Date of Approval: May 31, 1996

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-063

NUFLOR®

Injectable Solution

Cattle

For the treatment of bovine respiratory disease (BRD) associated with *Pasteurella haemolytica, Pasteurella multocida,* and *Haemophilus somnu*

Sponsored by:

Schering-Plough Animal Health

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I. GENERAL INFORMATION

A. File Number

NADA 141-063

B. Sponsor

Schering-Plough Animal Health Corporation
P.O. Box 529
Kenilworth, New Jersey 07033

C. Proprietary Name

Nuflor® Injectable Solution

D. Drug Product Established Name

Florfenicol

E. Dosage Form

Injectable solution

F. Amount of Active Ingredient

300 mg/ml

G. How Supplied

NUFLOR® Injectable Solution is a sterile non-aqueous solution available in 100-, 250-, and 500-mL glass vials. Each milliliter contains 300 mg florfenicol.

NUFLOR® injectable Solution should be stored at controlled room temperature (15 to 30 °C or 59 to 86 °F). Protect from freezing.

H. Dispensing Status

This is a prescription product and will include the caution statement as follows: Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian.

I. Dosage Regimen

NUFLOR® Injectable Solution should be administered by intramuscular injection to cattle at a dose of 20 mg/kg body weight (3 mL/100 lb). A second dose should be administered 48 hours later. Do not inject more than 10 mL at each site. The injection should be given only in the neck musculature

J. Route of Administration

Intramuscular injection in the neck

K. Species/Class

Cattle

L. Indication

NUFLOR® Injectable Solution is indicated for the treatment of bovine respiratory disease (BRD) associated with *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*

II. EFFECTIVENESS

Data from the following dose titration, dose confirmation and clinical field trials demonstrate that NUFLOR® Injectable Solution is effective for the treatment of bovine respiratory disease when administered intramuscularly, twice, at 20 mg/kg body weight (bw) with a 48-hour interval. The clinical trials were conducted in multiple geographic locations

A. Dosage Characterization

1. Type of Study: Dose titration study in cattle with naturally-occurring bovine respiratory disease (BRD).

2. Investigator: David T. Bechtol, D.V.M.
   Agri Research Center, Inc.
   Canyon, Texas 79015

3. General Design:

   a. Purpose: To establish the effective dose of florfenicol administered twice, 48-hours apart, in the treatment of naturally-occurring BRD.

   b. Animals: Forty-eight (48) head of feedlot cattle (12 per group) ranging in age from 5 to 7 months. The initial mean weight was approximately 160 kg.

   c. Controls: Negative control was a non-drug placebo vehicle administered intramuscularly at 0.5 mL/10 kg body weight.

   d. Diagnosis: The diagnosis of BRD was based on presence of acute clinical signs of pneumonia with an elevated rectal temperature (104.0 °F or greater) and respiratory rate (40 per minute or greater) and an illness index scored "moderately ill" or worse (illness index was based on presence of depression, coughing, inappetance, and overall clinical impression). Pretreatment naso-pharyngeal swabs were taken for bacterial examination.

   e. Dosage form: The dosage form was an injectable solution containing 300 mg florfenicol per mL.

   f. Route of administration: Intramuscular injection

g. Doses: The 10, 20 and 30 mg/kg groups were dosed twice with a 48-hour interval between doses.

h. Test Duration: 12 days

i. Pertinent Parameters Measured: Mortality, rectal temperature, and clinical illness index were measured and recorded daily from Day 0 to the day of necropsy. Lung consolidation was recorded and lung swabs taken for bacterial examination at necropsy upon natural death or sacrifice 7 days after end of treatment period.

4. Results:

Florfenicol prevented mortality from respiratory disease at all dose levels tested. Mortality in the non-medicated control group was 25% (3/12). As summarized in Table 4.1, the 20 mg/kg dose group showed the greatest drop in rectal temperature and the lowest lung consolidation score at necropsy among the 3 florfenicol dose groups.

Pasteurella haemolytica was isolated from 39 (81%) of the 48 pre-treatment nasopharyngeal swabs of the cattle used in this study. *Pasteurella multocida* was found in 16 (33%) of the same 48 swabs. *Pasteurella haemolytica* was isolated from 8 of the 60 post-treatment lung swabs. All pathogens (*Pasteurella*) isolated from either naso-pharyngeal or lung swabs were sensitive to florfenicol.

Table 4.1. Rectal temperature reduction and percent lung consolidation following treatment with florfenicol injectable solution in cattle with naturally - occurring BRD

	Rectal temperature (°F) Reduction since Day 0		**Percent Lung Consolidation**
Dose (mg/kg)	**1-day post-Tx**	**7 days post-Tx**	**7 days post-Tx (or at death)**
0	1.7	1.5	17.9
10	1.7	1.9	11.7
20	2.4	2.4	7.9
30	2.1	1.0	12.1

5. Conclusions:

Under the conditions of this study, the dose of 20 mg florfenicol/kg bw was shown to be an effective dose for the treatment of bovine respiratory disease when administered twice, 48 hours apart, by the intramuscular route.

6. Adverse Reactions: There were no adverse reactions in any treatment group.

B. Dose Confirmation

1. Type of Study: Field trial in cattle with naturally occurring bovine Respiratory disease (BRD)

2. Investigator: M.I. Wray, Ph.D.

   Horton Feedlot and Research Center
   Wellington, Colorado 80549

3. General Design:

   a. Purpose: To confirm the therapeutic efficacy of florfenicol administered twice, 48 hours apart, by the intramuscular route at the dose of 20 mg/kg body weight, for the treatment of naturally occurring bovine respiratory disease complex.

   b. Animals: Fifty (50) mixed-breed beef calves (25 per group) approximately 6 months old with an approximate initial mean weight of 190 kg.

   c. Control: Control was a non-drug placebo (normal saline) administered twice, 48 hours apart.

   d. Diagnosis: The diagnosis of BRD was based on acute clinical signs of pneumonia with an elevated rectal temperature (104 °F or greater) and respiratory rate (40 per minute or greater). Pretrial nasal swabs were taken for bacterial examination.

   e. Dosage Form: The dosage form was an injectable solution containing 300 mg florfenicol per mL.

   f. Route of administration: Intramuscular injection

   g. Doses: Negative control (0 mg/kg) and 20 mg/kg administered twice with a 48 hour interval.

   h. Test Duration: 29 days

   i. Pertinent Parameters Measured:

      Mortality from Day 0 to Day 28, and overall treatment on Day 28 were the primary variables for assessing efficacy.

4. Results:

   A significant reduction in mortality was observed in the florfenicol-treated group. Mortality in the florfenicol-treated group was 4% (1/25), whereas mortality in the non-medicated control group was 48% (12/25). Also, as

summarized in Table 4.2, significantly more treatment successes were observed in the florfenicol group (72%) than in the control group (24%).

Pasteurella haemolytica was isolated from 25 (50%) of the 50 pre-treatment nasopharyngeal swabs in this study. *Pasteurella multocida* was found in 6 (12%) of the same 50 swabs. *Haemophilus somnus* was isolated in only one swab.

All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol.

Table 4.2. Percent initial treatment success, relapse, and overall treatment success in cattle with naturally-occurring respiratory disease treated with intramuscular florfenicol in 2 doses 48 hours apart

Dose (mg/kg)	Initial Treatment Success (Days 4 to 10)	Relapse (Days 11 to 28)	Overall Treatment Success (Day 28)
0	32 (8/25)	8 (2/25)	24 (6/25)
20	84 (21/25)	12 (3/25)	72 (18/25)

5. Statistical Analysis:

   For qualitative variables, the exact p-values of the Wilcoxon Midrank test were computed using methods suggested by Mehta, et. Al. (*Biometrics* 40:819-825; 1984), which provided a one-tailed test for the equivalence of the florfenicol group against placebo with respect to the ordered categorical responses. For mortality the Fisher's Exact Test was used. All the analyses were performed separately for each observation day. The results of all statistical tests were declared significant at the alpha = 0.05 level.

6. Conclusion:

   Under the conditions of this study, florfenicol administered twice intramuscularly, 48 hours apart, at a dose of 20 mg/kg body weight, was a safe and effective treatment of bovine respiratory disease.

7. Adverse Reactions:

   There were no adverse reactions in either treatment group.

C. Field Investigations

1. Type of Study: Field trials were conducted at 4 locations in cattle with spontaneously occurring bovine respiratory disease (BRD).

2. Investigators:

M.I. Wray, Ph.D.
Horton Feedlot and Research Center
Wellington, Colorado 80549

J.C. Johnson, DVM
Schering-Plough Animal Health
Elkhorn Research Center
Elkhorn, Nebraska 68022

E.G. Johnson, DVM
Johnson Research Center
Parma, Idaho 83660

D. Bechtol, DVM
Agri Research Center
Canyon, Texas 79015

3. General Design:

a. Purpose: The efficacy of florfenicol, administered by intramuscular injection at a dose of 20 mg/kg body weight, repeated at 48 hr, for the treatment of naturally-occurring BRD was tested in positively-controlled field investigations at several locations.

b. Animals: One hundred five (105) mixed-breed beef calves that were approximately 6 months old and weighed an average of 193 kg were used at each trial location. At each trial; site, 70 calves were injected with florfenicol and 35 with the control product.

c. Control: The control product was an approved oxytetracycline Injectable solution administered for 4 consecutive days, at the dose of 10 mg oxytetracycline per kg body weight (4.5 mg/lb).

d. Diagnosis: The diagnosis of BRD was based on acute clinical signs of pneumonia with an elevated rectal temperature (104 °F or greater) and respiratory rate (40 per minute or greater). Pretreatment nasal swabs were taken for bacterial examination.

e. Dosage Form: The test article was an injectable solution containing 300 mg florfenicol per mL.

f. Test article: Florfenicol injectable solution was administered twice by intramuscular injection at a 48-hour interval at a dose of 20 mg/kg body weight.

g. Test Duration: 29 days

h. Parameters Measured: Mortality from Day 0 to Day 28, and overall treatment success on Day 28 were the primary variables for assessing efficacy.

4. Results:

Day 28 treatment success was significantly greater in the florfenicol-treated groups than in the oxytetracycline groups at all locations. Initial treatment success, relapse, and overall treatment success are summarized in Table 4.3.

Mortality was significantly lower in the florfenicol groups at the Nebraska and Texas trial locations. No significant difference in mortality was noted at the Colorado or Idaho trial locations. Percent mortality is summarized in Table 4.4.

Table 4.3. Percent initial treatment success, relapse, and overall treatment success in cattle with naturally-occurring respiratory disease treated with intramuscular florfenicol in 2 doses 48 hours apart

	Dose (mg/kg)	**Initial Treatment Success (Days 4 to 10)**	**Relapse (Days 11 to 28)**	**Overall Treatment Success (Day 28)**
Colorado	10 OTC[a]	49 (17/35)	0 (0/35)	49 (17/35)
Colorado	20 FFC[b]	74 (51/69)	0 (0/69)	71 (51/69)
Nebraska	10 OTC[a]	31 (11/35)	0 (0/35)	31 (11/35)
Nebraska	20 FFC[b]	91 (63/69)	12 (8/69)	80 (55/69)
Idaho	10 OTC[a]	31 (11/35)	0 (0/35)	31 (11/35)
Idaho	20 FFC[b]	71 (50/70)	9 (6/70)	63 (44/70)
Texas	10 OTC[a]	24 (8/33)	0 (0/33)	24 (8/33)
Texas	20 FFC[b]	83 (58/70)	9 (6/70)	74 (52/70)

[a](OTC) oxytetracycline; [b](FFC) florfenicol

Table 4.4. Percent mortality in cattle with naturally-occurring respiratory disease treated with intramuscular florfenicol in 2 doses 48 hours apart

Dose (mg/kg)	Colorado (% Mortality)	Nebraska (% Mortality)	Idaho (% Mortality)	Texas (% Mortality)
10 OTC[a]	9 (3/35)	17 (6/35)	0 (0/35)	15 (5/33)
20 FFC[b]	1 (1/69)	0 (0/69)	0 (0/70)	1 (1/70)

[a](OTC) oxytetracycline; [b](FFC) florfenicol

5. Microbiology

In the Colorado field trial, *Pasteurella haemolytica* was isolated from 81 (77%) of the 105 pre-treatment naso-pharyngeal swabs in this study. *Pasteurella multocida* was found in 11 (10%) of the same 105 swabs. All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol with an MIC_{90} of 2 and 1 µg/mL for *P. haemolytica* and *P. multocida*, respectively.

In the Nebraska field trial, *Pasteurella haemolytica* was isolated from 75 (71%) of the 105 pre-treatment naso-pharyngeal swabs of the cattle used in this study. *Pasteurella multocida* was found in 3 (3%) of the same 105 swabs. All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol with an MIC_{90} of 2 mcg/mL for *P. haemolytica*. The MIC_{90} values for the three *P. multocida* isolates were 1 |J.g/mL or less.

In the Idaho field trial, *Pasteurella haemolytica* was isolated from 40 (38%) of the 105 pre-treatment naso-pharyngeal swabs of the cattle used in this study. *Pasteurella multocida* was found in 50 (48%) of the same 105 swabs. All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol.

In the Texas field trial, *Pasteurella haemolytica* was isolated from 79 (75%) of the 105 pre-treatment naso-pharyngeal swabs of the cattle used in this study. *Pasteurella multocida* was found in 9 (9%) of the same 105 swabs. *Haemophilus somnus* was isolated in 3 of those swabs (3%). All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol. MIC_{90} values for florfenicol were 4 µg/mL for *P. haemolytica*, and 2 µg/mL for *P. multocida*.

6. Statistical Analysis:

For qualitative variables, the exact p-values of the Wilcoxon Midrank test were computed using methods suggested by Mehta, et. al. (in Biometrics, 40 (3), September 1984, 819-825), which provided a one-tailed test for the equivalence of the florfenicol group against positive control with respect to the ordered categorical responses. For mortality the Fisher's Exact Test was used.

All the above analyses were performed separately for each observation day.

The results of all statistical tests were declared significant at the alpha = 0.05 level.

7. Conclusion:

   Under the conditions of this study, florfenicol administered twice intramuscularly, 48 hours apart, at a dose of 20 mg/kg body weight, was a safe and effective treatment of bovine respiratory disease.

8. Adverse Reactions:

   There were no adverse reactions in either treatment group at any of the four field trial locations.

D. Field Investigation – *Haemophilus somnus*

1. Type of Study: Field trial in cattle with naturally-occurring bovine respiratory disease (BRD)

2. Investigator: Dr. Kee Jim
   Feedlot Health Management Services
   Postal Bag Service #5
   Okotoks, Alberta TOL ITO, Canada

3. General Design:

   a. Purpose: To confirm the therapeutic efficacy of florfenicol administered twice, 48 hours apart, by the intramuscular route at the dose of 20 mg/kg body weight, for the treatment of naturally-occurring bovine respiratory disease associated with *Haemophilus somnus*.

   b. Animals: One hundred twenty-five (125) auction-derived, 5 to 10 month old, mixed-breed beef steers, weighing an average of 278 kg, were used in the study. Eighty-four (84) steers were treated with NUFLOR Injectable Solution and 41 were treated with a control product.

   c. Control: The control product was saline administered according to the NUFLOR Injectable Solution dosage regimen.

   d. Diagnosis: Diagnosis of BRD and subsequent entrance into the study was based on rectal temperature ≥ 105 °F for two consecutive days and the absence of clinical signs referable to organ systems other the respiratory system.
   Exposure to *Haemophilus somnus* was confirmed at study completion based on histopathologic lesions characteristic of *H. somnus* infection, and the results of microbiological culture of blood, nasal swabs, and transtracheal washes.

   e. Dosage Form: The dosage form was an injectable solution containing 300 mg florfenicol per mL.

   f. Route of administration: Intramuscular.

g. Doses: Florfenicol injectable solution and the placebo were administered twice with a 48-hour interval at a dose of 20 mg/kg body weight.

h. Test Duration: 15 days

i. Pertinent Parameters Measured: Mortality was monitored daily from Day 0 to Day 15, rectal temperature was measured and recorded from Day -1 to Day 4 and on Day 15. Relapses were recorded from day 4 to day 15.

An animal was considered a treatment success if it met the entrance criteria, was subsequently treated, and did not relapse.

4. Results:

Microbiology: *Haemophilus somnus* was isolated in 12% (10/84) of the nasal swabs and/or transtracheal washes in the NUFLOR® Injectable Solution group and in 7% (3/41) of the controls. *Pasteurella haemolytica* was present in 26% (22/84) and 29% (12/41) of the nasal swabs and/or transtracheal washes from the NUFLOR Injectable Solution and saline treatment groups, respectively. *Pasteurella multocida* was isolated in 29% (24/84) of the NUFLOR® Injectable Solution group and 34% (14/41) of the saline group.

Haemophilus somnus exposure: Based on lung histopathology and positive *H. somnus* cultures from nasal swabs and/or transtracheal washes, 12% (10/84) of the calves in the florfenicol treatment group and 15% (6/41) of the calves in the control group were considered exposed to *H. somnus.*

Mortality: Overall study mortality was 1.2% (1/84) in the florfenicol treatment group and 34% (14/41) in the control group. In regard to the 16 animals considered exposed to *H. somnus*, there were no mortalities in the florfenicol-treated animals and 83% (5/6) mortality in control animals.

Treatment success: As summarized in Table 4.5, there were significantly more overall treatment successes in the florfenicol treatment group (62%) than in the control group (12%). In regard to animals considered exposed to *H. somnus*, 7 of the 10 florfenicol-treated animals were treatment successes, and 1 of the 6 control animals was a treatment success.

Table 4.5. Percent treatment success on Day 15 for all animals on study and for animals exposed to *Haemophilus somnus*

Treatment	**Success (all animals)**	**Success (*H. somnus*-exposed)**
saline	12 (5/41)	17 (1/6)
florfenicol	62 (52/84)	70 (7/10)

5. Statistical Analysis: Overall treatment success and mortality were analyzed using the Cochran-Mantel-Haenszel statistic. Treatment success and mortality for animals exposed to *H. somnus* were analyzed using the Fisher's Exact test for 2 X 2 tables.

6. Conclusion: Under the conditions of this study, florfenicol administered twice, 48 hours apart, by the intramuscular route at the dose of 20 mg/kg body weight was a safe and effective treatment of bovine respiratory disease associated with *Haemophilus somnus.*

7. Adverse Reactions: There were no adverse reactions in either treatment group.

E. Pharmacokinetics

The pharmacokinetic information provided in the labeling for NUFLOR® Injectable Solution was based on the following published study.

Lobell, R.D., Varma, K.J., Johnson, J.C., Sams, R.A., Gerken, D.F., Ashcraft, S.M. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. *J. vet. Pharmacol. Therap.* 17, 253-258, 1994.

The disposition of florfenicol after single intravenous and intramuscular doses of 20 mg of florfenicol/kg of body weight (b.w.) to feeder calves was investigated. Serum florfenicol concentrations were determined by a sensitive high performance liquid chromatographic method with a limit of quantitation of 0.025 µg/mL. The extent of serum protein binding of florfenicol was only 13.2% at a serum florfenicol concentration of 3.0 µg/mL. Serum concentration-time data after intravenous administration were best described by a triexponential equation. Total body clearance and steady state volume of distribution were 3.75 mL/min/kg b.w. and 761 mL/kg b.w., respectively. The terminal half-life after intravenous administration was 159 minutes. The absolute systemic availability after intramuscular administration was 78.5% (range: 59.3-106%) and the harmonic mean of the terminal half-life was 1098 minutes, indicating slow release of the florfenicol from the formulation at the intramuscular injection site.

F. Microbiology

Data from fifteen studies conducted between 1990 and 1993, in the United States, Europe, and Canada were used to establish an MIC data base for florfenicol against bacteria isolated from cattle with naturally occurring bovine respiratory disease. The minimum inhibitory concentrations (MICs) of florfenicol determined for isolates of *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus* are shown in Table 4.6.

Table 4.6. Minimum inhibitory concentrations (MICs) of florfenicol against isolates from natural infections in cattle

Organism	No. isolates	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Pasteurella hemolytica	398	0.5	1.0
Pasteurella multocida	3.50	0.5	0.5
Haemophilus somnus	66	0.25	0.5

III. TARGET ANIMAL SAFETY

A Drug Tolerance Test in beef calves was conducted to determine the tolerance to and clinical profile of a 10X overdose (200 mg/kg) of the intended clinical dose of NUFLOR® Injectable Solution. A Target Animal Safety Study in beef calves was conducted to address the safety of multiple injections of NUFLOR® Injectable Solution at the 1X (20 mg/kg), 3X (60 mg/kg), and 5X (100 mg/kg) dose levels. An Injection Site Irritation Study was conducted to determine the irritation potential of the intramuscular route of administration. In addition, a feed consumption study was conducted to provide additional information regarding the impact of florfenicol treatment on feed consumption in calves.

A. Drug Tolerance Test

1. Type of Study: This was a 17-day study in which 2 separate injections of florfenicol injectable solution were administered 48 hours apart to 4 cross-bred beef calves. Following the dosing period, the calves were observed and necropsied on Day 17 (15 days following the last injection of the test article).

2. Investigator: Dan C. Ronning
   Colorado Animal Research Enterprises
   Ft. Collins, Colorado

3. General Design:

   a. Purpose: To determine the clinical profile in beef calves following a 10X overdose of florfenicol injectable solution when administered intramuscularly.

   b. Animals: Four cross-bred beef cattle, 2 males and 2 females, weighing 213 to 244 kg at dose initiation

   c. Control: None

   d. Dosage Form: Florfenicol injectable solution, 300 mg/mL

   e. Route of Administration: Intramuscular injection

   f. Dose: 200 mg/kg (10X the therapeutic dose) administered as two separate treatments 48 hours apart.

   g. Test Duration: 17 days (includes 15-day post-dose period)

h. Pertinent Measurements/Observations: clinical observations, physical examinations, feed and water consumption, hematology, serum chemistries, urinalysis, fecal examinations, gross pathology, and histopathology

4. Results

a. Clinical Observations: Loose feces and decreased rumen activity were observed.

b. Feed and water consumption: Feed and water intake were decreased substantially following dosing, with a return to normal intake observed by the end of the post-dose period.

c. Body Weight: Body weight was decreased during dosing with a return to normal weight gain during the post-dose period.

d. Hematology/Serum Chemistry: Mild elevations in serum enzymes (LDH, SGOT, SGPT, and GGT) indicating liver and muscle changes were observed with a return to normal during the post-dose period.

e. Urinalysis: A slight amount of urinary acetone detected during dosing was attributed to slight ketosis due to anorexia.

f. Fecal Examination: No test article-related effects were observed.

g. Gross and Histopathologic Observations: Florfenicol-related changes were limited to the injection sites. Gross lesions included injection site swelling, discoloration, hardness, and loss of texture. Histopathologically, these lesions correlated with minimal to moderate edema, skeletal muscle degeneration/necrosis, hemorrhage, inflammation, fibrosis, and some mineralization. The injection site lesions appeared to be healing.

5. Statistical Analysis: none

6. Conclusions: Florfenicol-related changes included marked anorexia, decreased water consumption, decreased body weight, and elevated serum enzymes of liver and muscle origin during the dosing period. These parameters returned to normal during the post-dose period. Florfenicol-related local irritation at the injection site was noted.

B. Toxicity Text (1X, 3X, 5X Study)

1. Type of Study: This was a 13-day study in which six total intramuscular injections of NUFLOR® Injectable Solution at doses of 0, 20, 60, or 100 mg florfenicol/kg body weight were administered to cross-bred beef calves.

2. Study Director:

Dan C. Ronning

Colorado Animal Research Enterprises, Inc.
Ft. Collins, Colorado

3. General Design:

   a. Purpose: This study was designed to determine the toxicologic effects of florfenicol administered by intramuscular injection to cross-bred beef calves. Potential target organs and tissues were to be identified through clinical observations, gross necropsy, and histologic evaluation. Hematology, serum chemistry, urinalysis, and fecal blood examinations were other variables of interest.

   b. Animals: Twenty-four (24) cross-bred beef calves (12 males and 12 females) approximately 5 to 6 months of age and weighing 161 to 203 kg at initiation of dosing

   c. Control: Normal physiologic saline

   d. Dosage Form: Florfenicol injectable solution, 300 mg/mL

   e. Route of Administration: Intramuscular injection

   f. Dose: 0, 20, 60, or 100 mg/kg body weight every other day for a total of 6 doses

   g. Test Duration: 13 days

   h. Pertinent Parameters Measured: clinical observations, physical examinations, feed and water consumption, body weights, hematology, serum chemistry, urinalysis, fecal examinations, gross pathology, and histopathology.

4. Results

   a. Clinical Observations: Treatment-related changes observed at all dose levels included depression, soft feces, and slight dehydration. These effects were observed most frequently in the 60 mg/kg and 100 mg/kg dose groups, primarily near the end of dosing.

   b. Feed and water consumption: A slight decrease in feed and water intake was observed at the 20 mg/kg dose level. Decreased feed and water intake was observed at the 60 mg/kg and 100 mg/kg dose levels

   c. Body Weight: Decreased body weight gain was observed in the 60 and 100 mg/kg dose groups.

   d. Hematology/Serum Chemistry: Increased serum enzymes (LDH, SGOT, SAP, SGPT) were observed indicating liver and muscle changes in the 60 and 100 mg/kg dose groups.

   e. Urinalysis: Decreased urine pH in the 60 and 100 mg/kg dose groups.

   f. Fecal Blood: No treatment-related effects.

g. Gross and Histopathological Observations: Florfenicol related lesions were limited to the injection sites. All treated groups had gross lesions at the injection sites including swelling, discoloration, hardness, and loss of texture. The lesions were slightly larger in the 60 and 100 mg/kg dose groups. Histopathologically, these lesions correlated with minimal to moderate edema, skeletal muscle degeneration/necrosis, inflammation, fibrosis, and some mineralization.

5. Statistical Analysis: Study data were tabulated and, as appropriate, summarized through calculation of mean and standard deviation values.

6. Conclusions: Minor changes attributed to florfenicol intramuscular injection were seen in calves receiving the recommended dosage (20 mg/kg) when administered for three times the recommended duration of treatment. Florfenicol administered at the recommended dose and duration may produce transient inappetance, decreased water consumption, or diarrhea.

C. Injection Site Irritation Study I

1. Type of Study: This was a 57-day study in which four groups of calves were dosed with two injections of NUFLOR® Injectable Solution, 300 mg/mL in two sites (right side neck and rump musculature). Each dose was administered at a calculated dose of 20 mg/kg body weight. In addition, a similar volume of physiologic saline was injected in two sites (left side neck and rump musculature).

2. Investigator:

   Dan C. Ronning
   Colorado Animal Research Enterprises, Inc.
   Ft. Collins, Colorado

3. General Design:

   a. Purpose: To determine the injection site muscle irritation potential of NUFLOR® Injectable Solution, 300 mg/mL, when administered intramuscularly at a dose 20 mg/kg body weight in each of 2 separate sites (neck and rump musculature)

   b. Animals: 32 crossbred beef calves (189 to 269 kg), 16 males and 16 females

   c. Control: Sterile, normal physiological saline

   d. Dosage Form: florfenicol solution, 300 mg/mL

   e. Route of Administration: intramuscular injection

   f. Dose: 20 mg/kg bw; injection in two sites (right side neck and rump)

   g. Test Duration: 57 days

h. Pertinent Measurements/Observations: Clinical observations, body weight, gross and microscopic pathology. Injection sites were examined visually and by palpation prior to injection, at 4 hours post-dose, and then daily through the first 14 days post-injection and twice weekly thereafter until necropsy. Four animals of each gender were euthanized for collection and evaluation of injection site tissues at 28, 35, 42, and 56 days post-injection.

4. Results

a. Neck injection sites: Palpable swellings were observed at some neck injection sites. No post-mortem injection site lesions were observed at the four post-injection examination times

b. Rump injection sites: Only the rump injection sites showed florfenicol-related gross lesions including discoloration, hardness, and loss of texture. The severity of the gross lesions decreased as the post-dose period increased. Microscopically these lesions correlated with minimal to mild edema, skeletal muscle degeneration, inflammation, fibrosis, and some mineralization. By the end of the 56-day post-dose period, only mild fibrosis and inflammation persisted at the florfenicol injection site.

5. Statistical Analysis: none

6. Conclusions: NUFLOR® Injectable Solution when administered intramuscularly at a dose of 20 mg florfenicol/kg body weight causes local irritation at the injection site which tends to resolve over time. Irritation resulting from injection into the rump musculature took longer to resolve than did irritation resulting from injection into the neck musculature. Gross injection site lesions resolved within 28 days post-injection when florfenicol was administered in the neck musculature. However, gross and histopathologic changes were still apparent at 56 days post-injection when florfenicol was administered in the rump musculature.

D. Injection Site Irritation Study II

1. Type of Study: This was a 55-day study in which 16 beef calves were dosed with two injections of NUFLOR® Injectable Solution, 300 mg/mL, in two sites (right and left sides of the neck). A dose volume of 10 mL was administered at one site and a dose volume of 20 mL at the other site.

2. Investigator:

J.C. Johnson
Schering-Plough Animal
Elkhorn, Nebraska

3. General Design:

a. Purpose: To evaluate the effects of NUFLOR® Injectable Solution, 300 mg/mL, on muscle tissue of cattle following intramuscular administration of 10 mL and 20 mL in the cervical region.

b. Animals: 16 crossbred beef calves (mean weight 291 kg)

c. Dosage Form: florfenicol solution, 300 mg/mL

d. Route of Administration: intramuscular injection in cervical region

e. Dose: 10 mL and 20 mL injections on opposite sides of neck

f. Test Duration: 55 days

g. Pertinent Measurements/Observations: Following injection, the calves were observed daily for post-injection pain, changes in mobility, and visible swelling. Injection sites were examined visually and by palpation on days 0, 2, 5, 7, 9, and 14. Eight calves were euthanized on Day 28 and Day 55, respectively. Sixteen injection sites (2 per calf) were collected at each time point for gross and microscopic examination.

4. Results

a. Live animal observations: Injection site swellings were not observed, or palpable on Days 0, 2, 5, 7, 9, and 14 post-injection of 10 mL or 20 mL of NUFLOR® Injectable Solution.

b. Day 28 post-mortem evaluation: Five of the 8 sites injected with 10 mL had little or no grossly observable lesions at necropsy. Three sites were observed to have measurable lesions that included fascial discoloration, white streaks in muscle, and one lesion contained a small amount of clear fluid.

Three of the 8 sites injected with 20 mL had little or no grossly observable lesions at necropsy. Five sites were observed to have measurable lesions that included muscle discoloration, nodules in the fascia, granulomas, and two lesions contained small amounts of clear fluid.

c. Day 55 post-mortem evaluation: Fifteen of the 16 injection sites had no visible lesions present at 55 days post-injection. One lesion was observed in a 20 mL injection site, and was characterized as an area of accentuated fascia with adjacent muscle discoloration.

5. Statistical Analysis: none

6. Conclusions: NUFLOR® Injectable Solution when administered at a dose volume of 10 mL in the cervical musculature may cause local tissue reaction that persists beyond 28 days post-injection. No tissue reaction was observed at 55 days post-injection with 10 mL of NUFLOR® in the cervical musculature.

E. Feed Consumption Study

1. Type of Study: Controlled exploratory study on feed consumption in healthy animals

2. Investigator:

J.C. Johnson, D.V.M.
Schering-Plough Animal Health
Elkhorn Research Center
Elkhorn, Nebraska 68022

3. General Design:

   a. Purpose: To evaluate the effect of florfenicol administered twice, 48 hours apart, by the intramuscular route at the dose of 20 mg/kg body weight, on the weight gain and feed consumption of healthy feeder cattle.

   b. Animals: 16 mixed-breed beef cattle (8 males, 8 females) ranging in age from 6 to 8 months were housed in 16 individual pens. The initial mean weight was 220.8 kg. The 16 calves were randomized by sex and weight and assigned to one of two treatment groups of 4 females and 4 steers each.

   c. Control: The control product was a 0.9% sodium chloride solution injected twice, 48 hours apart, at the dose of 0.67 mL per 10 kg.

   d. Dosage Form of Test Article: A solution containing 300 mg florfenicol per mL.

   e. Route of administration: intramuscular injection

   f. Doses: Both the placebo and florfenicol injectable solution were administered as two doses at a 48-hour interval.

   g. Test Duration: 43 days

   h. Pertinent Parameters Measured: Weekly weight and daily feed intake.

4. Results:

   No significant difference was found between the treatment groups for cattle weight at the initial weighing (Day 0) or at any of the post-treatment time points.

   A statistically significant decrease in feed consumption was observed on Days 1 and 2 in cattle treated with florfenicol compared to saline treated cattle. No significant difference was apparent between the two groups at Day 0 or any day from Day 3 to Day 42.

5. Statistical Analysis:

   Cattle weight and daily feed consumption were analyzed for between treatment group differences with respect to changes from pretreatment using analysis of covariance. Analysis of variance was used to analyze pre-treatment levels. All analyses were blocked on sex, and type III sums of squares were used for the ANOVA/ANCOVA.

   Analysis of between treatment group differences for increase in mean cattle weight and daily feed intake were evaluated by a comparison of linear

regressions, following the method discussed in *Applied Linear Statistical Methods* (Neter and Wasserman; Richard D. Irwin Inc. [Illinois, 1974], pp. 160-167). All analyses were conducted separately for each sex. The level for statistical significance was defined as p=0.05.

6. Conclusion: A transient statistically significant decline in feed consumption was observed in the florfenicol treated cattle for two consecutive days (the day after the first injection and the day of the second injection). However, under the conditions of this study where florfenicol was administered twice, 48 hours apart, by the intramuscular route at the dose of 20 mg/kg body weight to healthy cattle, florfenicol had no effect on weight gain and no long term effect on feed consumption when compared to saline.

7. Adverse Reactions: There was no evidence of adverse reaction in either of the treatment groups except transient pain following the florfenicol injections

IV. HUMAN FOOD SAFETY

A. Toxicity Studies on Florfenicol (Active Ingredient)

Florfenicol was tested for genotoxic activity in a battery of *in vitro* and *in vivo* assays that evaluated a spectrum of endpoints. The results indicate that florfenicol is not genotoxic.

1. Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay (UDS)

   a. Report Number: D-17050

   b. Study Dates: January 25 to May 7,1983

   c. Study Director: Brian C. Myhr, Ph.D.

   d. Location of Study:

      Litton Bionetics, Inc.
      Kensington, Maryland

   e. Identification of Substance and Dosage Form: florfenicol (florfenicol) powder

   f. Species and Strain: rat hepatocytes (adult male Fisher 344 rats)

   g. Number of Animals per Sex per Treatment Group: not applicable

   h. Drug Levels Tested and Duration of Dosing: 10, 25, 50, 100, 250, 500, 1000, 2500 µg/mL; 18-hour treatment period (Note: test article was insoluble at doses ≥ 500 µg/mL in the aqueous vehicle employed.)

   i. Route of Drug Administration: not applicable

   j. Parameters Tested: The objective of this assay is to detect DNA damage caused by the test material, or an active metabolite, by measuring unscheduled DNA synthesis in primary rat hepatocytes in vitro.

Autoradiographic technique is used to determine the nuclear grain counts resulting from incorporation of [3H]-thymidine into the DNA.

k. Significant Toxicity Observed: The florfenicol dose groups did not meet any of the evaluation criteria for unscheduled DNA synthesis. None of the treatments induced nuclear labeling. The labeling remained very similar to that observed for the negative controls, and no evidence for a dose-related response was observed. Little or no toxicity was observed at doses ≤ 1000 µg/mL.

l. No Observed Effect Level: not applicable

m. Statistical Analysis: not applicable

n. Conclusions: Florfenicol did not induce significant changes in the nuclear labeling of primary rat hepatocytes. Florfenicol was evaluated as inactive in the Primary Rat Hepatocyte UDS Assay.

2. Mouse Lymphoma Forward Mutation Assay

a. Report Number: D-16766

b. Study Dates: December 17, 1982 to February 14, 1983

c. Study Director: Maria A. Cifone, Ph.D.

d. Location of Study:

Litton Bionetics, Inc.
Kensington, Maryland

e. Identification of Substance and Dosage Form: florfenicol powder

f. Species and Strain: Mouse lymphoma cell line, L5178Y TK+/- 3.7.2C, *in vitro*

g. Number of Animals per Sex per Treatment Group: not applicable

h. Drug Levels Tested and Duration of Dosing: Nonactivation phase and Activation phase: 125, 500, 1000, 1500, 2000, 3000 and 4000 mcg/mL of florfenicol. Aroclor 1254 induced rat liver S9 fraction was used. Exposure period of 4 hours was used. (Note: Of the doses of test article to which cells were exposed, doses ≤ 1500 µg/mL, appeared to be soluble in the aqueous cell culture medium.)

i. Route of Drug Administration: not applicable

j. Parameters Tested: The objective of this assay was to evaluate the ability of florfenicol to induce forward mutations at the thymidine kinase (TK) locus in the presence and absence of S9, as assayed by colony growth of L5178Y TK+/- mouse lymphoma cells in the presence of 5-trifluorthymidine (TFT).

k. Significant Toxicity Observed:

Under nonactivation conditions, moderate to high toxicities were induced by florfenicol. The induced mutant frequencies of the florfenicol treated groups ranged from 8.5 x 10^{-6} to 27.2 x 10^{-6}. The mutant frequencies did not exceed the required frequency of 33.1 x 10^{-6} in order to be considered mutagenic.

In the presence of metabolic activation, sporadic, small increases in the mutant frequency were induced at 125, 1000, and 2000 µg/mL, but the increases were not dose dependent and duplicates at the same concentrations were not mutagenic.

l. No Observed Effect Level: not applicable

m. Statistical Analysis: not applicable

n. Conclusions: The results of the assay were considered equivocal. Another mouse lymphoma assay was conducted to clarify the significance of the results of this assay.

3. Mouse Lymphoma Forward Mutation Assay

a. Report: A-22860

b. Start Date: May 23,1988

c. Termination Date: June 30, 1988

d. Study Director: Robert R. Young, M.S.

e. Location of Study:

Hazelton Laboratories America, Inc.
Kensington, Maryland

f. Identification of Substance and Dosage Form: florfenicol powder

g. Species and Strain: L5178Y mouse lymphoma cell tine (clone 3.7.2C) - *in vitro*

h. Number of Animals per Sex per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing: 62.5, 125, 250, 500, 1000, 1500, 2000 µg/mL; exposure period of 24 hours (Note: The test article dose of 2000 µg/mL appeared to be insoluble in the aqueous cell culture medium.)

j. Route of Drug Administration: not applicable

k. Parameters Tested: The objective of this *in vitro* assay was to evaluate the ability of florfenicol to induce forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line under S9 metabolic activation conditions.

l. Significant Toxicity Observed: Florfenicol produced dose-related toxicity in the S9 metabolic activation mutation assay. At concentrations ranging from 500 to 2000 µg/mL, the relative growth compared to controls had a mean reduction of 35 to 69%, respectively. The growth at the lower doses, 62.5 to 250 µg/mL, was considered comparable to controls. The mutant frequency of treated cultures varied randomly with dose and toxicity within a range comparable to the mutant frequencies of the vehicle controls.

m. No Observed Effect Level: not applicable

n. Statistical Analysis: not applicable

o. Conclusions: Florfenicol was evaluated as negative for inducing forward mutations at the TK locus in L5178Y mouse lymphoma cells under the S9 metabolic activation conditions used in the study.

4. Chromosomal Aberrations (CABs) with Chinese Hamster Ovary Cells *in vitro*

a. Report Number: D-23053

b. Start Date: August 1, 1988

c. Termination date: October 27,1988

d. Study Director: I.A. Leddy, B.Sc, Ph.D.

e. Location of study:

Inveresk Research International
Musselburgh, SCOTLAND

f. Identification of Substance and Dosage Form: florfenicol powder

g. Species and Strain: Chinese Hamster Ovary (CHO-10 B4) cells *in vitro*

h. Number of Animals per Sex per Treatment Group: not applicable

i. Drug Levels Tested and Duration of Dosing: A preliminary toxicity assay was conducted to help select the concentrations for the definitive assay. The toxicity assay was conducted at doubling concentrations ranging from 19.5 to 5000 µg/mL. Some precipitation was noted at concentrations ranging from 1250 to 5000 µg/mL. In the presence of S9, the cell numbers were reduced at concentrations ranging from 2500 to 5000 µg/mL. In the absence of S9, the cell numbers were reduced at concentrations ranging from 312 to 5000 µg/mL.

For the definitive tests, the following concentrations were selected (which were restricted due to the solubility limits of florfenicol):

In presence of S9: 313, 625, 1250, and 2500 µg/mL of florfenicol; 6-hr treatment

In absence of S9: 62.5, 125, 625, and 1250 µg/mL of florfenicol; 24-hr treatment

j. Route of Drug Administration: not applicable

k. Parameters Tested: Florfenicol was evaluated for clastogenic potential using Chinese hamster ovary (CHO) cells *in vitro* both in the presence and absence of a postmitochondrial supernatant fluid preparation (S9).

l. Significant Toxicity Observed: In the presence of S9 mix, florfenicol induced structural chromosomal aberrations at the highest dose tested of 2500 µg/mL. The aberrations were notably chromatid breaks and exchanges. This dose level was toxic in that the cell count of these cultures was much reduced. Levels of endoreduplicated cells observed in cultures treated with 1250 and 2500 µg/mL florfenicol were consistently raised.

In the absence of S9 mix, there was no evidence that florfenicol was capable of inducing either structural or numerical chromosomal aberrations. One culture treated at the highest dose of 1250 µg/mL had a slightly elevated level of chromatid gaps.

m. No Observed Effect Level: not applicable

n. Statistical Analysis: Statistical evaluation of in-house historical data from vehicle and untreated control culture has enabled acceptable aberration frequency ranges for a negative response to be defined, and are based on 95% and 99% confidence limits of mean values.

o. Conclusions: It was concluded that florfenicol, when tested with CHO cells, was clastogenic at toxic dose levels and was capable of inducing endoreduplication at both toxic and non-toxic dose levels. These effects were only seen when florfenicol was tested in the presence of a metabolic activation system (S9 mix).

5. Mouse Bone Marrow Cytogenetics Assay

a. Report Number: D-23845

b. Study Dates: November 8, 1989 to February 22, 1990

c. Study Director: James L. Ivett, Ph.D.

d. Location of Study:

Hazelton Laboratories America, Inc.
Kensington, Maryland

e. Identification of Substance and Dosage Form: florfenicol powder

f. Species and Strain: Adult mice, strain ICR

g. Number of Animals per Sex per Treatment Group: 5

h. Drug Levels Tested and Duration of Dosing: Acute: 500, 1667, 5000 mg/kg of florfenicol administered one time only. Harvest times were 6, 18 and 30 hours post dose. Subchronic: 500, 1667, 5000 mg/kg of florfenicol administered daily for 5 days. Harvest time was 6 hours after the last dose.

i. Route of Drug Administration: oral (gavage)

j. Parameters Tested: To verify the cytogenetic potential of florfenicol, in vivo cytogenetics were conducted in mouse bone marrow cells using both acute and subacute exposure regimens.

k. Significant Toxicity Observed: In the acute trial, two males in the 30 hour harvest group, one dosed with 500 mg/kg and one dosed with 1667 mg/kg died approximately 28 and 30 hours after dosing, respectively. All other mice appeared normal and remained healthy until the appropriate harvest times, florfenicol under either the acute or subchronic exposure conditions of the assay did not induce significant increases in the percentage of chromosomally aberrant cells in either sex, at any harvest time or at any dose level over the level of aberrations observed in the concurrent vehicle controls. In the subchronic trial there was a dose related decrease in the mitotic index in both sexes. These data provided evidence that higher doses could not have been used and that florfenicol induced systemic effects on the bone marrow.

l. No Observed Effect Level: not applicable

m. Statistical Analysis: The Kruskal-Wallis test was performed at the alpha = 0.05 level to determine whether any of the mean values among the PEG 400 vehicle control and treatment groups were significantly different from each other.

n. Conclusions: Florfenicol was considered negative for inducing chromosomal aberrations in bone marrow cells of male and female mice under both the acute and subchronic dosing conditions of this study, indicating that florfenicol was a non-clastogen in vivo. Therefore, the previous concerns for the clastogenic potential of florfenicol raised by the positive in the in vitro test were alleviated.

6. Micronucleus Test in Bone Marrow of CD-1 Mice

a. Report Number: A-23052 (IRI Report #4738)

b. Study Dates: May to November 1988

c. Study Director: L.M. Holstrom, B.Sc, M.Sc.

d. Location of study:

Inveresk Research International
Musselburgh, SCOTLAND

e. Identification of Substance and Dosage Form: florfenicol (florfenicol) powder in a polyethylene glycol (PEG 400) vehicle

f. Species and Strain: Mice, CD-1

g. Number of Animals per Sex per Treatment Group: 15 (5 for each of 3 timepoints)

h. Drug Levels Tested and Duration of Dosing: The treated group (florfenicol) received a single oral dose of 5000 mg/kg, the positive control group (cyclophosphamide) received a single oral dose of 80 mg/kg, and the negative control group received PEG 400.

i. Route of Drug Administration: oral (gavage)

j. Parameters Tested: Treated mice were sacrificed at 24, 48, and 72 hours for bone marrow harvest. Five thousand polychromatic erythrocytes per group/sex were scored for micronucleation.

k. Significant Toxicity Observed: No micronucleus induction was observed at any harvest time despite a depression of the PCE/NCE ratio at 72 hours post-dose.

l. No Observed Effect Level: not applicable

m. Statistical Analysis: not applicable

n. Conclusions: Florfenicol was devoid of clastogenic effects when tested for micronucleus inducing potential in bone marrow of male and female CD-1 mice at the standard maximum *in vivo* dose of 5 g/kg.

7. 13-Week Oral Toxicity Study in Rats With 4-Week Recovery Period

a. Report Number: A-23493

b. Study Completion: November 4, 1987

c. Investigator:

C.J. Perry
Inveresk Research International
Musselburgh, EH 21 7UB
SCOTLAND

d. Substance and Dosage Form: Florfenicol was prepared as a suspension in polyethylene glycol 400 (PEG 400).

e. Species and Strain: Sprague-Dawley (SD) rat

f. Number of Animals per Sex per Treatment Group: 20/sex/group, rats were approximately 6 weeks old at the start of dosing

g. Levels and Duration of Dosing: 0, 10, 30, or 100 mg/kg/day of florfenicol was administered orally once daily for 13 weeks.

h. Route of Drug Administration: oral gavage

i. Parameters Tested: Clinical signs, body weight, food and water consumption, ophthalmoscopy, clinical pathology, organ weights, gross and microscopic pathology were evaluated.

j. Significant Toxicity Observed: testicular toxicity resulting in severe atrophy of the seminiferous tubules and decreased testicular weight in the high dose males.

k. No Observed Effect Level: 30 mg/kg/day

l. Conclusions: Body weight gain was decreased in males and females in the 100 mg/kg dosage group. Testicular atrophy was observed in males in the 100 mg/kg dosage group.

8. 13-Week Oral Toxicity Study in Dogs

a. Report Number: A-23487

Study Completion: February 12, 1988

b. Investigator:

R. J. Greenough
Inveresk Research International
Musselburgh, EH 21 7UB SCOTLAND

c. Substance and Dosage Form: Florfenicol, a white powder administered orally in gelatin capsules.

d. Species and Strain: Beagle dogs

e. Number of Animals per Sex per Treatment Group: 4/sex/group. Dogs were approximately 4 months old at the start of dosing.

f. Levels Tested and Duration of Dosing: 0, 10, 30, or 100 mg/kg/day of florfenicol was orally administered daily for at least 13 weeks.

g. Route of Drug Administration: oral, gelatin capsules

h. Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, organ weights, gross and microscopic pathology were evaluated.

i. Significant Toxicity: was observed in the liver, testes, kidney, central nervous system, and bone marrow.

j. No Observed Effect Level: the NOEL for hematopoietic toxicity was 30 mg/kg/day.

k. Conclusions: Increased liver weight, hepatocyte hypertrophy, and/or hepatocellular vacuolation was seen at all doses. Dogs given 30 and 100 mg/kg also had central nervous system vacuolation. Males given 100

mg/kg had renal tubule dilation and testicular atrophy. Hematopoietic changes occurred in the 100 mg/kg/day group. While the NOEL for hematopoietic toxicity was 30 mg/kg/day, there was no NOEL established for hepatic toxicity.

9. 13-week Oral Toxicity Study in Dogs With a 4-Week Recovery Period

   a. Report Number: A-23805

   b. Study Completion: October 25, 1989

   c. Investigator:

      R. Goburdhun
      Inveresk Research International
      Musselburgh, SCOTLAND EH 21 7UB

   d. Substance and Dosage Form: Florfenicol powder was orally administered in gelatin capsules.

   e. Species and Strain: Beagle dog

   f. Number of Animals per Sex per Treatment Group: 4/sex/group plus additional 2/sex/group in control and high-dose group were carried through the four week recovery period. Dogs were approximately 6 months old at the start of dosing.

   g. Levels Tested and Duration of Dosing: 0,1, 3 and 12 mg/kg/day of florfenicol was orally administered daily for at least 13 weeks.

   h. Route of Drug Administration: oral, gelatin capsules.

   i. Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, organ weight and gross and microscopic pathology were evaluated.

   j. Significant Toxicity Observed: hepatotoxicity

   k. No Observed Effect Level: 3 mg/kg/day

   l. Conclusions: Increased liver weight was noted in males given 12 mg/kg/day. There was no evidence of hematopoietic toxicity at the dose levels tested.

10. Two-Generation Reproduction Study on Rats

   a. Report Number: A-24920

   b. Study Completion: May 10, 1990

   c. Investigator:

      S. J. Barton
      Inveresk Research International

Musselburgh, SCOTLAND EH 21 7UB

d. Substance and Dosage Form: florfenicol, a white powder was formulated daily as a suspension in polyethylene glycol (PEG 400)

e. Species and Strain: Sprague-Dawley rats

f. Number of Animals per Sex per Treatment Group: 28/sex/group in the F_0 generation, 24/sex/group in the F_1 generation.

g. Drug Levels Tested and Duration of Dosing: 0,1, 3 and 12 mg/kg/day. F_0 parental rats were dosed daily for 10 weeks prior to mating and continued for both sexes throughout mating, gestation and lactation for two successive litters. The F_1 parents were dosed daily starting when they were about 25 days old and continuing for about 15 to 17 weeks prior to mating and throughout mating, gestation and lactation for two successive litters.

h. Route of Drug Administration: oral gavage

i. Parameters Tested: Clinical signs, body weight, food consumption, reproductive parameters, organ weight, and gross and microscopic pathology were evaluated.

j. Significant Toxicity Observed: reduced epididymal weights

k. No Observed Effect Level: 1 mg/kg/day.

l. Conclusions: Biologically and statistically significantly reduced epididymal weights was noted in the FO and FI generation males. Also F2b pups had low viability index at all dosage levels, low lactation index at 3 and 12 mg/kg/day, and low overall survival index at all dosage levels.

11. Teratogenicity Study in Rats

a. Report Number: A-23769

b. Study Completion: July 4, 1988

c. Investigator:

S. J. Barton
Inveresk Research International
Musselburgh, SCOTLAND EH 21 7UB

d. Substance and Dosage Form: florfenicol, a white powder was formulated daily as a suspension in polyethylene glycol (PEG 400)

e. Species and Strain: Sprague-Dawley rats

f. Number of Animals per Sex per Treatment Group: 26 females per group

g. Drug Levels Tested and Duration of Dosing: 0, 4, 12, and 40 mg/kg/day. The rats were dosed once daily for 12 days from Days 6 through 17 of gestation.

h. Route of Drug Administration: Oral gavage

i. Parameters Tested: Maternal toxicity, embryotoxicity, and teratogenicity

j. Significant Toxicity: moderate maternal toxicity and embryotoxicity

k. No Observed Effect Level: 4 mg/kg/day.

l. Conclusions: Maternotoxicity (reduced food and water consumption) and embryotoxicity (reduced fetal weight and retarded ossification) were observed in rats given 12 or 40 mg/kg/day. No evidence of treatment related teratogenicity was noted at any dosage level employed in this study.

12. 104-Week Oral Toxicity Study in Rats with 52 week interim kill (Results from the 52-week kill animals).

a. Report Number A-23625

b. Study Completion: September, 1989

c. Investigator:

D.J. Everett
Inveresk Research International
Musselburgh, SCOTLAND EH 21 7UB

d. Substance and Dosage Form: Florfenicol was compounded daily into polyethylene glycol 400 (PEG 400) suspension.

e. Species and Strain: Sprague-Dawley rat

f. Number of Animals per Sex per Treatment Group: 20/sex/group

g. Drug Levels Tested and Duration of Dosing: 0, 3, 12, and 48 mg/kg/day for at least 52 weeks

h. Route of Drug Administration: oral gavage

i. Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, organ weights, gross and microscopic pathology were evaluated.

j. Significant Toxicity: Testicular toxicity

k. No Observed Effect Level: 3 mg/kg/day

l. Conclusions: Decreased body weight gains and testes weights were observed in the 12 and 48 mg/kg/day dosage groups. Testicular tubular

degeneration/atrophy occurred in the 12 and 48 mg/kg/day dosed males. Decreased erythroid parameters were seen in the 48 mg/kg dosage group.

13. 52-Week Oral Toxicity Study in Dogs

    a. Report Number: A-24922

    b. Study Completion: August 13, 1990

    c. Investigators:

       R. Goburdhun and F. MacNaughton,
       Inveresk Research International
       Tranent, SCOTLAND EH33 2NE

    d. Substance and Dosage Form: florfenicol powder

    e. Species and Strain: Beagle dogs

    f. Number of Animals per Sex per Treatment Group: 4/sex/group

    g. Drug Levels Tested and Duration of Dosing: 0, 1, 3, or 12 mg/kg/day was administered daily for at least 52 weeks.

    h. Route of Drug Administration: oral, by gelatin capsules

    i. Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopy, clinical pathology, organ weights, gross and microscopic pathology were evaluated

    j. Significant Toxicity Observed: hepatotoxicity

    k. No Observed Effect Level: 3 mg/kg/day

    l. Conclusions: Increased liver weight and slight increase in hepatocyte rarefaction was observed in males of the 12 mg/kg/day group.

14. 104-Week Oral Carcinogenicity Study in Rats

    a. Report Number: A-25233

    b. Study Completion: December 3, 1989

    c. Investigator:

       D. J. Everett
       Inveresk Research International
       Tranent, EH33 2NE
       SCOTLAND

    d. Substance and Dosage Form: Florfenicol powder was compounded daily into a polyethylene glycol 400 (PEG 400) suspension.

    e. Species and Strain: Sprague-Dawley rat

f. Number of Animals per Sex per Treatment Group: 50/sex/group

g. Drug Levels Tested and Duration of Dosing: 0, 3,12, and 48 mg/kg/day of florfenicol administered daily for at least 104 weeks.

h. Route of Drug Administration: oral gavage

i. Parameters Tested: clinical signs, body weight, food consumption, hematology, gross and microscopic pathology were evaluated

j. Significant Toxicities: testicular toxicity

k. Conclusions: Administration of florfenicol to SD rats for approximately 104 weeks had no carcinogenic effect. Florfenicol at 12 and 48 mg/kg/day increased incidence of grossly small/flaccid testes and microscopically with severe testicular tubular degeneration/atrophy. At 48 mg/kg/day, florfenicol is associated with induction of benign interstitial cell tumors in the testes of male rats without any evidence of malignancy. It was concluded that florfenicol is not a carcinogen in rats.

15. 104-Week Oral Carcinogenicity Study in Mice

a. Report Number: A-24921

b. Study Completion: April 8, 1990

c. Investigator:

D.J. Everett
Inveresk Research International
Tranent, EH33 2NE
SCOTLAND

d. Substance and Dosage Form: Florfenicol was compounded daily into a polyethylene glycol 400 (PEG 400) suspension.

e. Species and Strain: CD-1 mice

f. Number of Animals per Sex per Treatment Group: 50/sex/group

g. Drug Levels Tested and Duration of Dosing: 0, 20, 10 and 200 mg/kg/day for 104 weeks.

h. Route of Drug Administration: oral gavage

i. Parameters Tested: Clinical signs, body weight, food and water consumption, hematology, gross and microscopic pathology were evaluated.

j. Significant Toxicity Observed: testicular toxicity

k. Conclusion: Administration of florfenicol to CD-1 mice for approximately 104 weeks had no carcinogenic effect. Florfenicol at 48 mg/kg/day caused testicular degeneration/atrophy.

B. Toxicology Studies – N-methyl-2-pyrrolidone

The following studies were conducted to investigate the human food safety issues of the new excipient used in NUFLOR Injectable Solution, N-methyl-2-pyrrolidone

1. Mutagenicity Test on GMP M-Pyrol (93519-90) in the Salmonella/mammalian-Microsome Reverse Mutation Assay (Ames Test) with a Confirmatory Assay

   a. Report Number: A-25620

   b. Start Date: January 14,1991

   c. Termination date: February 28, 1991

   d. Investigator:

      Timothy E. Lawlor, M.A.
      Hazelton Laboratories America, Inc.
      5516 Nicholson Lane
      Kensington, MD 28095

   e. Substance and Dosage Form: N-methyl-2-pyrrolidone is an organic solvent which is used as an excipient in the NUFLOR® Injectable Solution formulation.

   f. Species and Strain: The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA 98, TA 100, TA 1535, TA 1537, and TA 1538.

   g. Drug Levels Tested and Duration of Dosing: The doses tested in the mutagenicity assay were selected based upon the results of a dose range finding study using tester strain TA 100 and ten dose levels of test article ranging from 5000 to 6.67 µg per plate, one plate per dose, both in the presence and absence of S9. The assay was conducted using three plates per dose level both in the presence and absence of S9. Six doses of test article were tested, from 5000 to 100 µg per plate both in the presence and absence of S9.

   h. Parameters Tested: This assay evaluates the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of specific *Salmonella typhimurium* tester strains both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from Aroclor-induced rat liver (S9).

   i. Significant Toxicity Observed: In the initial mutagenicity assay, all data were acceptable and no positive increases in the number of histidine revertants were observed with any of the tester strains either in the presence or absence of S9. In the confirmatory assay, all data were acceptable and no positive increases in the number of histidine revertants per plate were observed with any of the tester strains either in the presence or absence of S9.

j. Conclusion: N-methyl-2-pyrrolidone was not mutagenic under the conditions of this assay.

2. Mutagenicity Test on N-methyl-2-pyrrolidone in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

   a. HLA Study Number: 10519-0-447

   b. Study Dates: August 22 to October 8, 1988

   c. Location of Study:

      Hazelton Laboratories America, Inc.
      5516 Nicholson Lane, Suite 400
      Kensington, MD 20895

   d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone Squid

   e. Summary: In the *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis (UDS) Assay, the test material did not induce significant increases in UDS. Freshly prepared rat hepatocytes were exposed to N-methyl-2-pyrrolidone at concentrations ranging from 0.500 µg/mL to 5000 µg/mL in the presence of 5 mCi/mL ^{3}HTdr (20 Ci/mmole). Treatment at 5000 µg/mL was not analyzed for nuclear labeling due to high toxicity. Treatments from 4000 µg/mL to 250 µg/mL, which covered a good range of toxicity (75.3 to 97.6% survival), were selected for analysis. The test material was soluble in media at all concentrations tested. None of the criteria used to indicate UDS were approached by the chemical treatments and dose-related response was observed.

   f. Conclusions: N-methyl-2-pyrrolidone was evaluated as inactive in the Rat Primary Hepatocyte UDS assay

3. Mutagenicity Test on N-methyl-2-pyrrolidone in the CHO/HGPRT Forward Mutation Assay

   a. HLA Study Number: 10194-0-435

   b. Report Date: June 23, 1988

   c. Location of Study:

      Hazelton Laboratories America, Inc.
      5516 Nicholson Lane, Suite 400
      Kensington, MD 20895

   d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone liquid

   e. Summary: The objective of this *in vitro* assay was to evaluate the ability of N-methyl-2-pyrrolidone to induce forward mutations at the hypoxanthine-guanine phosporibosyl transferase (HGPRT) locus in Chinese

hamster ovary cells under conditions with and without metabolic activation.

The test material was soluble in F12 culture medium at 50.0 mg/mL. Preliminary range finding cytotoxicity testing found the test article to be nontoxic at all dose levels tested from 0.005 mg/mL to 5.0 mg/mL under both nonactivation and S9 metabolic activation test conditions. For each test condition, six dose levels that ranged from 0.5 mg/mL to 5.0 mg/mL were used in the mutation assays. The test article was not toxic at any dose level in the mutation assays.

Mutant frequencies of all cultures treated with test material varied randomly with dose within a range acceptable for negative control mutant frequencies. In the S9 metabolic activation mutation assay, one of the six treatment conditions achieved statistical significance. The culture that achieved statistical significance had a mutant frequency within the acceptable range for background mutant frequencies. The statistical significance was apparently due to normal assay variation. Without S9 metabolic activation, none of the six dose levels had mutant frequencies that achieved statistical significance. Therefore, N-methyl-2-pyrrolidone was considered negative for inducing forward mutation at the HGPRT locus in CHO cells under the S9 metabolic activation and nonactivation conditions of the assay.

4. Evaluation of the Safety of N-methyl-2pyrrolidone in Wistar-Derived Rats Following 90-Day Administration in the Diet

   a. Laboratory Number: 5026

   b. Report Date: December 9, 1976

   c. Location of Study:

      Food and Drug Research Laboratories, Inc.
      Route 17C
      Waverly, NJ 14892-0107

   d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone liquid

   e. Summary: This study was conducted to evaluate the safety of the compound N-methyl-2-pyrrolidone following a 90-day administration in the diet of weanling rats. Two hundred (200) weanling FDRL-Wistar rats (25/sex/dose level) were dosed with 0 (control), 800, 2000, and 5000 ppm of the compound. The 800, 2000 and 5000 ppm dosages produced a significant decrease ($p<0.05$) on female body weights, increase in male thyroid weight, and changes in the chemical properties of urine (pH in males and females, albumin and specific gravity in females, and SGPT in males at termination). It was concluded, however, that the 800 ppm dose level (which equated to 40 mg/kg consumption level) was the no effect level (NOEL) for the compound in this study.

5. 90-Day Feeding Study in Beagle Dogs with N-methyl-2-pyrrolidone

a. Laboratory Number: 6414

b. Report Date: September 3, 1980

c. Location of Study:

Food and Drug Research Laboratories, Inc.
Route 17C
Waverly, NJ 14892-0107

d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone liquid

e. Summary: This study was conducted to evaluate the potential systemic toxicity of dietary n-methyl-2-pyrrolidone when administered to beagle dogs for 90 days at dose levels of 0, 25, 79 and 250 mg/kg body weight. Forty-eight (24 male and 24 female) beagle dogs ranging from 5 to 6 months of age at the beginning of the study were included. Body weights and food consumption were measured weekly, animals were observed daily, blood samples for hematology and biochemical determination were collected and analyzed pretest, 60 days after initiation and at termination. At the termination of the study (90 days), gross and microscopic examinations were performed.

No signs of toxicity or behavioral abnormalities were observed during the study. Intermittent significant variations in total cholesterol, serum albumin and serum total protein were noted but toxicological significance was not supported by any histopathological finding. No test article related histopathological findings were noted in any organs and analysis of organ weight failed to reveal any significant treatment differences.

The only unusual finding was a very minimal growth rate of all dogs in the study. Although food consumption was similar among all groups, the high dose males gained almost no weight compared to the controls after the 90-day period. The NOEL for the study was 79 mg/kg body weight/day based on the lack of body weight gain of the high dose group.

6. Teratologic Evaluation of N-methyl-2-pyrrolidone After Dermal Application in Sprague-Dawley Rats

a. Laboratory Number: 6161

b. Report Date: November 18, 1979

c. Location of Study:

Food and Drug Research Laboratories, Inc.
Route 17C.
Waverly, NJ 14892-0107

d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone liquid

e. Summary: This study was conducted to evaluate the teratogenic potential of N-methyl-2-pyrrolidone administered dermally to pregnant Sprague-Dawley rats at 0 (negative, positive and aspirin control groups), 75, 237 or 750 mg/kg body weight per day on days 6 through 15 of gestation. Six (6) groups were utilized providing 25 female rats per group. On Day 20 of gestation, all animals were euthanized for uterine examination and fetal examination for skeletal and soft tissue abnormalities.

The frequency and severity of dry skin and bright yellow urine increased with dosage. Maternal toxicity was evidenced by significantly lower body weights on days 15 and 20, and reduced weight gains during gestation in the 750 mg/kg/day females. The high dose also resulted in fewer live fetuses per dam, an increase in the percentage of resorption sites (although the percentage of dams showing resorptions was unaffected), and a significant reduction in fetal weights.

Fetal examination revealed a variety of skeletal variations at the 750 mg/kg dose which indicates possible retardation in fetal development and possibly a teratogenic potential of the compound at high doses. Treatment with 750 mg/kg/day of the compound could be considered maternally toxic since it resulted in observations similar to those of the positive controls administered hexafluoroacetone solution. No maternal or fetal effects were seen in the animals treated with 75 or 237 mg/kg/day of the compound. No teratogenic effects were seen at the dose levels tested. The NOEL for maternal and fetal toxicity was 237 mg/kg body weight/day.

7. Developmental Toxicity Study of N-mediyl-2-pyrrolidone in New Zealand White Rabbits

a. Laboratory Number: 637-002

b. Report Date: December 17. 1991

c. Location of Study

International Research and Development Corporation
Mattawan, Michigan, U.S.A. 49071

d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone liquid

e. Summary: Inseminated New Zealand White SPF female rabbits were used to determine the development toxicity including the teratogenic potential of N-methyl-2-pyrrolidone. The rabbits were randomly assigned to one placebo control and three treatment groups (55, 175 and 540 mg/kg/day) consisting of twenty (20) animals each. The treatments were administered orally by gavage as a single daily dose on gestation Days 6 through 18 at a volume of 3.0 mL/kg. Cesarean section examinations were performed on all females on gestation Day-29, followed by teratologic examination of the fetuses.

Maternal toxicity was observed at 540 mg/kg/day. Statistically significant inhibition of maternal body weight gain and food consumption were observed at this level and an abortion at 540 mg/kg/day was confirmed. At 175 mg/kg/day, there appeared to be a dose related trend toward inhibited body weight gain; however, this inhibition was only statistically significant during first treatment subinterval (days 6-12). This, plus the fact that reduced feed consumption did not attain statistical significance when compared to the control group, and in the absence of any other overt signs of toxicity, does not clearly establish the 175 mg/kg/day dose as maternally toxic.

Developmental toxicity was observed at 540 mg/kg/day manifested by increased postimplantation loss, increased incidences of cardiovascular and skull malformations and developmental variations.

The no adverse effect level (NOAEL) based on this study was equivocally considered to be 55 mg/kg/day with respect to maternal toxicity, and 175 mg/kg/day with respect to developmental toxicity.

8. Multigeneration Rat Reproduction Study with N-methyl-2-pyrrolidone

   a. Laboratory Number: 236535

   b. Report Date: November 26, 1991

   c. Location of Study:

      Exxon Biomedical Sciences, Inc.
      Toxicology Laboratory
      Mettlers Road, CN2350
      East Millstone, NJ 08875-2350

   d. Identification of Substance and Dosage Form: N-methyl-2-pyrrolidone liquid

   e. Summary: A two generation reproduction study with two litters per generation was conducted with N-methyl-2-pyrrolidone at dose levels of 50, 160, and 500 mg/kg/day in the diet. No reproductive effects were seen in the PI generation. However, high dose Fib (P2) male mating indices and fertility indices were lower than controls for both litters. High dose female (P2) fertility and fecundity indices were lower than controls for both litters. Additionally, there were differences in survival indices and growth rate of all litters with 500 mg/kg/day of the compound. The NOEL for reproductive and developmental effects was established as 160 mg/kg/day of the compound.

   f. Conclusions: The 160 mg/kg/day dose was established as the parental, reproductive and developmental NOAEL in this study.

C. Safe Concentration Residues

1. No-Observed Effect Level (NOEL) - florfenicol

The safe concentrations of florfenicol total residues were determined from the lowest NOEL in the most sensitive species from the various toxicology studies conducted. A summary of the studies which can be used in determination of the Acceptable Daily Intake (ADI) follows in Table 6.1.

Table 6.1. Toxicology studies usable for determination of Allowable Daily Intake (ADI) for florfenicol

Study	**No-observed effect level**
13-Week Rat Study	30 mg/kg/day
13-Week Dog Study	3 mg/kg/day
13-Week Dog Study (w/recovery period)	3 mg/kg/day
2-Generation Reproductive Tox. Study in Rats	1 mg/kg/day
Teratology In Rats	4 mg/kg/day
104-Week Rat Study (52-wk Interim Kill)	3 mg/kg/day
52-Week Dog Study	3 mg/kg/day

The calculation of the Acceptable Daily Intake (ADI) for residues of florfenicol was based upon the NOEL of 1 mg/kg/day from the two-generation reproduction study in rats with an assigned safety factor (SF) of 100.

2. Calculation of Acceptable Daily Intake (ADI):

$$\text{Acceptable Daily Intake (ADI)} = \frac{\text{Lowest NOEL}}{\text{Safety Factor}}$$

A safety factor (SF) of 100 is used because the ADI is based on two-generation reproduction study data.

The lowest NOEL is 1 mg/kg, so $\text{ADI} = \frac{1 \text{ mg/kg/day}}{100}$

$= 0.01$ mg/kg or 10µg/kg

3. Calculation of Safe Concentrations (SC): The calculation of the safe concentrations is based on the *General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals* (FDA/CVM Revised July 1994):

$$\text{SC (muscle)} = \frac{10 \text{ µg/kg bw/day} \times 60 \text{ kg}}{300 \text{ g/day}} = 2.0 \text{ µg/kg} = 2.0 \text{ ppm}$$

$$\text{SC (fat)} = \frac{10 \text{ µg/kg bw/day} \times 60 \text{ kg}}{50 \text{ g/day}} = 12.0 \text{ µg/kg} = 12.0 \text{ ppm}$$

$$\text{SC (kidney)} = \frac{10\ \mu g/kg\ bw/day \times 60\ kg}{50\ g/day} = 12.0\ \mu g/kg = 12.0\ ppm$$

$$\text{SC (liver)} = \frac{10\ \mu g/kg\ bw/day \times 60\ kg}{100\ g/day} = 6.0\ \mu g/kg = 6.0\ ppm$$

Table 6.2 Safe concentrations for total residues of florfenicol in edible tissues from cattle using the revised food consumption factors

Tissue	Safe Concentration
Muscle	2.0 ppm
Liver	6.0 ppm
Kidney	12.0 ppm
Fat	12.0 ppm

4. Threshold Assessment: Florfenicol was negative in genotoxicity assays and was not carcinogenic in mouse and rat carcinogenicity bioassays.

5. N-Methyl-2-pyrrolidone

   The NOEL from the 90-day rat study (40 mg/kg) was used to calculate the safe concentration for total residues of N-methyl-2-pyrrolidone in edible tissues. Using a safety factor of 1000, and average consumption values for edible tissues, the calculated safe concentrations for the total residues of N-methyl-2-pyrrolidone in muscle, liver, kidney, and fat are 8, 24, 48, and 48 ppm, respectively. In the absence of chronic toxicity studies in the rodent and dog, the maximum allowable residue that can be assigned to muscle is 5 ppm.

D. Total Residue Depletion and Metabolism Studies – Florfenicol

1. Total Residue Depletion Study in Cattle Following Intramuscular Administration of ^{14}C-SCH 25298. Study No. 90708

   a. Name and Address of Investigator:

      Ronald J. Christopher, Ph.D.
      Hazelton Wisconsin, Inc.
      3301 Kinsman Boulevard
      Madison, Wisconsin 53707

      Analytical Laboratory:

      Schering-Plough Corp.
      Route 94 South
      Lafayette, New Jersey 07848

   b. Test animals: Twelve 7-to 9-month-old calves weighing from 188 to 256 kg were arranged in 4 groups of 3 animals each.

c. Route of Drug Administration and Time and Duration of Dosing: Test animals were given two intramuscular 20 mg/kg bw doses of florfenicol with a 48-hour interval between the doses. The animals were sacrificed by group at 0.5, 5, 15, and 30 days after the second dose of florfenicol.

d. Radioisotope: Uniformly ring-labeled ^{14}C-florfenicol was used in this study. The radiochemical purity of the test article was 98.7% by high performance liquid chromatography. The injectable solution used in this study was as the formulation to be marketed for use in cattle.

e. Total residue concentration: The following samples were collected in this study: blood, liver, kidneys, muscle, fat, and injection sites. Tissue samples were combusted and counted with a liquid scintillation counter, while urine, plasma, and cage washes were counted directly. Mean concentrations of radioactivity (total residues) are shown in Table 6.3.

Table 6.3. Mean concentrations (ppm) of radioactivity in tissues and plasma of cattle following two intramuscular doses of ^{14}C-florfenicol at 20 mg/kg bw/day

	Post dosing Interval to Sampling (days)			
Sample	**0.5**	**5**	**15**	**30**
liver	19.5 ±2.18	17.0±0.78	10.8 ±1.74	4.1 ±0.11
kidney	11.0 ±1.39	9.3±4.98	1.8 ±0.32	0.4 ±0.04
muscle	2.0 ±0.35	1.0±0.31	0.3 ±0.10	0.2 ±0.05
fat	0.2 ±0.27	0.9±0.40	0.1 ±0.05	ND
injection site 1	1860 ±1055	285.0±160.8	22.0 ±20.0	0.5 ±0.45
Injection site 2	1280 ±506.5	550.0±497.1	1.0 ±0.90	0.8 ±0.23
plasma	1.6 ±0.40	ND	ND	ND

ND = not detected. Limit of detection varied from about 1 ppm for the 0.5-day group to 0.8 for the 30-day group.

Radioassay of the urine and feces samples showed that the majority (63% to 71%) of the administered ^{14}C-florfenicol was excreted via the urine with only minor amounts (6%-9%) appearing in the feces.

2. Metabolism of Florfenicol in Cattle

The metabolism of ^{14}C-florfenicol was investigated as part of the total residue study discussed above (Study # 90708). Metabolites were isolated and identified in the urine, feces, liver, kidneys, and injection sites. Tissue and feces samples were initially extracted with methanol to determine the amount of free and bound residues. The methanol extracts were then analyzed by HPLC to determine the amounts and identities of the individual metabolites present. Urine samples were analyzed directly by HPLC. The residue present in liver and kidney tissue was predominantly non-extractable (bound) residue from which florfenicol amine can be released by strong acid hydrolysis.

Table 6.3 Percent of sample total radioactivity in metabolites of florfenicol in the livers and kidneys of cattle following two 20 mg/kg bw doses with a 48-hr interval (values listed are the percentages of the sample total radioactivity)

6.3.1 Liver Tissue

	Sample Time (days)			
Liver Tissue	**0.5**	**5**	**15**	**30**
Extractable:	28.89	13.04	9.38	8.90
Florfenicol	4.94	0.68	1.71	0.55
FFC Amine	10.92	5.98	2.33	4.08
FFC Alcohol	5.20	2.31	2.13	1.51
FFC Oxamic acid	ND	ND	0.53	0.31
Monochlorflor	ND	1.34	ND	0.49
Unknown 1	1.44	ND	ND	ND
Unknown 2	2.86	0.90	0.71	0.53
Other unknown*	3.52	2.01	1.97	1.42
Non-extractable:	71.11	86.96	90.62	91.10

6.3.2 Kidney Tissue

	Sample Time (days)			
Kidney Tissue	**0.5**	**5**	**15**	**30**
Extractable:	73.75	77.81	23.93	NR
Florfenicol	35.38	74.31	4.45	NR
FFC Amine	13.99	3.50	3.59	NR

	Sample Time (days)			
Kidney Tissue	**0.5**	**5**	**15**	**30**
FFC Alcohol	3.36	ND	1.47	NR
FFC Oxamic acid	5.08	ND	5.42	NR
Unknown 1	ND	ND	2.02	NR
Unknown 2	ND	ND	2.85	NR
Unknown 3	15.95	ND	ND	NR
Other unknown*	ND	ND	4.13	NR
Nonextractable:	26.25	22.19	76.07	NR

E. Total Residue Depletion and Metabolism Studies – N-Methyl-2-pyrrolidone

Because the depletion characteristics of the excipient used in NUFLOR® Injectable Solution were not known, a total residue study was conducted to determine whether the product would be regulated on the basis of the excipient, N-methyl-2-pyrrolidone, or on the marker residue for the active ingredient florfenicol.

1. Total Residue Depletion Study of [^{14}C]-M-Pyrol (N-Methyl-2-pyrrolidone) Residues in the Calf Following Intramuscular Administration of M-Pyrol with Florfenicol. Study No. 90714; P-Report 5743 (11 May 95).

   a. Name and Address of Investigator:

      Test Facility (In-Life Portion):

      Southwest Bio-Labs, Inc.
      401 N. 17th St., Suite 11
      Las Cruces, NM 88005

      Analytical Laboratory:

      Shawn F. Charles, M.S.
      Schering-Plough Research Institute
      144 Route 94 South
      Lafayette, NJ 07848

   b. Test animals:

The test animals consisted of 12 crossbred cattle arranged (6 steers and 6 heifers) in four groups of three cattle per sacrifice time point. At the time of dosing, the cattle body weight range was 155 to 207 kg.

c. Route of Administration and Time/Duration of Dosing:

The cattle were dosed intramuscularly on study days 1 and 3 with a solution of [^{14}C] N-methyl-2-pyrrolidone (^{14}C-NMP) containing florfenicol at a rate of 16.7 mg of NMP/kg body weight/day (reflecting the normal level of excipient NMP used in the NUFLOR Solution formulation). The cattle were sacrificed by group at 0.5, 5, 15 and 30 days after the last dose.

d. Radioisotope:

^{14}C-N-Methyl-2-pyrrolidone was uniformly ring-labeled. The specific activities of the dose preparations used in this study were 617-2781 dpm/µg. Radiochemical purities were 95.98-98.85% (HPLC) and 99.47-100% (TLC). The radiolabeled injectable solution used in this study was prepared according to the proposed florfenicol formulation intended for use in cattle.

e. Total Residue Concentrations:

Edible tissues (muscle, liver, kidney and fat) were collected at each sacrifice time point and analyzed for total ^{14}C-NMP residues (ppm calculated as NMP). The mean concentrations of total NMP residues are shown in Table 6.4.

Table 6.4. Total ^{14}C-N-Methyl-2-pyrrolidone Residues (ppm)

	Post dosing Interval to Sampling (days)			
Tissue	**0.5**	**5**	**15**	**30**
Liver	15.5 ±0.64	1.98 ±0.37	0.92 ±0.16	0.43 ±0.11
Kidney	17.2 ±1.08	1.19 ±0.35	0.57 ±0.12	0.26 ±0.08
Leg Muscle	12.0 ±0.51	0.59 ±0.14	0.40 ±0.09	0.43 ±0.10
Loin Muscle	12.0 ±0.65	0.59 ±0.12	0.44 ±0.05	0.43 ±0.10
Omental Fat	5.00 ±0.36	0.43 ±0.08	0.39 ±0.16	0.28 ±0.19
Perirenal Fat	6.14 ±1.91	1.04 ±0.27	0.88 ±0.44	0.44 ±0.35
Injection Site (R)	12.6 ±0.93	0.90 ±0.19	0.70± 0.17	0.50 ±0.11
Injection Site (L)	14.2 ±1.46	0.84 ±0.26	0.62 ±0.07	0.47 ±0.05

Urine was the primary route of elimination of total ^{14}C-NMP residues accounting for 65% of the administered dose, while residues in the feces accounted for 16% of the administered dose.

2. Metabolism of N-methyl-2-pyrrolidone

High performance liquid chromatography (HPLC) was used to determine profiles of metabolites in extracts of urine, feces, and liver. The major ^{14}C-component found in cattle urine, feces, and liver extracts was N-methyl succinimide, representing approximately 31, 23, and 42% of total sample extract radioactivity, respectively. ^{14}C-NMP was found to be a minor component in urine and feces extracts, while it represented approximately 18% of liver extract radioactivity. A major unknown metabolite was found in significant amounts: 32% in urine, 17% in feces, and 6% in liver extracts.

F. Comparative Metabolism in the Rat – Florfenicol

SCH 25298 (Florfenicol): Distribution, Metabolism, and Excretion of ^{14}C-SCH 25298 in Rats Following Seven Consecutive Oral Doses. Study No. 90717

1. Study Director: Ronald Christopher, Ph.D.

2. Testing Facility:

Department of Drug Metabolism and Pharmacokinetics
Safety Evaluation Center, Schering-Plough Research
Lafayette, NJ 07848

3. Study Date: October 31, 1991

4. Study Design: Five male and 5 female Sprague-Dawley rats were orally administered 20 mg of ^{14}C-florfenicol/kg bw once daily for 7 consecutive days. Urine and fecal samples collected during the in-life portion of the study were pooled by sex and analyzed by HPLC. The rats were sacrificed at two hours following the final dose, and the radioactivity was determined in excreta and selected tissues.

5. Results:

Table 6.5. Distribution of florfenicol and its metabolites in the urine and feces collected from rats during the 0-24 hour and 120-146 hour intervals of the dosing period. Values are expressed as the percentage of the administered dose.

Compound	Urine 120-146 hr	Urine 0-24 hr	Feces 0-24 hr	Feces 120-146 hr
Florfenicol	58.02	52.33	3.32	4.38
Florfenicol amine	5.26	5.49	4.55	3.66
Florfenicol oxamic acid	13.42	12.69	16.12	15.04
Florfenicol alcohol	4.01	4.64	10.77	8.77

Compound	Urine 120-146 hr	Urine 0-24 hr	Feces 0-24 hr	Feces 0-24 hr
FFC amine glucuronide	1.49	2.09	15.02	15.17
Monochloroflorfenicol	1.94	2.02	14.66	16.70
Diffuse	4.91	4.05	20.18	17.60
Not Recovered	10.95	16.69	5.69	8.69

Analysis of liver extracts from male and female rats demonstrated the presence of florfenicol, florfenicol amine, florfenicol alcohol, florfenicol oxamic acid, monochloroflorfenicol, and unknowns 1-6. The rat liver, urine and feces metabolite profiles were qualitatively similar to those observed in cattle. Thus, the rats used in the toxicity tests were exposed to all of the metabolites appearing in the profiles from cattle liver and kidney.

G. Comparative Metabolism in the Rat - N-Methyl-2-pyrrolidone

SCH 25298 (Florfenicol): Metabolism of ^{14}C-M-Pyrol in Rats Following Repeated Oral Administration. Study No. 93707; P-Report 6031 (8 Nov 95).

1. Investigator: Shawn F. Charles, M.S.

2. Test Facility (In-Life and Analysis):

   Schering-Plough Research Institute
   144 Route 94 South
   Lafayette, NJ 07848

3. Study Design:

   Twelve rats (6F, 6M: Crl:CD(SD)BR VAF/PLUS) were divided into two dose groups (1 and 2), each containing six rats (3M,3F). The rats in each group were dosed with 400 mg of ^{14}C-NMP/kg/day by gavage for three consecutive days. The ^{14}C-NMP was uniformly ring labeled and the specific activities of ^{14}C-NMP were 570 dpm/µg (Group 1) and 2137 dpm/µg (Group 2). Urine and feces were collecte at 24-hr intervals throughout the the study until sacrifice 48 hr following the final dose. Urine, feces, liver, kidneys, carcass and cage wash were analyzed for total radioactivity. HPLC ^{14}C-profiles were determined for extracts of urine, feces and liver.

4. Results:

   An average of 79% (males) and 76% (females) of the total ^{14}C-dose was eliminated in the urine; 4% was recovered in the feces. Unchanged ^{14}C-NMP was the major ^{14}C-component found in the liver (58% of the sample extract), the second largest component in feces (20% of the sample extract), and was detected in the urine (3% of the sample extract). N-Methylsuccinimide was found in extracts of urine (2%), feces (<2%), and liver (9%).

The HPLC metabolite profiles found in rat urine, feces and liver were found to be qualitatively similar to those previously determined in cattle urine, feces and liver (P-5743). The comparison of metabolites of NMP in rats following oral administration, and in cattle following intramuscular administration, indicates that the NMP-derived residues in the edible tissues of target animals (cattle) have been adequately tested in the toxicological species (rat).

H. Selection of a Target Tissue, Marker Residue, and Determination of a Tolerance

The data in total residue study # 90708 with ^{14}C-florfenicol in cattle established that liver is the edible tissue of cattle in which residues of florfenicol are highest and persist longest. Study # 90708 also showed that the majority of the total residue in liver is bound (non-extractable), and that strong acid hydrolysis will release florfenicol amine in high yield from the bound residue as well as from the extractable residues. These observations lead to the development of a regulatory assay for florfenicol (see Part J) based on florfenicol amine as the marker residue and liver as the target tissue.

The tolerance assignment for residues of florfenicol amine in cattle liver also was done with data from the livers of cattle dosed with ^{14}C-florfenicol in study # 90708. The radioassays for the livers and the parallel assay by the HPLC determinative procedure provided the data listed below and allowed calculation of the percentage of florfenicol amine in the total residue present in each liver as measured by the HPLC assay.

Table 6.6. Mean concentrations (ppm) of florfenicol total residues and marker residue (florfenicol amine) in the livers of cattle dosed twice with ^{14}C-florfenicol at 20 mg/kg bw.

Post dosing interval (days)	Total residue in liver	Marker residue (florfenicol amine)	Marker residue (percentage)
0.5	19.5	19.3	99
5	17.1	11.5	67
15	10.8	7.3	68
30	4.1	2.4	59

A graphical presentation of these data shows that, when florfenicol total residues in liver are at the safe concentration of 6.0 ppm, florfenicol amine will be at an average level of 3.7 ppm, as measured by the HPLC assay. Thus, 3.7 ppm (61% Of 6.0 ppm) is assigned as the tolerance for florfenicol amine in cattle liver.

I. Study to Establish the Withdrawal Time

Final Residue Depletion Study in Cattle Following Intramuscular Administration of SCH 25298. Study No. 90709

1. Study Author: R. Michael Bodden

2. Study Completion Date: November 26, 1991

3. Performing Laboratory:

   Hazelton Wisconsin, Inc.
   3301 Kinsman Boulevard
   Madison, Wisconsin 53704

4. Animals Used: Twenty five (25) Hereford and Hereford-cross cattle (mixed sexes) were used in this study. At the time of dosing, the animals were 7 to 8 months of age and 214 to 319 kg in body weight.

5. Route of drug administration: Each animal was given two intramuscular doses of florfenicol with a 48 hour interval in between.

6. Time and duration of dosing: Each animal received a 20 mg/kg bw dose of florfenicol followed by a second dose 48 hours later. The test animals were sacrificed in groups of five at intervals of 5, 10, 20, 30, and 40 days post-dosing and the following tissue samples were collected: liver, kidneys, muscle, fat, and injection sites.

7. Results: Samples were assayed using the high performance liquid chromatography determinative method described below under the section on the regulatory method. The assay of the liver samples yielded the mean values for the marker residue, florfenicol amine, shown in Table 6.7.

   Table 6.7. Mean concentrations (ppm) of florfenicol amine in the livers of cattle following two 20 mg/kg bw intramuscular doses of florfenicol.

Days Post-dosing	Florfenicol amine (ppm)
5	10.2
10	8.1
20	4.0
30	1.4
40	0.5

   Using these data, a withdrawal time of 28 days was calculated for use of florfenicol in cattie. The withdrawal time was calculated using the agency's statistical tolerance limit method (99% tolerance limit with a 95% confidence interval method).

   The results of total residue study, #90714, demonstrated that residues of N-methyl-2-pyrrolidone deplete to their safe concentrations in cattle by 5 days withdrawal. Because this is less than the 28 day withdrawal for florfenicol, NUFLOR® Injectable Solution will be regulated based on the depletion of residues of the active ingredient florfenicol.

J. Regulatory Method

1. Determinative Method

The determinative assay for florfenicol amine in the target tissue, liver, is a high performance liquid chromatography (HPLC) method which provides acceptable sensitivity for the routine monitoring of florfenicol residues. 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 column and eluted with ethyl acetate. The ethyl acetate elutes 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. Display the Method

   The validated regulatory method for detection of residues of florfenicol is filed in the Food Additives Analytical Manual on display in FDA's Freedom of Information Public Room (Room 12A-30), 5600 Fishers Lane, Rockville, MD 20857.

K. User Safety

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 the accidental injection or 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.

V. 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® Injectable Solution is effective for the treatment of bovine respiratory disease when administered intramuscularly as two injections of 20 mg florfenicol per kilogram body weight, 48-hours apart.

Based on a battery of toxicology tests, the safe concentrations for total florfenicol-related residues are 2.0 ppm in muscle, 6.0 ppm in liver, 12.0 ppm in kidney, and 12.0 ppm in fat. Based on metabolism studies in cattle, a tolerance (Rm) of 3.7 ppm for the marker residue, florfenicol amine, has been established in liver. The tolerance (Rm) refers to the residue measured by the regulatory method described herein.

A pre-slaughter withdrawal period of 28 days was calculated from a residue depletion study of florfenicol residues in cattle, following the administration of NUFLOR® Injectable Solution. 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.

Labeling restricts this drug to use by or on order of a licensed veterinarian. This decision was based on the following factors: (a) the product contains a new antimicrobial entity intended only for therapeutic purposes, (b) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat bovine respiratory disease, and (c) the frequency of violative tissue residues and possible emergence of resistant organisms will be reduced by the involvement of veterinarians in product use.

The agency has carefully considered the potential environmental effects of this action and has concluded that the action will not have a significant impact on the human environment and that and environmental impact statement is not required. The agency's finding of no significant impact (FONSI) and the evidence supporting that finding are contained in an environmental assessment, which may be seen in the Docket Management Branch (HFV-305), Park Building (Room 1-23), 12420 Parklawn Dr., Rockville, Maryland 20855.

Under Section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of approval because no active ingredient (including any ester or salt of the active ingredient) has been approved in any other application. NUFLOR® Injectable Solution is under U.S. patent numbers #4,235,892 and #5,082,863, which expire on November 25, 1997, and January 21, 2009, respectively.

VI. APPROVED PRODUCT LABELING

A copy of the draft facsimile labeling is attached to this document.

A. NUFLOR® Injectable Solution – Master Shipper (Case) Label

B. NUFLOR® Injectable Solution – Carton Label

C. NUFLOR® Injectable Solution – Vial Label

D. NUFLOR® Injectable Solution – Package Insert