Date of Approval: December 16, 2011

FREEDOM OF INFORMATION SUMMARY

SUPPLEMENTAL NEW ANIMAL DRUG APPLICATION

NADA 141-207

ADVOCIN
Danofloxacin Mesylate
Injectable Solution
Beef Cattle

Additional dosage regimen for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica* and *Pasteurella multocida* in beef cattle at a single subcutaneous dose of 8 mg danofloxacin/kg body weight (BW).

Sponsored by:

Pfizer, Inc.

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I. GENERAL INFORMATION:

A. File Number:	NADA 141-207
B. Sponsor:	Pfizer, Inc. 235 East 42^{d} St. New York, NY 10017 Drug Labeler Code: 000069
C. Proprietary Name:	ADVOCIN
D. Established Name:	Danofloxacin mesylate
E. Pharmacological Category:	Antimicrobial
F. Dosage Form(s):	Sterile injectable solution
G. Amount of Active Ingredient(s):	180 mg danofloxacin per mL
H. How Supplied:	100 mL and 250 mL bottles
I. How Dispensed:	Rx
J. Dosage(s):	Administered by subcutaneous injection at either 8 mg per kilogram of body weight once or 6 mg per kilogram of body weight with treatment repeated once approximately 48 hours following the first injection.
K. Route of Administration:	Subcutaneous (SC) injection
L. Species/Classes:	Cattle/beef
M. Indication:	For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica* and *Pasteurella multocida*.
N. Effect of Supplement:	This supplement provides for the administration of a subcutaneous dose of 8 mg/kg body weight given as a single dose.

II. EFFECTIVENESS:

A. Dosage Characterization:

The Freedom of Information (FOI) Summary for the original approval of NADA 141-207 (A180 Sterile Antimicrobial Injectable Solution) dated September 20, 2002, contains dosage characterization information for cattle and documents the effectiveness of a subcutaneous dose of danofloxacin 18% injectable solution administered at 6 mg/kg body weight (BW) and repeated once approximately 48 hours later. A dose determination study was performed in the United States, using a target dose of 8 mg/kg BW danofloxacin 18% injectable solution administered as a one-time subcutaneous injection in cattle. During the study, animals were evaluated for clinical signs of BRD and the effectiveness of treatment was based on the comparison of treated to control animals. A pooled analysis of the multiple sites in the study was conducted, with success rate determined on Study Day 12. Based on the results of this pilot study, a target dose of 8 mg/kg BW administered once through subcutaneous injection was selected for evaluation in pivotal clinical studies.

B.Substantial Evidence:

Effectiveness was confirmed in a four site well-controlled study of naturally acquired bacterial respiratory infections in feedlot age cattle. This study was conducted under commercial conditions at four locations in North America.

1. Title

"Efficacy of danofloxacin 18% against spontaneous bovine respiratory disease" Study Site Numbers 1133C-60-96-249; 1133C-60-96-250, 1133C-60-97-258, and 1133C-60-97-261. October 1996 to April 7, 1999.

2. Investigators and Study Locations

Study Site Number 1133C-60-96-249 – Ed G. Johnson, D.V.M. Johnson Research, Parma, ID

Study Site Number 1133C-60-96-250 – Terry TerHune, DVM, PhD; Corcoran Feedyard, Corcoran, CA

Study Site Number 1133C-60-97-258 – David Bechtol, DVM; Agri Research Center, Inc., Canyon, TX

Study Site Number 1133C-60-97-261 – Kelly Lechtenberg, DVM, PhD; Midwest Veterinary Services, Oakland, NE

3. Study Design

a. Objective: To confirm the effectiveness of danofloxacin 18% injectable solution against naturally-occurring bovine respiratory disease (BRD) under commercial conditions when administered subcutaneously at 8 mg danofloxacin/kg body weight as a single treatment.

b. Six to 11-month old male castrate crossbred calves were utilized in the study. Body weight ranged from 165 to 332 kg. Forty (40) animals were assigned to the danofloxacin treatment group at each of the four study locations for a total of 160 calves treated at 8 mg/kg with a single dose. Twenty additional animals were assigned to the negative control group at each site for a total of 80 calves. Control animals were handled in the same manner as those assigned to the drug treatment groups.

c. Study Duration: The study duration was 10 days from Day 0 (enrollment and first treatment) to Day 10.

d. Test Article: ADVOCIN (danofloxacin mesylate) 18% injectable solution dosed at 8mg/kg BW one-time subcutaneous injection. Sterile saline administered one-time subcutaneously feed was used as the control article.

e. Experimental Design: This study was conducted at four sites. The study was originally designed as a five-site study, but Study Site Number 1133C-60-96-248 was excluded from the effectiveness analysis due to a confirmed Bovine Viral Diarrhea Virus (BVDV) outbreak. The study used a randomized complete block design at each site, as animals met enrollment criteria; each was randomly assigned to treatments and pens according to a generalized complete block design. Study personnel were masked to treatment groups.

f. Measurements and Observations: Recently transported calves with clinical signs of acute BRD (compromised respiration and depression) and rectal temperatures >104.0 °F (40.0 °C) were selected for the study. At enrollment, a nasopharyngeal swab was obtained from each calf for bacteriologic analysis.

   The primary parameter for determining effectiveness was treatment success on Day 10. Treatment success (calves identified as responders during Days 3 to 5 that did not relapse by Day 10) was based on respiratory signs, attitude, and rectal temperature.

g. Statistical Analysis: Each study site was evaluated individually and then the data were pooled for analysis.

4. Results

Animals administered danofloxacin demonstrated significantly higher ($p = 0.0222$) Day 10 success rates across all four study sites when compared to animals administered the saline control. Summaries of the treatment success results by trial location are presented in Table 2.1 below.

One hundred-seven, ninety-six, and five of the pretreatment nasopharyngeal swabs were positive for *Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni (Haemophilus somnus)*, respectively.

Table 2.1 Percent responders and treatment success in cattle with spontaneously occurring BRD treated with danofloxacin.

Location	Treatment (mg/kg)	% Responders (Day 3-5)	% Success (Day 10)
Idaho	0	40.0% (8/20)	40.0% (8/20)
	8 (single dose)	97.5% (39/40)	90.0% (36/40)
Nebraska	0	10.0% (2/20)	10.0% (2/20)
	8 (single dose)	70.0% (28/40)	67.5% (27/40)
California	0	55.0% (11/20)	35.0% (7/20)
	8 (single dose)	90.0% (36/40)	90.0% (36/40)
Texas	0	75.0% (15/20)	75.0% (15/20)
	8 (single dose)	85.0% (34/40)	85.0% (34/40)
Total	0	45.0% (36/80)	40.0% (32/80)
	8 (single dose)	85.6%(137/160)	83.1%(133/160)

5. Adverse Events

There were no test article-related adverse events observed during the study.

6. Conclusion

This study demonstrates that ADVOCIN (danofloxacin mesylate) 18% injectable solution is effective for the treatment of BRD associated with *Mannheimia haemolytica* and *Pasteurella multocida* in beef cattle when administered at 8 mg/kg BW as a one-time subcutaneous injection.

III. TARGET ANIMAL SAFETY:

CVM did not require target animal safety studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-207 dated September 20, 2002, contains a summary of target animal safety studies for beef cattle.

IV. HUMAN FOOD SAFETY:

A. Microbial Food Safety:

Two studies were conducted by the sponsor to address outstanding exposure concerns associated with the administration of a single dose of danofloxacin 18% injectable solution at 10 mg/kg to cattle. These studies aimed to assess 1) the development of ciprofloxacin (CIP), nalidixic acid (NAL), and/or ceftriaxone resistance in *Salmonella typhimurium* and/or commensal *Escherichia coli* in the feces, and 2) the numbers of *S. typhimurium* and commensal *E. coli* in the feces following treatment.

Study 1
Pfizer Study 1430N-60-99-297
Effects of danofloxacin administration on ciprofloxacin susceptibility of fecal *Salmonella typhimurium* and *Escherichia coli* in calves experimentally infected with *S. typhimurium*
Colorado Animal Research Enterprises, Wellington, CO
Study director: Diane J. Fagerberg, Ph.D.

A total of 28, seven to nine-month old cross-bred beef calves (heifers) were divided into two groups: a control group (n=16) and a danofloxacin-treated group (n=12). Calves were individually housed in isolation rooms. Calves were tested and shown to be free of salmonellae prior to procurement. Calves were not tested for *E. coli* or susceptibility profiles of *Salmonellae* or *E. coli*.

The control group received sterile saline subcutaneously (SC) twice, 48 hours apart, on Days 0, 14, and 28. The treated group received a single SC injection of danofloxacin 18% injectable solution at 10 mg/kg body weight, on Days 0, 14, and 28.

Prior to treatment, all calves were challenged with a test strain of *S. typhimurium*, shown to be highly susceptible to ciprofloxacin (CIP - MIC≤0.015 µg/mL), but resistant to streptomycin. Colonization was shown to be successful, and calves were treated according to treatment allocation as described above. Fecal samples were collected by rectal retrieval on selected days until 56 days after the first dose. They were collected following each treatment regimen and were cultured for *Salmonella* and *E. coli* with appropriate selective media and identification schemes.

From each sample obtained from each animal, up to five isolated colonies each of the recoverable challenge strain of *S. typhimurium* were selected from MacConkey agar (MAC) plates supplemented with streptomycin. Similarly, up to five isolated colonies of *E. coli*, were selected from MAC. *S. typhimurium* and commensal *E. coli* isolates were subjected to CIP susceptibility testing using a broth microdilution method. Susceptibility testing was performed according to standards published by the National Committee for Clinical Laboratory Standards (now the Clinical and Laboratory Standards Institute - CLSI).

Results for *Salmonella* indicated no increase in shedding as a result of danofloxacin treatment. No significant (P≤0.05) change was observed in the susceptibility of the challenge strain of *S. typhimurium* to CIP following exposure to danofloxacin as judged by the total MIC range, and no discernable change among MIC_{50} or MIC_{90} values.

Results for commensal *E. coli* indicated there was no increase in shedding as a result of danofloxacin treatment. However, CIP-resistant *E. coli* were detected in 5/7 calves sampled within 48 hours after receiving the first dose, and 25/25 of those isolates had MICs > 16 µg/mL. CIP-resistance persisted in three calves to Day 42, when resistance decreased to an overall prevalence of 8% in one calf on Days 49 and 56.

Results indicated that the rapid onset, high prevalence, and persistence of CIP resistant commensal *E. coli* following a single SC injection of danofloxacin raised

concerns with regard to the microbial food safety at the proposed dose; therefore, additional data were required to mitigate concerns.

Study 2
Pfizer Study 1437N-60-10-828
Effects of a Single Administration of Danofloxacin 18% Injectable Solution on Ciprofloxacin Susceptibility of *Escherichia coli* isolated from Feces of Calves
Pfizer Animal Health Richland Farm, Richland, MI; and Micromyx, LLC, Kalamazoo,MI.
Study director: Colin J. Giles, BVetMed, PhD, MRCVS.

Forty beef cattle were selected from a herd with individuals that carried both CIP- and nalidixic acid (NAL)-resistant *E. coli*. Cattle were group-housed and acclimated to the facility for 17 days before randomization. The day of treatment (Day 0) was prospectively identified and fecal samples were collected by rectal retrieval from all cattle on Days -10, -7, and -4. On Day -4, 24 animals were randomly allotted to treatment group (danofloxacin or saline, 12 per group), weighed and moved to pens (two cattle per pen, one animal from each treatment group). An additional pen (two sentinel animals) was assigned as a biosecurity control that received neither treatment. On Day 0, animals received either a single SC injection of danofloxacin 18% injectable solution at 8 mg/kg or saline. Additional fecal samples were collected by rectal retrieval on Days 0, 2, 4, 6, 14, 21, and 28.

Fecal samples were analyzed for viable commensal *E. coli* in colony forming units (CFU)/g of feces on MAC for total *E. coli* counts, and for quinolone-resistant counts, on MAC with added 1 µg/mL CIP (MAC+CIP) and on MAC with added 16 µg/mL NAL (MAC+NAL). Fecal counts were analyzed on a dry weight basis and to account for the high frequency of non-detectable *E. coli* data points, a Kaplan-Meier method was used to test the difference of flipped and logtransformed counts between baseline and each sampling day for each test by treatment.

Representative colonies from MAC+NAL and MAC+CIP were subcultured for susceptibility testing against NAL, CIP and ceftriaxone by broth microdilution methods in accordance with CLSI standards. Susceptibility testing was only performed on isolates from MAC plates on Days -10, -7, -4, and 0; but performed on any *E. coli* colonies (limit of 3 per plate) present on MAC+NAL or MAC+CIP plates on all sampling days pre- and post dose. The identities of putative *E. coli* were confirmed using the BBL Crystal System. The few instances of non-confirmation were recorded and subsequently excluded from summaries and analysis.

Across all time points, the sentinel and saline-treated animals showed total *E. coli* counts that varied from 8.7 x 103 to 1.1 x 108 CFU/g of feces dry weight.

Total counts for the danofloxacin-treated animals were broadly similar, but there was a transient, approximate 2 log10 drop in mean count on Day 2 in response to danofloxacin treatment.

The counts derived from the MAC+NAL and MAC+CIP plates indicate that both NAL-resistant *E. coli* and CIP-resistant *E. coli* were present in the cattle population pre-treatment in both treatment groups. There was no evidence of transmission of resistant *E. coli* between pens.

On Day 2, two of the danofloxacin-treated and two of the saline-treated animals had both NAL-resistant and CIP-resistant *E. coli* in their feces. Thereafter, in the danofloxacin-treated group, there was no detectable NAL- or CIP-resistant *E. coli* present in the feces. For the purpose of data analysis, baseline was defined as the mean CFU/g dry weight of Day -4 and Day 0 (before treatment). Analysis of fecal CFU/g dry weight with baseline as the covariate for both NAL-resistant and CIP-resistant *E. coli,* indicated there was neither a significant treatment effect (P=0.4212, for NAL; P=0.4081 for CIP) nor a treatment by sampling point interaction effect (P=0.7984 for NAL; P=0.7291 for CIP). A significant correlation of total *E. coli* (not CIP- or NAL-resistant *E. coli*) between two pens of commingled animals, suggests transmission was more likely in these pens.

These data indicate that, at any time point and across time points post-treatment, there were no significant differences detected (P>0.1) in populations of NAL- or CIP-resistant *E. coli* carried in the feces of danofloxacin-treated cattle between the groups.

MIC data showed that *E. coli* isolates demonstrating resistance to both NAL and CIP were detectable within the cattle population both before and after either treatment. No NAL- or CIP-resistant *E. coli* isolates were detected in the danofloxacin-treated group after Day 2 post-treatment. None of the *E. coli* isolates, including those resistant to CIP or NAL, were resistant to ceftriaxone.

Qualitative Antimicrobial Resistance Risk Assessment

The microbial food safety assessment was based on a qualitative antimicrobial resistance risk assessment, and included a release assessment to describe the probability that danofloxacin and its use in beef production will result in the emergence of resistant bacteria or resistance determinants in treated cattle under the proposed conditions of use; an exposure assessment to describe the likelihood of human exposure to resistant bacteria or resistance determinants through consumption of edible products from treated cattle; and a consequence assessment to describe potential human health consequences arising from exposure to defined resistant bacteria or resistance determinants by considering the human medical importance of antimicrobials (e.g., CIP) used in the treatment of human infectious diseases. The microbial food safety of danofloxacin under the proposed conditions of use was judged by the Agency on the sponsor's hazard characterization and qualitative antimicrobial resistance risk assessment, as well as on the data from the studies described above. While Pfizer study, 1430N-60-99-297 showed a rapid onset, high prevalence, and persistence of CIP-resistant commensal *E. coli* following a single SC injection of danofloxacin, the subsequent Pfizer study, 1437N-60-10-828, was more robust, better targeted microbiological endpoints of concern, and was more appropriately designed to assess the effects of a single 8 mg/kg dose of danofloxacin on the development and persistence of quinolone-resistant populations of *E. coli*.

Decision Statement: The Agency's evaluation of this information, and additional consideration of the therapeutic use in beef cattle for treating individual animals with a single dose, and an overall low extent of use, resulted in the Agency's individual rankings of **Medium** for the release assessment, **Medium** for the exposure assessment, and **High** for the consequence assessment. The Agency determined that the overall risk estimation associated with the use of the

danofloxacin in beef cattle under the proposed conditions is **High**, corresponding with mitigation strategies assigned to Category 1 antimicrobial drugs for food-animal use. Risk management steps for a Category 1 antimicrobial drug include prescription marketing status, extra-label use restriction, use in individual diseased animals, and continued monitoring by the National Antimicrobial Resistance Monitoring System (NARMS). These are all applicable to the use of danofloxacin in beef cattle as described above.

B.Impact of Residues on Human Intestinal Flora:

The agency did not require additional information on the impact of residues of danofloxacin on human intestinal flora for this supplemental approval. The FOI Summary for the original approval under NADA 141-207, dated September 20, 2002, contains a summary of all information used to assess the impact of danofloxacin residues on human intestinal flora. The established toxicological ADI (144 µg/person/day or 0.0024 mg/kg BW/day) is protective against the effects of residues of danofloxacin on human intestinal flora.

C. Toxicology:

CVM did not require toxicology studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-207 dated September 20, 2002, contains a summary of all toxicology studies.

D. Assignment of the Final ADI:

The lowest NOEL for danofloxacin in toxicity studies was 2.4 mg/kg, established based on quinolone-induced lesions in articular cartilage at higher doses in a 3-month dog study. The desmethyldanofloxacin metabolite comprises an important concentration of the total residues and its toxicity is approximately 10-fold higher than that of danofloxacin; therefore, the standard Acceptable Daily Intake calculation for total residues using a safety factor of 1000 applies. Applying a safety factor of 1000 to this NOEL, an ADI is calculated as shown below.

$$\text{ADI} = \frac{\text{NOEL}}{\text{Safety Factor}}$$

$$\text{ADI} = \frac{2.4 \text{ mg/kg/day}}{1000 \text{ Safety Factor}} = 0.0024 \text{mg/kg/day}$$

E.Safe Concentrations for Total Residues (edible tissues and injection sites, if applicable):

A safe concentration in muscle is calculated from the acceptable daily intake, assuming the average weight of a man to be 60 kg and the daily human intake of muscle to be 300 g, as follows:

$$\text{Safe concentration in muscle} = \frac{(60 \text{ kg})\ (0.0024 \text{ mg/kg/day})}{300 \text{ g}} = 0.48 \text{ ppm}$$

The safe concentration of residues in liver, kidney and fat are determined from this number using appropriate food consumption values (food factors) for these tissues. Therefore, the safe concentrations are:

Liver: 0.48 ppm x 3 (food factor) = 1.44 ppm
Kidney: 0.48 ppm x 6 (food factor) = 2.88 ppm
Fat: 0.48 ppm x 6 (food factor) = 2.88 ppm

F.Residue Chemistry:

CVM did not require residue chemistry studies for this supplemental approval. The FOI Summary for the original approval of NADA 141-207 dated September 20, 2002, contains a summary of residue chemistry studies for cattle.

G. Analytical Method for Residues:

The FOI Summary for the original approval of NADA 141-207 dated September 20, 2002, contains the analytical method summaries for danofloxacin in cattle.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to ADVOCIN:

For use in animals only. Keep out of reach of children. Avoid contact with eyes. In case of contact, immediately flush eyes with copious amounts of water for 15 minutes. In case of dermal contact, wash skin with soap and water. Consult a physician if irritation persists following ocular or dermal exposures. Individuals with a history of hypersensitivity to quinolones should avoid this product. In humans, there is a risk of user photosensitization within a few hours after excessive exposure to quinolones. If excessive accidental exposure occurs, avoid direct sunlight.

VI. 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. The data demonstrate that ADVOCIN, when administered subcutaneously to beef cattle at a dose of 8 mg/kg body weight as a single injection, is safe and effective for the treatment of BRD associated with *Mannheimia haemolytica* and *Pasteurella multocida*. Additionally, data demonstrate that residues in food products derived from cattle treated with ADVOCIN will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

Labeling restricts this drug to use by or on order of a licensed veterinarian. This decision was based on the following factors: (a) adequate directions cannot be written to enable lay persons to appropriately diagnose and subsequently use this product to treat BRD associated with *Mannheimia haemolytica* and *Pasteurella multocida,* and (b) restricting this drug to use by or on order of a licensed veterinarian should help prevent indiscriminate use that could result in violative tissue residues.

B.Exclusivity:

Under section 512(c)(2)(F)(iii) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for THREE years of marketing exclusivity beginning on the date of the approval. The three years of marketing exclusivity apply only to the single dose administration at 8 mg/kg BW for which this supplement is approved.

C. Supplemental Applications:

This supplemental NADA did not require a reevaluation of the safety or effectiveness data in the original NADA (21 CFR 514.106(b)(2)).

D. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.