ENVIRONMENTAL ASSESSMENT
FOR
AQUAFLOR FOR FRESHWATER-REARED SALMONIDS

SECTION 1. DATE: January 31, 2006

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

SECTION 3. ADDRESS: 556 Morris Ave.
Summit, NJ 07901

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SECTION 4. DESCRIPTION OF THE PROPOSED ACTION:

A new animal drug approval has been requested for the use of Aquaflor® in freshwater-reared salmonids. Aquaflor® contains the active ingredient florfenicol, a synthetic, broad-spectrum antibiotic, effective in the control of mortality due to *Flavobacterium psychrophilum* (coldwater disease) in freshwater-reared salmonids.

The Aquaflor® is added to fish feed by incorporation prior to pelleting or coated onto pellets. The rate of administration of the premix to the feed will be dependent on the feeding rate. At a feeding rate of 1% body weight per day, 2.0 g of the medicated article containing 1.0 g florfenicol would be applied per kilogram of feed. The recommended dosage regimen in salmonids is 10 mg/kg body weight for 10 consecutive days. The amount of florfenicol being administered will be dependent on the quantity and weight of fish requiring treatment. The product is intended for use in cultured salmonids in the freshwater environment.

This product is currently being used for treatment and control of bacterial fish diseases in Japan, Norway, Chile, Canada, the UK, the US, Ecuador, Venezuela, Panama, Honduras, Nicaragua, El Salvador, Guatemala and Mexico.

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

5.1 PHYSICO-CHEMICAL CHARACTERISTICS:

Florfenicol is the 3-fluoro derivative of thiamphenicol which is a chloramphenicol analogue in which the p-nitro group on the aromatic ring is substituted with a sulfonylmethyl group (see structure below). The Tier A physico-chemical characteristics of florfenicol and its major metabolites have been determined, Table 1 (Appendix 1). Florfenicol has a molecular weight of 358.21 with solubility in water of 1.32 g/l at pH 7 and a log K_{ow} value of 0.37, the latter indicating little potential for bioaccumulation. In 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 pose problems in the environment due to locally elevated concentrations. Such problems would be expected with materials of low solubility or those that readily adsorb or accumulate, but compounds such as florfenicol that have substantial solubility with an extremely low Log K_{ow} would not be expected to accumulate in biota or other environmental matrices (sediments).

Florfenicol has a low molecular weight as do its metabolites which range from 69 - 89% of parent. The parent and metabolite solubilities and K_{ow} values differ. The metabolites are markedly more soluble (solubilities ranging from 49.7 to >500 g/L) and are markedly less lipophillic (i.e. have lower K_{ow}). These factors make the metabolites even more likely than florfenicol to enter and remain in water rather than partition to sediment, suspended particulates or biota.

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.

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

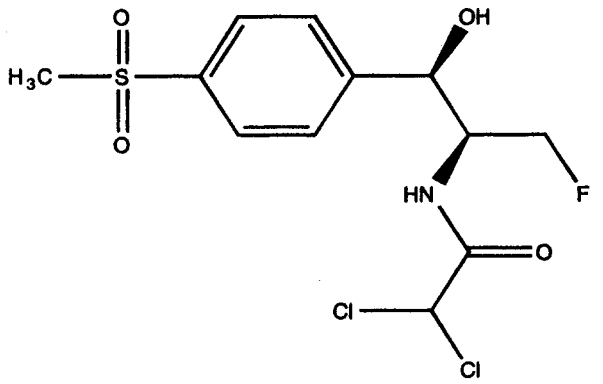
NA = Not applicable/available
* = With ionic strength correction

In addition, florfenicol is a nonvolatile solid, has a UV maxima at 224 nm, and florfenicol has a melting point of 153 - 154°C (Appendix 2).

5.2 CHEMICAL FORMULA AND NOMENCLATURE:

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

Figure 1: Structural Formula:



Florfenicol (SCH 25298)

SECTION 6. FATE OF THE EMITTED SUBSTANCES IN THE ENVIRONMENT:

The Tier A fate studies for florfenicol in the environment have been discussed in detail in support of its use in cattle. The results of those studies and additional studies relating specifically to this proposed action will be discussed below. The dissipation and degradation of florfenicol will be addressed in each of three compartments (soil, sediments and water). Three principal studies are: 1) a metabolism in soil (Appendix 3), 2) degradation in sediment:water systems (Appendix 4) and 3) degradation pig slurry (Appendix 5). The effect of these fate characteristics on the PEC values in receiving water/sediment environments will also be discussed.

6.1 FATE OF FLORFENICOL IN WATER:

The physicochemical properties of florfenicol and florfenicol-related residues are essential to understanding the fate of these chemicals in water. Studies on the susceptibility of florfenicol and its metabolites to photolysis and hydrolysis indicate that these are unlikely to play a significant role in the degradation of these compounds in the environment, Table 2 (Appendices 6-10). Hydrolysis of florfenicol has only been detected in synthetic humic water, where a half-life of 350 days was determined (Appendix 6). 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 (Appendix 6). However, in a recent study florfenicol and its metabolites exhibited abiotic degradation under anaerobic conditions (see section 6.4)

The ability of florfenicol and its metabolites to degrade under the conditions of the FDA 3.11/OECD ready biodegradation CO_2 evolution test has been investigated. None of the compounds was found to degrade to any marked degree as indicated by CO_2 evolution or loss of parent compound (Appendices 11-13). However, analysis of the test media at day 28 of the incubations indicated that while 81.4%, 98.6% and 70.7% of the florfenicol, oxamic acid and alcohol metabolites remained, only 25.4% of the amine metabolite was detected indicating degradation of this metabolite (Appendix 13). These data were inconclusive because no check was made of the potential for microbial inhibition by the antibiotic under the test conditions. It could not be concluded that the lack of degradation observed in these studies was not due to inhibition. A study has been conducted on the persistence of florfenicol, at an initial concentration of 3 mg/L, in synthetic seawater containing sand (Appendix 14). Bacterial growth inhibition was employed as a bioassay and 31% to 38% loss of activity was detected within one day and 82% to 93% was lost over 31 days. Anti-microbial activity was reduced by 50% in approximately 4 days (Appendix 14). A reduced rate of loss of activity was found in some incubations, which lacked added sediment, particularly where the incubation vessel was shaded from direct sunlight. This effect may be due to a reduced microbial biomass from the sediment in the incubation media (Appendix 14). Although this study was non-GLP and lacked adequate documentation it would appear unlikely that florfenicol will retain antimicrobial activity for extended periods in natural water/sediment systems. The timeframes for loss of activity reported in this study and for degradation in water generally support the findings of the aquatic biodegradation study (sediment:water systems) discussed in Section 6.3, below.

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 (Appendix)	7	8	9	10

NA = NOT APPLICABLE/AVAILABLE

NSR = NO SIGNIFICANT REGRESSION

6.2. FATE OF FLORFENICOL IN SOILS AND SEDIMENTS:

Studies on the adsorption and desorption of florfenicol and metabolites in three different soil types determined that florfenicol was generally classified as highly mobile while the metabolites were less so and classified as moderately to highly mobile. These results are summarized in Table 3 (Appendices 15-18). 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	Highly	Partially	Highly	Moderately
Reference (Appendix)	15	16	17	18

Degradation and mineralization studies of florfenicol, added at an initial concentration of 0.05 mg/kg, to three soil types amended with manure demonstrated that mineralization was extensive with mineralization half-lives ranging from 86 to 270 days (Appendix 3) and a mean value of 158 days at 22°C. Primary degradation, or transformation, of the florfenicol was considerably quicker and only 2.6% to 9% of the florfenicol could be recovered at the end of the 92-day study. Half-lives of 3.6 to 27.2 days were reported in this study. Based on these data, a conservative half-life of 27.2 days is taken for florfenicol in soils in calculations of environmental concentrations (Appendix 3). In a published paper, the uptake of active ingredients of Veterinary Medicinal Products into plants was examined in a laboratory study. Although not a degradation study, the authors indicated that the DT_{50} and DT_{90} values for florfenicol based on the initial and final actual concentrations at the two points were <103 and >152 days, respectively (Appendix 19). The initial actual

concentration was less than 50% of nominal, which may reflect degradation. The study was not designed to determine degradation rates. While degradation products appeared in the course of the soil degradation study (Appendix 3) they did not accumulate. On this basis, the same half-life has been adopted for the degradation metabolites. While no study has been undertaken on the degradation of florfenicol in manure alone, it is considered likely that the degradation rate would be faster in manure than in soils due to the microbial biomass associated with excreta. Degradation of florfenicol in a slurry of pig waste under anaerobic conditions was conducted and the results are discussed in section 6.4, below. This should ensure that the half-life of florfenicol in excreta is no longer than that in manure amended soils, unless the levels of florfenicol in excreta initially inhibit the microbial activity. In the anaerobic study, there was no evidence of inhibition at 8.2 ppm.

Results of studies on the toxicity of florfenicol to worms and plants provide corroborative evidence for the occurrence of degradation in soil. The concentrations of florfenicol in the soils were determined at the beginning and end of the respective 14 and 21-day exposure periods (Appendices 20-22). The analytical results indicate that the levels of florfenicol in the soils had declined by the end of the studies (Appendices 20 and 21). A similar trend was indicated in the earthworm study. The final concentration in soil represented 87.5% of the initial, nominal concentration of 1,000 mg/kg (Appendix 22). These data provide are qualitative and corroborate the soil degradation of florfenicol.

In studies on the persistence of florfenicol in marine sediment systems, the rate of loss of florfenicol and the amine metabolite indicated their potential to undergo transformation and elution from the sediments. A dissipation half-life of 5 days was

determined based on elution and degradation. The amine metabolite was found in deeper segments after the parent florfenicol had declined (Appendix 23). This is most likely due to it being a degradation product with a higher K_d/K_{oc} (i.e., absorption potential), and somewhat lower mobility than the parent compound (Appendices 15-18). The detection of the metabolite at the first sampling point indicates that the microbial flora of the sediment was able to degrade the florfenicol at concentrations between 1 and 50 mg/kg. In a subsequent publication where the persistence and impact of a number of antibacterial agents were examined half-lives of 1.7 and 7.3 days were determined for florfenicol at the two depths studied, 0-1 cm and 5-7 cm, respectively (Appendices 24 and 25). While it is possible that the more rapid reduction in concentrations in surface sediments was contributed to by greater washout of the florfenicol at the surface, the appearance of the amine metabolite demonstrates that degradation was occurring. The degradation and/or washout indicate that florfenicol and its metabolites are unlikely to accumulate in sediments. This finding concurs with the results from soil systems and predictions based on physicochemical properties. It is reasonable to include dissipation data in this assessment and it is particularly relevant with episodic and intermittent uses where dilution and degradation are both expected to occur. Results reported in these studies with sediments are consistent with the results reported for sediment:water systems (Section 6.3) discussed below.

6.3 DEGRADATION IN SEDIMENT:WATER SYSTEMS:

The results and conclusions of the studies discussed above are confirmed by the results of a recently completed guideline study: *Determination of the Aerobic Transformation of [^{14}C] Florfenicol in Aquatic Sediment Systems Based on OECD*

Guideline 308 (Appendix 4)[1] .This Tier A study (VICH Phase II) is the corner stone of the environmental fate data base relative to biodegradation in water and sediment. The remaining studies represent Tier B studies. They are designed to address very specific issues and they are outside the scope of the Tier A environmental fate studies (VICH Phase II).

Three sediments, two freshwater and one marine (Table 4) were used. The overlying water was collected concurrently with each sediment. The study was designed according to OECD 308 (Appendix 26). Radio-labeled florfenicol (ring-labeled) was added to the water fraction of sediment water systems. The systems were prepared from sediment and overlying water collected from three sites (see Table 4). 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. Samples were collected at regular intervals for 100 days. At each sampling time, duplicate test systems were assayed. Residues/degradates were measured in water and sediment using liquid scintillation counting (LSC) and high-pressure liquid chromatography with radiometric detection (HPLC/RAM).

[1] Aquatic transformation in aquatic sediment systems (Appendix 25).

Table 4: Degradation Times (DT) for Three Different Sediments

Source	Type of Site	Sediment type*	% Organic Carbon	Degradation Rates for Sediment:Water Systems (days)		
				DT_{50}**	DT_{75}	DT_{90}
Duxbury Marine (DM)	Marine	Loam	3.2	8.4	16.7	27.8
Goose River (GR)	Freshwater	Loam	2.4	13.0	26.0	43.1
Weweantic River (WR)	Freshwater	Sand	0.76	19.4	38.8	64.5

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

Results show rapid degradation of florfenicol to smaller more polar compounds in all sediments[2]. These smaller, more polar compounds were observed to degrade in both the water and the sediment portions of the system (Figure 2). The parent peak also declined with time in both water and sediment. The pattern of degradation and the subsequent decline in degradates is qualitatively similar for both water and sediment. The data presented in Figure 2 are for the Goose River sediment (half-life of 13 days) (Table 4) and the pattern of degradation presented here is consistent in all three of the sediments evaluated. Figure 3 presents a series of chromatographs from the Goose River (GR) sediment over time. Concurrent chromatographs of water (top) and sediment extract (below) show that a number of lower molecular weight compounds (i.e., eluting before the parent) appear and degrade with time (Figure 3).

[2] Samples were collected from three sediments based on sediment characteristics, but overlying water was also collected and used in the study as the water fraction of the sediment/water systems.

The similar pattern of appearance and decline of metabolites as shown in Figure 4 for GR sediment was observed for the other two sediments. Parent [^{14}C] florfenicol was observed to partition between and sediment and to degrade in both fractions of the test water system (Figures 2 and 3). Half-lives ranged from 8.4 to 19.4 days for three sediments (Table 4). [^{14}C] florfenicol degraded to smaller, more polar metabolites that were not persistent as shown in Figures 3 and 4. Metabolites were observed to degrade at similar or faster rates than the parent. The only metabolite above 10% Total Radioactive Residues (TRR)[3] was the monochloroflorfenicol which had a retention time of 18.4 min (Figure 4). 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 bound residues increased with time and ranged from 63% to 85% of TRR 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 and 9.1 to 32.9, respectively. These values are very low and indicate that florfenicol has a low potential to partition to sediments. Florfenicol related residues would not be

[3] TRR (Total Radioactive Residues): The TRR is the total radioactivity [^{14}C] extracted or collected from oxidation of specific tissues following dosing with [^{14}C]florfenicol. All TRRs in this study are assumed to be florfenicol or florfenicol metabolites or degradates, unless collected as CO_2.

expected to partition to sediments in lotic aquatic systems even though they partitioned in the closed, static system of this study. Any residues that did reach the sediment would degrade to compounds that associate strongly.

Mass balance was calculated by summing the percentage applied dose in volatiles, sediment extracts, aqueous extracts, and bound residues (following combustion of post-extracted sediment). 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% to 120.0%. Microbial biomass was measured at the beginning of the acclimatization phase and beginning and end of the test period.

Table 5: Recovery Rates 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%)

6.4 DEGRADATION IN A SLURRY OF PIG WASTE UNDER ANAEROBIC CONDITIONS

In a definitive GLP study, degradation of florfenicol was evaluated in anaerobic pig slurry. This Tier B fate study is very important to the risk assessment because it provides anaerobic data (Appendix 5) to complement the aerobic biodegradation study discussed above in section 6.3. These two 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 the parent 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. Parent florfenicol declined from 84% of TRR at time zero to 6.8% at three days and 1.3% TRR at 7 days. Florfenicol was observed to partition rapidly from water to biosolids as observed in time zero samples where 55.9% and 28.5% of the TRR were in water and biosolids, respectively. From day 7 to day 48 recovered[4] florfenicol residues remained at approximately 1.0%. No parent florfenicol was reported at day 90. Parent florfenicol was observed to partition between water and solids and to degrade rapidly in both compartments.

[4] Biosolids were extracted and water samples were analyzed directly.

The primary metabolite, monochloroflorfenicol, was present in the time zero samples at 2.3% and 1.9% TRR in water and biosolids, respectively. The metabolite reached a maximum of 34.9% TRR on day 3 and declined to 0.8% in water at 90 days and 1.0% in biosolids at 48 days. This indicates that the primary metabolite, monochloroflorfenicol, is rapidly formed (as shown in the zero time samples), does not accumulate, and degrades very rapidly as does the parent. Other metabolites (florfenicol amine and florfenicol oxamic acid) were observed, but did not exceed 4.0% TRR at any time interval. These observations were similar to the results of the aquatic biodegradation study discussed above in section 6.3.

This study also included a set of sterile systems run concurrently with the definitive non-sterile slurry study. These essentially microbe free systems were maintained under anaerobic conditions and florfenicol degraded from 90% of TRR at time zero to 5.0% at 90 days. The observed distribution between water and biosolids was similar to that observed in the non-sterile systems. These data indicate that abiotic degradation will occur under anaerobic conditions with florfenicol at 90.2% of TRR at time zero degrading to 5.0% by 90 days. Although the results at 90 days were similar to those of the biologically active system, the rate of degradation was much slower in the abiotic, sterile system.

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 DT_{50} and DT_{90} values of 2.4 and 8.1 days, respectively, and appeared to follow pseudo first order kinetics.

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 abiotic degradation under anaerobic conditions.

Two previous, less rigorous, non-GLP slurry studies produced similar results. In a study on the decomposition of florfenicol in chicken excreta suspended in water at 37°C it was found that 80% of the florfenicol had degraded by day 14 (Appendix 27) (Table 6). From the reported values, an estimate of the half-life of *ca.* 10 days can be made in the presence of chicken excreta. Similar data are reported for pig excreta suspended in water, Table 6 (Appendix 28).

6.5 SUMMARY OF ENVIRONMENTAL FATE STUDIES:

In summary, the degradation of florfenicol and the monochloro metabolite is rapid in soil, sediment:water systems, and anaerobic pig slurry as reported in the three principal studies including Tier A and Tier B studies (Appendices 3, 4, 5) (Table 6). The numerous supporting studies also report rapid degradation of florfenicol and its metabolites with the exception of studies where some treatment levels may have resulted in microbial inhibition (Appendices 11-13). Existing biodegradation data from a range of studies and study designs are summarized.

As discussed above the reliability of the studies in Table 6 ranges from the three principle studies which were highly reliable GLP studies of soil degradation, biodegradation in sediment/water systems, and anaerobic degradation, in slurried pig waste (Appendices 3-5) to uniquely designed experiments with a lower level of documentation and reliability (Appendix 14). The latter studies are included in this assessment in a qualitative manner. Due to the low reliability of some of these

studies and the lack of delineation between dissipation and degradation, the half-lives of florfenicol in these studies serve primarily to corroborate and support the definitive, principal biodegradation studies. However, data for the three principal studies and the supporting studies represent a weight-of-the-evidence that florfenicol and florfenicol-related residues degrade in environmental matrices and partition between water, and sediments and soils. In all these studies, from manure amended-soils to marine sediments, rapid degradation, dissipation and loss of biological activity is consistently observed.

Table 6: A summary of Tier A and Tier B degradation/dissipation studies in water, soil, and sediments with florfenicol

Study	Reference (Append.)	Matrix/System	Environmental Half-Lives (DT_{50} in days)
Principal Studies			
Aerobic Biodegradation in Manure-amended Soil	3	Manure amended soil	3.6 to 27.2
Determination of the Aerobic Transformation of [^{14}C] florfenicol in Aquatic Sediment Systems	4	Sediment:water systems	13.0 (range 8.4 to 19.4)
Anaerobic degradation in Pig Slurry	5	Pig slurry system	1.0 (florfenicol) 2.4 (monochloroflorfenicol metabolite)
Supporting Studies			
Persistence of anti bacterial agents in marine sediments	23, 24	Marine sediments	Dissipation half-lives of 1.7 and 7.43 days at 0-1 and 5-7 cm, respectively
The decomposition characteristics in seawater	14	A sediment: water system	50% loss of antibiotic activity in four days
Acute toxicity to the earthworm	22	Bioassay soil (14 day earthworm study)*	Half-lives for initial concentrations of 1, 10, 100 and 1,000 mg/kg were 8, 14, 37 and 73 days, respectively.
Florfenicol: Terrestrial plants, growth test	20, 21	Bioassay soil (21 day plant study)	After 21 days 16%, ca 34.6% and 67.2% remained in soil treated with 1, 10 and 100 mg/kg, respectively
Diluted chicken excreta	27	Diluted chicken excreta	80% degradation in 14 days @37°C
Diluted pig excreta	28	Diluted pig excreta	Residues of florfenicol not detected after 15 days @37°C
Up take of VMPs from soil	19	Agricultural soil	Florfenicol: DT_{50} <103d DT_{90} >152d

*Includes preliminary and definitive exposures. Preliminary exposures were not replicated.

SECTION 7. INTRODUCTION OF THE SUBSTANCES INTO THE ENVIRONMENT:

The levels of florfenicol and its metabolites in the receiving environment will be dependent on the quantities administered and consumed, and the fate of excreted florfenicol-related residues in water. This includes subsequent dilution within the facility and in the receiving environment. The principal route by which florfenicol reaches water is via absorption, metabolism and excretion from fish. Florfenicol may reach the sediment as uneaten, medicated feed or in feces. However, both florfenicol and its metabolites will enter the water column by leaching from feed and feces. Elimination from fish directly to the aqueous phase via the excreta is the primary route that florfenicol-related residues enter water. Florfenicol and the principal metabolites would be expected to remain in water based on the physico-chemical characteristics. However, data from the aerobic biodegradation study in sediment:water system shows that both parent and metabolites partition to sediments in static, closed systems. Degradation was observed in both sediment and water compartments.

Based on these data, the current assessment assumes that all florfenicol-related residues are excreted to and probably remain in the water column in lotic systems (i.e. rivers and streams). In static or lentic systems (ponds, quiescent areas of raceways), some portion of the florfenicol and metabolites would be expected to partition into the solid phase. The amount of partitioning in a closed static sediment:water system in the lab was approximately 60 to 30 (i.e. 30% in sediment). The amount expected to partition out of water in a lotic system would be difficult to quantify.

Therefore, partitioning to sediment is not considered in the initial estimates of the PECs and all florfenicol and florfenicol related residues are considered to remain in the water. However, florfenicol and breakdown products associated with sediments are strongly bound and are unlikely to dissociate. Raceways and settling ponds or other water treatment processes that rely on trapping of solids could provide a sink for a portion of the florfenicol in the raceway, or entering settling ponds or receiving waters. Furthermore, degradation would be expected to occur in association with the sediment and water phases.

7.1 TREATMENT REGIMEN AND USE PATTERN:

This is a worst-case scenario since some partitioning of florfenicol to the solid phase would be expected in settling ponds and quiescent areas of raceways, and potentially in receiving waters. The target disease for Aquaflor in fish is 'coldwater disease' or 'rainbow trout fry syndrome (RTFS). The causative agent formerly known as *Flexibacter psychrophilum* is *Flavobacterium psychrophilum*, which is referred to in this EA as "coldwater disease". This organism does not appear to be ubiquitous in the environment, but is found in the water and sediments of affected areas. The organism also may be transmitted between generations in the eggs from infected brood stock (Appendix 25, R. MacMillan pers. comm.[5]). The disease affects predominantly younger juvenile (fry and fingerling) salmonids that weight 0.2 to 10.0 g

[5] Dr.Randy MacMillan, Vice President with responsibilities for Environmental Affairs, Clear Springs Foods, Inc., Buhl, ID. Discussions occurred on and around August 17, 2006.

(Appendix 29, Appendix 25, R. MacMillan pers. comm.). This is why the disease is called "Rainbow Trout Fry Syndrome". Fish in grow out ponds are generally not affected (Appendix 29, Appendix 25, R. MacMillan pers. comm.).

Florfenicol will be administered to fish in the form of a premix applied to feed. The product can be mixed in un-medicated feed prior to pelleting or by dry coating the premix onto the feed and sealing it by over oiling. The medicated feed is administered to fish at a rate targeted to deliver a dose of 10 mg florfenicol per kg of fish per day for 10 days. Fish suffering from bacterial diseases are known to exhibit loss of appetite. To increase the opportunity for the fish to ingest sufficient medicated feed so that the MIC is maintained for a sufficient period, circa seven days, a ten-day treatment period has been selected. This treatment period has been established in numerous efficacy studies and should ensure that the potential for fish to be re-infected from other fish that are not feeding is reduced.

Aquaflor is applied to the same population or cohort of fish typically only one or two times per year with an expected maximum of five treatments (expected use pattern based on discussion of current practices that are associated with Veterinary Feed Directive (VFD) (D. Erdahl pers. comm.[6]) with a minimum interval of six weeks between treatments. However, it is unlikely that treatment of another outbreak of the disease in the same population soon after the initial treatment would utilize Aquaflor since the

[6] Dr. Dave Erdahl, Aquatic Animal Drug Approval Program (AADAP) Coordinator, U.S. Fish and Wildlife Services, Bozeman, MT. Discussions occurred August 22, 2006.

second outbreak could imply a lack of disease in this population by florfenicol. Based on the rapid elimination of florfenicol from fish (half-life of 8.8 h) and rapid degradation in water and sediment (half-life 13 days), sequential, episodic treatment with Aquaflor would not lead to accumulation in the environment. This product is subject to the VFD, and any application must have a prescription from a veterinarian, which limits its use compared to over the counter products such as oxytetracycline or Romet.

Prevention, treatment and control of bacterial diseases of fish in the U.S. are achieved through a combination of vaccines and antibiotic therapy. Aquaflor when approved would be one of three available antibiotics that are treatments for coldwater disease.

The introduction of florfenicol will likely result in a redistribution of market share for the antibiotics available for use in freshwater-reared salmonids. Presently, only oxytetracycline and Romet are approved for use in salmonids, both have been in use for >20 years and both have encountered significant resistance issues among aquatic pathogens. In conjunction with emerging regulation under the VFD, the introduction of florfenicol is not likely to increase the overall use of antibiotics in this use pattern (D. Erdahl pers. comm.).

Treatment of freshwater-reared salmonids will be prescriptive under the VFD requiring approval by a veterinarian for application only to actively infected, clinically diagnosed populations. Application is limited to 10

days and there is no prophylactic application (Appendix 30). The VFD for application of florfenicol to a specific population of fish requires that feed be prepared in a certified feed mill and the VFD for prepared feed expires after 15 days. Any expired medicated feed cannot be fed to fish.

The application of antibiotics to a specific population is generally limited in scope due to the need for an active infection. Only a portion of a facility (i.e. specific infected raceways) is treated at one time (R. McMillan, pers. comm.). The release of florfenicol-related residues is episodic, occurring over approximately 15 days during and after a 10-day application period. Such treatment generally occurs once or occasionally twice in a production cycle (D. Erdahl pers. comm.). Antibiotic treatments are most often made during the spring and early summer (Appendix 31) when water temperatures rise and the target pathogen is most active (D. Erdahl, pers. comm.), but may be used year around. The applications in the spring and early summer also correspond to the highest water flows and, therefore, the highest potential for dilution in receiving waters (D. Erdahl, pers. comm.). These factors related to the use pattern, combined, limit the magnitude and duration of exposure and must be considered in characterizing the over all environmental risk from florfenicol use in freshwater-reared salmonids.

Coldwater disease does not spread readily between raceways and outbreaks are generally limited to one or two raceways[7]. At Clear Springs[8] facilities, for example, the predominant occurrence is in fry in hatch houses. One or two (especially, if they are closely related populations) raceways would be treated and no other outbreak would be expected. Since raceways with fry are only a limited part of a hatchery's operation, only a fraction of the raceways would receive treatment with florfenicol. Thus 2 of 15 (single) raceways might be treated (R. MacMillan pers. comm.). This is consistent with other hatcheries where the incidence is observed to be much lower than a 20% estimated upper bound (Dr. D. Erdahl pers. comm.) This proportion of treatment is not addressed in any known publication. The figure of 20% is the result of a survey of current use practices and is the upper bound of the proportion of a facility that would be treated at one time. It is based on a thorough understanding of the disease being treated and the VMPs used. Dr. D. Erdahl is the INAD coordinator for the US Fish and Wildlife Service and is familiar with all of the government run hatcheries. R. MacMillan is the managing biologist for Clear Spring's aquaculture facilities in Idaho. Combined these two men have knowledge of how florfenicol, and other VMPs, would be used to treat coldwater disease and what proportion of raceways would be treated concurrently.

[7] Culture units made generally of concrete, plastic or stainless steel used to raise trout. Raceways are generally 0.5 - 1.0 m deep and of varying length. Raceways are generally gravity-fed open systems and are often constructed in sequence (e.g., 3 - 6 units).

[8] Clear Springs: Clear Springs Foods is a vertically integrated company. From their state-of-the-art farm, feed manufacturing, processing plant, and research facilities, that produces 20 million pounds of trout per year. Clear Springs' state-of-the-art Research and Development center produces vaccines, monitors water quality in the springs, the farms, and the Snake River, and provides an array of fish health services to the farms. Research projects are ongoing in the areas of nutrition, waste management, genetics, and fish culture. The research division provides a complement of quality assurance services to the other divisions of the company.

Brood stock (> 2.0 kg each) also present with this disease which is possibly stimulated by the stress of production of eggs and sperm. As with juvenile fish, brood stock are a limited part of a hatchery's operation and only a small proportion (≤20%) of the raceways might be infected and treated in any facility (R. MacMillan pers. comm.). The disease does not spread between parallel raceways, but is likely to spread in sequential raceways. In the current assessment, a basic unit of three sequential raceways is used in PEC calculations.

Coldwater disease is currently treated with oxytetracycline or Romet in the U.S. Resistance to antibiotics has been observed in some facilities (Appendix 25). Aquaflor will provide an alternative to oxytetracycline and Romet should resistance occur with either of these products in the US.

The following key factors related to use pattern, as discussed above, will be incorporated quantitatively in this EA. These are:

1. A prescribed dietary application (requiring veterinarian approval) designed to deliver 10 mg florfenicol per kg of fish per day for 10 days;
2. Due to the differential sensitivity of fish to coldwater disease (decreases with increasing age) (Appendix 25), the likelihood of treating young fish is much greater than for older fish (Appendix 25, J. Hinshaw pers. comm[9].). The current EA will include a non-

[9] Dr. Jeffrey Hinshaw, Associate Professor & Extension Specialist, College of Agriculture and Life Sciences, North Carolina State University, Fletcher, N.C. Former president U.S. Aquaculture Society. Discussions occurred August 1, 2006.

worst-case treatment of very young fish (i.e. 5 kg/m^3 density, flow rate of 22 L/s), a median level treatment more typical of production size pan fish (density 18 kg/m^3, flow rate of 22 L/s), and a worst-case treatment of production size fish at a density of 47 kg/m^3 and a low flow rate of 3 L/s.

3. Fish treated for coldwater disease are principally 0.2 - 10 g fish with densities ranging from 5 kg/m^3 for smaller fish (0.2 - 5 g) (Appendix 25, Dr. Erdahl pers. comm.) to 20 - 40 kg/m^3 for 5 - 10 g fish (J. Hinshaw, pers. comm.). Further, these sizes of fish occupy only 5 - 10% of the capacity of a typical trout farm (J. Hinshaw, pers. comm.).

7.2 PHARMACOKINETICS IN SALMONIDS:

Florfenicol and related residues enter the aquatic environment via ingestion and excretion by treated fish. Florfenicol is readily absorbed, distributed, metabolized and excreted by salmonids in both fresh and salt water and no bioconcentration is expected. Using various routes of administration (intravenous, gavage and dietary exposure) and a range of study designs, the following results demonstrate a consistent pattern of pharmacokinetics in trout and salmon (Appendices 32-40). The residues observed included the parent florfenicol and three primary metabolites (florfenicol amine, the alcohol, and the oxamic acid) and conjugates (e.g. glucuronides) of parent and metabolites. The results of metabolism and pharmacokinetic studies are similar to those conducted with other vertebrate species (e.g., cattle, rats, swine, poultry, and sheep).

Radio-labeled florfenicol administered in feed to salmon has been shown to have a bioavailability of 96.5% and 99% (Appendices 35, 36). The uptake is rapid with radioactivity being detected after three hours in tissue and urine, the latter indicating rapid distribution and elimination (Appendix 36). The main routes of excretion are via bile and urine, with the levels in bile and kidney peaking at day three. The metabolism and depuration of florfenicol are such that half-lives of 30 - 35 hours have been found for all organs, except kidney, following single doses (Appendix 38). The total residue levels were below the levels of detection in muscle, blood, brain and fat 28 days after administration but persisted at low levels for up to 56 days in the kidney. In multiple dose studies, half lives of 25, 34 and 21 hours for florfenicol and 64, 92 and 198 hours for the amine metabolite were determined for muscle, liver and kidney, respectively (Appendices 32, 33). In these studies, the amine metabolite was generally found at lower levels than the parent compound in the plasma. When administered intravenously the half-life was determined as 12.2 hours (Appendix 36).

Gavage studies with salmon show that while the florfenicol compound and the amine metabolite represent 90% and 7%, respectively, of the residues in muscle at 6 hours post dosing, the relative respective proportions were 20% and 70% after 3 days, indicating rapid metabolism (Appendix 33). Studies with parr and post-smolts in freshwater and seawater, respectively, resulted in higher serum concentrations in the parr (Appendix 32). The results indicate that the florfenicol was well absorbed, excreted rapidly in bile, feces and urine and rapidly

metabolized to florfenicol amine with florfenicol alcohol and oxamic acid being present as minor metabolites (Appendix 32).

Extensive metabolism was recorded (Appendices 32, 33) in residue depletion studies with salmon dosed orally with ^{14}C-labeled florfenicol and held at 5°C and 10°C. Florfenicol and its alcohol, oxamic acid, monochloro- and amine derivatives were identified together with a number of unidentified moieties, some of which were chromatographically similar to the glucuronides of the alcohol, amine and parent compound. These were found to be present in the tissues, bile and excreta and it was concluded that the metabolism of florfenicol in fish was similar to that in cattle and rats with the florfenicol being metabolized through the identified intermediates to florfenicol amine (Appendix 36).

In rainbow trout, an elimination half-life of 8.8 hours was determined following intravenous injection at 10°C (Appendix 37). Following oral intubation at 10°C and oral administration of medicated feed at 10°C and 16°C, bioavailabilities of 73.9% and 66.3%, respectively, were determined for rainbow trout. The residue levels in the plasma of trout fed medicated feed treated with florfenicol at 10°C were observed to peak at 12 hours after dosing and declined by 48 hours (Appendix 37). Finally, residue depletion studies were conducted in trout (*Oncorhynchus mykiss*) at 8 and 15°C (Appendices 39, 40). Residues in edible tissues declined to below the Safe Concentration (1.0 mg/kg) in 7 and 10 days, respectively.

The available data indicate that the pharmacokinetic distribution patterns for florfenicol in rainbow trout and salmon are similar although the trout had lower rates of absorption and shorter elimination times at 10°C. That the metabolism should be similar in salmon and trout might be expected as it has been found that the metabolic degradation of florfenicol in cattle, rats, poultry, swine, sheep and salmon are similar with the florfenicol being degraded to the alcohol, oxamic acid, monochloroflorfenicol and florfenicol amine.

The available data indicate that excretion via urine will be a major route for florfenicol and its metabolites to reach the environment. Given the rapid uptake of florfenicol from feed in salmon it is possible that some of the material excreted in the bile will be conjugated and have the glucuronide conjugate bonds cleaved and some of the freed florfenicol may be re-absorbed undergoing enterohepatic recirculation. However, given the rapid excretion of residues and the short half-life, enterohepatic recirculation is not playing a major role in the excretion of florfenicol residues. For the purposes of this analysis all florfenicol related residues are conservatively assumed to enter the water column and the magnitude of residues in uneaten feed or remaining in feces is not considered significant.

Finally, based on the physicochemical property data (high water solubility, low K_{ow}) and the pharmacokinetics data discussed above (i.e., rapid uptake and clearance), no significant bioaccumulation of parent florfenicol or the primary metabolites would be expected in aquatic organisms.

Using the U.S. EPA's estimation software (Appendix 41) EPIWIN (version 2.2), the estimated bioconcentration factor (BCF) is 3.16 (log BCF = 0.50), which is below any level of concern and supports a conclusion that a bioconcentration study, a Tier B study under VICH Phase II (Appendix 42), is not required for florfenicol.

7.3 PREDICTED ENVIRONMENTAL CONCENTRATIONS (PECs) IN THE WATER:

The quantity of florfenicol and related residues that would be released into the water during the treatment period and post-treatment period is dictated by the rate of absorption from the gut, metabolism and depuration/excretion by fish. Medicated feed is administered to the fish for 10 days. Based on metabolism data, most of the dose is excreted within about 5 days after the last treatment day. Based on these values the administered material would be mainly excreted over a total period (during treatment and post treatment) of 15 days. All residues of florfenicol and the principal metabolites enter water in excreta of fish. Fish pharmacokinetics and metabolism determine the rate and magnitude of these residues entering the water column. To be conservative, for the purposes of this assessment, all residues are assumed to be contained in the total water volume released from a typical raceway during the period of treatment (10 days) and depuration (5 days).

7.3.1 Environmental concentrations of florfenicol administered to salmonids reared in freshwater:

Representative Raceway Scenario

Assumptions

- Flow-through raceway facility
- 30.5 long concrete raceways
- 3.0 m wide
- 0.75 m water depth

- Raceways configured in two parallel series of three consecutive raceways each
- Flow rates of 22 and 3 L/s (Table A)
- Densities of 5, 18 and 47 kg fish/m^3 water (Section 16 Table A)

This "typical" raceway configuration includes an average flow rate of 22 L/s based on the median flow rate in raceways in 61 coldwater[10] facilities surveyed by the USGS (Appendix 43) (Table A). A worst-case value would be 3 L/s, based on the 5th percentile data for raceway flow rate from the same set of facilities (Appendix 43). This survey also shows that approximately 50% of facilities have settling ponds that vary in size. This is discussed in more detail in section 7.3.3 as part of a discussion of dilution and other mitigation factors.

Trout and other salmonids are raised commercially and sold as food. In addition, trout and other salmonids are reared by state and federal fish hatcheries as a source of game fish. Although a wide variety of rearing facilities have been used in the past for trout aquaculture, concrete raceway facilities are now used to rear approximately 90% of trout species cultured in the United States, i.e., rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) (D. Erdahl, pers. comm.). Typical concrete raceways are 30.5 m long, 0.75 m deep (1.0 m of raceway depth) and 3.0 m wide with a 15 cm gradient per 30.5 m length (Appendix 44). This corresponds to a mean

[10] The term coldwater is defined as systems with maximum annual temperatures below 75°F selected from a survey of 100 facilities as reported by U.S. Geological Survey (Appendix 43).

volume of 68.6 m^3 for individual raceways, which is the median value for raceways in the USGS hatchery survey data (Appendix 43). Raceways may be configured in single or parallel rows of raceways (Appendix 44). In this analysis, a sequential configuration of three raceways is used as representative. This allows for a total volume of:

30.5 m x 3.0 m x 0.75 m = 68.6 m^3 per single raceway, and;

68.6 m^3 x 3 raceways = 206 m^3 in 3 raceways

In general practice, the flow of water in a series of three raceways is 22 L/s to maintain the oxygen level (Appendix 43). This value is based on USGS survey data[11]. A flow of 3 L/s is used in the current assessment is a representative worst-case based on the 5th percentile value from of data for actual hatcheries and production facilities (Appendix 43) (Table A). The carrying capacity (biomass of fish per volume of water) of raceways is limited by oxygen consumption and waste accumulation, which are proportional to the quantity of feed consumed. In the majority of hatcheries/production facilities water passes through a series of raceways once (Appendix 45). The relationship of stocking density is not highly correlated with flow, but is likely determined by a range of factors. Based on the data from the USGS survey (Appendix 43), the densities for various facilities and sizes of raceways, is correlated with fish density and various other factors, e.g. age and size (rapidly growing fish consume

[11] This value is the average flow rate in size one raceways reported in the USGS Survey (Appendix 43).

more oxygen) of fish to be treated, temperature and oxygen content (i.e. some facilities supplement with O_2).

7.3.2 Concentration of florfenicol-related residues in raceway effluent:

The recommended dosage regimen of 10 mg/kg body weight for 10 consecutive days, and at stocking densities of 5, 18 or 47 kg fish per m^3 (Table A), a concrete raceway with a rearing volume of 68.6 m^3 (68,600 L) in one raceway and a total of 206 m^3 (206,000 L) in a set of 3 sequential raceways, would hold either 1030 kg, 3,708 kg, or 9,682 kg of fish. The biomass of fish per volume (density) for young fish is generally low (e.g. 5 kg/m^3). Densities for larger fish are generally in the range of 18 kg/m^3 for fish less than 2.0 kg and 47 kg/m^3 or greater for fish larger than 2.0 kg. In this assessment, a value of 47 kg/m^3 (95^{th} percentile density value) is used in combination with a 5% flow rate to represent a worst-case application to production size fish. Other than brood stock, trout of this weight range (>2.0 kg) would not be held in raceways and would be expected to be treated infrequently with florfenicol. Older fish (production fish and larger) are less likely to be susceptible to coldwater disease due to previous exposure, but production size fish are included here as a worst-case scenario.

Since most trout production is of pan fish size, 300 - 600 g (Appendix 25), the 18 kg/m^3 is considered as a typical case. Young fish in hatcheries which are more likely to be treated than more mature populations would be reared at a density close to 5 kg/m^3 for fish 0.2 - 5 g (Appendix 25).

Larger young fish (5 - 10 g) are reared at 18 kg/m^3. Young fish (0.2 - 10 g) have higher oxygen demand than larger fish resulting in lower densities (Appendix 25).

The data from the USGS survey (Appendix 43) do not differentiate between size and age of the fish, but provide a summary of data for 49 sites (Table A). The average density is 20.8 kg/m^3, the median is 18 kg/m^3, and the upper 95th percentile probability is 47 kg/m^3. The density of 5 kg/m^3 is included in this discussion as a typical (low-density) scenario for the smallest size fish that might be treated (0.2 - 5 g). Effluent concentrations of florfenicol in this assessment will be made using these three density scenarios for trout, but estimates of risk will be based primarily on a density of 47 kg/m^3, the worst-case scenario (Table 7), and 18 kg/m^3, the median or typical worst case for juvenile and larger fish.

Table 7: Fish Biomass Treated and Total Florfenicol Applied for Typical and Worst-Case Scenarios

Scenario	Typical Low Density	Typical Median Density	Worst-Case Density
Size of Fish Treated	Applied to juvenile fish	Applied to juvenile and adult fish	Applied to adult fish
Density (kg/m^3)	5	18	47
kg fish treated	1030	3,708	9,682
mg florfenicol/day	10,300	37,080	96,820
mg/ florfenicol/10 days of treatment	103,000	370,800	968,200

[a] Based on the median value reported for 49 sites.

For example:

- At a density of 18 kg fish/m^3 then 3,708 kg fish would be contained in three raceways.
- Under a treatment regime of 10 mg florfenicol/kg fish then 37,080 mg florfenicol would be used in 3 raceways in one day, and
- During 10 days of treatment, 370,800 mg of florfenicol is assumed to be released into water over a 240 hr (10-day) period plus a 120 hr (5 days) post-treatment period.

(The worst-case scenario is presented in Table 7, column 4)

The amount of florfenicol must be divided by the total amount of water flowing through the three raceways during the 15-day period to estimate water concentrations. The rapid uptake and elimination of florfenicol-related residues allows the simplified assumption that all residues will be eliminated in effluent during the 10-day treatment period and a 5-day post-treatment period, for a total of 15 days. In this assessment, the estimated florfenicol used (mg) is divided by the estimated total water volume (L) expected to pass through the race ways be excreted over the 15 day period. The flow rate of 22.0 L/s (Appendix 43) is the basis for estimating the total water volume over the 15-day period when time and flow are considered. Calculations of total flow are as follows:

- 22 L/s x 60 s/min x 60 min/hr x 24 hr/day = 1,900,800 L/day
- An additional factor of 15x is used to calculate the total flow over the 15 day treatment period (plus post-treatment) = 28,512,000 L = 15 days x 1,900,800 L/day or 1900.8 m^3/day (Table 8).

Table 8: Estimation of Water Flow

Scenario	Juvenile Case	Typical Case	Worst-case
Volume (m^3) for a single raceway	68.6	68.6	**68.6**
Volume (m^3) for a set of 3 raceways (Volume in L)	206 (206,000)	206 (206,000)	**206 (206,000)**
Flow rate (L/s)	22	22	**3**
Total Flow (L/day)	1,900,800	1,900,800	**259,200**
Total water flow for 15 d (L)	28,512,000	28,512,000	**3,888,000**

The worst-case scenario has a much slower flow rate (3 L/s) and smaller overall flow volume for the 15-day period of release.

This configuration of raceways would serve as a basic unit of a hatchery. Any changes in scale can be estimated by multiplying the values reported here.

7.3.3 Concentration of florfenicol-related residues in facility effluent:

The initial PEC_{water} values are presented in Table 9 and represent the concentrations that might occur in the effluent from the raceway without accounting for any internal or external dilution factors. PEC_{water} values are calculated for three representative density-levels and flow-rate combinations, and the worst-case scenario is emphasized in this assessment. This scenario is based on the 5^{th} and 95^{th} percentile values for flow rate and density, respectively (Table 8). The median flow rate of 22 L/s is used to calculate both the median density (18 kg/m^3) level (typical case) and the lowest density (5 kg/m^3) level (non-worst case)

values. These are the principal values used to refine the exposure estimate in the subsequent calculations.

The initial PEC_{water} values presented in Table 9 do not include any consideration of in-facility dilution due to treatments of only a portion of the total facility (generally < 20% in any one treatment). Dilution of florfenicol-related residues in settling ponds, or receiving waters, are not included in calculating initial PEC_{water} values. In addition, exposure to aquatic organisms would be transient and episodic based on degradation and dilution of released florfenicol-related residues.

Initial PEC_{water} (Environmental Introduction Concentration, $EIC_{aquatic}$) is calculated using the following calculations:

1) Worst-case Scenario:

Assumptions:

- Density (kg fish/m^3 water) = 47
- Flow rate (L/s) = 3
- 9,682 kg fish treated

968,200 mg/10 days÷3,888,000 L/15 days = 0.249 mg/L (249 µg/L) = Initial PEC_{water}

2) Typical Case Scenario:

Assumptions:

- Density (kg fish/m^3 water) = 18
- Flow rate (L/s) = 22
- 3,708 kg fish treated

Input (mg florfenicol/ 10 days) ÷ volume/15 days = Initial PEC_{water}

370,800 mg/10 days ÷ 28,512,000 L/day = 0.013 mg/L (13 µg/L) = Initial PEC_{water}

Table 9: Initial PEC_{water} Values for Three Exposure Scenarios Involving Three Densities of Fish and Two Flow Rates.

Fish Density/Flow Rate Scenarios	Density (kg/m^3)	Water Flow Rate (L/s)	Initial PEC_{water}[1] mg/L (µg/L)
Worst-case: 95th Percentile density and 5th percentile flow rate	47	3	0.249 mg/L (249 µg/L)
Typical Case: Median density with median flow rate	18	22	0.013 mg/L (13 µg/L)
Juvenile Case: Typical density for juvenile fish and median flow rate	5	22	0.0036 mg/L (3.6 µg/L)

[1] The initial PEC_{water} is calculated by dividing the mg florfenicol / 15 days by the total water volume for 15 days.

3) Juvenile Case Scenario

Assumptions:

- Density (kg fish/m^3 water) = 5
- Flow rate (L/s) = 22
- 1030 kg fish treated (0.2 - 5 g each)

Input (mg florfenicol/10 days) ÷ water volume over 15 days = Initial PEC_{water}

103,000 mg/10 days ÷ 28,512,000 L/15 days = 0.0036 mg/L (3.6 µg/L) = Initial PEC_{water}.

VICH: Phase I Considerations:

Up to this point, this assessment has followed the general approach outlined by the VICH[12] Environmental Impact Assessment (EIA's) for Veterinary Medicinal Products (VMPs) – Phase II Guidance (Appendix 42). A Phase II analysis is needed based upon the results of the Phase I Decision Tree in which the following points have been raised. Florfenicol:

- is not exempt from regulation;
- is not a natural product;
- will be used in food animals;
- is intended for use in a major new species;
- will be used to treat whole raceways (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 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 assessment is required based on the direct release into the environment. The worst-case release could potentially occur when all the fish in a hatchery, with a flow rate of only 3 L/s, are at the

[12] VICH stands for the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products.

high stocking density of 47 kg/m^3 and require treatment for coldwater disease. The risk characterization (Section 8.3) will specifically address why even in this worst-case situation there will be little or no impact on the environment from the use of Aquaflor. The risk assessment will discuss mitigating factors that result in reduced initial PEC_{water} values including treatment of only a portion (<20%) of a facility at one time and partitioning to solid phases.

Inside any given facility, only a portion of that facility (specific raceways) is treated at any one time (R. McMillan pers. comm.; D. Erdahl, pers. comm., J. Hinshaw pers. comm.). In this assessment a maximum of <20% of a given facility would be treated at one time. The <20% is a conservative upper bound estimate and the actual value is likely to be much lower (D. Erdahl, pers. comm.).

Finally, dilution and partitioning play key roles in the environmental fate of florfenicol discharged from salmonid facilities and reduce the incremental risk to aquatic organisms. Dilution occurs at various points in the pathway through the facility and in receiving waters. Bacterial diseases generally do not affect all fish in a hatchery at one time; therefore, in any single treatment period only a portion (e.g. <20%) would be treated (R. McMillan pers. comm.; D. Erdahl, pers. comm.). This results in an internal dilution factor of five within the hatchery. A given facility would be treated up to five times in one year and only a portion (approximately 20% or less) would be treated in any single treatment. A mitigation factor of 5 is used to account for the proportional treatment within the hatchery.

A 1.5 mitigation factor is included in Table 10. This is based on the following factors: 1) bioavailability of florfenicol, meaning that trout will excrete 30% of the dose in their feces (Appendix 37), 2) the K_d values derived from soil sorption/desorption studies (Table 3) (Appendix 15) and 3) the partitioning which occurred in a sediment/water biodegradation (OECD 308) study (discussed above in section 6.3). The soil binding study, supported by the results of the sediment:water degradation study, indicates that on average about one quarter to one third of the florfenicol in water will partition from water to sediment. In the raceway context approximately 33% of the florfenicol and its residues which have the same or higher K_d values (based on the bioavailability and partitioning in soil and sediment) will be associated with solids e.g., fecal matter. In the case of Clear Springs in Idaho, the raceways remove 80% of solids via quiescent zones in each raceway and the water continues to downstream raceways. Solids recovered are removed to offline settling ponds, aged and removed as slurry of 12% biosolids. This slurry is applied to agricultural lands. Any florfenicol excreted in or associated with feces would likely be removed directly from the treated raceway and rapid degradation would occur during the aging process in the slurry pond as shown in the pig slurry study discussed above in section 6.4.

Only the internal (in-facility) dilution factor of 5 and the mitigation factor of 1.5 are considered quantitatively in estimating the PEC values in this assessment. Dilution factors external to the facility, including settling

ponds, are discussed elsewhere but since not all facilities have them, they cannot be considered as the common situation.

Table 10: Dilution factors considered in estimating refined PEC_{water} values

Mitigation Factors	Mean Dilution or Reduction (n)	5th Percentile	95th Percentile	Range
In-Facility Mitigating Factors (Included in refined PEC_{water} Calculations)				
Proportion Treated[1]	5[1](NA)	--	--	--
Partitioning to Solid Phases	1.5 (1/0.667)			
Total In-Facility Mitigating Factor	**7.5**			
External Dilution				
Settling Pond	33.8 (46)	1.07	108	0.071 - 168.52
Receiving waters				
Low water flow	21 (52)	0.19	115	00.0 – 329.6
High water flow	33.7 (56)	0.80	214	0.10 – 461.6

[1] Based on a maximum of 20% of a facility being treated at one time.

A standard set of calculations of the concentration of florfenicol-related residues in raceway water yields initial PEC_{water} values in a range from 0.0036 - 0.249 mg/L (Table 11) when dilution and partitioning are considered.

Table 11: Refined PECs

Fish Density/Flow Rate Scenarios	Initial PEC_{water} Values (mg florfenicol residues/L)[a]	Density (kg/m^3)[a]	Water Flow Rate (L/s)[a]	Combined Mitigation Factor	Refined PEC_{water} Values (mg florfenicol residues/L)
Worst-case: 95th Percentile density and 5th percentile flow rate	**0.249**	**47**	**3**	**7.5**	**0.033**
Typical Case: Median density with median flow rate	0.013	18	22	7.5	0.0017
Juvenile Case: Typical density for juvenile fish and median flow rate	0.0036	5	22	7.5	0.00048

[a] Taken from Table 9.

In summary, the release of florfenicol-related residues to the environment (i.e. PEC_{water} concentrations) and exposure to non-target species is low, transient and episodic. In the worst-case situation of the 95th percentile stocking density and the 5th percentile flow rate, with all fish being treated, the PEC in the effluent from such a hatchery would be 0.249 mg/L. This value is conservative because stocking densities are usually lower and flow rates are higher and not all fish are treated at the same time. Once released to receiving waters, residues of florfenicol and florfenicol-related residues degrade rapidly in water, sediment and soil as shown in three principal environmental fate studies (Appendices 3, 4 and 5). The importance of these factors to the risk assessment is discussed in more detail in the Risk Characterization section (Section 8.3). The use-pattern and environmental fate characteristics indicate a low potential for exposure to non-target species.

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

8.1 TOXICITY DATA:

8.1.1 Microorganisms and Plants:

Florfenicol exhibits activity against a wide spectrum of prokaryotic microorganisms with MIC values ranging from 0.4 mg/l for *Bacillus subtilis* to 16 mg/l for *Serratia*, Table 12. 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 (Appendix 46). 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. 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. From the data on reductions in concentrations of florfenicol in soils in terrestrial organism toxicity studies (Appendix 20-22), and half-lives derived in Section 6, it is apparent that rates of reduction in concentrations are inversely proportional to the initial concentrations of florfenicol present.
From the data available on the rates of degradation of florfenicol at different concentrations, the results from the nutrient transformation study might be expected. The recovery in microbial activity indicates that, under the conditions of the study, the microbial populations responsible for transformation were partially inhibited, not killed, and were able to

resume processing when the florfenicol was degraded. As shown in Table 12, below, the metabolites were found to be 5 - >1,000 fold less toxic than the parent florfenicol (Appendices 49-51). 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) (Appendix 51) as the parent florfenicol for three microbial species (Table 12, Column 2 and 6). In this assessment, the total residues of florfenicol are conservatively treated as parent compound (i.e., as having the same rates of inhibition as parent compound) for the purposes of assessing risk. The potential inhibitory action the monochloro metabolite is similar to the parent moiety and does not pose any additional risk.

8.1.2 Aquatic plants:

Florfenicol is generally less active against eukaryotes than prokaryotes, although activity was tested with three species of algae: the green algae *Selenastrum capricornutum* (Appendix 52), the freshwater diatom, *Navicula pelliculosa,* (Appendix 53) and the marine diatom *Skeletonema costatum* (Appendix 54) (Table 13). The toxicity data for *S. costatum* indicate that it is the most sensitive of the eukaryotes to florfenicol with 72 hour IC_{50} and NOEC values of 0.0128 and 0.00423 mg/l, respectively, based on cell density. *S. costatum* is one to two orders of magnitude more sensitive than *S. capricornutum,* and two to three orders of magnitude more sensitive than *N. pelliculosa.* The degradation metabolites are similar or less active against prokaryotes for which the activity has been determined, Table 12.

Table 12 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]
Aspergillus niger	>1000	>1000	>1000	>1000	
Trichoderme viride	>1000	>1000	>1000	>1000	
Nostoc	4.0	20	200	400	
Bacillus subtilis	0.4	40	40	>1000	
Clostridium perfringens	1.0	80	40	>1000	
Moraxella	0.5	--	--	--	
Serratia	16	--	--	--	
Escherichia coli	8.0	--	--	--	4.0
Aeromonas salmonicida	0.3-2.5	--	--	--	
Vibrio sp.	0.8-1.6	--	--	--	
Pasteurella multocida	0.25[e]				0.5
Mannheimia haemolytica	1[e]				1

[a]Appendix 47, [b]Appendix 48, [c]Appendix 49, [d]Appendix 50, [e]Appendix 51.

Table 13 **Toxicity data for florfenicol and major metabolites against *Selenastrum capricornutum, Navicula pelliculosa* and *Skeletonema costatum***

		Principal Metabolites		
	Florfenicol	Amine	Alcohol	Oxamic acid
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
Navicula pelliculosa (mg/L)				
Maximum growth rate				
MIC	141			
NOEC	0.493			
Maximum cell density				
MIC	61			
NOEC	<0.493			
Selenastrum capricornutum (mg/L)[a]				
Maximum growth rate				
MIC	>2.9	>2.7	>0.98	80
NOEC	2.9	2.7	0.98	38
Maximum cell density				
MIC	1.5	2.7	0.26	80
NOEC	0.75	1.4	0.13	19
Skeletonema costatum (mg/L)[b]				
Maximum growth rate				
MIC	0.0336	--	--	--
NOEC	0.00423	--	--	--
Maximum cell density				
MIC	0.0128	--	--	--
NOEC	0.0106	--	--	--

[a]Appendices 55, 56 and 57; [b]Appendix 54.

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 *S. capricornutum* (Appendices 52, 55-57), Table 13. The differences in the MIC and NOEC values for S. *capricornutum*, 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 (Appendices 53-57). The same observation florfenicol effects algistatic, not algicidal based on growth data, is reported in the *N. pelliculosa* study (Appendix 53). It can also be concluded that the degradation products did not reach levels that were algistatic in the course of the study.

8.1.3 Invertebrates:

Insufficient immobilizations occurred with *Daphnia magna* exposed to florfenicol at concentrations up to 330 mg/l to enable an EC_{50} value to be determined (Appendix 58). Similarly, no EC_{50} values could be determined for the metabolites. The latter compounds were tested at lower levels due to limitations of available material. Values are presented here simply to show that these metabolites are of a similar order of toxicity or less toxic than the parent compound which is consistent with the order of toxicity observed for microbes and algae (see above), (Appendices 58-61). The NOEC was reported as <100 mg/l for florfenicol as sub-lethal effects, lethargy and erratic swimming, were observed among the survivors at all concentrations tested (Appendix 58), Table 14.

Table 14 EC_{50} and no observed effect concentration (NOEC) data (mg/L) for florfenicol and major metabolites against *Daphnia magna* over 48 hours

	Florfenicol	Amine	Alcohol	Oxamic acid
SPAH Code No.	SCH 25298	SCH 40458	SCH 45705	SCH 48057
EC_{50} (mg/L)	>330	>18	>14	>24
NOEC (mg/L)	<100	18	8.9	24
Reference (Appendix)	58	59	60	61

Table 15 Results of toxicity tests with early life stages of a *Litopenaeid* shrimp[a]

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

[a] Data is taken from Appendix 62.
* Not a larval stage, but a transitional stage between protozoea and mysis.
** The EC_{50} is defined as the total toxic levels (considering lethality and morbidity) of 50% of the exposed organisms.

Toxicity data for florfenicol are available for larval stages of the white shrimp, *Litopenaeus vannamei,* formerly known as *Penaeus vannamei,* (Appendix 62). This work was part of broader evaluation of 12 antibiotics being considered for potential use in shrimp mariculture. The results of acute bioassays with a range of larval or transitional stages of *L. vannamei.* This work indicated florfenicol was among six compounds considered safe and effective in shrimp (Table 15). In these studies florfenicol showed toxicity at part per million (mg/L) concentrations and the authors believed that toxicity values would be higher (i.e., show less toxicity) to later life stages of this species. In addition, this level of toxicity

is consistent with reported toxicity values for other animal species exposed to florfenicol or florfenicol-related residues.

Table 16 **LC_{50} and no observed effect concentration (NOEC) data (mg/L) for florfenicol and major metabolites against fish species over 96 hours.**

		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
Appendix	65	63	66	67
Lepomis macrochirus				
LC_{50} (mg/L)	>830	>20	>15	>25
NOEC (mg/L)	830	20	15	25
Appendix	64	68	69	70

8.1.4 Fish:

The available data for rainbow trout and bluegill sunfish indicate that florfenicol is not toxic to either fish with NOEC values of 780 and 830 mg/L (Appendices 63, 64), Table 16. While the metabolites were not tested at the same concentrations, no mortalities were observed in to either species when exposed to concentrations up to 20, 15, and 25 mg/L in the case of the amine, alcohol and oxamic acid metabolites, respectively (Appendices 63, 66-70). The data support that neither florfenicol nor its degradation products are likely to cause toxic effects to fish species that may be exposed in the environment.

8.1.5 Terrestrial species: plants and invertebrates:

Finally, terrestrial species are presented here to show the low level of toxicity to higher plants and soil invertebrates, compared to aquatic

plants. In the phytotoxicity studies, there was no effect on seedling emergence at any of the concentrations tested up to 100 mg/kg with either mustard or cress (Appendices 20, 21). In the case of wheat, the percentage emergence was reported as 40% at 100 mg/kg, but the control group only exhibited 85% emergence. Based on seedling emergence the LC_{50} value was reported as being >100 mg/kg for the three species tested. From the weights of the emerged seedlings EC_{50} values were estimated as 1.7, 0.5 and 6.7 mg/kg in the case of mustard, cress and wheat, respectively. Visible effects, e.g. chlorosis, were found at all treatment levels, 1-100 mg/kg, in all species throughout the test. The studies on toxicity to terrestrial organisms discussed above established that florfenicol was not toxic to *Eisenia foetida*, the earth or manure worm, at concentrations up to 1,000 mg/kg, with no repellency or other sublethal effects observed (Appendix 22).

8.2 PNEC CALCULATIONS:

The Predicted No Effect Concentrations (PNECs) are presented in Table 17a for key species of fish, invertebrates, algae and one microbial species. Toxicity values range over five orders of magnitude with fish, *O. mykiss* and *L. macrochirus*, having the highest reported NOEC values of 780 and 830 mg/L, respectively, and *S. costatum* having the lowest NOEC (for growth) of 0.0042 mg/L. This latter value indicates that the marine algae, *S. costatum* is the most sensitive species for which data are available, but as discussed below it is an extremely sensitive species and the only species that consistently exceeds a PEC:PNEC of 1.0 for all treatment scenarios. This NOEC is two orders of magnitude lower than

that of the green algae, *S. capricornutum*, and three orders below the freshwater diatom, *N. pelliculosa*. In addition, as discussed below the NOEC for *S. costatum* is two orders of magnitude below the most sensitive of twelve reported microbial species, *P. multocida*, four orders below the most sensitive invertebrate species *L. vannamei*, the white shrimp, and five orders of magnitude below the most sensitive fish species, *Oncorhynchus mykiss*.

Table 17a: Initial predicted no effect concentrations for aquatic organisms

Organism	EC_{50}/LC_{50} (mg/L)	NOEC (mg/L)	Assessment factor (AF)	PNEC (mg/L)
Oncorhynchus mykiss	>780	780	1000[a]	0.78
Lepomis macrochirus	>830	830	1000	0.83
Daphnia magna	>330	<100	1000	0.33
Litopenaeus vannamei	>64	4	1000	0.064
Navicula pelliculosa	61	<0.493	100[b]	0.00493
Selenastrum capricornutum	1.5	0.75	100	0.0075
Skeletonema costatum	0.0128	0.0042	100	0.000042
Pasteurella multocida	0.25[c]		1000	0.00025

[a] An AF of 1000 was used to account for inter-species and intra-species variation and extrapolation from acute to chronic data.
[b] An AF of 100 is used for algal species because these studies include data for chronic endpoints.
[c] MIC

Assessment Factors (AFs) are uncertainty factors used to account for interspecies variability, intraspecies variability or acute to chronic extrapolation. AFs are applied to NOECs and MICs as presented in column 4, Table 17a.

AFs are used to adjust for uncertainty in the data. The VICH approach includes a factor of 100 for fresh and saltwater algae and 1000 for all

other taxa as an initial screen for toxicity data (Table 17a). The initial PNECs (the ratio of the toxicity values over the AFs) for a range of species representing several phyla are 0.78 for fish down to 0.000042 for algae and 0.00004 for microbes. When used in predicting safe levels from laboratory data this AF of 1000 includes a factor of 10 to account for intra-species (10x) and inter-species (10x) variation and for extrapolation from acute to chronic data (10x).

The size of the AF is determined by the amount and quality of available data (Appendices 71-73). In estimating the refined PNEC values AFs of 1, 10, 200 and 1000 are used (Table 17b) which is principally consistent with the CVM discussions. These AFs can be used because the existing database (Tables 12-17) is substantial and high quality, and in the case of the most sensitive species (i.e., microbes and aquatic plants) data for chronic endpoints are available. Furthermore, the species tested are representative of the non-target species potentially at risk in aquatic environments, thus requiring less extrapolation.

A factor of 1000 is used for acute L(E)C_{50}s for the less sensitive fish and invertebrate species (*Daphnia*). These are the least sensitive species by two to three orders of magnitude, and a 10x factor is assumed adequate to account for intra-species variation, inter-species variation, and extrapolation to chronic endpoints. The resulting acute values from GLP laboratory studies are considered resilient, and reproducible and adequate to protect populations of these organisms. There are two

vertebrate and one invertebrate species for which there are acute values and these species are representative of the species potentially at risk.

There are differences in the AFs used in the current assessment and those used in previous submissions to the Center for Veterinary Medicine. A factor of 10 (for intra-species variation) is generally applied to chronic end points. For example, data for *L. vannamei*, a sensitive estuarine/marine shrimp species, include data for all of the five principal early-life stages of this organism. This is considered predictive of chronic toxicity especially since florfenicol does not bioconcentrate. Thus, an AF of 200 is used in calculating the PNEC (Table 17b) and the PEC:PNEC ratio (see Section 8.3.1, Tables 18a, b) based on a factor of 10x for inter-species variability, 10x for intra-species variability and 2x for extrapolation to chronic toxicity.

Toxicity Studies on two of the three aquatic plant species and the microbial species include partial or complete life cycles of the respective organisms and an AF of 10 is used in calculating the PNECs and PEC:PNECs for these species. As will be discussed further in the Risk Characterization section, the *S. costatum* data represents a toxicologically extreme species or the lower end of the distribution of sensitivities of all aquatic plant species. Therefore, inter-species and intra-species variation and extrapolation to chronic data are already accounted for in estimating the PNEC. Thus, an AF of 1.0 is used for this very sensitive species.

An AF of 10 is used to calculate the PNEC values for microbial species, as represented by *P. multocida* (Table 17a), from the MIC values. This PNEC represents the lowest reported MIC from 12 species; Therefore, there is no need to adjust for inter-species variation (i.e. protect for more sensitive species) based on a probabilistic presentation of microbial inhibition data (Figures 6, 7, 8). The data for these microbial species inherently incorporate a chronic endpoint (population growth); therefore, an AF of 10 is used to calculate the PNEC. This AF of 10 adjusts for potential intra-species variability.

The PNEC values extend over four orders of magnitude consistent with the range of toxicity values used in the calculations. The highest PNEC was for *L. macrochirus* (0.83 mg/L) and the lowest for *S. costatum* (0.0042 mg/L). The latter being one order of magnitude lower than that of the freshwater diatom, *N. pelliculosa* and, the green algae, *S. capricornutum*, the nearest plant species, and *P. multocida* which has the lowest toxicity value reported for 12 microbial species as discussed above. This indicates that this marine diatom, *S. costatum,* is an extreme value with respect to sensitivity to florfenicol exposure (See section 8.3.2 below for further discussion) among algal and microbial species.

Table 17b: Refined predicted no effect concentrations for aquatic organisms

Organism	EC_{50}/LC_{50} (mg/L)	NOEC (mg/L)	Assessment factor (AF)	PNEC (mg/L)
Oncorhynchus mykiss	>780	780	1000	0.78[a]
Lepomis macrochirus	>830	830	1000	0.83[a]
Daphnia magna	>330	<100	1000	0.33[a]
Litopenaeus vannamei	>64	4	200	0.32[b]
Navicula pelliculosa	61	<0.493	10	0.0493
Selenastrum capricornutum	1.5	0.75	10	0.075[c]
Skeletonema costatum	0.0128	0.0042	1	0.0042[d]
Pasteurella multocida	0.25[e]		10	0.025[f]

[a] An AF of 1000 was used to account for inter-species and intra-species variation and extrapolation from acute to chronic data.
[b] An AF of 200 was used to account for inter-species (10x) and intra-species variation (10x), and some prediction of chronic toxicity (2x).
[c] An AF of 10 was used to account for intra-species variation. These values already represent chronic end points.
[d] This is an extremely sensitive species with EC_{50} and NOEC values two orders of magnitude lower than other algal or microbial species. Chronic endpoints are covered in the studies and no intra-species or inter-species extrapolation is needed.
[e] MIC
[f] This MIC value was adjusted by an AF of 10 to account for intra-species variation in calculation of the PNEC. Chronic and inter-species variations are already accounted for in the data.

8.3 RISK CHARACTERIZATION:

The Risk Characterization is presented for four different scenarios: initial (Table18a) and refined (Table 18b) worst-case scenarios and initial (Table 18c) and refined (Table 18d) typical case scenarios. The initial PEC_{water} value for the worst-case situation used in Table 18a is based on fish densities of 47 kg/m^3 at a flow rate of 3 L/s. For the initial typical case scenario, the initial PEC_{water} value (Table 18c) is based on a fish density of 18 kg/m^3 and a flow rate of 22 L/s. In these calculations, it is assumed that all florfenicol and florfenicol-related residues are released into water

during a 15-day period, the 10-day treatment period and 5-day post treatment excretion/elimination period. The calculations of the refined PEC_{water} values (Table 19) included a combined mitigation factor of 7.5 which includes an upper estimate of the proportion of facility treated (20%) and a proportion (33%) for material bound to feces and solid matter in the raceway. Similar partitioning would be expected to occur in settling ponds, and possibly in receiving waters.

Release of florfenicol in facility effluents will be episodic and short-lived. Based on the use-pattern, Aquaflor would be expected to be applied two times to the same population of fish with a minimum six week interval between treatments and an expected maximum of five times in one year and only a portion (i.e., 20%) of the entire facility would be treated as part of one application. Florfenicol will be released from raceways or other facilities to settling ponds, when present, and receiving waters of various volumes. When released, florfenicol will rapidly disperse in the water column with partitioning to sediments where it will degrade rapidly. Degradation will occur in both compartments of the aquatic system. The half-life in water and sediment of receiving waters is expected to be 13.0 days (range of 8.4 - 19.4 days) based on laboratory studies with natural water and sediments (Appendix 4). In the laboratory, partitioning of florfenicol to sediments has been demonstrated in a small system. It has also been shown that the degradation of florfenicol results in the irreversible incorporation of florfenicol residues into the humin and fulvic acid fractions of sediments. Because florfenicol has a high water solubility and facility effluents discharge to lotic systems any absorption in

the field may be limited based on residence time. However, florfenicol residues enter the raceway via excretion from fish. The association of florfenicol with feces and excreta will be characterized by strong binding and low desorption from fecal material (Appendices 3, 4 and 5). However, degradation will be rapid in sediments and water, and residual degradates with lower molecular weights will be strongly bound to sediments in the humin fraction and not biologically available (Appendix 4). PEC values and PEC:PNEC ratios are not calculated for any environmental compartments other than water, the principal compartment for dissipation and degradation of florfenicol discharged from facilities. PEC:PNEC ratios are risk quotients calculated by dividing the PEC (predicted environmental concentration) for specific environmental compartments (e.g. water) by the PNEC (predicted no effect concentration).

8.3.1 PEC:PNEC ratios:

The PEC:PNEC ratios are presented for four scenarios (combinations of flow rate and fish density and the PNEC used to calculate the PEC:PNEC ratios in Tables 18a-d and 19. Tables 18 a-d provide a progression from the worst-case PEC/PNEC ratios to ratios based on refined (alternate) PNEC and PEC values. A discussion of the worst-case calculations (Tables 18 a, b) is provided prior to discussion of the refined typical PEC/PNEC's (Tables 18 c, d). Table 19 provides a summary comparison of worst-case and typical scenarios based upon refined PNEC and refined PEC values. The differences observed in PEC/PNEC values in this table are based on differences in fish density and flow rates.

8.3.1.1 Tier A: Worst-Case Risk Characterization:

Following the VICH Guidance for Tier A, PEC/PNEC ratios are used to compare worst-case exposure estimates (PEC_{water}) with benchmark effects values (PNECs). PNECs are provided for two fish species, two invertebrate species, three aquatic plant (algal) species and one microbial species (Tables 17a,b). The latter species, *P. multocida*, is the most sensitive of twelve microbial species for which data are provided in this assessment (Table 12). Toxicity data include fresh and saltwater organisms even though this assessment is for a freshwater use.

Initial Assessment

As part of an Initial Assessment, a risk characterization can be made by comparing the initial worst-case PEC_{water} values listed in Table 9 to the initial (Tier A) PNEC values (Table 17a). As shown in Table 18a, the worst-case (i.e. high fish density of 47 kg/m^3 and low flow rate of 3 L/s) PEC_{water} value (Column 4, Table 9) when divided by the PNEC values for representative organisms provide PEC:PNEC ratios that are below the threshold of 1.0 for all fish species and invertebrates. However, in this initial worst-case scenario, the PEC:PNEC ratios exceed 1.0 for all three algal and one microbial species. For the initial worst-case scenario, PEC:PNEC ratios greater than 1.0 are highlighted in gray (Table 18a, Col. 6). Ratios that exceed 1.0 for the worst-case initial estimates range from 33.2 - 5928. These Tier A PEC:PNEC ratios are considered to indicate potential impact except that this is an extreme worst-case scenario which incorporates high density (initial worst-case 95th percentile fish, density of

47 kg/m^3), low flow rate (5th percentile, flow rate of 3 L/s) and unrefined AFs used in PNEC calculations (Table 18a).

Refined Assessment

This case can be refined in two ways. The first is by refining the PEC values from their initial values (Table 11 vs. Table 9) or, second, by refining the PNEC values based on modified AFs (Table 17b vs.17a).

In Table 18b the refined worst-case PECs are compared to the refined PNEC values (based on different AF values discussed above, see Section 8.2). The PEC:PNEC ratios can be more accurately calculated using the refined PNEC values in Table 17b and the refined PEC_{water} value from Table 11.

The values in Table 18b are based on a continued worst-case exposure scenario (fish stocking density of 47 kg/m^3 and a flow rate of 3 L/s) with refined PEC_{water} and refined PNEC estimates. The results show that for the worst-case exposure scenario (col.6) the PEC:PNEC ratios are below one for two fish and two invertebrate species, two freshwater algal species, and one microbial species, but exceed a ratio of 1.0 for one species of aquatic plant species, *S. costatum.* The PEC:PNEC ratio for this species exceeds 1.0 by only 7.9 fold (i.e. less than an order of magnitude) and the bacterium, *P. multocida*, exceeds 1.0 by only 1.32 fold.

Table 18a Initial Worst-Case Risk Characterization for Freshwater Salmonids

Organism (End Point)	Results (mg/L)	AF[a,b]	Initial PNEC (mg/L)	Worst-Case Scenario	
				Initial Worst-Case PEC_{water} Value (mg/L)[c]	PEC:PNEC Ratio
Oncorhynchus mykiss **(96-h LC_{50})**	>780	1000	0.78	**0.249**	**0.319**
Lepomis macrochirus **(96-h LC_{50})**	>830	1000	0.83	**0.249**	**0.3**
Daphnia magna **(48-h LC_{50})**	>330	1000	0.33	**0.249**	**0.755**
Litopenaeus vannamei **(48-h NOEC)**	<64	1000	0.064	**0.249**	**0.203**
Navicula pelliculosa **(72-h NOEC)**	<0.493	100	0.00493	**0.249**	**50.51**
Selenastrum capricornutum **(14-d NOEC)**	0.75	100	0.0075	**0.249**	**33.2**
Skeletonema costatum **(72-h NOEC)**	0.0042	100	0.000042	**0.249**	**5928**
Pasteurella multocida **(MIC)**	0.25	1000	0.00025	**0.249**	**996**

[a] An AF of 1000 was used to account for intra-species variation, inter-species variation, and in the extrapolation from acute to chronic data.
[b] An AF of 100 was used to account for inter-species and intra-species variation when toxicity values for chronic endpoints are already accounted for in the data.
[c] Initial worst-case PEC_{water} value for a fish stocking density of 47 kg/m^3 and a flow rate of 3 L/s.

Table 18b Refined Worst-Case Risk Characterization for Freshwater Salmonids using Refined PNECs

Organism (End Point)	Results (mg/L)	AF	Refined PNEC (mg/L)	Worst-Case Scenario (based on refined density and flow rate)	
				Refined PEC_{water} (mg/L)[e]	PEC:PNEC Ratio
Oncorhynchus mykiss **(96-h LC_{50})**	>780	1000	0.780[a]	0.033[d]	**0.04**
Lepomis macrochirus **(96-h LC_{50})**	>830	1000	0.830[a]	0.033	**0.04**
Daphnia magna **(48-h LC_{50})**	>330	1000	0.330[a]	0.033	**0.10**
Litopenaeus vannamei **(48-h NOEC)**	<64	200	0.320[b]	0.033	**0.10**
Navicula pelliculosa **(72-h NOEC)**	<0.494	10	0.049[c]	0.033	**0.67**
Selenastrum capricornutum **(14-d NOEC)**	0.75	10	0.075[c]	0.033	**0.44**
Skeletonema costatum **(72-h NOEC)**	0.0042	1	0.0042[d]	0.033	**7.90**
Pasteurella multocida **(MIC)**	0.25	10	0.025[c]	0.033	**1.32**

[a] An AF of 1000 was used to account for intra-species variation, inter-species variation, and in the extrapolation from acute to chronic data.
[b] An AF of 100 was used to account for inter-species and intra-species variation. A factor of 2x is used for extrapolation to chronic endpoints based on the early life stages being predictive of chronic toxicity.
[c] An AF of 10 was used to account for intra-species variation.
[d] An AF of 1 was used for the most sensitive species tested including chronic endpoints.
[e] Refined PEC_{water} value for a fish stocking density of 18 kg/m^3 a flow rate of 3 L/s and treatment of a maximum of 20% of fish in the facility.(i.e., with a mitigation factor of 7.5)

A refined worst-case risk characterization was made using refined PNECs and based upon the refined PEC_{water} value (0.033 mg/L) listed in Table 11. The latter value is an estimate of the florfenicol concentration in water

leaving the treated raceway and does not account for receiving water dilution. The PEC:PNEC ratios greater than 1.0 (for *S. costatum* and *P. multocida*) are highlighted in gray (Table 18b).

8.3.1.2 Tier A: Typical Case Risk Characterization

Initial Assessment

Initial analysis of the typical case scenario with the initial PNEC values (Table 18c, col. 6) shows a similar number of species with PEC:PNEC ratios that exceed 1.0, as observed in Table 18a, but the magnitude of the ratios is much smaller. These differences in the initial PEC:PNEC ratios are based on the different densities and flow rates used to calculate the respective initial PECs (Table 9). The estimates are based on a density of 18 kg/m^3 which is the median value from a survey of 100 facilities (Appendix 43) (Table A) and much lower than the 95% percentile value of 47 kg/m^3 used above in the initial worst-case scenario above. This lower density is combined with a higher (median) flow rate (Table A) of 22L/s compared to the 5th percentile value of 3 L/s flow rate used in the initial worst-case scenario. These median values result in the four species with ratios that previously exceeded 1.0 by one to two orders of magnitude as shown by comparing PEC:PNEC ratios (col. 6) in Table 18b with Table 18c (col. 6). Values in column 6 Table 18c, the initial typical case values, include four ratios that exceed 1.0 by 0.7x to 309.52x.

Table 18c Initial Typical Case Risk Characterization for Freshwater Salmonids with Initial PNECs

Organism (End Point)	Results (mg/L)	AF	Initial PNEC (mg/L)	Typical Case Scenario (based on typical density and flow)	
				Initial PEC_{water} (mg/L)[c]	PEC:PNEC Ratio
Oncorhynchus mykiss (96-h LC_{50})	>780	1000[a]	0.78	0.013	0.167
Lepomis macrochirus (96-h LC_{50})	>830	1000[a]	0.83	0.013	0.157
Daphnia magna (48-h LC_{50})	>330	1000[a]	0.33	0.013	0.13
Litopenaeus vannamei (48-h NOEC)	<64	1000[a]	0.064	0.013	0.203
Navicula Pelliculosa (72-h NOEC)	<0.493	100[b]	0.00493	0.013	2.64
Selenastrum capricornutum (14-d NOEC)	0.75	100[b]	0.0075	0.013	1.733
Skeletonema costatum (72-h NOEC)	0.0042	100[b]	0.000042	0.013	309.52
Pasteurella multocida (MIC)	0.25	1000[a]	0.00025	0.013	52.0

[a] An AF of 10 was used to account for intra-species variation and 10 for inter-species variation, and a factor of 10 was used in the extrapolation from acute to chronic data.
[b] An AF of 10 was used to account for intra-species variation and 10 for interspecies variation. These toxicity values are considered chronic endpoints.
[c] Initial PEC_{water} value for a fish stocking density of 18 kg/m^3 and a flow rate of 22 L/s.

Table 18d Refined Typical Case Risk Characterization for Freshwater Salmonids using Refined PNECs

Organism (End Point)	Results (mg/L)	AF	Refined PNEC (mg/L)	Typical Case Scenario (based on typical density and flow)	
				Refined PEC_{water} (mg/L)[e]	PEC:PNEC Ratio
Oncorhynchus mykiss **(96-h LC_{50})**	>780	1000	0.780[a]	0.0017	0.0022
Lepomis macrochirus **(96-h LC_{50})**	>830	1000	0.830[a]	0.0017	0.0020
Daphnia magna **(48-h LC_{50})**	>330	1000	0.330[a]	0.0017	0.0051
Litopenaeus vannamei **(48-h NOEC)**	<64	200	0.32[b]	0.0017	0.0053
Navicula pelliculosa **(72-h NOEC)**	<0.494	10	0.0494[c]	0.0017	0.034
Selenastrum capricornutum **(14-d NOEC)**	0.75	10	0.075[c]	0.0017	0.023
Skeletonema costatum **(72-h NOEC)**	0.0042	1	0.0042[d]	0.0017	0.4
Pasteurella multocida **(MIC)**	0.25	10	0.025[c]	0.0017	0.068

[a] An AF of 10 was used to account for intra-species variation and 10 for inter-species variation, and a factor of 10 was used in the extrapolation from acute to chronic data

[b] An AF of 10 for intraspecies variation and a factor of 10 is used for inter-species variation. A factor of 2x is used for extrapolation to chronic endpoints based on the early life stages being of chronic toxicity.

c An AF of 10 was used to account for intra-species variation. These toxicity values are considered chronic endpoints.

[d] An AF of 1 is used for the most sensitive species tested including chronic endpoints.

[e] Refined PEC_{water} value for a fish stocking density of 18 kg/m^3 and a flow rate of 22 L/s and treatment of a maximum of 20% of fish in the facility (i.e., with a mitigation factor of 7.5).

Table 19 Comparison of Refined Typical and Refined Worst-Case Risk Characterization for Freshwater Salmonids

Organism (End Point)	Results (mg/L)	AF	Refined PNEC (mg/L)	Worst-Case Scenario (Refined Assessment)		Typical Case Scenario (Refined Assessment)	
				Refined PEC_{water} (mg/L)[f]	PEC:PNEC Ratio[g]	Refined PEC_{water} (mg/L)[e]	PEC:PNEC Ratio[g]
Oncorhynchus mykiss **(96-h LC_{50})**	>780	1000	0.780[a]	**0.033**	**0.042**	0.0017	0.0022
Lepomis macrochirus **(96-h LC_{50})**	>830	1000	0.830[a]	**0.033**	**0.0398**	0.0017	0.0020
Daphnia magna **(48-h LC_{50})**	>330	1000	0.330[a]	**0.033**	**0.1**	0.0017	0.0051
Litopenaeus vannamei **(48-h NOEC)**	<64	200	0.32[b]	**0.033**	**0.103**	0.0017	0.0053
Navicula pelliculosa **(72-h NOEC)**	<0.493	10	0.0494	**0.033**	**0.67**	0.0017	0.034
Selenastrum capricornutum **(14-d NOEC)**	0.75	10	0.075[b]	**0.033**	**0.44**	0.0017	0.0023
Skeletonema costatum **(72-h NOEC)**	0.0042	1	0.0042[c]	**0.033**	**7.857**	0.0017	0.4
Pasteurella multocida **(MIC)**	0.25	10	0.025[b]	**0.033**	**1.32**	0.0017	0.068

[a] An AF of 10 was used to account for intra-species variation and 10 for inter-species variation, and a factor of 10 was used in the extrapolation from acute to chronic data.

[b] An AF of 10 for intraspecies variation and a factor of 10 is used for inter-species variation. A factor of 2x is used for extrapolation to chronic endpoints based on the early life stages being predictive of chronic toxicity.

c An AF of 10 was used to account for intra-species variation. These toxicity values are considered chronic endpoints.

[d] An AF of 1 is used for the most sensitive species tested including chronic endpoints.

[e] Refined PEC_{water} value for a fish stocking density of 18 kg/m^3 and a flow rate of 22 L/s.

[f] Refined PEC_{water} value for a fish stocking density of 47 kg/m^3 and a flow rate of 3 L/s.

[g] PEC/PNEC rations are calculated by dividing the refined PEC_{water} by the refined PNEC.

Refined Assessment

When the refined PNEC values with refined AFs (Table 17b) are used (Table 18d) none of the PEC:PNEC values exceeds one. When typical case density and flow rates are used in combination with refined PNEC values there are no significant impacts indicated (Table 18d).

8.3.1.3 Refined Risk Characterization

The refined risk characterization is based on two mitigating factors that reduce the potential for florfenicol concentrations in effluent to reach the initial concentrations (Table 9, col. 4) as high as 0.249 mg/L. These factors are 1) the proportion of a facility treated at one time (≤ 20%), and 2) the fate characteristic of florfenicol by which related residues associate strongly with solid phase within aquatic systems (e.g., sediments, feces).

Key individuals from USGS, academia and commercial trout farms agree in a phone survey that 100% treatment of a farm for coldwater disease will not occur. In this assessment, a maximum of 20% of a given facility would be treated at one time. This 20% is a conservative upper bound estimate; the actual value is likely to be much lower (D. Erdahl, pers. comm.). The worst-case exposure would be lower by a factor of five or greater. These factors are shown Table 10 as an "in facility" mitigation factor.

A further consideration in characterizing risk from the use of Aquaflor for coldwater disease is the principal populations being treated. Inside any given facility, only a portion of that facility (specific raceways) is treated at any one time (R. McMillan pers. comm.; D. Erdahl, pers. comm., J. Hinshaw pers. comm.). The primary use for florfenicol is in treating fish of 0.2 - 10 g (Appendix 29) and only 5 -10% of production space would be allotted to this size of fish (J. Hinshaw pers. comm.). An entire facility would not be treated at one time. These facts support the use of an upper bound proportion of 20% of a facility being treated at one time.

Further, a portion of susceptible juvenile fish would be held at densities likely to be lower than used in the above PEC:PNEC calculations. Fish from 5 - 10 g would be covered under the typical case scenario as discussed above, but fish < 5 g would be expected to be held at lower densities such as 5 kg/m^3.

The second in-facility mitigation factor is that when florfenicol is released into raceways or from raceways or other facilities to settling ponds (when present) and receiving waters of various volumes, will rapidly disperse in the water column with some partitioning to sediments where it will degrade rapidly in both water and sediment compartments of the aquatic system. The half-life in water and sediment of receiving waters is expected to be 13.0 days (range of 8.4 - 19.4 days) based on laboratory studies with natural water and sediments (Appendix 4). In the laboratory partitioning of florfenicol to sediments has been demonstrated in a small system. It has also been shown that the degradation of florfenicol results in the irreversible incorporation of florfenicol residues into the humin and fulvic acid fractions of sediments.

Because florfenicol has a high water solubility and facility effluents discharge to lotic systems any absorption in the field may be limited based on residence time. However, florfenicol residues enter the raceway via ingestion and excretion by fish, and will be expected to demonstrate strong binding to and low desorption from fecal material (Appendices 3, 4 and 5). However, degradation will be rapid in sediments and water, and residual degradates with lower molecular weights will be

strongly bound to sediments in the humin fraction where they are not biologically available (Appendix 4). PEC values and PEC:PNEC ratios are not calculated for any environmental compartments other than water, the principal compartment for dissipation and degradation of florfenicol discharged from facilities.

The calculations of the refined PEC_{water} values (Table 19) included a combined mitigation factor of 7.5, which included an upper estimate of the proportion of facility treated (20%) and a proportion (33%) for florfenicol, and florfenicol related residues that will likely be bound to feces and solid matter in the raceway. Residue binding to solids serves to lower the residues in the water column. Similar partitioning would be expected to occur in settling ponds and possibly in receiving waters.

The PEC:PNEC ratios shown in Table 18b are all below 1.0, with the exception of *S. costatum* and *P. multocida* when the PEC_{water} used is the refined worst-case scenario (0.033 mg/L) and the PNEC based on the refined AF values (Table 17b). Only, the values of *S. costatum* and *P. multocida* exceed a ratio of 1.0 and only then by a factor of 7.9 and 1.3 fold, respectively (Table 19). When the refined PEC_{water} values and the PNECs based on refined AFs are used to calculate the PEC:PNECs for the typical-case scenario, these ratios are all below one ranging from 0.002 for bluegill sunfish (*L. macrochirus*) to 0.4 for *S. costatum* (Table 19). These calculations, based on median or average density and exposure, yield PEC:PNEC ratios that indicate that no significant impact

would be expected when fish are treated with Aquaflor according to label directions.

8.3.2 Discussion of Potential Risk:

The level of risk, presented in the PEC:PNEC ratios in Table 18b for the refined worst-case exposure scenario, indicates no significant impacts to receiving water communities are expected despite the extreme nature of the assessment due to the following considerations:

1. The PEC:PNEC ratio for the alga, S. *costatum*, exceeds 1.0 by less than 10 fold making the exceedence for this species, the worst-case scenario, insignificant. Risk quotients for other algal species are all less than one, even under the worst-case scenario.
2. The organism with a PEC:PNEC ratio that exceeds 1.0 is an algal species that can recover from transient effects due to the ubiquitous nature of the species.
    a. Algae are part of communities of species that are structurally and functionally redundant and if one species such as *S. costatum* was removed, other species would expand to maintain both structure and function of the phytoplankton community.
    b. Refugia within aquatic systems would allow reintroduction of a species where a transient or localized decline of that species might occur.

c. Florfenicol is algistatic (i.e., inhibits growth), but not algicidal. Cell growth recovers during laboratory studies (Appendices 53, 54) and a population of an algal or microbial species if impacted in ambient waters would be expected to recover growth in a short period of time.

3. *S. costatum* would be able to survive potential growth effects even with initial peak end-of-pipe estimates of release.
4. Exposure is intermittent due to the low frequency of application (2 - 4 applications, six weeks apart), and episodic release over a 15 day period.
5. No more than 20% of the fish at a hatchery are expected to be treated at any one time. This percentage is based on the dynamics of hatchery husbandry and disease susceptibility. The life stage most likely to be infected (fry) make up a limited part of the fish population..
6. Approximately 30% of the florfenicol will be excreted in feces and will have limited bioavailability. Partitioning from water to solids is approximately 25 – 33% for florfenicol and the same or higher for its metabolites.
7. In facilities that have settling or holding ponds there would be a further lowering of the concentration of florfenicol residues in the effluent due to partitioning to sediments.
8. The case where the effluent from a facility mixes with a receiving stream, the dilution, even if it was not instantaneous would eventually lower the concentration of florfenicol residues in the stream.

9. Once in the stream, the florfenicol residues would undergo degradation as shown by the sediment:water degradation study.

Thus, the assessment of the worst-case scenario using the refined PEC_{water} and refined PNEC values yields a refined risk characterization that indicates no significant impact would be expected. The risk characterization improves when the typical case density and flow are used to estimate the PEC_{water} (See Tables 18c, d).

The data for *S. costatum* warrant careful examination. Examination of the toxicity data alone shows that the biological response of *S. costatum* is extreme relative to other species. The *S. costatum* NOEC (0.0042 mg/L) is nearly two orders of magnitude below the next most sensitive aquatic plant (algal) species, *N. pelliculosa*; three orders of magnitude below the most sensitive invertebrate species (*L. vannamei*); and nearly five orders of magnitude below the most sensitive fish species (*O. mykiss*). In addition, this observed toxicity value (0.0042 mg/L) is almost two orders of magnitude below the most sensitive microbial species tested (*P. multocida*). The *S. costatum* NOEC, as shown in Table 19 is clearly an extreme value in the distribution of potential biological responses in aquatic organisms and contributes to an overly conservative assessment of risk (see subsequent discussion). Furthermore, florfenicol is algistatic not algicidal. The effect reported is for growth not mortality (see subsequent discussion).

The exposure of aquatic organisms to florfenicol and metabolites in receiving waters would be low in magnitude, episodic, and transient. Given the low K_d values of florfenicol and its metabolites, these residues would principally partition into the water column of receiving streams or rivers. The concentration in the water phase would be low to begin with as shown in the initial PEC_{water} values (Tables 10 and 19) and would be further diluted by mixing with stream or river (receiving) waters. Although not considered in previous submissions, new data demonstrate significant movement of florfenicol out of water into sediment (in a closed system) with rapid degradation occurring in sediments and water. Reported half-lives in sediment water systems range from 8.4 to 19.4 days with a median of 13.0 days. Thus, florfenicol residues would not be expected to persist in receiving water environments for extended periods. Therefore, should concentrations arise which affect sensitive species, such as diatoms, any effects would likely involve inhibition of growth (algistatic) and would be transient due to dissipation and degradation, recolonization from refugia, and presumably rotation among the three available antibiotics. It is unlikely that florfenicol would be the only antibiotic used for bacterial disease control when the over the counter antibiotics, oxytetracycline and Romet, are easier for fish producers to obtain (not regulated under the VFD) and less expensive.

Under the Veterinary Feed Directive Aquaflor will be used solely under a prescriptive use pattern, which limits the magnitude, frequency and timing of applications. Diagnosis of coldwater disease by a practicing veterinarian is required to obtain treated feed. These prescribed or

controlled applications are made only to populations with active infections and there is no prophylactic use allowed. In addition, application is episodic occurring usually once or twice per year and, consistent with prescriptive use solely for active infections (Appendix 30). Residues associated with these applications would result in release of florfenicol-related residues for only about 15 days including the 10-day treatment period and a depuration period of five days.

8.3.2.1 SPECIES SENSITIVITY DISTRIBUTIONS: AN ALTERNATIVE TECHNIQUE FOR RISK CHARACTERIZATION

As an alternate, analysis that is supplemental (but corroborative) to the VICH approach to evaluating the toxicity data for aquatic organisms can be compared by graphing the concentration of exposures for various toxicity endpoints (on a log scale) on the x-axis for individual species. These values are graphed using the probability scale on the y-axis. This provides a normal distribution of the sensitivities for species tested and this distribution is assumed representative of the normal distribution of all species that might potentially be exposed to florfenicol. This approach to risk characterization using species sensitivity distribution (SSD) has been used in the regulation of effluents and pesticides in the United States as well as in the European Union (Appendices 74, 75) and is used here to supplement the PEC:PNEC ratios.

As can be seen in Figure 5 there is a gradient of sensitivities for both acute (•) and chronic (○) distributions with the fish being the least sensitive and the algal species being the most sensitive. Sensitivities of

microbial species (▼) show a range of toxicological responses over four orders of magnitude similar to the eukaryotic species (fish, invertebrates and algae) (Figure 5). This is another way of viewing the pattern of biological response among organisms and reinforces the previous discussion (Section 8.3.2 above) showing *S. costatum,* a marine diatom, to be a more sensitive species relative to the rest of the species evaluated. The *S. costatum* NOEC is nearly two orders of magnitude below the next most sensitive aquatic plant (algal) species, *N. pelliculosa,* a freshwater diatom. The *S. costatum* acute and chronic toxicity values are also greater than three orders of magnitude below the most sensitive invertebrate species (*L. vannamei*); and nearly five orders of magnitude more sensitive than the most sensitive fish species (trout). In addition, this observed toxicity value is greater than an order of magnitude below the most sensitive microbial species tested (*P. multocida*). Based on the MIC and NOEC values for *S. costatum*, as shown in Figure 5, this species is clearly an extreme value in the distribution of potential biological responses and this contributes to an overly conservative assessment of risk.

Figure 6 provides a visual comparison of the distribution of measured toxicity values (estimates of relative sensitivity) for all relevant species compared to point estimate worst-case PEC_{water} and typical case PEC_{water} values. This graph includes both the initial and refined PEC_{water} values for both worst-case and typical scenarios (Table 9, Col 3 and 4, rows 3 and 4). As described above for Figure 6, acute toxicity values for fish (LC_{50}), invertebrates (EC_{50}) algae (NOEC) and bacteria (MIC), combined, are

distributed evenly across the probability scale (Y-axis). This is plotted against the florfenicol (and florfenicol-related residues) concentrations for each species. The initial worst-case scenario (vertical line at 0.249 mg/L) is below all microbial MICs, vertebrate and invertebrate toxicity values, and acute and chronic toxicity values for two of three species of algae. This worst-case exposure value exceeds the acute (EC_{50}) and chronic (NOEC) values for *S. costatum* by one to two orders of magnitude. The refined worst-case PEC_{water} at 0.038 mg/L is approximately the same as the EC_{50} values for *S. costatum*. The conclusion is that all toxicity values, acute and chronic (without any adjustments for AFs), are above the initial worst-case PEC_{water} values with the exception of *S. costatum*. The acute and chronic toxicity values or this species are bracketed by the initial worst-case PEC_{water} and the refined typical case PEC_{water} values (Figure 6).

Further, the regression of the acute and microbial toxicity data (●, ▼) cross the x-axis (5% species sensitivity) at approximately 0.001 mg/L (1.0 µg/L) indicating that this concentration which is slightly above the refined worst-case PEC_{water} value, would be protective of >90% of the potential species from acute toxicity. Alternatively, this could be stated as less than 10% of potentially exposed microbial species would be at risk of acute toxicity from florfenicol use in fish farms (Figure 6).

The NOECs from fish, invertebrates and algae (Table 19) are plotted (○) in the same manner and the regression shows that less than 5 percent of all species potentially exposed to florfenicol or florfenicol-related residues

would potentially be at risk from florfenicol use in fish farms. Similarly the MICs for twelve microbial species (Table 12) are plotted (▼) and the regression shows that less than 5% of microbial species would expect to be at risk at concentrations below 0.0009 mg/L (09 µg/L). Microbial species were included as a separate distribution to allow separate analysis the relative sensitivity of microbes to this antibiotic. This is more than an order of magnitude higher than the PEC_{water} (total florfenicol-related residues) as shown by the vertical hashed line. In most cases, the chemical residue data is plotted at the same time as the SSD. The 95% probability value for exposure concentration is then used as the benchmark (Appendices 74, 75). In the current analysis, we have used a point estimate (the vertical hashed line) for the initial and refined PEC_{water} values for worst-case and typical case scenarios.

The data for *S. costatum* warrants careful examination. The low NOEC relative to other species is reflected in the PEC:PNEC ratios that range from 5,928 - 7.86 for initial worst-case and refined worst-case scenarios, respectively. *S. costatum* PEC:PNEC values, are above 1.0 for all four estimates of PEC:PNEC values provided in Tables 18a-d and 19 (above). Only the remaining algal species and one microbial species have PEC:PNEC values above 1.0 for the initial worst-case scenario. For all other scenarios (initial and refined) and other species (other than algae and one microbial species) PEC:PNEC ratios are below 1.0 (Tables 18a-d and 19).

Examination of the toxicity data alone shows that the biological response of *S. costatum* is an extreme value relative to other species (Figures 5, 7). In Figure 7 SSDs are plotted for only algae and microbial species. It can be clearly seen in this figure that the initial worst-case PEC_{water} is below the toxicity values for all species of algae and microbes with the exception of *S. costatum*. The acute and chronic data for this species are approximately one and two orders of magnitude below the initial worst-case PEC_{water}. As can be seen in Figure 7 the data for *S. costatum* indicates it is an extremely sensitivity species, toxicologically, compared to the other species tested.

Finally, the calculations of exposure discussed above are for the actual effluent (end-of-pipe) for facilities. Figure 8 shows a plot of the initial and refined, typical case PEC_{water} values for the end-of-pipe and for the same scenario with a conservative estimate of dilution that would occur in receiving waters (low flow). This PEC_{water} is approximately an order of magnitude lower than the chronic (NOEC) data for *S. costatum*. This shows how that if an average dilution in receiving water is considered it would result in PEC:PNEC ratios for all species tested being below 1.0 and a conclusion of no significant impact.

In summary, use of the SSD evaluation as part of the risk characterization process allows a probabilistic comparison of toxicity and exposure data and shows that no significant impact would be expected for florfenicol when used to treat freshwater-reared salmonids according to label directions.

8.3.3 Summary:

The use of Aquaflor to treat coldwater disease in freshwater salmonids does not present any significant risk to aquatic ecosystems due to the following combination of factors:

- use of Aquaflor is limited;
    - the fact that 2 other over the counter antibiotics, oxytetracycline and Romet, are available as competitive products in a limited market;
    - to prescriptive application with no prophylactic use under the Veterinary Feed Directive;
    - to application in feed at 10 mg/kg feed/day for 10 days;
    - temporally because Aquaflor use occurs only two to four times per year and at least six weeks apart;
    - because, only a small portion ($\leq$ 20%) of the fish within a facility would be treated at one time.
- Florfenicol, the active ingredient in Aquaflor dissipates due to degradation and dilution, and will remain principally in water with some binding to solid phases where it will undergo rapid degradation in both matrices;
- Florfenicol release is from flow-through raceway systems with dilution in the facility prior to release and in receiving waters that serve to mitigate exposure;

- Exposures in aquatic systems are expected to be low and transient ;
- Florfenicol generally presents a low potential hazard based in toxicity studies with a wide range of non-target aquatic and marine species;
- All risk quotients based on refined PEC:PNEC ratios (Table 19) are less than one with the exception of that for the marine diatom, *S. costatum* and the bacterium, *P. multocida*, in the refined worst-case assessment.
    - *S. costatum* is the most sensitive species tested with an NOEC 100 times lower than the next most sensitive algal species (a freshwater diatom);
    - Results for *S. costatum* were based upon a reversible growth inhibition that requires sustained exposures to be achieved in the laboratory.
    - Specific species such as (*S. costatum*) within any phytoplankton community are redundant functionally and loss of a species may result in some structural change, but with no functional change in the community would be expected.
    - Refugia are unexposed areas within an aquatic community that provide the source material (algal cells) to recolonize areas where populations of a sensitive species (e.g., *S. costatum*) may be locally diminished by infrequent episodic releases of florfenicol.

- Repopulation occurs in natural systems as indicated by the return to normal growth rates in algae when exposure is removed in laboratory studies.
- Similar mitigations to those described above would apply for the bacterium, *P. multocida.*

- All PEC:PNEC ratios based on refined PEC_{water} values for the typical case scenario are less than one (Table 19).

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

8.4 CONCLUSIONS:

The use of Aquaflor ® (florfenicol) under Veterinary Feed Directive regulations for control of mortality in freshwater reared salmonids exposed to *Flavobacterium psychrophilum* (coldwater disease) is not expected to pose any significant risk of widespread or long-term adverse environmental impact. Any impact will be transient and not significant. Schering–Plough Animal Health believes that these data and analyses support a Finding of No Significant Impact (FONSI).

SECTION 9. USE OF RESOURCES AND ENERGY:

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

SECTION 10. MEDICATED FEED STORAGE, SPILL CLEANUP, DISPOSAL:

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 'EPA Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category EPA 821-B-05-001, March 2006, Chapter 10: Material Storage for Flow-through, Recirculating, and Net Pen Facilities' [Full document available at http://www.epa.gov/waterscience/guide/aquaculture].

Medicated-Feed Spill Cleanup:

Should medicated feed be spilled, then 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 EPA 821-B-05-001, March 2006, Chapter 10: Material Storage for Flow-through, Recirculating, and Net Pen Facilities'. Records of medicated feed spills will be maintained in accordance with 'EPA Compliance Guide for the Concentrated Aquatic Animal Production Point Source Category EPA 821-B-05-001, March 2006, Appendix O: Spills and Leaks Log'.

Medicated-Feed Disposal:

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

SECTION 11. MITIGATION MEASURES:

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

SECTION 12. ALTERNATIVES TO THE PROPOSED ACTION:

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

SECTION 13. LIST OF PREPARERS:

The following personnel from Schering-Plough Animal Health Corp. were responsible for the preparation of this Environmental Assessment:

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SECTION 14. CERTIFICATION:

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

Richard G. Endris

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

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SECTION 16. TABLE A:

Table A. Average flow and stocking density for facilities culturing freshwater salmonids. (Adapted from 2004 CVM Review).

	Average Flow (L/s)	Average Stocking Density (kg/m^3)
	33	36.9
	02	32.3
	09	03.0
	28	17.6
	44	10.7
	20	22.2
	16	41.3
	22	12.2
	25	10.7
	83	35.0
	19	08.9
	02	48.9
	38	06.3
	25	46.7
	28	18.4
	38	27.0
	03	08.1
	22	18.0
	16	14.7
	44	41.8
	10	20.0
	08	55.5
	03	21.7
	17	02.3
	88	27.4
	158	23.5
	38	33.8
	22	12.9
	32	12.9
	63	10.6
	53	08.9
	06	30.0
	38	20.2
	06	03.0
	06	05.8
	10	12.0
	22	10.2
	25	12.0
	135	28.0
Average	32.3	20.8
Median	22.1	16.0
Min	1.9	02.3
Max	157.5	55.5
5^{th} Percentile	3	
95^{th} Percentile		46.9

SECTION 17. FIGURES:

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

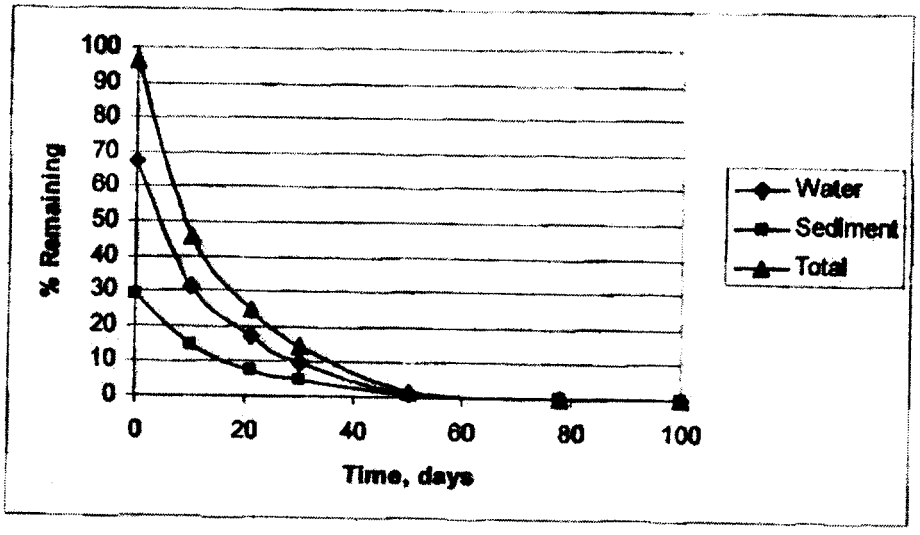
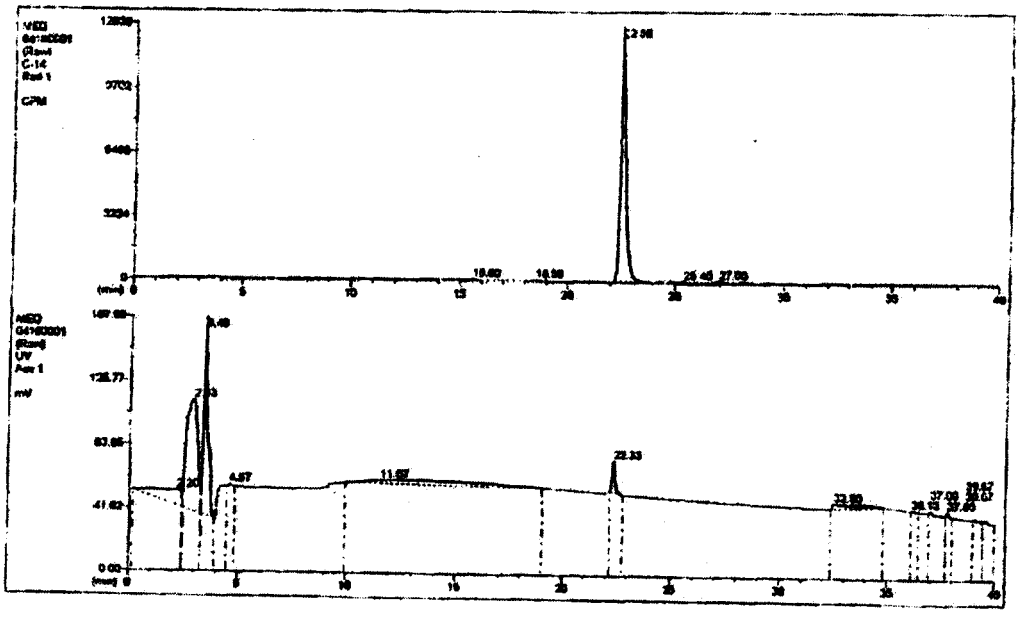


Figure 3 **HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

0 hour aqueous



0 hour sediment

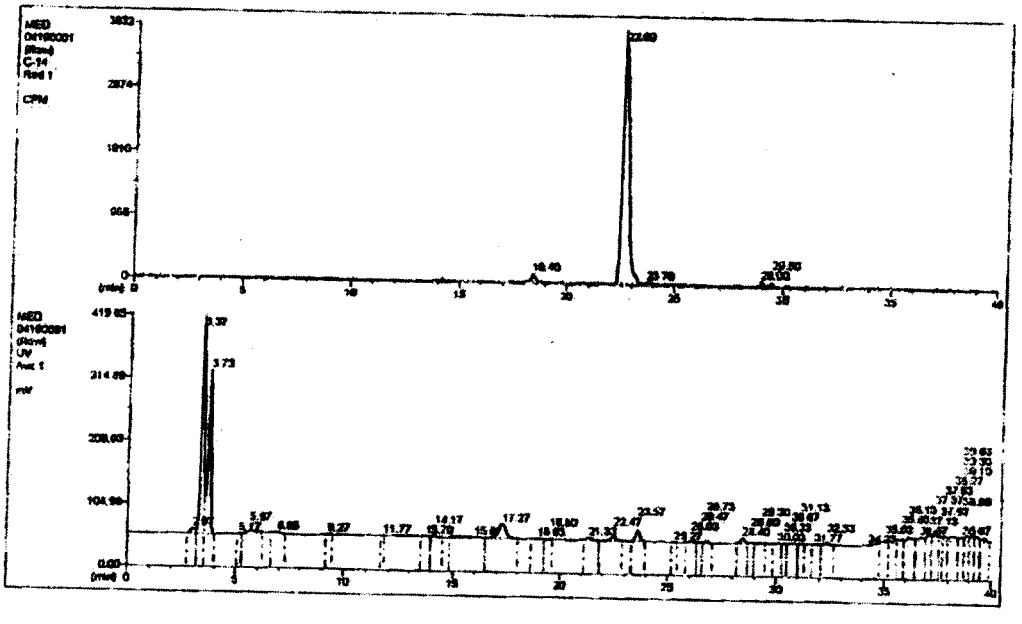
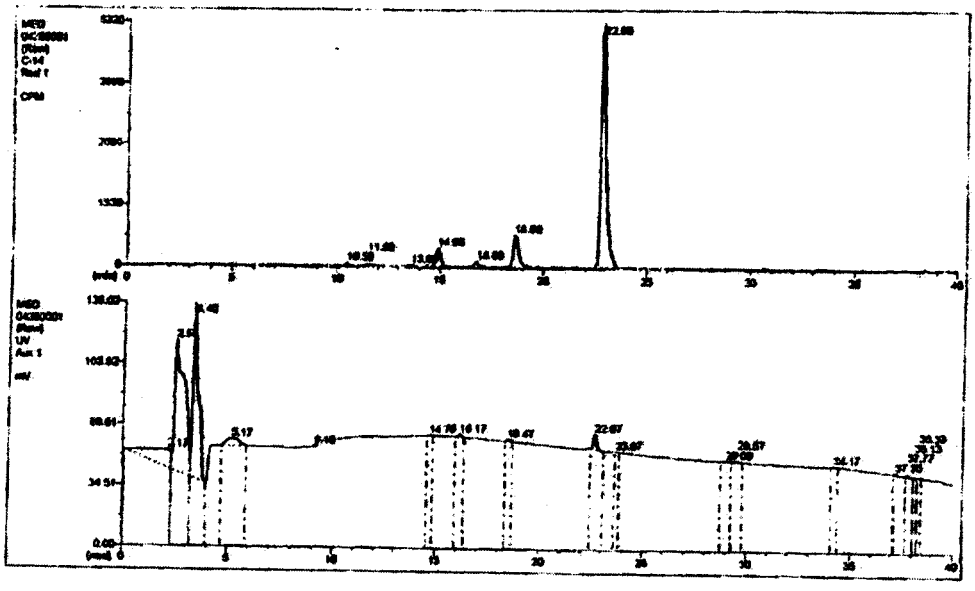


Figure 3 **Continued. HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

day 10 aqueous



day 10 sediment

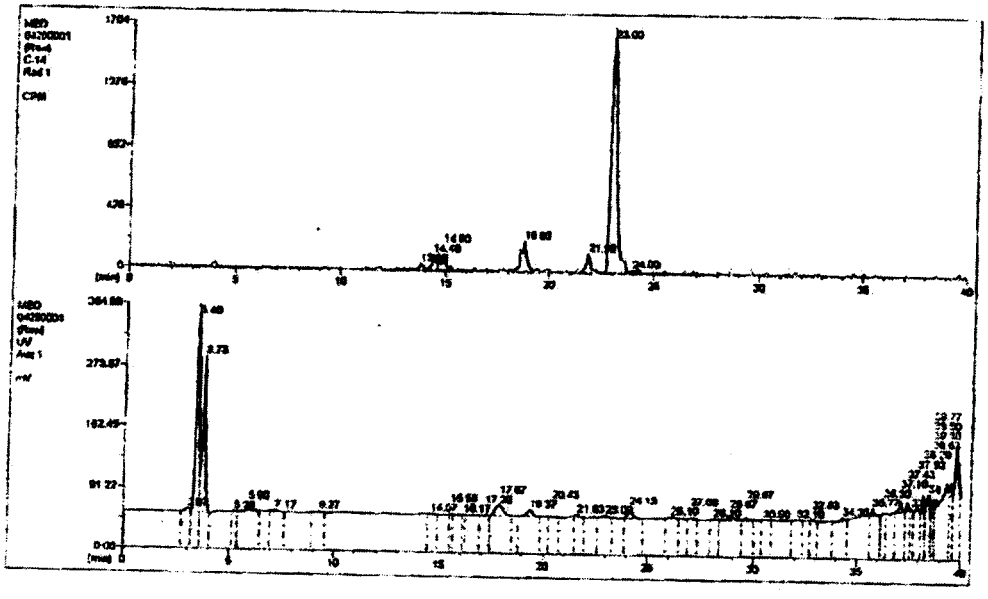
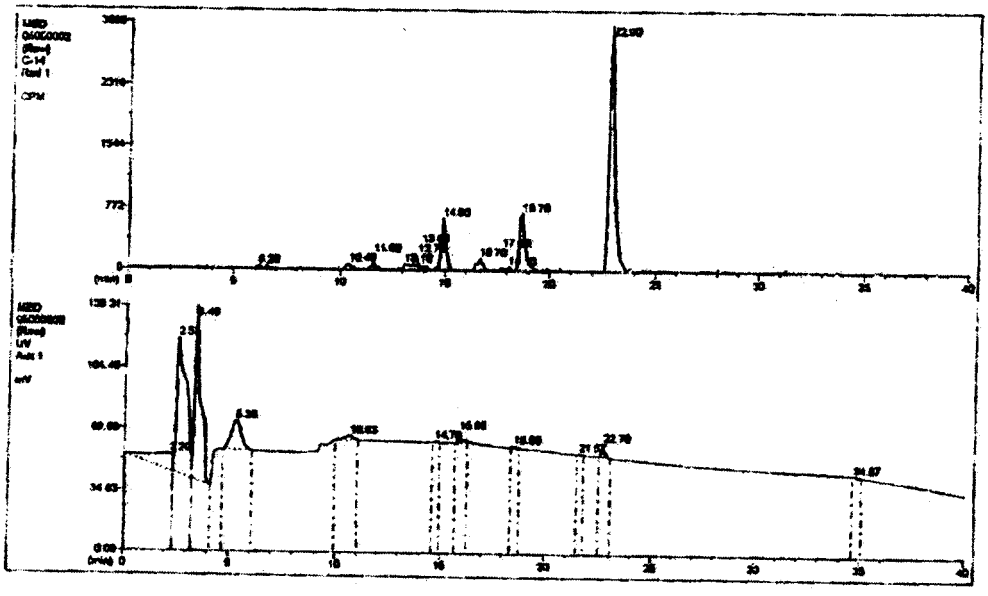


Figure 3 **Continued. HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

day 21 aqueous



day 21 sediment

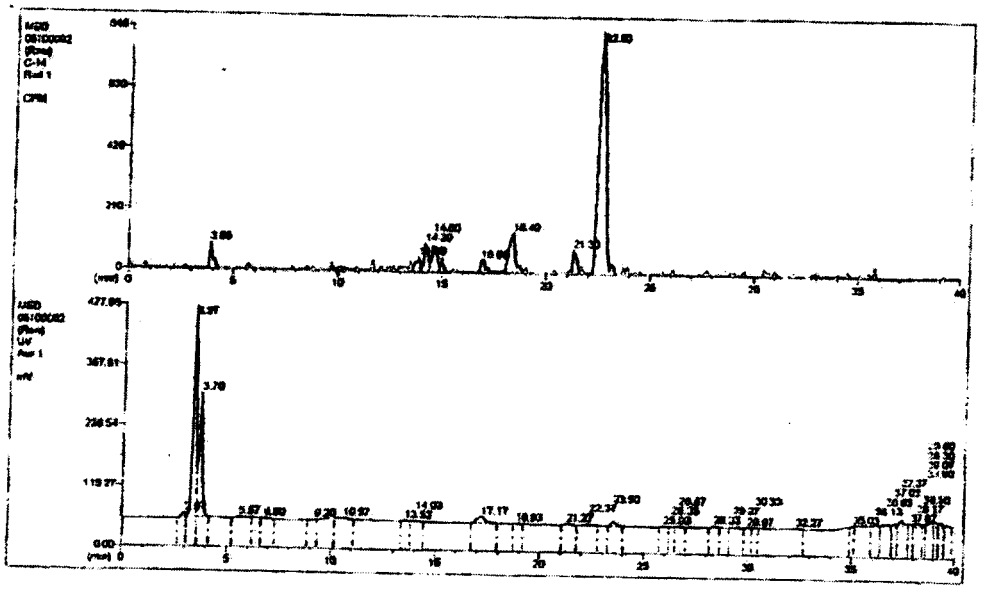
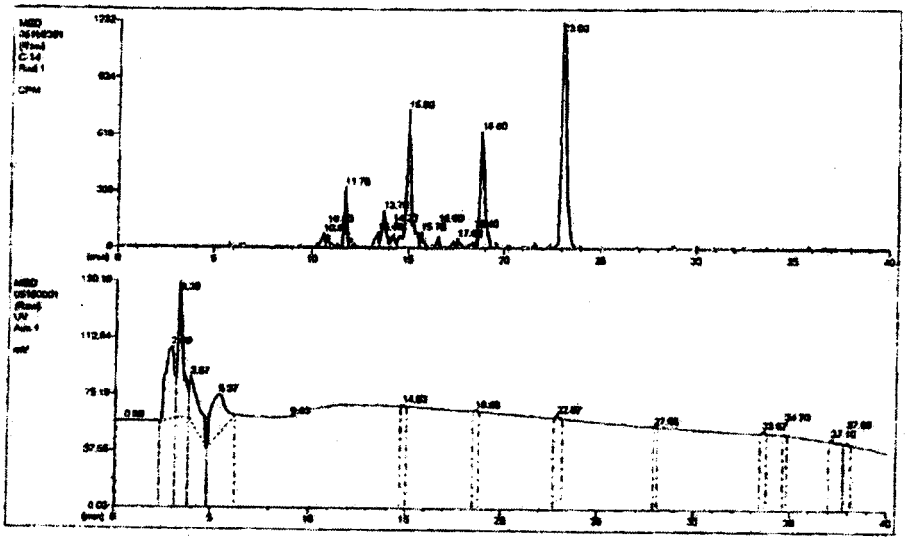


Figure 3 **Continued. HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

day 30 aqueous



day 30 sediment

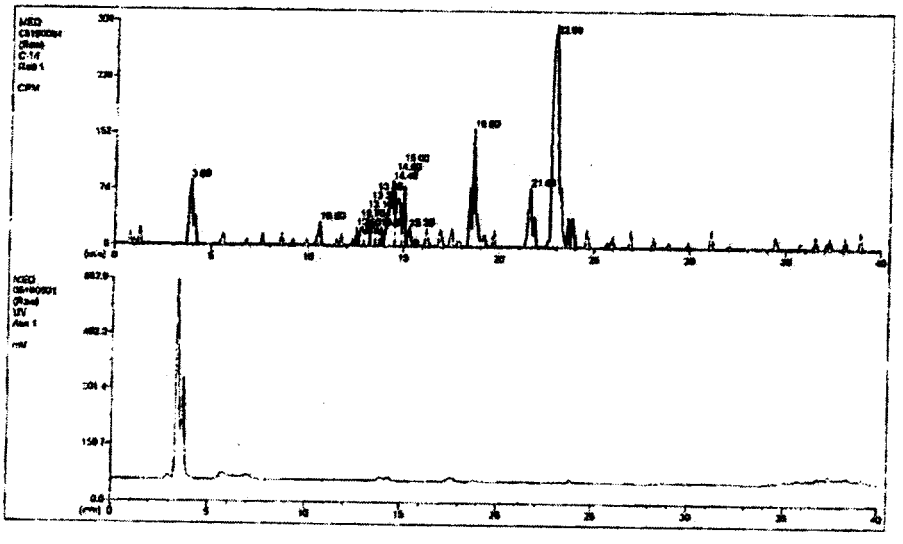
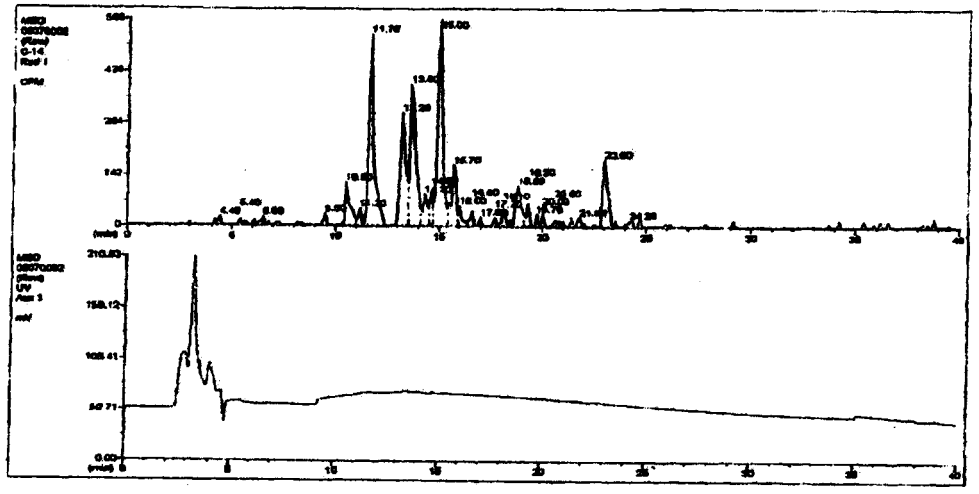


Figure 3 **Continued. HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

day 50 aqueous



day 50 sediment

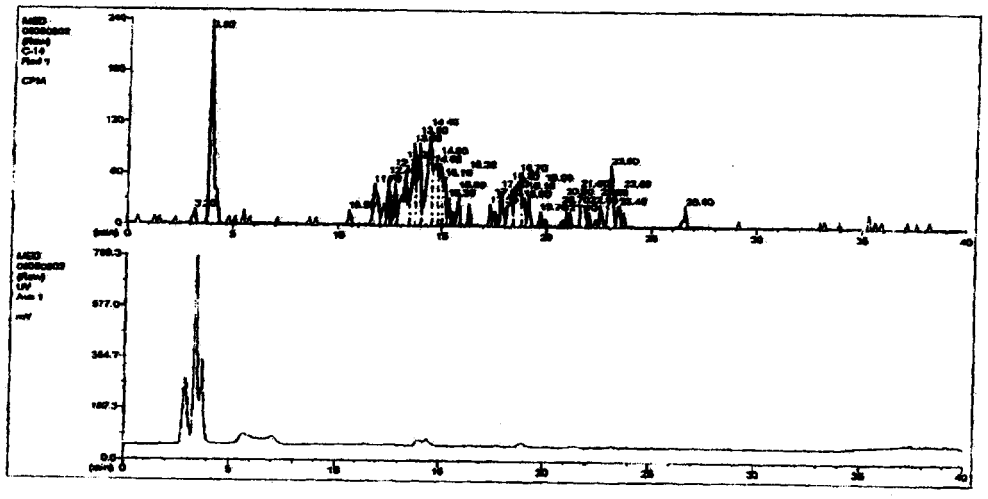


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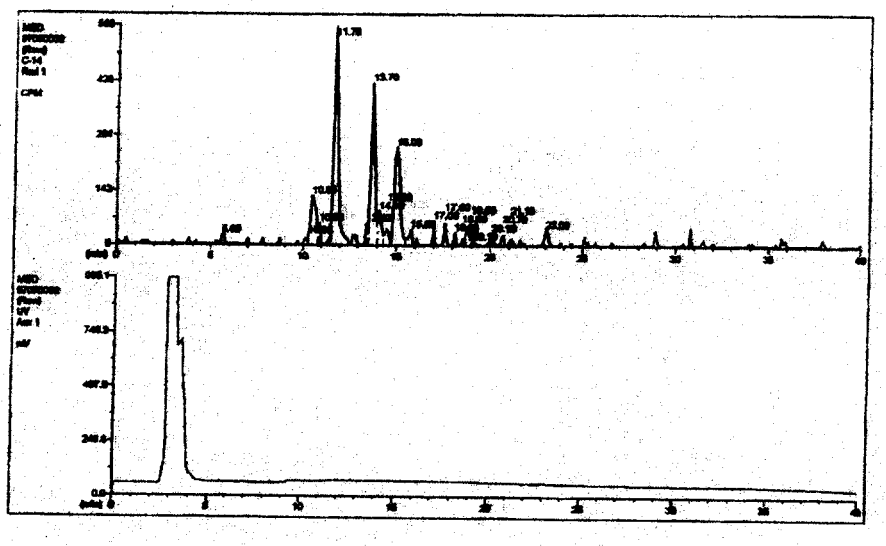
Figure 3 **Continued. HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

day 78 aqueous



day 78 sediment

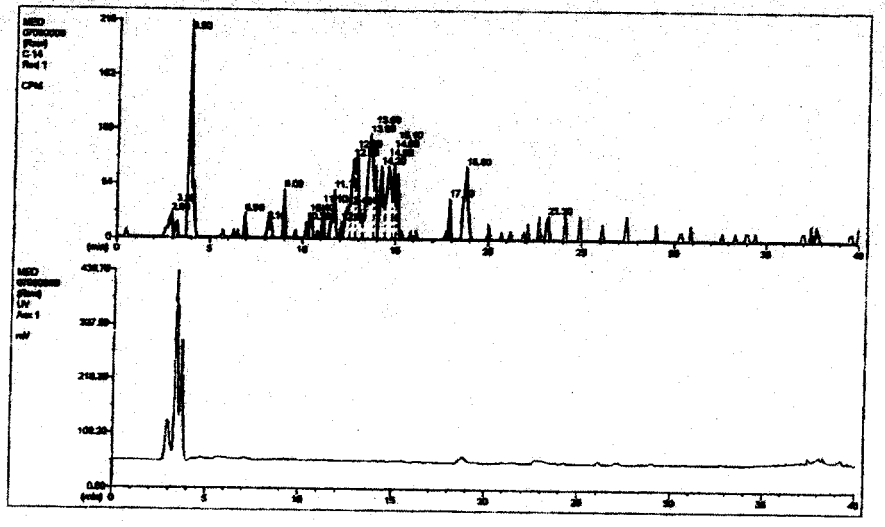


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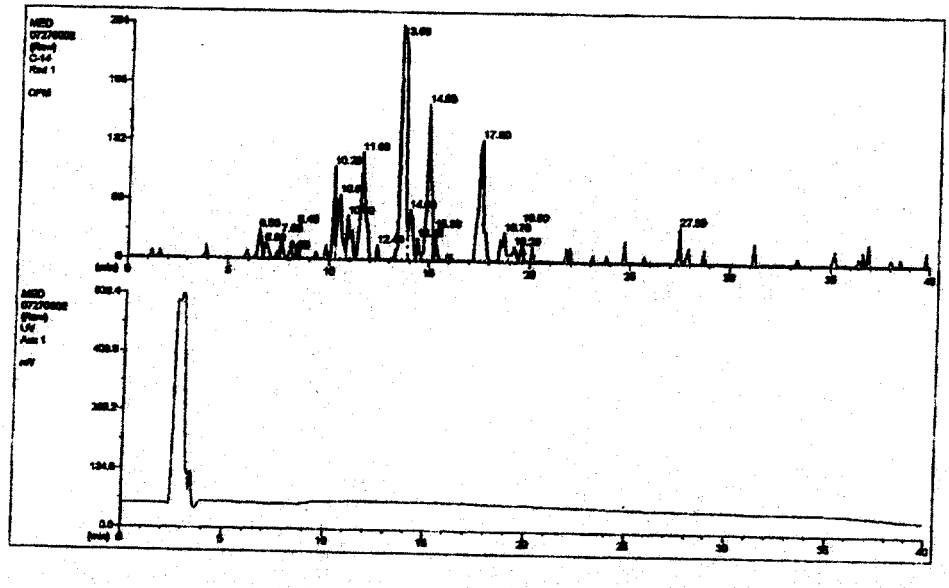
Figure 3 **Continued. HPLC/RAM chromatograms from GR sediment and water at 0 hour, and days 10, 21, 30, 50, 78 and 100.**

day 100 aqueous



day 100 sediment

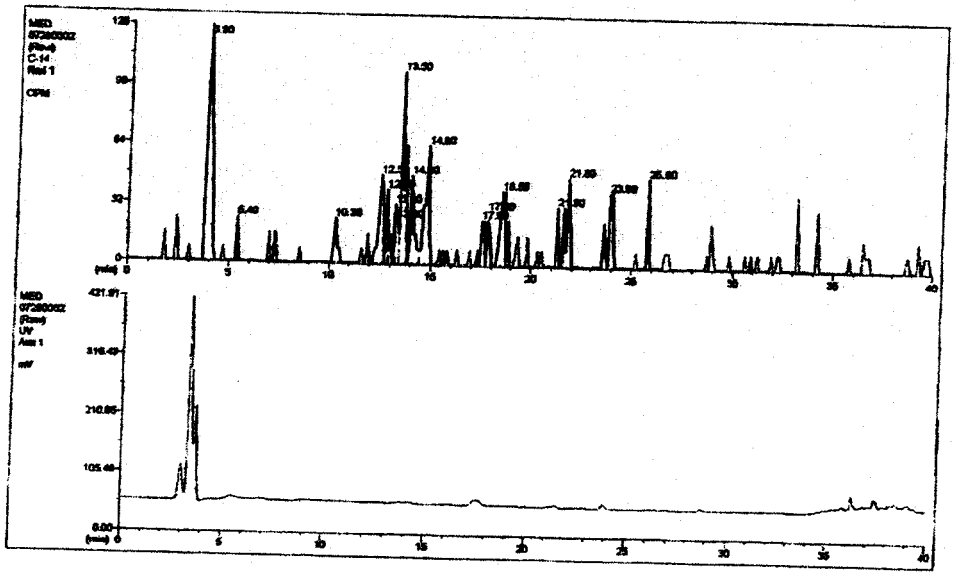


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Figure 4 **Metabolism of [^{14}C]florfenicol in GR sediment during the aerobic transformation study.**

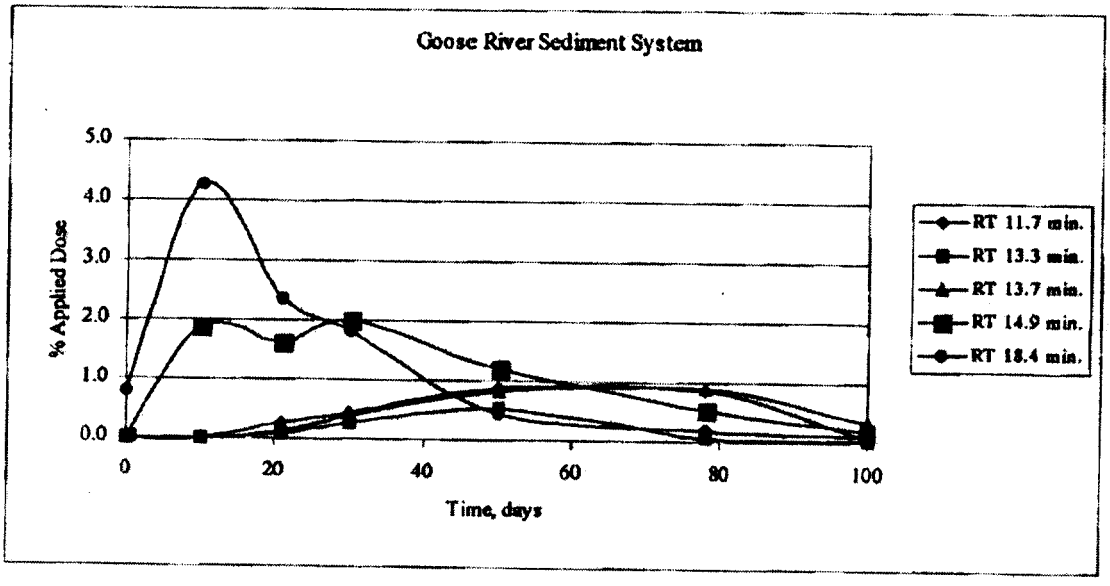

Figure 5: Species Sensitivity Distributions For Aquatic Organisms Exposed to Florfenicol in Water

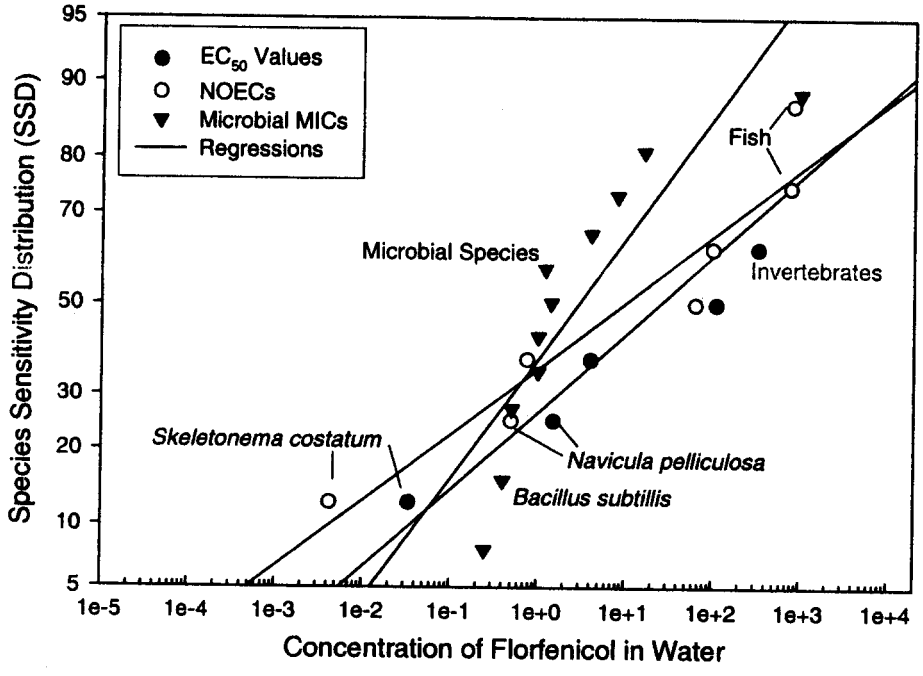

Figure 6. Species Sensitivity Distributions For Aquatic Organisms Exposed to Florfenicol in Water

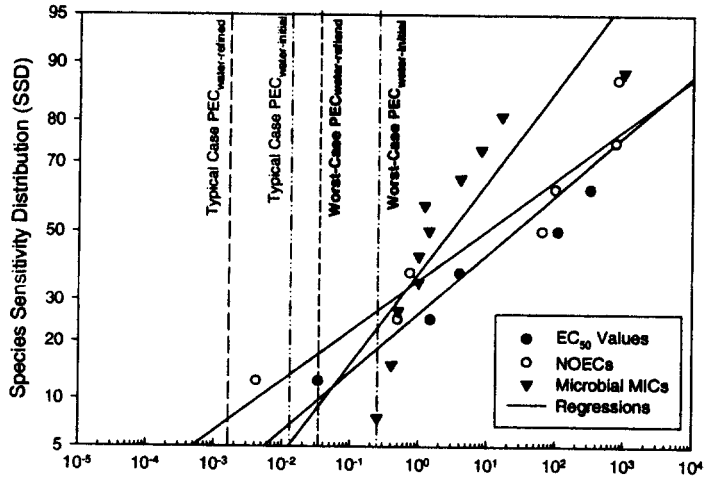

Figure 7. Risk Characterization of Aquatic Plant and Microbial Species Using Species Sensitivity Distributions to Florfenicol

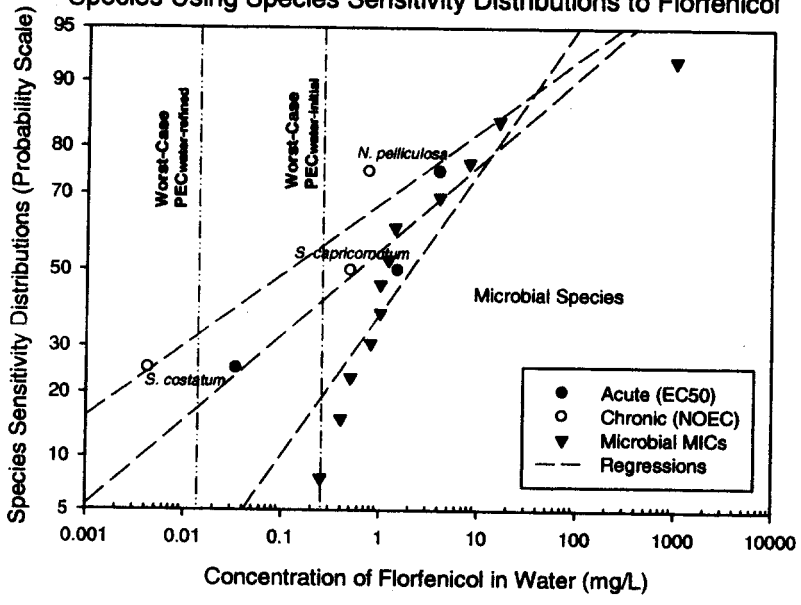

Figure 8. Species Sensitivity Distributions For Aquatic Organisms Exposed to Florfenicol in End-Of-Pipe and Receiving Waters.