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

in Recirculating Aquaculture Systems

Indications Supported:

(1) Enteric septicemia associated with *Edwardsiella ictaluri*
(2) Columnaris disease associated with *Flavobacterium columnare*
(3) Streptococcal septicemia associated with *Streptococcus iniae*
(4) Furunculosis associated with *Aeromonas salmonicida*
(5) Coldwater disease associated with *Flavobacterium psychrophilum*

Fish Culture Systems Supported:

(1) Recirculating systems

DATE:	**August 15, 2013**
NAME OF APPLICANT/PETITIONER:	**Intervet Inc d/b/a Merck Animal Health**
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1. DESCRIPTION OF PROPOSED ACTION(S) AND NEED

Aquaflor® Type A Medicated Article (premix) contains the active ingredient florfenicol, a synthetic, broad-spectrum antibiotic, effective in the control of a variety of fish pathogens. Aquaflor® 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.

An environmental assessment (EA) dated December 9, 2011, was prepared in support of the supplemental NADA approved on April 4, 2012, for the use of Aquaflor® (florfenicol) Type A Medicated Article in finfish feeds for control of mortality for the following indications:

- Enteric septicemia associated with *Edwardsiella ictaluri* in catfish up to 15 mg/kg for 10 consecutive days
- Columnaris disease associated with *Flavobacterium columnare* in freshwater-reared finfish up to 15 mg/kg for 10 consecutive days
- Streptococcal septicemia associated with *Streptococcus iniae* in freshwater-reared warmwater finfish up to 15 mg/kg for 10 consecutive days
- Furunculosis associated with *Aeromonas salmonicida* in freshwater-reared salmonids up to 10 mg/kg for 10 consecutive days
- Coldwater disease associated with *Flavobacterium psychrophilum* in freshwater-reared salmonids up to 10 mg/kg for 10 consecutive days

The 2011 EA supports uses of Aquaflor® in two types of aquaculture systems: ponds and flow-through water systems (e.g., raceways, tanks). This document serves as a supplement to the 2011 EA to evaluate environmental impacts from the proposed use of Aquaflor® in recirculating systems for freshwater-reared finfish at the same maximum dose rate, up to 15 mg florfenicol/kg body weight for 10 consecutive days.

This supplement has been prepared as a stand-alone document. Therefore, the information in Sections 2, 4, 5, 6, 8, 10, 11 and 12 is essentially the same as that presented in the December 9, 2011 EA. The information in Sections 3, 7, 9, and 13 is new or revised to reflect the exposure scenarios for recirculating systems.

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 *Edwardsiella ictaluri* when the waters are between 20ºC and 30ºC. 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 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 more susceptible to infection than juveniles, but fishes of all ages can be infected (Agnew, 2007). The disease can occur at temperatures 20-40ºC, with an optimal growth temperature of 37°C (Zhou et al., 2008).

Furunculosis is a contagious disease caused by *Aeromonas salmonicida*, a significant pathogen of salmonids, which in its atypical form has spread into cyprinids and marine flatfish. *A. salmonicida* has also been implicated in other conditions, notably ulcerative dermatitis (Austin and Austin, 2012).

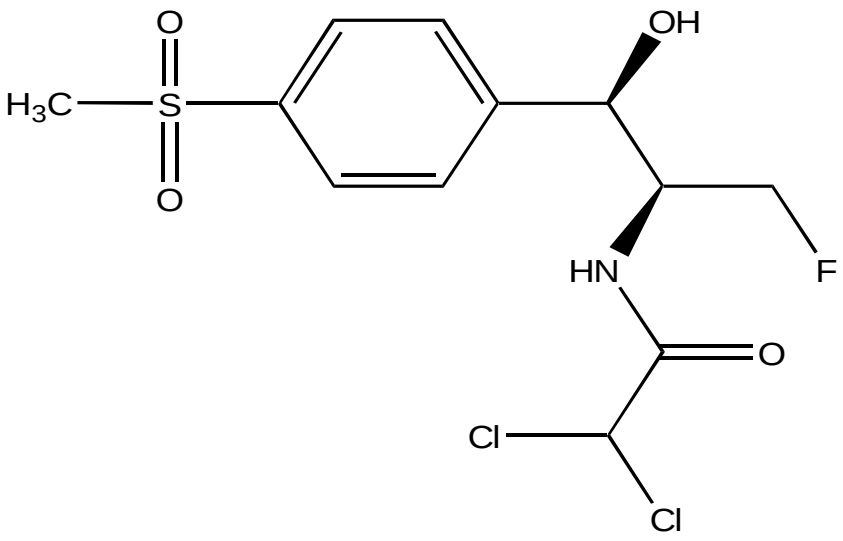
Coldwater disease, also called BCWD (bacterial coldwater disease) in North America and rainbow trout fry syndrome in Europe, and other infections caused by *Flavobacterium psychrophilum* are a world-wide concern, particularly for freshwater salmonid hatcheries. Juvenile rainbow trout and coho salmon are particularly susceptible, but *F. psychrophilum* infections have been reported in a wide range of both anadromous and non-anadromous salmonids of various sizes, and in other species as well (eel, carp, perch, etc.) (Barnes and Brown, 2011).

Aquaflor® is the first FDA approved drug for treatment of columnaris disease and streptococcal septicemia in freshwater-reared 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

The 2011 EA evaluated the risk of Aquaflor® in ponds and flow-through raceways. After the supplemental NADA was approved on April 4, 2012, restrictions were put on the product label: "Not for use in recirculating aquaculture systems. The effect of florfenicol on recirculating biofilters and water quality has not been evaluated." Therefore this EA supplement was prepared to evaluate effects of Aquaflor® in recirculating aquaculture systems.

The general risk assessment approach in this EA amendment follows the process described in CVM Guidance for Industry #166 (Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) – Phase II (CVM, 2006; VICH, 2004)[1] combined with EMEA Guidance (EMEA, 2008) as applied to aquaculture.

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.

[1] 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.

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 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.

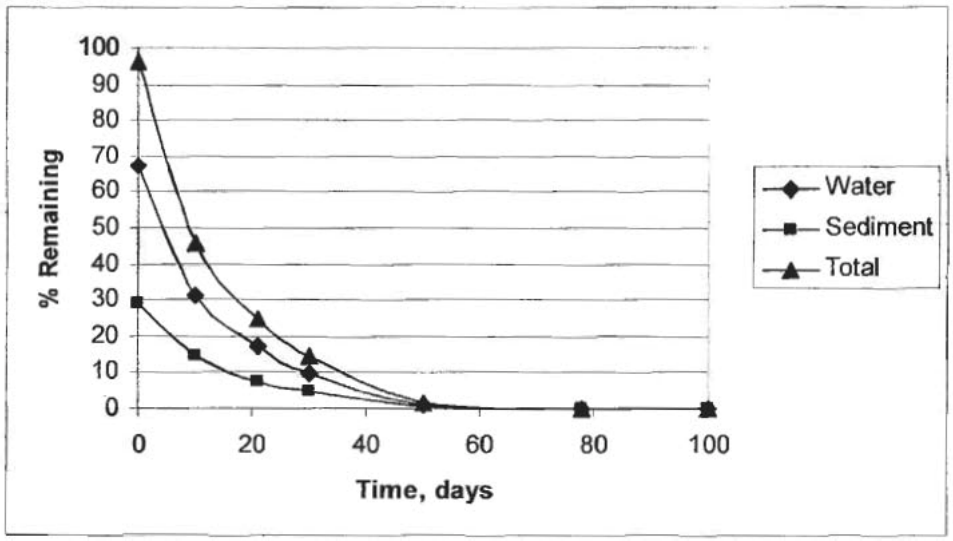
The similar pattern of appearance and decline of metabolites was observed for all three sediments. Parent [^{14}C]-florfenicol was observed to partition between water and sediment and to degrade in both fractions of the test system (Figure 2). Half-lives ranged from 8.4 to 19.4 days for the three sediment/water 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[2] 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.

[2] 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 (mg/L)	>2.9	>2.7	>0.98	80
NOEC (mg/L)	2.9	2.7	0.98	38
Maximum cell density, 14 days				
MIC (mg/L)	1.5	2.7	0.26	80
NOEC (mg/L)	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	
LOEC, 96-h	1.5	
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	
LOEC, 96-h	0.20	
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	
LOEC, yield (based on frond number), 7-d	0.94	
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[3] 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.

[3] 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.

However, reproduction was reduced at the highest test concentration. Thus, the NOEC, based on the most sensitive parameter, reproduction, was 1.5 mg/L.

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 Chironomid 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: Carbon Transformation 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 such that there was less than 25% effect relative to the control (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, 2.5	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 (in this case, recirculating 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 consecutive days. This dosing rate is at the upper end of the range requested to be approved for the five disease indications that are being evaluated in this EA amendment (i.e., enteric septicemia, columnaris, streptococcal septicemia, furunculosis, and coldwater disease). 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 8ºC and 100% feed consumption at 15ºC (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.

7.1 PHARMACOKINETICS AND RESIDUES IN FISH

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 salmonids 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 10ºC (Pinault, 1997a). Following oral intubation at 10ºC and oral administration of

medicated feed at 16ºC, bioavailabilities of 73.9% and 66.3%, respectively, were determined for rainbow trout (Pinault, 1997a). The residue levels in the plasma of trout fed medicated feed treated with florfenicol at 10ºC, 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 GLP-compliant residue studies were conducted with rainbow trout, one at 8ºC and one at 15ºC (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 8ºC and 15ºC, 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 8ºC. More rapid and more uniform depletion occurred at 15ºC. 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 8ºC and 1.48 mg/kg (15.1% of initial dose) at 15ºC. At four days post-treatment, mean residues are 26.4% and 5% of the initial dose at 8ºC and 15ºC, 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 8ºC and 0.29 mg/kg at 15ºC, 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).

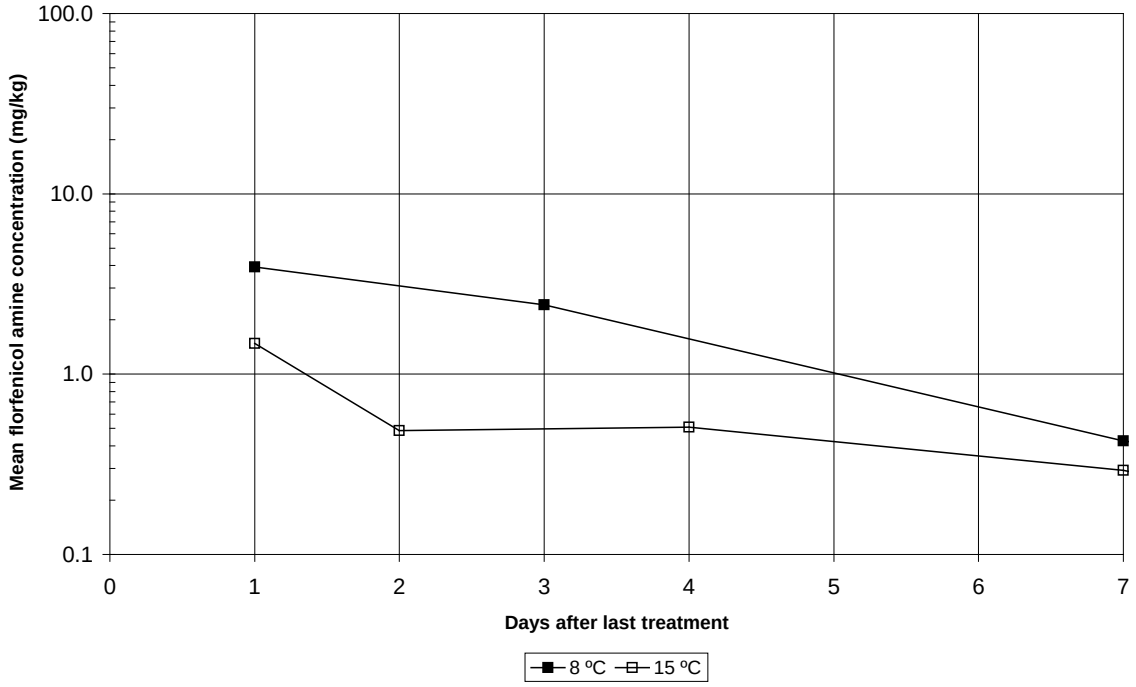
Table 20. Florfenicol-related residues in rainbow trout kept at 8 and 15 °C

Time after Last Treatment[2]	Florfenicol Amine Residue (mg/kg), Corrected for Recovery[1]			
	8ºC (n = 18–21) (Roy, 2002a)		15ºC (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 15ºC 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 8ºC and 15ºC, 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.

Recent studies performed to examine residue depletion in recirculating systems are germane to this EA amendment. Gaikowski et al. (2011) examined the depletion of florfenicol (measured as florfenicol amine) from tilapia (*Oreochromis* sp.) reared in a commercial size, dual rearing tank recirculating aquaculture system (RAS) following administration of florfenicol-medicated feed at a dose rate of 20 mg/kg body weight for 10 days. (This dose is above the 15 mg/kg dose that is the subject of this EA). The system consisted of two tanks of approximately 1,900 L each, which shared a mechanical (clarifier and suspended solids) filter and a biological filter where water commingled. The system recirculated 93.6% of the water, with an average daily replacement of 6.4%. Tilapia of near market size (average weight 447 g) were held at a stocking density of approximately 0.038 kg/L (38 kg/m^3), similar to that used at MinAqua, a large, commercial tilapia production facility which employs a recirculating system. The study was conducted at a temperature of 27 ± 2°C. Groups of 10 fish were collected from each tank prior to dosing and at nine time points after the completion of dosing for analysis of florfenicol amine (FFA) in the skin-

on fillets. Mean FFA levels, uncorrected for method recovery, decreased from 13.77 µg/g at 1 hour after the end of dosing to 0.39 µg/g ten days after the end of dosing.

Another study was conducted by the same group of investigators to determine the depletion of florfenicol in the tissues of rainbow trout (*Oncorhynchus mykiss*) maintained in a commercial RAS (Meinertz, 2012). The dual tank rearing system described above for the tilapia study was used. Rainbow trout (126 to 617 g; average weight 305–310 g) were treated at a nominal dose of 20 mg florfenicol per kg body weight per day for 10 days (again, above the 15 mg/kg/day dose that is the subject of this EA). In this study, however, fish were treated in only one of the two tanks (Tank A) in the dual rearing system, and the system recirculated 80.1% of the water, with 19.9% daily replacement. The treated tank contained 149 fish while the untreated tank contained 77 fish. Based upon these numbers, the average weight of the fish, and the reported amount of water in the system (3,195 L), the total density in the system was 21.7 kg/m^3 with the density in the treated tank at 14.7 kg/m^3. The study was conducted at a temperature of 12 ± 2 °C. Groups of 16 fish were collected from the dosed tank (Tank A) and groups of 8 fish collected from the non-dosed tank (Tank B) at nine time points after the completion of dosing. Analysis of FFA in the skin-on fillets was performed. Mean FFA levels were 11.6 µg/g at 12 hours and depleted to 0.25 µg/g at 240 hours (10 days) and further to 0.12 µg/g at 480 hours (20 days).

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 10-day 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 resuspension 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.

7.2 RECIRCULATING SYSTEMS

The use pattern considered in this EA Amendment is use of Aquaflor® in RAS. Although a variety of species may be grown in recirculating systems, tilapia are hardy and, as such, are suitable for culture at the high densities needed to make recirculating systems economically viable. Because florfenicol use is based on the biomass of fish treated, more of the compound is used with higher densities. Thus, tilapia aquaculture will serve as a conservative basis for assessing the environmental impact of Aquaflor® use in recirculating systems with any fish species.

Recirculating systems, though initially capital intensive, have reduced land and water requirements, can allow for year-round production, and can be located close to markets (Masser et al., 1999). As described by U.S. EPA (2004):

> Recirculating systems are highly intensive culture systems that actively filter and reuse water many times before it is discharged. These systems typically use tanks or raceways to hold the growing animals and have extensive filtration and support equipment to maintain adequate water quality. Recirculating systems use biological filtration equipment to remove ammonia from the production water. Solids removal, oxygenation, temperature control, pH management, carbon dioxide control, and disinfection are other common water treatment processes used in recirculating systems.

Typically, >90% of the water is recycled through the biofilters, while <10% of the water is replenished daily. The majority of RAS recirculate >90% of the water, with some as high as 99%. While 80% recirculation could occur, as used in the Meinertz (2012) study, this will not be the case at a majority of facilities. The volume replenished is associated primarily with waste removal (i.e., flushing waste from the bottom of the tank), and waste is usually removed to a settling or holding system (e.g., tank, pond) or directly to municipal sewers. This exposure assessment is based upon a recirculation rate of 95%, which is considered representative, worst-case, and also allows for comparison against the experimental data of Gaikowski et al. (2011).

7.3 PRODUCTION AND DISEASE TREATMENT OF TILAPIA IN RECIRCULATING SYSTEMS

The most common culture method for tilapia in the U.S. is the indoor recirculating system with a high level of water conservation. Recirculating systems accounted for 70% of U.S. tilapia production in 1997 (Kohler, 2000).

Aquaflor® is effective in the treatment of streptococcal septicemia in tilapia. As discussed previously, the quantities of florfenicol administered in the feed to obtain the desired dosage depend on the quantities and weight of fish requiring treatment.

Florfenicol and its metabolites enter the environment in excreta; entry through uneaten feed is inconsequential. Both florfenicol and its metabolites move into the water column through leaching from feces and by mixing of the aqueous phase of excreta into the water column. Nearly all tilapia feed is formulated as floating, extruded pellets. This 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. In addition, the amount of solid waste is carefully controlled and minimized in recirculating systems (Losordo et al., 1998; DeLong et al., 2009; Masser et al., 1999). Excess waste interferes with the function of the biofilters. Cichlids such as tilapia are aggressive feeders, and 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 solid (including solid waste or the solid phase of biofilters) and water phases and would degrade in each of these compartments.

Tilapia are warmwater fish that grow best when the water temperature is in the high-20°C range (approximately 27 to 29ºC; DeLong et al., 2009). As a result, most culture systems, specifically indoor re-circulating systems, are designed for water and heat conservation (Fitzsimmons,

2000). The indoor recirculating system using tanks is representative of most current tilapia production systems in the U.S. (Mel Stocks, personal communication)[4] and is used here as the principal example for estimating potential releases of florfenicol to the environment. These tanks require biofilters to clean the water primarily of nitrogen-containing compounds, especially ammonia, nitrate, and nitrite. There is much literature about the structure and design of various types of biofilters (Chen et al., 2002), but these are not discussed in detail in this EA.

Recirculating systems produce a small volume of effluent, mostly made up of solids removed by process equipment in the system, which are added to overtopping water. The overtopping water is system water displaced by make-up water (typically about 5% to 10% of the system volume each day) added to maintain water quality and replace water lost in evaporation and solids removal.

Recirculating systems in which warmwater species are cultured typically use 16 gallons of water for every pound of production (U.S. EPA, 2004). The density of fish in the tanks varies with their size. Table 21 is based on recommendations from Masser et al. (1999) on stocking rates for different size groups of tilapia in tanks in recirculating systems. These data were used to select a low, median or typical, and high stocking density for use in derivation of the PEC_{water}. The selected low, typical and high values are 0.16, 25, and 50 kg/m^3. These ranges account for treatment of juveniles, subadults and adults—any age group that may become infected with disease.

Table 21. Stocking rates, fish weights, and densities for different sizes of tilapia

Stocking Rate (no. fish/m^3)	Fish Weight (g)		Growth Period (days)	Weight Density (kg/m^3)	
	Initial	Final		Initial	Final
8,000	0.02	0.5 – 1	30	0.16	4 – 8
3,200	0.5 – 1	5	30	1.6 – 3.2	16
1,600	5	20	30	9	32
1,000	20	50	30	20	50
500	50	100	30	25	50
200	100	250	30	20	50
100	250	450	70	25	45

Reference: Masser et al., 1999. Stocking rates converted to no. fish/m^3 and rounded off. Final weight density reflects 100% survival.

Parameters for the recirculating systems used to construct the exposure scenarios are based on literature on recirculating systems, with an emphasis on systems for culturing tilapia (although

[4] Mel Stocks, President, MinAqua Fisheries, Renville, MN, July 26, 2011.

other species such as hybrid bass and even salmonids can be cultured in recirculating systems). Tanks can be of different shapes, but circular tanks are somewhat easier to clean, and they circulate water better. Sizes can range from 500 to 500,000 gallons and depend on stocking rates, species, water supply, water quality, and economic considerations. Tanks must be designed to correspond to the capacity of the other components of the system, particularly the size of the biofilter and sump (Helfrich and Libey, undated).

Some examples of recirculating systems, discussed in order of facility size (low to high), are summarized here and in Table 22. As noted by Losordo (1998), commercial operators often consider their systems as proprietary designs and do not release detailed information. For this reason, data from public university facilities are also included.

At Virginia Tech, there are nine 2,250-gallon rectangular tanks, each independent of the others (Helfrich and Libey, undated). Grayson Hills Farms in Illinois initially had eight 5,000-gallon tanks, but this was later reconfigured to four 10,000-gallon tanks (Brown et al., undated). Northern Tilapia, a small business in Lindsay, Ontario, has a growout system for tilapia of four 10,000-gallon tanks (Losordo, 1998). In the design specifications of Krause et al. (2006), which are based on the Cumberland County College Fish Barn, there are four 18,500-gallon tanks, of which two are linked together, as well as two 4,000-gallon quarantine tanks with a separate water treatment system. The North Carolina State University (NCSU) Fish Barn (Losordo et al., 2000) has four 15,850-gallon tanks, of which two are linked together. This facility also has a 1,350-gallon quarantine tank and a 3,500-gallon secondary quarantine/nursery tank. Although not specifically for tilapia, design specifications for RASs prepared by Hutchinson et al. (2004) present sample calculations using a tank volume of 53,000 gallons for barramundi. The ADM tilapia facility in Illinois has nine 10,000-gallon tanks, while Purdue University has three tanks of 80,000 gallons each (Brown et al., undated). Dakota Fish-N-Fillet in Binford, North Dakota, has two 6,500-gallon tanks used as nursery and harvest/holding tanks, and eight 10,000-gallon growout tanks; all of the water flows to one common treatment system (Losordo, 1998). Southern Farms Tilapia operates two growout units, one near Castalia, North Carolina, and one near Wilson, North Carolina, that are nearly identical. Each facility is composed of two separate recirculating systems, with each system having three 95-m^3 (approx. 25,000-gallon) tanks, for a total of approximately 75,000 gallons (DeLong and Losordo, 2006). AquaMar Industries, of Pocomoke City, Maryland, has 18 growout tanks of 30,000 gallons each, with individual treatment systems (Losordo, 1998). MinAqua Fisheries uses a recirculating system of 2,000,000 gallons, which is divided into 24 large tanks and 16 smaller tanks. Two to eight tanks are connected. In a smaller 8-tank connected system, there are 75,000 gallons (Mel Stocks, personal communication, July 26, 2011).

Using this information, which is tabulated in Table 22, a scenario for a typical facility was developed using a total volume of 100,000 gallons. (The median total facility volume from the tabulated data is 90,000 gallons).

Table 22. Tank volumes at recirculating facilities

Facility	Number of Tanks	Volume per Tank (gallons)	Number of Tanks Connected to a Single Biofilter	Volume of Water per RAS/Biofilter (gallons)	Total Facility Volume (gallons)
Virginia Tech	9	2,250	1	2,250	20,250
Grayson Hills Farms	4	10,000	NS[1]	-	40,000
Northern Tilapia	4	10,000	1[2]		40,000
Design specifications (Hutchinson et al., 2004)	1	53,000	-	-	53,000
Cumberland County College Fish Barn	4	18,500	2	37,000	82,000
	2	4,000	1	4,000	
NC State University	4	15,850	2	31,700	68,250
	2	1,300; 3,500	1	1,300 or 3,500	
ADM - Illinois	9	10,000	NS	-	90,000
Dakota Fish-N-Fillet	8	10,000	10	93,000	93,000
	2	6,500			
Southern Farms Tilapia #1	6	25,000	3	75,000	150,000
Southern Farms Tilapia #2	6	25,000	3	75,000	150,000
Purdue University	3	80,000	NS	-	240,000
AquaMar	18	30,000	1	-	540,000
MinAqua Fisheries	40		2 - 8	75,000 or more	2,000,000

[1]NS = not stated
[2] Although there are separate biofilters for each tank, the tanks share a common recirculating water supply

This information was also used to develop assumptions regarding the percentage of the fish (or tanks) treated at one time. Because a number of the facilities described above have two independent RAS (four tanks total with two connecting, or six tanks total with three connecting), a possible yet highly conservative estimate of the percentage of tanks treated at any one time is 50%. Treatment of 50% of the facility at one time would be highly unusual (Mark Gaikowski, personal communication).[5] Facilities having a multiple grow-out tank system are designed such that each RAS contains a different age group of fish, so the facility can stagger the production of market-ready fish. It is not likely that different age groups would become infected

[5] Mark Gaikowski, Branch Manager, Aquatic Ecosystem Health, USGS Upper Midwest Environmental Sciences Center, LaCrosse WI, June 17, 2011.

simultaneously. However, if they did, the maximum water volume that would be treated for the dual systems described is 50% of the total aquaculture facility volume.

Based on the literature, the water volume replaced in recirculating systems is cited as 5% to 10% per day (Masser et al., 1999; FAO, 2005-2010; Losordo et al., 2000); one turnover per hour (4%) (Helfrich and Libey, undated); <10% (Krause et al., 2006); and <10% by definition (Hutchinson et al., 2004). It is possible that a higher proportion of the water could be replaced, particularly if higher dissolved oxygen requirements are needed. However, by increasing the percentage of make-up water to maintain water quality, facilities may also be able to increase oxygen levels in the tanks by adding air stones or other forms of aeration. A recirculation rate of 95% was used to construct the exposure scenarios in this assessment. This is conservative, because it provides for a smaller volume of effluent (only 5% discharged) and thus a higher concentration of florfenicol in the effluent.

7.4 INITIAL PEC_{water} FOR RECIRCULATING SYSTEMS

The initial PEC_{water} was calculated using the assumptions in Table 23 for a low, typical, and high standing density. The initial PEC_{water} calculations assume complete equilibrium of the mass of florfenicol in the feed to the recirculating system and effluent, with no partitioning, degradation, or metabolism. No dilution in the receiving water is assumed.

Table 23. Assumptions used to calculate initial PEC_{water} for recirculating systems

Parameter	Value
Dose (mg/kg/day)	15
Treatment period (days)	10
Percent of feed consumed	100
Percent of florfenicol metabolized	0
Fish density at treatment, kg/m^3	
Low density	0.16
Typical density	25
High density	50
Volume of water in facility (total), gal	100,000
Percent of tanks treated	50
Water recirculation (% system volume)	95
Daily water release/replacement (% system volume)	5
Dilution factor in receiving water	1

Using a total facility volume of 100,000 gallons (= 378,541 L), and assuming that 50% of the tanks are treated, the mass of fish treated for each density is determined as follows:

Eq. 1: Mass of fish treated (kg) = Fish density (kg/m^3) × fraction treated × facility volume (L) × m^3/1,000 L

Using the low density scenario as an example:

Eq. 1a: Mass of fish treated (kg) = 0.16 kg/m^3 × 0.5 × 378,541 L × m^3/1000 L
= 30.3 kg

The amount of florfenicol used each day is determined by Eq. 2:

Eq. 2: Daily mass of florfenicol used (kg/day) = 15 mg/kg/day × mass of fish treated (kg) × 1 E-6 mg/kg

Using the low density scenario as an example:

Eq. 2a: Daily mass of florfenicol used (kg/day) = 15 mg/kg/day × 30.3 kg × 1E-6 mg/kg
= 0.00045 kg/day

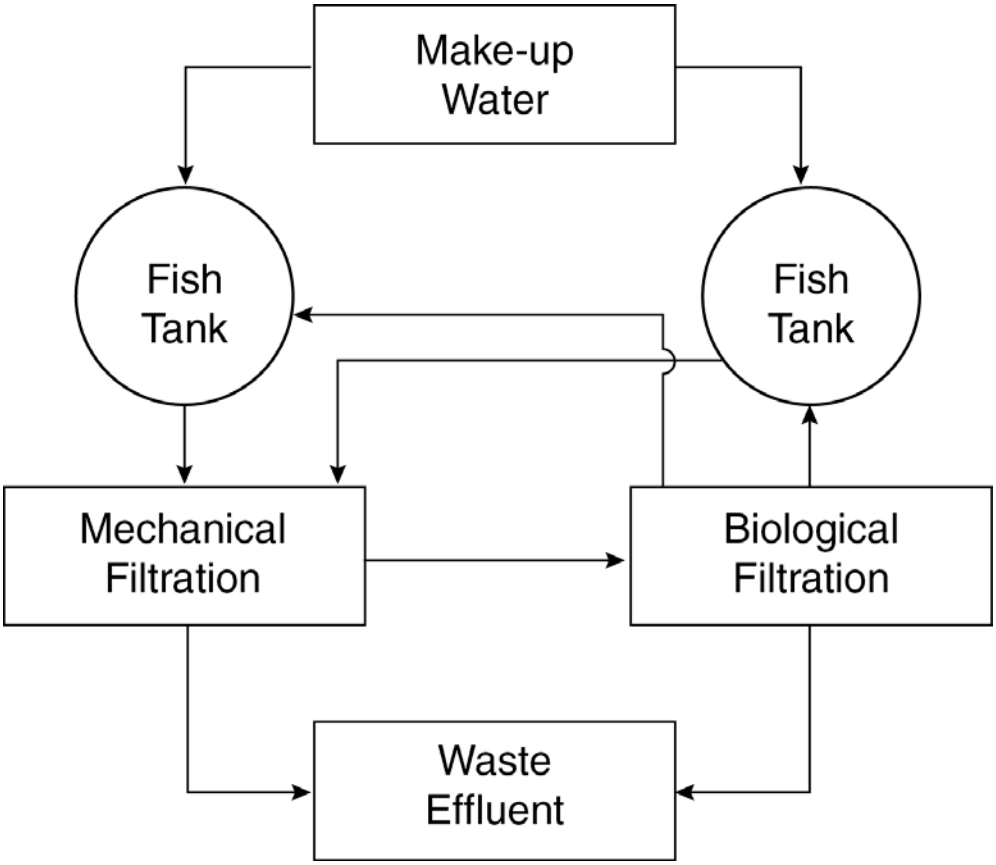
For the three densities of fish, these calculations result in the values given in Table 24.

Table 24. Florfenicol used for three fish densities assuming 50% treatment

Parameter	Low Density	Typical Density	High Density
Mass of fish treated, kg	30.3	4732	9464
Daily mass of FFC, kg	0.00045	0.0710	0.1420

The mass of florfenicol can be estimated in a recirculating tank system over a 10-day treatment period based upon the water recirculation rate. Figure 4 shows the system schematically and indicates the components of the mass balance.

Figure 4. Schematic of recirculating system



On day *n*, the amount of florfenicol in the wastewater emitted from the facility can be approximated based on the mass of florfenicol in the tank. The equations below incorporate the conservative assumption that the filters do not aid in the removal or degradation of florfenicol from the water; the calculation also reflects the assumption that the detention time in the recycle loop is approximately one day. Finally, although solids removal occurs, it is conservatively assumed for the initial calculations that all of the dosed florfenicol is excreted into the water compartment and remains there.

Eq. 3: $\text{Mass}_{\text{tank, day n}} = \text{Mass}_{\text{tank, day n-1}} + \text{Mass}_{\text{input, day n}} - \text{Mass}_{\text{effluent, day n}}$

Eq. 4: $\text{Mass}_{\text{recycle, day n}} = \text{Mass}_{\text{tank, day n-1}}$

The example used below in Table 25 is for the typical density scenario. As calculated in Table 24, a daily input of 0.071 kg florfenicol is made each day to a 100,000 gallon facility when 50% of the facility is treated at one time. On each day, the mass of florfenicol in the water is determined by (1) the amount of florfenicol excreted into the water by the treated fish (assumed to be 100% of the amount added in feed) plus (2) the amount already in the recycled water from previous treatment days because 95% of the water, and thus florfenicol, is recycled into the next day's total. Solids removal also occurs; however, it is conservatively assumed for the initial calculations that all of the dosed florfenicol is in the water compartment. Thus, there is a progressive increase in the total mass of florfenicol in the effluent during the 10-day treatment period, reflecting addition of medicated feed to the system, as well as dilution by daily water

replacements (~5%). After the 10-day treatment period, the mass of florfenicol in the water is expected to gradually decline. It should be noted that this simplistic yet conservative approach does not account for the florfenicol that would be lost from the fish to the water column during depuration (i.e., metabolism in the fish).

Table 25 presents the calculations for the typical density treatment scenario, carried through day 33. These calculations are presented graphically in Figure 5. The peak amount of florfenicol in the effluent occurs on day 10 of treatment and is estimated to be 0.028 kg for the typical density scenario. This peak value is used to calculate the PEC_{water} for the acute exposure scenario. The total amount of florfenicol released in effluent over 21 days for the typical density is estimated to be 0.402 kg, and the total over 33 days is estimated to be 0.543 kg. These longer-term values are used to calculate the PEC_{water} for the chronic exposure scenario. Calculations are similar for the low-density and high-density scenarios. Note that Table 25 and Figure 5 present data on mass, not concentration; concentrations are calculated in the next step.

A summary of the results of these calculations for the three fish densities is presented in Table 26.

Table 25. Calculation of florfenicol mass in effluent of recirculating system with 95% recirculation and 50% of fish treated: typical density scenario

Day	FFC in Feed per Day (kg)	FFC Excreted (kg)	FFC Input from Recycle (kg)	Total Mass FFC in System (kg)	Mass FFC Recycled (kg)	Mass FFC in Effluent (kg)
1	0.0710	0.0710	0.0000	0.0710	0.0675	0.0036
2	0.0710	0.0710	0.0675	0.1385	0.1315	0.0069
3	0.0710	0.0710	0.1315	0.2025	0.1924	0.0101
4	0.0710	0.0710	0.1924	0.2634	0.2502	0.0132
5	0.0710	0.0710	0.2502	0.3212	0.3052	0.0161
6	0.0710	0.0710	0.3052	0.3762	0.3574	0.0188
7	0.0710	0.0710	0.3574	0.4284	0.4069	0.0214
8	0.0710	0.0710	0.4069	0.4779	0.4540	0.0239
9	0.0710	0.0710	0.4540	0.5250	0.4988	0.0263
10	0.0710	0.0710	0.4988	0.5698	0.5413	0.0285
11	0.0000	0.0000	0.5413	0.5413	0.5142	0.0271
12	0.0000	0.0000	0.5142	0.5142	0.4885	0.0257
13	0.0000	0.0000	0.4885	0.4885	0.4641	0.0244
14	0.0000	0.0000	0.4641	0.4641	0.4409	0.0232
15	0.0000	0.0000	0.4409	0.4409	0.4189	0.0220
16	0.0000	0.0000	0.4189	0.4189	0.3979	0.0209
17	0.0000	0.0000	0.3979	0.3979	0.3780	0.0199
18	0.0000	0.0000	0.3780	0.3780	0.3591	0.0189
19	0.0000	0.0000	0.3591	0.3591	0.3412	0.0180
20	0.0000	0.0000	0.3412	0.3412	0.3241	0.0171
21	0.0000	0.0000	0.3241	0.3241	0.3079	0.0162
22	0.0000	0.0000	0.3079	0.3079	0.2925	0.0154
23	0.0000	0.0000	0.2925	0.2925	0.2779	0.0146
24	0.0000	0.0000	0.2779	0.2779	0.2640	0.0139
25	0.0000	0.0000	0.2640	0.2640	0.2508	0.0132
26	0.0000	0.0000	0.2508	0.2508	0.2382	0.0125
27	0.0000	0.0000	0.2382	0.2382	0.2263	0.0119
28	0.0000	0.0000	0.2263	0.2263	0.2150	0.0113
29	0.0000	0.0000	0.2150	0.2150	0.2043	0.0108
30	0.0000	0.0000	0.2043	0.2043	0.1940	0.0102
31	0.0000	0.0000	0.1940	0.1940	0.1843	0.0097
32	0.0000	0.0000	0.1843	0.1843	0.1751	0.0092
33	0.0000	0.0000	0.1751	0.1751	0.1664	0.0088

Figure 5. Florfenicol mass in effluent of recirculating system with 95% recirculation and 50% of fish treated: typical density scenario

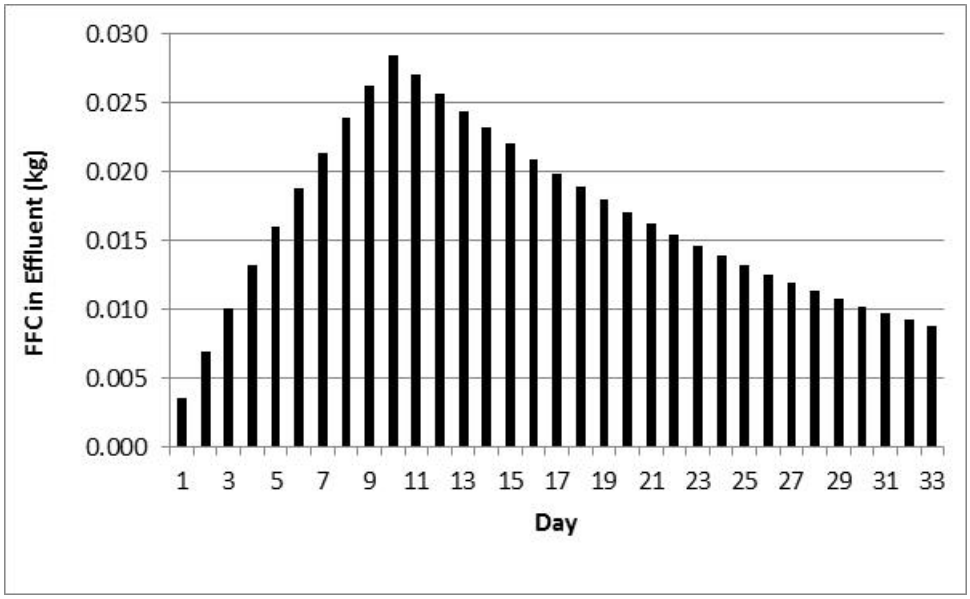


Table 26. Mass of florfenicol in effluent (peak and longer-term totals) for three fish density scenarios at 95% recirculation and 50% of fish treated

Mass of Florfenicol (kg)	Low Density	Typical Density	High Density
Peak (released on day 10)	0.00018	0.0284	0.0596
21-day total (total released over 21 days)	0.00257	0.4020	0.8039
33-day total (total released over 33 days)	0.00348	0.5435	1.086

Note: Calculations were done with more significant figures than presented here.

It can be seen that the florfenicol mass released is directly proportional to the dose rate, which in turn, is directly proportional to the biomass of fish treated. (For example, the 21-day total florfenicol released at a high density (50 kg/m^3) is twice the amount for a typical density (25 kg/m^3).

To determine the concentration of florfenicol released, the mass of florfenicol is divided by the volume of effluent discharged. For the acute PEC_{water}, the peak mass of florfenicol in effluent is divided by the daily discharge volume, as defined by Eq. 5:

Eq. 5: Acute PEC_{water} (mg/L) = peak mass FFC in effluent (kg) / daily discharge volume (L) $\times$ 1E6 (mg/kg)

With 95% of the water recycled and 5% discharged, the discharge volume is calculated as 5% of the total facility volume of 100,000 gallons, or 378,541 L × 0.05 = 18,927 L lost from the facility daily. Thus, Eq. 5 becomes:

Eq. 6: Acute PEC_{water} (mg/L) = peak mass of FFC (kg) / 0.05 x volume (L) × 1E6 (mg/kg)

Using the example of the typical density:

Eq. 6a: Acute PEC_{water} = 0.0284 kg / 18,927 L × 1E6 mg/kg = 1.51 mg/L.

The acute PEC_{water} is calculated similarly for the low density and high density (see the first row of information in Table 27).

For exposure periods of 21 days and 33 days (used in the Tier B assessment), the chronic PEC_{water} is calculated by taking the total mass of florfenicol in the effluent over those time periods and diluting in the total volume discharged over those time periods, respectively.

Eq. 7: Chronic PEC_{water} (mg/L) = total mass $FFC_{x\ days}$ (kg) / total discharge $volume_{x\ days}$ (L) × 1E6 (mg/kg)

As an example, for the typical density scenario averaged over a 21-day period, Equation 7 becomes:

Eq. 7a: Chronic PEC_{water} = 0.4020 kg / (18,927 L/day × 21 d) × 1E6 mg/kg = 1.01 mg/L

The initial acute and chronic PEC_{water} values are summarized for the three different densities in Table 27. All of these values are considered reasonable worst-case, because it has been assumed that100% of the florfenicol administered is in the water and that 50% of the fish in the facility are treated at the same time.

Table 27. Calculation of acute and chronic initial PEC_{water} for 95% recirculation and 50% of fish treated at three fish densities

	Low Density			Typical Density			High Density		
Period	**FFC Mass (kg)**	**Volume (L)**	**PEC (mg/L)**	**FFC Mass (kg)**	**Volume (L)**	**PEC (mg/L)**	**FFC Mass (kg)**	**Volume (L)**	**PEC (mg/L)**
Acute (Peak)	0.00018	18,927	0.0096	0.0284	18,927	1.51	0.0596	18,927	3.15
Chronic (21-day)	0.00257	397,467	0.0065	0.4020	397,467	1.01	0.8039	397,467	2.02
Chronic (33-day)	0.00348	624,591	0.0056	0.5435	624,591	0.87	1.086	624,591	1.74

Note: Calculations were done with more significant figures than presented here.

7.5 REFINED PEC_{water} FOR RECIRCULATING SYSTEMS

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_{water} values. Refinement occurs by considering the additional factors discussed below.

The assumption in the calculation of the initial PEC_{water} is that the total applied florfenicol is not metabolized, does not degrade, and does not partition to or bind to solids, but remains in water. The initial PEC_{water} values thus represent the worst-case concentrations of florfenicol in the effluent as it could be discharged, and do not account for metabolism or environmental fate processes. The refined PEC calculations consider each of these factors.

7.5.1 Reduction in concentration by metabolism, environmental fate processes, and dilution

The calculation of the initial PEC_{water} assumes that 100% of the feed is consumed, and all of the florfenicol is taken up and excreted by the fish into the aqueous compartment and then ultimately into the effluent from the recirculating system. Metabolism in the fish is not accounted for; however, many studies demonstrate that florfenicol is readily absorbed, distributed, metabolized, and excreted by fresh- and saltwater salmonids, and this is assumed to be true for other species of fish. Furthermore, there is no accounting for florfenicol in uneaten feed or feces and its subsequent removal with the solids-handling system. Some degradation is also likely to occur, based on the environmental fate studies summarized in Section 5. For example, a mean half-life of 13.6 days was determined in sediment/water systems (Gledhill, 2005).

The processes mentioned above were cited by Gaikowski et al. (2011) as being responsible for the observed difference between the theoretical and measured concentration of florfenicol in the water of a RAS. As described in section 7.1, a study was conducted with tilapia to determine the depletion of florfenicol in tissues; the concentration of florfenicol in water was also measured. This system consisted of two tanks which shared a mechanical and biological filter with tilapia at an approximate density of 38.0 kg/m^3, which is between the typical and high density scenarios used in this EA. There were an equal number of fish in each tank (n=144 fish per tank). The system recirculated 93.6% of the water, with an average daily water replacement of 6.4%. The test temperature was 27 ± 2 °C. Tilapia in both tanks were dosed at 20 mg/kg body weight/day for 10 days. Water samples were collected from the clarifier and from the suspended solids filter at numerous intervals during the 10-day dosing period and then daily on days 11 – 14, with a final sample taken on day 19 (9 days after dosing). The peak mean measured florfenicol concentration occurred on day 10, at 1.39 mg/L (mean of two samples). By day 19, the mean concentration was 0.847 mg/L.

Meinertz (2012) conducted a residue depletion study using rainbow trout (also described in Section 7.1). However, this system only recirculated 80.1% of the water, with 19.9% daily replacement. In addition, two connected tanks were used in this study, but only one tank of rainbow trout was dosed (n=144 fish), at 20 mg/kg body weight/day for 10 days. The other tank of fish in the system (n=77 fish) were fed unmedicated feed. The density of treated fish in the system was only approximately 14.2 kg/m^3, so this study is not representative of a worst-case or realistic scenario. In addition, more than half of the treated fish were removed from the system within 72 hours of the last dose, so the measured florfenicol concentrations during the rest of the study is not considered representative of actual RAS conditions. In this study, florfenicol concentrations at the end of the dosing period reached 0.384 mg/L in Tank A and 0.372 mg/L in Tank B. Water concentrations continued to remain relatively constant for the first 24 hours, but

began to decline thereafter, reaching concentrations of 0.009 mg/L in both tanks by day 29. The water concentrations in the rainbow trout study were much lower than those observed in the tilapia study, due to the lower biomass of treated fish in the system (and consequently lower mass of florfenicol dosed). The conditions used for the two residue depletion studies are summarized in Table 28. Because the rainbow trout study has significant limitations, only the results from the tilapia study are used to refine the PEC_{water}.

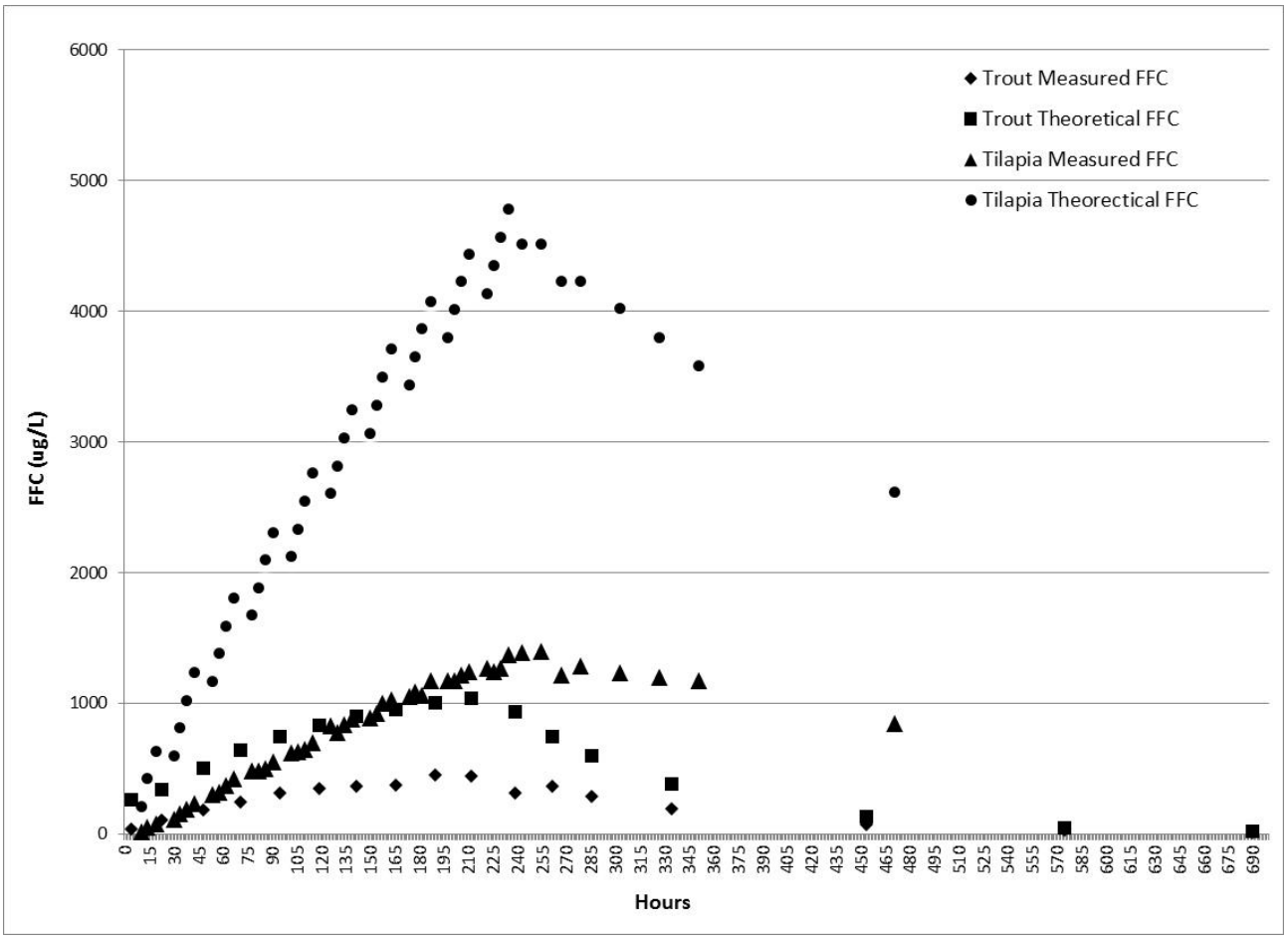
Table 28. A comparison of study conditions for two residue depletion studies

	Tilapia Study (Gaikowski et al., 2011)	**Rainbow Trout Study (Meinertz, 2012)**
Dosage	20 mg/kg bw/d for 10 consecutive days	20 mg/kg bw/d for 10 consecutive days
Water temperature	27°C	12 – 13°C
Percent recirculation	93.6%	80.1%
Tanks sharing a common biofilter	2	2
Tanks treated	2	1
Average fish size	447 g	305–310 g
Fish density in single RAS	Total 38.0 kg/m^3; Equal number of fish in both tanks (n=141 each)	Total 21.7 kg/m^3; Half as many fish in untreated tank (n_{RAC-A}= 149, n_{RAC-B} 77)
Total density of florfenicol-treated fish in the RAS at test initiation	38.0 kg/m^3	14.2 kg/m^{3*}
Number of fish sampled at each time point post-last dose	20 fish sampled (10 fish from each tank) at 1, 12, 24, 36, 48, 72, 96, 120, 240 h	24 fish sampled (16 treated and 8 untreated fish) at 6, 12, 24, 48, 72, 120, 240, 360, 480 h

* Density was not given in the study report but was calculated based on total system volume (3,195 L), number of fish per tank, and average fish weight per tank.

The measured water concentrations of florfenicol for these two studies are plotted against time in Figure 6. Figure 6 also shows the theoretical water concentrations of florfenicol for each study, which were calculated by the study authors based upon the concentration of florfenicol in the feed, the mass of feed offered daily, the water volume in the system, and the percentage of water replaced each day. The underlying data for the tilapia study are presented in Table 29; data for the rainbow trout study are not shown as these data are not used quantitatively to refine the PEC_{water}. The concentration of florfenicol in the water is predicted to increase during the dosing period and then decline post-treatment. The measured florfenicol concentrations indicate that, during the dosing period, the actual concentrations are only about a third of that predicted. After the 10-day dosing period (240 hours), concentrations of florfenicol in the water declined slightly slower than predicted.

Figure 6. Measured and theoretical concentrations of florfenicol in tissue residue depletion studies conducted with tilapia and rainbow trout dosed at 20 mg/kg body weight for 10 days



The data from the tilapia study can be used to refine the PEC_{water} values, because the measured concentrations integrate the effects of absorption, distribution, metabolism, and excretion of florfenicol by the fish, as well as potential degradation and removal within the RAS. The ratio of the measured-to-theoretical concentrations provides the basis for refinement.

This can be calculated by comparing the area under the curve for each measured time interval, according to Eq. 8:

Eq. 8: $AUC = ½ (C_1 + C_2)(t_2 - t_1)$

Where AUC = area under the curve, and C_1 and C_2 are the concentrations at the beginning (t_1) and end (t_2) of the time interval of measurement. Using the data in Table 29, the maximum and average overall ratios of measured-to-theoretical concentrations were determined. These values were then used to refine the acute and chronic PEC_{water} values.

Table 29. Measured and theoretical concentrations of florfenicol in a recirculating aquaculture system during a residue depletion study with tilapia

Day	Cumulative Sample Hour	Mean Measured FFC Concentration (µg/L)	Theoretical FFC Concentration (µg/L)	Measured AUC	Theoretical AUC	Ratio of Measured to Theoretical AUC
0	11	20	211	136	1266	0.11
0	15	48	422	305	2638	0.12
0	20	74	633	1040	6771	0.15
1	31	115	598	544	2816	0.19
1	35	157	810	700	3664	0.19
1	39	193	1,022	1075	5640	0.19
1	44	237	1,234	2976	13195	0.23
2	55	304	1,165	1244	5084	0.24
2	59	318	1,377	1386	5932	0.23
2	63	375	1,589	1995	8475	0.24
2	68	423	1,801	4983	19091	0.26
3	79	483	1,670	1930	7102	0.27
3	83	482	1,881	1970	7946	0.25
3	87	503	2,092	2645	10990	0.24
3	92	555	2,304	6474	24343	0.27
4	103	622	2,122	2498	8910	0.28
4	107	627	2,333	2556	9754	0.26
4	111	651	2,544	3380	13250	0.26
5	116	701	2,756	8432	29464	0.29
5	127	832	2,601	3222	10834	0.30
5	131	779	2,816	3234	11692	0.28
5	135	838	3,030	4295	15685	0.27
5	140	880	3,244	9735	34683	0.28
6	151	890	3,062	3626	12678	0.29
6	155	923	3,277	3846	13536	0.28
6	159	1,000	3,491	5063	17990	0.28
6	164	1,025	3,705	11413	39270	0.29
7	175	1,050	3,435	4280	14166	0.30
7	179	1,090	3,648	4310	15018	0.29
7	183	1,065	3,861	5600	19838	0.28
7	188	1,175	4,074	12925	43291	0.30
8	199	1,175	3,797	4690	15614	0.30
8	203	1,170	4,010	4770	16466	0.29
8	207	1,215	4,223	6150	21650	0.28
8	212	1,245	4,437	13833	47146	0.29
9	223	1,270	4,135	5030	16966	0.30
9	227	1,245	4,348	5020	17818	0.28
9	231	1,265	4,561	6600	23338	0.28
9	236	1,375	4,774	11040	37124	0.30
10	244	1,385	4,507	16710	54084	0.31
10	256	1,400	4,507	15690	52392	0.30
11	268	1,215	4,225	15000	50700	0.30
11	280	1,285	4,225	30180	98892	0.31
12	304	1,230	4,016	29160	93684	0.31
13	328	1,200	3,791	28440	88440	0.32
14	352	1,170	3,579	121020	371340	0.33
19	472	847	2,610			
						Mean = 0.27

Data in first four columns from Gaikowski et al. (2011).

The single highest ratio obtained between the measured and theoretical AUC was 0.33, while the overall average ratio was 0.27 (see Table 29). Thus a factor of 0.33 is used to refine the Tier A (acute) PEC_{water}, and a factor of 0.27 is used to refine the Tier B (chronic) PEC_{water}.

Eq. 9: Refined Tier A PEC_{water} = Initial Tier A PEC_{water} × 0.33

Eq. 10: Refined Tier B PEC_{water} = Initial Tier B PEC_{water} × 0.27

To be conservative, the refined PEC_{water} is presented as an "end-of-pipe" concentration (without dilution in receiving water) because there are some states where mixing zones are not allowed. However, this will be addressed later in the EA.

The results of the calculations for the refined PEC_{water} are presented (using Equation 9 for the acute period and Equation 10 for the chronic periods) at three fish densities in Table 30.

Table 30. Acute and chronic refined PEC_{water} for 95% recirculation and 50% of fish treated for three fish densities

Period	Low Density PEC, (mg/L)	Typical Density PEC, (mg/L)	High Density PEC, (mg/L)
Acute (Peak)	0.0032	0.494	1.04
Chronic (21-day)	0.0018	0.273	0.545
Chronic (33-day)	0.0015	0.235	0.470

Note: Calculations were done with more significant figures than presented here.

7.5.2 Multiple and sequential applications

The assumptions used to derive the initial and refined PEC_{water} values are based on a single application over a 10-day treatment period. Sequential applications to a single tank are not a significant factor because, based on the expected use pattern, Aquaflor® would not be reapplied to the same tank (i.e., cohort of fish) in less than 60 days, and a tank would not be treated more than two times during a grow-out cycle (approximately 8 to 9 months). This would allow for a maximum of three applications to a specific tank during a calendar year, with the third instance involving application to the next cohort of fish placed in the tank.

However, treatment of a portion of tanks in the facility at one time, followed by treatment of another portion of tanks later, could potentially occur at overlapping time intervals. This is because the occurrence of a disease outbreak is independent among tanks, unless the tanks are connected and share the same biofilter. Treatment of 100% of the fish at one time is highly unlikely, so this EA assumes that 50% of the facility is treated at one time for the initial PEC_{water}. Further refinements for the percent of a facility treated at one time were not considered.

7.6 *INITIAL PEC_{soil}* FOR RECIRCULATING SYSTEMS

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.

According to U.S. EPA (2004), recirculating system facilities use a variety of methods to treat, hold, or dispose of solids. Some facilities send the collected solids to a municipal wastewater treatment system, either directly or after pretreatment in a settling pond or other primary treatment system. Other facilities concentrate the solids and then apply the solids slurry to land. The latter handling method could result in exposure to terrestrial organisms and is addressed below.

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

Eq. 11: $$PEC_{soil} = \frac{ID * T * P * F_H * N_{SL}}{\rho * A * D * N_{pp} * N_{fish} * H}$$

where ID : individual dose [mg/kg]
T : number of treatments [n]
P : animals raised per place and year [n]
F_H : fraction of herd (stock) 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 continuously 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, 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. Using a 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, because 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 all of the stock is treated (F_H = 1), which is highly unlikely and represents an extreme 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 31. Using these values and solving Equation 11 results in an initial PEC_{soil} of 0.15 mg/kg.

Table 31. Assumptions used to calculate initial PEC_{soil} for recirculating systems

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 additional degradation in soil (half-life of 27.2 days). Metabolism in fish, degradation of florfenicol during holding of the slurry prior to application, or treatment of only a portion of the fish in the facility are also not considered.

7.7 REFINED PEC_{SOIL} FOR RECIRCULATING SYSTEMS

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. Each of these factors are discussed below.

Many studies exist to demonstrate that florfenicol is readily absorbed, distributed, metabolized, and excreted by fresh and saltwater salmonids, and this is assumed 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 constituted 19.9% of the total radioactivity, florfenicol amine constituted 71.5%, and other (less toxic) metabolites constituted 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 Phase II Guidelines.

The calculation of the initial PEC_{soil} was based on the assumption that 100% of the fish in the facility would be treated. This is highly unlikely. A reasonable yet conservative assumption, and that used in the determination of the PEC_{water}, is that 50% of the fish would be treated.

In the absence of data on degradation in tilapia biosolids (excreta), 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 tilapia 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) is not directly applicable for aquaculture but has been adapted as presented below:

Eq. 12 (Step 1): Mi [mg]

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

Eq. 13 (Step 2): Mt [mg]

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

Eq. 14 (Step 3): N_s [kg]

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

Eq. 15 (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 recirculating 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 those used in calculation of the initial PEC_{soil}. The mass of fish treated for the low-, typical-, and high-density scenarios is 0.160 kg/m^3, 25 kg/m^3, and 50 kg/m^3, respectively. With a facility volume of 100,000 gallons (= 378,541 L), this is equivalent to 60.6, 9464, and 18,927 kg, respectively (see Table 32, below). It is assumed that the solids are allowed to accumulate and then applied to land 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 32. Using these values, the refined PEC_{soil} for recirculating systems is determined to be 0.00018 mg/kg for all three scenarios.

Table 32. Assumptions used to calculate refined PEC_{soil} for recirculating systems

Parameter	Value
M_{fish}, Biomass of treated fish (kg)	
Low density	60.6
Typical density	9464
High density	18,927
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

The remaining assumptions are unchanged from those used to calculate the initial PEC_{soil} (Table 31).

7.8 TIER A RISK CHARACTERIZATION FOR RECIRCULATING SYSTEMS

7.8.1 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 values for representative species.

The initial PEC_{water} is based on a facility volume of 100,000 gallons, with 95% water recirculation. Three different fish densities were used (low, typical, and high), and it was conservatively assumed that 50% of the fish would be treated at one time, and that 100% of the

florfenicol fed to these fish would be in the water column, with no reductions for metabolism, environmental fate processes, or dilution. The initial PEC_{water} is assumed to be equivalent to the peak theoretical concentration of florfenicol released in the effluent. Although the peak value only occurs on day 10 of the exposure (see Table 29 and Figure 5), it is conservatively assumed that this exposure concentration is maintained for up to 96 hours, which is equivalent to the maximum duration of exposure in acute toxicity tests for aquatic receptors. Therefore, initial PEC_{water} values are 0.0096, 1.51, and 3.15 mg/L for the low-, typical-, and high-density scenarios, respectively (see Table 27).

The Tier A PNEC values are those presented in Table 16. The most sensitive toxicity result for a given species is used in the risk characterization. Comparing the PNEC values to the initial PEC_{water} values, it is evident that the PEC/PNEC ratios (risk quotients) exceed 1.0 for green algae, aquatic vascular plants, and cyanobacteria under both the typical-density and high-density scenarios (Table 33).

Table 33. Tier A aquatic risk characterization: Initial PEC/PNEC ratios for three densities at 95% recirculation and 50% of fish treated

Organism	PNEC, (mg/L)	Low Density PEC (mg/L)	Low Density PEC/PNEC	Typical Density PEC (mg/L)	Typical Density PEC/PNEC	High Density PEC (mg/L)	High Density PEC/PNEC
Oncorhynchus mykiss	7.8	0.0096	0.0012	1.51	0.19	3.15	0.40
Lepomis macrochirus	8.3	0.0096	0.0012	1.51	0.18	3.15	0.38
Daphnia magna	3.3	0.0096	0.0029	1.51	0.46	3.15	0.95
Navicula pelliculosa	6.1	0.0096	0.0016	1.51	0.25	3.15	0.52
Pseudokirchneriella subcapitata	0.1	0.0096	0.096	1.51	**15**	3.15	**32**
Lemna gibba	0.076	0.0096	0.13	1.51	**20**	3.15	**41**
Anabaena flos-aquae	0.023	0.0096	0.42	1.51	**66**	3.15	**137**

Note: PEC/PNEC ratios ≥ 1.0 are shown in **bold**.

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

7.8.2 Refined Tier A Aquatic Risk Characterization

The refined Tier A aquatic risk characterization uses the PEC/PNEC ratios based on acute exposure of aquatic organisms and the refined acute PEC_{water} values as determined for each standing density scenario. The Tier A PNEC values are those presented in Table 16. The refined PEC_{water} values were determined by accounting for metabolism and environmental fate processes based on the results of the study by Gaikowski et al. (2011) and presented in Table 30. The refined acute PEC_{water} values incorporate a factor of 0.33 and are 0.0032, 0.494, and 1.04 mg/L for the low, typical, and high density scenarios, respectively. The resulting risk quotients are presented in Table 34.

Table 34. Tier A aquatic risk characterization: Refined PEC/PNEC ratios for three densities (50% of fish treated) at 95% recirculation

Organism	PNEC, (mg/L)	Low Density PEC (mg/L)	Low Density PEC/PNEC	Typical Density PEC (mg/L)	Typical Density PEC/PNEC	High Density PEC (mg/L)	High Density PEC/PNEC
Oncorhynchus mykiss	7.8	0.0032	0.0004	0.494	0.06	1.04	0.13
Lepomis macrochirus	8.3	0.0032	0.0004	0.494	0.06	1.04	0.13
Daphnia magna	3.3	0.0032	0.0010	0.494	0.15	1.04	0.32
Navicula pelliculosa	6.1	0.0032	0.0005	0.494	0.08	1.04	0.17
Pseudokirchneriella subcapitata	0.1	0.0032	0.032	0.494	**4.9**	1.04	**10**
Lemna gibba	0.076	0.0032	0.04	0.494	**6.5**	1.04	**14**
Anabaena flos-aquae	0.023	0.0032	0.14	0.494	**21**	1.04	**45**

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

The VICH 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.

Risk quotients for green algae, aquatic vascular plants, and cyanobacteria still exceed 1.0 for the typical and high density scenarios, indicating a potential risk to these receptors under acute exposure conditions in the absence of any dilution of the effluent in the receiving water.

7.8.3 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 applied to land (calculated in Section 7.6).

Risk to soil microorganisms is evaluated based on the results of a laboratory study that evaluated effects on nitrogen and carbon transformation (Carter, 2002). Effects on nitrogen and carbon 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 ≥ 1.0, a refined Tier A terrestrial risk characterization was conducted.

Table 35. Tier A terrestrial risk characterization: Initial PEC/PNEC ratios

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**

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

7.8.4 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 in the fish, treatment of 50% of the fish in a facility, and degradation of the florfenicol in the accumulated solids (i.e., fish feces and uneaten feed) prior to spreading on agricultural land. Refining the PEC_{soil} (0.00018 mg/kg) results in risk quotients (PEC/PNEC) that are all below 1.0 (Table 36). Thus, no acute (Tier A) risks to terrestrial receptors are anticipated from exposure to fish biosolids containing florfenicol applied to agricultural soils.

Table 36. Tier A terrestrial risk characterization: Refined PEC/PNEC ratios

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

7.8.5 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 typical and high density scenarios for green algae, aquatic vascular plants, and cyanobacteria. The initial PEC_{water} assumed that all of the dosed florfenicol enters the water column and that 50% of the fish in the system would be treated, and did not consider metabolism of florfenicol in the fish, or any environmental fate processes that would remove florfenicol from the system prior to discharge in the effluent. Therefore, a refined PEC_{water} was calculated to include metabolism and environmental fate processes (e.g., removal with solids, degradation, etc.); but, Tier A risk quotients for green algae, aquatic vascular plants, and cyanobacteria still exceeded 1.0 for the typical- and high-density scenarios. This suggests that short-term impacts to these species could occur if the effluent were discharged directly to receiving waters without dilution. However, the effects data for these organisms are based on inhibition of growth, not mortality. Unless complete mortality of the population occurs, the remaining organisms can reproduce quickly, and the population regains its original density in a relatively short time. 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.

The refined PEC_{water} is presented as an end-of-the-pipe concentration to account for those receiving waters and states where a mixing zone is not allowed. However, recirculating systems discharge only a very small volume of effluent, and it is highly unlikely that this volume would remain undiluted in the environment. Dilution would be expected to occur if the effluent was discharged either directly into a receiving stream or to a wastewater treatment plant. If an additional refinement was made to the PEC to include a 10-fold dilution factor[6] some, but not all, acute risk quotients would fall below 1. Because potential risks to certain aquatic receptor groups cannot be ruled out even with dilution, risk mitigation measures for Aquaflor® are recommended, specifically the derivation of water quality benchmarks for florfenicol, as discussed in Section 7.10.

[6] An additional step in the December 2011 EA for Aquaflor® (for use in ponds and raceways) included the use of a 10-fold dilution factor to refine the PEC_{water}.

For exposure of terrestrial organisms at Tier A, the amount of florfenicol that would partition to the solids in the clarifiers or other solids-handling systems, 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, treatment of 50% of the fish, and degradation of florfenicol in the solids. 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 RAS facilities.

7.9 TIER B RISK CHARACTERIZATION FOR RECIRCULATING SYSTEMS

7.9.1 Initial Tier B Aquatic Risk Characterization

The initial Tier B risk characterization is presented as the ratio of the initial PEC_{water} to the Tier B (chronic) PNEC values for representative species.

As in Tier A, the initial Tier B PEC_{water} is based on a facility volume of 100,000 gallons, with 95% water recirculation, three different fish densities (low, typical, and high), and the conservative assumption that 50% of the fish would be treated at one time. In addition, it was assumed that 100% of the applied florfenicol would be in the water column, with no metabolism, degradation, or dilution. The initial chronic PEC_{water} is determined for both a 21-day period and a 33-day period. These periods correspond to the exposure periods used in the toxicity tests with the representative aquatic receptors. The 33-day PEC_{water} is used for comparison to effects data for *Pimephales promelas* (33-day test) and *Chironomus riparius*[7] (28-day test), while the 21-day PEC_{water} is used for comparison to the remaining aquatic receptor effects data, which all result from studies of 21 days in duration or less. The initial chronic PEC_{water} values (see Table 27) range from 0.0065 mg/L for the low-density scenario to 2.02 mg/L for the high-density scenario.

The Tier B PNEC values are those presented in Table 18 for chronic effects on aquatic organisms. Where more than one toxicity value was available, the lowest value (indicating the greatest toxicity) was selected. Comparing these PNEC values to the Tier B initial PEC_{water} values, it is evident that the PEC/PNEC ratios exceed 1.0 for several receptors under both the typical- and high-density scenarios (Table 37).

[7] Although *C. riparius* is a sediment-dwelling species and these data are not strictly required in the evaluation of the PEC/PNEC for water, the study was conducted using a water exposure, and therefore, the data are presented for completeness.

Table 37. Tier B aquatic risk characterization: Initial PEC/PNEC ratios for three fish densities at 95% recirculation and 50% of fish treated

Organism	PNEC (mg/L)	Low Density PEC (mg/L)	Low Density PEC/PNEC	Typical Density PEC (mg/L)	Typical Density PEC/PNEC	High Density PEC (mg/L)	High Density PEC/PNEC
Pimephales promelas	0.55	0.0056	0.0102	0.87	**1.58**	1.74	**3.16**
Chironomus riparius	2.5	0.0056	0.0022	0.87	0.35	1.74	0.70
Daphnia magna	0.15	0.0065	0.043	1.01	**6.7**	2.02	**13**
Brachionus calyciflorus	0.076	0.0065	0.086	1.01	**13**	2.02	**27**
Navicula pelliculosa	1.87	0.0065	0.003	1.01	0.54	2.02	**1.1**
Pseudokirchneriella subcapitata	0.075	0.0065	0.09	1.01	**13**	2.02	**27**
Lemna gibba	0.039	0.0065	0.17	1.01	**26**	2.02	**52**
Anabaena flos-aquae	0.011	0.0065	0.59	1.01	**92**	2.02	**184**

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

Because the risk quotients for many receptors were ≥ 1.0 under the typical and high density scenarios, a refined analysis was performed. The refined risk assessment uses the refined PEC_{water} and is presented below.

7.9.2 Refined Tier B Aquatic Risk Characterization

The refined risk characterization uses the refined PEC_{water} values for 21-day and 33-day exposure periods, which were determined by accounting for metabolism and environmental fate processes based on the results of the study by Gaikowski et al. (2011). The refined PEC_{water} values incorporate a factor of 0.27, and range from 0.0015 to 0.575 mg/L (see Section 7.5.1 and Table 30). Tier B PNEC values are presented in Table 18. The resulting risk quotients are presented in Table 38.

Table 38. Tier B aquatic risk characterization: Refined PEC/PNEC ratios for three densities at 95% recirculation and 50% of fish treated

Organism	PNEC (mg/L)	Low Density PEC (mg/L)	Low Density PEC/PNEC	Typical Density PEC (mg/L)	Typical Density PEC/PNEC	High Density PEC (mg/L)	High Density PEC/PNEC
Pimephales promelas	0.55	0.0015	0.0027	0.235	0.43	0.470	0.85
Chironomus riparius	2.5	0.0015	0.0006	0.235	0.09	0.470	0.19
Daphnia magna	0.15	0.0018	0.012	0.273	**1.8**	0.545	**3.6**
Brachionus calyciflorus	0.076	0.0018	0.024	0.273	**3.6**	0.545	**7.2**
Navicula pelliculosa	1.87	0.0018	0.001	0.273	0.15	0.545	0.29
Pseudokirchneriella subcapitata	0.075	0.0018	0.02	0.273	**3.6**	0.545	**7.3**
Lemna gibba	0.039	0.0018	0.05	0.273	**7.0**	0.545	**14**
Anabaena flos-aquae	0.011	0.0018	0.16	0.273	**25**	0.545	**50**

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

Risk quotients are greater than 1.0 for two species of invertebrates, green algae, aquatic vascular plants, and cyanobacteria under the typical and high fish densities. This indicates a potential risk for these receptors under chronic exposure conditions in the absence of any dilution of the RAS effluent in the receiving water.

7.9.3 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 that all of the dosed material was partitioned to the solids (Section 7.6). Effects data at Tier B for deriving PNECs are available for three species of plants: cress, cabbage, and mustard (Table 19). Where more than one toxicity value was available, the lowest value (indicating the greatest toxicity) was selected for PNEC derivation. These PNECs and the initial PEC_{soil} are presented in Table 39, along with the resulting PEC/PNEC ratios.

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

**Table 39. Tier B terrestrial risk characterization:
Initial PEC/PNEC ratios for recirculating systems**

Organism	PNEC (mg/kg)	Initial PEC_{soil} (mg/kg)	PEC/PNEC Ratios
Cress	0.016	0.15	**9**
Cabbage	0.0123	0.15	**12**
Mustard	0.0123	0.15	**12**

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

7.9.4 Refined Tier B Terrestrial Risk Characterization

The refined PEC_{soil} was calculated by factoring in metabolism of florfenicol in the fish, treatment of 50% of the fish in the facility, and degradation of florfenicol in the accumulated solids. Refining the PEC_{soil} (0.00018 mg/kg) results in risk quotients (PEC/PNEC) that are all below 1.0 (Table 40), indicating that chronic effects to terrestrial receptors from land application of florfenicol-containing solids to agricultural land is unlikely.

**Table 40. Tier B terrestrial risk characterization:
Refined PEC/PNEC ratios for recirculating systems**

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

7.9.5 Summary of Risk Characterization at Tier B

At Tier B, which evaluates chronic exposure and effects, the initial risk characterization produced PEC/PNEC ratios that exceeded 1.0 for fish, daphnids, rotifers, green algae, aquatic vascular plants, and cyanobacteria for the typical and high density scenarios. The initial PEC_{water} assumed that all of the dosed florfenicol enters the water column and that 50% of the fish in the system would be treated, and did not account for metabolism of florfenicol in the fish or any environmental fate processes that would reduce the concentration of florfenicol in the effluent water. Refinement of the PEC_{water} to account for these factors reduced the PEC/PNEC ratios, but not below 1.0. Therefore, it is possible, under certain circumstances, that florfenicol entering the aquatic environment from RAS effluent could have adverse effects on aquatic organisms. However, this assumes that no dilution of the effluent occurs in the receiving water. Dilution would be expected to occur if the RAS effluent was discharged either directly into a receiving stream or to a wastewater treatment plant. It is very unlikely that the effluent would constitute

the entire volume of even a very small stream. If an additional refinement was made to the PEC_{water} to include a 10-fold dilution factor, as used in the December 2011 EA for use of Aquaflor® in ponds and raceways, many, but not all, risk quotients would fall below 1. Therefore, risk mitigation measures described in this EA include derivation of water quality benchmarks, as discussed in Section 7.10.

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 recirculating system and subsequently would be removed for land application. The resulting risk quotients exceeded 1.0 for plants and earthworms. A refined PEC_{soil} was determined after consideration of metabolism in the fish, treatment of 50% of the fish, and degradation of florfenicol in the solids. 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 RAS facilities. 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}s) 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.

7.10 DERIVATION OF WATER QUALITY BENCHMARK FOR FLORFENICOL

An evaluation of potential acute and chronic risk associated with florfenicol in discharged effluent into surface water from RAS facilities indicates that adverse effects on aquatic life could potentially occur. However, the risk quotients (Tables 34 and 38 for acute and chronic exposures, respectively) are based on estimated “end-of-pipe” effluent concentrations of florfenicol, not potential concentrations in receiving waters downstream from the points of effluent discharge. Receiving water concentrations for most RAS discharges will likely be well below the effluent concentrations due to subsequent dilution and further degradation. However, some states do not allow the discharge of “toxic substances in toxic amounts” and, thus, incorporation of dilution in receiving waters for all facilities across the United States cannot be assumed, especially without assurance that incorporating a dilution factor is allowable under state and local water quality regulations.[8] Therefore, an alternative approach is needed to address the possibility for an individual RAS facility to discharge potentially toxic concentrations of florfenicol into a receiving stream.

Based on previously described risk quotients for some aquatic receptors (Section 7.8.2 and 7.9.2), it has been determined that some form of risk mitigation is needed to ensure that the use of Aquaflor® will not adversely impact aquatic life. Therefore, water quality criteria, or benchmarks, have been derived for florfenicol for the protection of aquatic life. These benchmark concentrations may be used by the appropriate National Pollution Discharge Elimination System (NPDES) or State effluent permitting authority.[9] If needed, this authority may establish

[8] The Clean Water Act allows individual states to set water quality standards, which include designated uses, criteria to protect those uses, and antidegradation policies. Some states allow toxicity within the mixing zone; those that do not evaluate toxicity at the end-of-the-pipe without consideration for dilution.

[9] The U.S. EPA is responsible for implementing the NPDES program but may delegate this authority to individual States, Territories, or Tribes to implement all or parts of the system, including issuing permits.

appropriate effluent discharge limits for an aquaculture facility, based on site-specific conditions (e.g., effluent treatment, in-stream dilution) that are in conformance with applicable State and Federal water quality regulations. Environmental statements will be added to the drug label for Aquaflor® that identify the water quality benchmark for its use by NPDES permitting authorities[10] and which require the drug user to report this information to the appropriate authority prior to the initial use of the drug.

The water quality benchmarks for Aquaflor® were derived using procedures in published EPA guidance. These procedures are dictated by the type and amount of available and well-documented toxicity data for a particular chemical. In instances where the existing database is not adequate to support the use of the standard EPA (Tier I) approach (Stephan et al. 1985, EPA 1991, 1994), the Tier II methodology described in the Great Lakes System Guidance (40 CFR Part 132, Appendix A; EPA, 1995) may be used for criteria development.[11]

7.10.1 Data and calculations for water quality benchmark

For the Tier I approach, acute toxicity endpoints should be available for at least one species of freshwater animal in at least eight different families to ensure a sufficient database on which to base the calculation of the "Final Acute Value" (FAV). These requirements are not met for florfenicol since only three families are represented (see Table 41). There is only one species represented for each genus, so the Genus Mean Acute Value (GMAV) is the same as the toxicity endpoint.

Table 41. Available florfenicol acute toxicity values for freshwater animals for derivation of final acute value

Species and Reference	Endpoint and Value (mg/L)	GMAV (Rank)
Daphnia magna (LeLievre, 1991a)	EC_{50} > 330	330 (1)
Oncorhynchus mykiss (LeLievre, 1991e)	LC_{50} > 780	780 (2)
Lepomis macrochirus (LeLievre, 1991f)	LC_{50} > 830	830 (3)

Because available florfenicol data are insufficient to meet Tier I requirements, the next step is to employ the Tier II procedure (EPA, 1995) and calculate a Secondary Acute Value (SAV). In order to calculate a SAV, the database must contain, at minimum, a GMAV for one of three genera in the family Daphnidae; this requirement is met with the data for *Daphnia magna*.

[10] Under Clean Water Act regulations (see 40 CFR 122.44(d)(1)(vi)(A)), information provided by FDA (such as water quality benchmarks) can be used by permitting authorities to derive numerical water quality criteria and establish appropriate effluent discharge limits.

[11] Criteria derived using the standard EPA approach are often referred to as Tier I criteria because the Great Lakes guidance describes several Tier I methodologies that are identical to the standard EPA approaches. The Great Lakes guidance defines these criteria as Tier I criteria while those developed using the Tier II methodologies are defined as Tier II "values" (not criteria).

The SAV is calculated as the lowest GMAV divided by the Secondary Acute Factor (SAF). The SAF corresponds to the number of satisfied minimum data requirements of the Tier I methodology (adjustment factors provided in Table A-1 of EPA, 1995). For 3 data requirements satisfied, SAF = 8.0. Therefore the SAV is defined by Eq. 16:

Eq. 16: SAV = GMAV / SAF = 330 mg/L / 8.0 = 41.25 mg/L

The Tier II procedure also provides a method for calculating a Secondary Chronic Value (SCV), which is based on the SAV and information on Acute-Chronic Ratios (ACRs). When chronic values are not available for the eight families, an ACR is calculated for studies where both an acute and chronic endpoint are available; however, no studies satisfy this requirement for florfenicol (see Table 42).

Table 42. Available florfenicol chronic toxicity values for determination of ACR

Species and Reference	Comment
Daphnia magna (Gallagher et al., 2008a)	Acute data exists for this organism, but from a different study
Brachionus calyciflorus (Sayers, 2009b)	No corresponding acute data
Chironomus riparius (Bradley, 2009)	No corresponding acute data
Pimephales promelas (Gallagher et al., 2008c)	No corresponding acute data

The EPA Tier II Guidance sates that if no experimentally determined ACRs are available from which a geometric mean can be calculated for the Secondary Acute-to Chronic Ratio (SACR), a default value of 18 is used, per Eq. 17:

Eq. 17: SCV = SAV/SACR = 41.25 mg/L / 18 = 2.29 mg/L

The Tier II value (water quality benchmark) consists of two concentrations: the Secondary Maximum Concentration (SMC), or acute benchmark value, and the Secondary Continuous Concentration (SCC), or chronic benchmark value. The SMC is calculated as ½ of the SAV, per Eq. 18:

Eq. 18: SMC = SAV/2 = 41.25 mg/L / 2 = 20.6 mg/L

The SCC is the lowest of either the SCV or the Final Plant Value (FPV). The SCV, as presented above, is 2.29 mg/L. A plant value is the result of a 96-hour test conducted with an alga (e.g., 96-h EC_{50} value) or a chronic test conducted with an aquatic vascular plant (e.g. geometric mean of the NOEC and LOEC values for a 7-day *L. gibba* test). The choice of these study endpoints is consistent with the EPA Tier II Guidance, which does not specify the specific endpoints that should be used for these studies, and those used historically for risk assessment for these receptors. The available data for calculating the FPV are presented in Table 43. Note that this table also includes data for a cyanobacteria (*Anabaena flos-aquae*), which in the past has been classified as a blue-green alga.

Table 43. Available florfenicol data for calculation of a Final Plant Value

Species and Reference	Endpoint Values	Plant value
Anabaena flos-aquae (Gallagher et al., 2008a)	96-h EC_{50}: 0.23 mg/L 96-h NOEC: 0.11 mg/L 96-h LOEC: 0.20 mg/L	0.23 mg/L
Lemna gibba (Softcheck, 2009)	7-day EC_{50}: 0.76 mg/L 7-day NOEC: 0.39 mg/L 7-day LOEC: 0.94 mg/L	0.605 mg/L (geometric mean of NOEC and LOEC)
Pseudokirchneriella subcapitata (Hoberg, 1991a)	96-h EC_{50}: 1.0 mg/L 96-h NOEC: 0.75 mg/L 96-h LOEC: 1.5 mg/L	1.0 mg/L
Navicula pelliculosa (Jenkins, 2005)	72-h EC_{50}: 61 mg/L 72-h EC_{10}: 18.7 mg/L	61 mg/L

The most sensitive species is *Anabaena flos-aquae*, with a 96-hour EC_{50} of 0.23 mg/L. Although this species is currently taxonomically classified as a cyanobacterium, it is considered here with the algae and aquatic plants because it has formerly been classified as a blue-green alga. Based on the data in Table 43, the FPV is 0.23 mg/L, which is lower than the SCV of 2.29 mg/L. Therefore, the FPV is selected as the SCC.

In summary, the water quality benchmarks for florfenicol consist of an acute benchmark value, of 20.6 mg/L, and a chronic benchmark value of 0.23 mg/L.

7.10.2 Proposed Aquaflor® Label for Environmental Safety

Based on the acute and chronic water quality benchmarks that have been derived for florfenicol, the Aquaflor® drug label should provide information that would enable its safe use in the environment and inform appropriate State and Federal effluent permitting authorities. The following risk mitigation language should be included on the Aquaflor® drug label:

> *LIMITATIONS AND CAUTIONS FOR ALL USES*
>
> *Before using this drug for the first time, you must inform the appropriate National Pollutant Discharge Elimination System (NPDES) permitting authority of your intentions and of the following information. Acute and chronic water quality benchmarks for the protection of freshwater aquatic life have been derived by FDA for florfenicol following EPA guidance for calculating Tier II water quality criteria for the Great Lakes System (40 CFR 132, App. A). The acute benchmark value (Secondary Maximum Concentration) is 20.6 mg/L (equivalent to one-half of the Secondary Acute Value). The chronic benchmark value (Secondary Continuous Concentration) is 0.23 mg/L (equivalent to the Final Plant Value). The NPDES authority may require an NPDES permit before you can discharge Aquaflor®. The water quality benchmark concentrations are not discharge limits, but may be used by the NPDES authority to derive such limits for the permit. Additional environmental information on Aquaflor® and the benchmark values are available in an environmental assessment posted at http://www.fda.gov/Animal Veterinary/ DevelopmentApprovalProcess/EnvironmentalAssessments/ucm300656.htm.*

7.11 SUMMARY OF AQUATIC AND TERRESTRIAL RISK CHARACTERIZATION FOR RECIRCULATING SYSTEMS

A Phase II Tier A and Tier B assessment was conducted, following VICH guidance, to examine the environmental risk of the use of Aquaflor® (florfenicol) 50% Type A Medicated Article in freshwater fish in recirculating aquaculture systems. The exposure assessment was based on the use of Aquaflor® at 15 mg/kg/day to treat tilapia, which are commonly grown in recirculating systems. Tilapia can be cultured intensively and present the highest potential biomass per volume in a RAS and, thus, the highest potential use rates for florfenicol.

Recirculating systems use mechanical and biological filtration to allow the water in the system to be reused with only a minimal discharge of aqueous effluent. The discharge may go to a holding pond or onsite lagoon, to a municipal wastewater treatment plant, or in some instances, directly to the receiving water (e.g., a river or lake). Solids are removed periodically and could potentially be land-applied. Thus, possible exposures to florfenicol of both aquatic and terrestrial receptors were evaluated. Because facility designs are highly variable, a representative design (100,000-gallon system) was selected, and three density scenarios were developed for this EA. It was conservatively assumed that the maximum percentage of fish to be treated at any one time would be 50% of the total facility biomass.

To calculate the initial PEC_{water} values, it was conservatively assumed that (1) all of the dosed medicated feed was eaten by the fish and excreted unmetabolized, (2)all residues in the water were the parent florfenicol compound, (3) florfenicol remained in the water and did not degrade or partition to solids, and (4) 50% of the fish in a facility were treated at the same time. A peak discharge concentration was determined for the acute (Tier A) assessment, and average discharge concentrations (over 21 or 33 days) were determined for the chronic (Tier B) assessment. Using the initial PEC_{water}, PEC/PNEC ratios at Tier A exceeded 1.0 for green algae, aquatic vascular plants, and cyanobacteria under the typical- and high-density scenarios. At Tier B, the risk quotients based on the initial PEC_{water} exceeded 1.0 for fish, invertebrates, algae, aquatic plants, and cyanobacteria under the typical- and high-density scenarios.

Therefore, refined PEC_{water} values were determined for both Tier A and Tier B. The refinements were based on measured florfenicol water concentrations collected during a residue depletion study in which tilapia were dosed at 20 mg/kg bw/day in a dual-tank RAS. The observed concentrations in this study reflected the totality of processes contributing to the reduction of florfenicol in the water: adsorption, distribution, metabolism, and excretion in the fish; degradation in the water; and partitioning to, and removal of, solids (i.e., feces and uneaten feed). Using the refined PEC_{water}, the PEC/PNEC ratios at Tier A exceeded 1.0 for green algae, duckweed, and cyanobacteria under the typical- and high-density scenarios. This suggests that short-term effects under these conditions and in the absence of any in-stream dilution could occur for these receptors; however, populations are expected to recover quickly. Using the refined PEC_{water} for Tier B, risk quotients still exceeded 1.0 for daphnids, rotifers, green algae, duckweed, and cyanobacteria under the typical- and high-density scenarios. This suggests that, under these conditions and in the absence of any in-stream dilution, effects could occur for these receptors.

The volume of water discharged from a RAS is very small; by definition, it is typically <10% of the total volume and, in some instances, can be much smaller. Because of the relatively small volume of effluent discharged, it is highly unlikely that there would be no dilution in the receiving water. Moreover, many facilities do not discharge directly into surface water, but rather send wastewater to a municipal treatment plant or to an onsite lagoon. Further degradation (and

dilution) of florfenicol would be expected to occur in these situations, thereby lowering the PEC_{water}. Because, even after refinement, concentrations of florfenicol in RAS effluent may pose a risk to aquatic organisms in a receiving stream, acute and chronic water quality benchmarks were developed as a form of risk mitigation. These benchmarks can be used in the NPDES permitting process for RAS facilities to aid in the protection the aquatic life. If needed, appropriate effluent discharge limits can be established on a facility-by-facility basis, taking into account site specific conditions (i.e., receiving water dilution) and other factors, to ensure that concentrations of florfenicol in receiving streams remain below these acute and chronic benchmarks values of 20.6 and 0.23 mg/L, respectively. Through this mechanism, the risk quotients greater than 1.0 are not anticipated to predict significant environmental effects because these risks will be dealt with at the locations of actual drug use by State or Federal effluent permitting authorities.

Solids removed from a RAS could potentially be applied to agricultural land. 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}, which considers metabolism, treatment of 50% of the fish at a facility, and some environmental fate processes, 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}. Therefore, risks to terrestrial receptors are highly unlikely.

7.12 RISKS TO BIOFILTERS

Most RAS include a biofiltration system (biofilter) of some type to remove ammonia-nitrogen, which if allowed to accumulate in the RAS, will result in toxicity to the fish. Biofiltration uses a substrate with a large surface area for the growth of nitrifying bacteria, specifically (1) *Nitrosomonas,* which oxidizes ammonia into nitrite and (2) *Nitrobacter,* which oxidizes nitrite into nitrate. Because florfenicol is an antimicrobial compound, its potential effect (i.e., reduction in bacterial populations) on these nitrifying bacteria in biofilters is of concern. If these bacteria populations are reduced to levels such that ammonia and nitrite are not efficiently oxidized, ammonia and nitrite concentrations in the water could elevate to toxic levels and result in fish morbidity and potentially mortality.

The impacts of florfenicol on biofilters and water quality parameters have been investigated in the studies of Gaikowski et al. (2011) and Meinertz (2012). Water samples were collected during the course of the residue depletion studies conducted in tilapia (Gaikowski et al., 2011) and rainbow trout (Meinertz, 2012) that were described previously in this EA (Sections 7.1 and 7.5.1). Water quality parameters were measured daily from the RAS and included temperature, pH, and total ammonia, nitrite, and nitrate levels. Concentrations of unionized ammonia (calculated from total ammonia concentrations) t remained at levels below 0.02 mg/L throughout the acclimation, dosing, and post-dosing periods of the study. Nitrate levels averaged 67, 81, and 75 mg/L during the acclimation, dosing, and post-dosing periods, respectively. Nitrite levels occasionally exceeded 2.0 mg/L, but only during the acclimation period and gradually increased to 2.0 mg/L during the first eight days of exposure. However, this increase appeared to be related to a buildup of biofilm material in the water supply line and not due to any adverse effects of florfenicol on the nitrifying bacteria in the biofilter because the nitrite levels rapidly decreased for the remainder of the test after the water supply lines were flushed on day 8. The study authors concluded that administering florfenicol at a dose of 20 mg/kg bw for 10 days in a RAS that recirculates 95% of the water did not alter the capacity of the biofilter to remove nitrogenous wastes.

Although there were no notable effects of florfenicol on biofilter function in the Meinertz study, the conditions of the study were not representative of what may occur in a majority of facilities. Most notably, (1) the system only recirculated 80% of the water and (2) fish in only one of the tanks in the dual-tank RAS was treated with florfenicol, for a total mass of treated fish of 14.2 kg/m^3. Therefore, the lack of effects on biofilter function noted in this study cannot be used conclude that florfenicol does not alter biofilter function in a cold water system.

Although these water quality data do not provide complete evidence that the biofilter performance of all types of RAS will not be adversely affected, there were no apparent effects noted under the conditions of these studies. The impacts of florfenicol on biofliters at commercial aquaculture facilities with different RAS parameters (e.g., higher recirculation rate, lower water temperature, higher biomass of treated fish) are not known.

Additional supporting information is available from studies conducted to examine the effects of florfenicol on two nitrifying bacteria, *Nitrobacter* sp. and *Nitrosomonas europaea* (Sayers, 2009a). Although there were methodological deficiencies, the results indicated a minimum inhibitory concentration (MIC) of 65 and 2.5 mg/L for each species, respectively. The highest calculated values for the refined PEC_{water} for acute exposure (1.04 mg/L) and chronic exposure (0.545 mg/L) are below these MIC values, suggesting that there are minimal risks to nitrifying bacteria.

7.13 CONCLUSIONS REGARDING RISKS OF AQUAFLOR® USE IN RECIRCULATING SYSTEMS

Based on the data, assumptions, and calculations presented in this EA, the use of Aquaflor® in recirculating aquaculture systems for freshwater finfish 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, and
  - to application in feed at up to 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 to a wide range of organisms based on toxicity studies.
- Exposures in aquatic systems are expected to be low and transitory.
- PEC_{water} values were calculated using conservative assumptions based on typical culturing practices of tilapia (i.e., high densities, low recirculation rate).
- Using the refined PEC_{water}, risk quotients for aquatic organisms exceed 1.0 at Tier A and Tier B in the absence of in-stream dilution. However, recirculating systems discharge very small volumes of effluent, so dilution in the environment is likely.
- Using the refined PEC_{soil}, no PEC/PNEC ratios exceed 1.0 for terrestrial organisms that could be exposed to florfenicol residues through land application of solids from recirculating systems.
- Toxicity to the most sensitive organisms—algae and cyanobacteria—is based on inhibitory effects, which are transitory and reversible when exposure is removed. Thus, any inhibited populations are expected to recover rapidly, widespread, ecologically significant, or long-lasting impacts are not expected.

- Because risk quotients for important receptor groups (i.e., *Daphnia*) were greater than 1.0 after refining the PEC_{water}, acute and chronic water quality benchmarks have been developed for florfenicol that will allow permitting authorities to establish appropriate effluent discharge limits for a facility, where needed, based on site-specific conditions (e.g., effluent treatment, in-stream dilution) and in conformance with applicable State and Federal water quality regulations. Environmental statements will be added to the drug label that identify the water quality benchmarks for use by NPDES permitting authorities.

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 recirculating 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

Risk mitigation measures for the use of Aquaflor® in recirculating aquaculture facilities include implementation of water quality benchmarks for the protection of aquatic life in the NPDES permitting process for such facilities. The water quality benchmarks are provided in the following label language:

> *LIMITATIONS AND CAUTIONS FOR ALL USES*
>
> *Before using this drug for the first time, you must inform the appropriate National Pollutant Discharge Elimination System (NPDES) permitting authority of your intentions and of the following information. Acute and chronic water quality benchmarks for the protection of freshwater aquatic life have been derived by FDA for florfenicol following EPA guidance for calculating Tier II water quality criteria for the Great Lakes System (40 CFR 132, App. A). The acute benchmark value (Secondary Maximum Concentration) is 20.6 mg/L (equivalent to one-half of the Secondary Acute Value). The chronic benchmark value (Secondary Continuous Concentration) is 0.23 mg/L (equivalent to the Final Plant Value). The NPDES authority may require an NPDES permit before you can discharge Aquaflor®. The water quality benchmark concentrations are not discharge limits, but may be used by the NPDES authority to derive such limits for the permit. Additional environmental information on Aquaflor® and the benchmark values are available in an environmental assessment posted at http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/EnvironmentalAssessments/ucm300656.htm.*

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 Exponent, Inc. under the direction of Jane P. Staveley. Experts at federal agencies that were consulted, other than those at the U.S. FDA/CVM, include Dr. Mark Gaikowski and Dr. Jeffery Meinertz at the U.S. Geological Survey, Upper Midwest Sciences Center, Biological Resources Division. Other experts consulted in the preparation of this document include Dr. Richard Endris and Dr. Gregor Scheef (Intervet Inc d/b/a Merck Animal Health).

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 G. Endris

15 August 2013

Richard G. Endris, PhD.
Associate Director, Project Management
Global Pharmaceutical Development
Intervet Inc d/b/a Merck Animal Health

Date

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