Approval Date: June 4, 1998

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

NADA 141-063

B. Sponsor

Schering-Plough Animal Health Corporation
1095 Morris Avenue
P.O. Box 3182
Union, New Jersey 07083-1982

C. Proprietary Name

NUFLOR® Injectable Solution

D. Established Name

Florfenicol

E. Dosage Form

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.

F. Dispensing Status

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

G. Dosage Regimen:

Nuflor® Injectable Solution should be administered by intramuscular injection to cattle at a dose rate of 20 mg/kg body weight (3 mL/100 lb). A second dose should be administered 48 hours later. Alternatively, Nuflor® Injectable Solution can be administered by a single subcutaneous injection to cattle at a dose rate of 40 mg/kg body weight (6 mL/100 lb.). Do not administer more than 10 mL at each site. The injection should be given only in the neck.

NOTES: Intramuscular injection may result in local tissue reaction which persists beyond 28 days. This may result in trim loss of edible tissue at slaughter. Tissue reaction at injection sites other than the neck are likely to be more severe.

H. Route of Administration

Nuflor® Injectable Solution should be administered by intramuscular or subcutaneous injection in the neck.

I. Indication

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

J. Effect of Supplement

Provides for the use of a subcutaneous route of administration for Nuflor® Injectable Solution in cattle.

II. EFFECTIVENESS

An original new animal drug application (NADA) for Nuflor® Injectable Solution (NADA 141-063) for intramuscular administration to cattle for the treatment of bovine respiratory disease was approved May 31, 1996.

Data from the following dose selection study and field trials demonstrate that Nuflor® Injectable Solution is also effective for the treatment of bovine respiratory disease when administered by a single subcutaneous injection to cattle at a dose rate of 40 mg/kg body weight.

A. Dose Selection Study - V95-263-01

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

2. Investigator:

   Dr. Kelly Lechtenberg
   Midwest Veterinary Services
   1443 Hwy. 77
   Oakland, NE 68045

3. General Design:

   i. Purpose: To establish an effective dose of florfenicol administered subcutaneously to cattle with naturally-occurring BRD.

   ii. Animals: 175 head of feedlot cattle (50 per each florfenicol treatment group; 25 saline controls) 5 months of age or older. The initial mean weight was approximately 250 kg.

   iii. Control: Saline (negative control) administered in a volume equivalent to a dose of Nuflor® Injectable Solution administered at 20 mg/kg body weight.

   iv. Diagnosis: The diagnosis of BRD was based on acute clinical signs of pneumonia: depression score of ³ 1 (using a scale of 0 to 3; with 0 being normal and 3 being severe depression), elevated rectal temperature (³ 104º F), and abnormal respiratory character (2 or more of the following scored

"abnormal"; respiratory rate, dyspnea, cough or nasal discharge). Pretrial naso-pharyngeal swabs were taken for bacterial examination.

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

vi. Route of Administration: Subcutaneous injection

vii. Doses: Negative control (0 mg/kg) dosed once, 20 and 30 mg/kg dosed twice with a 48 hour interval, and 40 mg/kg administered as a single injection.

viii. Test Duration: Twelve (12) days.

ix. Pertinent Parameters Measured: The pivotal variable was the Day 4 and Day 11 assessment of treatment success/failure. Mortality was measured and recorded daily from Day 0 to Day 11. Rectal temperature was measured and recorded Day 0 to Day 4 and again on Day 11. Depression and abnormal respiratory character were assessed from Day 0 to Day 4, on Day 11, and upon occurrence from Day 5 through Day 10.

4. Results: More treatment successes were observed in the florfenicol groups than in the control group on Day 4 and Day 11. Florfenicol prevented mortality from respiratory disease in 100% of the treated calves in all florfenicol treatment groups. An 8% mortality was observed in the non-medicated controls (p= 0.1081). Florfenicol significantly reduced pyrexia in all treatment groups.

   Pasteurella haemolytica was isolated from 52 (30%) of the 175 pre-treatment naso-pharyngeal swabs of the cattle used in this study. *Pasteurella multocida* was isolated in 24 (14%) of the same 175 swabs. *Haemophilus somnus* was isolated in 10 (6%) of the same 175 swabs. All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol. The criteria for measuring efficacy were: initial treatment success/failure on Day 4, and overall success on Day 11. The results are presented in Table 4.1.

   Table 4.1. Percent treatment success on Days 4 and 11 in cattle treated subcutaneously with various doses of florfenicol

Dose (mg/kg)	**Dose frequency**	**Percent treatment success**			
		Day 4		**Day 11**	
0	Single dose	28	(7/25)	20	(5/25)
20	2 doses, 48 hour interval	94	(47/50)	84	(42/50)
30	2 doses, 48 hour interval	94	(47/50)	82	(41/50)

Dose (mg/kg)	**Dose frequency**	**Percent treatment success**			
		Day 4		**Day 11**	
40	Single dose	94	(47/50)	90	(45/50)

5. Statistical Analysis: The pivotal variable for clinical evaluation was the rate of treatment success for each group. Analysis was by the Kruskal-Wallis Exact Test. Pairwise analyses were by the Fisher's Exact Test. Analyses were performed for Day 4 and Day 11.

   Individual calf was the experimental unit for all analyses. The results of all statistical tests were declared significant at the alpha = 0.05 level. Preliminary statistical significance was declared when $0.05 < a < 0.10$. Analyses used two tailed tests, and pairwise analyses to negative control used one-tailed tests. Software used was SAS version 6.08 (Cary, NC) and StatXact version 2.04 (Cambridge, MA).

6. Conclusion: Based on the results of this study, florfenicol administered subcutaneously once at a dose of 40 mg/kg body weight was determined to be a safe and effective dose for the treatment of BRD. This dose was selected for further testing in field trials.

7. Adverse Reactions: There was no evidence of adverse reaction in any of the treatment groups tested.

B. Field Investigations:

1. Type of Study: Field trials were conducted at 2 locations in cattle with spontaneously occurring bovine respiratory disease (BRD).

2. Investigators:

   Dr. Kelly Lechtenberg
   Midwest Veterinary Services
   1443 Hwy. 77
   Oakland, NE 68045

   Dr. Karen Rogers
   Veterinary Research and Consulting, LLC
   5626 W. 19th Street, Suite A
   Greeley, CO 80634

3. General Design:

   i. Purpose: To confirm the therapeutic efficacy of florfenicol administered once, subcutaneously at a dose of 40 mg/kg body weight for the treatment of naturally-occurring bovine respiratory disease complex (shipping fever).

ii. Animals: One hundred-fifty (150) mixed beef breed heifers approximately five (5) months old with an approximate initial mean weight of 200 kg.

iii. Control: Saline (negative control) administered in a volume equivalent to a dose of Nuflor® Injectable Solution administered at 40 mg/kg body weight.

iv. Diagnosis: The diagnosis of BRD was based on acute clinical signs of pneumonia: depression score of ³ 1 (using a scale of 0 to 3; with 0 being normal and 3 being severe depression), elevated rectal temperature (³ 104 ºF), and abnormal respiratory character (2 or more of the following scored "abnormal"; respiratory rate, dyspnea, cough or nasal discharge). Pretrial naso-pharyngeal swabs were taken for bacterial examination.

v. Dosage Form: The dosage form was an injectable solution containing300 mg/mL florfenicol.

vi. Test Duration: Twelve (12) days.

vii. Parameters Measured: The pivotal variable was treatment success/failure on Day 4 and Day 11. Mortality was measured and recorded daily from Day 0 to Day 11. Rectal temperature was measured and recorded from Day 0 to Day 4. Depression and abnormal respiratory character were assessed from Day 0 to Day 4 and thereafter upon occurrence through Day 11.

4. Results: The pivotal variables were the Day 4 and Day 11 assessment of treatment success or failure. A success was defined for each calf as: rectal temperature < 104 ° F, normal respiratory character, and a depression score £ 1. Any calf not meeting the success criteria, or dying prior to the observation day, was classified as a treatment failure.

   In both field trials, significantly more treatment successes were observed in the florfenicol group than in the control group on Day 4 and Day 11. Florfenicol treatment also significantly reduced pyrexia in both trials.

   In the Nebraska trial, florfenicol prevented mortality in 100% of the calves whereas a 14% mortality was observed in the non-medicated controls (p=0.0003). In the Colorado trial, florfenicol prevented mortality in 99% of the calves, whereas a 24% mortality was observed in the non-medicated controls (p=0.0004).

   Treatment success for both trial locations on Day 4 and Day 11 are summarized in Tables 4.2 and 4.3.

Table 4.2. Percent treatment success on Days 4 and 11 in cattle treated subcutaneously with florfenicol at the Nebraska trial site

Dose (mg/kg)	Dose frequency	Percent treatment success			
		Day 4		Day 11	
0	Single dose	26	(13/50)	16	(8/50)
40	Single dose	91	(91/100)	85	(85/100)

Table 4.3. Percent treatment success on Days 4 and 11 in cattle treated subcutaneously with florfenicol at the Colorado trial site

Dose (mg/kg)	Dose frequency	Percent treatment success			
		Day 4		Day 11	
0	Single dose	24	(12/50)	18	9/50)
40	Single dose	71	(71/100)	53	(53/100)

5. Microbiology: In the Nebraska field trial, *Pasteurella haemolytica* was isolated from 13 (65%) of the 20 pre-treatment naso-pharyngeal swabs of the cattle used in this study. *Haemophilus somnus* was isolated in 3 (15%) of the same 20 swabs. *Pasteurella multocida* and *Actinomyces pyogenes* were isolated in 2 (10%) of the same 20 swabs. All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol.

   In the Colorado field trial, *Pasteurella haemolytica*, exclusively, was isolated from 20 (100%) of the 20 pre-treatment nasal swabs. All pathogens isolated from the naso-pharyngeal swabs were sensitive to florfenicol.

6. Statistical Analysis: The pivotal variable for clinical evaluation was the rate of treatment success for each group. Analysis was by the Fisher's Exact Test. Analyses were performed for Day 4 and Day 11.

   Individual calves were the experimental unit for all analyses. The results of all statistical tests were declared at the alpha = 0.05 level. Preliminary statistical significance was declared when $0.05 < a < 0.10$. Analyses were one-tailed tests between Nuflor® and saline (except Day 0).

7. Conclusion: Under the conditions of both field trials, florfenicol administered once, subcutaneously at a dose of 40 mg/kg body weight, was a safe and effective treatment of bovine respiratory disease.

8. Adverse Reactions: There was no evidence of adverse reaction in either of the treatment groups.

III. ANIMAL SAFETY

A. Systemic Safety

The original approval of Nuflor® Injectable Solution provided for two intramuscular injections of florfenicol to cattle, administered 48 hours apart, at a dose of 20 mg/kg. The animal safety studies conducted in support of the original approval included a drug tolerance test conducted at a 10X (200 mg/kg) overdose level and a toxicity test which addressed the safety of multiple injections of Nuflor® Injectable Solution at the 1X (20mg/kg), 3X (60 mg/kg), and 5X (100 mg/kg) dose levels (see FOI summary for NADA 141-063 dated May 31, 1996).

To assess the systemic safety of florfenicol administered as a single subcutaneous injection at a dose of 40 mg/kg, data from three pharmacokinetic studies were evaluated. A comparison of the florfenical blood concentration data from these studies indicates that, intramuscular administration of florfenicol results in higher peak blood concentrations than does subcutaneous administration. However, due to prolonged drug absorption from the subcutaneous injection site, total drug exposure (expressed as area under the curve or AUC) associated with the two dosage regimens are not significantly different.

A linear extrapolation of blood concentrations resulting from intramuscular administration of florfenicol at doses of 20 mg/kg (1X), 60 mg/kg (3X), and 100 mg/kg (5X), administered as two doses 48 hours apart, were used to predict those florfenicol serum concentrations attained during the target animal safety study conducted to support approval of the original NADA. These estimates were then compared to those concentrations observed following a single 40 mg/kg subcutaneous dose.

Based upon an evaluation of the above data, it was concluded that animals receiving two 60 mg/kg intramuscular doses, 48 hours apart, experience significantly greater drug exposure (expressed as AUC and as time at which serum concentrations remained above 1.0 mcg/mL) and significantly higher florfenicol serum concentrations (expressed as maximum serum concentration or C_{MAX}) than animals receiving a single 40 mg/kg subcutaneous dose of Nuflor® Injectable Solution. Therefore, based upon these evaluations, it was concluded that the systemic safety of a single 40 mg/kg subcutaneous dose of Nuflor® Injectable Solution has been adequately demonstrated by the target animal safety study conducted for the original approval.

B. Injection site irritation study

1. Study Director:

   Ujjana B. Nandihalli, Ph.D.
   Covance Laboratories Inc.
   3301 Kinsman Blvd.
   Madison, WI 53704

2. General Design: This study was conducted in accordance with GLP regulations and CVM guidelines. Twenty beef calves were randomized into five groups (two animals/sex/group), and administered florfenicol (Nuflor®

Injectable Solution) subcutaneously at a dose of 40 mg/kg on Day 1. One group each was sacrificed at 14, 21, 28, 35, and 42 days post-dose. In addition, one steer was untreated and served as control, and was necropsied on Day 15.

This study utilized the same animals to establish the depletion profile of florfenicol residues (final residue study), and to evaluate injection site reactions (injection site irritation study). The 40 mg/kg dose of florfenicol was given in four to five sites per animal with a maximum volume of 10 mL of Nuflor® Injectable Solution per site. Two injection sites per calf were used for the pathologic evaluation portion of this study. Emphasis was placed on identifying gross lesions which would require trim-out at the time of slaughter if the animal were to be processed for food.

i. Purpose: The purpose of pathologic evaluation portion of this study was to determine the lesions associated with a single subcutaneous dose of 40 mg of florfenicol/kg of body weight in cattle, evaluated at specified post-dose time points. Each injection site received the maximum volume of 10 mL of Nuflor® Injectable Solution.

ii. Animals: 21 cross-bred beef calves with an initial mean weight of approximately 280 kg.

iii. Control: one steer

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

v. Route of Administration: Subcutaneous injection

vi. Test Duration: 43 days

vii. Pertinent Measurements and observations: Clinical observations, injection site palpation, gross and microscopic pathologic evaluation

3. Results:

i. Clinical Observation/Injection Site Palpation: Early in the post-dose period, test article-related swelling was noted at many of the injection sites, and usually correlated grossly at necropsy with thickening and discoloration at the injection sites.

ii. Gross Pathology Observations: The 14- and 21-day post-dose necropsies revealed injection site gross lesions, subcutaneous thickening, and/or discolored areas, in all animals. At the 14-day post dose necropsies, one gross lesion extended into the underlying skeletal muscle. In the 28-, 35-, and 42-day post-dose necropsies, injection site gross lesions were limited to subcutaneous thickening in one animal.

4. Conclusions: Subcutaneous administration of Nuflor® Injectable Solution at 40 mg/kg induced transient swelling that tended to resolve quickly. No gross

lesions involving muscle tissue were observed to persist beyond the 38-day withdrawal period established for the subcutaneous route of administration.

IV. HUMAN FOOD SAFETY

A. Toxicity Studies

The toxicity studies conducted with florfenicol in various test animal species are described in the FOI Summary for the original approval of NADA 141-063. No new toxicity studies were conducted in support of this supplement.

B. Safe Concentrations for Total Residues of Florfenicol

Safe concentrations for total residues of florfenicol in edible tissues of cattle were established with the original NADA 141-063 and are listed in Table 6.1.

Table 6.1. Safe concentrations for total residues of florfenicol in edible tissues of cattle

Tissue	Safe concentration (ppm)
Muscle	2
Liver	6
Kidney	12
Fat	12

C. Total Residue and Metabolism Studies and Marker Residue Tolerance

Total residue and metabolism studies with florfenicol administered intramuscularly to cattle are described in the FOI Summary for the original NADA 141-063. Those studies allowed the assignment of a tolerance of 3.7 ppm for florfenicol amine (the marker residue) in cattle liver (the target tissue). No new total residue or metabolism data were required for this supplement to NADA 141-063. As explained in Part F of this section of the FOI Summary, a muscle tolerance of 0.3 ppm florfenicol amine is established with this supplement as the tolerance for residues of florfenicol amine in cattle muscle.

D. Study Establishing Withdrawal Period

1. Type of Study: Tissue residue depletion

2. Study Director:

   Ujjana B. Nandihalli, Ph.D.
   Covance Laboratories Inc.
   3301 Kinsman Blvd.
   Madison, WI 53704

3. Study Design and Methods:

   Twenty one healthy, uniform crossbred calves, weighing approximately 234-322 kg, were randomly assigned to one of five sacrifice groups. One herdmate was used as a source of control tissue. Calves were dosed with

Nuflor® Injectable Solution subcutaneously once at a dose rate of 40 mg florfenicol/kg of body weight. The dose was administered using multiple sites of injection; with a maximum injection volume of 10 mL per site.

Groups were sacrificed at 14, 21, 28, 35, and 42 days after dosing. Skeletal muscle beneath the marked injection site, and the liver (target tissue) were collected from each animal and analyzed for the marker residue, florfenicol amine, by HPLC.

4. Results:

Samples were assayed using a high performance liquid chromatography determinative method that was approved with the original NADA. The assay of the liver and injection site samples yielded the mean values for the marker residue, florfenicol amine, shown in Table 6.2.

Table 6.2. Mean concentrations (ppm) of florfenicol amine in the muscle, livers, and injection sites of cattle following one 40 mg/kg subcutaneous dose of florfenicol.

Days Post-dosing	Florfenicol amine (ppm)		
	Muscle	**Liver**	**Injection Site**
14	0.14 ± 0.03	8.32 ± 0.03	135.49 ± 142.03
21	0.11 ± 0.01	5.97 ± 0.75	0.80 ± 0.87
28	0.05 ± 0.01	3.01 ± 0.36	5.53 ± 10.60
35	0.08 ± 0.01	2.12 ± 0.78	0.16 ± 0.06
42	0.00 ± 0.00	1.48 ± 0.41	0.14 ± 0.06

5. Calculation of a Withdrawal Time

Using the liver residue depletion data summarized in Table 6.2, and the tolerance of 3.7 ppm for florfenicol amine in cattle liver, a withdrawal time of 38 days was calculated. The calculation was made with the agency's statistical tolerance limit method (99% tolerance limit with a 95% confidence interval).

6. Assignment of a Tolerance for Residues in Cattle Muscle

In accordance with current CVM policy, a tolerance of 0.3 ppm is established for residues of florfenicol amine in cattle muscle. That value was obtained from a plot of the 99% upper tolerance limits (95% confidence) calculated from the residue values for florfenicol amine in cattle muscle reported in Study No. 96420 (Table 6.2). The plot showed that residues of florfenicol amine will be at or below 0.3 ppm in the muscle tissue of cattle at approximately three weeks post dosing. That interval is well within the 28-day withdrawal period established for florfenicol in cattle with intramuscular dosing, as well as the 38-day withdrawal period required with subcutaneous dosing.

7. Regulatory Method

A determinative assay procedure based on high performance liquid chromatography was approved with the original NADA 141-063 for the

measurement of residues of florfenicol amine in cattle liver. Validation data have also been provided to assure the method performs adequately in muscle. A copy of the procedure 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.

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 safe and effective for the treatment of bovine respiratory disease when administered subcutaneously as a single injection at a dose of 40 mg florfenicol per kilogram body weight. Under the Center's supplemental approval policy [21 CFR 514.106(b)(2)(iv)], this is a Category II supplement which required a re-evaluation of the safety and effectiveness data.

Based on a battery of toxicology tests conducted in support of the original approval of florfenicol in cattle, an acceptable daily intake of 10 m g/kg body weight/day was calculated. This further yielded safe concentrations for total florfenicol residues of 2 ppm in muscle, 6 ppm in liver, 12 ppm in kidney, and 12 ppm in fat. A tolerance of 3.7 ppm for florfenicol amine (the marker residue) was established in liver (the target tissue) based on metabolism studies conducted in support of the original approval of florfenicol in cattle. A tolerance of 0.3 ppm has been established with this supplement for florfenicol amine in cattle muscle based on the relationship of florfenicol amine to total residue and a safe concentration of 2 ppm total residue. The tolerance refers to the residue measured by the regulatory method described herein.

A pre-slaughter withdrawal period of 38 days has been assigned for cattle administered a single subcutaneous injection of 40 mg florfenicol per kilogram body weight. The withdrawal period 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. The Center for Veterinary Medicine (CVM) has concluded that this product shall continue to have prescription marketing status.

The agency has determined under 21 CFR 25.33 (a)(1) that this action is of the type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

Under Section 512(c)(2)(F)(iii) of the FFDCA, this approval for food producing animals qualifies for THREE years of marketing exclusivity beginning on the date of approval because the supplemental application contains substantial evidence of the effectiveness of the drug involved, any studies of animal safety, or, in the case of food producing animals, human food safety studies (other than bioequivalence or residue studies) required for the approval of the application and conducted or sponsored by the applicant.

The three years of marketing exclusivity applies only to the subcutaneous route of administration in cattle for which the supplemental application was approved. Nuflor® Injectable Solution is under U.S. patent number 5,082,863, which expires January 21, 2009.

The format of this FOI Summary document has been modified from its original form to conform with Section 508 of the Rehabilitation Act (29 U.S.C. 794d). The content of this document has not changed.