ENVIRONMENTAL ASSESSMENT FOR THE USE OF NUFLOR® INJECTABLE SOLUTION IN CATTLE

1. DATE: March 5, 1996

2. APPLICANT: Schering Plough Animal Health

3. ADDRESS: P.O. Box 529
Kenilworth, NJ 07033

4. DESCRIPTION OF THE PROPOSED ACTION:

A new animal drug approval has been requested for the use of NUFLOR® Injectable Solution in cattle. NUFLOR® Injectable Solution, which contains the active ingredient, florfenicol, will be used as a treatment for bovine respiratory disease. It will be administered by intramuscular injection to cattle at a dose of 20 mg/kg body weight. Treatment will be repeated at 48 hours after the initial injection for a total of two injections. Approval of this new animal drug would authorize production facilities in Union, NJ and Bao Ling, China to manufacture the drug substance, florfenicol. Formulation and packaging of NUFLOR® Injectable Solution will be done at the Rhone-Merieux (formerly Sanofi Animal Health, Inc.) facility in Fort Dodge, Iowa.

A complete food safety program has been conducted with florfenicol. A value of 6 ppm was determined to be the safe concentration for florfenicol residues in cattle liver. Based on the residue depletion data, a withdrawal time of 28 days has been established for cattle treated with NUFLOR® Injectable Solution. A description of the studies used in determining the safe concentration and withdrawal time may be found in the Freedom of Information Summary.

Based on the proposed action, florfenicol could potentially be introduced into the following environments:

a. The environment adjacent to the drug substance manufacturing plant and the formulating and packaging plant(s) .

b. Cattle feedlots where florfenicol residues may be found in animal waste.

c. Agricultural lands where waste products from cattle are used as fertilizer.

d. Aquatic systems where runoff may collect from sites receiving waste products from treated cattle.

5. IDENTIFICATION OF THE CHEMICAL SUBSTANCE

NUFLOR® Injectable Solution is a formulation of the active ingredient florfenicol in a sterile nonaqueous solution. The formulation will contain 300 mg of florfenicol /ml. Florfenicol is a synthetic broad spectrum antibiotic.

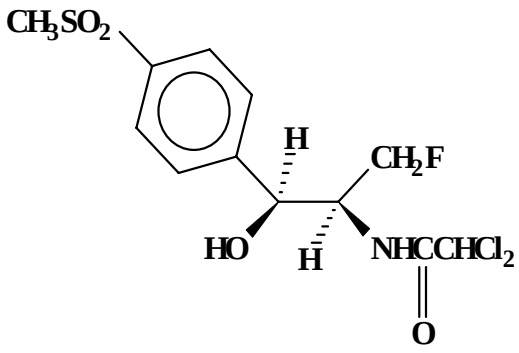
Chemical Name: [R-(R*,S*)]-2,2-Dichloro-N-[1-(fluoromethyl)-2-hydroxy-2-[4-(methylsulfonyl)phenyl]ethyl]-acetamide

CAS Registry Number: **73231-34-2**

Molecular Formula: **$C_{12}H_{14}Cl_2FNO_4S$**

Molecular Weight: **358.21**

Structural Formula:



Solubility:

Water (23 ± 1°C) at pH 7 = 1.32 ± 0.05 mg/ml (*Appendix 1*)

Melting Point: 153.5 - 154.5°C (*Appendix 2*)

UV Absorption:

Maximum molar absorptivity occurs at 224 nm in an aqueous solution containing 1% methanol *(Appendix 3*)

Vapor Pressure:

Florfenicol is a non volatile solid.

n-Octanol/Water Partition Coefficient:

The n-octanol/water partition coefficient for florfenicol was determined to be 2.36 at pH 7.0 *(Appendix 4*)

Density: 1.68 ± 0.01 g/cm^3 (*Appendix 5)*

Dissociation Constant:

The florfenicol molecule contains no functional groups which are ionized between pH 2 and pH 12. The florfenicol molecule contains the following polar functional groups: dichloromethyl amide, aliphatic alcohol, fluromethyl, and methyl sulfone. However, none of these groups are protonatated between pH 2 and pH 12. Therefore, florfenicol is a neutral compound which is not ionized in the range of pH 2 to pH 12.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

A. INTRODUCTION OF SUBSTANCES FROM THE MANUFACTURING SITES

Refer to *Appendix 6* for an assessment of potential environmental introduction of substances from the sites involved in manufacture of the drug substance, florfenicol and the dosage form, NUFLOR® Injectable Solution.

B. INTRODUCTION OF SUBSTANCE FROM THE USE SITE

NUFLOR® Injectable Solution will be used to treat bovine respiratory disease (BRD) in cattle. With a maximum dose rate of 20 mg/kg administered twice, a 275-kg calf would be injected with as much as 11 g of florfenicol. It is estimated that 26,000,000 calves enter feedlots in the U.S. on an annual basis. It is also estimated (worst case) that 34% may be treated for BRD (*Appendix 7*). If all of the treated cattle were treated with NUFLOR® Injectable Solution, up to 97,200 kg of florfenicol could be used annually. This represents a worst-case estimate since NUFLOR® Injectable Solution is not the sole treatment available for this condition.

i. Excretion of Florfenicol

The metabolism of ^{14}C-florfenicol was investigated in a total residue depletion study in cattle following administration of two 20 mg/kg doses of the intended commercial formula, intramuscularly, separated by a 48-hour interval (*Appendix 8*). Excreta were collected, and the metabolites were extracted and analyzed. Metabolic profiling of the sample extracts was accomplished by co-chromatography with florfenicol metabolite standards using TLC, HPLC, and GC/MS techniques. The structures of major metabolites were confirmed by GC/MS and thermospray liquid chromatography/MS.

The results indicated that an average of 66.5 to 68.5% of the radiolabeled dose was recovered in the urine , and that 7.4 to 9.0% was recovered in feces. In 0- to 120-hour pooled urine samples, the parent material, florfenicol represented 44% of the total radioactive dose administered. Florfenicol-related metabolites identified in the urine included the oxamic acid (8.2% of total dose), the amine (5.3% of total dose), the alcohol (4.7% of total dose), and trace amounts of the monochloro metabolite (1% of the total dose), and trace amounts of several unknown metabolites (4.6 - 5.6% of the total dose).

In 0 to 120-hour pooled fecal samples, up to 9% of the radioactive dose was recovered of which less than 0.3% was florfenicol, the parent material. Florfenicol-related metabolites identified in the feces included the monochloro metabolite (2.4% of total dose), the alcohol (1.4% of total dose) the oxamic acid (1% of total dose) and trace amounts of the amine (< 1% total dose), and trace amount of several unknowns (0 - 0.7% of total dose), and with 2-3% of total dose as unextractable ^{14}C-

residue.

The total average recovery of ^{14}C-residues in excreta was found to be 75.7% (77.5%, males; 73.9%, females). The analyzed tissues represented an average additional 5.0% of total dose (fat: 0.01-0.03%; inj. sites: 0.65-5.9%; kidneys: 0.03-0.08%; liver: 0.52-0.65%; muscle: 0.02-0.03%). Florfenicol was found to be a minor component in liver (< 1% of the total dose).

The total average recovery of ^{14}C-dose in sampled excreta and tissues was 80.7%. This recovery value is typical of studies of this type for two reasons, 1) tissues/fluids that are not analyzed (e.g., bile, G.I.-contents, carcass) may contain some radioactivity and 2) some loss of radioactivity occurs in the obtaining and handling of samples. The unaccounted for 19.3% (100%-80.7%) can be assumed to contain the same ratio of parent compound and metabolites as the 80.7%. Therefore the following calculations yield the extra % of parent compound which should be added to the 44% in order to present a correct estimate of worst case excreta concentration:

Urine: 67.5 ÷ 75.7 = 89.2% **Feces: 8.2 ÷ 75.7 = 10.8%**

89.2% x 19.3% = 17.2% **of the unaccounted for radioactivity would be excreted in the urine**

10.8% x 19.3% = 2.1% **of the unaccounted for radioactivity would be excreted in the feces**

(Urine) 17.2% x 44% **(urinary florfenicol excretion) = 7.57% additional florfenicol in the urine.**

(Feces) 2.1% x 0.3% (fecal florfenicol excretion) = 0.006% additional florfenicol in the feces

7.57% + 0.006% = @ 8% total additional florfenicol excretion

8% + 44% = 52% of the total dose is excreted as florfenicol (average case)

These results indicate that parent florfenicol is the major residue excreted in urine and that the three metabolites are excreted in feces. Each of the florfenicol metabolites represented less than 10% of the total administered dose and are thus not considered of environmental concern.

The major metabolites of florfenicol involve the dichloroacetamide group of florfenicol, and are shown in the following diagram:

ii. <u>Antimicrobial Activity of Metabolites</u>

Antimicrobial testing comparing florfenicol and its major metabolites has shown that the metabolites have much less antimicrobial activity. The comparative activity (*Appendix 9*) is summarized in the following table:

Table 1

Comparative Activity of Florfenicol (FF) and of its Metabolites

Bacteria	MIC µg/ml			
	FF	FF amine	FF alcohol	FF oxamic acid
Bacillus subtilis	**0.25**	**45.3**	**16**	**> 256**

E. coli	**6.8**	**150**	**71.2**	**> 512**
Str. faecalis	**4**	**> 512**	**128**	**> 512**
Enterobacter	**7**	**168.9**	**64**	**> 512**

The results in Table 1 indicate that the metabolites have much less antimicrobial activity than the parent florfenicol and are thus not of environmental concern.

iii. <u>Exposure Estimates</u>

The exposure estimates are based on the following information/assumptions:

a) A six acre feedlot will contain 1000 cattle.

b) Feedlot cattle weigh between 225-340 kg (500-750 lbs) and produce about 14-20 kg (30-45 lbs) of manure per day, respectively (Ref. 1). We will use an average weight of 275 kg (600 lbs), and produce 16.3 kg (36 lbs) manure/day.

c) Up to 60% (worst case) of the cattle will be treated for bovine respiratory disease (see *Appendix 7*), and only florfenicol will be used. This is an overestimate since several other products are also marketed to treat this condition.

d) Animals are most likely to require treatment soon after arrival: all animals requiring treatment will be treated within their first month after arrival, with an average of 15 days after arrival.

e) Florfenicol is administered by intramuscular injection to cattle at a dose

of 20 mg/kg body weight. Treatment will be repeated at 48 hours after the initial injection for a total of two injections.

f) Cattle metabolize florfenicol and only 52% of the total administered dose is excreted as florfenicol over a five-day period.

g) Florfenicol excretion is mostly in the urine. Feces contain only trace amounts of florfenicol.

h) The feedlot surface is constructed of concrete (worst case).

i) Feedlot cattle will urinate and defecate onto the concrete, and then trample/mix them together.

j) The primary biotransformation half-life of florfenicol in manure-amended soils ranged from 4-27 days. The ultimate half-life (mineralization to CO_2) averaged 5 months.

k) The cattle are in the feedlot for 120-150 days, then shipped to market. The accumulated manure (solid and liquids) are applied to agricultural fields as fertilizer at the rate of 20 tons per acre (worst case). The average incorporation rate is 5-10 tons per acre.

l) The Texas Cattle Feeders Association, whose members produce approximately 25 percent of the total U.S. beef cattle, described their common waste management practices. Only 25% of the manure go from the feedlot pen directly to an agricultural field. About 5-10% of the manure is composted. Most of the manure, 60-65%, is stockpiled for at least 3 months (or longer if cropland is seasonally unavailable for

fertilization). This is discussed further in *Appendix 10*.

m) Feedlot pens are cleaned about twice a year. The state water pollution control permits require that they be cleaned at least once a year. The pens are not cleaned while the cattle are in them, but are only cleaned after the cattle are shipped to market. In most cases, from the time the cattle enter the feedlot untill any of their manure is applied to cropland is at least 180 days and usually closer to 240 days (150 days in feedlot + 90 days of stockpiling).

n) The "worst possible case", resulting in the highest estimated florfenicol residues, would be to assume that the cattle are in the feedlot pen for only 120 days, and that the manure is immediately applied as fertilizer to agricultural fields.

o) Water runoff from cattle feedlots contains high concentrations of nutrients, salts, oxygen-demanding organic matter (biochemical oxygen demand -BOD), and bacteria (*Appendix 10*).

p) To discharge water, the feedlot would have to comply with the standards set by the U.S. EPA under the National Pollution Discharge Elimination System (NPDES) (Ref. 2) and specific standards set by the State (permitting agency) to prevent degradation of the receiving waters (Ref. 3).

q) It would be very costly to treat feedlot water runoff to meet NPDES standards for direct discharge into surface waters. Thus, feedlots use a water runoff control system to divert clean rainwater around the feedlot, and water runoff from the feedlot is diverted to a retention

basin for evaporation. In addition, in some areas, water from the retention basin may be used for irrigation. To be used for irrigation, some water treatments are done: settling pond/lagoons or serpentine waterways to reduce the total solids and BOD, and dilution with fresh water to reduce the salt concentration prior to application to agricultural fields for selected crops. (Ref. 3, 4 for U.S. EPA and Appendix 10)

r) **The Texas Cattle Feeders Association members use small settling basins before the water enters the retention basin. Any solids in these settling basins can easily be dredged out after a rainfall event and placed with the stockpiled manure. Thus, the retention basin rarely needs to be cleaned out. (Estimates place this as once every 20-25 years.)**

s) **The potential concentrations of florfenicol in the aquatic environment will be based on florfenicol residue concentrations in the rainwater runoff from agricultural fields fertilized with manure from treated cattle.**

t) **To estimate the "worst case", highest florfenicol residues in the aquatic environment, would be to assume that all of the florfenicol residues contained in the manure applied to an agricultural field were extracted from the manure amended soil by rainwater.**

The highest possible, "worst case", estimate of the concentration of florfenicol in the excreta from treated cattle is 340 ppm, based on the largest animal, a 340 kg animal, receiving an injection of 6.8 g of florfenicol which is 100% excreted. The florfenicol excreted in the urine is mixed with the 20 kg of manure excreted that day. However, metabolism studies show that only 52% of the administered dose is excreted as parent florfenicol. If we assume that the 52% of the administered dose which is usually excreted over 5 days, is excreted in one day, only 3.5 g of florfenicol is excreted and mixed with the 20 kg of manure. Thus, the excreta from this single animal could contain up to 177 ppm of florfenicol. The excreta from an average size animal with a weight of 275 kg would contain 175 ppm of florfenicol. The excreta from a smaller size animal with a weight of 225 kg would contain 167 ppm of florfenicol.

Worst Possible Case (100% of administered dose excreted as parent florfenicol):

340 kg X 20 mg/kg = 6800 mg of florfenicol administered

6800 mg / 20 kg of manure = 340 ppm in excreta

Realistic Worst Case (52% of administered dose excreted as parent florfenicol):

340 kg X 20 mg/kg = 6800 mg of florfenicol administered

6800 mg X 52% excreted = 3536 mg of florfenicol excreted

3536 mg / 20 kg of manure = 177 ppm florfenicol in excreta

Average Case (average size animal):

275 kg X 20 mg/kg = 5500 mg of florfenicol administered

5500 mg X 52% excreted = 2860 mg of florfenicol excreted

2860 mg / 16.3 kg of manure = 175 ppm florfenicol in excreta

(Note: the smaller animal produces less manure.)

Smaller Size Animal

225 kg X 20 mg/kg = 4500 mg of florfenicol administered

4500 mg X 52% excreted = 2340 mg of florfenicol excreted

2340 mg / 14 kg of manure = 167 ppm florfenicol in excreta

(Note: the smaller animal produces less manure.)

The florfenicol concentration in the excreta following both injections of florfenicol would be reduced to about 118 ppm, because it would be diluted by three days of manure excretion.

Two Injections, 48 hours apart, each dose excreted in 24 hours:

340 kg X 20 mg/kg/injection X 2 injections	**= 13600 mg of florfenicol administered in total**
13600 mg X 52% excreted	**= 7072 mg of florfenicol excreted**
7072 mg / (20 kg of manure/day X 3 days)	**= 118 ppm of florfenicol in the excreta following two injections.**

A maximum of 60% of the cattle in any large feedlot could exhibit signs of respiratory disease and potentially be treated with florfenicol. Assume a 6-acre feedlot with 1000 cattle, each weighs 340 kg and produces 20 kg of manure per day. The cattle are in the feedlot for 120 days. The total manure produced in 120 days is 2.4 X 10^6 kg.

20 kg of manure/day/animal X 1000 animals X 120 days = 2.4x10^6 kg of manure

If 600 animals (60% of all the cattle) are treated with florfenicol a total of 8200 g of florfenicol would be used, of which 2.4 X 10^6 mg of florfenicol is excreted in the urine and mixed with the manure. Thus, the concentration of florfenicol in the excreta would be a maximum of 1.8 ppm.

20 mg/kg X 2 injections X 340 kg/animal X 600 animals	**= 8.2 x 10^6 mg of florfenicol used**
8.2 x 10^6 mg X 52% excreted	**= 4.2 x 10^6 mg of florfenicol excreted**
4.2 x 10^6 mg of florfenicol / 2.4x10^6 kg of manure	**= 1.8 ppm**

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

A. BIODEGRADATION IN MANURE AMENDED SOIL

A 92-Day study was conducted with florfenicol in three soils each of which was amended with manure obtained from feedlot cattle. Soils were collected from Kansas (silty clay), Washington (sandy loam), and Wisconsin (loam). Manure was obtained from cattle raised in Georgia, Texas and Washington and mixed thoroughly before being amended to soils at a rate equivalent to 10 tons/acre. For 50 gram soil samples, 556 mg of manure was added (all weights are on a dry weight basis). Radiolabeled florfenicol was incorporated into the soils at 50 μg/kg, the lowest concentration possible which allowed for accurate analysis to be to be conducted and degradation to be observed. The study included both collection of radioactive carbon dioxide, and HPLC analyses of soil residues to demonstrate metabolic transformation of florfenicol. HPLC analysis was conducted at several intervals throughout the 92-day study period, and carbon dioxide trap analysis occurred at least once a week.

During this study, the mineralization of florfenicol was progressive over time. By day 92 of the study, over 50% of the applied radioactivity (^{14}C) was recovered as carbon dioxide in the Kansas and Washington soils. In the Wisconsin soil, 24% of the radioactivity was recovered as carbon dioxide during the course of the study. Half-lives for mineralization were 87, 118 and 270 days in the Kansas, Washington and Wisconsin soils, respectively. Thus, the average ultimate (mineralization to CO^2) was 158 days, or about 5 months.

Analysis of the soils by HPLC corroborated the biodegradation of florfenicol. By day 14 of the study, less than 40% of the radioactivity extractable from the soil was parent florfenicol. In addition, numerous metabolite peaks were seen in the chromatographic profiles. Examination of the HPLC profiles during the study indicated that polar

metabolites formed but did not accumulate. By day 92 of the study, 94-100% of the originally applied florfenicol had been metabolized in each of the three soils. Primary biotransformation half-lives for florfenicol in the three soil, based on the HPLC analysis of soil extracts, was 9, 4 and 27 days in the Kansas, Washington and Wisconsin soils respectively. Thus, the average biotransformation half-life was 13 days, or about 2 weeks.

The progressive mineralization of florfenicol to CO_2, the progressive reduction in extractable florfenicol over time, and the numerous metabolite peaks in the chromatographic profiles; all indicate biotransformation of florfenicol. This study conclusively demonstrated the ready biodegradability of florfenicol in the environment into which it will enter upon use. Thus there should be no detectable accumulation of florfenicol in the soil due to its rapid biotransformation and none of its metabolites should persist due to the relatively rapid rate of mineralization. A summary of this study is presented in *Appendix 11*.

Other studies were undertaken with florfenicol to determine whether or not other pathways also exist for the elimination of florfenicol from the environment.

B. AEROBIC BIODEGRADATION IN WATER

A 28-day aerobic biodegradation study in water was performed with florfenicol. The quantities of ^{14}C-carbon dioxide (CO_2) and ^{14}C-volatile products released as a result of microbial degradation of florfenicol in water were measured. HPLC measurements were also performed on a weekly basis to determine whether partial degradation had occurred. The cumulative $^{14}CO_2$ and ^{14}C-volatile products collected over the 28 day remained at less than 1.0% of the dose initially applied. However, HPLC conducted with UV detection determined that a small amount of florfenicol had degraded over the 28 days of testing. At test termination, HPLC conducted with

radiometric detection demonstrated that 81% of the total ^{14}C remaining in the test solutions was florfenicol. Thus, 19% of the florfenicol degraded over the 28 days. Under these test conditions, the half-life for florfenicol was determined to be greater than 28 days.

A summary report of this study may be found in *Appendix 12*.

C. PHOTODEGRADATION

Photolysis is defined as the chemical reaction produced by exposure to light or ultraviolet radiation. Direct photolysis involves the direct interaction of a compound with light, tested in a pure water. Indirect photolysis involves the interaction of light with substances in the water that may promote the degradation of a compound; tested with synthetic humic water. Dark controls (tubes wrapped with aluminum foil to block out light) were used to determine the degree of hydrolysis. Photodegradation estimates can then be corrected for hydolysis if necessary.

A 30-day direct and indirect photolysis study was conducted with florfenicol. The quantities of florfenicol and photodegradates were measured using HPLC-UV and photolytic half-lives were calculated. The photolytic half-life estimates were over 150 days (more than 5 times the study duration). Thus, neither direct or indirect photolysis were significant routes of degradation of florfenicol (*Appendices 13, 14*).

D. HYDROLYSIS

The dark controls used in the photodegradation study cited above were used to estimate the hydrolytic half-life of florfenicol. At pH = 7 in pure water, no significant hydrolysis occured. In synthetic humic water the hydrolytic half-life was about 350 days (more than 5 times the study duration). Hydrolysis is therefore not

a significant route of degradation for florfenicol.

E. SOIL SORPTION AND DESORPTION

A sorption/desorption study was conducted to evaluate the binding characteristics of florfenicol which may influence its fate in the environment.

The propensity for animal drug residues to be transported from sites of application is defined by an understanding of factors contributing to their mobility and persistence. Partitioning between soil and aqueous phases influences mobility by controlling leaching rates and ultimate disposition of residues as soil bound or freely soluble forms. The objective of this study was to determine the partitioning of florfenicol between sorbed and solution phases. The studies were conducted according to the methods and procedures published in the FDA Technical Assistance Document, Section 3-08. Adsorption coefficients were determined in three different soil types each for florfenicol. Soil types were characterized primarily by their texture and organic matter content.

Screening tests demonstrated that the presence of $CaCl_2$ did not significantly modify or alter the sorption of florfenicol to soil. However, the desorption of florfenicol was somewhat enhanced by the presence of $CaCl_2$. The degree of sorption was low in all soil types (less than 25% of applied test article sorbed), therefore, advanced tests were not required nor performed. Results of the study are summarized in Table 2.

Table 2

Sorption/Desorption with Florfenicol
Screening Test Results

	DDI Water[a]		0.01 M $CaCl_2$	
Soil Type	**K_d**	**K_{oc}**	**K_d**	**K_{oc}**
IALM#1[b]	**0.95**	**46**	**0.57**	**29**
CALSLM[c]	**0.16**	**24**	**0.07**	**10**
TXSLM[d]	**0.88**	**52**	**0.39**	**23**

[a] - Distilled, deionized water
[b] - Iowa Loam (9% sand, 59% silt, 32% clay, 3.5% organic matter, pH 7.4)
[c] - California Silt Loam (59% sand, 33% silt, 8.0% clay, 1.1% organic matter, pH 6.4)
[d] - Texas Silt Loam (14% sand, 60% silt, 26% clay, 2.9% organic matter, pH 8.0)

The values of K_d and K_{oc} presented above demonstrate that florfenicol binds slightly to soils, and can be classified as somewhat mobile.

A summary report of this study may be found in *Appendix 15*.

F. POTENTIAL ENVIRONMENTAL CONCENTRATIONS

Florfenicol could be introduced into the environment from manure used as fertilizer on cropland. Both the soil environment (direct exposure) or the aquatic environment (via runoff from agricultural fields) could be exposed to florfenicol. Florfenicol is a non-volatile solid. This was confirmed in the aerobic soil biodegradation study where no significant amount of volatile organic molecules were trapped over a 92-day period. Thus, measurable amounts of free florfenicol would not be expected in the atmosphere.

i. Potential concentrations of florfenicol in soil

The highest concentration of florfenicol in the soil would be in excreta in a feedlot. Most of the florfenicol excretion from treated cattle is through the urine. The cattle will urinate and defecate in the same area, and then trample/mix the excreta on the concrete feedlot floor (worst case). Assuming 60% of the 1000 head of cattle in a feedlot for 120 days are treated with florfenicol, the concentration of florfenicol in the accumulated excreta would be 1.8 ppm, assuming no biodegradation.

Subsequently, this manure could be spread over cropland as a fertilizer. Common practice is to apply cattle manure at the rate of 5-10 tons per acre (*Appendix 10)*. We will use 20 tons per acre (1.81×10^{4} kg of manure/acre). The application rate of florfenicol residue would be 3.3×10^{4} mg/acre.

(1.8 mg florfenicol/kg manure) X (1.81×10^{4} kg manure/acre) =

3.3×10^{4} mg of florfenicol/acre.

The manure is incorporated into the top six inches of topsoil in the field (9.1×10^{5} kg soil/acre). Thus, the final florfenicol residue once incorporated into cropland topsoil would be 36 ppb.

(3.3×10^{4} mg florfenicol/acre) / (9.1×10^{5} kg soil/acre) = 3.6×10^{-2} ppm = 36 ppb

This residue would then be biodegraded, since florfenicol has an average primary biotransformation half-life of about 13 days in manure-amended soils. Since the cattle requiring florfenicol treatment would most likely require the treatment shortly after arrival, they would receive florfenicol their during their first month

at the feedlot (with an average of within 15 days of arrival). The cattle are in the feedlot for 120 days, which gives an average of 105 days for biodegradation of florfenicol in the feedlot manure. Because florfenicol has been shown to rapidly biodegrade in manure amended soils, and because there are far greater numbers of bacteria in manure than in soil, it is likely that during the 105 days in the feedlot, significant biodegradation of florfenicol would ocurr, but this has not been quantitated. Biodegradation in the feedlot would reduce the florfenicol concentration to less than the 1.8 ppm cited above.

Based on the very low concentrations of florfenicol in the soil following use of the product, coupled with the rapid biodegradation of florfenicol in manure-amended soils, florfenicol should not persist or accumulate in soil. The florfenicol residues in the manure-soil admixture following incorporation into cropland will be so low, 3.6×10^{-2} ppm or 36 ppb, that they are negligible.

ii. <u>Potential concentrations of florfenicol in aquatic systems</u>

The potential movement of florfenicol through runoff into aquatic systems could occur from feedlots or from manure-fertilized cropland soils. However, water discharges from feedlots are regulated by federal and state laws and regulations (Ref. 5). In the United States, permits to control wastewater discharges into streams, rivers, and other bodies of water are issued by individual states in accordance with standards set by the U.S. Environmental Protection Agency under the National Pollutant Discharge Elimination System (Ref. 2). Water quality-based limits on the quantity and quality of discharged effluent from a feedlot are included in the NPDES permits. Individual states can establish very stringent specific limits for a particular discharge site to prevent degradation of high quality receiving waters. (Ref. 3,4)

Wastewater generated by runoff from a feedlot would require a very high level of effluent treatment before it could be discharged into any water body such as a river or stream. Two important indicators of allowable discharge quality are the carbonaceous or biochemical oxygen demand (BOD) and the wastewater nutrient level (measured as ortho-phosphorus and ammonia nitrogen). Typically, a wastewater effluent would require at least a 95% removal of the BOD (measured as the BOD_5) and the ammonia nitrogen to meet quality standards. Also feedlot wastewater would require chlorination to reduce coliform bacteria numbers, then dechlorination prior to final discharge to eliminate any potential toxic impact of chlorine on aquatic organisms. (*Appendices 16 and 17*)

A lagoon system to achieve a high quality effluent would require a series of staged operations. This system would consist of a first stage waste stabilization lagoon (anaerobic or facultative), followed by secondary and tertiary oxidation lagoons. The total detention time required would range from 50-70 days. In addition, the effluent would require chlorination and dechlorination prior to discharge. (*Appendix 18*)

The land requirements for such a lagoon system is estimated to be about three acres based on the following: (20 kg manure/animal/day) X (1000 animals) X (0.6 kg BOD_5/kg manure) = 12000 kg BOD_5 per day. The first stage waste stabilization lagoon would typically be 4 meters deep. The secondary and tertiary lagoons would typically be 1.3 meters deep. Since the feedlot is only six acres, this represents a 50% increase in land requirements. (*Appendix 18*)

An alternative to a lagoon system is a combination of a lagoon and a conventional biological system such as a waste stabilization lagoon followed by an oxidation ditch or aerated basin. Another alternative is a conventional biological activated

sludge system. Both alternatives require a much higher capital costs for construction. (*Appendix 18*)

In addition to capital expenditure, the costs for operating and maintaining the waste water treatment system are significant. These operational and maintenance costs include monitoring and sampling of wastewater effluent, maintaining mechanical equipment, administrative record keeping, and facility housekeeping. These activities would certainly warrant a least one wastewater control technician at the facility full time. (Ref. 6)

As a consequence of the above costs, it is not economically feasible for feedlots to invest in treatment facilities capable of achieving the high effluent standards required to directly discharge to natural water bodies such as a river or stream. Thus, feedlots do not discharge any wastewater (*Appendices 10, 16, 17, 18*). Rainwater on the feedlot and wastewater are diverted to a retention basin where it is allowed to evaporate. The retention basin has a water-impervious lining to prevent seepage into the groundwater. Typically, the retention basin is designed to hold all the feedlot wastewater and the rainfall associated with a 25-year storm. In Nebraska a 25-year storm equals 4-6 inches of rain in 24 hours (*Appendix 19*). Rainwater falling on land around the feedlot does not enter the feedlot, but is diverted around the feedlot using ditches and other water control devices. The runoff from the feedlot typically first goes through a series of small settling basins before it enters the retention basin. The solids in these settling basins are easily dredged and the dredged material placed with the stockpiled manure. Only rarely, once every 20-25 years, the retention basin area is cleaned, and this basin bottom material is mixed with the stockpiled manure and used as fertilizer on cropland.

The waste management practices of feedlots have to comply with a variety of local,

state, and federal regulations which were set up to protect the environment from the undesirable effects of cattle excreta. Direct runoff from a feedlot would contain high concentrations of organic matter and nutrients, which when entering surface waters would cause oxygen depletion, eutrophication, and lead to fish kills. Thus, the regulations were setup to prevent cattle wastewater runoff. Consequently, feedlot do not discharge any water runoff (*Appendix 10*).

Since feedlots do not discharge any wastewater, there is no potential for florfenicol movement into aquatic systems from feedlot runoff. However, rainwater runoff from cropland fertilized with manure may allow movement of florfenicol into aquatic environments. (*Appendix 10*)

In the previous discussion on the potential concentration of florfenicol in soil, it was shown that 3.3×10^4 mg florfenicol/acre could be applied to manure-fertilized cropland. A 2 inch rainfall would produce 2.05×10^7 liters of rain/acre. In the "worst possible scenario", if it rained just after the manure had been applied, and all of the florfenicol was extracted, the florfenicol concentration in the rainwater runoff would be 1.6×10^{-3} ppm or 1.6 ppb.

$$(3.3 \times 10^4 \text{ mg florfenicol/acre}) / (2.05 \times 10^7 \text{ l of rain/acre}) = 1.6 \times 10^{-3} \text{ ppm}$$
$$= 1.6 \text{ ppb}$$

This florfenicol concentration is far below the lowest NOEL of 0.75 ppm, of all the aquatic species tested. The aquatic safety factor is approximately 469 (0.75 ppm / 1.6×10^{-3} ppm). Thus, florfenicol containing runoff does not pose a threat to the aquatic environment.

TABLE 3
ESTIMATED FLORFENICOL CONCENTRATIONS IN CATTLE EXCRETA AND THE ENVIRONMENT

I. Florfenicol concentrations in excreta from a single treated animal

A. "Worst possible (highest) case" (Assume 100% florfenicol excretion)	340 ppm
B. Realistic worst case (52% florfenicol excretion)	177 ppm
C. Two injections, 48 hours apart (Diluted by 3 days of excreta accumulation)	118 ppm

II. Florfenicol concentrations in the excreta in a feedlot

A. 60% of cattle treated, in feedlot 120 days (Assume no biotransformation of florfenicol)	1.8 ppm

III. Florfenicol concentrations in the soil of a manure fertilized field

A. Florfenicol residue application rate (Assume 20 tons of manure/acre)	3.3×10^{4} mg/acre
B. Florfenicol concentration once manure is incorporated into the topsoil	4.0×10^{-2} ppm

IV. Florfenicol concentrations in rainwater runoff from a manure fertilized field

A. Assume 100% of florfenicol residue is extracted from the soil by the rain	**1.6×10^{-3} ppm**
B. Aquatic safety factor, based on the most sensitive species tested (Lowest NOEL = 0.75 ppm was observed in algae)	469

8. EFFECTS ON THE ENVIRONMENT OF RELEASED SUBSTANCES

A. MAMMALIAN TOXICITY STUDIES

A testing program has been completed with various laboratory animal species and florfenicol. Complete reports of all of these studies have been submitted to support the proposed action. Studies which help determine the safety of florfenicol to the public and the environment are summarized in the *Appendix 20*. Mammalian toxicology information is also summarized in a Freedom of Information Summary available for NUFLOR® Injectable Solution.

B. POTENTIAL ADVERSE EFFECTS OF THE PROPOSED ACTION ON HUMAN HEALTH

i. Human Exposure to Florfenicol During Production and Use of NUFLOR® Injectable Solution

Workers in the production areas are given specific instructions (as part of the production instructions) for the safe handling of both the florfenicol drug substance and the dosage form. All facilities involved in florfenicol or NUFLOR® Injectable Solution production comply with the appropriate federal/national, state, and local occupational safety laws and regulations. Additional information on practices followed at the specific manufacturing sites may be found in *Appendix 6*. A copy of the Material Safety Data Sheets for florfenicol is provided in *Appendix 21*.

The label for NUFLOR® Injectable Solution will instruct users that this formulation is available for use by or on the order of a licensed veterinarian, is not for human use and should be kept out of reach of children. Considering

the results of toxicity studies, the fact that florfenicol is neither a mutagen, teratogen or carcinogen, and that florfenicol will be in a liquid injectable formulation, it is concluded that users would not be adversely affected by the proposed action.

ii. Human Exposure to Florfenicol Via the Food Supply

A complete food safety program has been conducted with florfenicol. A value of 6 ppm was determined to be the safe concentration for florfenicol residues in cattle liver. Based on the residue depletion data, a withdrawal time of 28 days has been established for cattle treated with NUFLOR® Injectable Solution. Therefore, exposure of humans to large amounts of florfenicol via the food supply is quite unlikely. Since very little florfenicol residue will be released onto soil, it is highly improbable that measurable amounts of florfenicol would occur in drinking water from groundwater or surface water sources. Details of any exposure of humans to florfenicol in meat are listed in the Freedom of Information Summary for NUFLOR® Injectable Solution. The proposed action is not expected to adversely affect human health through the food supply.

C. POTENTIAL ADVERSE EFFECTS OF THE PROPOSED ACTION ON NONTARGET ORGANISMS

Use of NUFLOR® Injectable Solution in cattle should result in very little exposure of non-target organisms. Since florfenicol is injected into cattle and not mixed in their feed, avian species and nontarget mammals should have no opportunity to be exposed to florfenicol. Low concentrations (0.0027 ppb maximum) of florfenicol are expected in runoff water from manure fertilized cropland, and biodegradation in both manure and soil of florfenicol would result in even lower

exposure levels for aquatic organisms in surface water. Studies have been conducted to determine the effects of florfenicol on nontarget organisms and results of these studies are summarized below.

i. Aquatic Organisms

A series of studies were conducted to determine the acute toxicity (LC_{50}, EC_{50} or MIC) of florfenicol to the following species: bluegill sunfish (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus mykiss*), daphnids (*Daphnia magna*) and freshwater green algae (*Selenastrum capricornutum*). Along with LC_{50} (or EC_{50} or MIC) determinations, the no observed effect level (NOEL) was determined for each species. All studies were conducted according to the methods and procedures published by FDA in the Technical Assistance Document, Sections 4-01 (algae), 4-08 (daphnids) and 4-11 (fish).

The following minimum inhibitory concentrations (MIC), effect concentrations (EC_{50}), lethal concentrations (LC_{50}) and no observed effect limit (NOEL) were determined using HPLC to measure florfenicol concentrations in test solutions. Data is presented in Table 4 for each species tested and temperature.

Table 4

Acute Toxicity of Florfenicol in Aquatic Species

Species	LC_{50} (mg/L)	NOEL (mg/L)	Test Temp. (°C)	Summary Report
Selenastrum capricornutum	> 2.9[a]	2.9[a]	23-27	Appendix 22
Selenastrum capricornutum	1.5[b]	0.75[b]	23-27	Appendix 22
Daphnia magna	> 330[c]	< 100[d]	20-21	Appendix 23
Lepomis macrochirus	> 830	830	20-22	Appendix 24
Oncorhynchus mykiss	> 780	780	10-13	Appendix 25

[a] - minimum inhibitory concentration (MIC) or no observed effect concentration, based upon maximum growth rate

[b] - minimum inhibitory concentration (MIC) or no observed effect concentration, based upon maximum cell density

[c] - effect concentration for 50% immobilization (EC_{50})

d - The lowest concentration tested was 100 mg/l. Forty Daphnia magna organisms were exposed at this concentration. There were no totally immobilized organisms at this concentration. However, four organisms showed some lethargy; two were lethargic and on the bottom, one was lethargic but free swimming, and one organism was at the surface (probably trapped in the surface film). Due to the lethargy seen in a few of the Daphnia, the NOEL was stated as less than 100 mg/L.

ii. Terrestrial Organisms

For any chemical, the determination of the lowest concentration at which inhibition of microbial growth occurs is important because of possible ramifications if that concentration is exceeded in the environment. For florfenicol, five species were tested for the minimum inhibitory

concentrations (MIC). These studies were conducted according to the methods and procedures published by FDA in the Technical Assistance Document, Section 4-02.

Test article was incorporated into agar containing appropriate nutrients and incubated with each species for twenty four hours (longer for *Nostoc*). The MICs reported were defined as the lowest concentrations of test material that inhibited the growth of the test organism. During preliminary testing two species, *Aspergillus niger* and *Trichoderma viride* were unaffected by exposure to a wide range of concentrations and therefore, no definitive study was conducted with these species. The following table summarizes the findings.

Table 5

Minimum Inhibitory Concentration of Florfenicol

SPECIES	MIC (mg/L)
Aspergillus niger	> 1000
Trichoderma viride	> 1000
Clostridium perfringens	1.0
Bacillus subtilis	0.4
Nostoc	4.0

A summary report of this study may be found in *Appendix 26*.

9. UTILIZATION OF NATURAL RESOURCES AND ENERGY

Production and formulation of florfenicol will occur at facilities designated for production of pharmaceuticals. These operations do not require use of unusual

amounts of energy or natural resources.

10. MITIGATION MEASURES

The proposed action would not be expected to have any adverse effect on human health or the environment. Engineering controls, personal safety equipment, and personal hygiene precautions will be effective in minimizing exposure to florfenicol in production and formulation facilities. The label will instruct users in the safe use of the product.

11. ALTERNATIVES TO THE PROPOSED ACTION

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

12. LIST OF PREPARERS

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APPENDICES

1. **Solubility Testing of Florfenicol**

2. **Melting Temperature of Florfenicol**

3. **Ultraviolet-Visible Absorption Spectrum of Florfenicol**

4. **Partition Coefficient of Florfenicol**

5. **Density Measurements of Florfenicol**

6. **Environmental Assessment of Manufacturing Sites Involved in the Production of Florfenicol and NUFLOR Injectable Solution.**

7. **1995 Beef Cattle Report, The Agricultural Research Division of The Institute of Agriculture and Natural Resources, University of Nebraska**

8. **Total Residue Depletion Study in Cattle Following Intramuscular Administration of 14C-SCH 25298**

9. **Antibacterial Screening of Florfenicol Metabolites**

10. **Cattle Feedlot Waste Management Practices For Water and Air Pollution Control, Texas Agricultural Extension Service, Texas A&M University**

11. **Aerobic Biodegradation of SCH 25298 in Manure Amended Soil**

12. **SCH 25298 - Aerobic Biodegradation in Water**

13. **SCH 25298 - Determination of Aqueous Photolysis Rate Constant and Half-Life**

14. **SCH 25298 - Indirect Photolysis Screening Test with Synthetic Humic Water**

15. **SCH 25298 - Determination of the Sorption and Desorption Coefficients**

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20. Overview of Toxicological Studies Conducted with Florfenicol

21. Florfenicol Material Safety Data Sheet

22. SCH 25298 - Toxicity to Freshwater Green Algae

23. SCH 25298 - Acute Toxicity to Daphnids Under Static Conditions

24. SCH 25298 - Acute Toxicity to Bluegill Sunfish Under Static Conditions

25. SCH 25298 - Acute Toxicity to Rainbow Trout Under Static Conditions

26. SCH 25298 - Determination of Microbial Growth Inhibition