ENVIRONMENTAL ASSESSMENT

for

AQUAFLOR® (Florfenicol) 50% Type A Medicated Article

Fed at a Dose up to 15 mg florfenicol/kg body weight/day for

Control of Mortality Associated with Bacterial Diseases in

Freshwater-Reared Finfish

Indications Supported:

(1) Enteric septicemia associated with *Edwardsiella ictaluri*
(2) Columnaris disease associated with *Flavobacterium columnare*
(3) Streptococcal septicemia associated with *Streptococcus iniae*

Fish Culture Systems Supported:

(1) Freshwater ponds
(2) Flow-through water systems (raceways, tanks)

DATE: **December 9, 2011**

NAME OF APPLICANT/PETITIONER: **Intervet Inc. d/b/a Merck Animal Health**

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. DESCRIPTION OF PROPOSED ACTION(S) AND NEED

This environmental assessment (EA) was prepared in support of a proposed agency action(s) on one or more supplemental new animal drug applications (NADAs) for the use of Aquaflor® (Florfenicol) Type A Medicated Article in finfish feeds for control of mortality associated with bacterial diseases in freshwater-reared finfish. Aquaflor® contains the active ingredient florfenicol, a synthetic, broad-spectrum antibiotic, effective in the control of a variety of fish pathogens.

Aquaflor® Type A Medicated Article (premix) is incorporated into fish feed prior to pelleting or by surface coating (top coating) the premix onto the feed pellets and sealing it by over-oiling. The rate of administration of the premix to the feed will be dependent on the feed consumption rate.

This EA has been prepared to support the use of medicated feeds prepared from the Aquaflor Type A Medicated Article for control of mortality for the following indications when fed at a dose rate up to 15 mg florfenicol/kg body weight for 10 consecutive days[1]:

- Enteric septicemia associated with *Edwardsiella ictaluri* in catfish
- Columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish
- Streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish

In this EA, environmental impacts have been evaluated for the proposed uses of Aquaflor® in two types of aquaculture systems for freshwater-reared finfish: ponds and flow-through water systems (e.g., raceways, tanks). Use in open water net pens and recirculating systems has not been evaluated. Due to their extensive culture in the U.S., channel catfish are the primary example used to assess the environmental impacts of Aquaflor® use in ponds. However, information on other species is provided to illustrate that the example of channel catfish is sufficiently conservative to address the potential impacts of Aquaflor® use in ponds when other fish species are treated. For flow-through culture systems, tilapia are used as the primary example, as this species is very tolerant of high density and low dissolved oxygen conditions and thus would represent a likely "worst case" for florfenicol use in flow-through raceway systems.

Because of the wide range of species and rearing conditions considered herein, the EA is also considered sufficient to be used in support of future proposed actions on supplemental NADAs for use of Aquaflor® for additional indications and fish species beyond those disscussed, provided that the dose rate does not exceed 15 mg florfenicol/kg bw/day, the duration of feeding does not increase, and the fish species are reared in ponds or flow-through culture systems.

Aquaflor® Type A Medicated Article has previously been approved for use in catfish to control mortality due to enteric septicemia associated with *Edwardsiella ictaluri* at a dose rate of 10 mg a.i./kg bw/day for 10 days. It has also been approved for use in freshwater-reared salmonids at this same dose rate to control mortality due coldwater disease associated with *Flavobacterium psychrophilum* and furunculosis associated with *Aeromonas salmonicida*. Individual EAs were

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[1] Depending on food safety and other considerations, approvals for these indications may occur at lower dose rates (e.g., 10 mg/kg). A dose rate of 15 mg/kg body weight was used for the analyses in this EA as it represents the highest expected dose rate for all of the indications being evaluated, and thus represents a worst-case exposure scenario for use of Aquaflor®.

prepared in support of each of these Aquaflor® approvals. The EA for Aquaflor® use in catfish evaluated potential environmental effects resulting from treatment of catfish in ponds, while the EAs for freshwater-reared salmonids considered effects due to treatments in flow-through raceways. Each of these EAs resulted in preparation of a finding of no significant impact (FONSI) by FDA.

1.1 DISEASES SUBJECT TO TREATMENT UNDER THE PROPOSED ACTION

Enteric septicemia in catfish (ESC) is the leading cause of mortality and is caused by *E. ictaluri* when the waters are between 20°C and 30°C. In U.S. catfish production, this happens twice per year, lasting approximately 30 days in the fall (September/October) and 30 days in the spring (May/June). All age-classes of catfish are susceptible, but fingerlings are the most susceptible (Kelly, 2005).

Columnaris is a highly contagious disease caused by *Flavobacterium columnare*. It is the second leading cause of mortality in pond-raised catfish, after enteric septicemia. Most fish species are susceptible to columnaris disease and this disease commonly occurs when the water temperatures are are 20-25°C (Kelly, 2005).

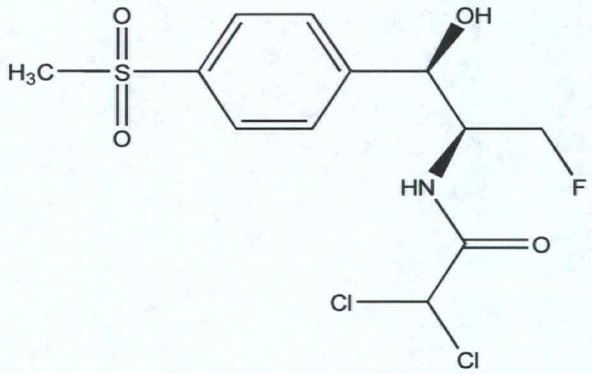
Streptococcus iniae infections can produce septicemia and affect multiple fish species. These infections are primarily a problem in intensive culture systems, including raceways and recirculating culture systems. *S. iniae* has emerged as a leading fish pathogen in aquaculture operations and tilapia and hybrid striped bass are the primary species affected in the U.S. aquaculture industry. Adult and subadult fish are groups more susceptible to infection than juvenile, but fishes of all ages can be infected (Agnew, 2007). The disease can occur at temperatures 20-40°C, with an optimal growth temperature of 37°C (Zhou et al., 2008).

There are currently no FDA approved drugs for treatment of columnaris disease or streptococcal septicemia in freshwater finfish.

2. IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

Florfenicol (CAS RN 73231-34-2) 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 structural formula of florfenicol is given in Figure 1.

Figure 1. Structural formula



Florfenicol (SCH 25298)

2,3-dichloro-N-[αS,βR)-α-(fluoromethyl)-β-hydroxy-p-(methylsulfonyl)-phenethyl]acetamide

The data relating to environmental toxicology have been derived with the florfenicol active ingredient or with the metabolites. The formulation of Aquaflor® consists of 50% Florfenicol, 47% Lactose Monohydrate, and 3% Povidone K29/32. It is believed that the excipients in the formulation will not affect the toxicity or environmental persistence of florfenicol.

3. INTRODUCTION TO ENVIRONMENTAL ISSUES

This EA is based in large part on prior EAs addressing the use of Aquaflor® in catfish in ponds at a dose rate of 10 mg/kg/day for 10 days (Schering-Plough Animal Health, 2004) and in freshwater-reared salmonids in flow-through raceways at a dose rate of 10 mg/kg/day for 10 days (Schering-Plough Animal Health, 2007). The general risk assessment approach in this EA follows the process described in CVM Guidance for Industy #166 (Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) – Phase II (CVM, 2006; VICH, 2004)[2] combined with EMEA Guidance (EMEA, 2008) as applied to aquaculture. Primary differences in this EA from the previous EAs prepared for Aquaflor® include 1) a consideration of additional fish species in the drug use pattern, 2) consideration of additional use indications and diseases, and 3) dosing at a higher rate, up to 15 mg/kg/day for 10 consecutive days.

A preliminary assessment was made following the Phase I decision tree as outlined in CVM Guidance for Industry #89 (Environmental Impact Assessment (EIA's) for Veterinary Medicinal Products (VMP's) – Phase I Guidance (CVM 2001; VICH, 2000). Utilizing the Phase I decision tree the following points have been raised. Aquaflor®:

- is not exempt from regulation;
- is not a natural product;
- will be used in food animals;
- is intended for use in a minor new species;
- will be used to treat whole systems (not isolated individuals);
- is extensively metabolized by fish, but significant amounts of parent compound will be excreted into the environment;
- will be used to treat aquatic organisms in confined facilities;
- is not an ecto- or endo-parasiticide;
- the initial Predicted Environmental Concentration in water (PEC_{water}; same as $EIC_{aquatic}$) is predicted to be released from an aquatic facility at a concentration > 1.0 µg/L.

Therefore, a Phase II Tier A assessment is required based on the direct release into the environment at a concentration > 1.0 µg/L (0.001 mg/L) as predicted in the Phase I assessment. Recently completed chronic toxicity testing also enabled a Phase II Tier B assessment to be conducted where required.

The initial assessment of the use of Aquaflor® in finfish is based on a VICH/CVM Phase II, Tier A assessment. This level of evaluation includes consideration of physicochemical properties, environmental fate studies, and acute environmental effects studies. Information on the use patterns of florfenicol is used to calculate the Predicted Environmental Concentrations (PECs). Initial PEC_{water} values are determined based on representative scenarios, including worst-case and typical scenarios, and compared to Predicted No Effect Concentrations (PNECs) for freshwater species as specified by VICH/CVM guidelines. Refined PEC_{water} values are determined after the inclusion of several additional factors affecting the concentrations of florfenicol in the environment. Similarly, PEC_{soil} values are determined where appropriate and compared to PNECs for terrestrial organisms.

[2] Referred to throughout this document as VICH/CVM guidance

Next, where necessary, an assessment of the use of Aquaflor® in freshwater-reared finfish is presented based on a VICH/CVM Phase II, Tier B assessment in which chronic environmental effects are evaluated and compared against the initial and refined PEC_{water} and PEC_{soil}, as appropriate.

Sections 4, 5, and 6 present information that is common to both use patterns being evaluated (ponds and flow-through water systems). In section 7, the assumptions used to calculate exposure for each use pattern (e.g., ponds and flow-through water systems), and the resulting risk quotients, are presented for each use pattern.

4. PHYSICO-CHEMICAL PROPERTIES

The Tier A physico-chemical characteristics of florfenicol and its major metabolites have been determined (Vincent, 1992) and are presented in Table 1. Florfenicol has a molecular weight of 358.21 with solubility in water of 1.32 grams per liter (g/L) at pH 7 and a log octanol-water partition coefficient (log Kow) value of 0.37, the latter indicating little potential for bioaccumulation according to the criteria presented in VICH/CVM Phase II in which substances with a log Kow of < 4.0 are not considered bioaccumulative. In view of these physico-chemical characteristics and those listed for the metabolites in Table 1, it is unlikely that florfenicol, or its metabolites/degradates, will accumulate in biota. Compounds such as florfenicol that have substantial water solubility with an extremely low Log Kow tend to remain in the water column.

Florfenicol has a low molecular weight, as do its metabolites, which range from 69 to 89% of parent mass. The parent and metabolite solubilities and Kow values differ. The metabolites are markedly more soluble (with solubilities ranging from 49.7 to >500 g/L) and are markedly less lipophilic (i.e. have lower Kow). Theoretically, these factors make the metabolites even more likely than florfenicol to enter and remain in water relative to sediment and not to bioaccumulate in biota.

In addition, florfenicol is a nonvolatile solid, has an ultraviolet (UV) light absorption maximum at 224 nanometers (nm), and has a melting point of 153−154°C (The Merck Index).

Table 1. Physico-chemical characteristics of florfenicol and major metabolites

	Florfenicol	Metabolites		
		Amine Metabolite	Alcohol Metabolite	Oxamic Acid Metabolite
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

5. ENVIRONMENTAL FATE

5.1 PHOTOLYSIS, HYDROLYSIS, AND ADSORPTION/DESORPTION

Studies on the susceptibility of florfenicol and its metabolites to photolysis and hydrolysis indicate that these mechanisms are unlikely to play a major role in the degradation of these compounds in the environment (Connor, 1995; Fackler, 1991a-d) (Table 2). Hydrolysis of florfenicol has only been detected in synthetic humic water, where a half-life of 350 days was determined (Connor, 1995). No significant regression could be determined for the degradation of florfenicol or its metabolites under the other conditions tested and as such no hydrolytic half-lives could be calculated (Connor, 1995). Pouliquen et al. (2007) also found that florfenicol was not degraded by hydrolysis or photolysis in deionized water, freshwater, or seawater either in darkness or at 1,400 lux when evaluated over 14 days at 8°C. However, in a recent study, parent and metabolites exhibited abiotic degradation under anaerobic conditions (see subsequent discussion in Section 5.2.4). Ge et al. (2009) observed that florfenicol dissolved in pure water (Millipore-Milli Q) did not photolyze under irradiation of sunlight or simulated sunlight; however, when dissolved in a natural fresh water, the solar photolytic half-life was 99 ± 16 hours. This recent information indicates that florfenicol may undergo some photolysis; however, it was conservatively assumed in this EA that this process would not be significant.

Table 2. Photolytic half-lives of florfenicol and its 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
Reference	Fackler (1991a)	Fackler (1991b)	Fackler (1991c)	Fackler (1991d)

NA = not applicable/available

Studies on the adsorption and desorption of florfenicol and metabolites in three different soil types determined that florfenicol was generally classified as very mobile to mobile, while the metabolites were less so and classified as slightly to very mobile. These results are summarized in Table 3 (Fackler, 1990; Weeden 1991a-c). K_d and K_{oc} values for florfenicol were determined to be 0.07–0.59 and 10–27, respectively, consistent with the low sorption characteristics.

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[1]	Very Mobile to Mobile	Moderately mobile	Very Mobile to Moderately Mobile	Mobile to Slightly Mobile
Reference	Fackler (1990)	Weeden (1991a)	Weeden (1991b)	Weeden (1991c)

[1] From classifications based on K_{oc}, as used to determine pesticide mobility in soils in the United Kingdom (Hollis, 1991).

5.2 DEGRADATION IN WATER, SEDIMENT, SOILS, AND ANIMAL WASTE SLURRY SYSTEMS

5.2.1 DEGRADATION IN WATER

The ability of florfenicol and its metabolites to degrade in a ready biodegradation CO_2 evolution test has been investigated (Weeden et al, 1991d-g). Testing was was conducted under the conditions described in the FDA Technical Assistance Handbook (FDA, 1987), Document 3.11 and according to Good Laboratory Practices (GLP). None of the compounds were degraded readily as indicated by CO_2 evolution or loss of parent compound. Analysis of the test media at day 28 of incubation indicated that the amine metabolite degraded to the greatest extent, with 25.4% remaining, while 81.4%, 98.6%, and 70.7% of the florfenicol, oxamic acid, and alcohol metabolites, respectively, remained. However, no check was made of the potential for microbial inhibition by the antibiotic under the test conditions, which employed high starting concentrations of florfenicol or its metabolites (about 20 mg/kg, well in excess of expected environmental concentrations). Therefore, it cannot be concluded that the lack of degradation observed in these studies was not due to inhibition.

5.2.2 DEGRADATION IN MANURE AMENDED SOIL

A soil degradation study in manure amended soils was conducted according to the FDA Technical Assistance Handbook (FDA, 1987) by Christensen (1995) in accordance with (GLP). 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 and a mean value of 158 days at 22°C. Primary degradation or transformation of the florfenicol was considerably faster and only 2.6–9% of the florfenicol could be recovered at the end of the 92-day study. Half-lives of 3.6–27.2 days were reported in this study. Based on these data, a conservative half-life of 27.2 days could be used for florfenicol in soils in calculations of environmental

concentrations. While degradation products appeared in the course of the soil degradation study, they did not accumulate (Christensen, 1995). The respective chromatograms demonstrate that peaks for florfenicol are substantially higher than those for the polar degradates at all time points. Hence, it can reasonably be concluded that the polar degradates degrade as fast or faster than florfenicol. Therefore the same half-life has been adopted for the degradation metabolites. Until recently no study had been undertaken on the degradation of florfenicol in manure alone. The newer studies on degradation of florfenicol in a slurry of cow manure under aerobic conditions (Button, 2007) and a slurry of pig waste under anaerobic conditions (Millais, 2005) are discussed below.

5.2.3 DEGRADATION IN CATTLE MANURE

A GLP Cattle Manure Study was conducted to evaluate the aerobic degradation of florfenicol in manure and urine (Button, 2007). Cattle manure from antibiotic-free cattle was mixed in a ratio of 2:1 feces:urine and added to test vessels which were flushed continuously with air at 60 mL/min. The vessels were acclimated for about 1 week prior to the addition of [^{14}C]-florfenicol at a nominal concentration of 5.5 mg/kg of manure. Control vessels contained sterile manure. Incubation of the florfenicol was initiated by the addition of [^{14}C]-florfenicol to the manure. The last sample was harvested on Day 92.

Unextractable or bound residues predominated from Day 7 onward. On Day 7 they were 51.6% of applied radioactivity (AR). They increased from Days 14 through 92 to account for 61.7 to 69.7% AR. In the sterile samples the unextractable residues were 3.9% and 4.1% at days 0 and 28, respectively. This indicates no abiotic degradation occurred.

In the test samples florfenicol decreased from 88% of AR at zero time to 41.9% by Day 3. Subsequently florfenicol declined to 5.5% AR at Day 7 and remained between 2.1% and 1.5% from Days 14 to 92. In the sterile samples florfenicol constituted 94% AR at zero time and 93.1% at Day 28. This indicates that, while biodegradation was occurring in the test systems, no abiotic degradation occurred in the sterile controls.

The overall recovery in the study was quantitative. Under sterile conditions there was limited degradation by Day 28 indicating the degradation was mainly mediated by the microorganisms present in the manure. The DT_{50} and DT_{90} values (degradation times for 50% and 90% degradation of florfenicol) were 2.4 and 8.0 days, respectively. For monochloroflorfenicol the same values were 3.0 and 10.0 days, respectively. The florfenicol DT values appear conservative in that although the florfenicol level dropped by about 50 % in the first 3 days, it declined to about 5.5% of AR by Day 7 reflecting about 4 half-lives or a half-life of about 1.75 days from zero time or about 1 day for the time period between Days 3 and 7. The degradates included known florfenicol metabolites, oxamic acid, amine, and alcohol, as well as a polar fraction. The metabolites reached a maximum of 4.1, 2.4, and 9.9% AR at different times of the incubation. The polar fraction reaches a level of 10.8% and most likely consists of more than one component based on the chromatogram presented with the study and therefore no one component would be present to the extent of greater than 10%. They are more polar than the metabolites (amine, oxamic acid, and alcohol) of florfenicol that have much reduced antimicrobial activity (Fackler, 1991e-h). These data indicate that both florfenicol and its monochloro metabolite degrade quite rapidly. Therefore, the degradation will start and proceed quickly once the florfenicol enters an aerobic system.

The unextractable degradates appeared to arise from both florfenicol and the monochloro-florfenicol since they are present early in the degradation process and cannot be totally accounted for by only the further degradation of monochloroflorfenicol (Button, 2007).

Unextractable residues are considered non-bioavailable. These residues would be released from the biosolids slowly, long after the extractable residues had moved from that area in the soil profile, and when released would be subject to the rapid degradation observed in soil and excreta (Christensen, 1995; Button, 2007). The other metabolites of florfenicol have undergone a number of fate and effect studies which demonstrate much lower biological activity (Fackler, 1991e-h), or as in the case of the monochloroflorfenicol, are assumed to have similar properties to florfenicol (Gledhill, 2005).

5.2.4 DEGRADATION IN SEDIMENT/WATER SYSTEMS

The results and conclusions of the studies discussed above are confirmed by the results of a guideline study: Determination of the Aerobic Transformation of [^{14}C]-Florfenicol in Aquatic Sediment Systems (Gledhill, 2005) conducted according to OECD Guideline 308 (Aerobic and Anaerobic Transformation in Aquatic Systems). This Tier A study (VICH/CVM Phase II) is the cornerstone of the environmental fate database relative to degradation in water and sediment. Because of observed aerobic degradation, the anaerobic portion of this study was not conducted. Anaerobic degradation data are available in the pig manure slurry study (Millais, 2005) (see below).

Briefly, three sediments, two freshwater and one marine (Table 4), were used with overlying water collected concurrently with each sediment. Radio-labeled florfenicol (ring-labeled) was added to the water fraction of sediment water systems. The concentration of [^{14}C]-florfenicol to be added to the water phase of the definitive test system was previously determined by a 21-day preliminary study which was conducted with two primary objectives: 1) to determine the exposure level for the definitive study which was below the lowest concentration where microbial inhibition was observed, and 2) to provide preliminary information on the rate of degradation as a basis for establishing the sampling regime for the definitive study.

The definitive study was initiated at an exposure level of 0.510 mg/L [^{14}C]-florfenicol in the water phase. This concentration was selected as non-toxic to the sediment microorganisms based on the results of a preliminary test. (In the preliminary test, 0.1, 1.0, and 10 mg/L treatments were used, resulting in no inhibition, 50% inhibition, and 99% inhibition, respectively. Notably even where 50% inhibition was seen, this was reversible by the 4th day). Samples were collected at intervals of 10, 23, 30, 50, 78, and 100 days. At each sampling time duplicate test systems were sacrificed. Residues/degradates were monitored in water and sediment using liquid scintillation counting (LSC) and high pressure liquid chromatography with radiometric detection (HPLC/RAM).

Results showed rapid partitioning between water and sediment phases and degradation of florfenicol to smaller more polar compounds in all three sediment types. These smaller, more polar compounds were observed to degrade in both the water and the sediment portions of the system. The parent peak also declined with time in both water and sediment. The pattern of degradation and the subsequent decline in degradates also is qualitatively similar for both water and sediment. The data presented in Figure 2 are for the Goose River (GR) sediment (half-life of 13 days) (Table 4) and the pattern of degradation presented here is consistent in all three of the sediment systems evaluated.

Table 4. Degradation of florfenicol in three different sediment-water systems

Source	Type of Site	Sediment Type*	% Organic Carbon	Degradation Rates for Sediment/Water Systems(days)		K_d	K_{oc}
				DT_{50}**	DT_{90}		
Duxbury Marine (DM)	Marine	Loam	3.2	13.0	43.1	0.293	9.1
Goose River (GR)	Freshwater	Loam	2.4	8.4	27.8	0.434	18.1
Weweantic River (WR)	Freshwater	Sand	0.76	19.4	64.5	0.250	32.9

Reference: Gledhill (2005)

* USDA textural type; ** DT in this table stands for degradation time.

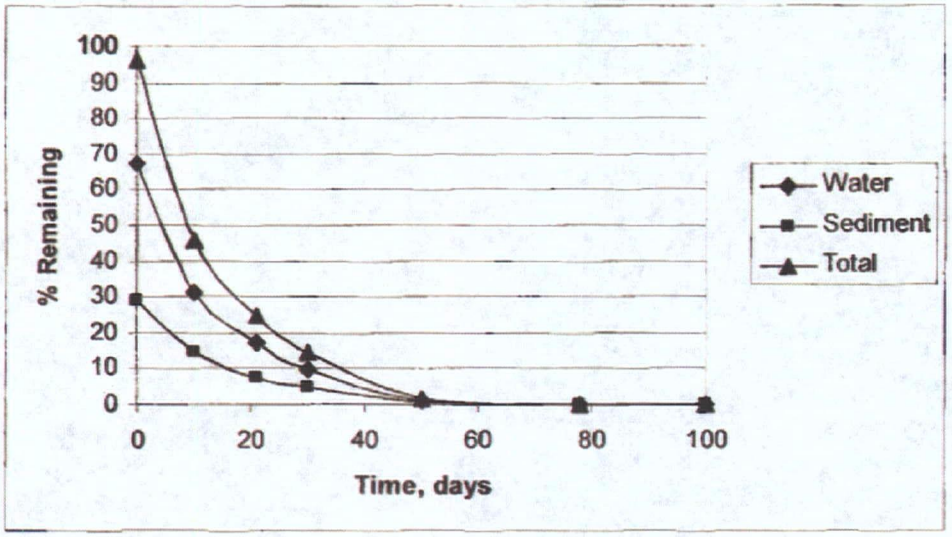
The similar pattern of appearance and decline of metabolites was observed for all three sediments. Parent [^{14}C]-florfenicol was observed to partition between water and sediment and to degrade in both fractions of the test system (Figure 2). Half-lives ranged from 8.4 to 19.4 days for the three sediment/water systems (Table 4) and the mean value of 13.6 days is used as the half-life in this assessment. [^{14}C]-florfenicol degraded to smaller more polar metabolites which were not persistent. Metabolites were observed to degrade at similar or faster rates than the parent. The only metabolite collected above 10% AR was the monochloroflorfenicol labeled with a retention time of 18.4 min. Identification of this metabolite was based on liquid chromatography and mass spectroscopy (LC/MS) compared against an analytical standard and only exceeded 10% in the Weweantic River water and sediment. Minimal mineralization (<6% conversion to CO_2) was observed in all three sediments.

Smaller or low molecular weight florfenicol-derived residues were observed to bind to sediments under the conditions of the study. These unextractable or bound residues increased with time and ranged from 63% to 85% AR at 100 days. Extensive multi-solvent extractions (with acidic and basic adjustments) did not yield any significant amount of additional parent florfenicol. Humic acid/fulvic acid/humin fractionation indicated the residues were incorporated into the latter two fractions and not readily desorbed. Although florfenicol and florfenicol-related residues partitioned to sediments and degraded, the reported K_d and K_{oc} ranged from 0.250 to 0.434 (average 0.33) and 9.1 to 32.9, respectively (Gledhill, 2005). These values are very low and indicate that florfenicol has a relatively low potential to partition to sediments. Any residues that did reach the sediment would degrade to compounds which associate strongly with the sediment and would not be bioavailable.

The mass balance was calculated by summing the % applied dose in volatiles, sediment extracts, aqueous extracts, and bound residues (following combustion of post extracted sediment). The mass balance for each sampling interval ranged from 97.1 to 107.1%, 92.3 to 113.7%, and 90.7 to 103.3% for water and sediment systems collected from Goose River, Duxbury Marine and Weweantic River, respectively. Analytical recovery rates (real-time analyses) are shown in Table 5. Quality control sample performance was set at 70.0–120.0%. Microbial biomass was measured at the beginning of the acclimatization phase and beginning

and end of the test period. The microbial biomass, expressed as organic carbon, at the end of the test period was greater than at the beginning of the acclimation phase for all three sediments, indicating that florfenicol at the selected test concentration did not cause any microbial inhibition.

Figure 2. Graphical illustration of the depletion of [^{14}C]florfenicol from GR sediment and water during the aerobic transformation study.



(from Gledhill (2005)

Table 5. Recovery of florfenicol in sediments and water using two different analytical methods

Matrix	LSC	HPLC/RAM
Water	96.9% (±2.36%)	101% (±7.70%)
Sediment (extracted)	90.4% (±6.98%)	87.0% (±10.2%)

Reference: Gledhill, 2005

5.2.5 DEGRADATION IN A SLURRY OF PIG WASTE UNDER ANAEROBIC CONDITIONS

In a definitive GLP study, degradation of florfenicol was evaluated in an anaerobic slurry of pig manure and urine (Millais, 2005). This Tier B fate study is important to the risk assessment because it provides anaerobic data to complement the aerobic biodegradation studies discussed above. Anaerobic conditions can occur in sediment of ponds. All of these studies show a consistent pattern of rapid degradation and a similar metabolic profile with monochloroflorfenicol being the primary metabolite.

The slurry of pig waste was allowed to incubate until the redox potentials indicated slightly reducing anaerobic conditions. These conditions were maintained throughout the 90-day test period at a temperature of 15 ± 2°C. At regular intervals duplicate vessels were sacrificed. Water and biosolids were separated and analyzed for radioactivity. Biosolids were extracted and the remaining material was dried and combusted to measure unextractable residues. Potential volatile radio-labeled compounds were monitored. Microbiological activity and anaerobic conditions were maintained throughout the study.

In this anaerobic, biologically-active system, degradation of florfenicol and the primary metabolite, monochloroflorfenicol, were rapid with DT_{50} values of 1.0 day and 2.4 days, respectively. [^{14}C]-florfenicol was added to the water phase at time zero. Florfenicol declined from 84% AR at time zero to 6.8% at three days and 1.3% AR at 7 days. The florfenicol was observed to partition rapidly from water to biosolids as observed in time zero samples where 55.9% and 28.5% AR were in water and biosolids, respectively. From Day 7 to Day 48 recovered[3] florfenicol residues remained at approximately 1.0%. Florfenicol was observed to partition between water and solids and to degrade rapidly in both compartments and no florfenicol was reported at day 90.

The primary metabolite, monochloroflorfenicol, was present in the time zero samples at 2.3% and 1.9% AR in water and biosolids, respectively. The metabolite reached a maximum of 34.9% AR on Day 3 and declined to 0.8% in water at 90 days and 1.0% in biosolids at 48 days. This indicates that monochloroflorfenicol is rapidly formed (as shown in the zero time samples), but does not accumulate, and degrades very rapidly as does florfenicol. Other metabolites (florfenicol amine and florfenicol oxamic acid) were observed but did not exceed 10% AR at any time interval. These observations were similar to the results of the aquatic biodegradation study (Gledhill, 2005) and the aerobic cattle slurry study (Button, 2007) discussed above.

This study also included a set of sterile control systems run concurrently with the definitive non-sterile slurry system. These essentially microbe-free systems were maintained under anaerobic conditions and the florfenicol degraded from 90% AR at time zero to 5.0% at 90 days. The observed distribution between water and biosolids was similar to that seen in the non-sterile systems. These data indicate that abiotic degradation will occur under anaerobic conditions with florfenicol. Although the results at 90 days were similar to the biologically active system, the rate of degradation was much slower in the abiotic, sterile system (Millais, 2005).

Biotic degradation of florfenicol was rapid in the pig slurry system and followed first order kinetics. The reported DT_{50} and DT_{90} values were 1.0 and 3.4 days, respectively, for florfenicol. The metabolite, monochloroflorfenicol, alone had reported DT_{50} and DT_{90} values of 2.4 and 8.1 days, respectively, and appeared to follow pseudo first order kinetics (Millais, 2005).

Unextractable, or bound, residues accumulated to 27.1% in sediment by 90 days. A similar level of bound residues, 23.2% AR, was found in the sediments of the sterile systems after 90 days indicating that abiotic degradation was occurring, although at a slower rate The polar fraction of AR that remained at the origin under TLC conditions increased with time and included the florfenicol oxamic acid and alcohol degradates and at least 10 other compounds. None of these compounds exceeded 10% AR. The oxamic acid metabolite was found at a concentration of 2% AR in this polar fraction.

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[3] Biosolids were extracted and water samples were analyzed directly

5.3 SUMMARY OF ENVIRONMENTAL FATE

Florfenicol is unlikely to degrade by hydrolysis or photolysis and has a low tendency to sorb to soil. The degradation of florfenicol and the monochloro metabolite is rapid in soil, sediment/water systems, aerobic cattle manure slurry, and anaerobic pig manure slurry as reported in the four principal environmental fate studies (Christensen, 1995; Button, 2007; Gledhill, 2005, and Millais, 2005) (Table 6). These four highly reliable GLP studies define the environmental fate of florfenicol. Despite slow rates of hydrolysis and photolysis, and the low K_{oc}, the four principal studies show that florfenicol and florfenicol-related residues degrade in environmental matrices and partition between water and solid matrices (e.g., sediments, manure and soils), respectively. Adsorption of florfenicol to soils and sediments may be via mechanisms unrelated to K_{oc}. Although accumulation of residues was not observed in the soil study, accumulated bound, or unextractable, residues were identified in sediments and biosolids (manure and slurry), respectively. In all four studies, from manure amended-soils to marine sediments, rapid degradation/dissipation/loss of biological activity was consistently observed. Peak unextractable residues ranged from 27.1% AR in the anaerobic study to 85% AR in the sediment/water study. These unextractable or bound residues are ultimately extracted by strong acid hydrolysis. Extracted residues consist of small polar metabolites of florfenicol. These residues are not biologically available which is important to the overall risk assessment (Button, 2007; Millais, 2005; Gledhill, 2005).

Results of the anaerobic pig slurry study are consistent with aerobic studies discussed above and presented in Table 6; however, this study is unique in demonstrating the occurrence of degradation under anaerobic conditions (Millais, 2005). The four studies listed in Table 6 all show rapid degradation under different experimental conditions with DT_{50}s (half-lives) ranging from 1.0 to 27.2 days. The mean value of 13.6 days for the sediment/water study (Gledhill, 2005) is used as the half-life for estimating degradation in water and solids. This is the most appropriate set of experimental conditions for making an estimation of degradation of florfenicol in uneaten feed and excreta from aquaculture facilities.

Table 6. Results of degradation studies

Principal Studies	Reference	Matrix/System	Environmental Half-Lives (DT_{50}) in days
Aerobic Biodegradation in Manure-Amended Soil	Christensen (1995)	Manure amended soil	3.6 to 27.2
Aerobic Degradation in Cow Manure Slurry	Button (2007)	Cow manure slurry system	2.4 (florfenicol) 3.0 (monochloroflorfenicol metabolite).
Determination of the Aerobic Transformation of [^{14}C]-Florfenicol in Aquatic Sediment Systems	Gledhill (2005)	Sediment/water systems	13.6[1] (range 8.4 to 19.4)
Anaerobic Degradation in Pig Manure Slurry	Millais (2005)	Pig manure slurry system	1.0 (florfenicol) 2.4 (monochloroflorfenicol metabolite).

[1] Mean of DT_{50} for three sediments.

6. EFFECTS ASSESSMENT

This section presents data on the acute and chronic effects of florfenicol (and its metabolites, where known) for microorganisms, fish, aquatic and terrestrial invertebrates, and aquatic and terrestrial plants. Data on microorganisms are discussed, followed by a discussion of the available data on aquatic plants, invertebrates, and fish. Finally, data on terrestrial plants and soil microbes and invertebrates are presented.

Both acute and chronic toxicity data are available for freshwater species representing three trophic levels of the aquatic ecosystem (plants, invertebrates, and fish). Data are available on some saltwater species, but since the use pattern evaluated in this risk assessment (florfenicol use on freshwater-reared finfish) does not encompass the marine environment, data on saltwater organisms are not included, as a marine/estuarine risk assessment is not required (CVM, 2006; VICH, 2004).

The primary focus in this section is on data generated in laboratory studies conducted according to FDA or OECD guidelines and under GLP. Additional data from the literature are presented as supporting information. The data are then used to calculate the PNECs for each species.

6.1 MICROORGANISMS

Florfenicol exhibits activity against a wide spectrum of prokaryotic microorganisms with minimum inhibitory concentration (MIC) values ranging from 0.25 mg/L for *Pasteurella multocida* to >1,000 mg/L for *Trichoderme viride* and *Aspergillus niger* (Table 7). Where comparative data are available, the parent moiety is more biologically active than the metabolites with the exception of the monochloro metabolite which has similar activity to the parent (Fackler, 1991e-h; Schuster, 2004).

In a study conducted according to FDA Technical Assistance Handbook Document 4.02, (FDA, 1987) microbial growth inhibition of florfenicol on two nitrifying bacteria, *Nitrobacter sp.* and *Nitrosomonas europaea*, was examined at concentrations up to 65 and 10 mg a.i./L, respectively (Sayers, 2009a). Microorganism growth was determined on days 14 and 17 for *Nitrobacter* sp. and *Nitrosomonas europaea*, respectively, and also at test termination (21 days). The MIC of florfenicol was determined to be 65 and 2.5 mg a.i./L for *Nitrobacter* sp. and *Nitrosomonas europaea*, respectively.

6.2 AQUATIC PLANTS

Algal toxicity tests were conducted on florfenicol and its principal metabolites according to FDA Technical Assistance Handbook Document 4.10 (FDA, 1987) and under GLP using *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) (Hoberg, 1991a-d). The MIC and no observed effect concentration (NOEC) obtained over the 14-day exposure period for each compound are presented in Table 8. The data for this green alga are similar to the data presented in Table 7, which shows that the degradation metabolites are similar or less active against prokaryotes for which the activity has been determined (Fackler 1991e-h; Schuster, 2004).

Table 7. Minimum inhibitory concentration (MIC) (mg/L) data for florfenicol and major metabolites against microorganisms

		Principal Metabolites			
	Florfenicol	**Amine**	**Alcohol**	**Oxamic Acid**	**Monochloro-florfenicol**
SPAH Code No.	**SCH 25298[a]**	**SCH 40458[b]**	**SCH 45705[c]**	**SCH 48057[d]**	**SCH 49435[e]**
Aeromonas salmonicida	0.3-2.5	--	--	--	--
Aspergillus niger	>1000	>1000	>1000	>1000	--
Bacillus subtilis	0.4	40	40	>1000	--
Clostridium perfringens	1.0	80	40	>1000	--
Escherichia coli	8.0	--	--	--	4.0
Mannheimia haemolytica	1[e]	--	--	--	1
Moraxella	0.5	--	--	--	--
Nitrobacter sp.	65[f]	--	--	--	--
Nitrosomonas europaea	2.5[f]	--	--	--	--
Nostoc	4.0	20	200	400	
Pasteurella multocida	0.25[e]	--	--	--	0.5
Serratia	16	--	--	--	--
Trichoderme viride	>1,000	>1,000	>1,000	>1,000	--
Vibrio sp.	0.8-1.6	--	--	--	

[a] Fackler (1991e), [b] Fackler (1991f), [c] Fackler (1991g), [d] Fackler (1991h), [e] Schuster, 2004 [f] Sayers (2009a)

Table 8. Toxicity data for florfenicol and major metabolites against *Pseudokirchneriella subcapitata*

		Principal Metabolites		
	Florfenicol	**Amine**	**Alcohol**	**Oxamic acid**
SPAH Code No.	**SCH 25298**	**SCH 40458**	**SCH 45705**	**SCH 48057**
Maximum growth rate, 14 days				
MIC	>2.9	>2.7	>0.98	80
NOEC	2.9	2.7	0.98	38
Maximum cell density, 14 days				
MIC	1.5	2.7	0.26	80
NOEC	0.75	1.4	0.13	19
Reference	Hoberg (1991a)	Hoberg (1991b)	Hoberg (1991c)	Hoberg (1991d)

Note : *Pseudokirchneriella subcapitata* is the updated nomenclature for *Selenastrum capricornutum.*

While the metabolites are generally less active than the parent compound toward eukaryotes, the alcohol metabolite has been found to be approximately six times more active against *P. subcapitata* (Hoberg, 1991a-d); however, this metabolite is the most transient of the major metabolites and would not be expected to accumulate in sufficient quantities to be of concern. The differences in the MIC and NOEC values for *P. subcapitata*, with regard to maximum growth rate and cell density, can be partially explained by exposure to 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 indicate that while florfenicol was algistatic, it was not algicidal from initial concentrations up to 2.9 mg/L (Hoberg, 1991a-d).

Other freshwater aquatic plant species that have been tested for florfenicol toxicity in GLP laboratory studies are duckweed, *Lemna gibba* (Softcheck, 2009); the diatom, *Navicula pelliculosa* (Jenkins, 2005); and the cyanobacterium or blue-green alga, *Anabaena flos-aquae* (Gallagher et al., 2008a). These organisms were exposed for 7 days, 72 hours, and 96 hours, respectively, to florfenicol. Procedures followed OECD Guideline 201, Freshwater Alga and Cyanobacteria Growth Inhibition Test and OECD 221, *Lemna* sp. Growth Inhibition Test, as indicated in Table 9. The results of these studies are compared to the results for *P. subcapitata* in Table 9. (Raw data from the *P. subcapitata* 14-day test were used to generate the 96-h EC_{50} values to enable the comparison). The same observation that florfenicol effects were algistatic, not algicidal based on growth data, is reported in the *N. pelliculosa* study (Jenkins, 2005). The concentrations of florfenicol were analytically verified during this study, indicating no loss of florfenicol during the test. Thus it can also be concluded that the degradation products did not reach levels that were algistatic in the course of the study. In the study with *Lemna gibba* (Softcheck, 2009), effect levels based on both yield and growth rate were calculated based on both frond number and dry weight. Results were expressed based on the mean measured concentrations, which ranged from 98 to 100% of nominal. Similar to the algal studies, the endpoints in the duckweed test based on growth rate were consistently higher than those based

on yield. Table 9 includes all of the EC_{50} values from the duckweed test but for simplicity, only presents the most conservative (lowest) NOEC or EC_{10} values.

Table 9. Toxicity of florfenicol to aquatic plants

Species, Reference, And Toxicity Endpoint	Toxicity Value, mg/L	Guideline
P. subcapitata (Hoberg, 1991a)		
EC_{50} biomass, 96-h[1]	1	FDA 4.01
EC_{50} growth rate, 96-h[1]	>2.9	
NOEC, 96-h[1,2]	0.75	
Navicula pelliculosa (Jenkins, 2005)		
EC_{50} biomass, 72-h	61	OECD 201
EC_{50} growth rate, 72-h	141	
EC_{10} biomass, 72-h[3]	18.7	
Anabaena flos-aquae (Gallagher et al., 2008a)		
EC_{50} biomass, 96-h	0.23	OECD 201
EC_{50} growth rate, 96-h	0.54	
NOEC, 96-h[2]	0.11	
Lemna gibba (Softcheck, 2009)		
EC_{50} yield (based on frond number), 7-d	0.76	OECD 221
EC_{50} growth rate (based on frond number), 7-d	1.8	
EC_{50} yield (based on dry weight), 7-d	0.82	
EC_{50} growth rate (based on dry weight), 7-d	3.3	
NOEC, yield (based on frond number), 7-d	0.39	
EC_{10}, yield (based on dry weight), 7-d[3]	0.28	

[1] Calculated from raw data presented in study report.

[2] The selected NOEC is based on the most sensitive test parameter.

[3] The EC_{10} was reported as a more accurate assessment of toxicity than the NOEC.

Lai et al. (2009) determined the toxicity of three antibiotics, including florfenicol, to two species of marine algae and one freshwater green alga, *Chorella pyrenoidosa*. The results for the marine species are not discussed here as they are not relevant to a freshwater risk assessment. It is not known if this study was conducted under GLP but it was reported that the methods in OECD 201 were followed, with modifications. The reported EC_{50} for florfenicol for *Chlorella pyrenoidosa* was 215 mg/L. It was not evident whether this result was determined based on biomass or growth rate. Since this result is higher than the results reported from the GLP studies, it is not used further in the aquatic risk characterization. Rather, the data from the more sensitive species are used.

The reported (or calculated) EC_{50} values based on both biomass/yield and growth rate are presented in Table 9; these are used in the Tier A risk characterization. In addition, NOEC values or EC_{10} values were either reported or calculated. The tabulated NOEC or EC_{10} values, which are based on the most sensitive response variable measured during the study, are used in the Tier B risk characterization. It is notable that *Anabaena flos-aquae* was more sensitive

than the other species. This is not unexpected, as *A. flos-aquae* is more appropriately classified with the cyanobacteria[4] rather than the green algae and other aquatic plants, and florfenicol is an anti-bacterial compound.

6.3 AQUATIC INVERTEBRATES

6.3.1 ACUTE TOXICITY

In an acute toxicity test conducted according to OECD Guideline 202 (*Daphnia* sp. Acute Immobilization Test) and under GLP, 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 (LeLievre, 1991a). Similarly, no EC_{50} values could be determined for the metabolites (LeLievre, 1991b-d). 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 which is consistent with the order of toxicity observed for microbes and algae (see previous discussion). The acute 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 (LeLievre, 1991a) as shown in Table 10.

Table 10. Acute toxicity of florfenicol and major metabolites to *Daphnia magna*

	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
Reference	LeLievre (1991a)	LeLievre (1991b)	LeLievre (1991c)	LeLievre (1991d)

6.3.2 CHRONIC TOXICITY

The effects of florfenicol on the survival, growth, and reproduction of *Daphnia magna* were evaluated in a 21-day static renewal test conducted according to OECD 211 (*Daphnia magna* Reproduction Test) and under GLP (Gallagher et al., 2008b). *D. magna* were exposed to five concentrations and a control (mean measured concentrations 0, 0.18, 0.38, 0.75, 1.5, and 3.0 mg/L). No significant effects on survival or growth were observed at any test concentration. However, reproduction was reduced at the highest test concentration. Thus, the NOEC, based on the most sensitive parameter, reproduction, was 1.5 mg/L.

[4] NCBI (National Center for Biotechnology Information) Taxonomy Browser, http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Tree&id=1166&lvl=3&lin=f&keep=1&srchmode=1&unlock, accessed 16 January 2009.

Chronic toxicity of florfenicol to the freshwater rotifer, *Brachionus calyciflorus*, was determined under static conditions in a 48-hour test (Sayers, 2009b). This study was conducted under GLP and in general accordance with Snell and Moffat (1992) and ASTM Standard Guide E 1440-91 (ASTM, 2004). Although the exposure duration is short, this is considered a life-cycle test because it measures reproduction from both parental females and F_1 females, with the relevant endpoint being the intrinsic rate of population increase. Organisms were exposed to five concentrations (mean measured concentrations of 0.40, 0.76, 1.6, 3.0, 6.9, and 14 mg/L), a control, and a solvent control. The NOEC was 0.76 mg/L and the LOEC was 1.6 mg/L, based on impairment of the intrinsic rate of increase at the four highest test concentrations.

A 28-day study investigating chronic effects of florfenicol-spiked water on emergence and development rate of the sediment-dwelling midge (*Chironomus riparius*) was conducted under GLP and according to OECD Guideline 219 (Sediment-Water Chrionomid Toxicity Test Using Spiked Water) by Bradley (2009). The water was spiked at nominal concentrations of 0.78, 1.6, 3.1, 6.3, 13, and 25 mg/L. No effects were observed; therefore, the 28-day NOEC was 25 mg a.i./L, the highest concentration tested. In this study, sampling of the overlying water, pore water, and sediment indicated that the spiked florfenicol remained largely in the water column with limited partitioning to sediment.

Results of the chronic toxicity tests with invertebrates are summarized in Table 11.

Table 11. Chronic toxicity of florfenicol to invertebrates

Species, Reference, and Toxicity Endpoint	Toxicity Value, mg/L	Guideline
Daphnia magna (Gallagher et al., 2008b) Survival, NOEC Reproduction, NOEC Growth (length), NOEC Growth (weight), NOEC	 3.0 1.5 3.0 3.0	OECD 211
Brachionus calyciflorus (Sayers, 2009b) Reproduction (intrinsic rate of increase), NOEC	0.76	Snell and Moffat (1992); ASTM E 1440-91
Chironomus riparius (Bradley, 2009) Percent emergence, NOEC Development rate, NOEC	 25 25	OECD 219

The EC_{50} from the acute toxicity test with *Daphnia magna* is used in the Tier A risk characterization. For the Tier B risk characterization, the lowest NOEC from the *D. magna* chronic toxicity test as well as the NOEC values from the toxicity tests with the rotifer and the midge were used.

6.4 FISH

6.4.1 ACUTE TOXICITY

The acute toxicity of florfenicol and its major metabolites was determined for two freshwater species, rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*), in GLP studies conducted under static conditions following FDA Guidance 4.11 (Freshwater Fish

Acute Toxicity) (LeLievre, 1991e-l). The results (Table 12) indicate that florfenicol is not toxic to either freshwater fish species with LC_{50} values > 780 and > 830 mg/L, respectively. While the metabolites were not tested at the same concentrations, no mortalities were observed in 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. The data support the concept that neither florfenicol nor its degradation products are likely to cause toxic effects to fish species which may be exposed at estimated environmental concentrations (i.e., PECs).

Table 12. Acute toxicity of florfenicol and major metabolites to freshwater fish

		Principal 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
Reference	LeLievre (1991e)	LeLievre (1991g)	LeLievre (1991h)	LeLievre (1991i)
Lepomis macrochirus				
LC_{50} (mg/L)	>830	>20	>15	>25
NOEC (mg/L)	830	20	15	25
Reference	LeLievre (1991f)	LeLievre (1991j)	LeLievre (1991k)	LeLievre (1991l)

6.4.2 CHRONIC TOXICITY

The effects of florfenicol on time to hatch, hatching success, survival, and growth in the fathead minnow (*Pimephales promelas*) during early life-stage development were evaluated. The test was conducted over a 33-day period (5-day embryo hatching and 28-day post-hatch juvenile growth period) under GLP and according to OECD 210 (Fish Early Life-Stage Toxicity Test) (Gallagher et al., 2008c). The fish were exposed under flow-through conditions to five concentrations and a control (average mean measured concentrations of 0, 0.68, 1.4, 2.8, 5.5, and 11 mg/L). No significant effects on time to hatch, hatching success, and larval survival were observed. No significant reductions in wet weight or dry weight were seen. However, total length was reduced at the highest test concentration. Thus, the NOEC, based on the most sensitive parameter, was 5.5 mg/L. The results of this study are presented in Table 13.

Table 13. Chronic toxicity of florfenicol to *Pimephales promelas*

Toxicity Endpoint	Toxicity Value, mg/L	Guideline
Survival, NOEC	11	OECD 210
Hatching success, NOEC	11	
Time to hatch, NOEC	11	
Growth (total length)	5.5	
Growth (dry weight)	11	
Growth (wet weight)	11	

Reference: Gallagher et al. (2008c)

The acute LC_{50} values for the rainbow trout and bluegill are used in the Tier A risk characterization, while the lowest NOEC from the chronic test with the fathead minnow is used in the Tier B risk characterization.

6.5 TERRESTRIAL PLANTS

The results of toxicity tests with terrestrial plants are presented in Table 14. These studies were conducted according to OECD 208 (Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test) and under GLP. In all of the phytotoxicity studies there was no effect on seedling emergence. The LC_{50} values, based on seedling emergence, were reported as being >100 mg/kg for cress, mustard, and wheat (Farrelly, 1999a), >10 mg/kg for cabbage and mustard (Gray, 2007), and >1 mg/kg in a second test with cress (Bealing et al., 1999). However, florfenicol did have an impact on the growth of the plants, as indicated by effects upon wet weight. From the weights of the emerged seedlings, EC_{50} values were estimated as 0.5, 1.7, and 6.7 mg/kg for cress, mustard, and wheat, respectively, in the study by Farrelly (1999a), as >1 mg/kg for cress in the study by Bealing et al. (1999), and as 0.705 and 0.859 mg/kg for mustard and cabbage, respectively, in the study by Gray (2007). The EC_{50} values for terrestrial plants are used in the Tier A risk assessment.

Table 14 also includes the NOEC values reported from the various terrestrial plant studies. The lowest NOEC value for a particular species, based on the most sensitive response variable, is used in the Tier B risk assessment.

Additional information, from a study by Boxall et al. (2006), indicates that florfenicol at 1 ppm had no effect on the growth of lettuce or carrots, based on wet weight, over a period in excess of 100 days.

Table 14. Toxicity of florfenicol to terrestrial plant species

Species, Reference, and Toxicity Endpoint	Toxicity Value (mg/kg dry weight)	OECD Guideline
Cress (Farrelly, 1999a)		
LC_{50} emergence	>100	208
EC_{50} weight	0.5	
NOEC	NR[1]	
Mustard (Farrelly, 1999a)		
LC_{50} emergence	>100	208
EC_{50} weight	1.7	
NOEC	NR	
Wheat (Farrelly, 1999a)		
LC_{50} emergence	>100	208
EC_{50} weight	6.7	
NOEC	NR	
Cress (Bealing et al., 1999)[2]		
LC_{50} emergence	>1	208
EC_{50} weight	>1	
NOEC (development and growth)	0.16	
Cabbage (Gray, 2007)		
LC_{50} emergence	>10	208
LC_{50} survival	>10	
EC_{50} height	16.7	
EC_{50} weight	0.859	
NOEC height	1.11	
NOEC weight	0.123	
Mustard (Gray, 2007)		
LC_{50} emergence	>10	208
LC_{50} survival	1.41	
EC_{50} height	1.75	
EC_{50} weight	0.705	
NOEC height	0.37	
NOEC weight	0.123	

[1] NR = not reported

[2] Calculated based on raw data in report

6.6 SOIL MICROBES AND INVERTEBRATES

Florfenicol was found to have a transient effect on the microbial transformation of nitrogen when added to soils at concentrations of 0.1, 0.5, and 2.5 mg/kg (Carter, 2002) in the Soil Microorganisms: Nitrogen Transformation Test (OECD 216). While the nitrate concentrations were similar to those in controls throughout the study, the ammonium levels rose significantly in soils treated at 0.5 and 2.5 mg/kg, before the rates returned to the control level by Day 28 (see

Table 15). Deviations in nitrogen transformation were less than 25% for both ammonium and nitrate at the end of the 28-day study period. In the Soil Microorganisms: CarbonTransformation Test (OECD 217), carbon transformation was reduced at all florfenicol concentrations but by Day 28 had recovered in soils treated at 0.1 and 0.5 mg/kg, with recovery of activity in the soils treated at 2.5 mg/kg by Day 56 (Carter, 2002). These values will be compared to predicted soil concentrations of florfenicol in the terrestrial risk characterization. From the data on reductions in concentrations of florfenicol in soils in terrestrial organism toxicity studies (Farrelly, 1991a; Farrelly, 1991b), it is apparent that rates of reduction in concentrations are inversely proportional to the initial concentrations of florfenicol present.

Table 15. Toxicity of florfenicol to soil microbes and invertebrates

Study	Toxicity Endpoint (mg/kg dry weight)	Guideline
Nitrogen[a] Transformation study (28 days)	0.1, 0.5, 2.5	OECD 216
Carbon[a] Transformation study (28 days)	0.1, 0.5	OECD 217
Earthworm[b] reproduction (NOEC)	1.56	OECD 222

[a] Carter (2002); [b] Porch et al. (2009)

The results of the nutrient transformation study might be expected based on the data available on the rates of degradation of florfenicol at different concentrations. The recovery in microbial activity indicates that, under the conditions of the study, the microbial populations responsible for transformation were partially inhibited, not killed, and were able to resume processing when the florfenicol was degraded. As shown in Table 7, the metabolites were found to be 5- to >1,000-fold less toxic than the parent florfenicol (Fackler, 1991e-h). This indicates that parent florfenicol is the chemical of concern in assessing the risks to microbial species. Recent work has shown that monochloroflorfenicol (SCH 49435) has essentially the same level of inhibition (MICs) (Schuster, 2004) as the parent florfenicol for three microbial species (Table 7, Column 2 and 6). In this assessment, the total residues of florfenicol in the water column are conservatively treated as parent compound (i.e., as having the same rates of inhibition as the parent compound) for the purposes of assessing risk. The potential inhibitory action of the monochloro metabolite is similar to the parent moiety and does not pose any additional risk (Table 7).

Table 15 also includes the results of toxicity testing with the earthworm, *Eisenia foetida*. This test was conducted under GLP and according to OECD Guideline 222 (Earthworm Reproduction Test) and examined the effects of florfenicol during an 8-week exposure in artificial soil (Porch et al., 2009). A negative control and six concentrations (1.56, 3.13, 6.25, 12.5, 25.0, and 50.0 mg a.i./kg dry soil, nominal) were tested. Analyses of the lowest, middle, and high test concentrations at the beginning and end of the test confirmed the dosing. The LC_{50} for adult mortality was > 50 mg/kg, while the EC_{50} for reproduction was 8.61 mg/kg. The LOEC and NOEC for production of juveniles were 3.13 mg/kg and 1.56 mg/kg, respectively (Porch et al., 2009).

6.7 TIER A ACUTE EFFECTS AND PNEC CALCULATIONS

The Tier A risk characterization considers the effects determined in short-term exposures, typically regarded as acute effects, upon the aquatic and terrestrial receptors; chronic effects

are addressed in Tier B. The data on acute effects are used with standard assessment factors from the VICH/CVM guidance to determine the Predicted No Effect Concentrations (PNECs).

6.7.1 TIER A AQUATIC PNECs

The Tier A PNECs are presented in Table 16 for key fish, invertebrate, and aquatic plant species. The PNEC is the ratio of the toxicity value divided by the assessment factor. Toxicity values range over three orders of magnitude, with fish (*O. mykiss* and *L. macrochirus*) having the highest reported acute values of >780 and >830 mg/L, respectively, and *A. flos-aquae* having the lowest acute value of 0.23 mg/L. This latter value indicates that the freshwater cyanobacterium, *A. flos-aquae*, is the most sensitive freshwater species for which Tier A data are available. This EC_{50} value is one order of magnitude lower than that for the freshwater green alga, *P. kirchneriella*, two orders of magnitude lower than that for the freshwater diatom, *N. pelliculosa*, and three orders below the values for *Daphnia* and fish species. The sensitivity of this cyanobacterium is not surprising given that florfenicol is designed to be effective against bacteria.

The PNECs for the key freshwater taxa as required under VICH/CVM Phase II Tier A were determined based on toxicity data and assessment factors (AFs). AFs are used to adjust for uncertainty in the data. The VICH/CVM approach includes a factor of 100 for algae and 1,000 for invertebrates and fish as an initial screen when using acute toxicity data to evaluate chronic exposures. This includes a factor of 10x to account for extrapolation from acute to chronic toxicity. Where the exposures are considered acute, it is relevant to compare them to effects based on acute toxicity data. In this case the 10x factor is removed. Table 16 thus presents the PNECs for use in comparing acute effects to acute exposures. Absent guidance, the same AF was used for the aquatic plant, *Lemna gibba,* as for algae. The initial PNECs (the ratio of the toxicity values divided by AFs) for a range of species representing several phyla are approximately 8 mg/L for fish, 3 mg/L for invertebrates, and down to 0.023 mg/L for cyanobacteria.

Table 16. Tier A PNECs for aquatic organisms

Species and Reference	EC_{50} or LC_{50} (mg/L)	Assessment Factor (AF)[1]	PNEC (mg/L)
Oncorhynchus mykiss (LeLievre, 1991e)	>780	100	7.8
Lepomis macrochirus (LeLievre, 1991f)	>830	100	8.3
Daphnia magna (LeLievre, 1991a)	>330	100	3.3
Navicula pelliculosa[2] (Jenkins, 2005)	61	10	6.1
Pseudokirchneriella subcapitata[2] (Hoberg, 1991a)	1	10	0.1
Lemna gibba[2] (Softcheck, 2009)	0.76	10	0.076
Anabaena flos-aquae[2] (Gallagher et al., 2008a)	0.23	10	0.023

[1] These assessment factors do not include extrapolation for acute to chronic effects and are not used for evaluation of chronic exposures.

[2] For algae, cyanobacteria, and duckweed, the EC_{50} for the most sensitive parameter was selected. In all cases, this was biomass/yield.

6.7.2 *TIER A TERRESTRIAL PNECs*

The Tier A PNECs are presented in Table 17 for terrestrial invertebrates and plants. The assessment factor used in each instance is according to the VICH/CVM guidance for Tier A assessment. The terrestrial plant studies examined both seedling emergence and growth. The latter was the more sensitive endpoint, so the PNECs are derived based on the growth data (wet weight). The PNECs (the ratio of the toxicity values divided by the AFs) for terrestrial organisms range from 0.005 mg/kg for cress to 0.156 mg/kg for earthworms.

Regarding soil microorganisms, nitrogen transformation in soil is transiently affected by florfenicol when added in concentrations of 0.1, 0.5, and 2.5 mg/kg (Carter, 2002). While the nitrate concentrations were similar to those in controls throughout the study, the ammonium levels rose significantly in soils treated at 0.5 and 2.5 mg/kg before the rates returned to the control level by Day 28. The deviation in measured activity in soils treated with florfenicol at all concentrations was <25% by Day 28 compared to the control. Carbon transformation was reduced at all florfenicol concentrations tested but by Day 28, recovery had occurred in soils treated at 0.1 and 0.5 mg/kg, with recovery by Day 56 at 2.5 mg/kg.

Table 17. Tier A PNECs for terrestrial organisms

Species and Reference	Toxicity Value (mg/kg)	Assessment Factor	PNEC (mg/kg)
Earthworm (Porch et al., 2009) NOEC reproduction	1.56	10	0.156
Cress (Farrelly, 1999a) EC_{50} weight	0.5	100	0.005
Mustard (Farrelly, 1999a) EC_{50} weight	1.7	100	0.017
Wheat (Farrelly, 1999a) EC_{50} weight	6.7	100	0.067
Cress (Bealing et al., 1999) EC_{50} weight	>1	100	>0.01
Cabbage (Gray, 2007) EC_{50} weight	0.859	100	0.009
Mustard (Gray, 2007) EC_{50} weight	0.705	100	0.007

For the terrestrial plant studies, the most sensitive toxicity result for a given species is used in the risk characterization. Thus, for cress, the PNEC of 0.005 mg/kg derived from the study by Farrelly (1999a) is used rather than the value resulting from the study by Bealing et al. (1999). For mustard, the PNEC of 0.007 derived from the study by Gray (2007) is used rather than the value resulting from the study by Farrelly (1999a).

6.8 TIER B CHRONIC EFFECTS AND PNEC CALCULATIONS

The Tier B risk characterization considers the effects determined in long-term exposures, typically regarded as chronic effects, upon the aquatic and terrestrial receptors. The data on chronic effects are used with standard assessment factors from the VICH/CVM guidance to determine the PNECs.

6.8.1 TIER B AQUATIC PNECs

Aquatic effects data at Tier B are available for three trophic levels: aquatic plants, invertebrates, and fish. The algal and cyanobacterial growth inhibition studies that were conducted (Hoberg, 1991a; Softcheck, 2009; Jenkins, 2005; Gallagher et al., 2008a) can be used to assess both acute and chronic effects, although different test endpoints and assessment factors are used in Tier B (chronic effects) as compared to Tier A (acute effects). For the invertebrates, data from a *Daphnia* life-cycle study (Gallagher et al., 2008b), a rotifer reproduction study (Sayers, 2009b), and a 28-day benthic midge study (Bradley, 2009) are available. For fish, an early life-stage study (Gallagher et al., 2008c) provides data for Tier B assessment. Table 18 presents the toxicity values and assessment factors used at Tier B, per the VICH/CVM guidance, along with the resulting Tier B PNEC values. Where more than one toxicity value was available, the lowest value (indicating the greatest toxicity) was selected.

Table 18. Tier B PNECs for aquatic organisms

Species and Reference	Toxicity Endpoint[1]	Toxicity Value (mg/L)	Assessment Factor	PNEC (mg/L)
Pseudokirchneriella subcapitata (Hoberg, 1991a)	NOEC, 96-h	0.75	10	0.075
Lemna gibba (Softcheck, 2009)	NOEC, 7-d	0.39	10	0.039
Navicula pelliculosa (Jenkins, 2005)	EC_{10}, 72-h	18.7	10	1.87
Anabaena flos-aquae (Gallagher et al., 2008a)	NOEC, 96-h	0.11	10	0.011
Daphnia magna (Gallagher et al., 2008b)	NOEC, 21-d	1.5	10	0.15
Brachionus calyciflorus (Sayers, 2009b)	NOEC, 2-d	0.76	10	0.076
Chironomus riparius (Bradley, 2009)	NOEC, 28-d	25	10	2.5
Pimephales promelas (Gallagher et al., 2008c)	NOEC, early life stage	5.5	10	0.55

[1] In each case, the most sensitive response parameter (lowest NOEC) was selected,

6.8.2 TIER B TERRESTRIAL PNECs

The Tier B PNECs for terrestrial organisms are presented in Table 19. According to the VICH/CVM guidelines, terrestrial effects studies at Tier B include nitrogen transformation studies extended to 100 days and terrestrial plant growth tests. Available data on the toxicity of florfenicol that meet these requirements are presented below. The study on cress by Bealing et al. (1999) found that the most sensitive effect measured was on the longest leaf of the primary and secondary leaf pairs, and the NOEC based on this effect was 0.16 mg/kg. The study by Gray (2007) provided NOEC values for cabbage and mustard based on weight, which was the most sensitive endpoint.

Table 19. Tier B PNECs for terrestrial organisms

Species and Reference	Toxicity Endpoint	Effect Level (mg/kg)	Assessment Factor	PNEC (mg/kg)
Cress (Bealing et al., 1999)	NOEC for development and growth[1]	0.16	10	0.016
Cabbage (Gray, 2007)	NOEC based on weight	0.123	10	0.0123
Mustard (Gray, 2007)	NOEC based on weight	0.123	10	0.0123

6.9 SUMMARY OF EFFECTS ASSESSMENT

Data are available from acute and chronic toxicity tests conducted following standard guidelines and under GLP on a variety of aquatic and terrestrial receptors, including bacteria, cyanobacteria, algae, aquatic vascular plants, aquatic invertebrates, fish, terrestrial plants, soil microbes, and earthworms. Bacteria and cyanobacteria are the most sensitive organisms, which is not unexpected given the antibacterial activity of florfenicol. Aquatic plants (algae and duckweed) are an additional group of organisms that are relatively sensitive to florfenicol. The available data indicate that florfenicol was algistatic, and not algicidal, meaning that populations of algae were inhibited but not killed. Especially for unicellular organisms (algae, bacteria, cyanobacteria), populations have the ability to re-grow rapidly if 100% of the organisms are not killed. PNEC values presented for cyanobacteria, algae, and duckweed are based on inhibition of growth, not mortality. Thus it can be expected that when the stressor is removed, populations that were inhibited from growth in the presence of the stressor are able to recover.

7. EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION

Florfenicol is released into the environment when used as an antibiotic administered in feed. It is this use pattern that determines the amount released to the environment. Factors such as the magnitude, timing, frequency, and duration of administration will be determined by the use pattern. These factors, coupled with metabolism, biomass treated, and characteristics of the aquaculture scenario (ponds or flow-through systems) will determine the predicted environmental concentrations (PECs). These and other factors are discussed in this section.

Florfenicol will be administered to fish in the form of a premix applied to feed. The product can be incorporated in unmedicated feed prior to pelleting or by dry coating the premix onto the feed and sealing it by over-oiling. The exposure assessment in this EA is based on the administration of medicated feed to fish at a rate targeted to deliver a dose of 15 mg florfenicol per kg of fish per day for 10 days. This dosing rate is at the upper end of the range expected to be approved for the three disease indications that are being evaluated in this EA (i.e., enteric septicemia, columnaris, and streptococcal septicemia). Fish are known to establish feeding hierarchies, and those suffering from bacterial diseases are known to exhibit reduced appetite. To increase the opportunity for each fish to ingest sufficient medicated feed to maintain tissue concentrations greater than the MIC for a sufficient period, a 10-day treatment period has been selected. This treatment period, established in numerous efficacy studies (e.g., Inglis et al., 1991), should ensure that the potential for fish to be re-infected from other fish is reduced, because consumption of the nominal dose has been shown to be effective in pathogen treatment.

The pharmacokinetics of florfenicol in target species is one factor determining the route, timing, and magnitude of residues entering the environment. The principal route of release of these residues is as excreted material, including parent florfenicol, metabolites, and conjugates. Although metabolism of florfenicol occurs in fish, it will be assumed initially that all material is excreted as parent florfenicol. This assumption is conservative, because the metabolites are (generally) less toxic to ecological receptors. Refined risk scenarios incorporate metabolism.

The other pathway for release of florfenicol to the environment is through uneaten feed. However, available data for trout indicate 97.3% feed consumption at 8°C and 100% feed consumption at 15°C (Roy, 2002a; Roy, 2000b). Other species of fish also consume a very high proportion of feed. Therefore, it will be assumed that uneaten feed presents an insignificant pathway of exposure and that all of the dosed florfenicol enters the environment through excreta.

A large body of evidence exists to show that florfenicol is readily absorbed, distributed, metabolized, and excreted by fresh and saltwater salmonids. Using various routes of administration (intravenous, gavage, and dietary exposure) and a range of study designs, the results demonstrate a consistent pattern of pharmacokinetics in these fish. The residues observed included the parent florfenicol and three metabolites (florfenicol amine, the alcohol, and the oxamic acid) and conjugates (e.g., glucuronides) of parent and metabolites. The results in fish are similar to results of studies with other vertebrate species (cattle, rats, humans) and can be assumed to be directly relevant to other fish species such as catfish and tilapia.

In rainbow trout, an elimination half-life of 8.8 hours was determined following intravenous injection at 10°C (Rodger, 2002; Pinault, 1997a). 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 (Rodger, 2002; Pinault, 1997a). The residue levels in the plasma of trout fed medicated feed treated with florfenicol at 10°C, when sampled after the final

dose of a 10-day treatment, were found to be reduced more than ten-fold relative to the peak recorded at 12 hours of treatment (Pinault, 1997b). The residues in the muscle and skin taken from the same fish were reduced more than ten-fold from the 12-hour peak value when sampled 8 days after the last dosing (Pinault, 1997b).

Two more recent GLP-compliant residue studies were conducted with rainbow trout, one at 8°C and one at 15°C (Roy, 2002a; Roy 2002b). In both studies, feed medicated with Aquaflor® premix was fed to trout for 10 consecutive days at a target dose rate of 10 mg florfenicol/kg body weight per day. The mean achieved daily dose rates were 9.2 and 9.8 mg/kg at 8°C and 15°C, respectively. Residues were measured in muscle and skin using a validated analytical method. Residues, measured as florfenicol amine, were higher and slower to deplete in the fish exposed at 8°C. More rapid and more uniform depletion occurred at 15°C. Based on the data presented in Table 20 and in Figure 3, it is evident that excretion is rapid, with mean residues at one day after the last treatment of 3.94 mg/kg (42.8% of initial dose) at 8°C and 1.48 mg/kg (15.1% of initial dose) at 15°C. At four days post-treatment, mean residues are 26.4% and 5% of the initial dose at 8°C and 15°C, respectively. By 7 days after treatment, only a very small amount of florfenicol is retained in the muscle and skin of trout (mean of 0.43 mg/kg at 8°C and 0.29 mg/kg at 15°C, or 0.4% and 0.3% of the total dose, respectively). These data support the assumption that florfenicol and its residues (>99% of the total dose) can be assumed to enter the receiving water environment in the excreta of the fish during a fairly small window of time (10 days of treatment plus 7 days post-treatment).

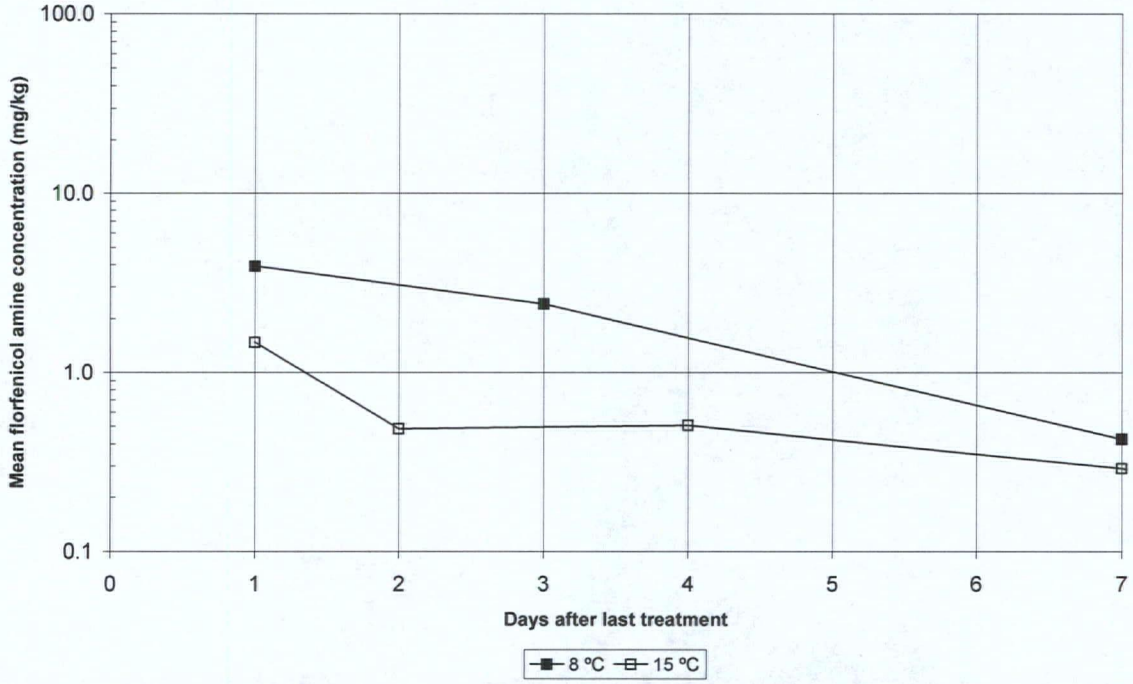
Table 20. Florfenicol-related residues in rainbow trout kept at 8 and 15°C

Time after Last Treatment[2]	Florfenicol Amine Residue (mg/kg), Corrected for Recovery[1]			
	8°C (n = 18–21) (Roy, 2002a)		15°C (n = 12–15) (Roy, 2002b)	
	Mean ± SD	Highest Individual Residue	Mean ± SD	Highest Individual Residue
1 day	3.94 ± 6.24	18.90	1.48 ± 4.13	15.10
2 days	—	—	0.49 ± 1.26	4.54
3 days	2.43 ± 2.94	9.25	—	—
4 days	—	—	0.51 ± 0.52	1.35
7 days	0.43 ± 0.56	1.96	0.29 ± 0.22	0.62
10 days	0.21 ± 0.27	0.94	0.10 ± 0.15	0.50
14 days	0.33 ± 0.18	0.63	0.10 ± 0.09	0.26
21 days	0.15 ± 0.16	0.43	0.09 ± 0.08	0.20
28 days	0.10 ± 0.11	0.35	0.07 ± 0.06	0.19
35 days	0.12 ± 0.11	0.31	—	—

[1] Residue in muscle with skin

[2] Fish were administered Aquaflor®-medicated diet daily for 10 consecutive days at a nominal dose rate of 10 mg florfenicol/kg bodyweight

Figure 3: Mean florfenicol-related residues, measured as florfenicol amine, in muscle with skin from rainbow trout kept at 8 and 15°C and administered Aquaflor®-medicated diet daily for 10 consecutive days at a nominal dose rate of 10 mg florfenicol/kg body weight



Note: When calculating means, results that were non-quantifiable (<0.102 and <0.117 mg/kg at 8°C and 15°C, respectively) or non-detectable (<0.0278 mg/kg at both temperatures) were assumed to be half of these respective values. Figure taken from Parker (2002).

Residue depletion studies conducted with two species of tilapia (Meinertz et al., 2006) and catfish (Wrzesinski et al., 2006) showed rapid depuration of florfenicol residues. Residues in fillets were below the 1.0-mg/kg tolerance by two days post-treatment and four days post-treatment for tilapia and catfish, respectively.

Based on the rapid elimination of florfenicol from fish (half-life of 12.2 h for Atlantic salmon, Martinsen et al., 1993; and 8.8 h for rainbow trout, Pinault, 1997a) and rapid degradation in water and sediment (mean half-life 13.6 days, Gledhill, 2005), sequential, episodic treatment with Aquaflor® would not lead to accumulation in the environment. Each treatment would be an independent event, as discussed further in subsequent sections of this assessment.

All residues of florfenicol and principal metabolites enter water in the urine and feces of treated fish, but due to the relative solubility and low K_{oc} of the parent and metabolites, 100% is assumed to enter the water column for the aquatic risk characterization. The actual dispersion, partitioning, settling, and re-suspension of florfenicol residues in the vicinity of each aquaculture facility will be determined by local hydrographic conditions and waste minimization procedures at the facility, as influenced by the environmental fate properties of florfenicol. To be conservative, for the purposes of calculating the initial PEC_{water}, all residues are assumed to be parent florfenicol and to be contained in water; partitioning of florfenicol to solid phases is

addressed in refinement of the PEC. The conservative assumption used in calculating the PEC_{soil} is that all residues partition to solid phases.

7.1 USE PATTERN: PONDS

The first use pattern considered in this EA is the use of florfenicol in ponds. The primary example of pond aquaculture is the culture of channel catfish, which represent more than half of the ponds in production in the United States (U.S. EPA, 2004). In addition to being representative of the majority of commercial pond aquaculture, catfish ponds are the largest relative to other species and would represent the worst case conditions with respect to disease treatment. Information on other species, though less extensive than the information available for catfish, is also presented to demonstrate that the catfish example is sufficiently conservative to address the use of florfenicol for other freshwater-reared species cultured in ponds.

7.1.1 PRODUCTION AND DISEASE TREATMENT IN PONDS

Aquaflor® is currently approved for use in ponds to treat enteric septicemia in channel catfish (*Ictalurus punctatus*) at a dose of 10 mg/kg body weight/day for 10 days. The frequency and timing of applications are limited both by regulation and biology. Under the Veterinary Feeds Directive, disease in the population must be confirmed by an aquaculture veterinarian prior to the prescribed use of florfenicol; prophylactic treatments are not allowed. This results in more controlled and minimized use. In addition, the use of florfenicol in fish is limited by the biological characteristics of the target pathogen (i.e., optimal conditions for disease outbreak).

Channel catfish are raised commercially in the southeastern portion of the United States, mainly in Mississippi, Alabama, Arkansas, and Louisiana. These four states represented 53.5% of all US catfish operations and 91.3% of the water surface acres used for catfish production in 2010 (USDA, 2010). Approximately 95% of domestic catfish production occurs in these states, with California and Missouri accounting for the remainder (Hargreaves et al., 2002). The following discussion is based upon the large amount of information available on the culture of channel catfish; however, it is considered applicable to pond culture in general. Depending on location, aquaculture ponds are typically earthen ponds of two general types: levee ponds or watershed ponds. In some areas, the two types may be combined due to local topography and hydrology. Levee ponds are built by removing soil from the area that will be the pond bottom and forming it into a levee around the pond perimeter. They are usually filled and maintained with groundwater or nearby surface water pumped into the pond. Watershed ponds are built by damming a small watercourse, and thus, they rely on inflow from the upstream drainage basin, stormwater runoff, and rainfall to maintain their water volume. Levee ponds are typically about 3.2–6 hectares (ha) in area and 1–1.5 m in average depth. Watershed ponds typically range from 4 to 5 ha in area and 1 –2 m in average depth. To reduce water loss via overflow and the release of effluent from the ponds, the ponds are usually managed by maintaining a water level below the overflow structure. Water levels maintained 20 cm below the overflow level are recommended so that rainfall would not normally cause the ponds to overflow. Generally, ponds are built in areas where the soil is dominated by clay; therefore, leakage is minimal. All of these factors serve to limit the release of pond water and any florfenicol-related residues into the environment. Because 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. Levee ponds are the most commonly used method of production for channel catfish, accounting

for more than 90% of all U.S. commercial catfish ponds by acreage, while the remaining 10% are watershed ponds (U.S. EPA, 2004).

It has been estimated that ESC and columnaris each caused the loss of about one-fifth of the catfish fry/fingerlings in 2009 (USDA, 2010). Susceptibility of fish to diseases may depend on production methods and previous exposure of the fish to the pathogen. The culturing of many fish species starts in brood-fish ponds 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 as well. Also, since the brood fish are older, they most likely have been exposed previously to the pathogen and are therefore possibly less susceptible to subsequent infection under the conditions in the brood ponds. For catfish, brood ponds only constitute about 8.8% of all operations of the catfish industry (USDA, 2010).

Catfish fingerlings are raised from eggs during about a 12-month period from the spring of one year to the spring of the next year. Being both naive to disease and under high-density production conditions, fingerlings may be the life stage most susceptible to infection. This is the case for catfish with ESC but may not be the case for all infections. Fingerling (nursery) ponds are drained each year to facilitate harvesting of the fish and this usually occurs in the spring before the potential for infection. The harvested fingerlings are then typically purchased for the stocking of production ponds. Catfish fingerling ponds take up approximately 13% of the total area used for catfish production, but because they are drained annually, they represent 30% of the annual discharge (Hargreaves et al., 2002).

In production ponds, the goal is to raise the fish to market weight in the shortest period of time. This usually takes about 6–12 months of additional growth after the fingerling stage, but this time is variable depending on weather conditions, water quality, fish species, growth rate characteristics of individual fish within the fish population, and the preferred market weight of the fish. Particularly for catfish, although the fingerlings can be grown to market size in a single cohort, most operations use the multiple cohort method, wherein production ponds contain fish of various ages and therefore sizes. Fish are harvested by seining on a "continual" basis, 1–3 times per year. Fingerlings are added each year, usually in the spring, to make up for the fish harvested the previous year, plus any losses. Larger fish in production ponds could also become infected, but the likelihood may be less depending on the disease if immunity was acquired as a result of previous exposure

Most ponds are managed so that waste inputs (fish waste products and organic matter produced by algae) do not exceed the capacity of the pond's 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 florfenicol, 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, catfish production ponds are drained every 6.5 years (Hargreaves et al., 2002), when the pond bottom and/or levees require renovation. At those times, the pond bottom is leveled and the soil is used to rebuild eroded levees.

To be conservative, the worst-case release scenario will involve draining a pond 12 days (the minimum pre-harvest interval) after the last of 10 daily applications. This allows a 12-day observation period to detect any recurrence of infection in the population. Harvest requires repeated seining over a 1- to 3-month harvest period (Hargreaves et al., 2002; Tucker, 1996).

This scenario produces the maximum amount of water potentially released after the minimum timeframe following application.

The target dose to the fish is 15 mg/kg/day for 10 consecutive days, for a total dose of 150 mg/kg of fish. The concentration of florfenicol in the feed is adjusted based on the feeding rate to the fish. The total amount dosed to a pond is determined by the biomass of fish in the pond. Feeding rate is used only to determine the florfenicol concentration in the feed. The biomass depends on the number and size of the fish in the pond and is usually estimated from the stocking density, food consumption in the pond over a period of time, and the temperature of the water. Using the dose and the biomass, the amount of florfenicol added to the system can be determined.

Catfish are maintained at high stocking densities relative to other pond-cultured species. Engle (undated) recommends the use of catfish fry stocking rates to yield 1,390 to 3,687 pounds per acre without thinning of the fingerlings, or 1,240 to 4,652 pounds per acre with thinning (i.e., thinning when the fry reach a size of approximately 2 inches and then re-stocking). Data in this publication show that larger fish result from lower stocking densities, and vice versa. Tucker (1996) presents stocking densities for fry as ranging from 250,000 to 500,000 per hectare. By late fall, these fingerlings weigh between 20 and 50 g each. Assuming the lower stocking density results in higher fish weight, and vice versa, the resulting densities are 11,250 and 9,000 pounds per acre. (A factor of 0.8924 is used to convert kg/ha to lb/acre based on 0.4047 ha/acre and 2.205 lb/kg). Tucker (1996) also reports that, in production ponds, stocking densities range from 10,000 to 25,000 or more fish per hectare. With a harvest size of 0.4 to 0.8 kg per fish, and larger size assumed to result from smaller stocking density, production ponds would contain 7,200 to 9,000 pounds per acre, according to data from Tucker (1996). SRAC (1998) presents data on ponds stocked at 15,000 fish per hectare, resulting in production of 4,990 to 6,683 kg/ha (4,553 to 5,964 pounds per acre). Masser et al. (1997) reports that experienced producers can produce up to 6,000 pounds of channel catfish per acre. Based on this information, the highest stocking density for catfish for either fingerling ponds or production ponds can be assumed to be approximately 10,000 pounds per acre.

The concentrations of florfenicol-related residues in the receiving environment (i.e., outside of a fish pond) depend on the quantities administered, the proportion consumed, the proportion excreted as parent compound and as metabolites, and the partitioning within the pond environment, as well as the volume, frequency, and timing of water releases to the receiving environment. As stated previously, despite evidence of metabolism in the treated fish, for the purposes of the initial assessment, a worst-case scenario will be presented by considering that all florfenicol-related materials reach the environment as unmetabolized florfenicol (parent compound). Nearly all pond fish feed is formulated as floating, extruded pellets. This floating feed has high water stability and does not sink into the sediments. Very little, if any, feed is unconsumed. Therefore, for the purposes of this assessment, it is assumed that 100% of the feed is consumed by the fish.

Florfenicol (and its metabolites) will enter the receiving environment in excreta; entry through uneaten feed in inconsequential. Leaching from feces and mixing of the aqueous phase of excreta serve as the sources of florfenicol in the water column. From the water column of fish ponds, florfenicol enters the receiving aquatic environment when the pond is drained, or via overflow resulting primarily from rainfall. Fingerling ponds are usually drained annually and a typical 10-acre pond with an average-size drain (12 inches) would take approximately 3–4 days to drain into an empty ditch. Catfish production ponds are drained only every few years (≥ 6 years on average) 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. Solids are not removed regularly from ponds for land application, and thus, it is not necessary to consider the PEC_{soil}.

7.1.2 PRODUCTION OF OTHER FISH SPECIES

7.1.2.1 Hybrid striped bass

Aquaculture of hybrid striped bass has increased over the years, with production growing from about 400,000 pounds in 1987 to about 10.6 million pounds in 2001 (Ludwig, 2004). Hybrid striped bass are a cross between striped bass (*Morone saxatilis*) and white bass (*Morone chrysops*). When the female parent is a striped bass, the offspring are called palmetto bass; when the female parent is a white bass, the offspring are called sunshine bass. Fingerling production usually takes place in ponds in the spring and early summer, with ideal ponds 3 to 5 acres in size and 1 to 2 m deep (Ludwig, 2004; Ohs et al., 2008). In the first phase of production, newly hatched fry are stocked into ponds at densities between 100,000 and 200,000 fish per acre (Ludwig, 2004; Ohs et al., 2008). Subsequent grow-out to market size can occur in two additional phases or following a "direct stock" method. In the former method, 1- to 5-g fingerlings are stocked at 8,000 to 18,000 fish per acre, grown until they reach 100 to 225 g, and then re-stocked at 3,000 to 4,400 per acre until they reach market size (D'Abramo and Frinsko, 2008; Ohs et al., 2008). The direct-stock method uses larger stocking fish (3 g) at a density of 3,750 to 4,000 per acre (D'Abramo and Frinsko, 2008). Typical production ponds are levee style and are 1 to 10 acres in size and <5 ft deep (Ohs et al., 2008).

From this information, and using the lower density range with the larger size fish, the maximum stocked biomass of hybrid striped bass can be calculated to be 200 pounds per acre for fingerling ponds and 1,667 pounds per acre for production ponds. At attainment of marketable size, the yield in a production pond is 6,500 to 7,000 pounds per acre (D'Abramo and Frinsko, 2008). These estimates are below the value used for catfish of 10,000 pounds per acre. Therefore, the PEC values determined based on catfish are sufficiently conservative for hybrid striped bass production in ponds.

7.1.2.2 Largemouth bass

The largemouth bass (*Micropterus salmoides*) is one of the most popular sport fish in the U.S., and thus, there is an increased demand for remedial stocking in sport-fish ponds (Kentucky State University, undated). After rearing for several weeks in nursery ponds, the fingerlings must be trained to accept prepared food. Tidwell et al. (1998) reported that largemouth bass yields could be as high as 4,598 kg/ha (4,103 pounds per acre) without adverse effects when 122 g fish were stocked at 12,350 fish/ha. It was also reported, however, that this may not be the maximum or optimum rearing density. But even if the yield were doubled it would still be lower than the 10,000 pounds per acre used to calculate PEC values, Kubitza et al. (1997) found that standing crops of 5,000 to 7,300 kg/ha were achieved in ponds without water exchange when emergency aeration was provided. The highest reported stocking density of those discussed above, 7,300 kg/ha, equals approximately 6,500 pounds per acre. These values are below the value used for catfish of 10,000 pounds per acre. Therefore, the PEC values determined based on catfish are sufficiently conservative for largemouth bass production in ponds.

7.1.2.3 Bluegill sunfish

Bluegill sunfish (*Lepomis macrochirus*) or hybrid bluegill (*L. macrochirus* x *L. cyanellus*) are often stocked for recreational purposes. A stocking density of 100,000 fish/acre for 50-mm fingerling production (900 fish/kg) is recommended by Wyatt et al. (2008). This translates to 247 pounds per acre for fingerlings. Borisova et al. (undated) states that the highest stocking density for profitable production of hybrid bluegill is 339 to 1,409 pounds per acre. A maximum of 3,560 pounds per acre was achieved, but only 68% of the fish were considered market size. The overall pond harvest density is actually 300-500 pounds per acre greater if largemouth bass and grass carp are considered since this was a polyculture (Borisorva et al., undated). These values are well below the stocking density used for catfish, and therefore, the PEC values determined based on catfish are sufficiently conservative for bluegill sunfish production in ponds.

7.1.2.4 Carp

The production of carp fingerlings normally occurs in semi-intensive culture in ponds with natural food and supplementary feeding. When stocked at 50,000 to 200,000 fry per hectare, a final weight of 30 to 100 g per fish can be obtained. In the second year, stocking densities as high as 20,000 fish per hectare can be used with supplemental feeding; fish of up to 400 g can be obtained (The Fish Site, 2009). Using this information and the combinations of higher stocking density with lower weight and lower stocking density with higher weight, the maximum stocked biomass of carp can be calculated to be 4,462 to 5,354 lb/acre. For second year fish, the maximum density is 8,000 kg/ha or 7,139 lb/acre. These values are below the value used for catfish of 10,000 pounds per acre. Therefore, the PEC values determined based on catfish are sufficiently conservative for carp production in ponds.

7.1.2.5 Yellow perch

Yellow perch (*Perca flavescens*) is a popular sport fish, especially in the Great Lakes area. Because of low supplies and high demand, interest in aquaculture of this species has increased. Ponds ranging in size from 0.1 to 1 acre and 6 to 8 ft deep are often used (Hart et al., 2006). Yellow perch can be raised from eyed eggs to fingerlings at production levels as high as 1,085 to 1,360 pounds per acre, although 159 to 319 pounds per acre is more typical; fingerlings can be raised to food-size fish at production levels at least as high as 4,229 pounds per acre (Hart et al., 2006). Trials conducted by Wallat et al. (2001) in ponds produced fingerling yields of 1,266 and 1,819 pounds per acre. In trials conducted at ponds in Wisconsin, Malison and Held (2008) reported production ranging from 1,300 to 5,400 kg/ha (1,160 to 4,819 pounds per acre) with an average of 2,141 kg/ha (1,911 pounds per acre). Hinshaw (2006) reports that in more northerly areas of the U.S., pond production of yellow perch may yield as much as 3,000 to 4,500 pounds per acre. Riepe (1997) reported that targeted production yields of yellow perch were 3,000 pounds per acre. The maximum reported production of 4,819 pounds per acre from the above information for yellow perch is below the value used for catfish (10,000 pounds per acre). Therefore, the PEC values determined based on catfish are sufficiently conservative for yellow perch production in ponds.

7.1.2.6 Tilapia

Ponds for growing tilapia are typically shallow (3 to 6 ft) and small (1 to 10 acres) (Rakocy and McGinty, 1989). An inverse relationship of stocking density with size exists, such that 1-g fry stocked in nursery ponds at 20,000 fry/acre will produce 100-g fingerlings in 18 weeks, while 40,000 fry/acre will produce 50-g fingerlings in 12 weeks, and 72,000 fry/acre will produce 27-g fingerlings in 9 weeks. These specifications are all equivalent to approximately 4,400 pounds

per acre. Rakocy and McGinty (1989) also state that to produce 500-g fish from 50-g fingerlings, a period of about 6 months is required, and stocking rates can vary from 4,000 to 20,000 fish per acre. (Assuming 500-g fish, this converts to 4,440 to 22,220 pounds per acre). However, at the highest density, supplemental aeration is required. Chapman (2000) provides a recommended stocking density of 4,000 to 8,000 fish per acre for young (50-g) hybrid tilapia in fed and aerated production ponds; this is equivalent to 440 to 880 pounds per acre. Therefore, at the time of harvest (~500 g) this density could be as high as 8,820 lbs/acre. Boyd (2004) reports that typical production in ponds treated with commercial fertilizer is 1,000 to 5,000 kg/ha, but ponds treated with a combination of feeding and fertilizer can increase production to 5,000 to 6,000 kg/ha in semi-intensive systems, and addition of water exchange or aeration can increase production to 20,000 kg/ha or more. This range of stocking densities is approximately 900 to 18,000 pounds per acre. FAO (2010) cites normal yields of 6 to 8 tonnes per hectare, ranging as high as 10 to 20 tonnes per hectare with ideal conditions and intensive management (high-quality feed, water exchange, continuous aeration, etc.). These values are equal to approximately 5,400 to 18,000 pounds per acre. Thus, at the higher end of the possible range of tilapia production, the maximum fish density exceeds that used to calculate the PEC values based on catfish production in ponds. However, the higher densities cite the use of intensive management procedures such as water exchange and continuous aeration. Rather than implementing these costly measures in ponds, it is more likely that recirculating systems will be used for tilapia culture in the U.S., because control of temperature is also required (tilapia growth diminishes below 68°F, and death occurs below 50°F; Rakocy and McGinty, 1989). In addition, production ponds of such high densities would likely be a rare occurrence in the overall production of fish in ponds. Thus, the catfish stocking density of 10,000 pounds per acre is still considered valid for pond culture of tilapia, as it is likely to be practiced in the U.S.

7.1.2.7 Salmonids

Salmonids can be cultured in ponds or raceways and can withstand a wide range of temperature variation (0-24 °C), but the optimum water temperature is below 20 °C. Spawning and maximum growth occurs in a narrower range (9-14 °C) where dissolved oxygen concentrations are higher. North Carolina ranks second to Idaho in the U.S. in commercial production of rainbow trout and most of the trout farms are cement raceway systems in series (NCSU, undated). Earthen ponds are used at some sites but they are more difficult to manage. In the Southern Appalacian region, the trout industry depends primarily on diverted streams for water supplies beause water temperature, dissolved oxygen and ammonia concentrations are the primary limiting factors (Hinshaw, 2000). For salmonids, the carrying capacity of an earthen pond depends, in part, on the water flow rate. Since salmonids raised in ponds generally require flowing water due to their water quality requirements, it can reasonably be assumed that if they were stocked in earthen ponds, the stocking density would not exceed 10,000 lbs per acre.

7.1.2.8 Ornamental fish

Ornamental fish production is accomplished primarily in the state of Florida, which accounts for 95% of all ornamentals produced in the U.S. (Hill and Yanong, 2006). Hill and Yanong (2006) report that earthen pond culture is most common in Florida, although some areas use above-

ground tanks and small ponds dug into the coral rock bed. These ponds vary from 0.04 to 0.06 acres in size and are 3 to 8 ft (1 to less than 3 m) deep (Roy Yanong, pers. commun[5].).

Tropical fish culture is highly varied, with some farms specializing in one or a few fish groups, while other farms produce a wide variety of aquatic livestock (Hill and Yanong, 2006). Live-bearing fish are the foundation of the tropical ornamental fish industry in Florida (Hill and Yanong, 2006) and include two common genera: *Poecilia* (guppies and mollies) and *Xiphophorus* (platies, swordtails, and variatus). Live-bearers are grown almost exclusively in outdoor pools in Florida (Watson and Shireman, 2002).

The swordtail (*Xiphophorus helleri*) is used as an example in this assessment, because it is an economically important species. Watson and Shireman (2002) report that stocking rates for breeding ponds vary greatly, from 50 to 1000 fish. In central Florida, ornamental fish ponds are typically stocked with 5,000 older juvenile swordtails (Roy Yanong, pers. commun.). Six-month-old swordtails about 6 cm in length would weigh about 1.4 g (Yanong et al., 2006). Thus, 5,000 fish weighing 1.4 g would result in biomass of 7,000 g. In a pond of 0.05 acres, this is equivalent to 307 pounds per acre. This is well below the stocking density used for catfish; therefore, the PEC values determined based on catfish are sufficiently conservative for ornamental fish production in ponds.

7.1.3 INITIAL PEC_{water} FOR PONDS

The underlying assumptions for calculating potential releases of florfenicol-related residues from ponds are listed in Table 21. The dosage regimen is 15 mg florfenicol/kg fish/day, and the fish are treated for 10 days, for a total amount of florfenicol of 150 mg/kg fish. Food consumption is assumed to be 100%. The pond size is assumed to be 10 acres, with a depth of 1 m. A standing density of 10,000 pounds per acre is used. Although the time it takes to drain a pond is influenced by a number of factors, including size, drain diameter, and drainage ditch conditions, a typical 10-acre pond with a typical 12-inch drain would take about 3–4 days to drain into a ditch that does not already have water in it. This therefore represents an acute exposure, comparable to acute effects data (generally 48- to 96-hour laboratory exposures). At this stage in the assessment, it is assumed that there is no dilution in the receiving stream.

[5] Roy P. Yanong, V.M.D., Associate Professor, School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, FL, September 2010.

Table 21. Assumptions used to calculate initial PEC_{water} for pond scenarios

Parameter	Value
Dose (mg/kg/day)	15
Treatment period (days)	10
Percent of feed consumed	100
Percent of florfenicol metabolized	0
Maximum biomass (lbs/acre)	10,000
Size of pond (acres)	10
Depth of pond (m)	1
Dilution in receiving stream	None (factor of 1)

The initial PEC_{water} is calculated assuming that the total concentration of florfenicol (FFC) added in the feed (with no losses through metabolism or environmental fate processes) is diluted in the total volume of the pond, which is then released all at once (3–4 days) into a receiving stream with no dilution.

Eq. 1: Total mass of florfenicol = 150 mg FFC/kg fish × 10,000 lb fish/acre × 0.45 kg/lb × 10 acres = 6,750,000 mg FFC

Eq. 2: Volume of pond = 10 acres × 1 m x 4,046.86 m^2/acre × 1,000 L/m^3 = 40,468,600 L

Eq. 3: Concentration in pond = mass FFC, mg / pond volume, L = 6,750,000 mg/ 40,468,600 L = 0.17 mg/L

Eq. 4: PEC_{water} = Concentration in pond × dilution factor = 0.17 mg/L × 1 = 0.17 mg/L

This initial PEC_{water} of 0.17 mg/L is derived based on the worst-case concentration of florfenicol-related residues from pond water. This assumes that 100% of the florfenicol-related residues (florfenicol and metabolites) are in the water column, none is partitioned to sediment, and none remains in the fish. This also assumes that no degradation occurred to any of the residues over the 10-day treatment period and the pre-harvest interval. No dilution in the receiving water is considered. A refined PEC_{water}, discussed subsequently, includes considerations of metabolism, environmental fate processes, and dilution.

7.1.4 REFINED PEC_{water} FOR PONDS

As presented later in the Risk Characterization section, the use of initial PEC_{water} values resulted in risk quotients greater than 1; thus, it was necessary to refine the PEC values as explained below.

Calculation of the initial PEC_{water} for the pond did not consider metabolism, environmental fate processes, or dilution. The refined PEC calculations consider each of these factors.

Many studies exist to demonstrate that florfenicol is readily absorbed, distributed, metabolized, and excreted by fresh and saltwater salmonids (Martinsen et al., 1993; Horsberg et al., 1996), and this is presumed to be true for other species of fish. The resulting residues include the parent florfenicol, florfenicol amine, florfenicol alcohol, and florfenicol oxamic acid. Based on studies in Atlantic salmon by Horsberg et al. (1994), at 3 days after oral administration the parent florfenicol comprised 19.9% of the total radioactivity, florfenicol amine comprised 71.5%, and other (less toxic) metabolites comprised the remainder (each less than 10%). Therefore, approximately 10% of the total florfenicol dosed in an aquaculture situation can be considered to be metabolized to less toxic products, and the PEC_{water} can be refined accordingly. Data presented in Section 6 show that the metabolites are, in general, less toxic than the parent compound. Moreover, metabolites representing less than 10% of the administered dose can be ignored per the VICH/CVM Phase II Guidelines.

Although florfenicol has a low tendency to partition to solids relative to the aqueous phase, it is still likely that some florfenicol would be removed from the ponds due to partitioning to sediments in the interval between treatment and discharge. This florfenicol would not be discharged with the aqueous effluent. The K_d determined in the sediment/water study (Gledhill, 2005; see Table 4) suggests that, given the right conditions, approximately 33% of the florfenicol would be bound to solids. Based on the period of potential sediment accumulation prior to pond draining and a relatively high organic matter content of the pond sediment, the K_d determined in this study can be used to refine the PEC_{water} due to sediment partitioning. Note that the pond is likely to be renovated periodically (after a number of years), at which time the bottom is leveled and any excess material is used to rebuild the levees. Due to the infrequent nature of this process and the limited amount of material removed, this is not considered to be a significant pathway of potential exposure to florfenicol for terrestrial receptors.

Finally, in the initial PEC_{water} calculations, it was assumed that there was no dilution in the receiving water, which is very conservative. Similarly, the refined PEC_{water} is presented as an "end-of-pipe" concentration (without dilution) to account for those receiving waters and states where a mixing zone is not allowed. In a separate additional step, the refined PEC_{water} is presented with dilution in receiving waters to reflect more realistic conditions. As stated in the VICH/CVM Phase II Guidance (CVM, 2006; VICH, 2004), calculations of the PEC for surface water should address "how much further dilution occurs in receiving waters such as running river/stream water when effluent is discharged from the fish farm." A factor of 10-fold dilution is considered for a typical pond (fingerling or production) that would be drained yearly or after many years for refurbishment, and for an extreme worst-case scenario pond which considers a pond that not managed for water storage and could overflow with any rainfall event. Under the extreme worst-case scenario, it is assumed that florfenicol treatment could occur simultaneously with a large rainfall in a pond with no storage capacity.

The additional assumptions (beyond those used to determine the initial PEC_{water}) that are used to calculate the refined PEC_{water} for a typical pond and an extreme worst-case scenario are presented in Table 22.

Table 22. Assumptions used to calculate refined PEC_{water} for pond scenarios

Parameter	Typical pond scenario	Extreme worst-case pond scenario
Metabolism of parent florfenicol	10%	10%
Partitioning to sediments	33%	0%
Half-life in water/sediment systems	13.6 days	Not applicable
Release of pond volume	42 days after end of treatment	Concurrent with treatment
Dilution	10-fold *	10-fold

* A higher dilution factor (100-fold) is more likely for a production-pond that is managed for storage because only heavy rainfall events that result in pond overflow would be associated with maximum runoff and stream flow.

The initial PEC_{water} value of 0.17 mg/L is a worst-case peak concentration in the receiving environment based on the draining of the water from ponds. To refine the initial worst-case PEC value, metabolism, partitioning, and degradation must be considered.

As discussed previously, approximately 10% of the total florfenicol administered can be assumed to be metabolized into less toxic products. This reduces the initial PEC_{water} to 90% of the initial concentration (0.9 times the initial value).

Although florfenicol has a low tendency to partition to solids relative to the aqueous phase, it is still likely that some florfenicol would be removed from the aqueous phase by partitioning to sediment. The mean K_d determined in the sediment/water study (Gledhill, 2005) suggests that approximately one-third of the florfenicol would partition to sediments, while two-thirds would remain in the water column. Thus, the initial loading of florfenicol to the water column and subsequently available for discharge can be reduced by two-thirds (0.67). Applying this factor to the total loading of florfenicol for the fingerling pond scenario of 6,750,000 mg (Eq.1) gives a value for the water column of 4,522,500 mg.

The study by Gledhill (2005) also indicates that degradation can be expected in the pond, with an average half-life ($T_{1/2}$) for sediment/water systems of 13.6 days.

Eq. 5: By definition, $T_{1/2} = 0.6931 / k$

Eq. 6: Thus, $k = 0.05\ days^{-1}$

For most ponds that are drained yearly, the florfenicol residues would remain in the pond for a minimum of 42 days (12 days of observation plus 30 days of harvesting[6]). Following the VICH/CVM Guidance Phase II Guidance (CVM, 2006; VICH, 2004, a degradation period of half the storage time, or 21 days, is used. The amount of florfenicol residues in the water column at that time (A_{21}) would be defined by:

[6] According to Hargreaves et al.(2002) and Tucker (1996), harvesting requires repeated seining over a 1- to 3-month harvest period. To be conservative, the shorter time frame was used for calculations.

Eq. 7: $A_{21} / A_0 = e^{-kt}$

Eq. 8: $A_{21} / A_0 = e^{-0.05 \text{ days-1} \times 21 \text{ days}}$

Eq. 9: $A_{21} / A_0 = 0.3499 = 0.35$

Eq. 10: $A_{21} = 4{,}522{,}500 \text{ mg} \times 0.35 = 1{,}582{,}875 \text{ mg}$

Thus the total mass of florfenicol available for discharge in the water column would be 1,582,875 mg. Note that, although a pond may be treated a second time during the season, each treatment is considered an independent scenario due to the expected lack of accumulation of florfenicol in the pond sediment (owing to the high water solubility and low octanol-water partition coefficient). A dilution factor of 1 (no dilution) is initially used.

Considering these factors, the PEC_{water} for a typical pond that is drained yearly (i.e., fingerling ponds) can be refined as follows:

Eq. 11: Refined PEC_{water} = [Mass in pond water after partitioning and degradation (see Eq. 10)] / Volume of pond (see Eq. 2) × metabolism factor × dilution factor

Eq. 12: Refined PEC_{water} = [1,582,875 mg / 40,468,600 L] × 0.9 × 1

Eq. 13: Refined PEC_{water} = 0.035 mg/L (without dilution)

Consideration of 10-fold dilution in a receiving stream results in further refinement of the PEC_{water} to 0.0035 mg/L.

To refine the initial PEC_{water} for ponds that are not drained yearly (e.g., catfish production ponds that are drained ≥ 6 years), similar factors (metabolism, partitioning, and degradation) are considered. However, many times levee ponds are managed to conserve water so the level of the pond is maintained below full capacity (e.g., sufficient to retain a 90th percentile rainfall event). As a result, only heavy rainfall events would result in pond overflow; but this will also be associated with maximum runoff and stream flow. Thus, a 100-fold dilution factor is deemed appropriate, as explained in the following paragraph. Therefore the refined PEC_{water} would be further reduced to 0.00035 mg/L.

The high-rainfall period in the region where a majority of ponds, especially catfish ponds, are located occurs in December through April (Hargreaves et al., 2002). This time period coincides with the time when discharges from ponds due to overflow from rain could occur, as well as harvesting of fish from a pond to be refurbished. Streams and rivers may be at high flow, and ditches may contain water where mixing of pond water would occur prior to movement into streams and rivers. As a result, the concentration of florfenicol and its metabolites would be further diluted. Times of the year when treatments for ESC and columnaris are expected to occur would be in periods of lower rainfall; i.e., May/June and September/October, with at least six weeks (42 days) likely to pass before a high-rainfall event occurred.

Finally, an extreme worst-case estimate of the PECwater can be made based on ponds that are not managed to conserve water (i.e., no storage capacity). No degradation or partitioning to sediment would be assumed, because the proximity of overflow to treatment is not known. The extreme worst-case estimate of the PECwater without dilution is thus defined by Equation 14:

Eq. 14: Refined PEC_{water} = [6,750,000 mg / (40,468,600 L] × 0.9 × 1

Eq. 15: Refined PEC_{water} = 0.15 mg/L (without dilution)

Because theoretically this scenario would produce overflow with any rainfall, and because this overflow would not necessarily be associated with an unusual rainfall event or high stream flow, in-stream dilution would be expected to be less. Application of a 10-fold dilution factor results in a refined PEC_{water} for the extreme worst-case scenario of 0.015 mg/L.

This last scenario reflects a worst-case event wherein florfenicol treatment occurs simultaneously with sufficient rainfall to cause the pond to overflow. It is highly unlikely that a pond is managed with maximum water level, treated with Aquaflor®, and subjected to a significant rain event within a short time from treatment. More likely, the release or overflow of pond water to receiving waters would mostly occur months after treatments were administered and during significant rain events (Hargreaves et al., 2002), wherein substantial dilution would occur in receiving waters.

7.1.5 TIER A RISK CHARACTERIZATION FOR PONDS

7.1.5.1 Initial Tier A Aquatic Risk Characterization

The initial Tier A aquatic risk characterization is presented as the ratio of the initial PEC_{water} to the Tier A (acute) PNEC. The initial PEC_{water} values are based on treatment of 15 mg/kg/day for 10 days in a 10-acre pond with a depth of 1 m. It is assumed that all of the feed is consumed, and the florfenicol is excreted unmetabolized. The standing density of fish is assumed to be 10,000 lbs/acre. No partitioning to sediments, degradation, or dilution are assumed. Under these conservative conditions, the initial PEC_{water} is 0.17 mg/L.

These PEC values are divided by the Tier A PNEC values (based on acute effects) for each species to obtain the risk quotients. The Tier A PNEC values for aquatic organisms are calculated, as described in Table 16, from the EC_{50} or LC_{50} and an appropriate assessment factor. PNEC values are provided for two species of fish, one invertebrate, a diatom, a green alga, an aquatic vascular plant, and a cyanobacterium. The aquatic PNEC values reflect acute exposures and are suitable for comparison with the PEC values calculated for ponds, because the release of florfenicol residues through pond drainage occurs over a short period of time (3–4 days).

Table 23 shows the Tier A (acute) PNECs, the initial PEC_{water}, and the resulting PEC/PNEC ratios. These ratios, also called Risk Quotients (RQ), are below 1.0, the threshold of concern, for fish, daphnids, and diatoms. However, the RQ values are ≥ 1.0 for green algae, aquatic vascular plants, and cyanobacteria. Therefore, the refined PEC_{water} is used in the next step. The refined PEC_{water} considers metabolism, partitioning to sediment, degradation, and ultimately, dilution.

Table 23. Tier A aquatic risk characterization: Initial PEC/PNEC ratios for ponds

Organism	PNEC (mg/L)	Initial PEC_{water} (mg/L)	Initial PEC/PNEC
Oncorhynchus mykiss	7.8	0.17	0.02
Lepomis macrochirus	8.3	0.17	0.02
Daphnia magna	3.3	0.17	0.05
Navicula pelliculosa	6.1	0.17	0.03
Pseudokirchneriella subcapitata	0.1	0.17	**1.7**
Lemna gibba	0.076	0.17	**2.2**
Anabaena flos-aquae	0.023	0.17	**7.4**

PEC/PNEC ratios ≥ 1.0 are bolded

7.1.5.2 Refined Tier A Aquatic Risk Characterization

The refined Tier A aquatic risk characterization for ponds is presented in Tables 24 and 25. The PNEC values are the same as for the initial Tier A risk characterization. The refined PEC_{water} values are presented both with and without dilution in a receiving stream. Without dilution, the refined PEC_{water} is 0.035 mg/L for the typical pond scenario, and 0.15 mg/L for the extreme worst-case pond scenario (Table 24). Incorporating a 10-fold in-stream dilution reduces the refined PEC_{water} to 0.0035 mg/L for the typical pond and 0.015 mg/L for the extreme worst-case pond scenario (Table 25).

Use of the refined PEC_{water} values for the typical pond scenario, without considering dilution in receiving streams, results in risk quotients below 1.0 for all organisms except cyanobacteria, for which an RQ value of 1.5 is calculated (Table 24). Risk quotients are also presented for the extreme worst-case pond scenario, in which the florfenicol treatment occurs simultaneously with a large rainfall event in a pond with no storage capacity, resulting in immediate overflow from the pond. Because ponds are generally managed to have an overflow capacity, this scenario (i.e., PEC_{water} value of 0.15 mg/L) is unlikely to occur at most locations. This extreme worst-case scenario results in RQ values ≥1.0 for green algae, aquatic vascular plants, and cyanobacteria. Further discussion about the risk characterization is provided in Section 7.1.6.

Table 24. Tier A aquatic risk characterization: Refined PEC/PNEC ratios for ponds (without dilution)

Organism	PNEC (mg/L)	Refined PEC_{water} for a typical pond (mg/L)	Refined PEC/PNEC for a typical pond	Extreme worst case Refined PEC_{water} (mg/L)	Extreme worst case Refined PEC/PNEC
Oncorhynchus mykiss	7.8	0.035	0.005	0.15	0.019
Lepomis macrochirus	8.3	0.035	0.004	0.15	0.018
Daphnia magna	3.3	0.035	0.011	0.15	0.045
Navicula pelliculosa	6.1	0.035	0.006	0.15	0.025
Pseudokirch-neriella subcapitata	0.1	0.035	0.35	0.15	**1.5**
Lemna gibba	0.076	0.035	0.46	0.15	**2.0**
Anabaena flos-aquae	0.023	0.035	**1.5**	0.15	**6.5**

PEC/PNEC ratios ≥ 1.0 are bolded

Table 25 presents the risk quotients that incorporate in-stream dilution likely to occur for a typical pond that is drained yearly and the extreme worst case scenario. With dilution in receiving streams, all PEC/PNEC ratios are less than 1.0, even for the extreme worst-case scenario.

Table 25. Tier A aquatic risk characterization: Refined PEC/PNEC ratios for ponds (with dilution)

Organism	PNEC (mg/L)	Refined PEC_{water} with dilution for typical pond (mg/L)	Refined PEC/PNEC with dilution for typical pond*	Extreme worst case Refined PEC_{water} with dilution (mg/L)	Extreme worst case Refined PEC/PNEC with dilution
Oncorhynchus mykiss	7.8	0.0035	0.00045	0.015	0.0019
Lepomis macrochirus	8.3	0.0035	0.00042	0.015	0.0018
Daphnia magna	3.3	0.0035	0.0011	0.015	0.0045
Navicula pelliculosa	6.1	0.0035	0.00057	0.015	0.0025
Pseudokirch-neriella subcapitata	0.1	0.0035	0.035	0.015	0.15
Lemna gibba	0.076	0.0035	0.046	0.015	0.20
Anabaena flos-aquae	0.023	0.0035	0.15	0.015	0.65

* These refined PEC/PNEC values take into consideration that the pond is drained yearly, using a 10-fold in-stream dilution factor. Many production ponds are not drained yearly (i.e., >6 years) and are managed for storage, so they would only overflow during very heavy rainfall and thus the risk would be further decreased if a 100-fold dilution in receiving streams was factored in.

The VICH/CVM guidance specifies that the $PEC_{sediment}$ should be determined if the risk quotient for invertebrates is above 1. The data for *Daphnia magna* in Table 24 (RQ = 0.011) indicate that the $PEC_{sediment}$ does not need to be determined.

Where some of the risk quotients are ≥1.0 following calculation of the refined PEC_{water}, a Tier B assessment is normally conducted per VICH/CVM guidance. The Tier B assessment evaluates chronic effects. However, the exposure to florfenicol from draining aquaculture ponds, or in the case of overflow due to significant rain events, occurs only over a short duration (3–4 days); thus, an evaluation of chronic effects from chronic exposures is not applicable for the pond use pattern.

7.1.6 SUMMARY OF AQUATIC RISK CHARACTERIZATION FOR PONDS

At Tier A, using data on acute effects to aquatic organisms to evaluate acute exposures to florfenicol, the initial risk characterization produced PEC/PNEC ratios that exceeded 1.0 for green algae, aquatic vascular plants, and cyanobacteria when using the initial PEC_{water}. Upon refinement of the PEC_{water} value to account for metabolism, partitioning to sediment, and degradation, the risk quotients exceeded the level of concern only for cyanobacteria (RQ = 1.5), with the exception of production ponds not managed for water storage (extreme worst case). Under the extreme worst-case conditions of a pond in which the florfenicol treatment occurred

simultaneously with a large rainfall in a pond with no storage capacity, resulting in immediate overflow from the pond, refined Tier A risk quotients exceeded 1.0 for green algae, aquatic vascular plants, and cyanobacteria.

This indicates that, if there were no in-stream dilution, peak concentrations of florfenicol could have short-term impacts on these organisms. However, cyanobacteria and other unicellular organisms have very short generation times and are able to recover quickly from brief exposures to toxicants. Studies with green algae (Hoberg, 1991a-d) and diatoms (Jenkins, 2005) demonstrate that these organisms are able to resume growth once florfenicol is removed from the system. With rapidly growing organisms, unless complete mortality of the population occurs, the remaining organisms can readily reproduce, and the population regains its original density in a relatively short period of time. Moreover, although algal toxicity data are limited, there is a wide range of sensitivity to florfenicol and many algal species will likely not be affected by florfenicol under the expected exposure condtions. In addition, because there is functional redundancy in algal communities, even if sensitive species are affected, the overall productivity of the phytoplankton is likely to remain relatively constant. In addition, the release of florfenicol from ponds will be short-lived and relatively rare. Therefore, any inhibitory effects are likely to be temporary, and thus, short-term exposures to florfenicol are unlikely to have any significant impact at the population level.

Pond effluents are mainly released into ditches, which would lead into streams or rivers 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 by 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. Dilution in the receiving water would further reduce florfenicol concentrations. Once in-stream dilution of 10-fold is incorporated into the assessment, the risk quotients for all receptors fall below the level of concern, even under the extreme worst-case scenario of a pond that is not managed for water storage and could overflow with any rainfall. Most ponds are managed to conserve water and would only overflow in conditions of heavy rainfall where there would be significant in-stream dilution (100-fold), further reducing the risk quotients.

As discussed in the VICH/CVM guidelines, the main criteria in an aquatic risk assessment for advancing to Tier B are when risk quotients are ≥1 or if the substance is bioaccumulative (log $K_{ow} \geq 4$). As noted in Section 4, log K_{ow} for florfenicol is <4. Another reason for performing a Tier B assessment is to evaluate the risks of chronic exposure using chronic-effects data. The release of water from ponds does not constitute a chronic exposure. Therefore, for these reasons, the Tier B assessment is not necessary.

Other factors must be considered in characterizing the risk to aquatic ecosystems from use of Aquaflor®. Under the Veterinary Feed Directive, Aquaflor® will be used solely under a prescriptive use pattern, that is, under a veterinarian's order. These prescribed or controlled applications are made only to fish populations with active infections, and no prophylactic use is allowed. In addition, application is episodic, occurring once or twice per year and consistent with prescriptive use solely for active infections. Thus, the time period and frequency of application are limited.

The risk characterization for pond scenarios was based on data for culture of channel catfish. However, the assumptions used are sufficiently conservative to address culture of other fish species, including hybrid striped bass, largemouth bass, bluegill sunfish, carp, yellow perch, salmonids, and ornamentals. Information is provided to indicate that tilapia stocking densities

can exceed 10,000 pounds per acre (the estimated upper limit for catfish ponds), but this would be a rare occurence in the overall pond aquaculture industry. Catfish make up a majority of the pond culture industry and 10,000 pounds per acre is still a conservative estimate. The PEC is based on the dose of florfenicol, which is determined by the biomass of fish treated. Because stocking densities for all mentioned fish species, except tilapia, are less than that used for channel catfish, less florfenicol would be used, and the PEC would be correspondingly lower, while the PNEC would be unchanged. This would result in lower risk quotients than those presented for catfish. Even if a stocking density of 20,000 pounds per acre for tilapia was considered in risk characterizations, risk quotients would double but no additional species would have quotients ≥1 that were not already ≥1. When dilution in receiving streams is taken into consideration, only under the extreme worst-case scenario would cyanobacteria would have a risk quotient of 1.3. As stated previously, ecological effects on cyanobacteria and related unicellular organisms in receiving waters are not expected because the release of florfenicol from ponds will be short-lived and relatively rare, and growth of any affected populations is rapid, resulting in a short recovery time.

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 that affect sensitive species, any effects would be transient due to dissipation and degradation.

While initial PEC values indicate a potential risk to green algae, aquatic vascular plants, and cyanobacteria, the established environmental mobility and relatively short half-life of florfenicol (half-life of 13.6 days in water/sediment systems) indicate that any potential risk would be short-lived. Moreover, unless entirely killed, populations of these organisms are likely able to recover in the absence of the inhibitory agent. Upon refinement of the PEC_{water} to account for metabolism, partitioning, and degradation, only the risk quotient for cyanobacteria is above 1.0 (e.g., 1.5) for the typical pond scenario. For an extreme worst-case pond scenario where there is no water storage capacity and florfenicol treatment coincided with an extreme rain event causing overflow, PEC/PNEC ratios in excess of 1.0 are estimated for green algae, aquatic vascular plants, and cyanobacteria. Such a scenario represents an extreme worst case that is highly unlikely, but again, would be of short duration and not represent a population-level risk to those receptors. After consideration of a 10-fold in-stream dilution, risk quotients for all receptors under both pond scenarios fall below 1.0.

7.1.7 CONCLUSIONS REGARDING RISKS OF AQUAFLOR® USE IN PONDS

Based on the data, assumptions, and calculations presented in this Environmental Assessment, the use of Aquaflor® in pond culture of fish does not present any significant risk to the environment, due to the following combination of factors:

- Aquaflor® is limited to:
  - Prescriptive application with no prophylactic use under the Veterinary Feed Directive
  - Application in feed at no more than 15 mg/kg feed/day for 10 days
- Florfenicol, the active ingredient in Aquaflor®, will remain primarily in water where it dissipates due to degradation and dilution, or will partition to solids where it readily degrades.
- Florfenicol release is limited by aquaculture practices (frequency and timing of pond draining, 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 transitory.
- PEC/PNEC ratios were based on scenarios developed using channel catfish, which are the most extensively cultured pond species and are cultured using the highest stocking densities. These scenarios are sufficiently conservative to evaluate florfenicol risks from use in other fish species cultured in ponds.
- Using realistic yet conservative assumptions, the only PEC/PNEC ratios that exceed 1.0 are for cyanobacteria under typical conditions and for green algae, aquatic vascular plants, and cyanobacteria under extreme worst-case conditions. This assumes no dilution in the receiving water. After consideration of in-stream dilution, no risk quotients exceed 1.0.
- Toxicity to the most sensitive organisms—algae and cyanobacteria—is based on inhibitory effects, which are transitory when the exposure is removed. Thus, any inhibited populations are expected to recover rapidly and widespread, ecologically significant or long-lasting impacts are not expected.

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 from the use of Aquaflor® at up to 15 mg/kg/day for freshwater-reared finfish culture in ponds is considered to be very small.

7.2 USE PATTERN: FLOW-THROUGH WATER SYSTEMS

The second use pattern considered in this EA is the use of florfenicol to treat fish cultured in flow-through water systems containing raceways and/or tanks. A variety of species can be cultured in flow-through systems using raceways and tanks, but freshwater speices that are commonly cultured in these systems include trout, catfish, and tilapia. Tilapia are now one of the most widely farmed fish in the world (Popma and Masser, 1999). Salmonid aquaculture is also a major U.S. economic contributer to the production of farmed fish, as is catfish aquaculture.

Although trout and salmon are more commonly reared in the U.S. in flow-through water systems, in this EA, tilapia culture in raceways is considered as a "worst-case" example for evaluation purposes because tilapia can be stocked at relatively high densities and reared with low water flow rates. This is due to their greater tolerance than most commonly farmed freshwater fish to diseases, high water temperatures, low dissolved oxygen, and high ammonia concentrations (Popma and Masser, 1999). As a result of these high stocking densities and low water flow rates, treatment of tilapia with Aquaflor® would be expected to result in higher florfenicol effluent concentrations than if other species such as trout were treated.

Aquaflor® can be used to treat streptococcal septicemia associated with *Streptococcus iniae* in fish. The frequency and timing of applications are limited by regulation: under the Veterinary Feeds Directive, disease in the population must be confirmed by an aquaculture veterinarian prior to the prescribed use of Aquaflor®, and prophylactic treatments are not allowed. This results in more controlled and minimized use.

As described by U.S. EPA (2004), raceways typically are long, rectangular tanks constructed of earth, concrete, plastic, or metal. The size varies depending on topography and the operational goals of the facility, but typical raceway dimensions are 100 ft long, 10 ft wide, and 3 ft deep. Flow-through raceway systems are constructed to reuse the flowing water several times by

passing the water through multiple raceway units arranged in series before discharging it. This is refered to as multi-pass water reuse. Water discharge is often reused after settleable solids are captured in quiescent zones at the end of each unit and the water is then reaerated as it cascades into a lower raceway (Wedemeyer, 2001).

7.2.1 PRODUCTION AND DISEASE TREATMENT IN FLOW-THROUGH RACEWAYS

Aquaflor® is incorporated into fish feed prior to pelleting. As discussed previously, the rate of administration of the premix to the feed will depend on the feed consumption rate. The recommended dosage regimen is 15 mg a.i./kg body weight for 10 consecutive days. For example, at a feeding rate of 1% body weight per day, a total of 3.0 g of the medicated article (1.5 g florfenicol) would be applied per kilogram of feed. The quantities of florfenicol being administered will depend on the quantities and weight of fish requiring treatment.

Florfenicol and its metabolites will enter the environment in excreta; entry through uneaten feed is considered inconsequential. 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 fish feed is formulated as floating, extruded pellets. Unlike sinking feed used in the culture of other aquatic animals, floating feed has high water stability and results in limited excess food at the bottom of tanks, where it may disintegrate or become unavailable to the fish. 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 bottom of tanks or settling ponds. For the purposes of this assessment, it is assumed that feed is 100% consumed by the fish. Based on the environmental fate characteristics discussed above, any florfenicol or its metabolites that would enter the water column of a tank would initially partition between the solids (including solid waste) and water phases and would degrade in each of these compartments.

7.2.2 INITIAL PEC_{water} FOR FLOW-THROUGH RACEWAYS

The amount of medication administered is based on appropriately fortified feed being provided to deliver 15 mg florfenicol per kilogram of fish per day for 10 days. The biomass to be treated is dependent on the capacity of the facility and the proportion of fish to be treated. The stocking density within any given facility will depend on the water flow and engineering provisions within the facility. No removal by effluent treatment systems is assumed in estimating environmental concentrations.

A variety of raceway configurations can be used. Raceways can be configured in a series, with the water flowing from one to the next. For this assessment, a configuration of 100 ft long, 10 ft wide, and 3 ft deep (U.S. EPA, 2004) is assumed, or roughly 30 m long, 3 m wide, and 1 m deep. For assessment purposes in this EA, it is also assumed that there are three raceways in a series, although it is acknowledged that this number is sometimes higher in facilities where water quantities are not limiting and higher than typical flow rates can be used.

Below are the assumptions for flow-through raceway scenarios for median and worst case scenarios:

- Median-case flow rate and fish density: 15 L/s and 25 kg/m^3
- Worst-case flow rate and fish density: 3 L/s and 50 kg/m^3

There is a relationship between flow rate and stocking density, such that higher flow rates are needed to maintain higher stocking densities. Boyd (2004) reports that the normal exchange

rate in tilapia raceway systems is 0.5 to 4 exchanges per hour, which would be 12.5 to 100 L/s for a 90-m^3 tank. Discussing tilapia in flow-through systems, DeLong et al. (2009) have stated that "as a rule, the amount of water needed for a facility is the amount required to replace 100 percent of the tank water every 90 to 120 minutes." For a tank of 30 m × 3 m × 1 m, this would constitute a flow rate of approximately 13 to 17 L/s, or an average of 15 L/s.

It has been reported that the maximum biomass density of tilapia in highly aerated tank systems can be 30 to 72 kg/m^3 (Delong et al., 2009), or an average of approximately 50 kg/m^3. Chapman (2000) reported that, in tanks or raceways, hybrid tilapia of 25 to 50 g are stocked at densities from 140 to 248 fish/m^3. Assuming that lower densities correspond to larger fish, and vice versa, these densities represent 6.2 to 7 kg/m^3. Thus, if 100% of the fish survive until harvest at an average weight of 500 g/fish, then the fish density would be approximately 62-70 kg/m^3. Even at 80% survival, fish density could be 56 kg/m^3.

Salmonids can also be stocked at densities of 30-40 kg/m^3 with proper feeding and water supply, although higher production is possible (FAO, 2011). At higher densities, however, physical crowding may produce social stress which will be reflected in survival. Increased losses are directly related to increased density and decreased water flow (Banks, 1994). A test was conducted using varying flow rates of 12, 25, and 38 L/s for at each of 3 densities of Chinook salmon at 15, 29, and 44 kg/m^3. It was noted that fish at the higest density and lowest level of water flow (i.e., 44 kg/m^3 with water flow of 12 L/s) had significantly higher mortality than fish in any other experimental cell (Banks, 1994) and that raceways with the highest water flow produced more adults than at the lower flow rates.

Given the wide ranges in possible combinations of raceway configuration, stocking density, and flow rate found in the literature, a median-case and a worst-case raceway scenario were created. The median case uses a median fish density (25 kg/m^3) with a higher flow rate of 15 L/s. The worst case employs a low flow rate (3 L/s) and high fish density (50 kg/m^3). Both values are within the bounds of acceptable aquaculture practice, although unlikely to be used in combination as the flow rate usually increases with increasing fish density. As a general rule, it is likely that a higher fish density would require a higher flow rate. Banks (1994) indicated that rearing salmonids at a density around 30 kg/m^3 with a flow rate of 15-25 L/s is normal for some trout hatcheries. However, tilapia can withstand lower dissolved oxygen concentrations and higher temperatures than salmonids, and thus lower flow rates are possible while still maintaining acceptable water quality conditions for this species. Overall, the fish densities in raceways can potentially exceed this worst-case scenario, but when taking the low flow rate of 3 L/s into consideration, a density of 50 kg/m^3 would still be considered very conservative for determining PECs to cover all potential fish species that could be raised in flow-through culture systems. Note that using the worst-case assumptions for all parameters concurrently, for example by including additional raceways in series, would create an unrealistic exposure scenario that would never occur in practice.

For the Tier A (acute) assessment, it is assumed that all of the dosed florfenicol enters the water column during the 10-day treatment period, and this corresponds to the potential exposure period. For the Tier B (chronic) assessment, it is assumed that all of the dosed florfenicol enters the water column and is distributed evenly over a 21-day period, a time frame that coincides with the exposure period of the Tier B (chronic) effects studies. For both Tier A and Tier B, it is also assumed that 100% of the feed is consumed and excreted into the water column. In calculating the initial PEC_{water}, it is assumed that florfenicol is not metabolized and is excreted unchanged to the water. No loss from the water column or partitioning to solids is assumed at this step.

The assumptions used to calculate the initial PEC_{water} for flow-through systems raceways are presented in Table 26.

Table 26. Assumptions used to calculate initial PEC_{water} for flow-though raceway scenarios

Parameter	Median Case	Worst Case
Dose (mg/kg/day)	15	15
Exposure period (days) for Tier A	10	10
Exposure period (days) for Tier B	21	21
Percent of feed consumed	100	100
Percent of florfenicol metabolized	0	0
Fish density (kg/m^3)	25	50*
Flow rate (L/s)	15	3
Dimensions of raceway (length x width x depth, m)	30 × 3 × 1	30 × 3 × 1
Raceways in series (number of)	3*	3*
Dilution in receiving water	None	None

*Fish density in a raceway can exceed 50 kg/m^3 and the number of raceways in a series can exceed 3, so these are not the worst case values for these parameters by themselves. However, PEC calculations also are driven by the flow rate. A worst case flow rate of 3 L/s has been used in this scenario. An increase in either the fish density or numbers of raceways in a series would require a proportional increase in the flow rate. Using the worst-case values for all parameters in combination would create a very unrealistic exposure scenario. Therefore, this is considered a "reasonable" worst-case scenario.

The volume of the raceway is determined as follows:

Eq. 16: Raceway volume = 30 m × 3 m × 1 m = 90 m^3 per single raceway, and

Eq. 17: Raceway volume for series = 90 m^3 × 3 raceways = 270 m^3 in three raceways

The amount of florfenicol applied, at a dosage regimen of 15 mg/kg body weight for 10 days, is calculated based on fish density according to Equation 18:

Eq. 18: Total FFC, mg = dose, mg/kg/day × 10 days × fish density, kg/m^3 × volume, m^3

For the median fish density:

Eq. 18a: Total FFC, mg = 15 mg/kg/day × 10 days × 25 kg/m^3 × 270 m^3 = 1,012,500 mg

For the worst case fish density:

Eq. 18b: Total FFC, mg = 15 mg/kg/day × 10 days × 50 kg/m^3 × 270 m^3 = 2,025,000 mg

The total amount of florfenicol determined by Equation 18 is assumed to be distributed evenly in the total water flow over 10 days for the Tier A assessment and 21 days for the Tier B assessment. The total amount of florfenicol must be divided by the total amount of water flow through the three raceways during those respective time periods to estimate water concentrations. Calculations of total flow are as follows:

Eq. 19: Total flow, L = flow, L/s × 60 s/min × 60 min/h × 24 h/day × days

At Tier A, for the median case scenario, with the higher flow rate:

Eq. 19a: Total flow, L = 15 L/s × 60 s/min × 60 min/hr × 24 h/day × 10 d = 12,960,000 L

At Tier A, for the worst-case scenario, with the lower flow rate:

Eq. 19b: Total flow, L = 3 L/s × 60 s/min × 60 min/hr × 24 h/day × 10 d = 2,592,000 L

The calculations are the same for Tier B, except that 21 d replaces 10 d. The resulting flow values are 27,216,000 L for the median-case scenario and 5,443,200 L for the worst-case scenario.

The initial PEC_{water} is then calculated by dividing the mass of florfenicol in the system by the total water volume during either 10 days (Tier A) or 21 days (Tier B). This is an end-of-pipe calculation and assumes no dilution in the receiving water:

Eq. 20: Initial PEC_{water} = Total FFC, mg / total flow, L

At Tier A, for the median-case scenario:

Eq. 20a: Initial PEC_{water} = 1,012,500 mg / 12,960,000 L = 0.078 mg/L

At Tier A, for the worst-case scenario:

Eq. 20b: Initial PEC_{water} = 2,025,000 mg / 2,592,000 L = 0.781 mg/L

Calculations for Tier B are similar, but the values for total flow that are used are based on 21 days instead of 10 days. These calculations are summarized in Table 27.

Table 27. Calculation of initial PEC_{water} for flow-through raceway scenarios

Scenario	Median Case		Worst Case	
	Tier A (Acute[a])	Tier B (Chronic[b])	Tier A (Acute[a])	Tier B (Chronic[b])
Stocking density, kg/m^3	25	25	50	50
Flow rate, L/s	15	15	3	3
Total dose of florfenicol, mg	1,012,500	1,012,500	2,025,000	2,025,000
Total flow (L)	12,960,000	27,216,000	2,592,000	5,443,200
Initial PEC_{water}, mg/L	0.078	0.037	0.781	0.372

[a] Tier A (acute) estimations take into consideration a 10-day exposure period to coincide with the duration of the treatment
[b] Tier B (Chronic) estimations take into consideration a 21-day exposure period to coincide with the duration of chronic toxicity tests

The worst-case scenario has a lower flow rate (3 L/s) and smaller overall flow volume. It also has a higher biomass (50 kg/m^3) and thus provides for the maximum amount of florfenicol in the smallest volume of water.

The configuration of raceways described above would serve as a basic unit of a facility. Any changes in scale can be estimated by multiplying the values reported here. Exposure of aquatic organisms would be expected to be episodic and transitory, based on infrequent uses, degradation, and dilution of released florfenicol-related residues. The intermittent nature of the exposure would not be specific to this raceway configuration.

The initial PEC_{water} values presented in Table 27 are determined by the total water flow and the biomass treated and are calculated for two representative stocking densities and flow-rate combinations for both Tier A (acute) and Tier B (chronic) scenarios. The worst-case scenario is based on the low end of the range of water flow (3 L/s) and the high end of the range of biomass (50 kg/m^3). A flow rate of 15 L/s is used in combination with the median density level (25 kg/m^3) to present a more common example. It should be noted that these calculations are end-of-pipe estimates and also assume that 100% of florfenicol is in the water column.

In addition, the initial PEC_{water} values presented in Table 27 do not include any consideration of mitigating factors such as metabolism by the fish or in-facility dilution due to treatment of only a portion of the total facility. Due to nature of disease occurrence, aquaculture practices, and the fact that Aquaflor® cannot be used in a prophylactic manner, it is highly unlikely that the entire facility will be treated at one time. Therefore, it is assumed that, at most, only 50% of the facility will be treated at one time. Neither dilution of florfenicol-related residues in settling ponds nor dilution in receiving waters is included in calculating initial PEC_{water} values. Moreover, exposure to aquatic organisms would be expected to be transient and episodic based on infrequent uses, degradation, and dilution of released florfenicol-related residues. These factors are considered in determination of the refined PEC_{water} values.

7.2.3 REFINED PEC_{water} FOR FLOW-THROUGH RACEWAYS

The initial PEC_{water} was determined using very conservative assumptions to create both a median case and a worst case. Refinement occurs by considering additional factors, including:

- Treatment of only a portion (50%) of a facility at one time (in-facility dilution)
- Metabolism of florfenicol
- Partitioning to solids
- Dilution in the receiving water.

Each of these factors is discussed below.

Inside any given facility, only a portion of that facility (specific raceways) is treated at any one time due to the nature of diseases. Bacterial diseases generally do not affect all fish in a facility at one time. Many times, the age of the fish determines its susceptibility to a particular disease, and a given facility will typically have a range in the ages of fish. For example, ESC typically affects younger fish, while streptococcal septicemia is predominant in subadult and adult fish. Moreover, it is feasible and practical to treat one series of raceways and not another series when there are no water connections between them; therefore, in any single treatment period, only a portion of the facility would be treated. In determining the refined PEC_{water}, it is assumed that a maximum proportion of 50% of a given facility would be treated at one time. This factor also considers that there could be multiple indications treated at one time, or overlapping non-synchronous treatments of a single pathogen in separate raceways. This factor is considered conservative because it is more likely that a smaller fraction of the fish would be treated. A factor of 50% (two-fold in-facility dilution) means that the overall aqueous discharge from the facility includes water that does not contain florfenicol residues (50%) and water that does contain florfenicol residues (50%).

Many studies exist to demonstrate that florfenicol is readily absorbed, distributed, metabolized, and excreted by fresh and saltwater salmonids, and this is presumed to be true for other species of fish. As discussed in Section 7.1.4, based on these studies it can be assumed that approximately 10% of the total florfenicol dosed in an aquaculture situation is metabolized to less toxic products (leaving 90%), and the PEC_{water} can be refined accordingly (multiplying by a factor of 0.9).

Although florfenicol has a low tendency to partition to solids relative to the aqueous phase, it is still likely that some florfenicol would be removed from the raceway system in the settling ponds, which trap feces and uneaten feed before discharging to the receiving water. This florfenicol would not be discharged with the aqueous effluent but would instead be applied to agricultural lands. The K_d determined in the sediment/water study (Gledhill, 2005) suggests that, given the right conditions, 33% of the florfenicol would be bound to solids. However, in the absence of knowledge about the hydraulic retention time of the settling ponds and the amount of organic matter present therein, this calculation is not performed with respect to the aqueous effluent. The conservative assumption is to ignore the effect of partitioning to solids and omit this factor in the calculation of the refined PEC_{water}.

Finally, in the initial PEC_{water} calculations, it was assumed that there was no dilution in the receiving water. This is very conservative. Similarly, the refined PEC_{water} is presented as an "end-of-pipe" concentration (without dilution) to account for those receiving waters and states where a mixing zone is not allowed. In a separate additional step, the refined PEC_{water} is presented with dilution to reflect more realistic conditions. As stated in the VICH/CVM Phase II

Guidance (VICH 2004), calculations of the PEC for surface water should address "how much further dilution occurs in receiving waters, such as running river/stream water, when effluent is discharged from the fish farm." Raceway facilities require relatively large volumes of water to operate efficiently and thus, dilution in the receiving stream is expected. Therefore, the use of in-stream dilution by a factor of 10-fold to modify the refined PEC_{water} is considered in a separate step.

The additional assumptions used to calculate the refined PEC_{water} for flow-through raceways, beyond those used for calculation of the initial PEC_{water}, are presented in Table 28. There is no difference between the median-case scenario and the worst-case scenario in the refinement of the PEC_{water}.

Table 28. Assumptions used to calculate refined PEC_{water} for flow-though raceway scenarios

Parameter	Median Case	Worst Case
Metabolism of parent florfenicol (%)	10	10
In-facility dilution (fraction of fish treated), %	50	50
In-stream dilution (considered separately)	10-fold	10-fold

The refined PEC_{water}, accounting for metabolism and in-facility dilution, is thus determined as:

Eq. 21: Refined PEC_{water} = Initial PEC_{water} × 0.90 (for metabolism) × 0.50 (for in-facility dilution)

For example, at Tier A, for the median case scenario:

Eq. 21a: Refined PEC_{water} = 0.078 mg/L × 0.9 × 0.5 = 0.035 mg/L

The refined PEC_{water} for both the median- and worst-case scenario, at Tier A and Tier B, is presented in Table 29.

Table 29. Calculation of Refined PEC_{water} for flow-though raceway scenarios

Scenario	Median Case		Worst Case	
	Tier A	Tier B	Tier A	Tier B
Fish density, kg/m^3	25	25	50	50
Flow rate, L/s	15	15	3	3
Refined PEC_{water}, mg/L	0.035	0.017	0.35	0.17
Refined PEC_{water}, mg/L (with in-stream dilution)	0.0035	0.0017	0.035	0.017

7.2.4 INITIAL PEC_{soil} FOR RACEWAYS

In the aquatic exposure assessment, it was assumed in the calculation of the initial PEC_{water} that all florfenicol and metabolites enter the water column. This assumption is justified based on the physicochemical properties of florfenicol and its metabolites. The high water solubility and low binding potential of florfenicol and its metabolites indicates that any active ingredient present on uneaten feed or excreta will be unlikely to remain associated with particulate material for long periods of time. However, for the terrestrial exposure assessment, it is assumed that some florfenicol residues would reach the terrestrial environment through the land application of solids removed from the aquaculture facility. Most flow-through raceway facilities have settling ponds where the uneaten food and excreta are collected for removal. Solids are removed at intervals generally ranging from 1 to 2 times per year; i.e., slurry is removed annually or semiannually, and can be applied to agricultural fields. While this was not factored into the calculation of PEC_{water} as a conservative measure with respect to that calculation, it is considered with respect to exposure to florfenicol in agricultural soil.

The approach used to calculate the initial PEC_{soil} was adapted from that provided for intensively reared animals (EMEA 2008) as follows:

Eq. 22: $$PEC_{soil} = \frac{ID * T * P * F_H * N_{SL}}{\rho * A * D * N_{pp} * N_{fish} * H}$$

where
- ID : individual dose [mg/kg]
- T : number of treatments [n]
- P : animals raised per place and year [n]
- F_H : fraction of herd treated [n]
- N_{SL} : EU nitrogen spreading limit [kg/ha*y]
- ρ : soil bulk density [kg/m^3]
- A : area of 1 hectare (ha) [m^2/ha]
- D : penetration depth in soil [m]
- N_{pp} : nitrogen production period [d/y]
- N_{fish} : nitrogen produced by fish [kg/kg*d]
- H : housing factor [n]

No value for the nitrogen produced per year is available in EMEA (2008) for fish aquaculture. Van Weerd et al. (1994) determined the average daily production of nitrogenous compounds by rainbow trout fed 1x/day, 2x/day, 4x/day, or continously to be 0.930 g/kg body weight/day (range 0.778-1.050 g/kg bw/day). To determine the value for annual nitrogen production, the daily amount of nitrogen produced (N_{fish}) (van Weerd et al., 1994) and a nitrogen production period of 365 days (equal to 1 year, N_{pp}) are used. Because van Weerd et al. (1994) presented the nitrogen production as g nitrogen per kg body weight/day, the factor for body weight needs to be included in the denominator of the equation. However, in doing so, the factor for body weight originally present in the numerator is eliminated, and the modified equation no longer contains the factor for body weight.

According to Masser et al. (1999), the grow-out period for tilapia to marketable size is approximately 8 months. The grow-out period for catfish and salmonids can be much longer. Using a conservative grow-out period of 8 months, the number of animals raised per place and year (P, equal to generations per place and year) is 1.5. This value represents the worst case, as extended grow-out periods will result in a reduced number of generations per place and year.

In the calculation of the initial PEC_{soil}, it is assumed that 100% of the stock is treated, which represents the worst case. It is also assumed that all of the excreted florfenicol is bound to solids, also a worst case.

The assumptions for calculation of the initial PEC_{soil} are presented in Table 30 and conservatively assume that all of the florfenicol enteringing the system is bound to solids.

Using these values and solving Equation 22 results in an initial PEC_{soil} of 0.15 mg/kg.

Table 30. Assumptions used to calculate initial PEC_{soil} for flow-through raceways

Parameter	Value
ID, individual dose (mg/kg)	15
T, number of treatments (n)	10
P, animals raised per place and year (n)	1.5
F_H, fraction of herd treated (n)	1.0
N_{fish}, nitrogen produced by fish (kg/kg*d)	0.000930
N_{pp}, nitrogen production period (d/y)	365
N_{SL}, nitrogen spreading limit (kg/ha*y)	170
H, housing factor (n)	1
D, penetration depth in soil (m)	0.05
ρ, soil bulk density (kg/m^3)	1500
A, area of 1 hectare (m^2/ha)	10,000

It should be noted that this PEC represents the concentration of florfenicol initially applied to land in slurry from the facility and does not account for degradation in soil (half-life of 27.2 days). Metabolism in fish or treatment of only a portion of the fish in the facility are also not considered.

7.2.5 REFINED PEC_{SOIL} FOR RACEWAYS

The calculation of the initial PEC_{soil} does not account for metabolism, treatment of only a portion of the fish in the facility, or degradation in fish excreta. The adjustment factors for metabolism (0.9) and treatment of a portion of the fish (0.5) have been discussed previously. In the absence of data on degradation in fish manure, refinement for degradation is based on the findings of a study investigating florfenicol degradation under anaerobic conditions in pig slurry (Millais 2005), which resulted in a half-life of 1.0 d. A factor of 10 was applied to this DT_{50} to account for

uncertainty in extrapolating between pig and fish excreta, resulting in a half-life of 10 d for use in refinement of the PEC_{soil}.

The equation for refinement of the PEC_{soil} provided in the EMEA (2008) guidance is not directly applicable for aquaculture but has been adapted as presented below:

Eq. 23 (Step 1): Mi [mg]

$$Mi = ID * M_{fish} * T * F_H * Fa$$

Eq. 24 (Step 2): Mt [mg]

$$Mt = Mi * e^{\left(\frac{(-\ln(2)*(Tst/2))}{DT_{50}}\right)}$$

Eq. 25 (Step 3): N_s [kg]

$$N_s = M_{fish} * N_{fish} * Tst$$

Eq. 26 (Step 4): refined PEC_{soil} [mg/kg]

$$refinedPEC_{soil} = \frac{Mt * N_{SL}}{\rho * A * D * N_s}$$

Where:

Mi	:	mass of active ingredient in manure [mg]
ID	:	individual dose [mg/kg]
M_{fish}	:	mass of treated fish in raceway system [kg]
T	:	number of treatments [n]
F_H	:	fraction of herd treated [n]
F_a	:	fraction of the dose considered to be active [n]
Mt	:	mass of active ingredient in manure after storage time [mg]
Tst	:	Length of time manure is stored [d]
DT_{50}	:	50% degradation time in manure [d]
N_{pp}	:	nitrogen production period [d]
N_{fish}	:	nitrogen produced by fish [kg/kg*d]
N_s	:	nitrogen produced during storage time [kg]
N_{SL}	:	EU nitrogen spreading limit [kg/ha*y]
ρ	:	soil bulk density [kg/m^3]
A	:	area of 1 hectare [m^2]
D	:	penetration depth in soil [m]

The values for ID, T, N_{fish}, N_{SL}, ρ, A, and D are the same as used in calculation of the initial PEC_{soil}. The additional assumptions for calculation of the refined PEC_{soil} are presented in Table 31. It is assumed that the settling pond is cleaned on a semi-annual basis, so the length of time that manure is stored is set to 180 days. The fraction of the dose considered to be active is set equal to 0.9 to account for metabolism, and the fraction of animals treated is set to 0.5. The additional assumptions for calculation of the refined PEC_{soil} are presented in Table 31. The

biomass of fish is calculated to be 25 kg/m^3 × 270 m^3 for the median case and 50 kg/m^3 × 270 m^3 for the worst case.

Using these values for both the median and worst-case flow-through raceway scenarios, the refined PEC_{soil} is 0.00018 mg/kg.

Table 31. Assumptions used to calculate refined PEC_{soil} for flow-through raceways

Parameter	Value
M_{fish}, Biomass of treated fish (kg)	6,750 for median case; 13,500 for worst case
F_A, fraction of the dose considered to be active (n)	0.9
F_H, fraction of herd treated (n)	0.5
Tst, length of time manure is stored (d)	180
DT_{50}, 50% degradation time in manure (d)	10

7.2.6 TIER A RISK CHARACTERIZATION FOR RACEWAYS

7.2.6.1 Initial Tier A Aquatic Risk Characterization

The initial risk characterization is based on a median and worst-case scenario, both with a representative facility design. The initial PEC_{water} is based on 100% of applied florfenicol in the water column, no removal of florfenicol in settling ponds, 100% facility treatment, and no dilution in the receiving water.

The worst-case scenario for release of florfenicol to the receiving water is based on the assumptions that all of the fish in the facility, at a high fish density of 50 kg/m^3, require treatment, and the facility uses a low flow rate of 3 L/s. The initial PEC_{water} for the worst-case scenario is 0.78 mg/L. For the median scenario, with a fish density of 25 kg/m^3 and a flow rate of 15 L/s, the initial PEC_{water} is 0.078 mg/L (as presented in Table 27).

For the Tier A assessment, the release of florfenicol from raceways was calculated using flow over a 10-day period. This is conservatively assumed to be an acute exposure relative to the aquatic organisms in the receiving stream. The appropriate Tier A PNEC values are based on the use of acute effects data with acute exposures (Table 16).

Table 32 shows the Tier A PNECs, the median case and worst-case initial PEC_{water} values, and the resulting PEC/PNEC ratios. The ratios for fish, invertebrates, and diatoms are less than 1.0,

indicating that the use of florfenicol does not pose a risk to these receptors, even using a worst-case calculation. However, the ratios for green algae, aquatic vascular plants, and cyanobacteria are greater than 1.0, both for the worst-case scenario and the median-case scenario.

Table 32. Tier A aquatic risk characterization: Initial PEC/PNEC ratios for flow-through raceways

Organism	PNEC (mg/L)	Median-Case Scenario PEC (mg/L)	Median-Case Scenario PEC/PNEC	Worst-Case Scenario PEC (mg/L)	Worst-Case Scenario PEC/PNEC
Oncorhynchus mykiss	7.8	0.078	0.010	0.78	0.10
Lepomis macrochirus	8.3	0.078	0.009	0.78	0.09
Daphnia magna	3.3	0.078	0.024	0.78	0.24
Navicula pelliculosa	6.1	0.078	0.013	0.78	0.13
Pseudokirchneriella subcapitata	0.1	0.078	0.78	0.78	**8**
Lemna gibba	0.076	0.078	**1.0**	0.78	**10**
Anabaena flos-aquae	0.023	0.078	**3.4**	0.78	**34**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

The Risk Quotients for some receptors were >1.0, so a refined analysis was performed. The refined risk assessment uses the refined PEC_{water} and is presented below.

7.2.6.2 Refined Tier A Aquatic Risk Characterization

Table 33 shows the PEC/PNEC ratios based on acute exposure of aquatic organisms using the refined PEC_{water} values determined for both the worst-case and the median-case scenarios, absent any dilution in the receiving water. The Tier A PNEC values used are those presented in Table 16. The refined PEC_{water} values were determined by taking into account in-facility dilution (because only a portion of the fish would be treated at any one time) and metabolism. The refined Tier A PEC_{water} values were determined to be 0.35 mg/L for the worst-case scenario and 0.035 mg/L for the median case (as presented in Table 29).

Table 33. Tier A aquatic risk characterization: Refined PEC/PNEC ratios for flow-through raceways (without dilution)

Organism	PNEC (mg/L)	Median-Case Scenario PEC (mg/L)	Median-Case Scenario PEC/PNEC	Worst-Case Scenario PEC (mg/L)	Worst-Case Scenario PEC/PNEC
Oncorhynchus mykiss	7.8	0.035	0.0045	0.35	0.045
Lepomis macrochirus	8.3	0.035	0.0042	0.35	0.042
Daphnia magna	3.3	0.035	0.0106	0.35	0.106
Navicula pelliculosa	6.1	0.035	0.0057	0.35	0.057
Pseudokirchneriella subcapitata	0.1	0.035	0.35	0.35	**3.5**
Lemna gibba	0.076	0.035	0.46	0.35	**4.6**
Anabaena flos-aquae	0.023	0.035	**1.5**	0.35	**15**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

Risk quotients for green algae, aquatic vascular plants, and cyanobacteria exceed 1.0 for the worst case scenario. For the median case, only the risk quotient for cyanobacteria exceeds 1.0, at a value of 1.5. This indicates a potential risk for these receptors under acute exposure condtions in the absence of any dilution of raceway effluent in the receiving water. Further discussion of the risk quotients is provided in Section 7.2.6.5.

The VICH/CVM guidance specifies that the $PEC_{sediment}$ should be determined if the risk quotient for invertebrates is above 1. The data for *Daphnia magna*, above, indicate that the $PEC_{sediment}$ does not need to be determined.

Table 34 presents the results of the refined Tier A aquatic risk characterization with the inclusion of a 10-fold dilution factor in the receiving water. The only risk quotient greater than 1.0 is that for cyanobacteria, and only under the worst-case scenario. Section 7.2.6.5 provides further discussion.

**Table 34. Tier A aquatic risk characterization:
Refined PEC/PNEC ratios for flow-through raceways (with dilution)**

Organism	PNEC (mg/L)	Median-Case Scenario PEC (mg/L)	Median-Case Scenario PEC/PNEC	Worst-Case Scenario PEC (mg/L)	Worst-Case Scenario PEC/PNEC
Oncorhynchus mykiss	7.8	0.0035	0.00045	0.035	0.0045
Lepomis macrochirus	8.3	0.0035	0.00042	0.035	0.0042
Daphnia magna	3.3	0.0035	0.00106	0.035	0.0106
Navicula pelliculosa	6.1	0.0035	0.00057	0.035	0.0057
Pseudokirchneriella subcapitata	0.1	0.0035	0.035	0.035	0.35
Lemna gibba	0.076	0.0035	0.046	0.035	0.46
Anabaena flos-aquae	0.023	0.0035	0.15	0.035	**1.5**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

7.2.6.3 Initial Tier A Terrestrial Risk Characterization

The initial Tier A risk characterization for terrestrial organisms uses the initial PEC_{soil} and the Tier A PNEC values for exposures of terrestrial organisms. The initial PEC_{soil} of 0.15 mg/kg was determined by assuming that all of the florfenicol entering the system is bound to solids, which are then land applied.

Risk to soil microorganisms is evaluated based on the results of a laboratory study that evaluated effects on nitrogen transformation and carbon transformation (Carter, 2002). Effects on nitrogen transformation were transient and <25%, even at concentrations of 2.5 mg/kg, which is well above the PEC_{soil} of 0.15 mg/kg. Thus, no risk to soil microbes is anticipated.

The PNEC values at Tier A for terrestrial organisms are given in Table 17. The most sensitive toxicity result for a given species is used in the risk characterization. The appropriate PNEC values are tabulated with the initial PEC_{soil} and the PEC/PNEC ratios given in Table 35. Because all of the PEC/PNEC ratios were above 1.0, a refined Tier A terrestrial risk characterization was conducted.

Table 35. Tier A terrestrial risk characterization: Initial PEC/PNEC ratios for flow-through raceways

Organism	PNEC (mg/kg)	Initial PEC_{soil} (mg/kg)	PEC/PNEC Ratios
Earthworm	0.156	0.15	**1.0**
Cress	0.005	0.15	**30**
Wheat	0.067	0.15	**2.2**
Cabbage	0.009	0.15	**17**
Mustard	0.007	0.15	**21**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

7.2.6.4 Refined Tier A Terrestrial Risk Characterization

The refined Tier A risk characterization for terrestrial organisms uses the refined PEC_{soil}, which considers metabolism of florfenicol, treatment of only a portion of the fish in the facility, and degradation of the florfenicol in the feces produced by the fish and subsequently spread on agricultural land. Comparing the refined PEC_{soil} of 0.00018 mg/kg to the Tier A PNEC values from Table 17, it is evident that all of the risk quotients are below 1.0 (Table 36). Thus, no acute (Tier A) risks to terrestrial receptors are anticipated from exposure to florfenicol applied to agricultural soils.

Table 36. Tier A terrestrial risk characterization: Refined PEC/PNEC ratios for flow-through raceways

Organism	PNEC (mg/kg)	Refined PEC_{soil} (mg/kg)	PEC/PNEC Ratios
Earthworm	0.156	0.00018	0.0012
Cress	0.005	0.00018	0.036
Wheat	0.067	0.00018	0.0027
Cabbage	0.009	0.00018	0.020
Mustard	0.007	0.00018	0.026

7.2.6.5 Summary of Risk Characterization at Tier A

At Tier A, the initial risk characterization produced PEC/PNEC ratios that exceeded 1.0 under both the median-case and the worst-case scenarios for aquatic vascular plants and cyanobacteria, and also for green algae under the worst-case scenario. The calculation of the PEC_{water} assumed all of the dosed florfenicol enters the water column. The initial PEC_{water} did not consider in-facility dilution resulting from treatment of only a portion of the facility (some raceways) at any one time, or florfenicol metabolism in the fish. Therefore, a refined PEC_{water} was determined. With factors for metabolism and in-facility dilution included, Tier A risk quotients for green algae, aquatic vascular plants, and cyanobacteria exceed 1.0 for the worst-case scenario. For the median case, only the risk quotient for cyanobacteria exceeded 1.0, at a value of 1.5. With the inclusion of dilution of raceway effluent in receiving waters, the Tier A risk quotients were below 1.0 in all instances except for cyanobacteria under the worst-case scenario.

This indicates that peak concentrations of florfenicol could have short-term impacts on cyanobacteria. However, cyanobacteria and other unicellular organisms have very short generation times and are able to recover quickly from brief exposures to toxicants. Studies with green algae (Hoberg, 1991a–d) and diatoms (Jenkins, 2005) demonstrate that these organisms were able to resume growth once florfenicol was removed from the system. With rapidly growing organisms, unless complete mortality of the population occurs, the remaining organisms can rapidly reproduce, and the population regains its original density in a relatively short period of time. Moreover, although algal toxicity data are limited, there is a wide range of sensitivity to florfenicol and many algal species will not likely be affected by florfenicol under the expected exposure conditions. In addition, because there is functional redundancy in algal communities, even if sensitive species are affected, the overall productivity of the phytoplankton is likely to remain relatively constant. In addition, the release of florfenicol will be short-lived. Therefore, any inhibitory effects on these groups of organisms are likely to be temporary, and thus, short-term exposures to florfenicol are unlikely to have any significant impact at the population level.

For exposure of terrestrial organisms at Tier A, the amount of florfenicol that would partition to the solids in the settling ponds and subsequently be removed for land application was determined based on the assumption that all of the dosed florfenicol is bound to solids. Using the initial PEC_{soil}, risk quotients exceeded 1.0 for plants and earthworms. A refined PEC_{soil} was determined after consideration of metabolism, in-facility dilution, and degradation of florfenicol. The resulting risk quotients were all below 1.0. No risks to soil microbes were predicted using either the initial or refined PEC_{soil}. It can be concluded that no short-term risks to terrestrial receptors are anticipated from florfenicol in land-applied wastes from raceways.

7.2.7 TIER B RISK CHARACTERIZATION FOR FLOW-THROUGH RACEWAYS

7.2.7.1 Initial Tier B Aquatic Risk Characterization

As in Tier A, the Tier B risk characterization uses both a worst-case and a median scenario for fish reared in flow-through raceway systems. Both scenarios employ a representative raceway-type facility design. In the worst-case scenario, a high fish density (50 kg/m^3) and low flow rate (3L/s) were used to determine the PECs. The median scenario employs more realistic values for fish density (25 kg/m^3) and flow rate (15 L/s). The PEC_{water} for Tier B is determined based on the florfenicol in the system over 21 days of system flow and assumes that concentration is representative for chronic exposure.

The initial Tier B PEC_{water} values are divided by the Tier B PNEC values (based on chronic effects) for each species to obtain the risk quotients. The Tier B PNEC values for aquatic organisms are calculated as described in Table 18, from the NOEC or EC_{10} and an appropriate assessment factor. PNEC values are provided for one species of fish, three invertebrates, a diatom, a green alga, an aquatic vascular plant, and a cyanobacterium.

For the initial PEC_{water}, no metabolism or in-facility dilution are assumed. As a result, in the median-case scenario, the risk quotient exceeded 1.0 for cyanobacteria. In the worst-case scenario, risk quotients exceeded the level of concern for a number of organisms (Table 37). Thus, the Tier B PEC_{water} was refined in the next step of this assessment. The refinement considers metabolism and in-facility dilution, and subsequently, dilution in the receiving water.

Table 37. Tier B aquatic risk characterization: Initial PEC/PNEC ratios for flow-through raceways

Organism	PNEC (mg/L)	Median-Case Scenario PEC (mg/L)	Median-Case Scenario PEC/PNEC	Worst-Case Scenario PEC (mg/L)	Worst-Case Scenario PEC/PNEC
Pimephales promelas	0.55	0.037	0.067	0.372	0.68
Chironomus riparius	2.5	0.037	0.015	0.372	0.15
Daphnia magna	0.15	0.037	0.25	0.372	**2.5**
Brachionus calyciflorus	0.076	0.037	0.49	0.372	**4.9**
Navicula pelliculosa	1.87	0.037	0.020	0.372	0.20
Pseudokirchneriella subcapitata	0.075	0.037	0.49	0.372	**5.0**
Lemna gibba	0.039	0.037	0.95	0.372	**9.5**
Anabaena flos-aquae	0.011	0.037	**3.4**	0.372	**34**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

7.2.7.2 Refined Tier B Aquatic Risk Characterization

In the refined risk characterization, florfenicol metabolism (10% metabolism) and in-facility dilution (50% of facility treated at one time) are considered in order to more accurately determine the PEC_{water}. The calculation of the refined PEC_{water} for tilapia raceways is described in Section 7.2.3. The Tier B refined PEC_{water} values are 0.017 mg/L for the median scenario and 0.167 mg/L for the worst-case scenario. These values do not account for any dilution in the receiving water.

These refined PEC_{water} values are divided by the Tier B (chronic) PNEC values, providing the risk quotients in Table 38. Risk quotients exceed 1.0 (i.e., 1.6) only for cyanobacteria under the median-case scenario. Under the worst-case scenario, risk quotients slightly exceed the level of concern for *Daphnia magna* (1.1), *Brachionus calyciflorus* (2.2), and *Pseudokirchneriella subcapitata* (2.2). Exceedances are also noted for duckweed and cyanobacteria. However, these calculations do not account for in-stream dilution.

Once the assumption of 10-fold dilution in the receiving water is taken into consideration, the only risk quotient exceeding 1.0 is that for cyanobacteria under the worst-case scenario (Table 39). Further discussion of the risk quotients is provided in Section 7.2.7.5.

Table 38. Tier B aquatic risk characterization: Refined PEC/PNEC ratios for flow-through raceways (without dilution)

Organism	PNEC (mg/L)	Median-Case Scenario PEC (mg/L)	Median-Case Scenario PEC/PNEC	Worst-Case Scenario PEC (mg/L)	Worst-Case Scenario PEC/PNEC
Pimephales promelas	0.55	0.017	0.031	0.167	0.30
Chironomus riparius	2.5	0.017	0.0068	0.167	0.067
Daphnia magna	0.15	0.017	0.11	0.167	**1.1**
Brachionus calyciflorus	0.076	0.017	0.22	0.167	**2.2**
Navicula pelliculosa	1.87	0.017	0.0091	0.167	0.089
Pseudokirchneriella subcapitata	0.075	0.017	0.23	0.167	**2.2**
Lemna gibba	0.039	0.017	0.44	0.167	**4.3**
Anabaena flos-aquae	0.011	0.017	**1.6**	0.167	**15**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

Table 39. Tier B aquatic risk characterization: Refined PEC/PNEC ratios for flow-through raceways (with dilution)

Organism	PNEC (mg/L)	Median-Case Scenario PEC (mg/L)	Median-Case Scenario PEC/PNEC	Worst-Case Scenario PEC (mg/L)	Worst-Case Scenario PEC/PNEC
Pimephales promelas	0.55	0.0017	0.003	0.017	0.030
Chironomus riparius	2.5	0.0017	0.0007	0.017	0.0067
Daphnia magna	0.15	0.0017	0.011	0.017	0.11
Brachionus calyciflorus	0.076	0.0017	0.022	0.017	0.22
Navicula pelliculosa	1.87	0.0017	0.0009	0.017	0.0089
Pseudokirchneriella subcapitata	0.075	0.0017	0.023	0.017	0.22
Lemna gibba	0.039	0.0017	0.044	0.017	0.43
Anabaena flos-aquae	0.011	0.0017	0.16	0.017	**1.5**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

7.2.7.3 Initial Tier B Terrestrial Risk Characterization

To evaluate terrestrial risks at Tier B, first the initial PEC_{soil} was compared to the chronic (Tier B) PNECs for terrestrial receptors. The initial PEC_{soil} of 0.15 mg/kg was determined by calculating the amount of florfenicol that could be in the solids removed from an aquaculture facility, assuming all of the dosed material partitioned to the solids. Effects data at Tier B for deriving PNECs are available for three species of plants: cress, cabbage, and mustard (Table 19). These PNECs and the initial PEC_{soil} are presented in Table 40, along with the resulting PEC/PNEC ratios.

The initial Tier B risk quotients are all above 1, triggering a refined Tier B assessment.

Table 40. Tier B terrestrial risk characterization: Initial PEC/PNEC ratios for flow-through raceways

Organism	PNEC (mg/kg)	Initial PEC_{soil} (mg/kg)	PEC/PNEC Ratios
Cress	0.016	0.15	**9.3**
Cabbage	0.0123	0.15	**12**
Mustard	0.0123	0.15	**12**

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

7.2.7.4 Refined Tier B Terrestrial Risk Characterization

The refined PEC_{soil} was calculated by factoring in metabolism of florfenicol in the fish (10% metabolism), treatment of only a portion of the facility (50% treated), and degradation of florfenicol in the accumulated solids (DT_{50}=10 d; storage time 180 d). The refined PEC_{soil} was 0.00018 mg/kg. Comparing this value to the effects data at Tier B resulted in all PEC/PNEC ratios below 1.0 (Table 41), indicating that chronic effects to terrestrial receptors from land application of florfenciol-containing solids to agricultural land is unlikely.

Table 41. Tier B terrestrial risk characterization: Refined PEC/PNEC ratios for flow-through raceways

Organism	PNEC (mg/kg)	Refined PEC_{soil} (mg/kg)	PEC/PNEC Ratios
Cress	0.016	0.00018	0.011
Cabbage	0.0123	0.00018	0.015
Mustard	0.0123	0.00018	0.015

PEC/PNEC ratios ≥ 1.0 are shown in **bold**

7.2.7.5 Summary of Risk Characterization at Tier B

At Tier B, using data on the chronic exposure of aquatic organisms and chronic effects, the initial risk characterization produced PEC/PNEC ratios that exceeded 1.0 for daphnids, rotifers, green algae, aquatic vascular plants and cyanobacteria for the worst-case scenario, which was based on the highest stocking density and the lowest flow conditions. Risk quotients also exceeded 1.0 for cyanobacteria for the median scenario. The initial PEC_{water} did not account for in-facility dilution resulting from treatment of only a portion of the facility (some raceways) at any one time, nor did it account for metabolism of florfenicol in the fish. Also, the initial PEC_{water} was an end-of-pipe estimate and did not include any dilution in the receiving water. Therefore, a refined PEC_{water} was determined. With the inclusion of factors to account for metabolism and in-facility dilution, under the worst-case scenario, risk quotients exceeded the level of concern for duckweed, cyanobacteria, daphnids, rotifers, and green algae, but only slightly exceeded 1.0 for

the latter receptors (*Daphnia magna* at 1.1; *Brachionus calyciflorus*, and *Pseudokirchneriella subcapitata* at 2.2). Using the more typical conditions of stocking density and flow rate, the refined risk quotients at Tier B exceed 1.0 only for cyanobacteria (1.5). After consideration of a 10-fold dilution that the raceway effluent would be expected to undergo in the receiving stream, all risk quotients are below 1.0 for both scenarios, with the exception of cyanobacteria for the worst-case scenario. Note that both the initial PEC_{water} and the refined PEC_{water} are still conservative, because they do not account for partitioning of solids in the settling ponds prior to effluent discharge, nor any degradation in the settling ponds or in the receiving water.

The Tier B aquatic risk characterization thus indicates the possibility of adverse effects under worst-case conditions absent any in-stream dilution or additional fate processes (e.g., partitioning to solids, degradation), on green algae, aquatic vascular plants, and cyanobacteria. However, the effects data for these organisms are based on inhibition of growth, not mortality. Especially with rapidly growing organisms, unless complete mortality of the population occurs, the remaining organisms can reproduce rapidly, and the population regains its original density in a relatively short time. As discussed previously, studies with green algae (Hoberg, 1991a–d) and diatoms (Jenkins, 2005) demonstrate that these organisms were able to resume growth once florfenicol was removed from the system. Therefore, any inhibitory effects on these groups of organisms are likely to be temporary, and thus, short-term exposures to florfenicol are unlikely to have any significant impact at the population level. Even under the assumptions of a chronic (21-day) exposure scenario, florfenicol is used only for a limited time, and exposures would not be continuous. The slight exceedance of risk quotients for invertebrates was calculated only for the worst-case scenario of high stocking density and low flow, coupled with treatment of as much as 50% of the facility at once, and the absence of any dilution or degradation in the receiving stream. This combination of circumstances is highly unlikely.

For the aquatic risk characterization, it was conservatively assumed that there would be no partitioning of florfenicol to solids. However, for exposure of terrestrial organisms at Tier B, it was assumed that all of the florfenicol would partition to the solids in the settling ponds and subsequently would be removed for land application. The initial resulting risk quotients exceeded 1.0 for plants and earthworms. A refined PEC_{soil} was determined after consideration of metabolism, in-facility dilution, and degradation of florfenicol. The resulting risk quotients were all below 1.0. No risks to soil microbes were predicted using either the initial or refined PEC_{soil}. Thus, no chronic risks to terrestrial receptors are anticipated from florfenicol in land-applied wastes from flow-through raceways. It should be noted that, although the PEC_{soil} was determined based on degradation of florfenicol in the slurry prior to land application, the terrestrial risk assessment did not consider the further degradation that would occur once these solids were applied to land. The rapid degradation rates (DT_{50}) in the soil degradation study (27.2 d), the aerobic cow manure slurry study (2.4 d), and the anaerobic pig manure slurry study (1.0 d) all indicate that further degradation would occur, and thus, there would be very low risk from application of slurry containing florfenicol to agricultural lands.

7.2.8 SUMMARY OF AQUATIC AND TERRESTRIAL RISK CHARACTERIZATION FOR FLOW-THROUGH RACEWAYS

A Phase II Tier A and Tier B assessment was conducted, following VICH/CVM guidance, to examine the environmental risk of the use of Aquaflor® for culture of fish in flow-through raceways. The exposure assessment was based on the use of Aquaflor® at 15 mg/kg/day to treat tilapia. Raceway aquaculture systems typically have parallel series of one or more raceways and are operated on a flow-through basis, with settling ponds at the tail end of the raceways for solids removal prior to discharge to the receiving water. Because facility design is

highly variable, a representative design was selected and two scenarios were developed: a worst-case scenario using a higher fish stocking density with a lower flow rate, and a median scenario using a lower stocking density and higher flow rate.

To calculate the initial PEC_{water}, a number of conservative assumptions were used. All of the dosed medicated feed was assumed to be eaten, and all residues were assumed to be parent florfenicol and contained in the total water volume released from a typical facility during 10 days for the acute (Tier A) assessment, or during 21 days for the chronic (Tier B) assessment. Additionally, the potential loss of florfenicol from the water column due to partitioning to solids or potential biodegradation was not considered. Using the initial PEC_{water}, PEC/PNEC ratios at Tier A exceeded 1.0 for aquatic vascular plants and cyanobacteria under both scenarios, and additionally for green algae under the worst-case scenario. At Tier B, the risk quotients based on the initial PEC_{water} exceeded 1.0 for invertebrates, algae, aquatic plants, and cyanobacteria under the worst-case scenario, and for cyanobacteria for the median-case scenario. It should be emphasized that the initial PEC_{water} did not account for in-facility dilution that would result from the standard practice of treating only a portion of the facility at any one time, nor did it account for metabolism of florfenicol in the fish. In addition, the initial PEC_{water} did not consider in-stream dilution.

Therefore, a refined PEC_{water} was determined for both Tier A and Tier B for the median and worst case scenarios. Using the refined PEC_{water} considering metabolism and dilution, the PEC/PNEC ratios at Tier A exceeded 1.0 for cyanobacteria under the median case and for green algae, duckweed, and cyanobacteria under the worst-case scenario. Short-term effects under worst-case conditions and in the absence of any in-stream dilution could occur for these receptors; however, populations are expected to recover quickly. Once in-stream dilution was considered, the only risk quotient greater than the level of concern at Tier A was for cyanobacteria. At Tier B, the risk quotients exceeded 1.0 for cyanobacteria under the median case and for daphnids, rotifers, green algae, duckweed, and cyanobacteria under worst-case conditions. The risk quotients for daphnids, rotifers, and green algae only slightly exceeded 1.0, and moreover, in-stream dilution was not included in this step of the calculations. Once dilution of raceway effluent in the receiving water was considered, all risk quotients were below 1.0, with the exception of that for cyanobacteria under the worst-case conditions. Thus, the aquatic risk assessment indicates that effects on cyanobacteria are possible from chronic exposure to florfenicol, but even these are expected to be of short duration because florfenicol is not persistent in the environment and populations of microorganisms can recover quickly. Also, although treatment of infected fish could occur more than once during a growing season, each treatment event can be considered independent, as >99% of the total dose would enter the receiving water environment over a small window of time (10 days of treatment plus 7 days of depuration). The use of a factor of 50% for in-facility dilution is also considered conservative and provides for the possibility of overlapping non-synchronous treatments.

It should be emphasized that even the refined PEC_{water} is conservative, because it does not take into consideration any florfenicol degradation in settling ponds, effluent treatment systems that may be present, or the aquatic environment. An additional factor that would reduce the PEC_{water} and that was not considered quantitatively in the aquatic risk assessment is the partitioning of at least some florfenicol to the solids that are retained in the settling ponds and not discharged in the effluent.

Partitioning to solids was addressed, however, in the terrestrial risk assessment. The amount of florfenicol that would partition to the accumulated solids in the settling ponds at the tail end of the raceways and be subsequently removed for land application was determined. Using the

initial PEC_{soil}, risk quotients for plants and earthworms exceeded 1.0 at both Tier A and Tier B. However, using the refined PEC_{soil}, the PEC/PNEC ratios were below 1.0 at both Tier A and Tier B. There were no risks to soil microbes, even using the initial PEC_{soil}. It is thus concluded that there is no risk to terrestrial receptors. Additional conservatism in the terrestrial risk assessment results from the lack of consideration of the additional degradation that would occur once these solids were applied to land.

7.2.9 CONCLUSIONS REGARDING RISKS OF AQUAFLOR® USE IN FLOW-THROUGH WATER SYSTEMS

Based on the data, assumptions, and calculations presented in this Environmental Assessment, the use of Aquaflor® in flow-through aquaculture systems (i.e., raceways and tanks) for fish culture does not present any significant risk to the environment, due to the following combination of factors:

- Application of Aquaflor® is limited to:
  - Prescriptive application with no prophylactic use under the Veterinary Feed Directive
  - Application in feed at 15 mg/kg body weight/day for 10 days.
- Florfenicol, the active ingredient in Aquaflor®, will remain in water, where it dissipates due to degradation and dilution, or will partition to solids where it readily degrades.
- 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 transitory.
- PEC/PNEC ratios were based on flow-through raceway scenarios using tilapia, which are likely to be the species subject to the highest stocking densities and lowest flow rates, presenting the most conservative assessment.
- Using the refined PEC_{water} prior to consideration of in-stream dilution, risk quotients exceed 1.0 only for cyanobacteria at Tier A and Tier B under typical conditions.
- Under the worst case scenario, risk quotients exceed 1.0 in the absence of consideration of any dilution in the receiving water. After consideration of in-stream dilution, the only risk quotient exceeding 1.0 under worst-case conditions is for cyanobacteria.
- Using the refined PEC_{soil}, all PEC/PNEC ratios are below 1.0 for terrestrial organisms that could be exposed to florfenicol residues through land application of solids from the raceway settling ponds.
- Toxicity to the most sensitive organisms—algae and cyanobacteria—is based on inhibitory effects, which are transitory when exposure is removed. Thus, any inhibited populations are expected to recover rapidly, and widespread, ecologically significant or long-lasting impacts are not expected.

Based on this assessment and the factors listed above, the probability of a combination of circumstances resulting in any sustained adverse impacts on aquatic or terrestrial ecosystems from the use of Aquaflor® at 15 mg/kg/day for freshwater-reared finfish culture in flow-through water systems is considered to be very small.

8. MEDICATED FEED STORAGE, SPILL CLEANUP, AND DISPOSAL

8.1 Medicated Feed Storage

Medicated fish feed should be administered as soon as possible after delivery to the farm from the feed mill in accordance with the Veterinary Feed Directive. If medicated feed must be stored at the farm prior to administration, then such storage should comply with the information in "EPA Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category, Chapter 10: Material Storage for Flow-through, Recirculating, and Net Pen Facilities" (U.S. EPA, 2006).

8.2 Medicated Feed Spill Cleanup

Should medicated feed be spilled, the farm should have instituted a spill response plan developed in accordance with "EPA Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category, Chapter 10: Material Storage for Flow-through, Recirculating, and Net Pen Facilities" (U.S. EPA, 2006). Records of medicated feed spills are to be maintained in accordance with "EPA Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category, Appendix O: Spills and Leaks Log" (U.S. EPA, 2006).

8.3 Medicated Feed Disposal

Waste medicated feed (including any feed dropped or spilled) or unused feed in culture facilities is to be disposed of in accordance with local regulations, i.e., composted, incinerated, or placed in municipal landfills.

9. MITIGATION MEASURES

Because the use of Aquaflor® at 15 mg/kg/day in accordance with label directions poses no unacceptable short-term or long-term risks to aquatic or terrestrial ecosystems associated with commercial aquaculture of freshwater-reared fish using ponds or raceways, no mitigation measures will be required.

10. ALTERNATIVES TO THE PROPOSED ACTION

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

11. LIST OF PREPARERS

This document was prepared by Jane P. Staveley of Exponent, Inc. Other than the U.S. FDA/CVM, no state or federal agencies were consulted. Other experts consulted in the preparation of this document include Dr. Richard Endris (Intervet Inc. d/b/a Merck Animal Health) and Dr. Gregor Scheef (Intervet Innovation GmbH).

12. CERTIFICATION

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

Richard Endris

12 Dec 2011

Richard G. Endris, PhD.
Research Program Manager
Intervet Inc. d/b/a Merck Animal Health

Date

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