Date of Approval Letter: September 4, 2002

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-206

NUFLOR® 2.3% Concentrate Solution
(florfenicol)

"For use in the treatment of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Salmonella choleraesuis* and *Streptococcus suis* Type 2."

Sponsored by:
Schering-Plough Animal Health

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1. GENERAL INFORMATION:

a. NADA Number:	141-206
b. Sponsor:	Schering-Plough Animal Health Corporation 1095 Morris Avenue Union, New Jersey 07083 Drug Labeler Code: 000061
c. Established Name:	Florfenicol
d. Proprietary Name:	NUFLOR® 2.3% Concentrate Solution
e. Dosage Form:	Oral concentrate solution
f. How Supplied:	One-gallon plastic bottles (2.2 liter fill)
g. How Dispensed:	Rx
h. Amount of Active Ingredients:	23 mg florfenicol per mL
i. Route of Administration:	Oral. For use in swine drinking water only.
j. Species/Class:	Swine
k. Recommended Dosage:	400 mg per gallon of water (100 ppm), provided in the drinking water over 5 consecutive days.
l. Pharmacological Category:	Antimicrobial
m. Indications:	For use in the treatment of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Salmonella choleraesuis* and *Streptococcus suis* Type 2.

2. EFFECTIVENESS:

a. Dosage Characterization:

"Efficacy of NUFLOR® in Treating Swine Respiratory Disease" (Study No. 1370E-61-V96-280-01)

A seeder pig challenge study at a single location was used to evaluate the efficacy of NUFLOR® (florfenicol), an oral concentrate solution containing 23 mg of florfenicol per mL, administered in drinking water for 5 days for treatment of acute *Actinobacillus pleuropneumoniae* (APP) respiratory disease in swine. The investigator was Kelly Lechtenberg, DVM, Ph.D. The diagnosis of pleuropneumonia was based on acute clinical signs of pneumonia, dyspnea score of 1 or more and a rectal temperature of 104.5°F or higher. Test animals were divided into four treatment groups and given either non-medicated water or water medicated with 50, 100 or 200 mg/gallon florfenicol.

Pivotal variables were lung consolidation, rectal temperature and mortality. Rectal temperature and mortality were measured and recorded daily from Day 0 to Day 12. Lung consolidation was assessed on day of mortality or at study termination for survivors. Other variables measured and recorded were body weight, dyspnea, depression, body weight gain and perianal irritation. Lung cultures were taken from pigs at death or at necropsy performed at study conclusion.

Actinobacillus pleuropneumoniae (APP) was isolated at necropsy from 16/19 (84.2%) of the placebo group. APP was isolated from 13/20 (65%) of the 50 mg/gal group, 11/20 (55%) of the 100 mg/gal group and 12/20 (60%) of the 200 mg/gal group. In addition, *Streptococcus suis* was isolated from pigs in each group. All isolates were sensitive to florfenicol.

The placebo group had the highest mean total lung consolidation (29.5%) while the 200 mg/gal group had the lowest total consolidation (8.7%). The lung consolidation percentages for the 50 mg/gal group and the 100 mg/gal group were 17.7% and 14.6%, respectively. The percent mortality was 25% (5/20) in the placebo group and 15% (3/20) in the florfenicol 50 mg/gal group. There were no mortalities in the florfenicol 100 mg/gal and 200 mg/gal groups.

Under the conditions of this study, NUFLOR® administered in the drinking water at 100 and 200 mg/gal was effective in the treatment of acute APP respiratory disease in swine.

The following adverse reactions were observed: Pigs were observed daily for signs of perianal irritation, anal edema and rectal prolapse. The incidence of perianal irritation was statistically higher in the 200 mg/gal group (50% on Day 2 and Day 3) than in all other groups. All perianal irritation resolved spontaneously by Day 6. There were no occurrences of anal edema or rectal prolapse during the study.

b. Substantial Evidence:

1. "Florfenicol Water Medication Dose Confirmation Study for Treatment of Swine Respiratory Disease" (Study Nos. 1370C-61-V96-301 -01 and -02)

a. Type of Study: Two-location field trial in swine with naturally occurring pleuropneumonia.

b. Investigators:

Study 301-01
Kelly F. Lechtenberg, D.V.M., Ph.D., Midwest Veterinary Services, Inc., Rural Route # 2, Box 49, Oakland, Nebraska 68045

Study 301-02
Gary W. Davis, D.V.M., Ph.D., Greenbriar Veterinary Services, Inc., 6040 Dublin Road, Delaware, Ohio 43015

c. Study Design:

1) *Objective:* To evaluate the effectiveness of NUFLOR® (florfenicol) administered in drinking water for 3 or 5 days at 200 or 400 mg/gallon in comparison to a negative control for the treatment of naturally occurring swine respiratory disease.

2) *Experimental Animals:* Six studies were conducted at four different locations, using 149 (Site 301-01) and 95 (Site 301-02) castrated male crossbred swine with an approximate mean initial weight of 26.7 kg.

The diagnosis of pleuropneumonia was based on acute clinical signs of pneumonia with a rectal temperature of 104.5°F or higher. Pretrial nasal swabs and lung tissue samples from pigs that died were taken for bacterial examination.

3) *Test Article Administration:* The dosage form was a water-soluble formulation of florfenicol containing 23 mg florfenicol per mL (2.3% florfenicol activity), which was administered *ad libitum* in drinking water at 200 or 400 mg/gallon for 3 or 5 consecutive days, starting on Day 0. The control group received non-medicated water provided *ad libitum* throughout the study. Study duration (treatment and post-treatment observation period) was 12 days.

4) *Measurements and Observations:* Decision variables included lung consolidation (note: all pigs were necropsied), rectal temperature, mortality, body weight, dyspnea, cough, and depression. Other observations were recorded to evaluate safety of the drug, based on adverse reactions from the dose selection study. These included perianal inflammation (i.e., the present or absence of) and fecal consistency.

The investigator, who was blinded to treatment assignments, assessed clinical response variables. Rectal temperature was assessed daily from Day 0 to 7. Dyspnea, cough and depression were assessed daily from Day 0 to Day 11. The concurrent observations, i.e., perianal inflammation and fecal consistency were made daily from Day 0 to study termination. Dyspnea, cough and depression were evaluated based on numerical scores using the following scores: 0=absent (normal), 1=mild, 2=moderate, 3=severe.

Success/failure rates were assessed using temperature, dyspnea and depression scores on Days 5, 7, and 11. Pigs treated for 3 days were evaluated for success/failure two days post-treatment on Day 5, and on Days 7 and 11. Pigs treated for 5 days were evaluated two days post-treatment on Day 7, and also on Day 11. To qualify as a success, animals needed to have a rectal temperature of 104.0°F or less and clinical scores for dyspnea and depression of either 0 or 1. Once an animal was declared a failure, it was removed from the study, euthanized and necropsied.

d. Statistical Methods: The pen was the experimental unit. For each variable, each day was evaluated separately.

Rectal temperature and body weights were analyzed as a mixed model analysis of variance, with a separate analysis for each day of observation. Treatment, Site, Site by Treatment, and Pen nested in Treatment were factors in the model. For analyses beyond Day 0, a covariate was added to the model for the Day 0 measurement. Mortality, the occurrence of cases with <10% lung consolidation and the occurrence of clinical successes were all evaluated by Fisher's Exact Test. Lung consolidation was analyzed by the Kruskal-Wallis Exact Test.

Dyspnea, cough, depression and perianal inflammation were evaluated by the Stratified Cochran-Mantel-Haenszel Test (stratified by site) and pairwise by the Wilcoxon Exact Rank Sum Test. If statistical differences were found on Day 0, subsequent days were stratified based on Day 0 results using the Friedman Test.

Fecal consistency was analyzed by Fisher's Exact Test.

Statistical significance was declared when $p \leq 0.05$ was achieved. Analyses were performed on SAS PC version 6.12 and StatXact version 2.11.

e. Results:

1) *Mortality*: Mortality was insufficient to provide a significant difference between treated and control groups.

2) *Rectal temperature and lung consolidation:* Table 2.1 provides data for these variables.

Table 2.1. Lung Consolidation, Temperature and Treatment Success of Dose Confirmation Study 1370C-61- V96-301-01, 02

Treatment Group	% Cases with <10% Lung Consolidation		Day 5 Rectal Temperature (°F)	% Success 2 Days Post-Treatment
	Palpation visualization of Lobes	Digitized Score		
NUFLOR® 200 mg/gal x 3 days	8%	15%	103.5	67%
NUFLOR® 400 mg/gal x 3 days	22%	24%	103.1	76%
NUFLOR® 200 mg/gal x 5 days	40%	40%	102.8	78%
NUFLOR® 400 mg/gal x 5 days	41%	33%	102.8	93%
Non-medicated	10%	8%	103.9	42%

The rectal temperature scores of all treatment groups of animals, except the controls, returned to normal limits within the first several days of the trials, but there was a significant difference between all medicated groups and the non-medicated controls on Day 5.

There was significant improvement in lung consolidation in the 5-day treatment NUFLOR® groups (both the 200 and 400 mg/gal levels) and the 3-day 400 mg/gal NUFLOR® treatment compared with the 3-day 200 mg/gal treatment and the non-medicated controls groups. There was significant improvement in the success rate of the 5-day 400 mg/gal level therapy groups compared with all other therapy groups.

f. Adverse Reactions: The 400 mg/gal group had a statistically significantly higher incidence of perianal inflammation than the 200 mg/gal dosages on Days 1 through 6, while the 200 mg/gal dosages were not statistically different from the non-medicated group.

g. Conclusions: Florfenicol administered orally for 5 days at 400 mg/gal was effective in the treatment of SRD in swine.

2. "Efficacy of NUFLOR® (Florfenicol) in Drinking Water" [Study Nos. 1370C-61-V96-294-(01, 02, 03, 05, 06 and 07)]

Note: V96-294-04 investigation was not initiated due to low incidence of disease at this site.

a. Type of Study: Multi-location field trial in swine with naturally occurring pleuropneumonia.

b. Investigators:

Studies 294-01 and -06
Kelly F. Lechtenberg, D.V.M., Ph.D., Midwest Veterinary Services, Inc.,
1443 Highway 77, Oakland, Nebraska 68045

Studies 294-02 and -07
Gary W. Davis, D.V.M., Ph.D., Greenbriar Veterinary Services,
6040 Dublin Road, Delaware, Ohio 43015

Study 294-03
Martin F. Mohr, D.V.M., Swine Veterinary Center
1608 Minnesota Ave., St. Peter, Minnesota 56082

Study 294-05
Monte W. Fuhrman, D.V.M., Rural Technologies, Inc.
224 Main Avenue, Brookings, South Dakota 57006

c. Study Design:

1) *Objective:* This study was conducted to evaluate the effectiveness of florfenicol administered in drinking water at 400 mg/gallon, for the treatment of naturally occurring swine respiratory disease in comparison to a negative control treatment regimen.

2) *Experimental Animals:* Six trials were conducted at four different locations. Four hundred fifty six (456) crossbred swine, ranging in age from 8 to 13 weeks, with mean initial weights (per study) of 20.0 kg to 34.5 kg were enrolled. Two hundred thirty (230) swine were enrolled in the florfenicol treatment groups, and 226 were enrolled in the control groups.

The diagnosis of swine respiratory disease was based on pyrexia (≥104.5ºF rectal temperature) in animals from herds with confirmed histories of swine respiratory disease associated with *Actinobacillus pleuropneumoniae* (APP). Pretrial nasal swabs and lung tissue samples from pigs that died were taken for bacterial examination.

3) *Test Article Administration:* The dosage form was a water-soluble formulation of florfenicol containing 23 mg florfenicol per mL (2.3% florfenicol activity), which was administered *ad libitum* in drinking water. Florfenicol-treated water was administered for 5 consecutive days, starting on Day 0. The control group received non-medicated water provided *ad libitum* throughout the study. Study duration (treatment and post-treatment observation period) was 29 days.

4) *Measurements and Observations:* The pivotal variables were treatment success and cumulative mortality. The supportive variables were individual clinical response variables, feed consumption and weight gain.

Mortality was recorded daily from Day 0 to Day 28.

The investigator, who was blinded to treatment assignments, assessed clinical response variables. Rectal temperature was assessed daily from Day 0 to 7. Dyspnea, cough and depression were assessed daily from Day 0 to Day 28.

Dyspnea was assessed using the following scores: 0=absent (normal character of breathing), 1=mild (mild distress in breathing with minor abdominal effort), 2=moderate (moderate distress in breathing; intermittent gasping/thumping with noticeable abdominal effort after exercise), 3=severe (severe distress in breathing; continual gasping/thumping with extreme abdominal effort).

Cough was assessed using the following scores: 0=absent (no coughing), 1=mild (isolated shallow coughs), 2=moderate (repeated but intermittent coughing of variable intensity), 3=severe (persistent deep coughing).

Depression was assessed using the following scores: 0=absent (no depression; animal is bright, alert, responsive. Rises when investigator enters pen), 1=mild (still responsive but less alert; may not arise when investigator enters pen), 2=moderate (only partially responsive to stimuli, reluctant to rise under most circumstances), 3=severe (animal recumbent, essentially non-responsive and very reluctant to move).

Treatment success was calculated on both Days 5 and 7 using rectal temperature and depression and dyspnea scores. A pig was classified as a treatment success if the pig's rectal temperature was <104°F and its dyspnea and depression scores were both <2. Pigs not meeting the criteria for success were classified as treatment failures.

Body weights and feed consumption were recorded weekly. All animals that died or were euthanized during the study had their weight recorded at the time of death. All surviving animals were weighed on Day 28.

d. Statistical Methods: The pen was the experimental unit. For each variable, each day was evaluated separately.

Rectal temperature and body weight were analyzed by nested mixed model ANOVA (Day 0) or ANCOVA (using Day 0 as the covariate for subsequent days; site was the random variable and pen was nested within treatment), and pairwise contrasts used least squares means.

The Fisher's Exact Test (cumulative mortality) and the Stratified Cochran-Mantel-Haenszel Test (stratified by site) evaluated cumulative mortality and success rates. The Logrank Exact Test was also used to assess cumulative mortality, as was ANOVA using binomial responses from SAS macro GLIMMIX, using site as a blocking factor (random variable).

Dyspnea, cough, depression and perianal inflammation were evaluated by the Stratified Cochran-Mantel-Haenszel Test (stratified by site) and pairwise by the Wilcoxon Exact Rank Sum Test. If statistical differences were found on Day 0, subsequent days were stratified based on Day 0 results using the Friedman Test.

Statistical significance was declared when $p \leq 0.05$. Analyses were performed on SAS PC version 6.12 and StatXact version 2.11.

e. Results: Pooled results for the pivotal variables are summarized in Table 2.2.

Table 2.2. Summary of Mortality Data and Overall Treatment Assessment

Treatment Group	Cumulative Mortality Rate	Overall Assessment (Failure Rate)			
		Day 5		Day 7	
		Percentage Failure	p-value	Percentage Failure	p-value
Florfenicol 400 mg/gallon	7.4%	9.6%	---	29.6%	---
Nonmedicated	9.7%	50.9%	0.0001	45.6%	0.0125

Mortality in the field studies was insufficient to provide a significant difference between treated and control groups. NUFLOR® treatment at 400 mg/gallon for 5 days resulted in significantly more clinical successes than the control treatment with all sites in the model.

f. Adverse Reactions: Rectal eversion was reported in up to 6% and perianal inflammation was reported for up to 36% of the NUFLOR® treated swine during the therapy period. The rectal eversion or the perianal inflammation resolved in the post treatment period without medical intervention.

g. Conclusions: Under the conditions of this study, florfenicol administered in drinking water at a dose of 400 mg/gallon for 5 consecutive days is an effective treatment for swine respiratory disease.

c. Pharmacokinetics:

The pharmacokinetic information provided in the labeling for NUFLOR® 2.3% Concentrate Solution was based on the following study.

"Pharmacokinetics of Florfenicol in Swine Following Intravenous, Oral Gavage and Drinking Water Administration" (Study No. 1270C-61-V97-369)

1. Objectives:

To determine the pharmacokinetic profile of florfenicol after single intravenous, repeated oral gavage dosing and ad libitum (5 days) drinking water administration to swine.

To determine the bioavailability of florfenicol after oral administration.

2. Study Personnel:

Study Monitor: James A. Jackson, DVM, Schering-Plough Animal Health, Elkhorn, Nebraska.

Director, Clinical Phase: Michael S. Hanna, DVM, CSRC, Inc., Oakland, Nebraska.

Director, Analytical Phase: R.A. Sams, Ph.D., The Ohio State University, College of Veterinary Medicine, Analytical Toxicology Lab, Columbus, Ohio.

3. Study Design:

a) *Experimental Animals:* A total of 17 female and castrated male crossbred pigs, approximately 10 weeks of age and weighing approximately 20 kg, were used in the study.

b) *Test Article Administration:* Animals were assigned to one of three treatment groups. Five pigs were given a single intravenous injection of 15 mg florfenicol per kg bodyweight (NUFLOR® Injectable Solution, 300 mg/mL). Seven pigs received a 15 mg/kg bodyweight dose of florfenicol via oral gavage daily for five consecutive days (florfenicol drinking water concentrate, 23 mg/mL). Five pigs received 100 ppm of florfenicol as an oral administration via the drinking water provided *ad libitum* for five consecutive days (florfenicol drinking water concentrate, 23 mg/mL).

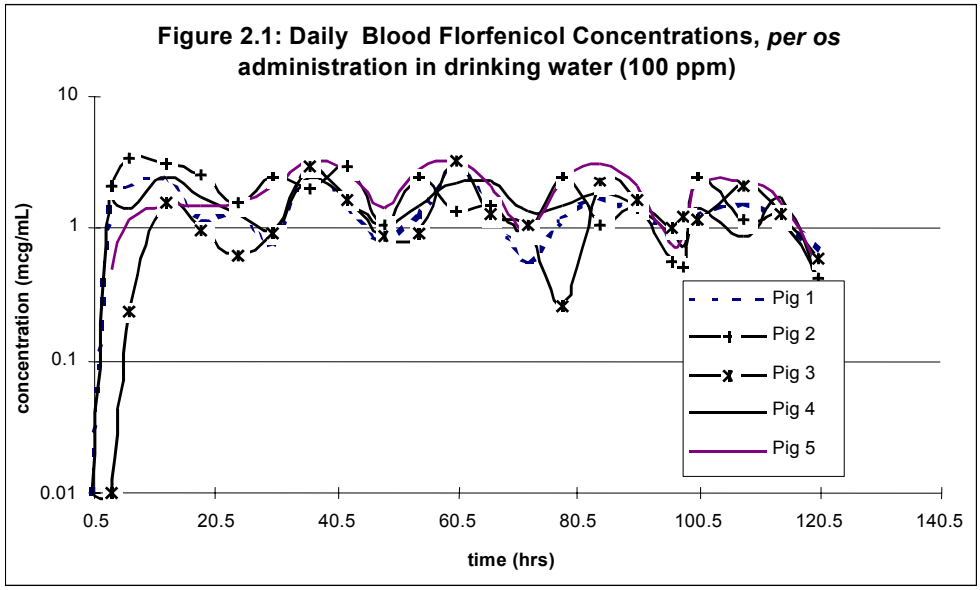
c) *Parameters Measured:* Serum concentrations of florfenicol were determined in blood samples collected at specific intervals for each treatment group. Florfenicol concentrations in serum were measured using a reversed phase high performance liquid chromatographic system with internal standardization (chloramphenicol) and ultraviolet detection.

4. Results: Mean pharmacokinetic parameters are summarized in Table 2.3 below. The serum florfenicol concentrations achieved in each individual swine following 100 ppm of florfenicol administered via the drinking water provided *ad libitum* for five consecutive days is provided in Figure 2.1.

Table 2.3. Pharmacokinetic Parameter Values of Florfenicol following IV or Gavage Dosing

Parameter	Mean Value (%CV)
Vd_{ss}[a] (L/kg)	0.95 (6)
CL_B[a] (mL/kg/min)	5.57 (11)
$T_{1/2}$[a] (hrs)	2.2 (14)
F[b] (%)	24-97

[a]parameter estimate based on intravenous data
[b]parameter range based upon a single oral gavage dose



Despite the rapid elimination seen after IV injection or oral gavage dosing, when administered in medicated drinking water, florfenicol concentrations in serum were maintained well above the targeted MIC value for the majority of the 5-day dosing interval. These results are consistent with product effectiveness when administered in drinking water in concentrations of 100 ppm over a 5-day dosing period.

Although the extent of oral drug absorption (F) tended to be variable (24 to 97% following a single oral gavage dose), florfenicol was rapidly absorbed. Its terminal elimination half-life ($T_{1/2}$) was also rapid, ranging between 2 to 3 hours. The average systemic clearance (CL_B) following IV administration was 5.6 mL/min/kg. Since the florfenicol steady state volume of distribution (VD_{SS}) closely approximates that of total

body water, peripheral tissue concentrations are expected to be similar to those concentrations observed in serum.

5. Adverse Reactions: One animal had watery diarrhea on Day 1 and 2 after IV administration. Six animals in the oral gavage group had diarrhea or loose stools. Regurgitation (two animals), coughing (two animals), and hypersalivation with open-mouth breathing (one animal) were reported following dosing via oral gavage. Three animals in the drinking water treatment group had diarrhea or loose stools at some time during the trial. One animal in the drinking water treatment group had perianal inflammation on Day 3.

6. Conclusions: Based on the pharmacokinetic data in this study, florfenicol serum concentrations will be maintained above 1 mcg/mL when administered *per os* in the drinking water for five consecutive days at concentrations of 100 ppm. Since florfenicol activity is dependent upon time above MIC, these results are consistent with product effectiveness.

d. Microbiology:

The minimum inhibitory concentration (MIC) of florfenicol was determined for isolates from diagnostic laboratory and clinical field efficacy studies conducted between 1990 and 2001 in the United States. Susceptibility testing followed the methods of the National Committee of Clinical Laboratory Standards [NCCLS. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standards. NCCLS Document M31-A (ISBN 1-56238-377-9). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087 USA, 1999]. Reference strains included *Escherichia coli* ATCC 25922 with a QC range of 2 to 8 mcg/mL and *Actinobacillus pleuropneumoniae* ATCC 27090 with a QC range of 0.25 to 1 mcg/mL. These MIC data were combined with similar data from other Schering-Plough Animal Health studies of US isolates of swine respiratory disease to provide a concise summary, which is shown in Table 2.4.

Table 2.4. MIC Values of Florfenicol Against Bacterial Isolates from Swine

Organism	Isolate Numbers	MIC_{90}* (mcg/mL)	MIC Range (mcg/mL)
Actinobacillus pleuropneumoniae	360	0.50	≤ 0.125 to 2.0
Pasteurella multocida	335	0.50	≤ 0.125 to 2.0
Salmonella choleraesuis	46	4.0	2.0 to 4.0
Streptococcus suis Type 2	203	2.0	0.5 to 2.0

*The minimum inhibitory concentration for 90% of the isolates.

3. TARGET ANIMAL SAFETY:

Data from the following Target Animal Safety study demonstrate that NUFLOR® 2.3% Concentrate Solution is safe when administered orally to swine at doses as high as 10X the recommended label dose.

"Target Animal Safety Study of SCH 25298 (florfenicol) Administered Orally via Drinking Water in Swine" (Study No. 96319)

a. Type of Study: A target animal safety study was conducted to evaluate the tolerance and effects of NUFLOR® 2.3% Oral Solution (florfenicol) when orally administered to swine via the drinking water at 1X, 3X, and 5X the clinical dose for three times the clinical duration, and at 10X the clinical dose for the clinical duration. This study was conducted in accordance with Good Laboratory Practice Regulations (21 CFR 58).

b. Study Director: Robert J. Harman, D.V.M., HTI Bio-Services, Inc., 10326 Roselle Street, San Diego, California 92121

c. Study Design:

1) *Objectives:* To determine the safety of florfenicol oral solution administered in drinking water at 400 mg/gal (1X), 1200 mg/gal (3X), and 2000 mg/gal (5X) for 15 or 16 consecutive days (3X duration) in growing swine, and to evaluate the effects of florfenicol oral solution administered in drinking water at 4000 mg/gal (10X) for 5 or 6 consecutive days in growing swine.

2) *Experimental Animals:* Forty (20 castrated males and 20 females) crossbred swine, approximately 4 months old with a weight range of 29.85 kg to 66.30 kg were used in the study. The test animals were representative of genetic stock currently in the United States that are used as finishing swine. Four male and four female pigs were randomly assigned to each of the five treatment groups.

3) *Test Article Administration:* The dosage form was a water-soluble formulation of florfenicol containing 23 mg florfenicol per mL (2.3% florfenicol activity). Water containing 400 mg florfenicol/gal, 1200 mg florfenicol/gal, or 2000 mg florfenicol/gal was provided for 16 consecutive days (Days 0-15). Water containing 4000 mg florfenicol/gal was provided for six consecutive days (Days 10-15). The control group received non-medicated water provided *ad libitum* throughout the study. The overall measured mean concentrations of florfenicol in the drinking water during the dosing period were 290, 1124, 1843 and 3565 mg/gal for the 400, 1200, 2000 and 4000 mg/gal target doses respectively. With 1X = 400 mg/gal, the measured concentrations correspond to 0.7, 2.8, 4.6 and 8.9X the clinical dose. The study duration was 37 days, including acclimation (Day -21 to -15), pre-treatment (Day -14 to -1 or Day -14 to Day 4), and treatment periods (Day 0-15 or Day 10-15).

4) *Measurements and Observations:* Clinical signs were observed daily during the entire study. Body weights were recorded once weekly from beginning of acclimation until necropsy. Feed and water intake were measured daily throughout the pre-treatment and treatment periods. All animals underwent a physical exam, and collection of specimens for hematology, coagulation, serum chemistry, urinalysis, and fecal analysis on specified study days. Gross and histopathological observations were made following necropsy of all animals on Day 15 or 16.

d. Statistical Methods: Variables measured multiple times during study were analyzed using repeated measures analysis of covariance (ANCOVA) with the average of baseline values as covariates. Histopathology variables were analyzed using analysis of variance (ANOVA) models. Randomization weight block was included in each model as a random effect.

An alpha level of 0.10 was used to determine significance of the treatment by time interaction terms in the repeated measures analysis. If the interaction term was significant, contrasts of each treatment group to control were tested at each time, also with an alpha level of 0.10. If the time by treatment interaction was not significant at the 0.10 level an alpha level of 0.10 was used to compare each treatment group to control, averaged over all time points. In the models without repeated measures, an alpha level of 0.10 was used to compare each treatment group to control.

Effects of gender were tested using an alpha level of 0.05. When significant gender effects were found, contrasts among treatments and control were conducted within each gender.

e. Results:

1) *Clinical Signs:* Test article-related constipation and anal swelling were seen in the 3X, 5X, and 10X treatment groups. The constipation in these groups was attributed in part to decreased water consumption during the medication period. Two animals in the 1X treatment group also showed signs of constipation with decreased water consumption.

2) *Body Weights:* Body weights were measured upon arrival of the pigs, and on Days 5, 10, and 14 (or 15) of the study. Weight gains were similar for the control and 1X groups. There was a decrease in weight gains for the 3X, 5X, and 10X treatment groups compared with the control group.

3) *Feed and Water Consumption:* Feed was available to swine *ad libitum*. Pen feed consumption was measured and calculated daily. Feed consumption was similar for the control and 1X groups. Test article-related decreases in feed consumption were seen in the 3X, 5X, and 10X treatment groups compared with the control group.

Test article-related decreases in water consumption were noted in all test article-treated groups (1X, 3X, 5X, and 10X) compared with the non-medicated control group. However, due to variation in water consumption the decreased water consumption in the 1X and 3X groups were considered of equivocal biological significance.

4) *Hematology and Serum Chemistry:* Blood samples for hematology and serum chemistries were collected on Days -14, -1, 5, 10, and 14 or 15. There were no test-article-related hematology changes. Increased serum sodium and chloride were seen in the 5X and 10X groups. The 10X group also had increases in serum total protein, albumin, and globulin. These changes were likely a result of the decreased water consumption and indicate mild dehydration in these animals. Increases in alanine aminotransferase (ALT) (1X, 3X, 5X, and 10X groups), calcium (3X and 5X groups), creatinine (1X and 5X groups), and lymphocytes (1X, 3X, 5X, and 10X groups) were not associated with abnormal clinical signs or pathological changes.

5) *Urine and Fecal Analysis*: Urine and feces were collected on Days -14, -1, 5, 10, and 14 or 15. Increased urine specific gravity was seen in the 10X group, and was likely related to decreased water consumption. An increased incidence of compacted feces and dark brown fecal color was seen in all treatment groups (1X, 3X, 5X, and 10X).

6) *Gross and Histopathology:* At the completion of the study, all animals were euthanized and necropsied. No test article-related gross lesions were noted.

   Organ weights of the brain, liver, ovaries, testes, and spleen, were found to be within normal limits. An increase in kidney weights was noted in the 10X group. Slight increases in absolute heart weights were noted in the 1X and 3X groups, and in liver weights for all dose groups, but the increases were not accompanied by histologic changes.

   Histopathological evaluation was performed on all tissues collected from the control and 5X group, and from the heart and liver for all groups. Other tissues collected from the 1X, 3X, and 5X groups were retained for possible further evaluation. There were no test article-related histopathological lesions observed.

f. Conclusions: NUFLOR® 2.3% Concentrate Solution can be safely administered to swine according to the recommended clinical regimen of 400 mg/gal in the drinking water for 5 consecutive days.

4. HUMAN SAFETY:

a. Toxicity:

Summaries of all toxicology studies supporting NUFLOR® 2.3% Concentrate Solution are incorporated by reference to approved NADA No. 141-063 for NUFLOR® Injectable Solution.

b. Safe Concentration of Total Residues – Determination of No Observed Effect Level (NOEL):

The determination of the NOEL supporting NUFLOR® 2.3% Concentrate Solution is incorporated by reference to approved NADA No. 141-063 for NUFLOR® Injectable Solution.

c. Safe Concentration of Total Residues – Calculation of the Acceptable Daily Intake (ADI) and the Safe Concentration (SC):

Assignment of safe concentration (SC) for NUFLOR® 2.3% Concentrate Solution is incorporated by reference to approved NADA No. 141-063 for NUFLOR® Injectable Solution.

d. Total Residue Depletion and Metabolism Study:

"SCH 25298 (Florfenicol): A Total Residue Depletion Study in Swine Following Oral Administration of ^{14}C-SCH 25298" (Study No. 96618, Report No. P-6853)

1. Study Director/Investigators:

Louis S. Crouch, Ph.D., Schering-Plough Research Institute, P. O. Box 32, 144 Route 94 South, Lafayette, N. J. 07848

In-Life Testing Facility: Charles Heird, Ph.D., Southwest Bio-Labs (SBL), 401 N. 17th St., Suite 11, Las Cruces, NM 88005

Analytical Facilities:
Louis Crouch, Ph.D., Schering-Plough Research Institute, P. O. Box 32, 144 Route 94 South, Lafayette, N. J. 07848

Lynda Farthing, B.S., EN-CAS Analytical Laboratories, 2354 Farrington Point Drive, Winston-Salem, NC 27107

Note: Treatment Groups I-III were allocated to a pilot study. The results of that pilot are not considered pivotal to the NUFLOR® 2.3% Concentrate Solution approval and are not reported here.

2. Animals: 19 swine (10 male and 9 female) 90 days old and weighing 38-48 kg.

3. Route of Drug Administration and Time/Duration of Dosing: Animals were dosed orally once daily for five consecutive days with 20 mg of ^{14}C-SCH 25298/kg body weight. Animals were assigned to one of six sacrifice times. A single animal served as an untreated control.

4. Radioisotope: ^{14}C-Florfenicol (SCH 25298) was universally labeled in the benzene ring. Radiochemical purity ranged from 97% to 100% by HPLC and TLC analyses for the dose compounds. The specific activity of the dose compounds was 0.4085 μCi/mg which corresponds to 907 dpm/μg florfenicol for Groups IV-V and 1.2741 μCi/mg which corresponds to 2830 dpm/μg florfenicol for Groups VI-IX.

5. Metabolism of ^{14}C-Florfenicol in Swine: At sacrifice time points of 3, 6, 9, 12, 15 and 19 days post final dose the following edible tissues were collected: liver, kidney, muscle, skin with intact fat and fat alone. Combustion and quantitation for ^{14}C-content by liquid scintillation analysis afforded the results as shown in Table 4.1.

   A majority of the radioactive dose was in the urine (57%– 70%) and feces (17%-22%) as shown in Table 3. At the last time point (day 19), the highest concentrations of ^{14}C-florfenicol-equivalent residues were found in the liver tissue. Total radiolabeled residues in the edible tissues are summarized in Table 4.2.

   The residue present in liver, kidney, muscle and skin with intact fat and fat alone was predominately non-extractable (bound) residue from which florfenicol amine was released by strong acid hydrolysis. The bound residue (percent of total radioactive residue) ranged from 94% to 97% for liver, 88% to 91% for kidney, 56% to 89% for muscle, 70% to 78% for skin with intact fat and 65% to 76% for fat alone.

   Details of metabolite distribution in liver and muscle tissues as determined by HPLC radio-chromatography are shown in Tables 4.3 and 4.4**,** respectively.

Table 4.1. Recovery of Administered Radioactivity in Segment II

		Feces		Urine		Excreta		Tissues	Cage Wash	Total
Group	**Days**	**Mean%**	**SD**	**Mean %**	**SD**	**Mean %**	**SD**	**Mean %**	**Mean %**	**Mean %**
IV	3	20.98	5.89	70.45	12.59	91.43	3.02	0.41	0.48	92.32
V	6	22.35	5.89	62.90	3.46	85.25	7.10	0.28	0.23	85.76
VI	9	17.14	2.52	70.66	3.46	87.80	5.41	0.17	0.12	88.09
VII	12	17.64	5.06	62.62	5.43	80.26	7.87	0.13	0.12	80.51
VIII	15	22.14	1.82	67.50	5.31	89.64	6.74	0.09	0.06	89.79
IX	19	21.72	3.94	57.91	18.15	79.63	21.98	0.06	0.18	79.87

Table 4.2. Total Radioactive Residues in Tissues: STUDY NUMBER 96618		
LIVER		
Days	mean-all	SD-all
3 (IV)	15.355	1.289
6 (V)	10.029	1.277
9 (VI)	5.936	1.044
12 (VII)	4.273	0.938
15 (VIII)	3.078	0.342
19 (IX)	2.155	0.162
KIDNEY		
Days	mean-all	SD-all
3 (IV)	4.929	0.340
6 (V)	3.096	0.142
9 (VI)	2.021	0.150
12 (VII)	1.619	0.259
15 (VIII)	0.954	0.032
19 (IX)	0.540	0.020
MUSCLE		
Days	mean-all	SD-all
3 (IV)	0.574	0.015
6 (V)	0.559	0.117
9 (VI)	0.398	0.082
12 (VII)	0.371	0.111
15 (VIII)	0.279	0.012
19 (IX)	0.229	0.012
SKIN		
Days	mean-all	SD-all
3 (IV)	0.471	0.063
6 (V)	0.553	0.041
9 (VI)	0.283	0.102
12 (VII)	0.239	0.109
15 (VIII)	0.177	0.122
19 (IX)	0.160	0.029
FAT		
Days	mean-all	SD-all
3 (IV)	0.223	0.009
6 (V)	0.172	0.025
9 (VI)	0.083	0.026
12 (VII)	0.046	0.036
15 (VIII)	0.030	0.016
19 (IX)	0.016	0.012
* Values below Limit of Detection		

Table 4.3. HPLC Analysis of Methanol-Extractable Residues: Liver
STUDY NUMBER 96618

Post-Final Dose (Group)	Portion of Eluted Radioactivity	
3 Days (IV)	Mean	SD
Unknown 1	3.5%	2.8%
Florfenicol Amine[a]	13.4%	4.5%
Florfenicol Oxamic Acid	34.4%	13.8%
Florfenicol Alcohol	10.2%	2.1%
Unknown 5	12.7%	4.0%
Florfenicol	3.3%	2.7%
Undefined[b]	22.6%	10.8%
6 Days (V)	Mean	SD
Unknown 1	1.7%	2.0%
Florfenicol Amine	10.2%	7.5%
Florfenicol Oxamic Acid	40.9%	13.7%
Florfenicol Alcohol	11.9%	1.2%
Unknown 5	12.9%	3.6%
Florfenicol	2.2%	2.2%
Undefined	20.3%	14.7%
9 Days (VI)	Mean	SD
Unknown 1	4.7%	0.5%
Florfenicol Amine	11.4%	1.4%
Florfenicol Oxamic Acid	32.4%	3.8%
Florfenicol Alcohol	20.0%	4.2%
Unknown 5	12.4%	0.5%
Florfenicol	1.0%	0.9%
Undefined	23.8%	7.5%
12 Days (VII)	Mean	SD
Unknown 1	4.9%	1.8%
Florfenicol Amine	14.1%	5.3%
Florfenicol Oxamic Acid	28.1%	3.4%
Florfenicol Alcohol	10.1%	2.6%
Unknown 5	11.3%	1.7%
Florfenicol	2.4%	0.4%
Undefined	29.1%	9.7%

a: florfenicol amine not resulting from acid hydrolysis
b: sum of eluted radioactivity not corresponding to florfenicol or indicated metabolites

Table 4.4. HPLC Analysis of Methanol-Extractable Residues: Muscle STUDY NUMBER 96618		
Post-Final Dose (Group)	**Portion of Eluted Radioactivity**	
3 Days (IV)	Mean	SD
Unknown 1	5.0%	2.4%
Florfenicol Amine[a]	17.0%	10.6%
Florfenicol Oxamic Acid	29.2%	16.2%
Florfenicol Alcohol	6.3%	0.8%
Unknown 5	3.6%	1.1%
Florfenicol	9.1%	3.4%
Undefined[b]	29.8%	8.5%
6 Days (V)	Mean	SD
Unknown 1	6.4%	NA[d]
Florfenicol Amine	4.3%	NA
Florfenicol Oxamic Acid	51.3%	NA
Florfenicol Alcohol	4.8%	NA
Unknown 5	1.3%	NA
Florfenicol	5.4%	NA
Undefined	26.7%	NA
9 Days (VI)	Mean	SD
Unknown 1	11.0%	1.5%
Florfenicol Amine	4.7%	1.5%
Florfenicol Oxamic Acid	45.6%	8.2%
Florfenicol Alcohol	3.4%	1.1%
Unknown 5	4.0%	1.3%
Florfenicol	3.7%	1.3%
Undefined	29.1%	9.2%
12 Days (VII)	Mean	SD
Unknown 1	14.5%	2.7%
Florfenicol Amine	5.5%	1.1%
Florfenicol Oxamic Acid	40.8%	8.8%
Florfenicol Alcohol	4.8%	0.3%
Unknown 5	2.3%	1.2%
Florfenicol	5.3%	1.4%
Undefined	26.7%	9.4%
a: florfenicol amine not resulting from acid hydrolysis b: sum of eluted radioactivity not corresponding to florfenicol or indicated metabolites c: not quantified, insufficient radioactivity in extract for analysis d: not applicable		

e. Comparative Metabolism Studies:

Metabolism studies conducted with florfenicol in the rat are incorporated by reference to approved NADA 141-063 for NUFLOR® Injectable Solution.

The same florfenicol metabolites were identified in the swine and the rat. The major metabolite in swine, rats and cattle was florfenicol amine. A number of minor metabolites were also present. The metabolic profiles in the rat and the swine were qualitatively similar. The similarities of the metabolic profiles in the rat and the swine demonstrate that the residues present in the edible tissues of swine have been adequately characterized toxicologically.

f. Assignment of the Tolerance:

Based on the depletion characteristics of the total radioactive residues in the edible tissues, the liver is determined to be the target tissue. Florfenicol amine is assigned as the marker residue. Using the validated assay for florfenicol amine residues in the edible tissues of swine, the following marker residue to total residue ratios were determined (Table 4.5).

Table 4.5: Total Radioactive Residues (μg ^{14}C-Florfenicol Equivalent/g) and Marker Residue Concentrations (μg Florfenicol Amine/g) in Swine Tissues (ppm ± standard deviation) STUDY NUMBER 96618

Group	Withdrawal Time	Liver		Muscle	
		Total Residue	Marker Residue	Total Residue	Marker Residue
IV	3 days	15.355 ± 1.289	6.71 ± 0.356	0.574 ± 0.015	0.161 ± 0.0475
V	6 days	10.029 ± 1.277	4.10 ± 1.30	0.559 ± 0.117	0.173 ± 0.0825
VI	9 days	5.936 ± 1.044	2.56 ± 0.517	0.398 ± 0.082	0.161 ± 0.0415
VII	12 days	4.273 ± 0.938	1.72 ± 0.151	0.371 ± 0.111	0.153 ± 0.0491

When total residues of florfenicol in the target tissue (liver) have depleted to the safe concentration of 6 ppm, mean residues of florfenicol amine (marker residue) in liver were 2.5 ppm as measured with the determinative HPLC assay. Therefore 2.5 ppm is established as the tolerance for marker residue, florfenicol amine, in the target tissue, swine liver. Based on the residue data contained in this NADA and NADA 141-063 (NUFLOR® Injectable Solution), a tolerance of 0.2 ppm is established for florfenicol amine in swine muscle.

g. Withdrawal Time:

Study to Establish Withdrawal Time: "SCH 25298 (Florfenicol): A Final Residue Depletion Study in Swine Following Oral Administration of SCH 25298" (Study No. 97418, Report No. P-6781)

1. Study Director/Investigators:

   Alice M. Bova, B.S. (1/19/98 to 2/25/98), William F. Feely, M.S. (2/25/98 to 7/21/98), Schering-Plough Research Institute, P. O. Box 32, 144 Route 94 South, Lafayette, N. J. 07848

   In-Life Testing Facility: Karol Bice-Godwin, D.V.M., HTI Bio-Services, Inc., 26578 Old Julian Highway, Santa Ysabel, California 92070

   Analytical Facility: Lynda Farthing, B.S., EN-CAS Analytical Laboratories, 2359 Farrington Point Drive, Winston-Salem, NC 27107

2. Animals: Forty four cross-bred swine (22 males and 22 females), 3 months old and weighing 32 to 59 kg: 42 (M, F) test, 2 (M, F) control.

3. Route of Drug Administration and Time/Duration of Dosing: Animals were assigned to one of seven sacrifice times. Untreated control animals were sacrificed prior to the slaughter of the first treatment group.

4. Test Article: Florfenicol (2.3% Concentrate Solution) administered at the intended final concentration of 400 mg/gal in drinking water.

5. Edible Tissue Residue Concentrations: At sacrifice time points of 1, 3, 6, 9, 12, 15 and 21 days post final dose the following edible tissues were collected: liver, kidney, muscle, and skin with intact fat. Samples were assayed using the validated determinative HPLC method and results are shown in Table 4.6.

Table 4.6. Marker Residue Concentrations in the Edible Tissues STUDY NUMBER 97418

Group #	Sac Time		Liver (ppm)	Kidney (ppm)	Muscle (ppm)	Skin w/Fat (ppm)
I	-1 Day		0.0759 a	0.0148 a	0.000 a	0.0184 a
II	1 Day	Mean:	9.86	3.27	0.527	0.884
		Std. Dev.	1.65	0.836	0.240	0.226
III	3 Days	Mean:	5.35	1.16	0.181	0.333
		Std. Dev.	0.743	0.188	0.021	0.046
IV	6 Days	Mean:	3.31	0.672	0.177	0.289
		Std. Dev.	0.688	0.055	0.011	0.092
V	9 days	Mean:	2.41	0.385	0.092	0.218
		Std. Dev.	0.555	0.104	0.046	0.053
VI	12 Days	Mean:	1.57	0.250	0.055	0.237
		Std. Dev.	0.328	0.051	0.060	0.100
VII	15 Days	Mean:	1.51	0.198	0.030	0.142
		Std. Dev.	0.209	0.020	0.047	0.057
VIII	21 Days	Mean:	0.674	0.123	0.000	0.122
		Std. Dev.	0.095	0.052	0.000	0.070

a -The reported control value represents an average of 5-6 analyses (reinjection values were not included in the average).
b -The original value (6.67 ppm) and duplicate reanalysis values (7.25 ppm and 7.21 ppm) are averaged and reported.
c -The original analysis yielded 0.666 ppm. This sample was reanalyzed in duplicate and the average of the duplicate reanalyses is reported.
d -The original value (0.828 ppm) and duplicate reanalysis values (0.811 ppm and 0.795 ppm) are averaged and reported.
e -The original value (0.197 ppm) and duplicate reanalysis values (0.218 ppm and 0.211 ppm) are averaged and reported.

6. Calculation of Withdrawal Period: On the basis of a tolerance of 2.5 ppm in liver, the withdrawal period for NUFLOR® 2.3% Concentrate Solution administered orally via the drinking water for 5 consecutive days was calculated using the Agency's statistical tolerance limit method (99% tolerance limit with a 95% confidence interval method). The withdrawal period calculated was 16 days.

h. Regulatory Method for Residues:

1. Determinative Assay Procedure:

The HPLC determinative procedure approved under NADA 141-063 for bovine tissues was successfully validated according to the Agency's guidelines for the quantitation of florfenicol amine (marker residue) residues in the edible tissues (liver, kidney, muscle, skin with attached fat) of swine receiving NUFLOR® 2.3% Concentrate Solution (INAD 009-750).

The determinative assay for the marker residue, florfenicol amine, in the edible tissues, is a high performance liquid chromatography (HPLC) method that provides acceptable sensitivity, specificity, accuracy and precision for the routine monitoring of florfenicol residues in swine. Florfenicol residues (and those of related metabolites) are converted to the marker residue, florfenicol amine, by acid-catalyzed hydrolysis. The hydrolysate is washed with ethyl acetate, centrifuged, and pH adjusted to 12.5 or greater. The pH-adjusted solution is poured through a solid phase extraction column and eluted with ethyl acetate. The ethyl acetate eluates are combined and evaporated to dryness. The dried residue is dissolved in buffer (10 mMolar potassium phosphate), pH 4.0, containing 1% (v/v) acetonitrile, filtered and analyzed by HPLC.

2. Confirmatory Procedure:

The LC/MS/MS confirmatory procedure submitted under NADA 141-063 for bovine tissues was successfully validated according to the Agency's guidelines for the confirmation of florfenicol amine (marker residue) residues in the livers (target tissue) of swine receiving NUFLOR® 2.3% Concentrate Solution (INAD 009750).

The confirmatory assay for florfenicol amine in the target tissue, liver, utilizes a liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) methodology applied to the purified solution obtained from the determinative method work-up. Daughter ion (*m/z* 248) mass spectrometry yielded confirmatory ions at *m/z* 130 (base peak), and *m/z* 151, *m/z* 197 and *m/z* 230.

3. Results of the Method Trial:

Interlaboratory Method Trial results for the determinative and confirmatory assays of florfenicol amine in cattle liver are incorporated by reference to approved NADA 141-063. Since the procedures are similar it was not necessary to repeat the interlaboratory trial.

4. Display of the Method:

The validated regulatory method for detection and confirmation of residues of florfenicol is available from the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855.

i. User Safety Concerns:

Florfenicol, with an oral LD50 in rats of >2000 mg/kg is classified as slightly hazardous via the oral route. Dermal exposure of 0.5 cc of florfenicol powder (moistened with saline) was shown to be non-irritating to rabbit skin. Ocular exposure of 0.1 cc of florfenicol powder in the rabbit eye was considered essentially non-irritating with slight conjunctival redness at 24 hours post-injection.

User safety concerns associated with direct contact have been satisfactorily addressed by establishing label warnings. In addition, a toll-free telephone number will be available on the label to inform users of where they can obtain additional information concerning user safety relative to the MSDS and to report adverse events.

5. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR Part 514 of the implementing regulations. The data demonstrate that NUFLOR® 2.3% Concentrate Solution is safe and effective for the treatment of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Salmonella choleraesuis* and *Streptococcus suis* Type 2.

Labeling restricts this drug to use by or on order of a licensed veterinarian. This decision was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat swine respiratory disease, (b) restricting this drug to use by or on order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues, and (c) the rate of emergence of florfenicol-resistant organisms may be reduced by the involvement of veterinarians in product use.

Based on toxicology studies, the acceptable daily intake (ADI) for total florfenicol-related residues is 10 micrograms per kilogram body weight per day. Based on metabolism studies in swine, a tolerance of 2.5 ppm for the marker residue, florfenicol amine, has been established in swine liver, the target tissue. The tolerance refers to the residue measured by the regulatory method described herein.

A pre-slaughter withdrawal period of 16 days was calculated from a residue depletion study of florfenicol residues in swine, following the oral administration of medicated water containing NUFLOR® 2.3% Concentrate Solution at a dose rate of 400 mg florfenicol/gallon of drinking water for 5 consecutive days. The withdrawal was based on a statistical analysis of the depletion data, using an upper tolerance limit containing 99 percent of the population with a 95 percent confidence limit.

Under section 512(c)(2)(F)(ii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval. The application contains investigations conducted or sponsored by the applicant that demonstrate animal safety and substantial evidence of effectiveness.

No patents were submitted with this application.

6. ATTACHMENTS:

Facsimile Labeling is attached as indicated below.

A. NUFLOR® 2.3% Concentrate Solution - Bottle Label
B. NUFLOR® 2.3% Concentrate Solution - Package Insert

Applicable labels may be obtained by writing to the following:

Freedom of Information Staff (HFI-35)
Food and Drug Administration, Room 12A16
5600 Fishers Lane
Rockville, Maryland 20857