

FREEDOM OF INFORMATION SUMMARY

I. GENERAL INFORMATION

A. File Number

NADA 141-096

B. Sponsor

Fort Dodge Animal Health
9401 Indian Creek Parkway
Overland Park, KS 66210

C. Proprietary Name

Dicural® Tablets

D. Established Name

difloxacin hydrochloride

E. Dosage Form, Route of Administration, and Recommended Dosage(s)

The dose range of difloxacin in dogs is 5 to 10 mg/kg (2.3 to 4.6 mg/lb) once a day for 2-3 days beyond cessation of clinical signs to a maximum of 30 days. Three tablet sizes are available for ease of administration:

DICURAL® Tablet	Tablet Color	Body Weight of Dog
11.4 mg	Blue	5 lb (2.3 kg)
45.4 mg	White	20 lb (9.0 kg)
136 mg	Orange	60 lb (27.2 kg)

The 11.4 mg and the 45.4 mg tablets are single scored and the 136 mg tablet is double scored for more accurate dosing.

Dogs should be administered difloxacin hydrochloride at 5 to 10 mg/kg body weight. For the treatment of skin and soft tissue infections, DICURAL® TABLETS should be given for at least two to three days beyond the cessation of clinical signs for a maximum of 30 days. For treatment of urinary tract infections, DICURAL® TABLETS should be administered for at least 10 consecutive days. If no improvement is noted after at least five days of therapy, then the clinical case should be re-evaluated and a different course of therapy should be considered.

Dose Rationale: The dose range of 5 to 10 mg/kg was established based upon the clinical efficacy demonstrated at the 5 mg/kg dose and by the linear kinetics of difloxacin up to 60 mg/kg. In addition, the 10 mg/kg dose was found to be a dose well within the established safety margin of the drug. The negligible drug accumulation evident in the pharmacokinetic studies coupled with the lack of adverse reactions evident in the clinical field and target animal safety studies

support administration of a dose within the dose range up to a maximum of 30 days duration of therapy.

F. Dispensing Status

Prescription

G. Indication

DICURAL[®] (difloxacin hydrochloride) TABLETS are indicated for the management of diseases in dogs associated with bacteria susceptible to difloxacin.

Efficacy Confirmation: Clinical efficacy was confirmed in dogs with skin and soft tissue infections (wounds and abscesses) and urinary tract infections (cystitis).

Specific bacterial pathogens isolated in clinical field trials are listed in Table 4 in the Clinical Efficacy section of the FOI Summary.

II. EFFECTIVENESS

A. Dose Titration

Identification: Dose Titration Study for Testing the Effect of Difloxacin (Abbott Compound A56619) in Dogs (*Escherichia coli* - *Klebsiella pneumoniae* Model).

Study Director:

John N. Berg, DVM, PhD

College of Veterinary Medicine

University of Missouri-Columbia

Columbia, MO

General Design:

- Purpose: To determine the effective dose of difloxacin for the treatment of an infected dermal wound.
- Animals: Forty-eight mixed breed dogs, male and female, weighing 14.5 to 24.5 kg, were placed into four replicates (12 dogs/replicate) and each replicate was randomly allotted into four treatment groups.
- Dose Levels and Regimen: Two surgical wounds (lateral neck and abdominal midline) were created in the dogs and were inoculated with a mixed culture broth of *Escherichia coli* and *Klebsiella pneumoniae*. The four treatment groups received a placebo or difloxacin capsule at 2.5, 5, or 7.5 mg/kg body weight, respectively, once a day for seven consecutive days, beginning eight hours after inoculation of the wounds.
- Pertinent Observations and Measurements: Individual clinical appearance, rectal temperature, appetite, and wound appearance were monitored for 13 days following inoculation of the wound. The parameters were scored, with each score tailored to the parameter according to severity. In addition, individual blood samples were collected for the determination of total and differential WBC count, total RBC count and hematocrit. The primary parameter of efficacy was lesion healing.

Scoring System:**Lesion Scores:**

- 0 = no signs of inflammation
 - 1 = slight swelling and/or pain on palpation
 - 2 = moderate swelling, pain on palpation, erythema
 - 3 = severe swelling, extreme pain on palpation, erythema
 - 5 = death of dog
- Added scores:
- purulent discharge, 1 point added to daily score
 - dehiscence of suture line, 1 point added to daily score

Clinical Appearance Scores:

- 0 = alert, afebrile, normal appetite
 - 1 = slight depression yet responsive to stimuli
 - 2 = depression poor response to stimuli
 - 3 = severe depression, recumbent, no response to stimuli
 - 5 = death of dog
- Added Scores: elevated temperature ($>$ or $= 1^{\circ}\text{F}$), 1 point added to score

Culture Scoring System:

- 0 = No growth
- 1 = Growth from TSB subculture only
- 2 = Colony log count 2
- 3 = Colony log count 3
- 4 = Colony log count 4
- 5 = Colony log count 5
- 6 = Colony log count 6
- 7 = Colony log count 7

The lesions on the neck were examined culturally on post-infection days 1, 2, 3, 6, 9, and at necropsy (day 13). The abdominal lesions were examined at necropsy only. The isolated colonies were identified as either *Escherichia coli* or *Klebsiella pneumoniae* and given a score based on their respective log counts. At the conclusion of the study the dogs were euthanatized and necropsied. The incisions were re-opened and the tissues were examined grossly and via culture for signs of residual infection.

Results: At the end of the study the clinical and culture scores were combined and the results from the four groups were compared statistically. The summary of the mean lesion healing scores were:

Lesion Healing Scores (Combined) *

Treatment Group (mg / kg)	Mean Clinical Score	Mean Culture Score <i>E. coli</i> & <i>K. pneumoniae</i>
0	3.96 ^a	8.08 ^a
2.5	3.44 ^{ab}	6.52 ^b
5.0	2.92 ^b	4.44 ^c
7.5	3.23 ^b	4.46 ^c

*The mean clinical combined score was based on the neck and abdominal lesion scores plus clinical signs. Different letters (a, b, or c) within a column indicate the means are significantly different ($P < 0.05$).

Difloxacin administered orally at 5.0 mg/kg bodyweight was found to be an effective dose for the treatment of *Escherichia coli* and *Klebsiella pneumoniae* induced dermal wound infections.

B. Dose Confirmation

Identification: Trial no. P146 - Dose Confirmation Study for Testing the Efficacy of Difloxacin (Abbott Compound A56619) Against Skin Wound Infection in Dogs (*E. coli* - *Klebsiella pneumoniae* Model).

Study Director:

Robert J. Harman, DVM, MPVM
HTI Bio-services, Inc.
Ramona, CA

General Design:

- Purpose: To confirm the effectiveness of difloxacin administered at 5.0 mg/kg against induced dermal wound infections in dogs.
- Animals: Twenty-four dogs, male and female, weighing 13.6 to 24.5 kg, were allotted randomly into two treatment groups of 12 dogs each.
- Dose Levels and Regimen: Two surgical wounds (lateral neck and abdominal midline) were induced in the dogs and were infected with a mixed culture of *Escherichia coli* and *Klebsiella pneumoniae*. The two treatment groups were administered a placebo or difloxacin in capsules at 5.0 mg/kg body weight once a day beginning eight hours post-infection for seven consecutive days.

Pertinent Observations and Measurements: The parameters that were observed daily beginning on day 1 and continuing through day 13, and scored according to a standardized scoring method (See Dose Determination Study) were general clinical appearance, appearance of wounds, wound cultures, rectal temperature and appetite. The lesions on the neck were examined culturally on post-infection days 1, 2, 3, 6, 9, and at necropsy (day 13). The abdominal lesions were examined at necropsy only. The dogs were monitored for 13 days following inoculation and then euthanatized and necropsied. The primary parameter of efficacy was lesion healing.

Results: Lesion healing for the difloxacin-treated dogs was significantly different by Day 5 and Days 5 & 6 combined ($p < 0.05$) using the Wilcoxon Rank Sum Test for treatment differences. However, after Day 6, the control animals were self-curing and differences in lesion healing scores were not seen. The summary of the lesion healing scores were:

Mean Clinical Scores (Combined) *

Treatment Group (mg / kg)	Day 5	Day 6	Day 5 & 6
0	4.7 ^a	4.3 ^a	4.5 ^a
5.0	3.5 ^b	3.2 ^a	3.3 ^b

* The mean clinical scores were based on the neck and abdominal lesion scores plus clinical signs for the 12 dogs in each group. The higher the score the more severe was the case. Different letters (a or b) within a column indicate that the means are significantly different ($P < 0.05$).

The administration of difloxacin at 5.0 mg/kg bodyweight was determined to be efficacious for the treatment of *Escherichia coli* and *Klebsiella pneumoniae* infections in skin wounds when compared to placebo treated dogs based on combined lesion healing scores.

C. Bioequivalence of Difloxacin HCl Capsules and Tablets

Identification: Study no. 92-304-085 - Bioequivalence of Difloxacin HCl Capsules and Tablets Following Oral Administration to Dogs.

Study Director:

In-Life Phase:

Beth Reagan, AAS
Liberty Research
Waverly, NY

Analytical Phase:

John Byrd, Ph.D.
Southwest Bio-Labs
Las Cruces, NM

Statistical Phase:

Thomas Keefe, Ph.D.
Envirostat Associates
Fort Collins, CO

General Design:

- Purpose: To determine the relative blood level bioavailability of the tablet and capsule formulations when equal doses of each were administered.
- Animals: Two test groups of 10 dogs (each consisting of 5 males and 5 females), ages 11 to 15 months, weighing 6.8 to 10.4 Kg, were used for this study.
- Dose Levels and Regimen: A two-period crossover design was used for the study. Dogs were fasted 12 hours prior to dosing until 6 hours after dosing. Water was available ad libitum. All animals were administered equivalent single doses of difloxacin capsules or tablets at 5 mg base equivalent per kilogram body weight. The start of each test period was separated by a 14-day washout period.

Pertinent Observations and Measurements: Plasma from blood samples collected before dosing (0 hour) and at 11 sampling times after dosing (0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 hours) were assayed by HPLC methods to determine circulating plasma concentrations of difloxacin.

Results: Based on a criterion that the 90% confidence interval for the difference between product means be within plus or minus 20% of the capsule product mean, the difloxacin HC1 tablet product was found to be bioequivalent to the difloxacin capsule product with respect to the Area Under the Concentration curve (AUC_{tau}) from zero through the time (tau) corresponding to the last quantifiable concentration. This criterion was also satisfied by the supplementary pharmacokinetic parameters: elimination half-life (T_{1/2}), AUC_{infinity}, and MRT_{infinity}. Thus, the rate and extent of bioavailability of the tablet and capsule formulations were determined to be equivalent. Therefore, the dose determination data, originally generated with the capsule formulation, was determined to be applicable to the tablet formulation.

Table 1. Mean Plasma Pharmacokinetic Parameters of the Tablet and Capsule after a Single Oral Administration at 5 mg/kg.

Pharmacokinetic Parameter	Units	Mean Value	
		Tablet	Capsule
AUC _{infinity}	mcg·hr/mL	14.5	16.2
AUC _{tau}	mcg·hr/mL	13.7	15.4
AUMC _{infinity}	mcg·hr ² /mL	162.5	183.4
AUMC _{tau}	mcg·hr ² /mL	120.1	140.6
MRT _{infinity}	hr	10.7	10.9
Elimination Half-Life (T _{1/2})	hr	9.3	9.4
Maximum Concentration (C _{max})	mcg/mL	1.8	2.1
Maximum Time (T _{max})	hr	2.8	2.3

D. Therapeutic Bioequivalence of Difloxacin HCl Tablets and Capsules

Identification: Study no. 93-204-001 - Therapeutic Bioequivalency of Difloxacin HCl Tablets and Capsules Against Induced *Escherichia coli* Urinary Infection in Dogs.

Study Director:

Martin Gilman, PhD
Liberty Research
Waverly, NY

General Design:

- Purpose: To compare the efficacy of the difloxacin capsules to tablets using an induced urinary infection in dogs.
- Animals: Thirty-six (36) Beagle dogs one and a half to three years of age, weighing 7.2 to 12.1 kg, were used in this study.
- Dose Levels and Regimen: The dogs were randomly allocated to one of three treatment groups; placebo, difloxacin tablet, or capsule. Each study dog's bladder was primed for infection, and, through a sterile urethral catheter, rinsed with sterile saline and then inoculated with *Escherichia coli*. The animals were administered 5 mg difloxacin/kg body weight beginning on day 8 post-inoculation.

Pertinent Observations and Measurements: Urine was collected by cystocentesis and cultured prior to inoculation and on post-inoculation (PI) days 7, 12, 15 and 19. Because urine is usually sterile, bacterial elimination was used as the primary parameter of efficacy.

Results: On day 7 post infection (PI), it was determined statistically that the dogs were uniformly infected. The rates of *E. coli* elimination (based on urine cultures) were compared on the 5th day of treatment (day 12 PI) and one day following the end of the seven day treatment schedule (day 15 PI). The active treatments, difloxacin tablets and capsules, administered on days 8-14 post-infection significantly eliminated *E. coli* on days 12 and 15 when compared to the placebo treated dogs. There was not a significant difference between the two difloxacin treatment groups when elimination rates of *E. coli* on days 12 and 15 PI were compared.

Dogs Positive for Induced *E. coli* Urinary Tract Infections

Treatment Group	Days Post Infection			
	7	12	15	19
Placebo	9 ^a	6 ^a	6 ^a	3 ^a
Tablet	10 ^a	1 ^b	0 ^b	0 ^a
Capsule	11 ^a	0 ^b	1 ^b	4 ^a

* Treatment administered on days 8-14 post-infection. Different letters (a or b) within a column indicate the numbers are significantly different (P<0.05).

This study demonstrated the therapeutic bioequivalence of difloxacin tablets and capsules administered at 5 mg/kg/day for seven consecutive days to treat *E. coli* urinary tract infection in dogs.

E. Pharmacokinetics and Metabolism

1. Study no. Abbott-56619:2

Identification: The Metabolism and Pharmacokinetics of ¹⁴C-Difloxacin Base in Dogs.

Study Director:

Ilmar Merits, PhD
Abbott Laboratories
North Chicago, IL

General Design:

- Purpose: To study the metabolism and pharmacokinetics of ^{14}C -difloxacin (radiolabeled difloxacin) base in beagle dogs.
- Animals: Two male and four female beagle dogs weighing between 6 to 10 kg and 10 to 16 months old were used in the study.
- Dose Levels and Regimen: The dogs were administered 10 mg difloxacin base/kg body weight (30 $\mu\text{Ci}/\text{dog}$) orally by gavage and 3 weeks later by intravenous administration. All administrations were made following an overnight fast. Fasting was continued for an additional 12 hours postdosing.

Pertinent Observations and Measurements: After oral and intravenous administration of 10 mg base/kg doses, both the total radioactivity and parent drug levels in the plasma, urine and feces were measured. Whole blood and fecal samples were combusted and the resulting $^{14}\text{CO}_2$ was trapped in Carbo-Sorb and was measured by liquid scintillation. Plasma, bile and urine samples were added directly to the liquid scintillation cocktail and were counted. To assess the concentrations of metabolites in the blood, plasma samples were analyzed by High Performance Liquid Chromatography (HPLC).

Results: After oral administration of a 10 mg base/kg dose, both the total radioactivity and parent drug levels in the plasma peaked at 2.4 mcg/mL by 1.5 hours. Within five days after oral administration of ^{14}C -difloxacin base, 16% of the ^{14}C dose was excreted in the urine and 80% was eliminated in the feces.

The unchanged parent drug accounted for 64% of the ^{14}C dose and was primarily excreted in the feces. The two major metabolites were the ester glucuronide of difloxacin and the desmethyl derivative of difloxacin, each representing about 12% of the ^{14}C dose.

Since 80% of the ^{14}C dose was eliminated in the feces after intravenous administration of ^{14}C -difloxacin, biliary secretion appeared to be an important factor in the disposition of the compound. Renal clearance accounted for less than 5% of the total systemic clearance. The ester glucuronide of difloxacin accounted for 72-80% of the radioactivity in the bile and only 6-9% was free parent drug. The difference between the fecal and biliary metabolic patterns suggested that the glucuronide of difloxacin was hydrolyzed in the gastrointestinal tract and that the parent drug undergoes enterohepatic cycling.

2. Study no. Abbott-56619:16

Identification: The Distribution of Radioactivity in the Tissues of Dogs after Oral Administration of ^{14}C -Difloxacin Base.

Study Director:
Barbara A Bopp, PhD
Abbott Laboratories
North Chicago, IL

General Design:

- Purpose: To determine the tissue concentrations of difloxacin in dogs over a 24-hour timeframe.

- **Animals:** Tissue distribution studies were conducted in four male beagle dogs, weighing 10 to 12 kg and approximately one year old.
- **Dose Levels and Regimen:** The dogs were given a single oral 10 mg/kg dose of ^{14}C -difloxacin base (47-51 $\mu\text{Ci}/\text{dog}$) following an overnight fast.

Pertinent Observations and Measurements: Selected tissue samples were obtained from two dogs at two hours after drug administration, from one dog at six hours after dosing, and from one dog at 24 hours after dosing. Tissue samples were homogenized and combusted. The resulting $^{14}\text{CO}_2$ was assayed in quantified via liquid scintillation procedures.

Results: The results are presented in Table 2.

Table 2: Levels of Radioactivity in Tissues of Dogs 2, 6, and 24 Hours after Single Oral Administration of 10 mg/kg BW ^{14}C -Difloxacin Base

Tissue	mCg equivalents/g or mL		
	2 hr.	6 hr.	24 hr.
Stomach	66.69	8.47	9.89
Small Intestine	18.26	38.69	16.41
Large Intestine	4.68	7.82	102.1
Liver	10.68	7.79	4.58
Kidneys	5.00	2.75	1.48
Heart	3.80	1.61	1.05
Lungs	3.22	0.91	0.76
Spleen	3.48	1.11	1.08
Pancreas	4.50	2.19	2.17
Adrenals	2.71	1.74	1.65
Prostate	3.36	1.46	1.41
Testes	3.07	1.56	0.78
Bladder	2.98	1.84	1.70
Thyroid	4.24	2.56	2.06
Lymph Nodes	3.06	2.46	1.71
Salivary Glands	4.62	2.32	1.32
Pituitary	3.68	1.59	1.03
Brain	1.30	0.62	0.48
Cerebral Spinal Fluid	1.10	0.58	0.39
Bone	6.45	7.23	6.51
Synovial Fluid	1.12	1.75	1.52
Muscle	4.12	1.17	1.12
Fat	0.73	0.84	0.80
Blood	2.26	1.05	0.60
Plasma	2.57	1.24	0.75
Bile	1231.00	1874.00	1624.00

3. Study number Abbott-56619:15

Identification: Plasma Difloxacin Base Levels After Oral Administration of 5, 20, and 60 mg/kg/day Doses to Dogs for 3 Months.

Study Director:
G. Richard Granneman, PhD

Abbott Laboratories
North Chicago, IL

General Design:

- Purpose: To study the plasma levels of difloxacin following oral administration of 5, 20 and 60 mg (base)/kg/day doses of difloxacin in dogs for 3 months.
- Animals: Forty-four purebred beagle adult dogs (22 males, 22 females) were used in this study.
- Dose Levels and Regimen: Dogs were assigned into one of four treatment groups: vehicle control (14 animals), 5 mg difloxacin base/kg/day (8 animals); 20 mg/kg/day (8 animals) or 60 mg/kg/day (14 animals). Dogs were fed once daily approximately 2 hours after dosing, and the food was removed the following morning at least 1.5 hours prior to dosing. Tap water was available ad libitum.

Pertinent Observations and Measurements: Plasma samples from the treated dogs were measured for difloxacin base prior to and three hours after dosing on days zero, 6, 28, 56 and 84. Plasma concentrations of the difloxacin base were quantified using HPLC procedures.

Results: Plasma drug levels prior to and three hours after dosing were linearly proportional to the administered doses. The hour 3 difloxacin concentrations averaged across the five sampling days are shown in the table below. The slightly lower plasma drug concentrations and somewhat greater variability associated with the 60 mg/kg dose is attributable to the higher frequency of emesis in the 60 mg/kg group. Except for random fluctuations in steady-state difloxacin concentrations, difloxacin kinetics appeared to remain constant, regardless of the duration of dosing. In addition, it appears that the drug does not accumulate in the plasma after repeated dosing.

Table 3: Mean plasma difloxacin concentrations

DOSE (mg/kg)	MEAN CONCENTRATIONS (µG/mL) + / - SD		% CV
	OBSERVED *	DOSE NORMALIZED **	
5	1.39 +/- 0.44	16.68	31.5
20	5.46 +/- 2.32	16.38	42.5
60	15.13 +/- 9.19	15.13	60.75

SD Standard Deviation

%CV Coefficient of Variation (SD/mean concen.)

* hour 3 plasma difloxacin concentrations across the 5 day study

** average hour 3 plasma difloxacin concentrations normalized to a 60 mg/kg daily dose

F. Clinical Field Trials

The safety and efficacy of difloxacin tablets administered to dogs at 5.0 mg/kg body weight was confirmed in a multi-center clinical field program. Dogs presented to the investigators with clinical signs of bacterial infections as defined in the protocol were admitted into the study. Eighteen veterinarians located in four different geographical areas of the U.S. (Southeast, Midwest, Northwest, and West) conducted the clinical efficacy and safety evaluations.

Study Investigators:

Michael Andrew, DVM, Portland, OR
Robert Blomme, DVM, Audubon, IA
Steve Callahan, DVM, Corvallis, OR
Glenn Finnell, DVM, Orlando, FL
Paul Glouton, DVM, Lilburn, GA
Richard Heers, DVM, Tulare, CA
Ken Krawford, DVM, Snellville, GA
Steven Krome, DVM, Walnut Creek, CA
Ted Lamp, DVM, Bellville, TX
Rick Lungo, DVM, Spokane, WA
Lonna Nielsen, DVM, St. Cloud, MN
Rob Privette, DVM, Kennewick, WA
Harmon Rogers, DVM, Snohomish, WA
Brian Scott, DVM, Lake Mary, FL
Roger Tsuruda, DVM, Fresno, CA
Ron White, DVM, Osceola, IA
Jim Winsor, DVM, Inver Grove Heights, MN
Robin Woodley, DVM, Oakland, CA

General Design:

- Purpose: To confirm the safety and efficacy of difloxacin tablets when administered to dogs using enrofloxacin (Baytril®) as a positive control.
- Animals: Difloxacin was administered to 141 dogs of various breeds, weights and ages. The variety of breeds depicted the U.S. canine population. Body weights and ages ranged from 1.4 to 55.9 kg and 9 months to 16 years, respectively. No breed, weight or age susceptibilities were observed. Enrofloxacin was used in a group of 131 dogs, as a positive control for comparative purposes. Fifty-six (56) enrofloxacin-treated animals and 55 difloxacin-treated animals were excluded from clinical and bacterial response evaluations primarily due to no initial pathogen isolation.
- Dose Levels and Regimen: Dogs were randomly treated with enrofloxacin (Baytril®) tablets at 2.5 mg/kg body weight twice daily (positive control) or difloxacin (DICURAL®) tablets at 5.0 mg/kg body weight once daily. Before dosing with either antibacterial tablet, a pretreatment culture sample was obtained to identify the bacterial pathogen and to perform susceptibility testing. Each dog was administered the respective antibiotic for 5 to 10 days (depending on severity of clinical signs). Concurrent therapy involved heartworm prevention, thyroid augmentation, flea control, anesthetics, anti-seizure, topical antibiotic/anti-inflammatory and anti-histamine medication.

Pertinent Observations and Measurements: Bacterial elimination was used as the primary parameter of efficacy for the urinary tract, while the resolution of the clinical condition was used as a primary parameter for the dermal system. After approximately five days of treatment each dog was returned to the investigator for a follow up evaluation.

Resolution or persistence of dermal wound clinical signs was evaluated and a bacterial culture obtained if the lesion was not healed. If clinical signs had abated then the treatment was continued for another 2 to 3 days. If the clinical signs had not abated, the treatment was continued for a maximum of 5 more days. Dogs

treated for 10 days were given a final examination 3 to 5 days following the last day of treatment. Resolution or persistence of clinical signs was again evaluated and a culture taken to determine if the pathogen was eliminated.

Resolution of urinary tract infections was evaluated through the use of a second bacterial culture performed on a cystocentesis urine sample at day 5. The treatment was discontinued when the second culture was negative. If the urine culture was positive at day 5, the dog was treated for an additional five days. Dogs treated for 10 days had an additional culture taken three to five days following the last day of treatment.

For both dermal and urinary tract infections, the dog was taken off the study and treated according to the best judgment of the investigator if the clinical condition had deteriorated. Any untoward reactions or clinical signs not expected to occur with the respective clinical syndrome were recorded by the investigator.

Results: Clinical and bacterial (i.e., pathogen elimination) responses for the dogs treated with enrofloxacin and difloxacin tablets were determined. The results from the cases that were evaluated for clinical efficacy are provided in Table 4.

Table 4. Clinical Efficacy

Enrofloxacin				Difloxacin		
		Clinically Resolved*				Clinically Resolved*
	No. of Cases	Resolved Day 5	Resolved 3-5 days post treatment	No. of Cases	Resolved Day 5	Resolved 3-5 days post treatment
Urinary Infection**						
<i>E. coli</i>	10	9	9(90%)	20***	17	20(100%)
<i>Proteus spp.</i>	7	7	7(100%)	5	5	5(100%)
<i>Staph. spp.</i>	1	0	1(100%)	7	6	6(86%)
Dermal Infection**						
<i>Staph. spp.</i>	34	14	32(94%)	38	12	36(95%)
<i>E. coli</i>	2	1	2(100%)	1	0	1(100%)
<i>Kleb. spp.</i>	0	0	0	2	1	1(50%)

* Clinically resolved after 5 days of treatment or 3-5 days after the last treatment. Numbers are cumulative.

** Bacterial elimination was the parameter of efficacy for the urinary system, while resolution of the clinical condition was the parameter of efficacy for the dermal system.

*** The therapeutic bioequivalence study provided 10 cases. Nine were clinically resolved (bacterial elimination) after 5 days of treatment.

Bacteria isolated during the clinical field trial were cultured for identification and susceptibility testing at Iowa State University. The testing was performed on all efficacy and safety cases for both enrofloxacin and difloxacin cases. All microbial sensitivity testing was performed to National Committee for Clinical Laboratory Standards (NCCLS) standards. The minimum inhibitory concentrations (MICs) for difloxacin from the isolates collected during the clinical trial are listed in Table 5.

Table 5. MIC values* ($\mu\text{g/mL}$) of difloxacin for bacterial pathogens isolated from skin and soft tissue infections and urinary tract infections in dogs enrolled in clinical studies conducted during 1991-1993.

Organism	No. of Isolates	MIC ₅₀	MIC ₉₀	MIC Range (mcg / mL)
<i>Enterobacter</i> spp.	9	0.11	3.66	< or = 0.05-3.66
<i>Escherichia coli</i>	28	< or = 0.05	0.11	< or = 0.05-7.3
<i>Klebsiella</i> spp.	8	0.11	0.11 0	0.11-0.23
<i>Pasteurella</i> spp.	8	< or = 0.05	< or = 0.05	< or = 0.05
<i>Proteus</i> spp.	15	0.92	1.83	0.11-1.83
<i>Pseudomonas</i> spp.	5	0.11	0.92	< or = 0.05-0.92
<i>Staphylococcus</i> spp.	193	0.23	0.46	< or = 0.05-1.83
<i>Streptococcus</i> spp.	56	1.83	3.66	0.11-7.3

* The correlation between the in vitro susceptibility data (MIC values) and clinical response has not been determined.

Nineteen of 131 (14.5%) enrofloxacin-treated dogs and 17 of 141 (12.1%) difloxacin-treated dogs were noted by the investigators to have experienced side effects such as: emesis, anorexia, diarrhea and inappetance.

G. MIC Study:

Identification: Study 95-500-001: In Vitro Antibacterial Activity of Difloxacin Against Canine Bacterial Isolates

Study Director:

Robert Walker, MS, PhD
Michigan State University

General Design: Three hundred canine clinical isolates consisting of *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp. or *Staphylococcus intermedius* were obtained from seven different geographical regions of the United States. The in vitro activity of difloxacin was determined using the microbroth dilution and disk diffusion test procedures in accordance with NCCLS, M31-P. Each isolate was also tested against enrofloxacin.

Results: Cumulative percent: Total number of organisms with MICs equal to or less than the indicated dilution divided by the total number of that species of bacteria tested. Difloxacin was most active against *E. coli*. MIC values against *K. pneumoniae* had the greatest variability. *Proteus* spp. was the least susceptible to difloxacin in

vitro. The body site from which the organisms were isolated did not influence the susceptibility to difloxacin. In addition, within a bacterial species, there was no difloxacin susceptibility difference depending on the geographic source of the isolate.

Table 6. MIC values ($\mu\text{g/mL}$) of difloxacin for canine bacterial isolates collected during a comprehensive study conducted in the United States during 1995-1996.

Bacteria Name	Number of Isolates	MIC₅₀	MIC₉₀	MIC Range
<i>Escherichia coli</i>	78	0.11	0.23	0.01 to >0.91
<i>Klebsiella pneumoniae</i>	20	0.46	0.46	0.03 to >0.91
<i>Proteus</i> spp.	38	0.91	0.91	0.46 to 1.82
<i>Staphylococcus intermedius</i>	164	0.91	0.91	0.11 to >0.91

*The correlation between the *in vitro* susceptibility data (MIC values) and clinical response has not been determined.

III. ANIMAL SAFETY

A. Ten-Day Drug Tolerance Test with Difloxacin Hydrochloride Tablets in Adult Beagle Dogs

Study Director:

William Voss, DVM
Hazelton Research Products, Inc.
Kalamazoo, MI

- Purpose: To evaluate the safety of 50 mg/kg body weight difloxacin administered daily to beagle dogs for 10 consecutive days.
- Animals: Two adult male and two adult female Beagle dogs at 9 to 11 months of age, weighing 9 to 11 kg, were administered difloxacin tablets.
- Dose Levels and Regimen: Difloxacin tablets were administered
- to dogs once daily for 10 consecutive days at a dosage level of 50 mg/kg body weight/day.

Pertinent Observations and Measurements: Data acquisition consisted of physical examinations, appearance and behavioral characteristics, mortality/morbidity observations, post dose observations for overt toxicity, body weights, food consumption, hematology, clinical chemistry, urinalysis and fecal analysis values, gross necropsy, organ weights, and histopathological evaluation.

Results: No treatment-related findings were observed in any male or female dogs with regard to physical examinations, mortality/morbidity observations, hematology and urine analyses, organ weights, and histopathological evaluation.

Isolated treatment-related findings of orange/yellow colored feces, emesis and/or excessive salivation were observed in some dogs when administered 50 mg/kg/day

of difloxacin tablets during the 10 days of dosing. Mean food consumption during the dosing period decreased by 17% for males and 35.8% for females as compared to mean food consumption during the acclimation period. Individual animal weight loss during the dosing period ranged from 2.4% to 14%. Gross necropsy revealed granular-like particles of the glucuronide ester of difloxacin in the bile within the gall bladder in three of the four dogs administered 50 mg/kg/day BW for 10 days. The same three animals also showed clinical signs of orange colored emesis and feces. All study animals survived to study termination.

B. Target Animal Safety Study with Difloxacin HCl Tablets in Dogs.

Study Director:

Clare M. Salamon
Hazleton Wisconsin, Inc.
Madison, WI

General Design:

- Purpose: To evaluate the effect of difloxacin when administered to dogs at 5, 15, and 25 mg/kg body weight.
- Animals: Difloxacin tablets were administered to three groups of skeletally-mature, 9.5 to 11.5 months old, beagle dogs (four/sex/group) weighing between 9.5 and 17.6 kg.
- Dose Levels and Regimen: Dogs were dosed at levels of at 5, 15 or 25 mg/kg of body weight/day (mg/kg) for 30 days. A control group (four/sex) received placebo tablets for 30 days.

Pertinent Observations and Measurements: The animals were observed twice daily for signs of toxicity, morbidity and mortality. Examinations to detect lameness and carpal flattening were conducted daily by two independent observers who were blinded to treatment group assignment. Food consumption was measured daily and body weights were recorded weekly. Electrocardiographs and ophthalmic examinations (including electroretinograph recordings) were conducted before initiation of treatment and four weeks after treatment initiation. Samples for hematology, clinical chemistry and urinalysis evaluations were collected prior to treatment and on days 9 and 31.

After 30 days of treatment, the animals were necropsied and the required tissues were weighed and collected. Histopathology was performed on all tissues from two dogs/sex from the nonmedicated control and 25 mg/kg difloxacin treatment groups. Articular cartilage was examined histologically from the animals from all treatment groups.

Results: All animals survived to scheduled sacrifice. Antemortem observations included diarrhea and transient erythema/edema. Erythema and edema (red/warm) were observed on the ears, face, lips, and around the eyes. The erythema and edema were transient and were not corroborated as specific lesions by histopathology. Transient erythema and edema have been noted in dogs administered fluoroquinolones for extended periods with dosages above the suggested usage rate.

All dogs were considered sound when examined by either of two independent observers or by a laboratory animal veterinarian. There were no confirmed occurrences of flattening (hyperextension) of the radiocarpal joint. There were no articular cartilage lesions characteristic of quinolone-induced arthropathy of juvenile dogs.

No ocular abnormalities were observed and all electroretinographic recordings were considered to be within normal range. There were no consistent arrhythmias or cardiac abnormalities that appeared to be difloxacin related.

C. Thirteen (13)-Week Capsule Toxicity Study with Difloxacin in Dogs with a 4-Week Recovery Period.

Study Director:

Matthew J. Palazzolo, Ph.D.
DABT Hazleton Wisconsin, Inc.
Madison, WI

General Design:

- Purpose: To establish the subchronic toxicity of difloxacin when administered orally to young dogs.
- Animals: Purebred Beagles (four to six/sex/group), 3.5 to 3.8 months old and weighing 4.2 to 7.3 kg, were used in this study.
- Dose Levels and Regimen: Difloxacin was administered orally to dogs at 0, 5, 25, 35, 50, or 125 mg/kg body weight per day by gelatin capsule once daily for 13 weeks, followed by a four week recovery period.

Pertinent Observations and Measurements: Parameters that were observed were clinical signs, food consumption, clinical pathology, physical examinations, electrocardiograms, electroretinograms, ophthalmic exams, gross pathology, and histopathology.

Results: Observations seen at different dose levels included periorbital swelling, red skin, red hair coat, or both; elevated third eyelid; and swelling of the ear, conjunctiva, and/or muzzle. The erythema and edema (e.g., swelling, red skin) were readily reversible, sporadic, and could not be correlated with other findings that were considered toxicologically significant. Two of eight nontreated dogs developed erythema on two different days, suggesting the possibility of other pre-disposing factor(s). At dose levels greater than 35 mg/kg there was an increased incidence of emesis, which appeared to diminish in frequency with continued exposure. Body weights were significantly lower during weeks 1 through 12 for females given 50 mg/kg. At 125 mg/kg, tremors, twitching, convulsions, dehydration, recumbency, tachycardia, increased incidence of lameness, loss of weight and weakness were also observed.

Clinical pathology observations included: lower total protein and globulin in males and females given 25, 35, 50, or 125 mg/kg; lower urine pH and fewer bacteria in urine sediment; higher aspartate aminotransferase and gamma glutamyl-transferase for males and females given 125 mg/kg; and higher alanine aminotransferase for males given 50 or 125 mg/kg and for females given 125 mg/kg.

An increased incidence of bilateral flattening (hyperextension) of the radiocarpal joint was noted at dose levels greater than 5 mg/kg and was dose related. Lameness was noted for a male and female dog given 50 mg/kg at Week 2.

Drug-related effects were found in the articular cartilage of the femur and proximal tibia, principally in the animals given 50 or 125 mg/kg. Infrequently, similar changes were noted in males given 5, 25, or 35 mg/kg. In addition, increased incidence of hepatic biliary hyperplasia was noted in the animals given 35, 50, or 125 mg/kg.

Difloxacin Safety Summary:

Adult/Skeletally Mature Dogs:

At 50 mg/kg for 10 days (5X the upper end of the dose range for one-third the recommended treatment) orange/yellow colored feces, emesis, excess salivation, decrease food consumption and deposition of difloxacin in the gall bladder occurred.

At levels of 5, 15 or 25 mg/kg for 30 days (maximum of 2.5X the upper end of the dose range for the labeled duration of dosing) diarrhea, erythema and edema of the face and ears were observed.

At 5 mg/kg once a day for ten days (as was administered in the clinical field trial) side effects such as emesis, anorexia, diarrhea and inappetance were seen in 12% of the dogs.

Immature dogs:

In puppies 3.5 - 4 months of age, erythema and edema of the face and ears were observed at doses of 5 mg/kg and above. Lameness was observed at doses of 25 mg/kg and above. At doses of 35 mg/kg emesis was seen. At 125 mg/kg, tremors, weight loss, weakness and convulsions were noted. Histologic evidence of articular cartilage defects were seen at doses of 50 mg/kg and above in female puppies and at doses of 5 mg/kg and above in male puppies. Hepatic biliary hyperplasia was noted in animals given doses higher than 35 mg/kg.

IV. HUMAN FOOD SAFETY

Data on human food safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. The drug is to be labeled for use in dogs, which are non-food animals.

V. AGENCY CONCLUSIONS

The data in support of this NADA comply with the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and section 514.111 of the implementing regulations. The data demonstrate that DICURAL[®] TABLETS (difloxacin hydrochloride), when used under labeled conditions, is safe and effective.

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is judged to be critical in the diagnosis and proper treatment of urinary and soft tissue infections.

Under section 512(c)(2)(F)(iv) of the FFDCA, this approval for non-food producing animals qualifies for three years of marketing exclusivity beginning on the date of approval because the applicant has elected to waive section 512(c)(2)(F)(i) of the Act.

Patent Information: Difloxacin hydrochloride - Patent number 4,730,000. Expiration date is March 8, 2005.

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