is principally in the water column of catfish ponds, enters the receiving aquatic environment via pond draining or overflow resulting primarily from rainfall. Two scenarios are considered in this assessment one representative of a worst-case release of florfenicol-related residues, i.e., fingerling ponds, and a more typical release of residues, i.e., levee production ponds. Scenario I involves fingerling ponds, which represent a worst-case release of florfenicol-related residues into receiving waters. These younger stages of catfish are more susceptible to *E. ictaluri* and are more likely to be treated with Aquaflor. In addition, fingerling ponds are drained more frequently (annually) allowing for potentially greater release within a shorter period of time. Although, fingerling ponds may present the potential for the highest rate of input of florfenicol-related residues to receiving waters it represents only 13% of the total pond acreage of catfish production (Appendix 3).

Scenario II involves production ponds, which represent a lower potential for release of florfenicol-related residues, but a much higher proportion of the total acreage of catfish ponds. Production ponds are not drained but every few years (an average of over six years) for pond maintenance. Levee ponds are

emphasized in this scenario, because watershed ponds having a similar (possibly slightly higher) potential for treatment have greater annual effluent and therefore greater potential for dilution of any released florfenicol-related residues. Levee ponds are less frequently treated than fingerling ponds. The latter is partially due to previous exposure of some portion of the population in production ponds to *E. ictaluri* and a lower susceptibility of older fish.

7.3.2 Scenario I: Fingerling Pond

The underlying assumptions for calculating potential releases of florfenicol–related residues from fingerling ponds are listed below followed by calculations.

Assumptions: Initial PEC Calculations

- A 10 acre pond, 1 m in depth
- Density of fish ranges from 3,000 to 6,000 lb/acre
- Treatment 10 mg/kg fish/day for 10 days or 100 mg/kg fish total application
- Pond is drained 42 days after the last (10^{th}) day of application.
- Food pellets are 100% consumed by catfish
- 1 acre = 4046.86 m^2
- 1 hectare = 2.47 acre =10,000 m^2
- 1 hectare, 1 meter in depth = 10,000 m^3
- 1 kg = 2.2 lb or 1lb = 0.45 kg

Calculations:

1. 10 acres 1 meter deep = 40468.6 m^2 1 meter deep or 40468.6 m^3
2. 40468.6 m^3 x 1,000 L/m^3 = 40,468,600 L pond volume
3. 6,000 lb fish biomass/acre x 10 acres = 60,000 lb fish in pond
4. 60,000 lb fish x 0.45 kg/lb = 27,000 kg fish/ in pond (10 acres).

5. Treatment is 100 mg florfenicol/kg x 27,000 kg fish = 2,700,000 mg florfenicol
6. 2,700,000 mg florfenicol/40,468,600 L in pond = 0.067 mg/L
7. Initial PEC_{water} is 0.067 mg/L for fingering ponds.

This preliminary PEC_{water} of 0.0067 mg/L represents the peak worst-case concentration of florfenicol-related residues in fingerling pond water. This assumes 100% of the florfenicol-related residues (florfenicol and metabolites) are in the water column, none is partitioned to sediment, and none is remaining in the fish (at 12 days post final treatment). Based on HPLC profiling of bile, the main components of the residues that will be excreted are florfenicol (41.3%), florfenicol amine (2.12%), florfenicol alcohol (10.37%), and florfenicol oxamic acid (4.1%), but for the purposes of this assessment all florfenicol-related residues will be considered as florfenicol. This assumes that no degradation occurred to any of the residues over the 10-day treatment period and a 12-day excretion period or pre-harvest interval. A refined worst-case PEC_{water} (Scenario I) includes considerations of additional environmental fate processes (biodegradation), dilution and dissipation (see Section 7.3.4 below).

7.3.3 Scenario II: Production pond

Assumptions:

- A 10 acre pond, 1 m in depth
- Density of fish is approximately 11,200 kg/hectare
- Treatment 10 mg/kg fish/day for 10 days or 100 mg/kg fish total application
- Pond is drained 6 months after the treatment.
- Food pellets are 100% consumed by catfish
- 1 acre = 4046.86 m^2
- 1 hectare = 2.47 acre

- 1 hectare, 1 meter in depth = 10,000 m^3
- 1 kg = 2.2 lb or 1lb = 0.45 kg

Calculations:

1. 10 acres 1 meter deep = 40468.6 m^2 1 meter deep or 40468.6 m^3
2. 40468.6 m^3 x 1,000 L/m^3 = 40,468,600 L
3. 11,200 kg fish biomass/hectare x 0.405 acre/hectare 4534.4 kg/acre
4. 4534.4 kg/acre x 10 acres = 45,344 kg fish in 10 acres total biomass
5. Treatment is 100 mg florfenicol/kg x 45,344 kg fish = 4,534,400 mg florfenicol
6. 4,534,400 mg florfenicol/40,468,600 L in pond = 0.112 mg/L
7. Preliminary PEC_{water} is 0.112 mg/L for production pond water on the last day of treatment.

This preliminary PEC_{water} assumes 100% of the florfenicol-related residues (florfenicol and metabolites) are in the water column, none is partitioned to sediment, and none remaining in the fish at 12 days post final treatment. A refined worst-case PEC_{water} (Scenario II) includes considerations of additional environmental fate processes (biodegradation), dilution and dissipation (see Section 7.3.4 below).

7.3.4 Refined PEC_{water} Values

In Scenario I above the initial PEC_{water} value of 0.067 mg/L is a worst-case peak concentration in the water column of fingerling ponds. These ponds represent only 13% of the total acreage of catfish ponds, but because they are drained annually fingerling ponds represent 30 percent of the annual discharge. Furthermore, the conservative scenario being employed includes the initiating of

the harvest of fingerlings 14 days after the last day of Aquaflor treatment. A period of up to 14 days is included to observe for reoccurrence of ESC.

To refine this initial worst-case PEC value degradation, dissipation, and dilution must be considered. Degradation of 30 days in water and sediment will be used as discussed above (Section 7.1 above). Fingerling ponds are harvested by seining over a 1 to 3 month period (Appendix 2). The pond is drained at the end of the harvest period. Based on a 14 day period of observation for reoccurrence and 30 days of harvest activities draining would occur no less than 44 days after the last treatment. A water concentration of 0.067 mg/L degrading with a 30-day half-life for a 42-day period of time yields an estimated concentration (PEC_{water}) of 0.0268 mg/L. If this is then diluted 1:10 into receiving waters then the refined PEC_{water} is 0.00268 mg/L. (See Table 5, below).

The refined PEC_{water} for levee production ponds must include consideration of ponds managed to conserve water or not managed in this respect. In the former case the level of the pond is maintained below full capacity (e.g., sufficient to retain a 90^{th} percentile rainfall event). In the latter case the pond is maintained at a level that allows overflow volume essentially equal to any rainfall event.

Table 5 Refined PEC_{water} values for different scenarios

Scenario	Pond Type	Assumptions	Refined PECwater
Scenario I	Fingerling Ponds	Drained 42 days[a] after treatment, and a 10 fold dilution in receiving waters	0.00268 mg/L
		Drained in six months, and 10 fold dilution in receiving waters	0.0000523 mg/L
Scenario II	Production pond (levee type)	No storage all rain is overflow	0.0104 mg/L
		Storage capacity for the 90th percentile volume of rainfall events	0.00042 mg/L

[a] Fingerling ponds are harvested by seining over a 1 to 3 month period (Appendix 2). The pond is drained at the end of the harvest period. Based on a minimum pre-harvest interval of 12 days and a minimum 30 days of harvest activities. Draining would occur no less than 42 days after the last treatment. The degradation half-life is 30 days.

The high rainfall period in the region where catfish farms are located is in December through April (Appendix 3). This time period coincides with the time when discharges from ponds due to overflow from rain and harvesting of production fish from a pond to be refurbished could take place. Streams and rivers would be at high flows. Also, ditches would contain water where mixing of pond water would occur prior to movement into streams and rivers thereby lowering the concentration of florfenicol and its metabolites even more. Periods when treatment for ESC is expected to occur would be in periods of lower rainfall, i.e. May / June and September / October.

The overflow from a pond can be calculated using the following hydrological budget equation (Appendix 3):

[1] $OF_w = WD_{d1} - WD_{d7} - P_w$

OF_w = overflow (cm) for a one week period

WD_{d-1} = pond water level (cm) at the beginning of day one of a one week period

WD_{d7} = pond water level (cm) at the end day seven of a one week period.

P_w = precipitation (cm) in a one week period

This equation is adapted from Appendix 3 and ignores losses due to evaporation and seepage or any input of ground water. A one-week time frame, not a one-day time frame, is used to estimate weekly rainfall. This allows the use of estimates of 90th percentile weekly rainfall amounts for Northwest Mississippi. This data was based on 30 years (1961-1990) historical rainfall records and used to estimate how much storage capacity would be needed to retain all rainfall for a given week 90% of the time. The later value is 7.6 cm of storage capacity.

In Scenario II this equation can be used to estimate the release of florfenicol-related PEC_{water} for this scenario.

[2] $OF_w = 92.4 \text{ cm} - 100 \text{ cm} + 7.60 \text{ cm} = 0$

For 90 percent of weeks the OF_w will be zero. The water level in the pond is held at a level that is at least 92.4 cm in depth (based on a 100 cm pond depth)

to retain all rainfall 90% of the time (period of one week). Assuming a 90th percentile rain event or rain week of 7.6 cm (Appendix 3) the final pond volume at the end of the week would be 100 cm. Rain events that exceed the 90th percentile level (7.6 cm) would most likely occur in the rainy season (i.e., late October to early December). Since application would most likely occur between August and September at least six weeks would likely pass before a >90th percentile rain event would be expected to occur.

During the six week period after treatment degradation in water would occur with a half-life of 30 days (see Section 7.1). The initial PEC_{water} for Scenario II of 0.112 mg/L (Section 7.33 above) would be reduced to 0.042 mg/L in the pond water. The >90th percentile rain events would be associated with maximum runoff and stream flow. Therefore, a conservative 100-fold dilution factor is applied to the pond water value to obtain the final, refined PEC_{water} for Scenario II of 0.00042 mg/L (Table 5).

Finally, a very extreme, worst-case estimate of the PEC_{water} (Alternate Scenario II) can be made based on the production pond scenario used above, but assuming that the pond was managed with no storage capacity, i.e., the water level in the pond was level with overflow structure. If a week with a 90% average rainfall occurred and similar assumptions used above are included this scenario could be described by the following equation (3).

[3] OF_w = 100 cm –100 cm + 7.60 cm = 7.60 cm

Under this scenario (Alternate Scenario II) the level at time zero and at the end of the week would be the same. For this illustration ponds are considered to be 100 cm (1 m) in depth. If mixing were assumed during the rain event the distribution within the water column reached and the concentration would be diluted by the additional 7.6% in volume. This would reduce the concentration of florfenicol related residues to 0.104 mg/L. Additional dilution would occur when mixing with receiving waters. Because theoretically this scenario would produce overflow with any rainfall, and because this over flow would not necessarily be associated with an unusual event or high stream flow associated with a >90th percentile event (or total rainfall for a week), a 10- fold dilution factor is employed (instead of 100x). The extreme worst-case estimate is of the PEC_{water} is 0.0104 mg/L (Table 5). No degradation of the florfenicol-related residues is considered in this extreme worst-case estimate of the PEC_{water}, because the proximity to treatment with Aquaflor is not known.

SECTION 8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES TOXICITY TO NON-TARGET ORGANISMS

8.1 Microorganisms and Plants

Florfenicol exhibits activity against a wide spectrum of prokaryotic microorganisms with MIC values ranging from 0.25 mg/l for the catfish pathogen *Edwardsiella ictaluri* to 16 mg/l for *Serratia*, Table 6. Florfenicol was found to

have a transient effect on the microbial transformation of nitrogen when added to soils at concentrations of 1, 5 and 25 mg/kg (Appendix 34). While the nitrate concentrations were similar to those in controls throughout the study the ammonium levels rose significantly in the soils treated at 5 and 25 mg/kg, before the rates returned to the control level by day 28. Carbon transformation was reduced at all florfenicol concentrations but by day 28 had recovered in soils treated at 1 and 5 mg/kg with activity recovering in the soils treated at 25 mg/kg by day 56. From the data on reductions in concentrations of florfenicol in soils in terrestrial organism toxicity studies (Appendix 33), and half-lives derived in Section 7, it is apparent that rates of reduction in concentrations are inversely proportional to the initial concentrations of florfenicol present.

Table 6 Minimum inhibitory concentration (MIC) (mg/l) data for florfenicol and major metabolites against microorganisms

		Principle Metabolites		
	Florfenicol	**Amine**	**Alcohol**	**Oxamic acid**
SPAH Code No.	**SCH 25298**	**SCH 40458**	**SCH 45705**	**SCH 48057**
Aspergillus niger	>1000	>1000	>1000	>1000
Trichoderme viride	>1000	>1000	>1000	>1000
Nostoc	4.0	20	200	400
Bacillus subtilis	0.4	40	40	>1000
Clostridium perfringens	1.0	80	40	>1000
Moraxella	0.5	--	--	--
Serratia	16	--	--	--
Escherichia coli	8.0	--	--	--
Aeromonas salmonicida	0.3-2.5	--	--	--
Vibrio sp.	0.8-1.6	--	--	--
Edwardsiella ictaluri	0.25	-	-	-

From the half lives of 3.6 to 27.2 days determined in the soil degradation studies at an initial concentration of 0.05 mg/kg, with a mean half-life 13 days, and the value determined from the plant studies of 8 and 14 days at initial concentrations of 1 and 10 mg/kg it would appear that concentrations up to and including 10 mg/kg have little or no effect on florfenicol degradation rates. At concentrations of 100 and 1,000 mg/kg the half-lives were greater and 10 mg/kg would appear to be a reasonable estimate of the NOEC for microbial function as indicated by florfenicol degradation. From the data available on the rates of degradation of florfenicol at different concentrations the results from the nutrient transformation study might be expected. The recovery in microbial activity indicates that under the conditions of the study the microbial populations responsible for transformation were inhibited and not killed and were able to resume processing when the florfenicol was degraded.

Florfenicol is generally less active against eukaryotes than prokaryotes although activity has been found with the green algae *Selenastrum capricornutum* (Appendix 35) and the marine diatom *Skeletonema costatum* (Appendix 36), Table 7. The toxicity data for *S. costatum* indicates that it is the most sensitive of the eukaryotes to florfenicol with 72 hour IC_{50} and NOEC values of 0.0128 and 0.00423 mg/l, respectively, and is more than one to two orders of magnitude more sensitive than *S. capricornutum*. The degradation metabolites are between five and a thousand fold less active against prokaryotes for which the activity has been determined, Table 6.

Table 7 Toxicity data for florfenicol and major metabolites against *Selenastrum capricornutum* and *Skeletonema costatum*

		Principle Metabolites		
	Florfenicol	Amine	Alcohol	Oxamic acid
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
Selenastrum capricornutum (mg/l)				
Maximum growth rate				
MIC	>2.9	>2.7	>0.98	80
NOEC	2.9	2.7	0.98	38
Maximum cell density				
MIC	1.5	2.7	0.26	80
NOEC	0.75	1.4	0.13	19
Skeletonema costatum (mg/l)				
Maximum growth rate				
MIC	0.0336	--	--	--
NOEC	0.00423	--	--	--
Maximum cell density				
MIC	0.0128	--	--	--
NOEC	0.0106	--	--	--

While the metabolites are generally less active than the parent compound towards eukaryotes the alcohol metabolite has been found to be approximately six times more active against *S. capricornutum* (Appendix 37-39), Table 7. The differences in the MIC and NOEC values reported with regard to maximum growth rate and cell density for *S. capricornutum* can be partially explained by degradation of the florfenicol over the 14 days of the study. This would enable the algae that were initially inhibited to achieve maximum growth rate even at the highest concentrations tested while the biomass would not reach the same level due to the initial inhibition. The data reported indicates that while florfenicol was algistatic it was not algicidal at initial concentrations up to 2.9 mg/l (Appendix 36). It can also be concluded that the degradation products did not reach levels that were algicidal, or algistatic in the course of the study.

Insufficient immobilizations occurred with *Daphnia magna* exposed to florfenicol at concentrations up to 330 mg/l to enable an EC_{50} value to be determined (Appendix 40). Similarly, no EC_{50} values could be determined for the metabolites. The latter compounds were tested at lower levels due to limitations of available material. Values are presented here simply to show that these metabolites are of a similar order of toxicity or less toxic than the parent compound consistent with the order of toxicity observed for microbes and algae (see above). (Appendices 41-43). The NOEC was reported as <100 mg/l for florfenicol as sub-lethal effects, lethargy and erratic swimming, were observed among the survivors at all concentrations tested (Appendix 40), Table 8.

Table 8 EC_{50} and no observed effect concentration (NOEC) data (mg/l) for florfenicol and major metabolites against *Daphnia magna* over 48 hours

	Florfenicol	Amine	Alcohol	Oxamic acid
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
EC_{50} (mg/L)	>330	>18	>14	>24
NOEC (mg/L)	<100	18	8.9	24

Toxicity data for florfenicol is available for larval stages of the white shrimp, *Litopenaeus vannamei* formerly known as *Penaeus vannamei*, (Appendix 44). This work was part of broader evaluation of 12 antibiotics being considered for potential use in shrimp mariculture. The results of acute bioassays with a range of larval or transitional stages of *L. vannamei* indicated that florfenicol was among six compounds considered safe and effective in shrimp (Table 9). In these studies florfenicol shows toxicity at parts per million (mg/L) concentrations

and the authors believed that toxicity values would be higher (i.e., show less toxicity) to later life stages of this species. In addition, this level of toxicity is consistent with reported toxicity values for other animal species exposed to florfenicol or florfenicol-related residues.

Table 9 Results of toxicity tests with early life stages of a *Litopenaeid shrimp*[a]

Life Stage	Duration (hrs)	LC_{50} (mg/L)	EC_{50}** (mg/L)
Nauplius I	24	>64	>64
Protozoea I	48	>64	>64
Protozoea III (mysis interface)*	48	95.2	110
Mysis I	48	>64	>64
Postlarva I	48	>84	>84
Overall Range (all larvae)	NA	>64 to 95.2	>64 to 110

[a] Data is taken from Appendix 44.
* Not a larval stage, but a transitional stage between protozoea and mysis.
** The EC50 is defined as the total toxic levels (considering lethality and morbidity) of 50% of the exposed organisms.

The available data for rainbow trout and bluegill sunfish indicates that florfenicol is not toxic to either fish with NOEC values of 780 and 830 mg/l (Appendices 45 and 46)(Table 10). While the metabolites were not tested at the same concentrations no mortalities were caused to either species when exposed to concentrations up to 20, 15, and 25 mg/l in the case of the amine, alcohol and oxamic acid metabolites respectively (Appendices 47-49 and 50-52). The data supports that neither florfenicol nor its degradation products are likely to cause toxic effects in the environment to fish species, which may be exposed.

Table 10 LC_{50} and no observed effect concentration (NOEC) data (mg/l) for florfenicol and major metabolites against fish species over 96 hours.

		Principle Metabolites		
	Florfenicol	Amine	Alcohol	Oxamic Acid
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
Oncorhynchus mykiss				
LC_{50} (mg/L)	>780	>19	>15	>23
NOEC (mg/L)	780	19	15	23
Lepomis macrochirus				
LC_{50} (mg/L)	>830	>20	>15	>25
NOEC (mg/L)	830	20	15	25

Finally, terrestrial species are presented here to show the low level of toxicity to higher plants and soil invertebrates, compared to aquatic plants. In the phytotoxicity studies there was no effect on seedling emergence in the case of any of the concentrations tested up to 100 mg/kg with either mustard or cress (Appendices 31 and 32). In the case of wheat the percentage emergence was reported as 40% at 100 mg/kg but the control group only exhibited 85%. Based on seedling emergence the LC_{50} value was reported as being >100 mg/kg for the three species tested. From the weights of the emerged seedlings EC_{50} values were estimated as 1.7, 0.5 and 6.7 mg/kg in the case of mustard, cress and wheat respectively. Visible effects, chlorosis, were found at all treatment levels, 1-100 mg/kg, in all species throughout the test. The studies on toxicity to terrestrial organisms discussed above established that florfenicol was not toxic to *Eisenia foetida*, the earth or manure worm, at concentrations up to 1,000 mg/kg, with no repellency or other sublethal effects observed (Appendix 33).

7.3 PNEC Calculations

The Predicted No Effect Concentrations (PNECs) are presented in Table 11 for key species of fish, invertebrates, algae and one microbial species. Toxicity values range over four orders of magnitude with fish, *O. mykiss* and *L. macrochirus*, having the highest reported NOEC values of 780 and 830 mg/L, respectively, and *S. costatum* having the lowest NOEC (for growth) of 0.0042 mg/L. This latter value indicates that *S. costatum* is the most sensitive species for which data is available. This NOEC is two orders of magnitude lower than the green algae, *S. capricornutum*, and the most sensitive reported microbial species, *B. subtilis.*

Assessment factors applied to NOECs and MICs are presented in column 4, Application factors (AFs) are used to adjust for uncertainty in the data. An AF of 100 is used for acute toxicity data to account for intraspecies variation (10x) and extrapolation from acute to chronic data (10x). A factor of 10 (for intraspecies variation) is applied to chronic end points. For example, data for *L. vannamei*, a sensitive estuarine marine shrimp species, includes data for all of the principal early-life stages of this organism. This is considered chronic data and thus an AF of 10 is used in calculating the PNEC (Table 11 below) and the PEC:PNEC (see Section 8.4, Tables 12-14). The two aquatic plant species and the microbial species include partial or complete life cycles of the respective organisms and an AF of 10 is also used in calculating the PNECs and PEC:PNECs for these species.

Table 11 Predicted no effect concentrations for aquatic organisms

Organism	EC_{50}/LC_{50} mg/L	NOEC mg/L	Application Factor	PNEC mg/L
Oncorhynchus mykiss	>780	780	100	7.8
Lepomis macrochirus	>830	830	100	8.3
Daphnia magna	>330	<100	100	1.0
Litopenaeus vannamei	>64	4	10	0.4[b]
Selenastrum capricornutum	1.5	0.75	10	0.075[b]
Skeletonema costatum	0.0128	0.0042	10	0.00042[b]
Bacillus subtilis	0.4[c]		10	0.04[b]

[a]An application factor of 10 was used to account for intraspecies and a factor of 10 was used in the extrapolation from acute to chronic data.
[b]An application factor of 10 was used to account for intraspecies variation. These values already represent chronic end points.
[c] MIC

The PNEC values range over five orders of magnitude consistent with the range of toxicity values used in the calculations. The highest PNEC was for *L. macrochirus* (8.3 mg/L) and the lowest for *S. costatum* (0.00042 mg/L). The latter being two orders of magnitude lower than that of the green algae, *S. capricornutum*, the nearest plant species indicating that *S. costatum* may be an outlier with respect to sensitivity to florfenicol exposure.

8.4 Risk characterization

Risk characterization is summarized in column 7 of Tables 12-14 as PEC:PNEC ratios for representative aquatic and terrestrial species and three exposure scenarios. Toxicity values are discussed in more detail in Section 6. Application factors (AFs) are used to adjust for uncertainty in the data (Tables

12-14, col. 4) and are discussed in detail in Section 7.3 above. An AF of 100 is used to account for intraspecies variation (10x) and extrapolation from acute to chronic data (10x) for the fish species and *Daphnia*. A factor of 10 (for intraspecies variation) is applied to chronic endpoints and is used in calculating PNECs for: *L. vannamei*, an estuarine/marine shrimp species; two aquatic plant species; and the microbial species.

The refined PEC_{water} values used in Tables 12-14 are worst-case scenarios. Scenario I, based on release of florfenicol-related residues following application to fingerlings is a reasonable worst-case due to annual draining of this type of pond. The second scenario is the most typical worst-case scenario and involves the release of residues from levee production ponds managed for water storage. The third scenario is an extreme worst-case and involves release of residues from levee production ponds with water levels maintained at maximum depth (i.e., at the top of overflow structure). These PEC_{water} values are compared to the same PNECs to provide PEC:PNEC ratios (Tables 12-14).

Sediments are not included in this analysis. Due to its physicochemical and environmental fate properties florfenicol will not enter or remain in sediments in significant amounts, but will move into the water phase. Any florfenicol that is released from ponds will remain in the water phase and will not partition to sediments. Therefore, sediments are not considered a significant source of exposure or risk and PEC sediment values are not included in this assessment.

8.4.1 *PEC:PNEC ratios: Scenario I, Fingerling Ponds*

As shown in Table 12 the PEC:PNEC ratios are based on PNECs discussed in Section 7 (Table 11). PNECs are provided for two fish species, two invertebrate species, two aquatic plant (algal) species and one microbial species. The latter species, *B. subtilis*, is the most sensitive of ten microbial species for which data is provided in this assessment. These species include fresh and saltwater organisms even though this assessment is for a freshwater use. Ratios for Scenario range from 0.000344 to 6.38 with the most sensitive species being the aquatic plant species *S. costatum* and the microbial species, *B. subtilis*.

The exposure scenario (Scenario I) uses the initial PEC_{water} for fingerling ponds of 0.067 mg/L. Harvest is initiated after a minimum observation period of 14 days[3] following the end of treatment. Normal practice allows for harvest over 1 to 3 months with draining of the pond at the end of this harvest period. To estimate the refined PEC_{water} for Scenario I, a one-month (30d) time period is added to the observation period for a total of 44 days. Incorporating degradation (a 30d t ½) (see Section 7.1 and 7.2) and minimal dilution (10x) in receiving waters results in the refined PECwater value of 0.00268 mg/L. For further details see Section 7.3.4. This value is used in generating all PEC:PNEC ratios in Table 12.

[3] The fingerlings are held long enough for observation of possible reoccurrence before the fingerlings are transferred to production ponds. An observation period of 14 days is used in these calculations.

The data for *S. costatum* warrants careful examination. The low NOEC relative to other species is reflected in the PEC:PNEC ratio, which with this exposure scenario (Scenario I) exceeds a ratio of 1.0, the threshold of concern, by a factor of six. All other ratios are well below 1.0 ranging from 0.000344 to 0.067 (Table 12). Examination of the toxicity data alone shows that the biological response of *S costatum* is an extreme outlier relative to other species. The *S. costatum* NOEC (0.0042 mg/L) is nearly two orders of magnitude below the next most sensitive aquatic plant (algal) species, *S. capricornutum*; three orders of magnitude below the most sensitive invertebrate species (*L. vannamei*); and

Table 12. Risk Characterization, Scenario I: Summary for fingerling ponds

Organism	End Point (mg/L)	Results (mg/L)	AF	PNEC (mg/L)	Refined PECwater (mg/L)	PEC:PNEC Ratio
Oncorhynchus mykiss	96-h LC_{50}	>780	100	7.8[a]	0.00268	0.000344
Lepomis macrochirus	96-h LC_{50}	>830	100	8.3[a]	0.00268	0.000323
Daphnia magna	48-h LC_{50}	>330	100	3.30[a]	0.00268	0.000812
Litopenaeus vannamei	48-h NOEC	<4	10	0.4[b]	0.00268	0.0067
Selenastrum capricornutum	14-d NOEC	0.75	10	0.075[b]	0.00268	0.0357
Skeletonema costatum	72-h NOEC	0.0042	10	0.00042[b]	0.00268	6.38
Bacillus subtilis	MIC	0.4	10	0.04[b]	0.00268	0.067

[a] An application factor of 10 was used to account for intraspecies variation and a factor of 10 was used in the extrapolation from acute to chronic data.
[b] An application factor of 10 was used to account for intraspecies variation. These toxicity values are considered chronic endpoints.

nearly five orders of magnitude below the most sensitive fish species (*O. mykiss*). In addition, this observed toxicity value (0.0042 mg/L) is almost two orders of magnitude below the most sensitive microbial species tested. The *S.*

costatum NOEC, as shown in Table 12 is clearly an outlier in the distribution of potential biological responses in aquatic organisms and contributes to an overly conservative assessment of risk (see subsequent discussion). Furthermore the effect reported is for growth not mortality (see subsequent discussion).

8.4.2 *PEC:PNEC Ratios: Scenario II, Production Ponds*

PEC:PNEC ratios for production ponds (Scenario II) are presented in Tables 13 and 14 below. PNEC values are the same as for Table 12 above, but in Tables 13 and 14 the PEC_{water} values are presented for a reasonable worst-case (Scenario II) and extreme worst-case (Alternate Scenario II), respectively. Due to pond management practices that provide water storage capacity and due to less frequent draining than fingerling ponds, production ponds present less of a worst-case scenario (Table 13). An additional extreme worst-case scenario for production ponds without water storage capacity is presented in Table 14. These tables use different PECwater values (Column 6).

The resulting PEC:PNEC ratios are lower than for fingerling ponds (Table 12) and range from 0.0000539 for *O. mykiss* to 1.0 for *S. costatum*, the most sensitive organism for which data is available. None of the PEC:PNEC ratios for this reasonable worst-case (Scenario II) exceed the threshold of 1.0. However, the relative pattern of sensitivity does not differ from the ratios based on fingerling pond data (Table 12).

Table 13 Risk Characterization, Scenario II: Summary for levee production ponds with water storage

Organism	End Point (mg/L)	Results (mg/L)	AF	PNEC (mg/L)	Refined PECwater (mg/L)	PEC:PNEC Ratio
Oncorhynchus mykiss	96-h LC_{50}	>780	100	7.8[a]	0.00042	0.0000539
Lepomis macrochirus	96-h LC_{50}	>830	100	8.3[a]	0.00042	0.0000506
Daphnia magna	48-h LC_{50}	>330	100	3.30[a]	0.00042	0.000127
Litopenaeus vannamei	48-h NOEC	<4	10	0.4[b]	0.00042	0.0011
Selenastrum capricornutum	14-d NOEC	0.75	10	0.075[b]	0.00042	0.0056
Skeletonema costatum	72-h NOEC	0.0042	10	0.00042[b]	0.00042	1.0
Bacillus subtilis	MIC	0.4	10	0.04[b]	0.00042	0.0105

[a] An application factor of 10 was used to account for intraspecies variation and a factor of 10 was used in the extrapolation from acute to chronic data.
[b] An application factor of 10 was used to account for intraspecies variation. These toxicity values are considered chronic endpoints.

The extreme worst-case example of production ponds (Alternate Scenario II), not managed for water storage, results in the largest PEC:PNEC ratios (Table 14) even though this exposure scenario would not be expected to ever occur in real life. The range of species and their relative sensitivities are the same for Table 14 as Tables 12 and 13, but the PEC:PNEC ratios for this extreme worst-case scenario are larger consistent with the larger PEC_{water} value of 0.0104 mg/L (Table 14, column 6). The PEC:PNEC ratios for Alternate Scenario II without water management are higher for all species compared to the scenarios presented in Tables 12 and 13. Yet the only species for which the PEC:PNEC ratio exceeds the level of concern (i.e., a ratio of 1.0) is the PEC:PNEC of 24.7 for *S. costatum*. All other ratios are below 1.0 even with this extreme worst-case PEC_{water} value used in calculating these PEC:PNEC ratios.

The worst-case scenario of a production pond not managed for water storage is extreme because the probability of this combination of use conditions and weather is extremely unlikely to occur. Ponds are not managed to maintain water levels at the overflow structure. These ponds, even if not managed for water storage, do not add water constantly to maintain maximum depth. Pumping water, generally ground water, costs money and is not an economically viable practice. Aquaflor is only applied prescriptively to a small portion of production ponds, and the potential that one of these ponds is managed with maximum water level, treated with Aquaflor, and subject to a significant rain event close to treatment is highly unlikely. Finally, under this scenario, the release or overflow of pond water to receiving waters would mostly occur months after treatments were administered and during significant rain events (Appendix 3) and would not result in any substantial exposure to diatoms or other aquatic species similar to *S. costatum* (a marine species) due to dissipation in receiving waters.

In this risk assessment worst-case estimates of peak exposure are compared to the most sensitive species for each environmental matrix and use scenario evaluated. The PEC:PNEC values for the selected species ranging from fish to microbes are all $\leq$ 1.0 with the exception of *S. costatum* for fingerling ponds (Scenario I) and for production ponds not managed for water storage (Alternate Scenario II). The PEC:PNEC ratios for these two scenarios are 6.38 and 24.7, respectively (see Tables 12 and 14). All three worst-case scenarios presented above (Tables 12 –14) represent acceptable levels of risk, including the two *S. costatum* PEC:PNEC values that exceed 1.0.

Table 14 Risk Characterization, Scenario II: Summary for levee production ponds without water storage

Organism	End Point (mg/L)	Results (mg/L)	AF	PNEC (mg/L)	Refined PECwater (mg/L)	PEC:PNEC Ratio
Oncorhynchus mykiss	96-h LC_{50}	>780	100	7.8[a]	0.0104	0.00133
Lepomis macrochirus	96-h LC_{50}	>830	100	8.3[a]	0.0104	0.00125
Daphnia magna	48-h LC_{50}	>330	100	3.30[a]	0.0104	0.00315
Litopenaeus vannamei	48-h NOEC	<4	10	0.4[b]	0.0104	0.026
Selenastrum capricornutum	14-d NOEC	0.75	10	0.075[b]	0.0104	0.139
Skeletonema costatum	72-h NOEC	0.0042	10	0.00042[b]	0.0104	24.7
Bacillus subtilis	MIC	0.4	10	0.04[b]	0.0104	0.26

[a] An application factor of 10 was used to account for intraspecies variation and a factor of 10 was used in the extrapolation from acute to chronic data.
[b] An application factor of 10 was used to account for intraspecies variation. These toxicity values are considered chronic endpoints.

The results of this risk assessment must be taken in context. First, PEC:PNEC ratios indicate acceptable risk with the exception of two PEC:PNECs for *S. costatum.* Although PEC:PNEC ratios of >1.0 may appear to indicate unacceptable risk to receiving aquatic ecosystems four issues must be considered: 1) the nature of the observed effect; 2) differences in exposure in laboratory and field situations; 3) the nature of the phytoplankton community; and 4) the consideration of *S. costatum* as a biological outlier with respect to sensitivity to florfenicol.

Florfenicol is not algicidal but algistatic (i.e., inhibitory) and even in the study discussed above (Appendix 36) an algistatic concentration (i.e., completely algistatic) was not reached in a static system. Similar inhibition would not be

expected in dynamic receiving waters with episodic release of florfenicol related residues due to obvious differences when comparing field to laboratory exposures. This inhibition is reversible; therefore, as the material released from the pond is diluted then any inhibited algae would be able to resume normal growth, as observed in laboratory studies. The approximate hundred fold difference in sensitivity of algal species indicates that even if there was reduction in growth of the most sensitive species there would be little impact on algal biomass with any temporarily inhibited algae being replaced by other species. The localized nature of any impact can be expected to be short-lived as there will be refugia both up and downstream from any pond discharges from which temporarily inhibited algae will be replenished.

The NOEC for *S. costatum* appears to be a significant outlier compared to the other species tested. The *S. costatum* NOEC is two orders of magnitude below the next most sensitive aquatic plant (algal) species, *S. capricornutum*; greater than four orders of magnitude below the most sensitive invertebrate species (*L. vannamei*); and more than five orders of magnitude lower than the most sensitive fish species (*O. mykiss*). In addition, this observed toxicity value is greater than an order of magnitude lower than the most sensitive microbial species tested (*B. subtilis*). The *S. costatum* MIC and NOEC are clearly outliers and contribute to an overly conservative assessment of risk. The PEC:PNEC ratios are low enough (6.38 and 24.7) to provide an adequate margin of safety even with this overly conservative risk characterization.

There are other factors that must be considered in characterizing the risk to aquatic ecosystems from use of Aquaflor. Under the fish culture conditions, the most likely time for infection by *E. ictaluri* would be in the autumn of the first year in nursery ponds (Scenario I). This would be the first time that these fish would be exposed to the disease organism under conditions conducive to infection. *E. ictaluri* is present in sufficient concentrations in water to be pathogenic only when the water temperature is between 20°C and 30°C (Appendix 2), which occurs generally within a 30 to 40 day window in the spring (May – June) and fall (September – October) (Appendix 2). Since Aquaflor treatment is prescriptive this restricted window for potential active infections limits the time period and frequency of application.

The fingerlings are naive with respect to ESC and would be the most susceptible at this time. In the fingerling production situation, the ponds would be drained to facilitate the harvest of the fingerlings and to recontour the pond bottom prior to restocking of fry. This would occur in the spring after all the fingerlings have been removed for addition to catfish production ponds.

In the production farm situation (Scenario II), the ponds are not emptied on a yearly basis but rather when renovation of the pond is required which averages about every 6.5 years (Appendix 3). Since any remaining fish in the ponds would have to be harvested at the end of the growing season, it would be unlikely that the pond would have required treatment for ESC since young and potentially susceptible fingerlings would not have been added to the pond in the

previous spring. Therefore, in the production pond situation, environmental exposure would only occur when excessive rainfall caused the level of the pond to rise to the point where overflow could occur during or shortly after the end of treatment. The rainy season starts in December, and treatment for ESC outbreaks occurs usually between mid-September and mid-October (Appendix 3). Therefore, there would be at least a six-week period between treatment and the potential for an overflow situation. During the rainy season, rivers and streams would be at high flow rates so overflow would be diluted to a large extent. In the case of levee production ponds, if overflow occurred, it would happen at times of high rainfall when dilution into streams and rivers would occur. Therefore, the nursery pond situation is the worst-case situation with regard to frequency of treatment (most susceptible life stage) and potential for pond water release from yearly pond draining. This scenario is used in estimating the worst-case PEC:PNEC ratios (see Tables 12-14).

Effluents are mainly released into ditches, which would lead into rivers or streams where further dilution would occur. Ponds are usually emptied intermittently over a several day period to eliminate the loss of sediment from the pond due to currents created upon emptying, to permit suspended solids to settle and to facilitate seining of remaining fish. Therefore, a large bolus of pond water would not enter a ditch on its way to a stream or river. A dilution factor of 10 was conservatively used in estimating the refined PECs with the exception of Scenario II where a dilution factor of 100 is used.

The degradability of florfenicol and metabolites demonstrate that these residues are not persistent and will not be present in the pond or receiving water environments for extended periods. Therefore, should concentrations arise which affect sensitive species, any effects would be transient due to dissipation and degradation.

Given the low K_d values of florfenicol and its metabolites, very little of these residues, if any, will equilibrate into the sediment of streams or rivers into which the water is released. The equilibrium would be strongly toward the aqueous phase. The concentration in the water phase would be low to begin with and would be further diluted by mixing with stream or river waters.

Under the Veterinary Feed Directive Aquaflor will be used solely under a prescriptive use pattern. These prescribed or controlled applications are made only to populations with active infections and there is no prophylactic use allowed. In addition, application is episodic occurring once or twice per year and, consistent with prescriptive use solely for active infections.

The existing toxicity data indicates that florfenicol is, in general, more active against prokaryotic than eukaryotic organisms with the exception of the marine diatom *S. costatum*. However, the likelihood of environmental effects to even prokaryotic organisms would appear to be limited, based on the MIC:PEC and PEC:PNEC ratio values calculated from the intended use-patterns and limited consideration of dispersion in the receiving environments. While initial PEC values indicate a potential risk to some sensitive species the established

environmental mobility and relatively short half-life of florfenicol in the environments (estimated conservatively to have a half-life of 30 days) for which data are available indicate that any potential risk would be short-lived.

Specifically any exceedences of the PNEC for the PEC_{water} must be evaluated in the context of this conservative risk assessment. Schering-Plough Animal Health believes that the limited magnitude of these exceedences is acceptable given the conservative nature of the risk characterization. In the reasonable worst-case scenario (Scenario I, fingerling ponds) exposure concentration is above the PNEC ratio, but remains below the lowest NOEC (*S. costatum*), which is an order or magnitude above the PNEC (using an AF of 10). Even for the extreme worst-case scenario (production ponds not managed for water storage), which is not expected to ever occur, the PEC_{water} exceeds the lowest NOEC (*S. costatum*) by only a factor of 2.5x. The effect in this case is partial inhibition (i.e., florfenicol is algistatic) and even 2.5 times the NOEC of 0.0042 mg/L (0.0105 mg/L) is still well below the MIC of 0.0336 mg/L (Table 7).

The use of Aquaflor to treat ESC in catfish does not present any significant risk to aquatic ecosystems due to the following combination of factors:

- Aquaflor is limited:
    - to prescriptive application with no prophylactic use under the Veterinary Feed Directive;
    - to application in feed at 10 mg/kg feed/day for 10 days; and
    - temporally to a 30 to 40 day window in the both spring and fall when water temperatures are appropriate for *E. ictaluri*

populations to potentially reach pathogenic levels in catfish ponds;

- Florfenicol, the active ingredient in Aquaflor, will remain in water where it dissipates due to degradation and dilution;
- Florfenicol release is limited by catfish culture (frequency and timing of pond draining) and water management practices (water storage);
- Florfenicol presents a low potential hazard based on toxicity studies with a range of organisms;
- Exposures in aquatic systems are expected to be low and transient
- Using three worst-case scenarios, the only two PEC:PNEC ratios that exceed 1.0, both involve a marine diatom that is:
    - the most sensitive species tested with an NOEC 100 times lower than the next most sensitive species (a green algae);
    - based upon a reversible growth inhibition that requires sustained exposures to be achieved in the laboratory; and,
    - based on peak, worst-case concentrations that are expected to be transient and most often associated with rain events.

Based on this assessment and the factors listed above, the probability of a combination of circumstances resulting in any sustained adverse impacts on aquatic ecosystems is considered to be very small.

Section 9. CONCLUSIONS:

The use of florfenicol under Veterinary Feed Directive regulations for control of mortality in channel catfish caused by infection with *Edwardsiella ictaluri* is not expected to pose any significant risk of widespread or sustained adverse environmental impact. Schering–Plough Animal Health believes that this data and analysis supports a Finding of No Significant Impact.

SECTION 10. USE OF RESOURCES AND ENERGY

Manufacturing Aquaflor® 50% Type A Medicated Article will require an amount of energy similar to that used to produce and package any conventional pharmaceutical product for animals. Disposal of wastewater and materials from the manufacturing process will not require use of unusual amounts of energy or natural resources. There will be no effects upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

SECTION 11 MITIGATION MEASURES

As there are no known or expected adverse effects of the proposed action, no mitigation measures will be required.

SECTION 12. ALTERNATIVES TO THE PROPOSED ACTION

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Therefore, alternatives to the proposed action do not need to be considered.

SECTION 13. LIST OF PREPARERS

The following consultant for Schering-Plough Animal Health Corp. was responsible for the preparation of this Environmental Assessment:

James F. Hobson, Ph.D., DABT
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SECTION 14. CERTIFICATION

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

Richard G. Endris, 16 Jan 04

Richard G. Endris, Ph.D.
Research Program Manager
Schering-Plough Animal Health Corp.

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REFERENCES:

Please refer to this INAD submission as well as the approved NADA #141-063 for the Nuflor® injectable for all pertinent information including information as provided in 21 CFR 511.1.

LIST OF APPENDICES (ABSTRACTS):

APPENDIX 1

Vincent W. 1992. Report No. 25973. SCH 25298: Environmental fate testing technical assistance document. Florfenicol amine metabolite (SCH 40458). Florfenicol alcohol metabolite (SCH 45705). Florfenicol oxamic acid metabolite (SCH 48057).

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APPENDIX 17

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APPENDIX 18

Fackler, P. 1991. Report No. A-25470. SCH 48057: Determination of aqueous photolysis rate constant and half-life.

APPENDIX 19

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APPENDIX 47

LeLievre, M. 1991. Report No. A-25394. SCH 25298: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static conditions.

APPENDIX 48

LeLievre, M. 1991. Report No. A-25398. SCH 45705: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static conditions.

APPENDIX 49

LeLievre, M. 1991. Report No. A-25403. SCH 48057: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static conditions.

APPENDIX 50

LeLievre, M. 1991. Report No. A-25395. SCH 40458: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under static conditions.

APPENDIX 51

LeLievre, M. 1991. Report No. A-25400. SCH 45705: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under static conditions.

APPENDIX 52

LeLievre, M. 1991. Report No. A-25401. SCH 48057: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) under static conditions.