Date of Approval: September 20, 2002

FREEDOM OF INFORMATION SUMMARY

Original New Animal Drug Application

NADA 141-207

A180® Sterile Antimicrobial Injectable Solution
(danofloxacin mesylate)

"……for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia* (*Pasteurella) haemolytica*, and *Pasteurella multocida*."

Sponsored by:

Pfizer Animal Health

1. GENERAL INFORMATION:

a. File Number:	NADA 141-207
b. Sponsor:	Pfizer Inc. 235 East 42nd Street New York, NY 10017 Drug Labeler Code 000069
c. Established Name:	Danofloxacin
d. Trade Name:	A180®
e. Dosage Form:	Sterile injectable solution
f. How Supplied:	100 mL and 250 mL bottles
g. How Dispensed:	Rx
h. Amount of Active Ingredients:	180 mg danofloxacin per mL
i. Route of Administration:	Subcutaneous (SC) injection
j. Species/Class:	Beef cattle
k. Recommended Dosage:	Administer a subcutaneous dose of 6 mg/kg body weight (1.5 mL/100 lb). Treatment should be repeated once at approximately 48 hours following the first treatment. Volume administered should not exceed 15 mL per injection site.
l. Pharmacological Category:	Antimicrobial
m. Indications:	For the treatment of bovine respiratory disease (BRD) associated with *Mannheimia* (*Pasteurella) haemolytica* and *Pasteurella multocida*

2. EFFECTIVENESS:

The effectiveness of danofloxacin 18% injectable solution was established in clinical dose range-finding studies conducted to evaluate the efficacy of danofloxacin against spontaneous bovine respiratory disease (BRD).

Effectiveness was confirmed in four well-controlled studies of naturally acquired bacterial respiratory infections in feedlot age cattle. These studies were conducted under commercial conditions at four locations in North America.

a. Dosage Characterization:

Clinical field studies using varying dosage regimens were conducted to evaluate the efficacy of danofloxacin against spontaneous BRD.

Dose Range-Finding Studies

Recently transported steers with clinical signs of acute BRD (increased rate and/or abnormal character of respiration and depression) and rectal temperatures $\geq$ 40.3°C (104.5°F) were used. At enrollment, a nasopharyngeal swab was obtained from each calf for bacteriologic analysis. Calves were clinically assessed by a veterinarian unaware of treatment assignments (masked studies), and rectal temperatures were determined daily for 10 days after enrollment. Treatment success (calves identified as responders during days 3-5 that did not relapse by day 10) was based on observations of respiratory signs, attitude, and rectal temperature.

Two hundred calves were randomly assigned to one of four treatments which received either saline (n=20), 4 mg danofloxacin/kg twice at an interval of 24 hours (n=60), 4 mg danofloxacin/kg once daily for 72 hours (n=60), 6 mg danofloxacin/kg twice at an interval of 48 hours (n=60).

One hundred-eight (108), and nine (9) pretreatment (Day 0) respiratory tract samples were positive for *Pasteurella haemolytica* and *Pasteurella multocida,* respectively. Twenty-four hours after the first treatment, and through Day 6, the rectal temperatures of the animals in the danofloxacin treatments were significantly lower than the negative controls. Based upon clinical results of this dose finding study, the possible regimens considered were 4 mg/kg 3 times, 24 hrs apart or 6 mg/kg twice, 48 hrs apart. Upon evaluating the pharmacokinetic profiles associated with danofloxacin, the 6-0-6 regimen was found to be most consistent with dose optimization of fluoroquinolones (i.e., minimizing the frequency and duration of administration while maximizing systemic drug exposure). Therefore, the 6-0-6 dosing regimen was selected for testing in the pivotal trials.

b. Substantial Evidence:

Pivotal Studies, Effectiveness Confirmation

i. Type of Study: Multi-location, masked, clinical effectiveness and safety study involving four locations and a total of 240 animals.

ii. Investigators:

Ed G. Johnson, D.V.M.
Johnson Research
Parma, ID 83660

Terry TerHune, D.V.M., Ph.D.
Corcoran Feedyard
Corcoran, CA 93212

Kelly Lechtenberg, D.V.M., Ph.D.
Midwest Veterinary Services, Inc
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David Bechtol, D.V.M.
Agri Research Center, Inc.
Canyon, TX 79015

iii. General Design:

Purpose: To confirm the effectiveness and safety of danofloxacin 18% injectable solution against spontaneous bovine respiratory disease (BRD) under commercial conditions when administered subcutaneously at 6 mg danofloxacin/kg body weight every 48 hours for two treatments.

Animals: 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 groups at each of the four study locations for a total of 160 treated calves. 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.

Control: Sterile saline administered subcutaneously.

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

1) Dosage Form: Proposed commercial formulation - aqueous solution containing 180 mg danofloxacin per milliliter.

2) Route of Administration: Subcutaneous injection.

3) Dose: 6 mg danofloxacin/kg twice at an interval of 48 hours.

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

5) Pertinent Variables Measured: The primary parameter for determining effectiveness was treatment success on Day 10. Treatment success (calves identified as responders during days 3-5 that did not relapse by day 10) was based on observations of respiratory signs, attitude, and rectal temperature.

iv. Results:

There were two mortalities in the negative controls. There were no mortalities in the danofloxacin treatment group. The danofloxacin treatment had significantly higher frequencies of responders compared with the negative control treatment. The frequency of relapses for danofloxacin treated calves was significantly lower than for the negative controls. The frequency of treatment success for danofloxacin treated calves were significantly higher compared with the negative controls. 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 *Pasteurella haemolytica*, *Pasteurella multocida* and *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)
	6 (twice, 48h apart)	94.7% (36/38)*	89.5% (34/38)*
Nebraska	0	10.0% (2/20)	10.0% (2/20)
	6 (twice, 48h apart)	87.5% (35/40)	87.5% (35/40)
California	0	55.0% (11/20)	35.0% (7/20)
	6 (twice, 48h apart)	95.0% (38/40)	95.0% (38/40)
Texas**	0	75.0% (15/20)	75.0% (15/20)
	6 (twice, 48h apart)	85.0% (34/40)	77.5% (31/40)
Total	0	45.0% (36/80)	28.3% (32/80)
	6 (twice, 48h apart)	90.5% (143/158)*	87.3% (138/158)*

* Two animals were excluded by protocol and were not included in the study results.

** Outbreak was atypical with saline treated animals exhibiting a high spontaneous cure and a low rate of isolation of target pathogens.

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

vi. Conclusions: In field outbreaks of BRD under actual use conditions, danofloxacin at a dose of 6 mg/kg given subcutaneously and repeated 48 hours later for a total of two doses, was safe and effective in reducing the clinical signs of acute BRD.

vii. Adverse Reactions: No adverse reactions were reported in this study.

Microbiology Studies

i. Type of Study: *In Vitro* Susceptibility Study - The *in vitro* activity of danofloxacin against selected cattle pathogens was assessed.

ii. Investigator: Don Bade
Colorado Animal Research Enterprises, Inc.
Fort Collins, CO 80524

iii. Purpose: To determine the minimum inhibitory concentrations (MICs) of danofloxacin for pathogens known to cause cattle respiratory disease.

iv. Samples: Bacterial isolates were obtained pretreatment from clinical cases of acute spontaneous BRD enrolled in various clinical studies conducted in North America from 1994 to 1997, including the isolates from the pivotal effectiveness confirmation studies.

v. Procedure: The identity of each isolate was confirmed and susceptibility tested using the standardized microdilution technique (Sensititre/Alamar, Accumed International). Data for the *in vitro* activity of danofloxacin against *Mannheimia* (*Pasteurella) haemolytica*, *Pasteurella multocida* and *Haemophilus somnus* are presented in Table 4.3.

Table 2.2 *In vitro* activity of danofloxacin against field isolates of bacterial pathogens of cattle (1994-1997).

		Minimum Inhibitory Concentration (MIC)			
Species	Number of Isolates	Min. (μg/mL)	Mode (μg/mL)	Max. (μg/mL)	MIC_{90} ** (μg/mL)
Mannheimia	363	≤0.015	0.06	0.12	0.06

(Pasteurella) haemolytica					
Pasteurella multocida	301	≤0.015	≤0.015	0.12	≤0.015
Haemophilus somnus*	32	≤0.015	0.03	0.06	0.06

* The clinical significance of these *in vitro* data have not been demonstrated.
** The minimum inhibitory concentration for 90% of the isolates.

Pharmacology Studies

Pharmacokinetic Evaluation: The pharmacokinetics of danofloxacin have been described on the basis of the following investigations:

i. Study #1, Evaluation of plasma and lung pharmacokinetics of a single dose of subcutaneously (SC) administered danofloxacin 18% injectable solution in cattle.

The objective of this investigation was to characterize the plasma and lung pharmacokinetics of danofloxacin after subcutaneous injection of a 6 mg/kg dose to ruminating cattle. Heath Management Services of Tulare, California conducted the study under the guidance of Terry TerHune, DVM, Ph.D. The test material was 180 mg danofloxacin/mL (18% w/v) injectable solution.

The study employed twenty-two male castrate beef cattle, approximately 6 months of age, weighing between 228 to 257 kg. Animals were individually housed and fed. All animals were healthy and without prior history of exposure to fluoroquinolones.

On study Day 0, the danofloxacin treatments were administered to all animals SC in the lateral neck. Dose volumes were based upon Day zero body weights. Duplicate 10 mL samples of blood were collected in evacuated heparinized tubes via jugular venipuncture immediately prior to slaughter and at hrs 1, 2, 8, 12, 24, 36, and 48 post-injection (3 animals per time point). Plasma was separated by centrifugation and transferred to two plastic vials labeled with study number, animal number, date, and time of collection. All samples were stored frozen at approximately -20^0C, pending shipment for analysis. Following blood collection, calves were euthanized by captive bolt, followed by exsanguination.

Lungs were removed intact, and a representative sample was taken from each lung lobe. For each animal, the lung tissue samples were homogenized in a food processor in two separate batches. The two batches were

combined to obtain a final, uniformly blended lung homogenate. Approximately 500 g of lung homogenate was taken from the processor and divided into two sub-samples of 250 g each. These samples were stored frozen (-20^{0}C), pending shipment for analysis.

BAS Analytics, a division of Bioanalytical Systems, Inc, West Lafayette, Indiana, generated the quantitative analysis of danofloxacin concentrations in the lung and plasma samples using a validated High Pressure Liquid Chromatographic (HPLC) method with fluorescence detection.

The concentrations at each timepoint (lung or plasma) were averaged and the resulting composite curves were used as the basis for the analysis. The area under the curve (AUC) was estimated using the linear trapezoidal rule from time zero to the last quantifiable drug concentration. The terminal elimination rate constant (β) was estimated from the slope of the Ln concentration versus time curve. Terminal elimination half-life (T½) was calculated as 0.693/β. AUC was estimated from time zero to time infinity ($AUC_{0\text{-inf}}$) by adding the terminal triangulated area (estimated as the last quantified plasma drug concentration divided by β) to the AUC. Cmax was defined as the peak observed plasma danofloxacin concentration, and Tmax was the time when that peak concentration was observed.

Results:

Danofloxacin is rapidly absorbed following subcutaneous injection, and lung levels promptly exceed concentrations observed in plasma (Figure 1). The following noncompartmental approximations were generated from the composite blood and lung profiles (Table 2.3).

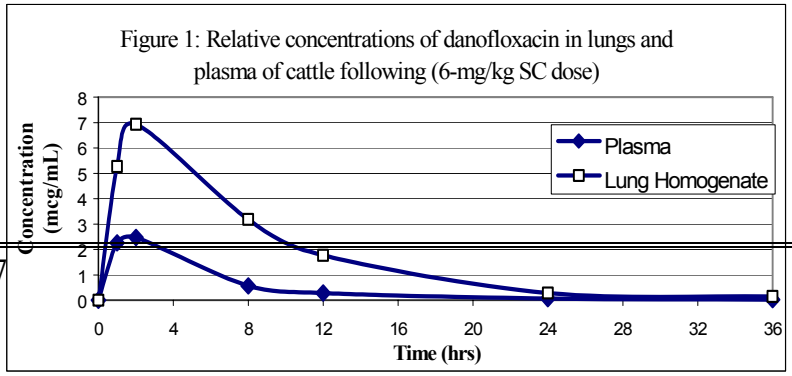
Table 2.3 Noncompartmental parameters based upon composite curves

Tissue	Tmax (hr)	Cmax (μg/mL)	T½ (hr)*	$AUC_{0\text{-inf}}$ (μg* hr/mL)
Lung	2.0	6.9	6.6	65.0
Plasma	1.0	2.2	5.3	16.0

* T1/2 = terminal elimination half-life

Based upon these data, it is evident that the lung concentrations are higher than are those in the plasma. The experimental conditions do not allow for

Figure 1: Relative concentrations of danofloxacin in lungs and plasma of cattle following (6-mg/kg SC dose)



differentiation between intracellular vs. extracellular drug concentrations, or the degree of binding that may have occurred between drug and pulmonary tissues.

ii. Study #2, Plasma pharmacokinetics of a single dose of subcutaneously administered danofloxacin in cattle.

The objective of this investigation was to determine whether or not danofloxacin exhibits linear pharmacokinetics following a single subcutaneous injection (SC) of the 18% solution. Fifteen crossbred beef heifers of approximately 6 months of age and about 214 kg were employed in this investigation. All animals were in good health and had uniform body weights.

The fifteen animals were randomly assigned to pens and treatment groups (5 animals per treatment). The doses administered were 1.25 mg/kg, 6.0 mg/kg, and 10.0 mg/kg. The test article was administered as a single subcutaneous injection into the right cervical region of the neck.

Duplicate 10-mL blood samples were collected in evacuated heparinized tubes via jugular venipuncture at 0.5, 1, 2, 4, 8, 12, 18, 24, 36, 48, and 72 hours post injection. Plasma was separated by centrifugation and stored frozen in plastic vials. Samples were sent to Pfizer Animal Health Drug Metabolism in Groton, Connecticut for assay. The analytical method was identical to that described in Study 1, "Evaluation of plasma and lung pharmacokinetics of a single dose of subcutaneously (SC) administered danofloxacin 18% injectable solution in cattle". Pharmacokinetic measures were estimated for each subject in accordance with the procedures described above. Comparisons of dose-normalized means were based upon an Analysis of Variance procedure. When significant differences were observed, individual treatment means were compared using the pairwise t-test. All cells were balanced in this study.

Results:

The data indicate that although dose-normalized peak danofloxacin concentration following SC administration of the 1.25 mg/kg dose is significantly higher than that associated with a 10 mg/kg dose, all other metrics do not differ significantly. Comparable rates of absorption are observed with a 6 mg/kg and a 10 mg/kg dose, and the extent of exposure (expressed as AUC) is dose proportional across the dosing range examined. Accordingly, the resulting plasma drug concentrations are concluded to vary in a manner proportional to the administered dose.

The corresponding pharmacokinetic measures are summarized in Table 2.4 below:

Table 2.4 Dose proportionality in danofloxacin plasma concentrations

Parameter	1.25 mg/kg		6.0 mg/kg		10.0 mg/kg	
	Mean	%CV**	Mean	%CV**	Mean	%CV**
Tmax (hr)	1.6	34	1.60	34	2.2	50
Cmax (µg/mL)	0.34 (1.65)*[a]	13	1.35 [a b]	18	2.07 (1.24)*[b]	12
T½ (hr)	3.6	13	3.80	3	3.7	5
AUC*** (µg x hr/mL)	2.24 (11.7)*	28	9.42	18	15.67 (9.4)*	15

* Values in parenthesis represent parameters that have been normalized to a 6-mg/kg dose
** Coefficent of variation (%) = Standard deviation/mean • 100
*** Values represent AUC to the last quantifiable dose.
[a,b] Values with like letters are not statistically significantly different ($p<0.05$).

iii. Study #3, Plasma pharmacokinetics of two doses of subcutaneously administered danofloxacin in cattle.

In this investigation, a 4 mg/kg dose of danofloxacin was administered twice (48 hours apart) to five female crossbred beef calves (4-0-4 dosing regimen). Calves were approximately 6 months of age and had an average body weight of 228 kg. Injections were placed SC into the right cervical region of the neck. Blood was collected from each calf immediately prior to administration, hour zero, and at 0.5, 1, 2, 4, 8, 12, 18, 24, 36, and 48 hours after the first and second injection. Plasma samples were analyzed for danofloxacin using the HPLC procedure previously described. Pharmacokinetic metrics were determined as defined in Study #1, above.

Results:

The mean data are provided in Table 2.5. From these data, danofloxacin pharmacokinetics do not vary after repeat SC injections. Furthermore, as evidenced by the similarity in Cmax values, drug accumulation does not occur when danofloxacin is administered twice at a 48 interval.

Table 2.5 Mean noncompartmental parameters obtained after repeated danofloxacin administrations

Parameter	Day 0		Day 2	
	Mean	%CV	Mean	%CV
Tmax (hr)	1.4	36	1.4	36
Cmax (μg/mL)	0.89	11	0.85	16
β (hr^{-1}) [T½]	0.2218 [3.1]	14	0.2021 [3.4]	16
$AUC_{0\text{-inf}}$ (μg x hr/mL)	5.19	15	5.13	14

When correcting these parameters to a 6 mg/kg dose, values are similar to those reported in Study 2 (Study 3: Cmax (Day 0,2) = 1.34 μg/mL and 1.28 μg/mL; $AUC_{0\text{-inf}}$ (Day 0,2) = 7.79 μg x hr/mL and 7.70 μg x hr/mL. Study 2: Cmax = 1.34 μg/mL; AUC = 9.42 μg x hr/mL).

iv. Study #4, Bioavailability of danofloxacin 18% injectable solution when administered as a single subcutaneous dose to ruminating cattle.

In this crossover study, cattle were administered danofloxacin as either a SC or an intravenous (IV) injection. The objective of this investigation was to characterize the pharmacokinetics of danofloxacin in ruminating cattle. Values for systemic clearance (CL) and volume of distribution at steady state (VDss) were based upon the IV data. By using a two-period crossover design, the bioavailability (F) of the danofloxacin 18% injectable solution (6 mg/kg, SC injection) was determined.

Twelve healthy calves (6 castrated males, 6 females) weighing 217 to 286 kg were selected on the basis of normal health and body weight. Each animal was randomly assigned to either sequence 1 (SC, period 1; IV, period 2) or sequence 2 (IV, period 1; SC, period 2). SC injections were administered into the lateral neck region. IV injections were administered into the left jugular vein. Administrations were separated by a 14-day washout interval.

Duplicate 10 mL blood samples were collected immediately prior to injection (hr zero) and at hrs 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 post injection. Plasma was separated by centrifugation and transferred to two plastic vials. All samples were stored frozen (approximately -20^0C) and shipped to BAS Analytics, West Lafayette, Indiana for analysis of danofloxacin content. The analytical method was identical to that used in all other previously described pharmacokinetic studies.

A noncompartmental analysis of the data was generated using the WinNonLin Software Program. From the IV datasets the parameters of

interest were CL[1] and the VDss[1], the later being indicative of the extent to which the drug can partition into systemic tissues. These parameters were evaluated with respect to potential gender differences. The IV data from one male and one female were excluded from the analysis because of perivascular administration. Within each subject, the SC $AUC_{0\text{-}inf}$ values were compared to those obtained after IV administration to determine product bioavailability (F) after SC injection.

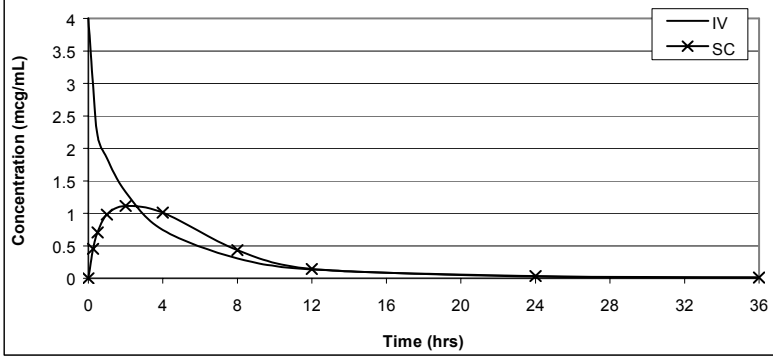
Results**:**

No statistically significant period or sequence effects were observed. Therefore, drug carryover effects did not bias the data, and comparisons could proceed as planned.

Mean pharmacokinetic parameters (by treatment and gender) are provided in Table 2.7 and Figure 2. Danofloxacin distributes extensively throughout the body, as evidenced by VDss exceeding 1-L/kg.

The percent bioavailability of the SC dose was determined by the ratio: $AUC_{0\text{-}inf}$(SC)/$AUC_{0\text{-}inf}$(IV). From these results, we see a slightly earlier time to peak concentrations after SC administration in steers as compared to heifers. The small but significant difference in $AUC_{0\text{-}inf}$ values after IV administration in steers and heifers may be due to the slight (but not significant) difference in CL observed in castrated males versus females. There were no statistically significant gender effects associated with Cmax, $AUC_{0\text{-}inf}$(SC) or T½ (after IV or SC administration), indicating that dose can be administered irrespective of gender. Moreover, there were no statistically significantly differences in the terminal elimination rate constants observed after SC versus IV administration.

Figure 2: Profile of Plasma Danofloxacin Concentrations Following IV and SC Administration (6 mg/kg)



1. Where CL= dose/$AUC_{0\text{-}inf}$ and VDss = dose*AUMC/AUC^2 and AUMC = the area under the first moment curve (extrapolated to time infinity) and AUC = $AUC_{0\text{-}inf}$

Table 2.7 Pharmacokinetic parameter estimation following SC vs. IV danofloxacin administration in castrated male (steers) and female calves (heifers)

Parameter	Units	IV ADMINISTRATION				SC ADMINISTRATION			
		Steer		Heifer		Steer		Heifer	
		Mean	%CV	Mean	%CV	Mean	%CV	Mean	%CV
$AUC_{0\text{-}inf}$*	μg x hr/mL	11.3	11	9.74	9	9.63	12	8.73	9
F						92	5	87	3
Cmax	μg/mL					1.2	16	1.3	13
Tmax**	hr					3.4	42	1.6	31
CL	L/hr	0.54	12	0.62	9				
VDss	L/kg	2.7	7	2.6	4				
$T_{1/2}$	hr	4.8	18	4.2	7	4.6	18	4.0	4

*$AUC_{0\text{-}inf}$: statistically significant gender effect after IV
**Tmax: statistically significant gender effect after SC administration

Conclusion:

On the basis of this investigation, danofloxacin is concluded to exhibit the following pharmacokinetic characteristics when administered to cattle:

1) Although the volume of distribution in steers and heifers is similar, females tend to exhibit a slightly greater CL. This difference contributed to the small but significant gender effect in the total danofloxacin exposure observed after IV administration. However, no gender effects were noted in the total drug exposure following SC administration.

2) Danofloxacin is highly bioavailable when administered as a SC injection into the cervical region of the neck. This high level of bioavailability is consistent between animals. Following SC administration, no statistically significant gender effects were observed for either AUC or Cmax. This indicates that the dose can be administered without adjusting for differences in gender.

3) The rate of danofloxacin elimination is equivalent following IV and SC injection. Thus, the injectable formulation is not associated with absorption-rate limited depletion (flip-flop kinetics) after SC administration.

Pharmacodynamic Evaluation

Bactericidal agents may be classified as either time-dependent or concentration-dependent. The fluoroquinolones have been shown to exhibit concentration

dependent killing (Craig, 1998). Both the Cmax/MIC and AUC (from time zero to hr 24 postdose)/MIC have been indicated as predictors of clinical efficacy (Drusano, et al., 1993; Craig and Dalhoff, 1998; Lode, et al., 1998). The corresponding ratio targeted for AUC/MIC is approximately 125, and that for Cmax/MIC is approximately 8 (Blaser, et al., 1987; Lode et al., 1998).

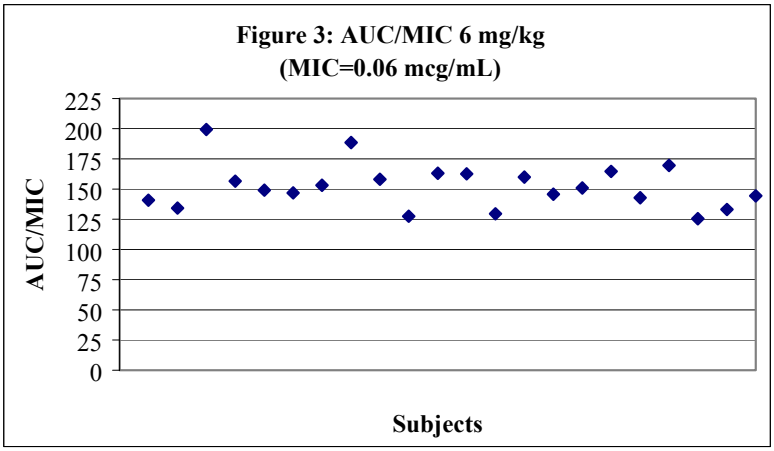
To explore the AUC/MIC and Cmax/MIC ratios associated with a 6 mg/kg SC dose of danofloxacin in cattle, the following data sets were employed:

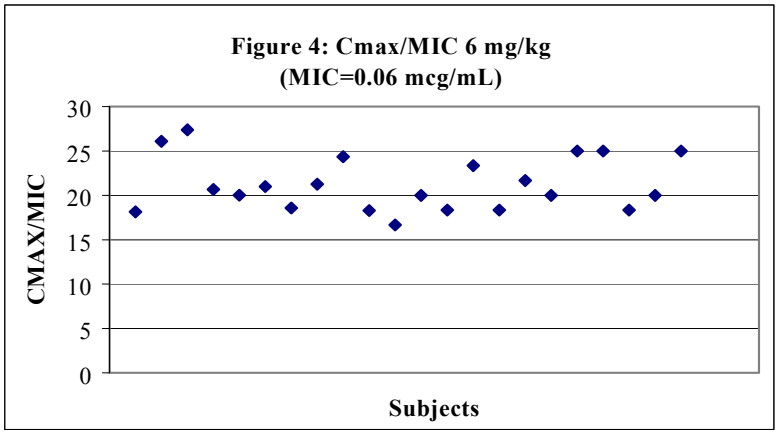
i. Study # 2, plasma pharmacokinetics of a single dose of subcutaneously administered danofloxacin in cattle, including the 5 cattle administered a 6 mg/kg SC injection and the 5 cattle administered a 10 mg/kg SC injection. For those animals receiving a 10 mg/kg dose, the corresponding pharmacokinetic data were normalized to a 6 mg/kg dose by multiplying parameter values by a factor of 0.6.

ii. Study #4, all AUC and Cmax values associated with the 6 mg/kg SC injection. Use of all SC bioavailability data, regardless of dosing period, is justified on the basis of absence of any statistically significant period or sequence effects.

Based upon the susceptibility data generated in support of this application, an MIC_{90} of 0.06 μg/mL was used as the pharmacodynamic variable. The results of this analysis are provided in Figures 3 and 4.

Figure 3: AUC/MIC 6 mg/kg (MIC=0.06 mcg/mL)





The ratio of AUC/MIC was equal to or greater than 125 and the Cmax/MIC ratio exceeded 15 for all twenty-two subjects included in this analysis. Accordingly, for the pathogens associated with the label indication (MIC values $\leq$ 0.06 µg/mL), the proposed dosing regimen of 6 mg/kg administered every other day is consistent with pharmacokinetic and pharmacodynamic ratios that have been associated with minimizing the risk of selecting for resistant microbial strains.

REFERENCES

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Craig, WA (1998). Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clinical Infectious Diseases*, 26: 1-12
Craig, W. and Dalhoff, A. (1998). Pharmacodynamics of fluoroquinolones in experimental animals. In: *Handbook of Experimental Pharmacology*. Born GVR, Cuatrecas P, Ganten D, Herken H, Melmon KL, and Starke K (eds). Springer Verlag, Berlin pp208-232.

Drusano, et al., Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of Pseudomonas sepsis. *Antimicrobial Agents Chemotherapy*, 1993,37: 483-490

Lode E, Borner K, and Koeppe P (1998). Pharmacokinetics of fluoroquinolones. *Clinical Infectious Diseases*, 27: 33-39.

3. ANIMAL SAFETY

All testing was conducted in full compliance with Good Laboratory Practice (GLP) Regulations (21CFR 58).

Pivotal Studies

a. Drug Tolerance Study:

i. Type of Study: This study evaluated the systemic tolerance of A180® (danofloxacin 18% injectable solution)

ii. Study Director: Karol Bice Godwin, D.V.M.
HTI Bio-Services, Inc.
Santa Ysabel, CA 92070

iii. General Design:

1) Purpose: To determine the toxic potential of danofloxacin 18% injectable solution when administered to cattle at 60 mg/kg body weight for three consecutive days.

2) Animals: Six healthy calves (three female and three castrated males) weighing between 192 and 254 kg. Four animals (2/sex) were assigned to the danofloxacin treatment group. One male and one female were assigned to the negative control group.

3) Control: Sterile saline administered subcutaneously.

4) Dosage Form: The proposed market formulation containing 180 mg danofloxacin per milliliter.

5) Route of Administration: Subcutaneous injection

6) Dose: 60 mg danofloxacin/kg body weight for three consecutive days.

7) Study Duration: 6 days

8) Pertinent Parameters: Clinical signs, body weight, hematology, serum chemistry, gross and histopathology.

iv. Results:

1) Clinical Observations: Clinical signs of toxicity were decreased feed consumption and loss of body weight. One animal had signs of depression. Lameness examinations and directed clinical observations did not detect lameness in any study animal. Injection site inflammation was noted in treated males, but not treated females or control animals.

2) Mortality: One of four danofloxacin-treated animals was found moribund one day after the final treatment administration and was euthanized before scheduled slaughter. All other animals survived to scheduled slaughter.

3) Body Weight: Loss of body weight was observed in all three danofloxacin treated animals that survived to study completion.

4) Feed consumption: Four of four danofloxacin-treated calves had decreased feed consumption.

5) Hematology: An increased white blood cell concentration and increased absolute segmented neutrophils were noted, possibly reflecting injection site inflammation.

6) Clinical Chemistry: Clinical pathology results were highly variable and showed no treatment related changes except post treatment absolute neutrophil counts about the reference range (600 - 4000 per microliter) in all danofloxacin-treated calves, and total white blood cell counts above the reference range (4,000 - 12,000 per microliter) in three of four danofloxacin treated calves. Serum magnesium concentration was below the laboratory reference range (2.0 - 2.8 mg/dl) in five of six animals before treatment and in four of six at slaughter.

7) Gross and Histopathological Observations: Two of the four danofloxacin treated calves had gross and histopathologic lesions consistent with injection site inflammation. All four danofloxacin treated calves demonstrated articular cartilage lesions typical of fluoroquinolone chondropathy.

v. Statistical Analysis: none

vi. Conclusions: Subcutaneous administration of danofloxacin administered at 60 mg danofloxacin/kg for three consecutive days can induce inappetance, loss of body weight, articular cartilage lesions, clinical pathology abnormalities, and injection site abnormalities.

b. General Safety Study

i. Type of Study: Margin of safety of danofloxacin 18% injectable solution in ruminating cattle.

ii. Study Director: Karol Bice Godwin, D.V.M.
HTI Bio-Services, Inc.
Santa Ysabel, CA 92070

iii. General Design:

1) Purpose: To assess the safety of danofloxacin 18% injectable solution when administered to cattle by subcutaneous injection for six consecutive days at 10, 20, and 30 mg/kg body weight.

2) Animals: Twenty-four healthy cattle (twelve females and twelve castrated males) weighing between 190 and 254 kg, randomly assigned among 4 treatment groups (6 animals each, 3 female and 3 male).

3) Control: Sterile saline administered subcutaneously.

4) Dosage Form: The proposed market formulation containing 180 mg danofloxacin per milliliter.

5) Route of Administration: Subcutaneous injection

6) Dose: 10 mg/kg, 24 hours apart for six consecutive days
20 mg/kg, 24 hours apart for six consecutive days
30 mg/kg, 24 hours apart for six consecutive days

7) Study Duration: 8 days

8) Pertinent Parameters: Clinical signs, body weight, hematology, serum chemistry, gross and histopathology.

iv. Results:

1) Clinical Observations: No clinical signs of toxicity were observed at the 10 mg/kg and 20 mg/kg dose levels. In animals receiving the 30 mg/kg dose, two had clinical signs of lameness; two exhibited signs of ataxia, nystagmus, depression, and became recumbent. Observation of the injection site revealed swelling in one animal in the 10 mg/kg dose level and five animals each in the 20 mg/kg and 30 mg/kg dose groups.

2) Mortality: Two animals receiving the 30 mg/kg dose were humanely euthanized on Day 4 of the study. All other cattle survived to study termination.

3) Body Weight: Cattle in all treatment groups had comparable weight gain.

4) Feed consumption: No treatment-related abnormalities were identified in animals receiving 10 mg/kg or 20 mg/kg. Decreased appetite was noted on Day 3 for one animal in the 30 mg/kg dose group.

5) Hematology: No treatment-related abnormalities were identified at the 10 mg/kg dose level. Increased white blood cell concentrations were noted in two animals receiving 20 mg/kg and four animals receiving 30 mg/kg. Increased absolute monocytes were noted in two animals in the 30 mg/kg dose level.

6) Clinical Chemistry: No treatment-related abnormalities were identified at the 10 mg/kg dose level. Test article related abnormalities in serum chemistry were noted as slightly increased sodium in two animals receiving 20 mg/kg, and slightly increased chloride in two animals receiving 30 mg/kg.

7) Gross and Histopathological Observations: No treatment-related abnormalities were identified at the 10 mg/kg dose level. Gross and histopathology demonstrated articular cartilage lesions typical of quinolone chondropathy in one calf in the 20 mg/kg group and five calves in the 30 mg/kg group. Inflammation was noted at subcutaneous injection sites.

v. Statistical Analysis: Study data were summarized and daily feed intake for each animal was analyzed using a linear model and *a priori* contrasts among least squares means at the 5% level of significance ($P \leq 0.05$*).*

vi. Conclusions: There were no observable adverse effects, including fluoroquinolone-induced chondropathy, at 10 mg/kg for 3 times the recommended label treatment duration.

c. Articular Cartilage Safety Study

i. Type of Study: Articular cartilage margin of safety of danofloxacin 18% injectable solution in cattle.

ii. Study Director: Terry N. Terhune, D.V.M., Ph.D.
Health Management Services
Tulare, CA 93274

iii. General Design:

1) Purpose: To evaluate the effect of danofloxacin 18% injectable solution on articular cartilage of growing cattle when administered by subcutaneous injection at 18 and 24 mg/kg body weight once daily for three days.

2) Animals: Fifteen healthy calves (castrated males) weighing between 264 to 328 kg.

3) Control: Sterile saline administered subcutaneously.

4) Dosage Form: The proposed market formulation containing 180 mg danofloxacin per milliliter.

5) Route of Administration: Subcutaneous injection

6) Dose: 18 mg/kg body weight once daily for three days.
24 mg/kg body weight once daily for three days.

7) Study Duration: 4 days

8) Pertinent Parameters: General health observations, clinical observations, gross pathology, histopathology, feed intake, and body weight.

iv. Results:

1) Clinical Observations: No clinical signs of toxicity were observed. Observation of the injection site revealed Zenker's degeneration of muscle, edema, hemorrhage, acute inflammation, and necrosis in animals receiving 18 and 24 mg/kg dose ranging in severity from minimal to moderate.

2) Mortality: All the cattle survived to study termination.

3) Body Weight: Cattle in all treatment groups had comparable weight gain.

4) Feed consumption: No treatment-related abnormalities were identified in animals receiving 18 mg/kg or 24 mg/kg and were similar between all groups.

5) Hematology: No treatment-related abnormalities were observed.

6) Clinical Chemistry: No treatment-related abnormalities were observed.

7) Gross and Histopathological Observations: No treatment-related abnormalities were identified at the 24 mg/kg dose level. Gross histopathology demonstrated articular cartilage lesions typical of quinolone chondropathy in one calf in the 18 mg/kg group. Cartilage changes unrelated to treatment were documented in one animal in the placebo group and one animal in the 18 mg/kg group.

v. Statistical Analysis: The study data were tabulated and, as appropriate, summarized through calculation of mean and standard deviation values.

vi. Conclusions: Subcutaneous administration of danofloxacin was associated with articular change in one of five calves administered 18 mg/kg for three consecutive days. No evidence of treatment related articular cartilage change was found in five calves administered danofloxacin at 24 mg/kg for three days.

d. Injection Site Toleration

i. Type of Study: Injection site toleration of danofloxacin 18% injectable solution administered subcutaneously in ruminant cattle.

ii. Study Director: Dan C. Ronning, M.S.
Colorado Animal Research Enterprises, Inc.
Fort Collins, CO 80524

iii. General Design:

1) Purpose: To investigate the injection site toleration of a subcutaneous injection of 15 mL danofloxacin 18% injectable solution in cattle.

2) Animals: Sixteen male castrate calves

3) Control: Sterile saline administered subcutaneously.

4) Dosage Form: The proposed market formulation containing 180 mg danofloxacin per milliliter.

5) Route of Administration: Subcutaneous injection of danofloxacin and control article in contralateral sides of the neck.

6) Dose: 15 mL of danofloxacin 18% (0.8 to 12.3 mg danofloxacin per kg body weight) on each of preslaughter days 35, 28, 21 and 5.

7) Study Duration: 35 days

8) Pertinent Parameters: General health observations, injection site observations, clinical observations and body weight.

iv. Results:

1) Clinical Observations: Palpation of injection sites revealed no swelling in saline-injected sites at any time point or in danofloxacin injected sites at five days after dosing. Detectable swellings were present after seven days in most danofloxacin-injected sites. A reduction in size of injection site swelling was recorded with increased time after injection.

2) Mortality: All animals survived to scheduled slaughter.

3) Body Weight: Cattle in all treatment groups had comparable weight gain.

4) Feed consumption: Not measured

5) Hematology: Not measured

6) Clinical Chemistry: Not measured

7) Gross and Histopathological Observations: In 4 out of 4 animals necropsied at 5 days post-treatment, danofloxacin-injected sites had localized serosanguinous fluid in the fascia and discoloration. In 2 of the 4 animals at this timepoint, the discoloration extended into underlying muscle. Histopathologic examination of subcutaneous danofloxacin injection site tissues revealed minimal to mild inflammation, edema, hemorrhage, fibrosis, and necrosis in subcutaneous fascia, with progressive resolution through 35 days post-treatment. Danofloxacin-injected sites were histologically within normal limits in 2/4 animals at 28 days and 2/4 animals at 35 days post treatment.

v. Conclusion: Subcutaneous injection of danofloxacin 18% administered as directed to cattle may induce a transient local reaction in the subcutaneous and underlying tissue that may result in trim loss of edible tissue at slaughter.

Corroborative Study

a. General Safety Study

i. Type of Study: Margin of safety of danofloxacin 18% injectable solution in preruminant calves.

ii. Study Director: Robert J. Harman, D.V.M.
HTI Bio-services, Inc.
San Diego, CA 92121

iii. General Design:

1) Purpose: To assess the safety of danofloxacin when administered to preruminant calves by subcutaneous injection at 6 mg/kg body weight once every other day for three consecutive treatment cycles, or 18 mg/kg body weight administered once every other day for one treatment cycle. One cycle was equal to two injections 48 hours apart.

2) Animals: Twenty-four healthy Holstein calves (12 male, 12 female), approximately 21 days old on treatment commencement.

3) Control: Sterile saline administered subcutaneously

4) Dosage Form: The proposed market formulation containing 180 mg danofloxacin per milliliter

5) Route of Administration: Subcutaneous injection

6) Dose: 6 mg/kg body weight once every other day for three consecutive treatment cycles and 18 mg/kg body weight once every other day for one treatment cycle. One cycle was equal to two injections 48 hours apart.

7) Study Duration: Nine days.

8) Pertinent Parameters: Body temperature, body weight, general health observations, clinical observations, lameness, and mortality.

iv. Results:

1) Clinical Observations: The only test article related finding was scleral/nasal erythema seen during the clinical observations in the high dose group calves. The significance of this finding is unknown and it was not seen in the control group. The erythema was less noticeable on non-dosing days and returned upon re-dosing. One calf in the low dose group had pre-treatment scleral erythema, and developed nasal erythema after treatment that may or may not have been treatment-related. No lameness was seen in study calves. No other clinical or pathological findings were recorded in this study.

2) Mortality: None

3) Bodyweight: Bodyweights were not used as a variable.

4) Feed Consumption: Feed consumption was not used as a variable.

5) Hematology: Hematologic parameters were not measured.

6) Clinical Chemistry: Clinical pathology parameters were not measured.

7) Gross and Histopathological Observations: No test article-associated lesions were found on gross and histopathological examination of liver, kidney, bone, and joint tissues.

v. Conclusion: Subcutaneous injection of danofloxacin 18% administered as directed in preruminant calves may cause mild transient nasal erythema.

4. HUMAN FOOD SAFETY

All pivotal testing was conducted in full compliance with the Good Laboratory Practice (GLP) Regulations (21 CFR 58). In all studies, the reported dose levels or concentrations tested are expressed in terms of the free base form of danofloxacin or desmethyldanofloxacin.

Toxicity Studies - Danofloxacin Mesylate

a. Three month oral gavage study with *in utero* exposure in rats

Protocol No: 89-607-19

Starting Date: September 1989

Termination Date: December 1989

Study Director: D.O. Fisher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in aqueous oral solution

Species and Strain: Long-Evans rats [Crl: (LE)BR]

Number of Animals Per Treatment Group: 20/sex/group

Drug Levels Tested and Duration of Dosing: 0, 1.0, 2.5 and 6.25 mg/kg administered daily for three months, beginning at weaning, following *in utero* and lactation exposure.

Route of Drug Administration: Oral gavage

Parameters Tested: clinical signs, body weight, food consumption, ophthalmoscopic examinations, clinical pathology, gross and histopathology, organ weights.

Significant Toxicity Observed: Higher incidence of proteinuria (females) and hematuria (males) in the high dose group.

No Observed Effect Level (NOEL): 2.5 mg/kg/day

Conclusion: The high dose females had a higher incidence of proteinuria. Hematuria was observed in the high dose males. The NOEL was 2.5 mg/kg/day.

b. Three month oral gavage study in Beagle dogs

Protocol No: 88-607-14

Starting Date: September 1988

Termination Date: December 1988

Study Director: D.O. Fisher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in gelatin capsules

Species and Strain: Beagle dogs

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

Drug Levels Tested and Duration of Dosing: 0, 5, 10 and 25 mg/kg administered daily for three months.

Route of Drug Administration: Oral capsule

Parameters Tested: clinical signs, body weight, food consumption, ophthalmoscopic examinations, blood pressure recordings and electrocardiographic tracings, clinical pathology, gross and histopathology, organ weights.

Significant Toxicity Observed: Articular cartilage lesions

No Observed Effect Level: A NOEL could not be established

Conclusion: Typical fluoroquinolone-induced articular cartilage lesions were observed in both genders at all dose levels. The severity and incidence of lesions was dose-related. A NOEL could not be established in this study.

c. Three month oral gavage study in Beagle dogs

Protocol No: 88-607-15

Starting Date: November 1988

Termination Date: March 1989

Study Director: D.O. Fisher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in gelatin capsules

Species and Strain: Beagle dogs

Number of Animals Per Treatment Group: 4/sex/group

Drug Levels Tested and Duration of Dosing: 0, 1.0, and 2.4 mg/kg administered daily for three months

Route of Drug Administration: Oral capsule

Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopic examinations, blood pressure recordings and electrocardiographic tracings, clinical pathology, gross and histopathology, organ weights.

Significant Toxicity Observed: None

No Observed Effect Level: 2.4 mg/kg/day

Conclusion: Combining the results of the study 88-607-14 and the present study, the toxicological effects of the drug are clearly defined in this species. Consequently, a NOEL of 2.4 mg/kg/day has been established for dogs.

d. Fetotoxicity oral gavage study in rats

Protocol Nos: 88093 and 88094

Starting Date: June 1988

Termination Date: July 1988

Study Director: M.J. Kessedjian

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in aqueous solution

Species and Strain: albino rat, [CrI:COBS-VAF-CD(SD)BR]

Number of Animals Per Treatment Group: For the main part of the study (88093), 20 inseminated females per treatment group; in the pharmacokinetic study (88094), 5 inseminated females received danofloxacin mesylate.

Drug Levels Tested and Duration of Dosing: In the main study, 0, 50, 100 and 200 mg/kg administered daily for 10 days (days 6 to 15 of gestation); in the pharmacokinetic study, 200 mg/kg administered daily for 10 days (days 6 to 15 of gestation).

Route of Drug Administration: Oral gavage

Parameters Tested: In the main study, maternal toxicity, embryotoxicity and teratogenicity; in the pharmacokinetic study, drug concentrations in maternal blood, amniotic fluid and fetuses.

Significant Toxicity Observed: Maternal effects (decreased body weight gain and food consumption) were observed at 100 and 200 mg/kg. Fetal effects at those doses included decreased mean body weights and a slightly increased incidence of abdominal closure defects.

No Observed Effect Level: 50 mg/kg/day

Conclusion: Maternotoxicity (reduced weight gain and food consumption) and embryotoxicity (delayed fetal development) were observed at doses of 100 and 200 mg/kg/day. Therefore, the NOEL for this study is 50 mg/kg/day.

e. Fetotoxicity study in mice by the oral route

Protocol No: 88115/88116

Starting Date: August 1988

Report Date: March 1989

Study Director: M. J. Kessedjan

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in aqueous solution

Species and Strain: Albino mouse, Cri:COBS-VAF_CD1(1CR)BR

Number of Animals per Sex per Treatment Group: 20 female

Drug Levels Tested and Duration of Dosing: 0, 50, 100 and 200 mg/kg/day for 8 days

Route of Drug Administration: Oral gavage

Parameters Tested: potential maternal toxicity, embryotoxicity, teratogenicity

Significant Toxicity Observed: Danofloxacin mesylate (CP-76, 136) did not induce any death, clinical signs or adverse effects on the reproductive parameters. However, a toxicological effect resulted in decreased body weight gain in the 200 mg/kg/day females and lower mean body weight in the fetuses of the same group.

No Observed Effect Level:100 mg/kg/day

Conclusion: Based on the results of this study, a NOEL of 100 mg/kg/day for danofloxacin mesylate is established in pregnant mice.

f. Three generation oral gavage study in Long-Evans rats

Protocol No: 89-607-16

Starting Date: April 1989

Termination Date: July 1990

Study Director: S.W. Stadnicki

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in aqueous solution

Species and Strain: Long-Evans rats, [CrI: (LE)BR]

Number of Animals per Sex per Treatment Group: 30/sex/group in the F_O generation. 25/sex/group in the F_1 and F_{2b} generations.

Drug Levels Tested and Duration of Dosing: 0, 1.0, 2.5, 6.25 and 150 mg/kg. Doses of 0, 1.0, 2.5, and 6.25 mg/kg were administered to F_O parental male and female rats daily for 9 weeks and 2 weeks, respectively, prior to mating and continued for both sexes throughout mating, gestation, parturition and weaning of F_1 offspring. F_1 offspring were continued on treatment while mating, producing and nursing F_{2a} and F_{2b} generations. The F_{2b} offspring were mated within dose groups and continued on drug to produce F_3 litters that were sacrificed at weaning. A 150 mg/kg/day group was similarly maintained, but terminated prior to the second F_1 matings.

Route of Drug Administration: Oral gavage

Parameters Tested: Clinical signs, body weight, food consumption, reproductive parameters

Significant Toxicity Observed: At 150 mg/kg, reductions in litter size at birth, pup weight and survival of pups in the F_1 and F_{2a} generations; reductions in copulatory and pregnancy rate and prolongation of gestation length in F_1 animals cohabited to produce the F_{2a} generation.

No Observed Effect Level: 6.25 mg/kg/day

Conclusion: The NOEL for neonatal and reproductive parameters over three generations of rats is 6.25 mg/kg/day of danofloxacin mesylate based on the results for copulatory rate, pregnancy rate, and litter parameters found at the 150 mg/kg/day dose level.

g. Reproductive study III (teratology) in New Zealand White Rabbits

Protocol No: 93-607-34

Starting Date: September 1993

Termination Date: December 1993

Study Director: M. Tassinari

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136) in aqueous solution.

Species and Strain: Pregnant female New Zealand White Rabbits

Number of Animals Per Treatment Group: Control 32 animals; low dose 29 animals; intermediate dose 33 animals; and high dose 39 animals

Drug Levels Tested and Duration of Dosing: 0, 2.5, 7.5 or 15 mg/kg/day for 15 days (days 6-20 of gestation).

Route of Drug Administration: Oral gavage

Parameters Tested: Maternal clinical signs, body weight, food consumption, reproductive parameters and fetal examinations following cesarean section on gestation day 28.

Significant Toxicity Observed: Decreased food consumption was observed in 17/39 does in the 15 mg/kg group. Eleven of these 17 subsequently aborted secondary to maternal toxicity. No other significant toxicity was observed in dams or fetuses in this study.

No Observed Adverse Effect Level: Maternal NOAEL = 7.5mg/kg; NOAEL for teratogenicity in fetuses = 15 mg/kg, the highest dose tested.
Conclusion: Danofloxacin caused maternal toxicity (decreased food consumption and subsequent abortions) in the high dose group of 15 mg/kg. Danofloxacin was not teratogenic at any dose tested.

h. *In Vitro* Human Lymphocyte Cytogenetics Assays

Protocol No: 89-607-18

Starting Date: June 1989

Report Date: October 1990

Study Director: D.E. Amacher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) in culture medium.

Species and Strain: Human lymphocytes

Drug Levels Tested and Duration of Dosing:

Initial direct assay: 25 to 70 µg/mL; second direct assay (with 400 µg/mL $MgSO_4 \cdot 7H_2O$ added): 50 to 70 µg/mL

Initial S9 activation assay: 100 to 700 µg/mL; second activation assay (with extra washes): 100 to 700 µg/mL; third activation assay (with extra washes and 400 µg/mL $MgSO_4 \cdot 7H_2O$ added): 300 to 700 µg/mL

Parameters Tested: Clastogenic activity *in vitro* in human lymphocyte cultures with and without exogenous metabolic activation. Because of the known chelating activity of danofloxacin, the additional assays were conducted with magnesium sulfate in the culture medium.

Significant Toxicity Observed: In the initial direct assay, the mitotic suppression (%) ranged from 78.3% to 31.6% over a dose range of 70 to 25 µg/ml in the absence of a metabolic activation and 70.5 to 38.6% over a dose-range of 600 to 400 µg/ml. The 70 µg/mL was toxic to the human lymphocytes used in the assay. Cell viability was improved with the addition of $MgSO_4.7H_2O$ as indicated by the reduction of toxicity.

Conclusion: Danofloxacin mesylate can induce chromosomal breakage among human lymphocyes, under the conditions of the assay as performed and that the

positive, statistically significant responses of danofloxacin mesylate are indicative of genotoxic activity of concern.

i. *In Vivo* Mouse Bone Marrow Cytogenetic Assay

Protocol No: 89-607-18

Starting Date: August 1989

Report Date: October 1990

Study Director: D.E. Amacher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) in aqueous solution

Species and Strain: CD-1 mice [Crl:COBS CD-1(ICR)BR]

Number of Animals Per Treatment Group: 5/sex/group; 3 groups sacrificed at 6, 24 or 48 hours after dosing.

Drug Levels Tested and Duration of Dosing: 1000 mg/kg; single dose

Route of Drug Administration: Oral gavage

Parameters Tested: Induction of chromosomal aberrations in metaphase cells of mouse bone marrow.

Significant Toxicity Observed: No evidence of toxic effect related to the treatment was observed both to the bone marrow or the target animals.

Conclusions: The *in vivo* cytogenetics test as performed was inadequate to evaluate the clastogenic potential of danofloxacin. Due to the lack of evidence of cytotoxic effect of danofloxacin, it is not certain that the test article reached the target tissue.

j. CHO/HGPRT Mammalian Mutation Assay

Protocol No: 92-607-26

Starting Date: April 1992

Report Date: August 1992

Study Director: P.J. Guzzie

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) DMSO solvent.

Species and Strain: Chinese hampster ovary (CHO) cells ($HGPRT^+$ subclone CHO-K,-BH_4)

Drug Levels Tested and Duration of Dosing: In the nonactivation phase, concentrations of 141 to 1070 μg/mL were used; in the S9 activation phase, concentrations of 465 to 2500 μg/mL were used. Five hour exposure.

Parameters Tested: Induction of forward mutations at the hypoxanthine-guanine phophoribosyl transferase (HGPRT) locus in the presence and absence of S9, as assayed by colony growth of chinese hampster ovary cells in the presence of 6-T; cytotoxicity (based on cell counts, cell lysis, cell morphology).

Significant Toxicity Observed: Cytotoxicity was observed at concentration ≥ 713 μg/mL without metabolic activation and at ≥ 1786 μg/mL with metabolic activation.

Conclusions: Danofloxacin mesylate did not produce a mutagenic response in the presence or absence of rat S9 in the CHO/HGPRT mammalian mutation assay.

k. Microbial Reverse Mutation Assays (Ames Tests)

Protocol No: 86-607-01

Starting Date: January 1987

Report Date: February 1990

Study Director: D.E. Amacher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) DMSO solvent; urine collected from mice dosed with test material.

Species and Strain: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100.

Drug Levels Tested and Duration of Dosing: Dose levels ranged from 0.0005 to 100 μg/plate with and without S9 metabolic activation. Cells from the above strains were also exposed to urine collected from mice dosed intraperitoneally with 5, 50 or 100 mg/kg danofloxacin mesylate. All cells were incubated at least 60 hours at 37°C.

Parameters Tested: Induction of *in vitro* bacterial mutations from histidine auxotrophic *Salmonella* cells in the presence and absence of S9 metabolic activator and excretory products in urine.

Significant Toxicity Observed: The test article was excessively bacteriostatic to the *Salmonella typhimurium* strains used in the assay at greater than 1 µg/plate (-S9) and 0.5 µg/plate (mouse liver S9). Because of the excessive bacteriostatic nature of the test article, the maximum dose using rat live S9 was restricted to 0.2 µg/plate.

Conclusion: There was no evidence of mutagenic excretory products found in urine from mice dosed intraperitoneally with danofloxacin mesylate. Due to the excessive bacteriostatic effect of danofloxacin, the results for the Ames Tests could not be evaluated.

l. *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

Protocol No: 87-607-02

Starting Date: August 1986

Report Date: December 1990

Study Director: D.E. Amacher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) DMSO solvent

Species and Strain: Primary cultures of rat hepatocytes (male [Cdf (F-344)/CrI BR Charles River] rat)

Drug Levels Tested and Duration of Dosing: 50, 100, 200, 400, and 600 µg/mL; 18-20 hour incubation period.

Parameters Tested: detection of DNA damage by measuring unscheduled DNA synthesis (UDS) in primary rat hepatocytes *in vitro*. Autoradiographic technique was used to determine the nuclear labeling resulting from incorporation of [3H]-thymidine into the DNA.

Significant Toxicity Observed: Acceptable viability with slight to moderate cytotoxicity was encountered in the range of 50 to 400 µg/mL. The highest concentration, 600 µg/mL, produced excessive cytotoxicity and could not be evaluated for induction of UDS. There was a substantial reduction of cell viability at 200 and 400 µg/mL.

Conclusion: Danofloxacin mesylate does not induce UDS in primary cultures of rat hepatocytes at levels that cause substantial reductions in cell viability.

m. Mouse Lymphoma L5178Y/TK ± S9 Assay

Protocol No: 86-607-02

Starting Date: August 1986

Report Date: December 1990

Study Director: D.E. Amacher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) serial dilutions in saline.

Species and Strain: L5178Y 3.7.2C mouse lymphoma cells heterozygous at the thymidine kinase locus (TK+/-)

Drug Levels Tested and Duration of Dosing: In the nonactivation phase, concentrations of 51-287 μg/mL were used; in the S9 activation phase, concentrations of 16-215 μg/mL were used. Three hour exposure.

Parameters Tested: Induction of 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)

Significant Toxicity Observed: The cell viability results for the clonable treatment conditions indicated that exposure to danofloxacin resulted in a range of growth from 22% Relative Total Growth (RTG) to 95 % RTG over a dose-range of 281 to 51μg/ml (-S9), and 0.16% RTG to 74% RTG over a dose-range of 215 to 16 μg/ml (+S9).

Conclusion: Danofloxacin mesylate did not produce a mutagenic response in the absence or presence of rat S9 in the L5178Y/TK mutation assay.

n. Two year oncogenicity study in ICR mice

Protocol No: 93-76-31

Starting Date: June 1993

Termination Date: June 1995

Study Director: S. Takatsu

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) as a feed admixture

Species and Strain: ICR mice

Number of Animals Per Treatment Group: 50/sex/group

Drug Levels Tested and Duration of Dosing: 0, 10, 50, and 100 mg/kg/day

Route of Drug Administration: Oral, admixed in the diet

Parameters Tested: Survival, clinical signs, body weight, food consumption, clinical pathology, gross and histopathology, organ weights

Significant Toxicity Observed: None

Conclusion: The administration in feed of danofloxacin to ICR mice for 24 months produced no evidence of treatment related oncogenicity.

o. Two year oncogenicity study in Long-Evans rats

Protocol No: 93-607-31

Starting Date: April 1993

Termination Date: April 1995

Study Director: D.O. Fisher

Identification of Substance and Dosage Form: Danofloxacin mesylate (CP-76, 136-27) as a feed admixture

Species and Strain: Long-Evans rats [Crl:LE (BR)]

Number of Animals Per Treatment Group: 50/sex/group

Drug Levels Tested and Duration of Dosing: 0, 10, 50, and 100 mg/kg/day

Route of Drug Administration: Oral, admixed in the diet

Parameters Tested: Survival, clinical signs, ophthalmoscopic examinations, body weight, food consumption,clinical pathology, gross and histopathology, organ weights

Significant Toxicity Observed: Survival was unaffected by treatment. Reduced body weight (less than 10%) was observed in high dose males. Clinical pathology changes included depressions in various leucocyte counts in both sexes in all treated groups. Also observed were decreases in globulin values, corresponding elevations in albumin/globulin ratios, and elevations in sorbitol dehydrogenase and aspartate aminotransferase in the high dose males. Decreased testes weight relative to body weight was observed in high dose males. In high dose males, tubular atrophy of the testes, oligospermia in the epididymis, and abnormal content in the epididymis were consistent with the known effects of quinolones on the testes.

Conclusion: The administration in feed of danofloxacin to Long-Evans rats for 24 months produced no evidence of treatment related oncogenicity.

Toxicity Studies - Desmethyl Danofloxacin Mesylate

a. Three month oral gavage study with *in utero* exposure in rats

Protocol No: 89-576-06

Starting Date: September 1989

Termination Date: December 1989

Study Director: S.W. Stadnicki

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) in aqueous oral solution

Species and Strain: Long-Evans rats [Crl: (LE)BR]

Number of Animals Per Treatment Group: 20/sex/group

Drug Levels Tested and Duration of Dosing: 0, 1.0, 2.5 and 6.25 mg/kg administered daily for three months, beginning at weaning, following *in utero* and lactation exposure.

Route of Drug Administration: Oral gavage

Parameters Tested: clinical signs, body weight, food consumption, ophthalmoscopic examinations, clinical pathology, gross and histopathology, organ weights.

Significant Toxicity Observed: None

No Observed Effect Level: 6.25 mg/kg/day

b. Three month oral capsule study in Beagle dogs

Protocol No: 89-576-03

Starting Date: March 1989

Termination Date: June 1989

Study Director: S.W. Stadnicki

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) in gelatin capsules

Species and Strain: Beagle dogs

Number of Animals Per Treatment Group: 3/sex/group

Drug Levels Tested and Duration of Dosing: 0, 2.5, 5 and 10 mg/kg administered daily for three months

Route of Drug Administration: Oral capsule

Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopic examinations, blood pressure recordings and electrocardiographic tracings, clinical pathology, gross and histopathology, organ weights.

Significant Toxicity Observed: Articular cartilage lesions were observed microscopically in one high dose dog; clinical signs indicative of sporadic joint pain were observed in one high dose and one low dose animal.

No Observed Effect Level: A NOEL could not be established

Conclusion: Typical fluoroquinolone-induced articular cartilage lesions were observed in one high dose dog. Due to sporadic articular-related clinical symptoms observed in one low dose dog, a NOEL could not be established in this study.

c. Three month oral capsule study in Beagle dogs

Protocol No: 89-576-07

Starting Date: January 1990

Termination Date: April 1990

Study Director: S.W. Stadnicki

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) in gelatin capsules

Species and Strain: Beagle dogs

Number of Animals Per Treatment Group: 3/sex/group

Drug Levels Tested and Duration of Dosing: 0, 0.25, and 0.5 mg/kg/day administered daily for three months.

Route of Drug Administration: Oral capsule

Parameters Tested: Clinical signs, body weight, food consumption, ophthalmoscopic examinations, blood pressure recordings and electrocardiographic tracings, clinical pathology, gross and histopathology, organ weights.

Significant Toxicity Observed: Articular cartilage erosion in one high dose dog

No Observed Effect Level: A NOEL of 0.25 mg/kg/day has been established

Conclusion: The two three-month oral capsule studies in dogs were conducted under similar conditions of animal age and dosage levels. Based on the results of both studies, a NOEL of 0.25 mg/kg/day can be established for desmethyldanofloxacin in dogs.

d. Three generation oral (gavage) study in Long-Evans rats

Protocol No: 89-576-04

Starting Date: April 1989

Termination Date: August 1990

Study Director: S.W. Stadnicki

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) in aqueous solution

Species and Strain: Long-Evans rats, [CrI: (LE)BR]

Number of Animals Per Treatment Group: 30/sex/group in the F_O generation. 25/sex/group in the F_1 and F_{2b} generations.

Drug Levels Tested and Duration of Dosing: Doses of 0, 1.0, 2.5, and 6.25 mg/kg were administered to F_O parental male and female rats daily for 9 weeks and 2 weeks, respectively, prior to mating and continued for both sexes throughout mating, gestation, parturition and weaning of F_1 offspring. F_1 offspring were continued on treatment while mating, producing and nursing F_{2a} and F_{2b} generations. The F_{2b} offspring were mated within dose groups and continued on drug to produce F_3 litters that were sacrificed at weaning.

Route of Drug Administration: Oral gavage

Parameters Tested: Clinical signs, body weight, food consumption, reproductive parameters

Significant Toxicity Observed: none

No Observed Effect Level: 6.25 mg/kg/day

Conclusion: The NOEL for neonatal and reproductive parameters over three generations of rats is 6.25 mg/kg/day of desmethyldanofloxacin mesylate. The results of this study indicate that none of the reproductive parameters examined were adversely affected by compound administration.

e. Microbial Reverse Mutation Assays (Ames Tests)

Protocol No: 90-576-09

Starting Date: January 1990

Report Date: January 1991

Study Director: D.E. Amacher

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) in DMSO solvent

Species and Strain: *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100.

Drug Levels Tested and Duration of Dosing: Dose levels ranged from 0.001 to 5 μg/plate. All cells were incubated at least 60 hours at 37°C.

Parameters Tested: Induction of *in vitro* bacterial mutations from histidine auxotrophic *Salmonella* cells in the presence and absence of S9 metabolic activator.

Significant Toxicity Observed: The test article was excessively bacteriostatic to the *Salmonella typhimurium* strain TA98 at a level of 1 μg/plate (-S9). Due to excessive toxicity of the test article, the maximum dose tested in the presence of metabolic activation was restricted to 0.5 ug/plate
Conclusion: Due to the excessive bacteriostatic effect of desmethyl danofloxacin, the results for the Ames Tests could not be evaluated.

f. Mouse Lymphoma L5178Y/TK ± S9 Assay

Protocol No: 86-576-01

Starting Date: January 1986

Report Date: August 1988

Study Director: H.E. Holden,

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate(CP-74, 416-27) in serial dilutions in saline

Species and Strain: L5178Y 3.7.2C mouse lymphoma cells heterozygous at the thymidine kinase locus (TK+/-)

Drug Levels Tested and Duration of Dosing: In the nonactivation phase, concentrations of 90-388 μg/mL were used; in the S9 activation phase, concentrations of 63-269 μg/mL were used. Three hour exposure.

Parameters Tested: Induction of 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)

Significant Toxicity Observed: The cell viability results for the clonable treatment conditions indicated that exposure to desmethyl danofloxacin resulted in a range of growth from 2% Relative Total Growth (RTG) to 92 % RTG over

a dose-range of 388 to 90µg/ml (-S9), and 7% RTG to 68% RTG over a dose-range of 269 to 63 µg/mL (+S9).

Conclusion: Desmethyldanofloxacin mesylate did not produce a mutagenic response in the absence or presence of non-induced rat S9 in the L5178Y/TK mutation assay.

g. *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay with Mg^{++}

Protocol No: 90-576-12

Starting Date: July 1990

Report Date: January 1991

Study Director: D. E. Amacher,

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) DMSO solvent

Species and Strain: Primary cultures of rat hepatocytes (adult male [Cdf (F-344)/CrI BR Charles River] rat)

Drug Levels Tested and Duration of Dosing:

Initial assay (without added magnesium): 62.5, 125, 187.5 and 250 µg/mL; second assay (1.62 mM magnesium): 62.5, 125, 187.5, 250 and 500 µg/mL; 18-20 hour incubation period

Parameters Tested: Detection of DNA damage by measuring unscheduled DNA synthesis (UDS) in primary rat hepatocytes *in vitro*. Autoradiographic technique was used to determine the nuclear labeling resulting from incorporation of [^{3}H]-thymidine into the DNA.

Significant Toxicity Observed: In the initial UDS assay (without MG++), there was slight reduction in the cell viability at 250 µg/mL. In the second UDS assay (with MG++), there was substantial reduction in the cell viability at 500 µg/mL.

Conclusion: The test material, desmethyldanofloxacin, induced positive UDS in primary cultures of rat hepatocytes in the presence and absence of added magnesium supplement to the culture medium.

h. *In Vitro* Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

Protocol No: 12180-0-447R

Starting Date: April 1990

Report Date: December 1990

Study Director: M. E. McKeon

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) DMSO solvent

Species and Strain: Primary cultures of rat hepatocytes (adult male Fischer 344 rats)

Drug Levels Tested and Duration of Dosing: 2.5 to 500 μg/mL

Parameters Tested: Detection of DNA damage by measuring unscheduled DNA synthesis (UDS) in primary rat hepatocytes *in vitro*. Autoradiographic technique was used to determine the nuclear labeling resulting from incorporation of [^{3}H]-thymidine into the DNA.

Significant Toxicity Observed: In trial 1, desmethyl danofloxacin was highly toxic to the hepatocytes at concentrations of 508 μg/mL and 254 μg/mL. Moderate toxicity with cellular morphologies acceptable for analysis was observed at 102 μg/mL (77.5% survival). In the second trial, the desmethyl danofloxacin was highly toxic to the hepatocytes at concentrations of 251 μg/mL and 176 μg/mL. Moderate toxicity with cellular morphologies acceptable to analysis was observed at 100 μg/mL (71.0% survival). When the concentration was decreased to 75.3 μg/mL, little toxicity (83.5%) was observed. Dose levels below 50.2 μg/mL were non-toxic.

Conclusion: The test material, desmethyldanofloxacin, induced positive UDS in primary cultures of rat hepatocytes at concentration ≤ 25 μg/mL.

i. *In Vitro/In Vivo* Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes

Protocol No: 12180-0-494

Starting Date: August 1990

Report Date: January 1991

Study Director: M. E. McKeon

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27) sterile deionized water

Species and Strain: Primary cultures of rat hepatocytes from adult male Fischer 344 rats exposed *in vivo* by oral gavage to the test article

Drug Levels Tested: 250, 500, 1000, or 2000 mg/kg administered by oral gavage

Parameters Tested: Detection of DNA damage by measuring unscheduled DNA synthesis (UDS) in primary rat hepatocytes exposed *in vivo* to the test material. Autoradiographic technique was used to determine the nuclear labeling resulting from incorporation of [^{3}H]-thymidine into the DNA *in vitro* giving a UDS measurement of repair of DNA.

Significant Toxicity Observed: In the early time point, perfusions were initiated 3.7 to 4.5 hours after administration of desmethyl danofloxacin by oral gavage. The hepatocytes collected for the UDS assay ranged in viability (determined by trypan blue exclusion) from 86.3% to 97.2% of the total cells collected. The attachment efficiency varied from 62.7% to 93.1% and the viability of the attached cells ranged from 88.6% to 97.0%. For the latter time point, perfusions were initiated 15.9 to 16.1 hours after administration of desmethyl danofloxacin by oral gavage. The hepatocytes collected for the UDS assay ranged in viability (determined by trypan blue exclusion) from 78.2% to 95.5% of the total cells collected. The attachment efficiency varied from 68.6% to 94.0% and the viability of the attached cells ranged from 86.6% to 96.5%.

Conclusion: The UDS assay, as performed, was inadequate to evaluate the UDS-inducing potential of desmethyldanofloxacin *in vivo*. Due to the lack of evidence of cytoxic effect, the test material may not have reached the target tissue.

j. *In Vivo* Micronucleus Assay

Protocol No: 90-576-10

Starting Date: May 1990

Report Date: February 1991

Study Director: D.E. Amacher,

Identification of Substance and Dosage Form: Desmethyldanofloxacin mesylate (CP-74, 416-27)

Species and Strain: male and female CD-1 mice [Crl:COBS CD(ICR)BR]

Drug Levels Tested and Duration of Dosing: 250, 500 and 1000 mg/kg/day; once daily three days by oral gavage, 5/sex/group

Parameters Tested: Clastogenic activity *in vivo* in mouse erythrocyte precursors as detected either by direct observation of bone marrow cells undergoing mitosis (metaphase analysis) or by observation of numbers of micronuclei in interphase of the daughter cells.

Significant Toxicity Observed: None noted.

Conclusion: The test material, desmethyldanofloxacin, does not induce micronuclei in male or female mouse bone marrow and peripheral blood PCEs. Reduction in the proportion of PCE in the bone marrow of both sexes indicates that the dose levels used were at the maximum tolerated dose.

Safe Concentrations of Residues

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.

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

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

The amount of microbiologically active residues of danofloxacin reaching the colon following an ADI of 144 μg/person/day (0.0024mg/kg/day) would most likely not cause adverse effects on consumer intestinal microflora.

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

Total Residue Depletion and Metabolism Studies

a. Radiotracer residue depletion study in edible tissues and injection site of cattle treated subcutaneously with [^{3}H]-danofloxacin.

Investigator: James E. Risk, M.S.
Animal Health Research Center
Pfizer Inc.
Terre Haute, Indiana 47808

Test Animals: Twenty two crossbred beef cattle (11 male castrates and 11 females) with an average body weight of 195 kg on Day 0. Two additional animals were used to provide nonmedicated tissues.

Test Substance: 18% Danofloxacin Injectable formulation containing [^{3}H]-danofloxacin.

Treatment Regimen: Three daily subcutaneous injections at a dose of 10 mg/kg body weight. The animals were sacrificed by group at 12, 24, 48, 72, and 120 hours post administration of the third dose. Three animals per sex were sacrificed at the 12 hour post administration period. Two animals per sex were sacrificed at all other post administration time periods.

Tissues Collected: Liver, kidney, muscle, fat, and the third (last) injection site.

Tissue Collection Schedule: All tissues were collected at 12, 24, 48, 72, and 120 hours after the final dose.

Total Residue Concentration: Tissue samples from each of the animals at all sampling intervals were analyzed to determine the [^{3}H] total residue depletion.

Results: The results from this study are summarized in Table 4.1

Table 4.1 Mean total tritium concentrations (± one standard error) in edible tissues and injection site of cattle treated with [^{3}H] danofloxacin subcutaneously at a dose of 10 mg/kg/day for three consecutive days.

Hours Post Final Treatment (ppm)					
Tissue	12 Hours	24 Hours	48 Hours	72 Hours	120 Hours
Fat	0.080 ± 0.017	<0.064 ± NA	LOD	LOD	<0.03 ± NA
Liver	5.7 ± 0.50	1.57 ± 0.15	0.70 ± 0.07	0.55 ±0.05	0.39 ± 0.04
Kidney	4.90 ± 0.40	0.95 ± 0.11	0.35 ± 0.04	0.23 ± 0.03	0.147 ± 0.016
Muscle	1.26 ± 0.11	0.108 ± 0.012	<0.02 ± NA	<0.02 ± NA	<0.02 ± NA
Injection Site	22.00 ± 11.00	0.28 ± 0.18	00.15 ± 0.09	0.09 ± 0.06	0.07 ± 0.05

Tissues from the study above were further analyzed using the determinative HPLC procedure for unchanged danofloxacin. In liver, the ratio of unchanged drug to total residues ranged from 40% at 12 hours after the final injection to 11% at 72 hours after the final injection. The ratio of unchanged drug to total residues was 15% at 48 hours after the final injection, the time point closest to the time that total residue concentrations fall below the safe concentration.

b. The metabolic profile of danofloxacin in cattle liver, muscle, and bile.

The concentrations of danofloxacin-related residues in liver, muscle, and bile following three daily subcutaneous doses of 10 mg/kg [^{3}H]-danofloxacin to metabolically mature ruminating cattle were determined. Tissue samples (liver, muscle, and bile) obtained at 12 hours post the last dose of [^{3}H]-danofloxacin were profiled in replicates of three, for metabolic fate. Four metabolites were identified in the tissue samples using radiochemical chromatography and mass spectrometry: N-desmethyl danofloxacin (I), danofloxacin acyl-glucuronide (II), hydration of the quinone ring (III), and danofloxacin N-oxide (IV), see Table 4.2. N-desmethyldanofloxacin was identified as the major metabolite in liver and muscle tissues. Together danofloxacin and desmethyldanofloxacin comprised 82-100% of total residues in liver and muscle tissue. Danofloxacin acyl-glucuronide was the most abundant metabolite in bile.

Table 4.2 Mean percentage of danofloxacin and metabolites in liver, muscle, and bile.

	Analyte (%)		
Compound	**Liver**	**Muscle**	**Bile**
Danofloxacin	35	74	47
N-desmethyl	47	26	---
acyl-glucuronide	---	---	26
hydration of the quinone ring	-	-	14
N-oxide	---	---	13
Unknown A	9	---	---
Unknown B	9	---	---

c. Comparative metabolism study in dog and rat

The metabolism of danofloxacin in dogs and rats was compared to cattle. The study was conducted to demonstrate exposure of danofloxacin to the laboratory species is comparable to residues derived from cattle.

The major residues derived from cattle liver were comprised of unchanged danofloxacin (24-29%) the N-desmethyl metabolite (41-43%) and traces of other metabolites 12 hours after treatment. Similar results were obtained in both the dog and rat liver tissue. Analysis of fecal extracts, urine, and bile obtained from dog and rat further demonstrated comparable residues derived from cattle.

A good match was demonstrated between the profile of extractable danofloxacin metabolites in cattle liver and the profiles obtained from the dog and rat. The findings confirm that the dog and rat were appropriate species for determining potential toxicology risks to humans associated with consumption of tissues from cattle containing danofloxacin or its metabolites.

Selection of Target Tissue and Marker Residue

Using the data from the total residue study summarized above (Total Residue Depletion and Metabolism Studies), liver was assigned as the target tissue and danofloxacin was assigned as the marker residue for cattle subcutaneously treated with 18% danofloxacin solution.

a. Target Tissue Determination

The data from the total residue study summarized in Total Residue Depletion and Metabolism Studies demonstrate that injection sites and the liver contain the highest concentrations of total danofloxacin-related residues. By 24 hours after the last dose, total danofloxacin-related residues are below the established safe concentrations in all edible tissues, except liver. Total residue concentrations in liver samples are below the safe concentration by 48 hours after the last dose. Since the liver is the edible tissue with the highest total residue concentrations and the tissue from which residues deplete the slowest, the liver is the most appropriate target tissue for danofloxacin in cattle.

b. Marker Residue Determination

The parent drug, danofloxacin and the primary metabolite, N-desmethyldanofloxacin, comprise the majority of the residues found in edible tissues. No other metabolite is present in significant quantities. Together, danofloxacin and N-desmethyldanofloxacin represent up to 80% of total residues in the target tissue, the liver. Unchanged danofloxacin is an appropriate marker residue since it represents 11-40% of the total residues in the target tissue.

Tolerance for Marker Residue

The tolerance for danofloxacin in liver of cattle was set using data obtained in the pivotal total residue depletion study. Because the total residue of danofloxacin in liver was below the safe concentration of 1.44 ppm by 48 hours after the last dose, the tolerance was calculated with the 48-hour marker:total ratio of 15%. Thus, a tolerance of 0.2 ppm is established for parent danofloxacin in liver of cattle (i.e., 15% of 1.44 ppm = 0.216, which is rounded to 0.2 ppm).

In addition, to provide guidance to FSIS/USDA, which administers a national residue monitoring program, FDA is establishing a tolerance of 0.2 ppm in muscle of cattle. This tolerance is identical to the MRL recommended by the joint WHO/FAO Expert Committee on Food Additives (JECFA). The muscle tolerance should both ensure the safe use of danofloxacin and limit trade problems.

Determination of the Withdrawal Time

a. Marker residue depletion study in edible tissues and injection site of cattle treated subcutaneously with danofloxacin.

Investigator: James E. Risk, M.S.
Animal Health Research Center
Pfizer Inc.
Terre Haute, Indiana 47808

Test animals: Thirty crossbred beef cattle (15 male castrates and 15 females) with an average body weight of 219 kg on Day 0. Two additional animals were used to provide nonmedicated tissues.

Test Substance: 18% Danofloxacin Injectable formulation.

Treatment Regimen: Three daily subcutaneous injections at a dose rate of 10 mg/kg body weight. Six animals were sacrificed by group at 12, 24, 48, 72, and 120 hours post administration of the third dose.

Tissues Collected: Liver, kidney, muscle, fat, and the third injection site.

Results: Danofloxacin residues in liver depleted from a mean of 3.9 μg/g 12 hours after the last treatment and depleted to a mean of <0.068 μg/g 120 hours after the last treatment. The highest concentrations of N-desmethyldanofloxacin residues were found in liver samples, 2.65 μg/g 12 hours after the last treatment and depleted to a mean of <0.056 μg/g 120 hours after the last treatment. Danofloxacin was the most abundant residue in all tissues examined. The results are summarized in Table 4.3.

Table 4.3 Mean danofloxacin μg/g of tissue ± one standard error) in cattle edible tissues and injection site.

Hours Post Final Treatment					
Tissue* μg/g	12 Hours	24 Hours	48 Hours	72 Hours	120 Hours
Fat	0.091 ± 0.01	<0.043 ± NA	<0.043 ± NA	<0.043 ± NA	<0.043 ± NA
Liver	3.9 ± 0.60	0.87 ± 0.13	0.17 ± 0.02	0.064 ±0.009	<0.068 ± 0.017
Kidney	7.00 ± 1.00	1.20 ± 0.18	0.15 ± 0.02	0.072 ± 0.11	<0.048 ± NA
Muscle	1.40 ± 0.20	0.20 ± 0.03	<0.046 ± NA	<0.043 ± NA	<0.043 ± NA
Injection Site	87.00 ± 57.00	6.00 ± 4.00	<0.80 ± 0.60	<1.6 ± 2.20	<0.043 ± NA

* The NTX for all tissues is <0.043 ± NA

b. Withdrawal Calculation

Liver residue depletion data from study 1531N-60-96-239 were statistically analyzed utilizing CVM's statistical tolerance limit algorithm. A liver tolerance of 0.2 ppm has been assigned (see page 54). The analysis of the data resulted in a calculated withdrawal time of 4 days. A withdrawal period of 4 days is assigned for the use of danofloxacin 18% injectable solution when administered subcutaneously up to 10 mg/kg/day for 3 consecutive days.

Regulatory Method

The determinative procedure for danofloxacin in tissues uses liquid chromatography (LC) with fluorescence detection. Tissues are homogenized in methanol/water solution containing perchloric and phosphoric acids. The extract is incubated at 50° C then centrifuged. An aliquot is analyzed using LC with fluorescence detection. For confirmation, a portion of the extract from the determinative procedure is analyzed by LC/MS/MS. The method is on file with the Center for Veterinary Medicine, Food and Drug Administration, 7500 Standish Place, Rockville, MD 20857.

Human Health Impact Assessment

Assessment of the impact on human health of danofloxacin 18% injectable to be used in cattle:

Pursuant to Guidance for Industry #78 *Consideration of the Human Health Impact of the Microbial Effects of Antimicrobial New Animal Drugs Intended for Use in Food-Producing Animals*, a study was conducted to assess the impact of the administration of danofloxacin 18% injectable solution to typical feedlot cattle on the development of resistance in a challenge strain of *Salmonella typhimurium*, and among resident *Escherichia coli*.

Pfizer study 1430N-60-99-297, titled "Effects of danofloxacin administration on ciprofloxacin susceptibility of fecal *Salmonella typhimurium* and *Escherichia coli* in calves experimentally infected with *S. typhimurium*," was conducted at Colorado Animal Research Enterprises. The study director was Diane J. Fagerberg, Ph.D. The study was conducted between January and April, 2000.

Forty, seven to nine month-old cross-bred beef calves (heifers) were acclimated and divided into two groups: a control group and a treated group. The control group received a sham injection of sterile saline SC twice, 48 hours apart, and repeated for three regimens 14 days apart (Days 0, 14, and 28). The treated group received 6 mg/kg body weight SC twice, 48 hours apart, and repeated for three regimens 14 days apart (Days 0, 14, and 28). Calves were individually housed in isolation rooms in two buildings. The calves were tested and shown to be free of salmonellae prior to procurement.

Prior to treatment, all calves were challenged with a test strain of *S. typhimurium*, shown to be highly susceptible to both ciprofloxacin and danofloxacin, but resistant to streptomycin. Colonization was shown to be successful, and calves were treated as described above. Fecal grab samples were collected on Days 2, 4, 7, and 14 following each treatment regimen, on Day10 following the first regimen, and on Days 21 and 28 following the third regimen (15 post-treatment samplings in total).

For each sample obtained from each animal, up to five isolated colonies of *E. coli* or the recoverable test strain of *S. typhimurium* were selected for microbroth dilution testing of their MICs against ciprofloxacin and danofloxacin (doubling dilutions between 0.015 and 16 μg /ml).

Results indicated that there was no remarkable change in the susceptibility of the test strain of *S. typhimurium* to ciprofloxacin following exposure to danofloxacin as judged by only a slight increase in MICs (one or two doubling dilutions) within the total MIC range, and no discernable change among MIC_{50} or MIC_{90} values. This slight change occurred early in the treatment regimen, and was gone by Day 7 of the 56 day study. Results further indicated that susceptibility of resident *E. coli* to ciprofloxacin following exposure to danofloxacin decreased sporadically as judged

by increases in MICs early in the study; however, the MIC_{90} stabilized at 0.06 μg/ml (two doubling dilution increase) by Day 21 of the 56 day study, and dropped to ≤ 0.015 by day 49.

Results from this study indicate that the use of danofloxacin at 6 mg/kg body weight injected twice 48 hours apart was safe with respect to microbial food safety, as determined by its negligible impact on ciprofloxacin resistance development among a challenge strain *Salmonella* and resident *E. coli* in treated beef cattle.

User Safety

The establishment of label warnings has satisfactorily addressed user safety concerns associated with danofloxacin. In addition, a toll-free number has been established and displayed on the label to report any adverse reactions or obtain a Material Safety Data Sheet.

5. AGENCY CONCLUSIONS

The data submitted in support of this NADA satisfy the requirements of Section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR 514 of the implementing regulations. The data demonstrate that A180® Sterile Antimicrobial Injectable Solution, a fluoroquinolone antibiotic, when administered subcutaneously to cattle at a dose of 6.0 mg/kg body weight, repeated once after 48 hours, is safe and effective for the treatment of bovine respiratory disease (BRD) associated with *Mannheimia* (*Pasteurella) haemolytica* and *Pasteurella multocida*.

Based on a battery of toxicology tests, an acceptable daily intake of 144 µg/person/day was calculated, which further yielded safe concentrations for total danofloxacin related residues of 0.48 ppm in muscle, 1.44 ppm in liver, 2.88 ppm in kidney, and 2.88 ppm in fat. The tolerance was determined following the evaluation of both liver and injection site residues from single dose and multiple dose residue studies. A tolerance of 0.2 ppm for danofloxacin, the parent compound, (the marker residue) in liver (the target tissue) is established for the subcutaneous treatment of beef cattle with danofloxacin. The tolerance refers to the residue measured by the regulatory method described herein. Using a tolerance of 0.2 ppm, liver and injection site residues following three consecutive daily subcutaneous injections of 10.0 mg/kg body weight of danofloxacin support a 4-day withdrawal period. Injection site residues resulting from the subcutaneous administration of the 18% danofloxacin solution at doses up to 10 mg/kg for up to 3 consecutive days require 2 days to deplete to their safe concentration. Following the subcutaneous administration of the 18% danofloxacin solution at doses up to 10 mg/kg for up to 3 consecutive days, liver residues deplete to 0.2 ppm by 4 days post dose.

The established tolerance of 0.2 ppm in muscle of cattle is identical to the MRL recommended by the Joint WHO/FAO Expert Committee on Food Additives (JECFA). The muscle tolerance should both ensure the safe use of danofloxacin and limit trade problems.

The Agency has concluded the amount of microbiologically active residues of danofloxacin reaching the colon following an ADI of 144 µg/person/day would most likely not cause adverse effects on consumer intestinal microflora.

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 bovine respiratory disease (BRD) associated *Mannheimia* (*Pasteurella) haemolytica* and *Pasteurella multocida*, (b) restricting this drug to use by or on order of a licensed veterinarian should help prevent indiscriminate use which could result in violative tissue residues, and (c) the rate of emergence of danofloxacin-resistant organisms may be reduced by the involvement of veterinarians in product use.

The Agency has established conditions of use that minimize potential adverse effects of the antimicrobial treatment, thereby reducing the number of fluoroquinolone-resistant zoonotic pathogens that may persist in treated animals until slaughter. These use conditions also minimize excretion of drug and fluoroquinolone-resistant zoonotic pathogens into the environment. In accordance with 21 CFR 530.41, extra-label use of this product in food-producing animals is prohibited.

Public health concerns associated with potential increases in fluoroquinolone-resistant bacteria have been satisfactorily addressed. The Agency has determined fluoroquinolone resistance in human health pathogens is very low when the drug is used according to the proposed approved conditions for use.

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval for food-producing animals qualifies for five years of marketing exclusivity beginning on the date of approval because no active ingredient of the new animal drug has been previously approved.

A180® Injectable Solution is under patent numbers U.S. 4,861,779 and 5,811,130, expiring August 19, 2006 and December 19, 2016, respectively.

6. LABELING

a. A180® - Package Insert (100 mL/250 mL)
b. A180® - Vial Label (100 mL/250 mL)
c. A180® - Box Label (100 mL/250 mL)