ENVIRONMENTAL ASSESSMENT

Danofloxacin 18% Injectable Solution for the Treatment
of Respiratory Disease in Cattle

1. DATE: May, 2000

REVISED: March, 2002

APPLICANT: Pfizer Inc
(Sponsor #000069)

ADDRESS: 235 East 42nd Street
New York, N.Y. 10017

2. DESCRIPTION OF THE PROPOSED ACTION:

Requested Approval and Need for the Action

Pfizer Inc is filing a New Animal Drug Application requesting approval for the use of danofloxacin 18% injectable solution (Advocin® 180) in cattle for the treatment of bovine respiratory disease. Bovine respiratory disease (BRD) continues to be a primary cause of production losses in all cattle producing regions of the United States. Danofloxacin 18% injectable solution will provide for effective treatment of BRD associated with *Pasteurella (Mannheimia) haemolytica* and *Pasteurella multocida.* Advocin® 180 will be used on cattle ranches and in cattle feedlots.

Danofloxacin is a fluoroquinolone antimicrobial agent that will be used only under prescription for the therapeutic treatment of cattle. Although drugs intended for use under prescription or veterinarian's order for therapeutic use in cattle could be categorically excluded for the requirement for preparation of an Environmental Assessment pursuant to 21 CFR 25.33 (d)(5), an Environmental Assessment is required for this product pursuant to 21 CFR 25.21, Extraordinary Circumstances, due to the public controversy associated with fluoroquinolone use in veterinary practice.

Advocin® 180 will be formulated at the Pfizer Inc manufacturing facility, Lee's Summit, Missouri, USA. The manufacture of danofloxacin and Advocin® 180 and the disposal of solvents, reaction products and byproducts will be conducted in accordance with local regulations to minimize environmental impact.

Disposal of unused drug product may result during manufacturing activities or from discarding of returned goods. The above referenced manufacturing facility provides for the ultimate disposal of the product through incineration or through landfilling. No special precautions are necessary for end-user disposal of individual units of empty or partly empty finished product containers.

The manufacturing site selected to formulate Advocin® 180 is a facility currently manufacturing other animal health drug products. The facility is in full compliance with all applicable environmental laws and regulations. The addition of Advocin® 180 will not have a material impact on the overall status of the facility.

3. IDENTIFICATION OF SUBSTANCES THAT ARE THE SUBJECT OF THE PROPOSED ACTION

3.1 Type of Product

Advocin® 180 is a sterile injectable 22.8% aqueous solution of danofloxacin mesylate, equivalent to 18% danofloxacin activity. Danofloxacin is a broad-spectrum fluoroquinolone antimicrobial that is active against a wide range of Gram-negative and Gram-positive bacteria and mycoplasmas of veterinary importance. Advocin® 180 is indicated for the treatment of bovine respiratory disease associated with bacteria susceptible to danofloxacin. Advocin® 180 has been shown to be effective in the treatment of bovine respiratory disease associated with *Pasteurella (Mannheimia) haemolytica* and *Pasteurella multocida*. The product will be administered as a subcutaneous dose of 6 mg/kg bodyweight with a repeat treatment once approximately 48 hours following the first injection.

3.2 Chemical, Physical and Pharmacological Properties

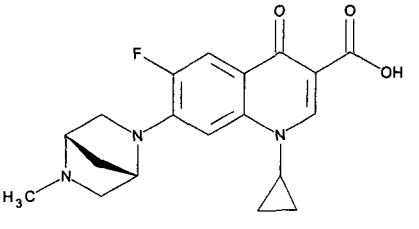
Active ingredient:	mg/mL
Danofloxacin (as mesylate)	180.0

Other ingredients:

2-pyrrolidone	200.0
Povidone C-15	50.0
Magnesium oxide, heavy	20.2
Phenol	2.5
Monothioglycerol	5.0
Conc HCl	variable
NaOH	variable
Water	588.5

Properties of Danofloxacin mesylate:

Structure:



• CH_4O_3S

Chemical class: fluoroquinolone
Chemical name: (1S)-1-Cyclopropyl-6-fluoro-1,4-dihydro-7-(5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl)-4-oxo-3-quinolinecarboxylic acid methanesulfonate
Molecular weight: 453.49 (inner salt: 357.37)
Empirical formula: $C_{19}H_{20}FN_3O_3 \bullet CH_4O_3S$
CAS registry number: 119478-55-6 (inner salt: 112398-08-0)

Aqueous solubility (mg/mL): 156.0, 0.0656, 1.060 at pH 5, 7, 9, respectively
n-Octanol-water partition coefficient (K_{ow}): 0.14, 0.39, 0.22 at pH 5, 7, 9, respectively
Dissociation constants (pKa): 6.22 ± 0.01 and 9.43 ± 0.02
Vapor pressure: < 7 x 10^{-7} torr
Melting Temperature: 328°C (inner salt: 263°C)
Soil-water distribution coefficient (K_d): 2280-3800 (mean value 2837)

Danofloxacin exerts its activity by inhibiting the bacterial DNA gyrase enzyme, thereby blocking DNA replication. Inhibition of DNA gyrase is lethal to bacteria and danofloxacin has been shown to be rapidly bactericidal. Danofloxacin is rapidly absorbed from the site of injection and has a high volume of distribution, indicating wide distribution in tissues.

Other Components:

Ingredient	Chemical Name	Empirical Formula	Molecular Wt	CAS No.
2-pyrrolidone	γ-aminobutyric acid lactam	C_4H_7NO	85.11	616-45-4
Povidone	1-ethenyl-2-pyrrolidinone homopolymer; poly(N-vinyl-2-pyrrolidinone)	$[C_6H_9NO]_n$	$[111.1]_n$ (approx)	9003-39-8
Magnesium Oxide		MgO	40.32	1309-48-4
Phenol	hydroxybenzene	C_6H_5OH	94.11	108-95-2
Monothioglycerol	3-mercapto-1,2 propanediol	$C_3H_8O_2S$	108.16	96-27-5
Hydrochloric acid		HCl	36.47	7647-01-0
Sodium Hydroxide		NaOH	40.01	1310-73-2

2-pyrrolidone is a normal component of some foods and is found endogenously in mammalian systems. It is extensively metabolized following parenteral administration and residual parent and metabolites, accounting for about 40% of the administered dose, are excreted in the urine. Metabolites consist primarily of 5-hydroxy-2-pyrrolidone and succinimide. These metabolites, as well as the parent chemical, are readily biotransformed, biodegraded and hydrolyzed and will not persist or accumulate in the environment (Soong, 1998; Verschueren, 1996). Povidone is a chemically inert polymer used extensively in the pharmaceutical, medical, cosmetic, food and textile industries. Residues will be excreted in the urine (Adeyeye, 1993). Magnesium is the second most plentiful cation of intracellular fluids and is a natural cofactor of many enzymes. It is part of a normal diet and is absorbed and distributed throughout the body, with a small percentage excreted daily in urine. Phenol is a normal constituent of animal tissues and occurs in urine, feces, saliva and sweat. It is rapidly metabolized and excreted in urine. Use of these excipients in the formulation under consideration will not present a significant hazard to the environment. Therefore, only the active ingredient, danofloxacin, will be considered in the following environmental assessment.

4. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

4.1 Metabolism and Excretion

Danofloxacin will be introduced into the environment intermittently and in low concentrations through the feces and urine of medicated cattle. In a total residue excretion study using a 2.5% injectable solution of danofloxacin, tritiated danofloxacin as the mesylate salt was administered subcutaneously for 5 consecutive days at 1.25 mg/kg b.w. to nine steers averaging 186 kg (Study No. 1535N-60-88-008 and 1538N-60-88-008 study supplement). About equal amounts of drug-related material were eliminated in urine and feces. Between 79 and 86% of the daily dose was recovered in urine and feces within the 24 hour period after dose administration. Peak concentrations (30 mg/kg in manure, 27 mg/kg in urine) were observed on the third day of dosing using this regimen. Levels declined to 4 mg/kg in manure and 2 mg/kg in urine by the second day after dosing was completed, accounting for only 7% of the daily dose. Between 50-54% of drug-related residues in feces during dosing days 4-5 consisted of unchanged drug. The metabolite N-desmethyldanofloxacin was detected but at a concentration too low to measure quantitatively. Uncharacterized polar metabolites constituted the balance of residues. In urine, during the same time frame, danofloxacin accounted for 88-94% of the radioactivity while the N-desmethyl metabolite accounted for the remainder. No other metabolite in urine or feces constituted greater than 10% of the total drug-related residues.

Pharmacokinetic parameters for the danofloxacin 18% injectable solution appear to correlate closely to those for danofloxacin 2.5% solution, indicating that excretion patterns will be similar. Lung and plasma pharmacokinetic profiles indicated that $AUC_{0-\infty}$ and C_{max} appeared to be dose-dependent whereas T_{max} and $t_{1/2}$ were dose independent (Study 1931E-60-95-206). Metabolism of danofloxacin in cattle is qualitatively and quantitatively similar over the dose range of 1.25 - 10 mg/kg/day (Study 1535N-60-96-243), thus the percentage of the N-desmethyl metabolite in excreta will be low (i.e., < 10% of drug-related residue), as previously quantitated for the 2.5% injectable solution (see above). Therefore, the residue excretion pattern following administration of danofloxacin 18% solution to ruminating cattle will be assumed to be similar to the pattern determined for the danofloxacin 2.5% solution, with total residues excreted corresponding to 100% of the administered dose. A conservative approach for assessing ecotoxicity of these residues is to assume that the entire dose administered is excreted as the equivalent of unchanged drug.

4.2 Predicted Environmental Concentration of Danofloxacin in Soil

The predicted environmental concentration (PEC) of active residues introduced into soil as a result of product use is based upon the total amount of product administered, including dose and frequency of use; the manure output of the animals; husbandry and agricultural practices; and manure storage and spreading practices. Advocin® 180 will be labeled for treatment of bovine respiratory disease (BRD) and could be administered to both pastured and feedlot cattle. Since the latter represent a denser population, they will be used to estimate upper limits for the amount and concentration of danofloxacin introduced into the environment. Estimates of the incidence of BRD in feedlot cattle vary from as low as 6% (T. Edwards, Midwest Feedlot Services, Inc., personal communication) to as much as 38% (D. Dargatz, USDA, personal communication). A national feedlot study conducted in 1994 as part of the USDA National

Animal Health Monitoring System (NAHMS) reported that 13.5% of cattle on feed were given a long-lasting (label specifies effect greater than 24 hours) antibiotic between arrival and exiting the feedlot and 15.4% were given a regular antibiotic (label specifies effect 24 hours or less), for an overall treatment rate of about 30% of feedlot animals (Cattle on Feed Evaluation, 1995). Different uses for antibacterials labeled as effective for less than vs. greater than 24 hours would be consistent with the need to treat some high-risk animals upon arrival at feedlots and to treat others during their feedlot confinement as disease symptoms dictate. Fluoroquinolones are not the first drug of choice for all uses, particularly for calves requiring treatment on arrival (Pfizer market survey data), and it is likely that only a fraction of animals requiring treatment for BRD will be treated with Advocin® 180. In order to accommodate a possible but conservative estimate for introduction of residues into feedlot manure and subsequently into soil, we will assume for this assessment that as many as 20% of feedlot animals may be treated with Advocin® 180. This estimate assumes the product may be used to treat a small percentage of animals upon arrival (e.g., 5%) as well as a larger percentage of animals requiring treatment at other times (e.g., 15%).

4.2.1 Danofloxacin Administration to Cattle

The average amount of drug administered to a single animal can be estimated as follows. Feedlot cattle at greatest risk for developing BRD are those newly arrived in a feedlot, in a weight range of approximately 230 – 320 kg (Overcash et al, 1983). Assuming an average body weight at treatment of 270 kg (see Baytril® EA, 1996) and a total dose of 12 mg/kg (6 mg/kg per dose x 2 doses), a typically treated animal will receive 3240 mg of danofloxacin:

$$270 \text{ kg} \times 12 \text{ mg/kg} = 3240 \text{ mg}$$

4.2.2 Concentration of Danofloxacin in Excreted Cattle Wastes

Sick animals may be treated in hospital pens and either returned to their home pens immediately or held for observation for a few days. Manure from hospital pens will be mixed into the bulk of feedlot manure collected from feedlot surfaces and stockpiled for later spreading (see, e.g., Sweeten, 1979). Excreted drug residues will therefore be diluted into the bulk manure collected from throughout the feedlot.

An 850 lb (385 kg) feedlot animal typically produces about 24 kg of wet waste (feces + urine) per day (Sweeten, 1979) and over the course of a typical 150 day stay in the feedlot (Overcash et al, 1983) would produce a total of 3600 kg wet waste:

$$24 \text{ kg wet waste/day} \times 150 \text{ days} = 3600 \text{ kg wet waste}$$

As discussed above, a conservative estimate assumes that 20% of feedlot animals might be treated with Advocin® 180. About half the administered dose is excreted in feces with the remainder excreted in urine. For maximum residue estimates it will be assumed that drug residue excreted in urine will partition to fecal matter, due to the strong sorption properties of danofloxacin, as discussed later. Therefore, a conservative approach is to assume that 100% of the dose will be excreted and associated with the feces. The average maximum concentration of drug residues in feedlot wet waste would thus be about 0.2 ppm:

$$[(3240 \text{ mg drug})/(3600 \text{ kg wet waste})] \times 0.20 = 0.18 \text{ mg/kg or } 0.2 \text{ ppm}$$

4.2.3 Concentration of Danofloxacin in Aged Feedlot Wastes

Fresh cattle excreta contains about 85% water by weight, whereas after aging on the feedlot, moisture content is reduced to about 25-40% (Sweeten, 1979). Assuming an average moisture content of 30% in aged feedlot waste and no degradation of danofloxacin residues in the manure, the concentration of danofloxacin residues would be increased by a factor of 2.8 (0.85/0.30) over that expected in wet waste, giving maximum expected concentrations in aged feedlot waste of approximately 0.5 mg/kg (ppm) (0.18 mg/kg x 2.8).

4.2.4 Potential Concentration of Danofloxacin in Soil Amended with Feedlot Wastes

Use of feedlot manure with residues of danofloxacin as fertilizer would result in introduction of the drug into the soil. The resulting concentration of drug in soil can be estimated from the concentration of drug in aged manure and the rate of application of aged manure to soil.

Manure is incorporated into the top 15 cm of soil at a rate of 5-20 tons aged waste/acre/year (Sweeten and Withers, 1990). At a density of 1.5×10^3 kg/m^3, 15 cm of soil weighs about 9.1×10^5 kg/acre; therefore, using an average rate of incorporation of 15 tons (13.6 metric tons) manure/acre/year, use of aged manure containing 0.5 ppm danofloxacin residues would result in a maximum concentration in soil of only about 8 ppb drug residue:

$$(0.5 \text{ mg/kg})(13.6 \times 10^3 \text{ kg/acre}) = 6.85 \times 10^3 \text{ mg/acre}$$

$$(6.85 \times 10^3 \text{ mg/acre}) \div (9.1 \times 10^5 \text{ kg/acre}) = 0.0075 \text{ mg/kg or 8 ppb}$$

Note that this estimate assumes no degradation of danofloxacin in the excreta prior to incorporation into soil.

4.3 Potential Concentration of Residues in Pasture Soils and Water Bodies

Introduction of danofloxacin residues onto pasture land by grazing cattle is not expected to be significant. Treatment of one or a few individual animals that might be allowed to return to grazing after treatment would introduce only minimal residues excreted for only about 24 hours after dose administration. Residues would be excreted in urine, which would be dispersed into soil, and in feces, where they would be bound and would not be expected to leach readily into soil (see Section 5.1.2). Stocking density for pastured cattle in the US is less than 1 animal unit per acre (see, e.g., NAHMS Beef 97, Part III). With an incidence of BRD in pastured animals of only about 1-2% (NAHMS Beef 97, Part II), there would be no more than one or two treated animals per 100 acres. Since all residue is excreted within about 24 hours after dosing, only a fraction of the manure excreted annually over this large land area would contain residues. Likewise, introduction of residues by excretion directly into pasture streams or ponds would be insignificant, given the low disease incidence and treatment rates, the limited excretion period, and the low stocking densities for pastured animals. Thus, this route of introduction of residues into soil or into surface waters is not considered further.

4.3.1 PEC of Danofloxacin in Ground Water

Entry of danofloxacin residues into ground water or into surface water by runoff from soil where manure has been applied is not expected to occur. Danofloxacin, like other fluoroquinolones, sorbs tightly to organic matter. Soil-water distribution coefficients (K_d) were 2280, 2430, and 3800 for sandy loam, clay loam and silty clay loam, respectively; corresponding distribution coefficients based on organic carbon content, K_{oc}, were 74600, 134000, and 644000, respectively (Study No. 2438-0688-6122-710; see Section 5.1.2). Such tight binding to soils precludes danofloxacin from partitioning into the interstitial pore water of soil. An estimate of the maximum pore water concentration, C_w, can be made using the conservative mean soil PEC value (C_s) of 8 µg/kg from application of manure from feedlot cattle (Section 4.2.4), the mean K_d value of 2837 and the following relationship:

$$C_w = C_s/K_d$$
$$C_w = (8)/2837 = 0.0028 \text{ µg/L} = 2.8 \text{ ng/L}$$

This soil pore water concentration can be assumed to be a conservative estimate of the maximum concentration that might occur in ground water, assuming no further dilution, and indicates that contamination of ground water with danofloxacin will not be likely.

5. ENVIRONMENTAL FATE AND EFFECTS OF DANOFLOXACIN

5.1 Fate of Danofloxacin in the Environment

5.1.1 Studies Conducted

The following physical-chemical and environmental fate studies have been conducted with danofloxacin and will be used in the current assessment:

Study		Study Number
Physical-Chemical Properties:	Dissociation Constants	NA
	UV-Visible absorption	
	Melting Temperature	
	Vapor Pressure	
Aqueous Solubility		2438-0690-6120-700
n-Octanol-Water Partition Coefficient		2438-0588-6121-705
Soil Sorption and Desorption		2438-0688-6122-710
Soil Column Leaching		PFZ 492/921630
Soil Column Leaching (low pH)		PFZ 671/952490
Hydrolysis		2438-0588-6123-715
Aquatic Photodegradation		4470-N-001-93
Aerobic Soil Biodegradation		SC910201
Sorption/Desorption in Chicken and Cattle Manure		SC930011
Microbial Transformations		NA

Physical -chemical properties are presented in Section 3.2 above; results of fate studies are briefly summarized below. Full report summaries are presented in Attachment 1 of this document.

Soil Sorption:	Soil Type	Kd	Koc
	Iowa sandy loam	2280	74,600
	California clay loam	2430	134,000
	Mississippi silty clay loam	3800	644,000

Fecal Sorption:	Feces Type	Kd	Koc
	Cattle	541	1036
	Chicken	138	323

Soil Column Leaching:	Soil Type	pH	% leached
	Thoresby loamy sand	7.2	ND*
	Alconbury sandy clay loam	7.9	ND
	Warwickshire sandy loam	5.2	ND
	Warwickshire clay loam	4.8	ND

* ND, none detectable

Hydrolysis: Stable to hydrolysis at pH 5-9

Aquatic Photodegradation:	pH	Half-life (minutes)
	5	24
	7	4.3
	9	2.6

Aerobic Soil Biodegradation:	Soil Type	Transformation Half-life (days)
	Ohio sandy loam	143
	Ohio clay loam	91
	Ohio loam	110

Microbial Transformations: Biotransformed by 12 soil microorganisms representing 8 genera; apparent mineralization by the fungus *Curvularia lunata*

5.1.2 Sorption Properties

Like other fluoroquinolones, danofloxacin binds very tightly to soil. Indeed, experimentally determined soil/water distribution coefficients indicate it is immobile in soils despite its relatively high water solubility. In the soil sorption/desorption study (Study 2438-0688-6122-710), three soils varying significantly in pH (5.6-7.6), cation exchange capacity (12.5-29.4) and organic carbon content (0.59-3.1%), all bound ^{3}H-danofloxacin rapidly and tightly; soil:water distribution coefficients (K_d) for adsorption were 2280, 2430, and 3800 and desorption K_d values were 2540, 2640 and 8180 for soils of pH 5.6, 7.6 and 7.0, respectively. Corresponding distribution coefficients based on the soil organic carbon content (K_{oc}) were 74500, 133500, and 644000, respectively. Chemicals with K_{oc} values greater than 1000 are considered immobile in the soil, with no significant leaching potential (Kenaga, 1980; Hamaker and Thompson, 1972).

Although danofloxacin readily dissolves in water, particularly at low pH, this property is not expected to affect its movement in soils. It is important to note that the low pH soil (pH 5.6) had sorption and desorption distribution coefficients comparable to those obtained for the pH 7.0 and 7.6 soils, suggesting that any differences in danofloxacin solubility at the various soil pHs did not impact the strong partitioning into the soil phase. Indeed, the relationship between water solubility and sorption that has been defined for nonionic organic molecules does not hold true

for those that ionize (Kenaga, 1980; Briggs, 1981; Nicholls, 1991). For example, the following equation has been used to estimate K_{oc} values for non-ionizing chemicals with reasonable reliability (Kenaga, 1980):

$$\log K_{oc} = 3.64 - 0.55 \log S, \text{ where S is solubility in mg/L}$$

This equation would predict that the K_{oc} values for danofloxacin would range from about 6 to 120, whereas they in fact range from 74,500 to 644,000. This is due to binding to soil components by cation exchange mechanisms and strong electrostatic interactions. Danofloxacin and related fluoroquinolones are amphoteric molecules and exist in the protonated form at pHs below their pKa values. For danofloxacin, with a pKa of 9.43 for the piperazine nitrogen and a pKa of 6.22 for the carboxylic acid moiety, ionic interactions with soil are expected. At low pH, e.g. pH < 6, both the piperazine nitrogen and the carboxylic acid moiety will be protonated to varying degrees, giving predominantly cationic and dipolar zwitterionic species which can interact with cation exchange sites in soils, particularly those with clay components. Such interactions of several fluoroquinolones, including enrofloxacin and ciprofloxacin, with charged sites in soil matrices have recently been described in some detail (Nowara et al, 1997). Although soluble in water at 250-1100 mg/L (Baytril® EA, 1996), enrofloxacin bound tightly to five different soils ranging in pH from 4.8 to 6.6, with K_d values of 260 to 5612 and K_{oc} values of 16506 to 768740. Furthermore, it was shown that the binding was due to interaction of the ionized fluoroquinolone molecules with cations on the surfaces of clay minerals and in the interlamellar spaces; organic carbon content had a secondary influence on the binding. This may explain why binding to feces, which are high in organic carbon relative to soils, is less tight than binding to soil, although still significant. Similarly, sarafloxacin binding to/immobility in soil is ascribed to ionic and electrostatic interactions with the soils' cation exchange sites (Saraflox® EA, 1995).

The soil binding properties of danofloxacin indicate that it will be found in only minimal or negligible concentrations in the aqueous phase of the soil compartment, i.e., the soil pore water. It can be estimated, based on sorption coefficients, that the soil pore water concentration in soils amended with manure containing maximum danofloxacin residues will not exceed 2.8 ng/L (Section 4.3.1). This is the maximum concentration of danofloxacin to which terrestrial organisms would be directly exposed, as the remaining residue would be bound to the soil and essentially non-bioavailable. Furthermore, the danofloxacin residues will not move with surface water or intercalate into ground water, as any soluble residue would be quickly re-adsorbed as it began moving through the soil. This assumption was verified in two soil column leaching studies using soils of low pH (4.8 and 5.2; Study PFZ 671/952490), where danofloxacin solubility is maximum, and more neutral soils (pH 7.2 and 7.9; Study PFZ 492/921630), with cation exchange capacities ranging from 5.0 (loamy sand) to 33.6 (clay loam). Danofloxacin did not move from the top 5 cm of the columns of any of the soils tested during passage of the equivalent of a 50 cm rainfall. Thus, danofloxacin is not expected to contaminate ground water or surface waters following application of residue-containing manure to soils.

5.1.3 Soil Transformation of Danofloxacin

A soil biodegradation study conducted using three soils of differing characteristics demonstrated that danofloxacin is biotransformed in soil to several minor metabolites (Study SC910201). Soils (sandy loam, clay loam, and loam) supplemented with ^{14}C-danofloxacin at approximately 27 mg/kg were monitored for 64 days for production of $^{14}CO_2$ or other labeled volatile products. Little mineralization of danofloxacin was observed. However, extraction of soils and analysis of extracts for degradates revealed that danofloxacin had been transformed to several minor

metabolites in all three soils. The metabolites accounted collectively for as much as 35% of the extracted residues, although none individually accounted for more than 10% of the applied radioactivity and, therefore, they were not identified. Estimated times to 50% transformation of danofloxacin to metabolites in the three soils were 91, 110 and 143 days (average 115 days). Like danofloxacin, neither enrofloxacin nor sarafloxacin showed evidence of extensive mineralization in soil biodegradation studies (Baytril® EA, 1996; Saraflox® EA, 1995; Marengo et al, 1997). Enrofloxacin was biotransformed in three soils, but at slower rates than observed for danofloxacin, with estimated transformation half-lives ranging from 359 to 696 days (Baytril® EA, 1996). By contrast, sarafloxacin was not biotransformed in soils under the study conditions reported; only unchanged parent drug and a metabolite which was formed abiotically and was acid-hydrolyzable to sarafloxacin were detected in soil extracts after 66 to 80 days of incubation (Marengo et al, 1997). Therefore, it appears that danofloxacin may be somewhat more susceptible to soil biotransformation reactions than are these other two fluoroquinolones.

The apparent susceptibility of danofloxacin to soil biotransformation reactions was further substantiated by investigation of metabolism of danofloxacin by pure cultures of soil microorganisms (Study: Microbial Transformations; see also Chen et al, 1997). Results demonstrated that danofloxacin is susceptible to degradation by a variety of soil microbes. Twelve organisms, representing eight different genera, biotransformed danofloxacin under *in vitro* culture conditions. Identified metabolites included N-desmethyldanofloxacin as well as the 7-amino danofloxacin derivative, formed by degradation of the piperazine ring. Importantly, one fungal culture mineralized ^{14}C-danofloxacin labeled in the quinolone ring, with 31% of the radiolabel evolved as $^{14}CO_2$ within 24 hours, thus demonstrating the lability of the molecule to complete microbial degradation. These results are consistent with studies recently reported for the closely related fluoroquinolones sarafloxacin, enrofloxacin and ciprofloxacin. Sarafloxacin was readily biotransformed by pure cultures of the white rot fungus *Phanerochaete chrysosporium,* with approximately 90% transformed into six unidentified components within seven days and 17.3% mineralized after 35 days; an estimated time to 50% mineralization by this organism in culture was 104 days (Saraflox® EA, 1995). When enrofloxacin labeled with ^{14}C in the carbonyl position was incubated with cultures of wood-rotting basidomycetes, including both white rot and brown rot fungi, as much as 53% of the ^{14}C-label was liberated as $^{14}CO_2$ within 8 weeks (Martens et al, 1996), demonstrating metabolism of the heterocyclic ring of the molecule, as was observed for danofloxacin. The brown rot fungus *Gloeophyllum striatum* degraded enrofloxacin at a faster rate than *P. chrysosporium* under the conditions of the study. Ciprofloxacin was also degraded by *G. striatum* in liquid culture (Wetzstein et al, 1997a). Metabolite identification following degradation of both enrofloxacin and ciprofloxacin revealed numerous intermediates in 2- to 3-day old cultures, including hydroxylated congeners, dihydroxylated congeners indicative of degradation of the homoaromatic portion of the molecule, the 7-amino and desethylene derivatives, indicating degradation of the piperazine ring, and products derived from cleavage of the heterocyclic core (Wetzstein, 1997a and 1997b). The authors proposed that four principal pathways of degradation, possibly reflecting initial attack of hydroxyl radicals at different sites on the molecule, were operative, including oxidative decarboxylation, defluorination, hydroxylation at position C-8 and oxidation of the piperazinyl moiety. Defluorination is an important metabolic activity to have demonstrated, as removal of the fluorine eliminates the xenobiotic structural element from the molecule, likely increasing the susceptibility of the residual structure to degradation by a wide variety of soil microbes.

Since danofloxacin is structurally similar to enrofloxacin and ciprofloxacin, it is likely that these same mechanisms of degradation will occur. Although metabolites were not identified in the danofloxacin soil biodegradation study, it can be presumed on the basis of data collected from animal metabolism studies as well as identification of metabolites from the microbial

transformation study that one of the soil metabolites is N-desmethyldanofloxacin. As discussed in the preceding paragraph, the microbial transformation study indicates that common soil microbes possess the capacity to degrade the piperazine ring and the quinolone ring of danofloxacin; N-desmethyldanofloxacin would be subject to the same degradative processes and therefore would be present only transiently in the soil. Other minor metabolites observed in soil extracts could represent small amounts of a variety of alterations to the piperazine ring, of which there are numerous possibilities (e.g., Wetzstein, 1997; Sorgel, 1989). Many of these metabolites are themselves unstable and subject to further metabolism. According to Sorgel (1989) and Wetzstein et al (1997), except for the demethylated metabolites, metabolites of fluoroquinolones that have been evaluated have little or no antimicrobial activity.

It is important to point out that danofloxacin and other related fluoroquinolones such as enrofloxacin are tightly bound to soil, and therefore bioavailability will be relatively low, impeding the ready degradation of residues in the soil. For example, when enrofloxacin was preadsorbed to soil, rates of degradation by *G. striatum* cultures were significantly reduced compared to cultures incubated without soil (Martens et al, 1996). However, as the authors pointed out, sorption may slow degradation but is not prohibitive, particularly if the primary mechanisms involve nonspecific hydroxyl radical-mediated reactions where diffusion of the radicals to molecules unavailable to direct attack by microbes (i.e., sorbed) may mediate the initial degradative steps (Martens et al, 1996; Wetzstein et al, 1997a and 1997b). Furthermore, fungi physiologically similar to the wood-rotting Basidiomycetes and capable of degrading fluoroquinolones are found in animal dung as well as in soil and plant litter (Wetzstein et al, 1998). For danofloxacin, binding to animal feces has been shown to be less tight than sorption to soil, with K_d values of 541 and 138 for cattle and chicken feces, respectively, compared to K_d values of 2280-3800 for soils, suggesting that there may be greater bioavailability in livestock feces and therefore a more rapid degradation of residues in manure than has been observed in soil. This was, in fact, demonstrated for sarafloxacin. In contrast to the lack of biotransformation of sarafloxacin in soil (Marengo et al, 1997), the drug was extensively degraded in turkey manure, with approximately 68% of the extractable residues biodegraded after 7 days and a total of 35 metabolite peaks detectable in the extracts (Saraflox® EA, 1995). If this is the case with danofloxacin, the initial levels of danofloxacin residues in soil amended with manure from danofloxacin-treated animals would be significantly less than the estimates made assuming that no degradation occurs in the manure prior to field application.

In addition to undergoing transformations by soil microbes, danofloxacin residues in manure exposed to sunlight in the feedlot or after field application may be photodegraded. Danofloxacin was rapidly degraded to more than 20 minor products in aqueous solution upon exposure to natural sunlight (Study 4470-N-001-93), with calculated surface water half-lives of only 2.6-24 minutes. This lability to sunlight has been reported for other fluoroquinolones (e.g., Burhenne et al, 1997a; Hidalgo et al, 1993), including loss of antibiotic activity upon photodegradation of ciprofloxacin (Phillips et al, 1990). Enrofloxacin and ciprofloxacin in aqueous solution are both readily degraded to numerous minor products upon exposure to sunlight; half-lives for enrofloxacin were 20.6, 3.4 and 14.3 minutes at pH 5, 7, and 9, respectively and half-lives for ciprofloxacin were 46.4, 9.0 and 23.1 minutes, respectively (Baytril® EA, 1996). These results for danofloxacin, enrofloxacin and ciprofloxacin are consistent with half-lives of 20.6, 36.2, and 90.2 minutes, respectively, determined upon irradiation of aqueous solutions of unreported pH with a xenon lamp (Burhenne et al, 1997a). Analysis of irradiated solutions indicated that degradation likely proceeds through a 7-aminoquinolone derivative to polar pyridone dicarboxylic and tricarboxylic acids which are subsequently photometabolized to CO_2 (Burhenne et al, 1997a, 1997b). Sarafloxacin in aqueous solution was also readily degraded in natural sunlight with half-lives of 0.9, 0.15 and 0.21 hours (= 54, 9 and 12.6 min) at pH 5, 7, and 9, respectively, with a

minimum of 15 degradates detected at each pH upon irradiation (Davis et al, 1993; Saraflox® EA, 1995). Importantly, a study with sarafloxacin that examined the photodegradation of the drug in turkey manure revealed that ^{14}C-sarafloxacin was degraded into six components after 8 to 48 hours of irradiation, with degradation of approximately 19% of the drug residue after 48 hours (Saraflox® EA, 1995). Thus, even though sorbed to manure or to soil, these drugs may be labile to photodegradation events that will contribute to depletion from terrestrial as well as from aquatic environments.

The transformation half-life of danofloxacin in soil can be used to assess whether danofloxacin will accumulate in soils amended annually with residue-containing manure. Assuming first order kinetics for degradation, an assumption useful for approximating the degradation of chemicals present in soil at very low concentrations (Alexander and Scow, 1989), and using the highest soil PEC of 8 µg/kg danofloxacin as the initial concentration, it can be calculated that one year after application the concentration of danofloxacin remaining in soil will be less than 1 µg/kg, as follows:

The concentration, C_t, of danofloxacin in soil at any defined time after its application to soil can be determined by the following equation assuming the initial drug concentration (C_0) in soil and the depletion half life are known:

$$C_t = C_0 e^{-kt}$$

The depletion rate constant (k) can be calculated from the estimated time (t) to 50% biotransformation by converting the above equation to logarithms and rearranging:

$$\log C_t = \log C_0 - kt/2.3$$

$$k = \frac{(2.3)(\log 2)}{t} = \frac{0.693}{t}$$

Using the average biotransformation half life of 115 days (Section 5.1.3) for further calculations, the average depletion rate constant can be calculated:

$$k = 0.693/115 = 0.006026 \text{ days}^{-1}$$

With the maximum mean initial concentration of danofloxacin in manure-amended soil equal to 8 µg/kg (Section 4.2.4) and the average depletion rate constant of 0.006026, less than 1µg/kg will remain in the soil 365 days after application (log C = log 8 -[0.006026 x 365/2.3] = -0.053; C = 0.885). The table below indicates that a maximum concentration of approximately 9 µg/kg danofloxacin in soil is reached after two or three successive annual applications of manure:

Number of successive reapplications	Concentration (µg/kg) of danofloxacin residues in soil
0	8
1	0.885 + 8 = 8.885
2	0.983 + 8 = 8.983
3	0.993 + 8 = 8.995
4	0.995 + 8 = 8.995

Thus, annual field application of feedlot manure containing danofloxacin-related residues would not be predicted to lead to concentrations of danofloxacin greater than about 9 µg/kg in soil; these maximum concentrations would deplete to less than 1 µg/kg between manure

applications. Furthermore, if the annual manure allotment were applied in two semi-annual applications, introducing only half the drug-related residues at each application, concentrations would be even less.

This again is a worst case projection, assuming no degradation in manure prior to soil application. It is likely that in practice maximum levels will be only a fraction of these estimates. Maximum concentrations could only be found shortly after manure application to soils and would be intermittent and transient, depleting over the interval of time from one annual application until the next. No risk management measures are therefore needed to avoid persistent residues in soil.

5.1.4 Potential Release of Danofloxacin to Surface and Ground Water

Only insignificant amounts of danofloxacin would be expected to partition into surface waters in runoff from open lots or paddocks due to the strong sorption of drug to feces and to soil (Section 5.1.2). The strong sorption indicates that the danofloxacin residues will remain associated primarily with the solid phase of any surface runoff. Furthermore, such runoff from open lots must be controlled following local guidelines, generally by collection and direction to settling and storage basins (see, e.g., EPA Feedlot Effluent Guidelines, 1995). Danofloxacin residues would be expected to partition almost exclusively into the solids phase of the settling basins, where they would ultimately be disposed of by application to soil, discussed in the preceding section.

Danofloxacin present in soils to which manure has been applied is not expected to move into surface water. The maximum soil pore water concentration estimated from partitioning properties is only 2.8 ng/L (Section 4.3.1). During a potential run-off event, this concentration would be diluted to even lower levels. A rainfall event might be expected to increase the soil moisture by at least 10%, reducing the pore water concentration to 2.5 ng/L. If this surface water enters streams or ponds during a run-off event, dilution into the receiving water body will reduce danofloxacin levels at least 3-fold, to only 0.8 ng/L. Residues in solution in surface runoff reaching a stream or pond would partition onto suspended particulates and sediments in the receiving water body, significantly reducing concentrations in the aqueous phase. Residues that might be excreted directly into pasture streams or ponds by grazing cattle (Section 4.3) would be similarly adsorbed to particulates and sediments. Furthermore, danofloxacin residues in solution would be expected to rapidly decline as low concentrations of the drug in aqueous solution are degraded within a matter of hours by sunlight. Aqueous solutions of danofloxacin exposed to simulated sunlight were degraded to numerous minor metabolites with a half-life of 2.6 - 24 minutes (Study No. 4470-N-001-93). Even in turbid waters, such a short half-life would contribute to a continuous reduction of residues in solution exposed at the surface of the water body. Consequently, it is unlikely that more than inconsequential trace concentrations of danofloxacin would ever be present in solution in streams or ponds.

The strong sorption of danofloxacin further precludes leaching into ground water. Even a worst case assumption that the maximum concentration of residue in ground water is equal to that in undiluted soil pore water predicts that only negligible amounts of danofloxacin ($\leq$ 2.8 ng/L) would be present. The predicted immobility of danofloxacin was verified in two soil column leaching studies using ^{14}C-danofloxacin and four soils of differing pH, organic carbon content and cation exchange capacity (Study PFZ 492/921630 and Study PFZ 671/952490). With a rainfall equivalent of 50 cm passing through the columns, no appreciable leaching was observed. In fact, all of the ^{14}C-radioactivity recovered (90 - 100%) was found in the top 5 cm of the columns, with leachates containing no detectable ^{14}C radioactivity (< 0.9% of the applied radioactivity, limit of detection). This observation is consistent with an estimate of danofloxacin's leaching

potential based on calculation of its relative mobility (R_f) on soil thin layer chromatography plates. The R_f value for a chemical can be determined using the following equation (EPA, 1982; Hamaker, 1975; Helling and Turner, 1968):

$$R_f = \frac{1}{1 + (K_d)(d_s)(1/\theta^{2/3} - 1)}$$

where K_d = soil sorption coefficient
d_s = density of soil solids
θ = pore fraction of the soil

Using the lowest K_d value measured for danofloxacin in the soil sorption and desorption study (2280) as a conservative estimate of the sorption of danofloxacin to soils, $\theta = 0.5$ and the mean soil density for the four soils that were used in the soil column leaching studies, an R_f value can be estimated as follows:

mean d_s = 1.1 (range 0.83-1.42)

$$R_f = \frac{1}{1 + (2280)(1.1)(1/0.5^{2/3} - 1)} = 6.8 \times 10^{-4}$$

This value indicates the distance in cm that the bulk of applied danofloxacin could move through the soil for every cm of water percolating through the soil. The 50 cm rainfall equivalent used in the soil column leaching study would then be expected to move the danofloxacin only about 0.03 cm (50 cm x R_f), consistent with the results obtained. To extrapolate to field conditions, if half the volume from a 25 cm rainfall percolates to the water table, the applied danofloxacin will move less than 0.01 cm (0.5 x 25 cm x R_f); even 10 times this amount of rainfall (i.e., 250 cm or 100 inches) would not lead to significant movement of danofloxacin through the soil.

Given the low concentration of danofloxacin in soil following repeated application of manure (maximum 8-9 µg/kg), the low concentration in soil pore water equilibrated with manure-amended soils (maximum 2.8 ng/L), the very high K_{oc} values, and the susceptibility of danofloxacin to biotransformation and degradation in soil, danofloxacin is not expected to leach into ground water to any significant extent.

5.2 Environmental Effects Studies with Danofloxacin

The following environmental effects studies have been conducted with danofloxacin and will be used in the current assessment:

Study	Study Number
Effects on Soil Microbes	2438-0189-6144-790
Anaerobic Digester Inhibition	260E-101
Subacute Toxicity in Earthworms	2438-1088-6127-630
Effects on Seed Germination and Root Elongation of Six Plant Species	SC920086; PRT-12-5PFF-05-003
Effects on Seedling Growth of Six Plant Species	PRT-13-2PFF-02-008; PRT-11-5PFF-04-004

Results of these studies are tabulated below; full report summaries are presented in Attachment 1 of this document.

Organism	Endpoint	
Soil Microbes	Minimum Inhibitory Concentration (MIC), mg/L	
	Without soil	With soil
Clostridium perfringens	1.0	6.0
Aspergillus flavus	80	600
Pseudomonas aeruginosa	6.0	100
Nostoc	0.8	1.0
Chaetomium globosum	6.0	8.0
Microbial Methane Production	EC_{50} > 100 mg/L	
Earthworm (*Lumbricus terrestris*)	28 day LC_{50} > 1200 mg/kg	

Crop Seeds	NOEC (mg/kg)	
	Germination	Root Elongation
Corn	1000	1000
Soybean	1000	400
Cucumber	1000	< 0.032
Rye grass	1000	< 0.125
Wheat	1000	2.0
Pinto bean	1000	20

Crop Seedlings	NOEC (mg/kg)	
	Sand	Soil
Corn	26	> 150
Soybean	2.9	ND*
Cucumber	1.6	ND
Rye grass	83	> 150
Wheat	46	46
Tomato	83	> 150

* ND, not determined

5.3 Effects of Danofloxacin Residues in the Environment: Exposure:Toxicity Ratios

Results of effects tests can be compared with the PEC to characterize the environmental risk presented by the proposed use of Advocin® 180 in cattle. Risk characterization can be presented in a variety of ways, but most often is presented as a quotient or ratio of exposure and effects levels. For this assessment, we will use the Quotient Method (Barnthouse et al, 1986), as used by the EPA Office of Toxic Substances, to assess risk of organisms to exposure to chemicals in the environment. In this approach, the PEC is divided by a toxicity endpoint, e.g., LC_{50} or NOEC. When the ratio is equal to or greater than one, then the hazard indicated may occur in the natural environment; the greater the ratio, the higher the probability that the adverse effect will occur. Conversely, the lower the ratio, the less likely that an adverse effect will occur.

5.3.1 Effects on soil microorganisms:

The lowest Minimum Inhibitory Concentration (MIC) of danofloxacin reported for the five representative species of soil microorganisms tested was 0.8 mg/L or 800 µg/L. Using the highest expected soil PEC value of 9 µg/kg (Section 5.1.3), the PEC/MIC ratio is 9/800 = 0.01. In the presence of 10% sandy loam soil incorporated into the medium (0.1 g/mL), MICs increased as much as 17-fold, ranging from 1 to 600 mg/L. PEC/MIC ratios, using MIC values with soil, range from 0.009 to 0.000015, with an overall average ratio for all five tested organisms of 0.0024. These are acceptable ratios for effects on soil microorganisms, particularly since most residues in the environment will be bound and activity significantly attenuated. This assumption is supported by the results of the study measuring microbial function in anaerobic digester sludge. The EC_{50} for inhibition of gas production by danofloxacin was greater than 100 mg/L. Thus, the complex metabolic processes occurring in this matrix were not adversely affected by concentrations of danofloxacin more than 10,000-fold above the maximum soil PEC and more than 500-fold above the maximum concentrations that might be found in excreta from feedlots where cattle are treated with danofloxacin (0.2 mg/kg; Section 4.2.2).

5.3.2 Earthworm toxicity

The LC_{50} for the earthworm *Lumbricus terrestris* was greater than 1200 mg/kg, the highest concentration tested, yielding a PEC/LC_{50} ratio of $9/(1.2 \times 10^{6}) = 7.5 \times 10^{-6}$. Thus adverse effects on soil fauna are not expected and no further evaluation of such effects are necessary.

5.3.3 Phytotoxicity to terrestrial plants

In seed germination and root elongation studies, no effects were observed on seed germination of six plant species at 1000 mg/kg, the highest concentration tested. The PEC/NOEC ratios are therefore $9/(1 \times 10^{6}) = 9 \times 10^{-6}$ for seed germination. For root elongation, NOECs ranged from 2 – 1000 mg/kg for 4 species, giving PEC/NOEC ratios of 4.5×10^{-3} to 9×10^{-6} for these species. The NOEC values for root elongation for cucumber and rye grass were below the lowest concentrations tested (0.032 and 0.125 mg/kg, respectively). The PEC/NOEC values for these two species would be > 0.28 and > 0.072, respectively. However, it should be noted that these effects tests were conducted with danofloxacin in aqueous solution; it is expected that soil will attenuate these effects and no adverse impact from residues in soil would be expected. This was verified by seedling growth studies. The most sensitive species was cucumber in this test as well, with a NOEC value of 1.6 mg/kg in a sand matrix. The PEC/NOEC ratio of $9/(1.6 \times 10^{3}) = 0.006$ is acceptable. Similarly, the ryegrass NOEC values were 83 mg/kg in sand and > 150 mg/kg in soil, giving acceptable PEC/NOEC ratios of 1×10^{-4} to $< 6 \times 10^{-5}$. The most sensitive species of four tested in soil was wheat, with a NOEC of 46 mg/kg, giving a maximum soil PEC/NOEC ratio of $9/(4.6 \times 10^{4}) = 1.96 \times 10^{-4}$. These ratios for seedling growth indicate that danofloxacin residues will not have an adverse effect on terrestrial plants and further evaluation of such effects is not needed.

6. ENVIRONMENTAL RISK ASSESSMENT

The active ingredient of the product is danofloxacin, a synthetic antimicrobial agent belonging to the fluoroquinolone class. The product is for therapeutic treatment of bovine respiratory disease. The target animals considered in the application are beef cattle for which individual animal treatment would be expected. Advocin® 180 injectable solution would be administered by subcutaneous injection at the recommended dose level of 6 mg/kg b.w. with a second dose administered 48 hours later. Essentially 100% of the administered dose will be excreted in urine and feces, primarily as parent drug with less than 20% of the administered dose excreted as

minor metabolites which do not need further evaluation for ecotoxicity. Estimated maximum residue concentrations in soil following field application of manure containing danofloxacin residues are 8-9 µg/kg. Introduction of residues onto pasture land by individually treated grazing cattle will not lead to significant environmental concentrations.

6.1 Effects on soil microorganisms

As discussed in detail in Section 5.1.2 above, danofloxacin will be tightly sorbed to soil, significantly limiting its bioavailability. Therefore, although danofloxacin is a potent antibacterial agent, its presence in soil should not have a significant impact on soil bacteria. The *in vitro* MIC study (Study 2438-0189-6144-790) demonstrates the attenuating effect that even a small amount of soil added to the culture medium has on the antimicrobial activity of danofloxacin; MICs were increased up to 17-fold for the organisms tested. PEC/MIC ratios do not exceed 0.01 for even the most sensitive species tested. Furthermore, in the soil microbes would be impacted primarily by the danofloxacin residue partitioned into the soil pore water. The maximum concentration in the pore water, and therefore the PEC for exposure of the soil microbes, has been estimated to be only 2.8 ng/L. Using the MIC for the most sensitive microorganism in the absence of soil, 0.8 mg/L or 8×10^5 ng/L, the PEC/MIC ratio is only 3.5×10^{-6}, clearly a satisfactory safety margin. This projection is consistent with results reported for other fluoroquinolones. For example, enrofloxacin was inhibitory to three species of soil bacteria at 1.3 mg/L in the absence of soil; when tested against two of these species in the presence of soil there were no inhibitory effects observed at up to 500 mg/kg, the highest concentration tested (Baytril® EA, 1996). Similarly, sarafloxacin was lethal to a variety of pond sediment bacteria at ≤ 3 mg/L when tested in aqueous suspension but in the presence of pond sediment no inhibition was observed at up to 300 mg/L (Saraflox® EA, 1995). More specifically, the sarafloxacin MIC for the catfish pathogen *Edwardsiella ictaluri* was 0.03 mg/L in nutrient agar whereas no inhibition was observed in sediments amended with as much as 300 mg/kg, 10,000 times the *in vitro* MIC (Saraflox® EA, 1995).

Importantly, the low and transient levels of danofloxacin residues and lack of bioavailability of these residues in soil will preclude the selection of microorganisms resistant to fluoroquinolones in the terrestrial environment. The maximum exposure level in soil pore water, 2.8 ng/L, is nearly 300,000-fold below the MIC for the most sensitive soil microbe tested. Even considering possible exposure of more sensitive gram-negative bacteria such as *Escherichia coli*, with danofloxacin MICs in the range of 0.03 – 0.06 µg/mL, exposures would still be >10,000-fold below the MIC. Thus, selective pressure for resistance development in this environmental compartment will be insignificant.

Danofloxacin has also been tested for inhibitory effects on microbial methane production (Study 260E-101), a process that can be important in some areas where manure is used for biogas production. Gas production from anaerobic digester sludge incubated with 0.1, 1.0, 10 and 100 mg/kg danofloxacin was monitored over 28 days. No inhibition was observed at 0.1 and 1.0 mg/kg and only about 10% inhibition relative to controls was observed at the highest concentration tested, 100 mg/kg; the EC_{50} was >100 mg/kg. Since the highest predicted concentration of danofloxacin residue in manure from feedlots where cattle are treated with Advocin® 180 is only 0.2 mg/kg the PEC/EC_{50} value is < 0.002 (0.2 mg/kg/>100 mg/kg). It is likely that the sorption properties of danofloxacin limit its effects on microbes involved in methane production from sludge, as discussed above for soil microbes. Some species of methanogenic bacteria are reportedly quite susceptible in culture to inhibition of both growth and gas production by fluoroquinolones (Hippe and Wetzstein, 1996). For example, *Methanosarcina barkeri* growth was inhibited by 75% and gas production by 50% in the presence of only 1 µg/mL

enrofloxacin in liquid culture. The lack of such inhibition by danofloxacin in the sludge matrix further supports the contention that sorption attenuates the antibacterial effects of these agents in the environment.

In summary, although danofloxacin is inhibitory to soil bacteria cultured in laboratory media, residues in livestock manure or in soil amended with such manure will be bound to a significant extent and are therefore not expected to impact the metabolic activities of either the aerobic or anaerobic soil or fecal microflora. The PEC/MIC ratio for soil microbes, particularly when estimated on the basis of danofloxacin concentrations in the soil pore water, provides for a sufficient safety margin to preclude both adverse effects on and selection of resistance in bacteria residing in the soil compartment.

6.2 Phytotoxicity to terrestrial plants

Terrestrial plants are not likely to be damaged by danofloxacin residues introduced into the soil with manure from treated animals. Several studies and considerations support this conclusion, as follows:

1) In the seed germination and root elongation studies (Study SC920086 and PRT-12-5PFF-05-003) germination of seeds of six plant species was not affected at levels of up to 1000 mg/L danofloxacin in solution. The NOEC for root elongation for 4 of 6 species ranged from 2 mg/L to 1000 mg/L. NOECs for cucumber and rye grass root elongation were not obtained in this study, where effects were observed at 0.032 and 0.125 mg/L, respectively, the lowest concentrations tested. However, this study was conducted by exposing seeds to danofloxacin in aqueous solution. As discussed above, the soil sorption properties of danofloxacin will reduce the amount of bioavailable residue, mitigating effects on crop seeds. A comparison of the effects levels measured in the aqueous test system with predicted soil pore water concentrations may be helpful. The maximum pore water concentration was estimated as 2.8 ng/L. The minimum test concentrations where effects were observed, 0.032 and 0.125 mg/kg, are > 11,000 to > 44,000-fold above these predicted exposure concentrations. Therefore, although root elongation NOEC values were not obtained for two of the species tested, consideration of actual exposure levels strongly support the conclusion that danofloxacin residues in soils will not adversely impact seed germination and root growth. This conclusion is consistent with results reported for enrofloxacin and sarafloxacin. As with danofloxacin, cucumber seeds were the most sensitive of six crop species exposed to enrofloxacin, with a NOEC of 0.27 mg/kg for root elongation in the absence of soil; in the presence of soil the NOEC for this species was 9.1 mg/kg, an increase of more than 30-fold compared to exposure without soil (Baytril® EA, 1996). In the absence of soil, sarafloxacin NOEC levels for root elongation were 12.5 mg/kg for soybean and ryegrass; 10 mg/kg for lettuce; 2.5 mg/kg for tomato and cucumber and 1.3 mg/kg for wheat; in the presence of soil, NOECs were 100 mg/kg for soybean, ryegrass, lettuce and tomato and 50 mg/kg for cucumber and wheat, increases of 8 to 40-fold due to reduced bioavailability in the presence of soil (Saraflox® EA, 1995).

2) In the danofloxacin seedling growth studies with six plant species (Study PRT-13-2PFF-02-008 and PRT-11-5PFF-04-004), both sand and soil support media were used and seedlings were subirrigated with nutrient solutions containing various concentrations of danofloxacin mesylate. Shoot lengths, shoot dry weights and root dry weights were measured. Results with cucumber may be particularly relevant in view of the discussion presented in the preceding paragraph. Although effects on cucumber root elongation were observed at 0.032 mg/kg danofloxacin in solution in the seed germination/root elongation study (Study PRT-12-5PFF-05-003), no effects on any parameter of cucumber seedling growth, including root dry weights, were

observed at 50 times this concentration (1.6 mg/kg) in a sand support matrix (Study PRT-11-5PFF-04-004). Similarly, whereas effects were observed at 0.125 mg/kg on rye grass root elongation in aqueous solution, no effects were observed on rye grass seedling growth in sand at 83 mg/kg or in soil at up to 150 mg/kg, the highest concentration tested (Study PRT-13-2PFF-02-008). For three of four species tested in soil, effects of danofloxacin on seedling growth were clearly mitigated in soil compared to sand, likely due to stronger sorption of danofloxacin to soil compared to sand. These NOEC values are significantly above the 9 µg/kg maximum soil PEC for danofloxacin, ranging from about 200-fold (cucumber, sand) to over 16,000-fold. For plants grown in soil there were also no differences in morphological observations (dwarfed, chlorotic or mottled growth) for control plants and those treated at levels of at least 46 mg/kg danofloxacin, 5000 times the maximum estimated soil concentration. These results are again consistent with those reported for enrofloxacin and sarafloxacin. Wheat was the most sensitive of six plant species exposed to enrofloxacin in a sand support matrix with a NOEC of < 0.13 mg/kg; when grown in the presence of soil the NOEC was 4.7 mg/kg, an increase of at least 36-fold compared to growth in the absence of soil (Baytril® EA, 1996). Ryegrass was the most sensitive of six species in a seedling growth study with sarafloxacin, with a NOEC of 2.1 mg/kg in sand which increased 15-fold, to 32 mg/kg, when seedlings were grown in soil; soil likewise mitigated the effects of sarafloxacin on all five of the other species tested, increasing NOEC values 9- to 55-fold above the values observed in sand (Saraflox® EA, 1995).

Finally, as discussed earlier, danofloxacin will not persist in the environment. Biotic as well as abiotic depletion mechanisms will reduce exposure concentrations for plant species to considerably less than those assumed in these worst case scenarios. Exposure will be intermittent and transient, yet, as results described above show, even maximum estimated soil levels will not cause damage to cultivated plants. Therefore, no further studies are necessary and no mitigation measures need be considered.

6.3 Residues in the Aquatic Environment

The intended use of Advocin® 180 for individual animal treatment and the strong sorption properties of danofloxacin preclude the need to test effects against aquatic organisms. Direct introduction of residues by grazing cattle will be inconsequential. Worst-case estimates for run-off from agricultural fields amended with manure containing danofloxacin residues indicate that concentrations would not exceed about 0.8 ng/L if all such residues were partitioned into the aqueous compartment. However, the high K_d and K_{oc} values for danofloxacin indicate that less than 1% of the residues are likely to partition into the aqueous phase and levels would therefore not exceed 0.008 -10 ng/L. Moreover, various biotic and abiotic depletion mechanisms, including susceptibility to rapid photolysis, will ensure only transient presence in surface waters. Toxicity data reported for related fluoroquinolones that may be used in aquaculture indicate that these agents have low toxicity for sensitive aquatic tester species. The *Daphnia magna* acute toxicity EC_{50} for sarafloxacin was 210 mg/kg; the 21 day chronic toxicity EC_{50} and NOEC were >170 mg/kg and 21 mg/kg, respectively (Saraflox® EA, 1995). For enrofloxacin the *D. magna* acute toxicity LC_{50} was 79.9 mg/kg and NOECs were 23 mg/kg for acute and 9.8 mg/kg for 21-day chronic exposure; the acute LC_{50} for *Hyalella azteca* was > 206 mg/kg (Baytril® EA, 1996). Toxicity for fish species is also low. The acute toxicity NOECs of sarafloxacin for Atlantic salmon, rainbow trout, and channel catfish were >300 mg/kg (Stamm, 1991). The enrofloxacin LC_{50} for trout was >196 mg/kg and for bluegill 79.5 mg/kg; NOECs were 33.5 and 18.6 mg/kg, respectively (Baytril® EA, 1996). Enrofloxacin reportedly has no toxic effects toward rainbow trout in amounts up to 400 mg/kg bw as a single oral dose or in feed at 50 mg/kg bw per day for 30 days (Hsu, 1993).

6.4 Conclusions

Use of Advocin® 180 for treatment of bacterial infections in cattle is not expected to present a significant hazard to non-target species or to the environment. Introduction of danofloxacin into the environment will be intermittent through the field application of aged cattle manure containing excreted drug residue, with some limited introduction also possible from treated grazing cattle. A comprehensive battery of environmental fate, ecotoxicity and physical-chemical studies have been conducted to address the environmental impact of danofloxacin use in livestock. The very low vapor pressure, low octanol:water partition coefficients and strong sorption to feces and to soil preclude residues from partitioning from soil into the atmosphere or into surface or ground water. Danofloxacin is not expected to bioaccumulate in non-target terrestrial or aquatic organisms. Unbound danofloxacin residues in soil will be biotransformed and degraded and will not accumulate. Intermittent maximum predicted concentrations of danofloxacin residues in soil of less than 10 µg/kg from field application of cattle manure is not expected to affect non-target species. Small amounts of danofloxacin residues that might enter surface water bodies through run-off or by excretion from pastured animals will be intermittent and transient. Strong sorption to particulates and to sediments will result in negligible levels in the water column that are not expected to affect non-target aquatic organisms. Susceptibility of danofloxacin to photolysis as well as to biodegradation will contribute to depletion of any residues that enter the aquatic environment. The manufacture of danofloxacin and Advocin® 180 and the disposal of solvents, reaction products and byproducts will be conducted in accordance with federal and local regulations to minimize environmental impact. The product, used as directed for treatment of livestock, does not present any significant risk to the environment.

7. ALTERNATIVES TO THE PROPOSED ACTION

The proposed action would not be expected to have any substantial adverse effect on human health or the environment. Therefore, alternatives to the proposed action do not need to be considered.

8. LIST OF PREPARERS

This Environmental Assessment was prepared by the following member of Pfizer Inc.

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9. CERTIFICATION

The undersigned official certifies that the information presented in this Environmental Assessment is true, accurate and complete to the best of his knowledge.

Robert G. Chesebrough Date: 3/22/02

Robert G. Chesebrough
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ATTACHMENT 1

STUDY REPORT SUMMARIES

Report Summary: Physical-chemical Properties of Danofloxacin and Danofloxacin Mesylate

Dissociation Constants: The dissociation constants (pKa's) of danofloxacin were determined by potentiometric titration, in triplicate, of 0.0125 M aqueous solutions of danofloxacin mesylate with 0.6 N sodium hydroxide solution at ambient temperature. The Automatic Potentiometric Titrator used in this experiment plotted the measured pH values against the volume of base added, and identified the inflection points of the resulting titration curve (Graph A). It calculated the pKa's as the pH values halfway to the first inflection point and between the first and second inflection points, respectively. Values of 6.22 $\pm$ 0.01 and 9.43 $\pm$ 0.02 were obtained.

Ultraviolet-Visible Absorption Spectrum: The absorption spectrum of danofloxacin mesylate in aqueous buffers at about 22-25°C was determined in triplicate with a diode array spectrophotometer (Graphs B, C and D). The following maxima were observed:

	Wavelength(nm)	Molar Absorptivity
pH 5	208	12090
	234	8553
	282	47796
	310	9957
	350	10682
pH 7	234	10612
	276	43268
	336	13244
pH 9	234	11392
	277	41978
	328	13877

Melting Temperature: A capillary tube containing danofloxacin (as the inner salt) or danofloxacin mesylate and another tube containing a reference standard were placed into the heating well of a melting point apparatus. The temperature was raised at a constant rate, and the temperatures were noted at which changes were observed in either material. The determinations were carried out in triplicate. The danofloxacin replicates all melted at 263°C. The danofloxacin mesylate replicates gave a range of 326.6 to 328.7°C, with an average melting temperature of 327.9°C. The standards showed the expected melting points: Thomas Standard H, 326.0 to 328.6°C; caffeine, 237°C.

Thermogravimetric Analysis: A sample of the test material, danofloxacin (as the inner salt) or danofloxacin mesylate, was heated in a commercial, Perkin-Elmer TGA instrument, which continuously and accurately monitored the weight of the sample. Samples were run in triplicate. Danofloxacin mesylate exhibited an average 3.86% loss of weight over the range of 77.5 to 156.3°C, corresponding to a water content of 3.87% as determined by the Karl Fischer method. There was no further loss of weight until decomposition set in at about 330°C. The TGA plot is shown in Graph E.

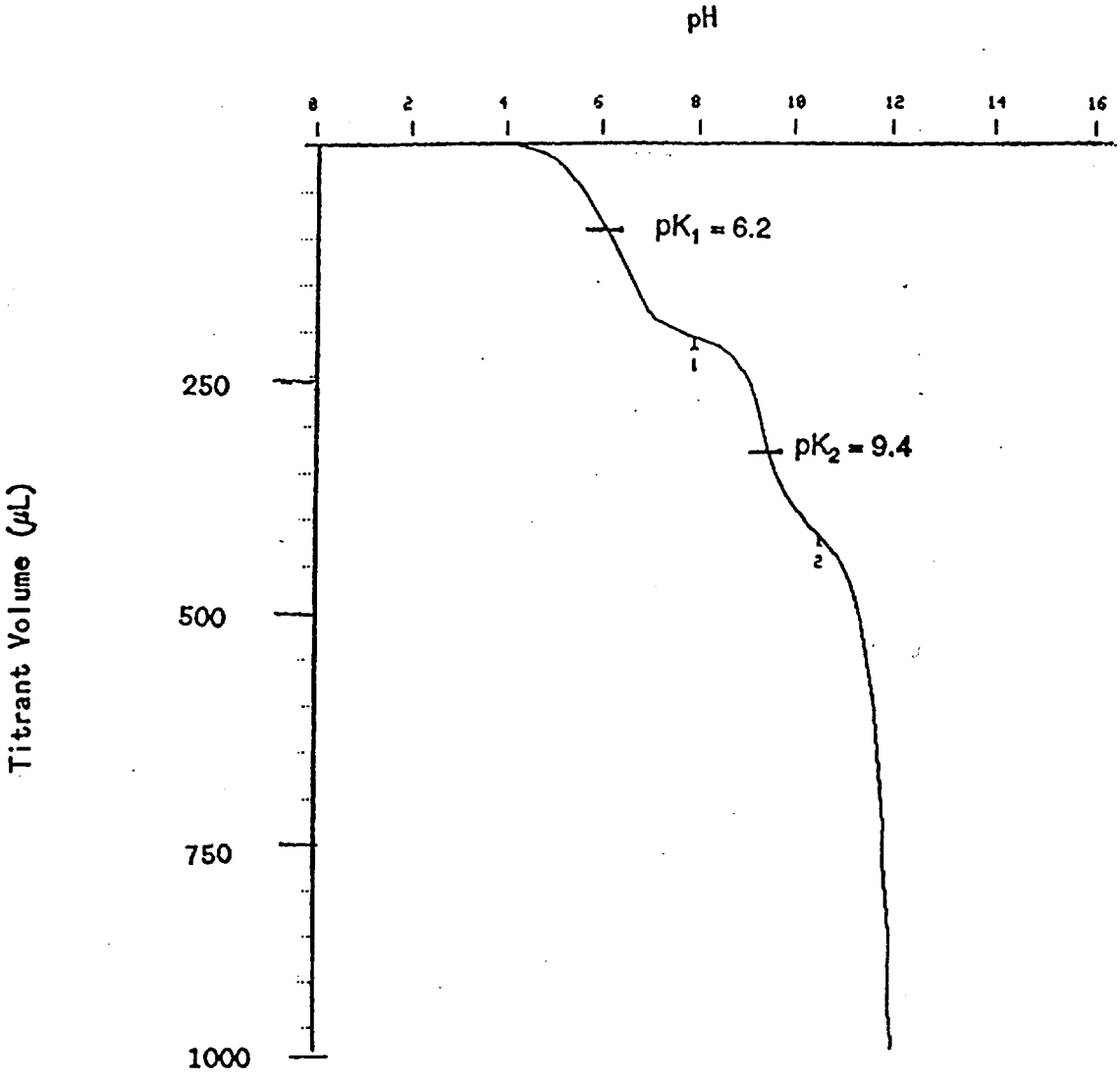
Danofloxacin exhibited an average 1.91% loss of weight up to about 143°C, in agreement with the sum (1.8%) of the water content (Karl Fischer) and isopropanol content of the sample. There was no further loss of weight until decomposition set in at about 330°C. The TGA plot is shown in Graph F.

The above results indicate that both danofloxacin mesylate and danofloxacin (inner salt) have very low vapor pressures and are non-volatile. Additional thermogravimetric data were obtained which confirm that the vapor pressures must be less than 10^{-8} torr at 20°C. One gram samples of danofloxacin mesylate, danofloxacin, and pyrene (for which the vapor pressure has been reported as 7×10^{-7} torr at 20°C), were examined for weight loss at a severe challenge condition of 100RC for 24 hours under vacuum. Neither the danofloxacin mesylate nor the danofloxacin (inner salt) samples lost any significant weight beyond solvated water, but the pyrene sample was nearly completely volatilized.

GRAPH A

POTENTIOMETRIC TITRATION PLOT FOR

DANOFLOXACIN MESYLATE

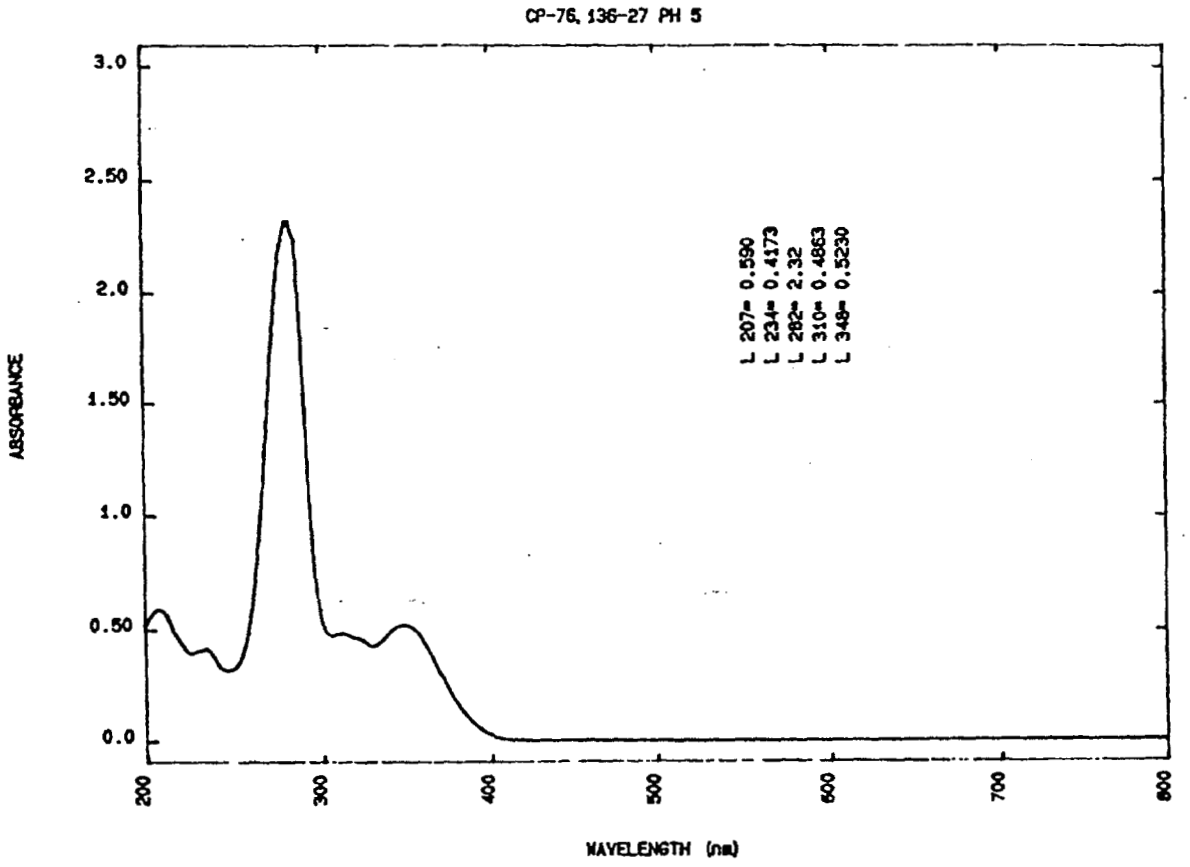


GRAPH B

UV-VIS ABSORPTION SPECTRUM OF

DANOFLOXACIN

pH 5.0 Buffer

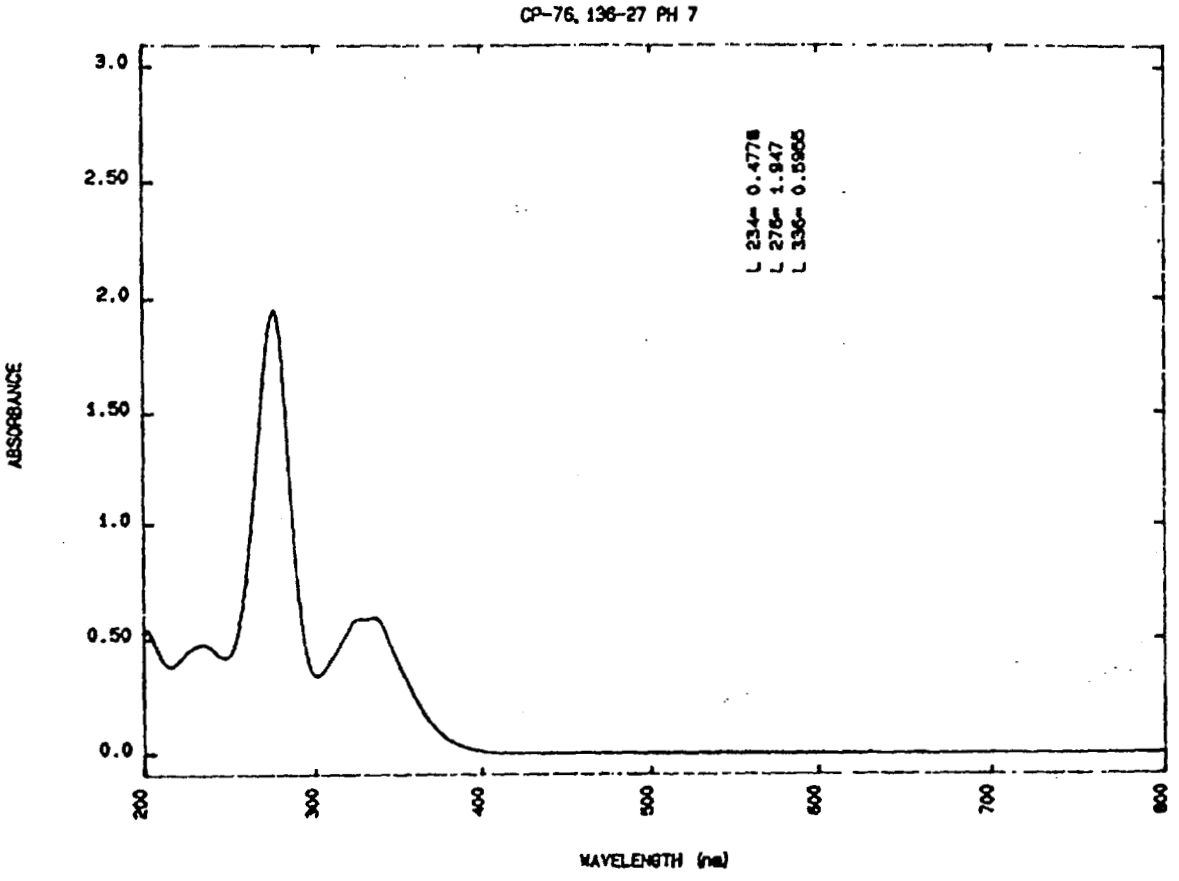


GRAPH C

UV-VIS ABSORPTION SPECTRUM OF

DANOFLOXACIN

pH 7.0 Buffer

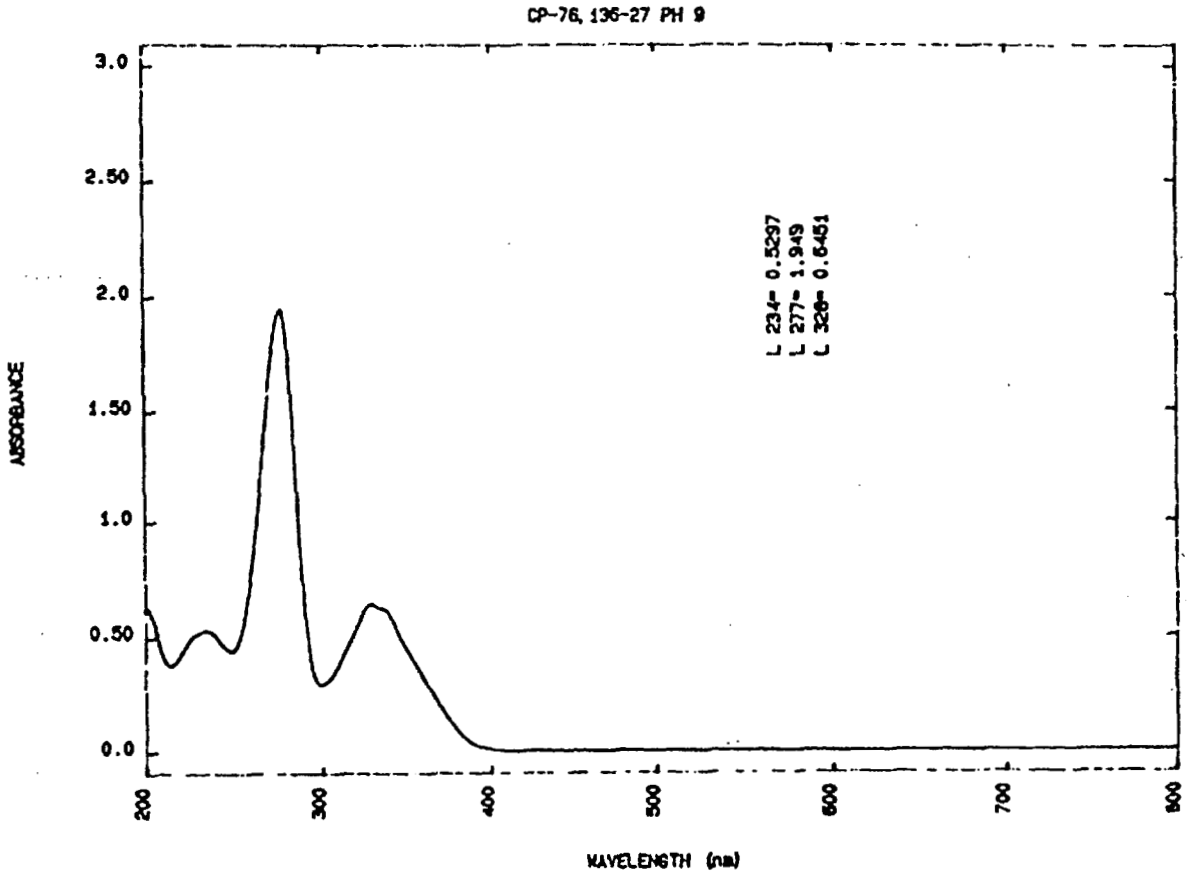


GRAPH D

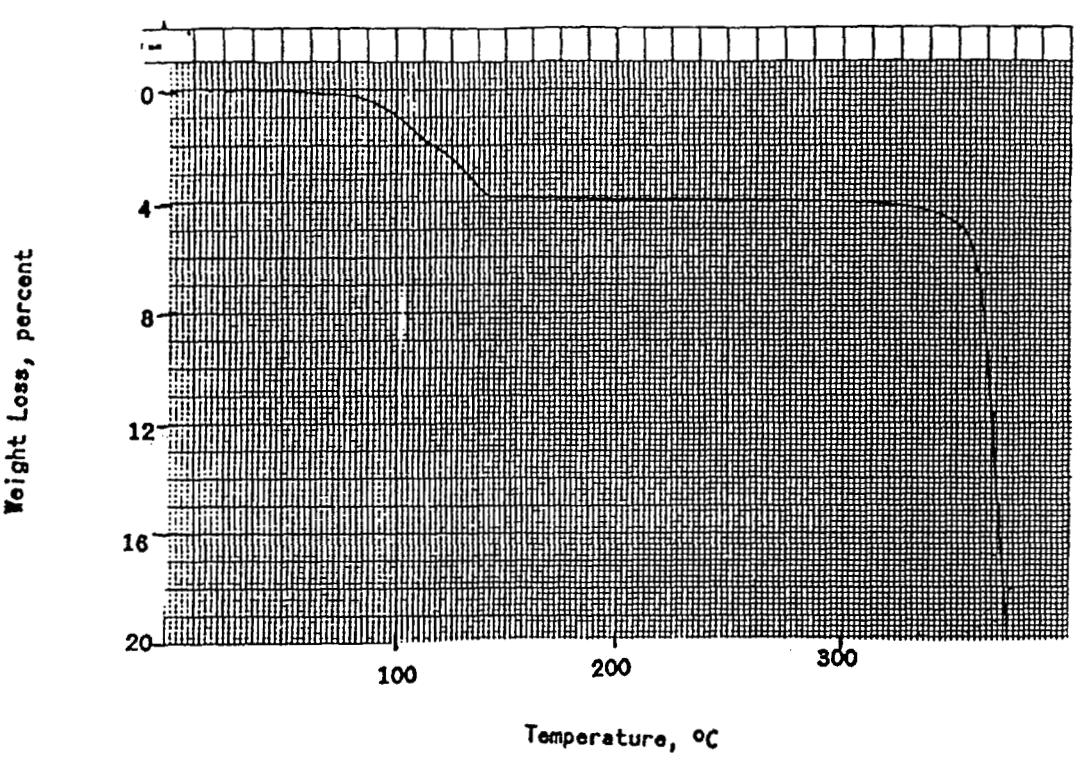
UV-VIS ABSORPTION SPECTRUM FOR

DANOFLOXACIN

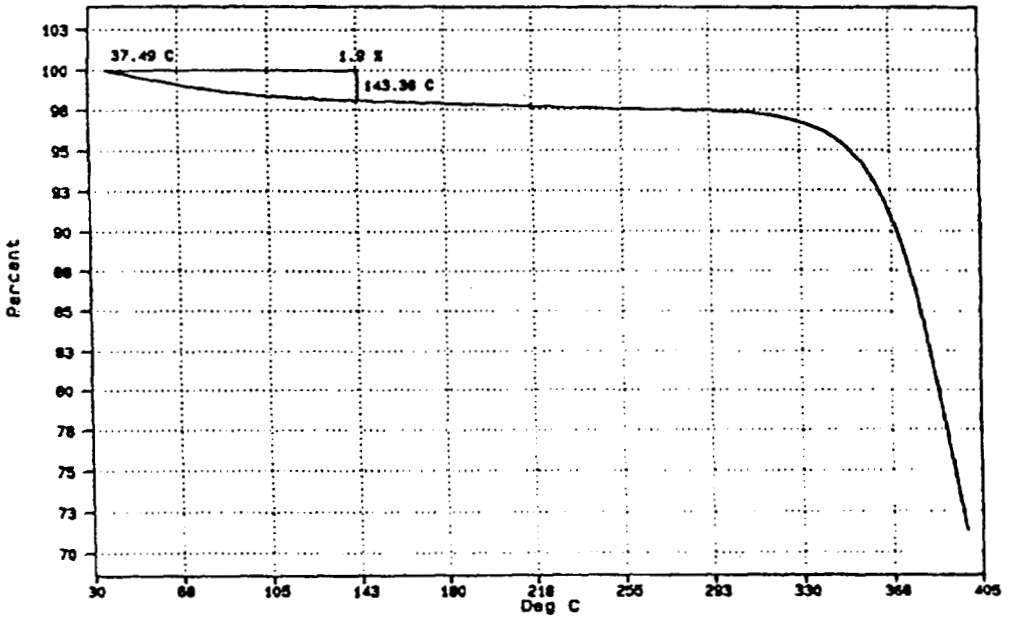
pH 9.0 Buffer



Graph E. TGA plot for Danofloxacin Mesylate



Graph F. TGA plot for Danofloxacin



Report Summary: Solubility of Danofloxacin in Aqueous Buffer

Study Number: 2438-0690-6120-700

Test System: pH 5, 7, and 9 aqueous buffers.

Summary of Experimental Design: Three aqueous buffers were prepared: pH 5, 1.07 M acetate; pH 7, 0.08 M phosphate; and pH 9, 0.07 M borate. Triplicate samples of each buffer were shaken with excess solid danofloxacin in centrifuge tubes maintained at 21.1 ± 0.4°C and shielded from decomposition by light. At daily intervals, an aliquot of each replicate was filtered to remove undissolved danofloxacin and assayed for danofloxacin concentration by a specific high performance liquid chromatography procedure. Equilibration at each pH was continued until the concentration of danofloxacin remained constant for at least three days.

The inner salt rather than the mesylate salt of danofloxacin was used because in preliminary experiments, the buffering capacity of buffer solutions was overwhelmed during attempts to saturate the buffers with the mesylate. This problem did not arise in experiments conducted at lower concentrations. At the low concentrations at which the drug would be introduced into the environment (i.e. in animal excreta, soil, water) through use, the salt form would be determined by the pH of the immediate environment and the concentration in the latter of naturally occurring anions, such as chloride, nitrate, and carbonate, which would far exceed any concentration of the mesylate anion.

Calculations:

The octanol-water partition coefficient (Kow), bioconcentration factor for fish (BCF), and soil sorption coefficient (Koc) of danofloxacin were estimated from the aqueous solubility (S) by means of standard equations:

log Kow = 5.00 - 0.67 log S, where S is in µg/L equation (1)

log Kow = 0.710 - 0.862 log S, where S is in moles/L equation (2)

log BCF=2.791-0.564 log S, where S is in mg/L equation (3)

log Koc = 3.64 - 0. 55 log S, where S is in mg/L equation (4)

Summary of Results:

Based on the measured concentrations shown in Table 1, the following values were obtained for the solubility in pH 5, 7, and 9 buffers:

pH 5: 156,000 ± 33,000 mg/L
pH 7: 656 ± 50 mg/L
pH 9: 1,060 ± 130 mg/L

As can be seen from Table 2, equations (1), (2), and (4) did not provide useful estimates of the partition coefficient and soil sorption coefficient of danofloxacin. This is not surprising, since these equations were derived from sets of primarily non-ionic and some weakly ionic compounds, whereas danofloxacin contains both acidic and basic functional groups and would be expected to ionize within the entire pH range of 5-9.

Table 1. Measured Concentrations of Danofloxacin (mg/L)

Sampling Interval (days)	pH 5			pH7			pH 9		
	A	B	C	A	B	C	A	B	C
0	171,000	117,000	125,000	656	694	716	1,080	1,080	1,080
1	408,000	344,000	350,000	867	835	796	835	1,020	1,280
2	155,000	147,000	158,000	782	760	890	807	764	650
3	169,000	271,000	134,000	763	668	658	2,320	1,420	1,690
4	143,000	159,000	151,000	637	646	630	2,110	1,670	1,840
5	145,000	136,000	137,000	623	615	569	827	544	729
6	146,000	147,000	142,000	720	671	667	812	804	804
7							1,360	1,350	1,350
8							1,030	1,100	1,040
9							1,250	1,150	906
10							932	929	1,220

Table 2. Parameters Calculated from Solubility vs. Actual Measured Values

Property	Source	pH 5	pH 7	pH 9
Kow	Equation (1)	0.32	13	9.2
	Equation (2)	10	1200	770
	Found	0.14	0.39	0.22
BCF				
	Equation (3)	0.73	16	12
Koc				
	Equation (4)	6.1	120	95
	Soil 1(pH 5.6)	74,600		
	Soil 2(pH 7.0)		134,000	
	Soil 3(pH 7.6)			644,000

Report Summary: The Octanol-water Partition Coefficient of Danofloxacin Mesylate

Study Number: 2438-0588-6121-705

Test System: Two-phase solvent system

Summary of Experimental Design: Solutions of radiolabelled danofloxacin mesylate were prepared at approximately 10^{-3} and 10^{-4} molar concentrations in pH 5, 7, and 9 aqueous buffers. Each combination of pH and concentration was prepared in triplicate. A 40 ml volume of each solution was shaken gently at 20 $\pm$ 1°C in a capped centrifuge tube, shielded from light, with 5 ml of n-octanol. Shaking was continued for 2 hours in the case of the solutions in pH 9 buffer and for 16 hours at pH 5 and 7. It had been determined in a preliminary experiment that these lengths of time were sufficient to attain equilibrium.

The amount of radiolabelled danofloxacin mesylate present in each phase was then determined by liquid scintillation counting of aliquots. The radiocount for each aliquot was divided by the volume of the aliquot, and the resulting radiocount per unit volume of the phase was divided by the specific radioactivity of the test material to obtain the final concentration of danofloxacin mesylate in the phase.

The partition coefficient (K_{ow}) for danofloxacin mesylate in each system was calculated by dividing the final concentration of danofloxacin mesylate in the octanol phase by its final concentration in the aqueous buffer phase. The partition coefficient was converted to its logarithm, log K_{ow}.

The radiometric mass balance was checked by multiplying the radiocount per unit volume of each phase by the volume of the phase, summing the resulting total radiocount per phase for the two phases of each system, and dividing the total by the amount of radioactivity originally added to the system in the buffer solution. The result was expressed as a percentage.

Summary of Results: The table below lists the mean values of the final concentration in the octanol phase, final concentration in the aqueous phase, partition coefficient, logarithm of partition coefficient, and percent radiolabel recovered, for each combination of pH and initial concentration in the aqueous buffer.

	Concentration (mg/ml)					
pH	Initial	Octanol	Water	K_{ow}	log K_{ow}	% recovery
5	0.0444	0.007	0.043	0.17	-0.78	98
5	0.444	0.049	0.43	0.12	-0.92	96
7	0.0444	0.020	0.039	0.52	-0.29	93
7	0.444	0.115	0.44	0.26	-0.58	101
9	0.0444	0.013	0.044	0.28	-0.55	102
9	0.444	0.073	0.44	0.17	-0.78	101

Combining the results obtained at each pH, the following values were calculated for the partition coefficient:

pH	Kow	Standard Deviation
5	0.14	0.03
7	0.39	0.14
9	0.22	0.06

These partition coefficients of less than one mean that danofloxacin mesylate remained preferentially in the aqueous phase at all three pH's and only partitioned into the octanol phase to a very limited extent. Based on these low values, danofloxacin mesylate is unlikely to bioconcentrate significantly in living organisms or sorb to neutral organic matter in soil or sediment.

Report Summary: Soil Sorption and Desorption of Danofloxacin Mesylate

Study Number: 2438-0688-6122-710)

Test System: Three types of soil in contact with aqueous solutions.

Summary of Experimental Design: The same general procedure was used to conduct a screening test, a soil kinetics test, and an isotherm determination. All tests were conducted in triplicate. Three different types of soil were used: an Iowa Sandy Loam, a California Clay Loam, and a Mississippi Silty Clay Loam. The characteristics of these soils are shown in Table 1.

To study sorption, samples of each soil were shaken in capped centrifuge tubes with solutions of radiolabelled danofloxacin mesylate in 0.01 M aqueous calcium chloride. The ratio of solution to soil was 5:1 in the screening test and 1,000:1 in the soil kinetics and isotherm tests. For every combination of soil type and initial concentration, the concentration remaining in the aqueous phase (C_e) was determined by radioassay, and the amount sorbed onto soil (x) was calculated from the difference between the initial and final concentration in the aqueous phases.

To study desorption, soil samples containing sorbed danofloxacin mesylate were equilibrated twice in succession with fresh 0.01 M aqueous calcium chloride, and the concentrations in the aqueous phases were again determined by radioassay. In the screening test, a separate set of sorption and desorption experiments was carried out with deionized water as the aqueous vehicle.

Calculations: The logarithm of the experimentally determined equilibrium concentration, log C_e, was plotted against log (x/m) for each soil, where x/m is the concentration in the soil. The points on the graph were fitted to a logarithmic transformation of the Freundlich isotherm equation:

$$\log (x/m) = \log (K_d) + 1/n \log (C_e)$$

where K_d is the Freundlich sorption coefficient and 1/n, an empirical constant, is the slope of the graph. Log K_d was read off the graph as the intercept. The antilog, K_d, was then calculated and converted to K_{oc}, the sorption constant adjusted for the organic carbon content of the soil, according to the equation:

$$K_{oc} = (K_d \times 100)/\% \text{ organic carbon}$$

The percent of the initially added danofloxacin mesylate that would be sorbed from aqueous solution onto each of the soils at a solution ratio of 5:1 was calculated from the K_d values determined for sorption in the isotherm test:

$$\% \text{ Sorbed} = [K_d /(K_d + 5)] \times 100$$

Similarly, the percent of the danofloxacin mesylate sorbed onto soil that would be desorbed when the soil is exposed to fresh 0.01M calcium chloride solution, at a solution:soil ratio of 5:1, was calculated from the K_d values determined for desorption:

$$\% \text{ desorbed} = [5(K_d+5)] \times 100$$

Summary of Results: The results of the screening test indicated that danofloxacin is strongly sorbed to all three soil types, suggesting that it would be advisable to conduct subsequent tests at a high ratio of solution to soil so the low concentrations in the aqueous phases could be determined accurately. It also showed that the presence of calcium chloride, which simulates natural conditions, did not interfere with the sorption of danofloxacin to soil and, in fact, enhanced it.

In the soil kinetics test, the concentration of danofloxacin mesylate in the aqueous phase declined initially, but remained essentially the same after 24 hours of contact with soil as after 16 hours, indicating that exchange of danofloxacin between solution and soil had reached equilibrium (Table 2).

The results of the isotherm test confirmed that danofloxacin mesylate is strongly though reversibly sorbed to soil (Table 3, 4 and 5). The value of K_d, the Freundlich sorption coefficient, in these three soils ranged from 2,280 to 3,800 for sorption (Table 4) and from 2,540 to 8,180 for desorption (Table 5). The corresponding ranges of K_{oc} were 74,600 to 644,000 (Table 4) and 82,900 to 1,390,000 (Table 5), respectively. Compounds having a K_{oc} value of 1,000 or larger are considered relatively immobile in soil and have a low potential for leaching into the water table or into runoff water. It was calculated that at a solution:soil ratio of 5:1, more than 99% of the initially added danofloxacin mesylate will be sorbed onto each of the soils from aqueous solution, and in all cases less than 1% will be desorbed upon subsequent exposure of the soils to fresh calcium chloride solution.

The sorption coefficients may not be very accurate because there were indications that the sorption sites on the soils were becoming saturated at the concentrations tested. However, if this was the case, studies at lower concentrations would give even higher values for the sorption coefficients, and not change the overall conclusions.

A mass balance was calculated at one concentration for each soil by adding the amounts measured in the three aqueous phases (one sorption phase plus two desorption phases) plus amounts in combusted soil samples following the second desorption phase. In the case of Iowa Sandy Loam, the mean of the resulting totals for the three replicates was 131 % of the amount present in solution before sorption. The corresponding means for Mississippi Silty Clay Loam and California Clay Loam were 73% and 162%, respectively. When corrected for the observed recovery from soil-less controls, the totals were 108 $\pm$ 20%, 60 $\pm$ 6%, and 128 $\pm$ 81% for the Iowa, Mississippi, and California soils, respectively.

The basic amino group in danofloxacin mesylate has a pKa of 9.43 and consequently is predicted to exist predominately in its protonated form throughout the pH range of 5-9 normally encountered in the environment, including the pH's of the soils used in this study: 5.6, 7.0, and 7.6 (Table 1). Thus, at these pH's the molecule contains a cationic group, which is likely to be responsible for the strong sorption to soil. In agreement with this interpretation, the sorption constants increased in the same order as the cation exchange capacity of the soils (Table 1). The low partition coefficient (see The Octanol-Water Partition Coefficient of Danofloxacin Mesylate) suggests that affinity for organic matter in soil is unlikely to contribute significantly to sorption, a conclusion supported by

the lack of correlation between the organic matter content of the soils (Table 1) and the sorption constants. The ionic nature of the sorption probably accounts for the non-linearity of the Freundlich isotherms, as indicated by values of n ranging from 1.66 to 22.4 (Tables 4 and 5).

Table 1. Soil Characterization of the Three Soils Used in the CP-76,136-27 Sorption/Desorption Coefficient Determination

Source/ Texture	**Iowa Sandy Loam**	**California Clay Loam**	**Mississippi Silty Clay Loam**
% Sand	53.2	43.0	13.6
% Silt	37.6	24.0	55.0
% Clay	9.2	33.0	31.4
%Organic Matter	5.2	3.1	1.0
pH	5.6	7.6	7.0
Cation Exchange capacity (meq/100g)	12.5	19.6	29.4

Table 2. Soil Kinetics Test for Sorption of Danofloxacin Mesylate to Three Soils

Time Interval (hours)	Mean Measured Concentration (mg/L) in Solution Iowa Sandy Loam	California Clay Loan	Mississippi Silty Clay Loan
0	25.0 (Nominal)	25.0 (Nominal)	25.0 (Nominal)
2	20.29 ± 1.28	14.02 ± 3.19	13.36 ± 2.72
4	20.13 ± 0.95	10.18 ± 0.28	7.98 ± 0.36
16	15.44 ± 1.39	10.59 ± 0.84	7.51 ± 0.37
24	14.96 ± 0.98	9.70 ± 1.27	7.39 ± 1.56

Table 3. Isotherm Test: Concentrations of Danofloxacin in Soil and in Solution
(Mean of three values and standard deviation)

Mean Measured Initial Concn. µg/ml		Soil type/concentration					
		IOWA SANDY LOAM		CALIFORNIA CLAY LOAM		MISSSSIPPI SILTY CLAY LOAM	
		Aqueous,µg/ml	Soil, µg/g	Aqueous, µg/ml	Soil, µg/g	Aqueous, µg/ml	Soil, µg/g
				SORPTION STUDY			
2.70	Mean	0.53	2176.03	0.65	2052.70	0.34	2366.03
	SD	0.04	38.0	0.23	231	0.03	30.55
5.11	Mean	1.67	3434.97	1.67	3303.33	1.02	3946.67
	SD	0.06					
9.79	Mean	6.58	3211.13	5.21	4581.13	4.06	5724.47
	SD	0.08	81.45	0.21	209.84	0.22	215.02
23.3	Mean	19.68	4182.73	10.54	13322.73	9.71	14149.40
	SD	0.29	291.43	3.79	3791.63	0.08	81.85
				DESORPTION STUDY			
		1 Des. 2 Des.		1 Des. 2 Des.		1 Des. 2 Des.	
2.70	Mean	0.10 0.08	2004.98	0.21 0.09	1768.07	0.04 0.02	2305.52
	SD	0.01 0.02	43.85	0.01 0.01	242.50	0.00 0.00	29.08
5.11	Mean	0.23 0.19	3029.50	0.33 1.63	1347.85	0.11 0.07	37773.73
	SD	0.01 0.04	72.30	0.01 0.06	637.75	0.02 0.01	56.30
9.79	Mean	0.34 0.29	2639.38	0.89 0.24	3497.47	0.44 0.22	5095.20
	SD	0.04 0.01	62.89	0.07 0.06	282.00	0.04 0.07	224.79
23.3	Mean	1.23 0.62	2334.73	3.30 0.76	9315.38	1.09 0.60	12515.72
	SD	0.26 0.07	280.53	0.10 0.01	3929.31	0.04 0.17	160.86

Table 4. Linear Regression Analysis of the Sorption Data Using the Freundlich Isotherm $Log_{10}(x/m) = \{log_{10}(K_d) + 1/n\ log_{10}(C_e)\}$ for CP-76,136-27 with Three Soil Types.

Mean Measured Concentration µg/mL	Iowa Sandy Loam		California Clay Loam		Mississippi Silty Clay Loam	
	$log_{10}C_e$	$log_{10}X/m$	$log_{10}C_e$	$log_{10}x/m$	$log_{10}C_e$	$log_{10}x/m$
2.70	-0.28	3.34	-0.19	3.31	-0.47	3.37
5.11	0.22	3.34	0.22	3.52	0.01	3.60
9.79	0.82	3.54	0.72	3.66	0.61	3.76
23.3	1.29	3.62	1.02	4.12	0.99	4.15
Correlation:	0.953		0.940		0.965	
Slope (1/n):	0.20		0.602		0.491	
Int ($log_{10}K_d$):	3.36		3.39		3.58	
n:	5.06		1.66		2.04	
K_d	2280		2430		3800	
K_{oc}	74600		134000		644000	

* % Organic carbon = organic matter/1.7

Table 5. Linear Regression Analysis of the Desorption Data Using the Freundlich Isotherm $Log_{10}(x/m) = \{log_{10}(K_d) + 1/n\ log_{10}(C_e)\}$ for CP-76,136-27 with Three Soil Types.

Mean Measured Concentration µg/mL	Iowa Sandy Loam		California Clay Loam		Mississippi Silty Clay Loam	
	$log_{10}C_e$	$log_{10}X/m$	$log_{10}C_e$	$log_{10}x/m$	$log_{10}C_e$	$log_{10}x/m$
2.70	-0.74	3.30	-0.52	3.25	-1.22	3.36
5.11	-0.38	3.48	0.29	3.13	-0.74	3.58
9.79	-0.20	3.42	0.05	3.54	-0.18	3.71
23.3	0.27	3.37	0.61	3.97	0.23	4.10
Correlation:	0.246		0.604		0.963	
Slope (1/n):	0.045		0.473		0.471	
Int ($log_{10}K_d$):	3.40		3.42		3.91	
n:	22.40		2.11		2.12	
K_d:	2540		2640		8180	
K_{oc}:*	82900		145000		1390000	

* % Organic carbon = organic matter/1.7

Report Summary: Soil Column Leaching of Danofloxacin

Study Number: PFZ-492

Test System: ^{14}C-danofloxacin mesylate admixed with two soils at a rate equivalent to 0.6 kg/ha (183 ppb) in 30 cm glass columns.

Summary of Experimental Design:

Characteristics of 2 soils employed in the study are as follows:

	Cation Exchange capacity (meq/100g)	Organic Matter (%)	pH	Bulk density (g/cm^3)
Thoresby Loamy sand	5.0	1.2	7.2	1.42
Alconbury Sandy clay loam	18.7	2.7	7.9	1.06

A leaching study was conducted to estimate the mobility of danofloxacin mesylate in two soils representative of those employed in agriculture. Two glass columns per soil, 5 cm in diameter and 30 cm in height, were packed with dried, sieved soil. Soil containing ^{14}C-danofloxacin mesylate at a rate equivalent to 0.6 kg/ha soil (183 ppb) formed the top 20 g of air dried soil in the column. After formation, the columns were saturated with 0.01 M calcium chloride solution and the void volume determined by addition of ^{36}Cl-sodium chloride. Leachate was then collected in fractions following addition of 1L of water. ^{14}C-danofloxacin mesylate and ^{36}Cl-sodium chloride content in leachate was quantitated by liquid scintillation counting; radioactivity in soil was determined by combustion analysis after dismantling the column into 5 cm sections.

Summary of Results: No appreciable leaching of danofloxacin mesylate was observed in either of the two soils evaluated. Total mean recoveries of ^{14}C-radioactivity and ^{36}Cl-radioactivity were 94-95% and 103-104%, respectively, of applied amounts (table).

Recovery of radioactivity from soil columns after elution following application of ^{14}C-danofloxacin mesylate

Recovery expressed as % applied ^{36}Cl or ^{14}C

Fraction	Alconbury sandy clay loam		Thoresby loamy sand	
	Column A	Column B	Column A	Column B
^{36}Cl in leachate	101.5	108.0	103.8	103.7
^{14}C in leachate	<0.7	<0.9	<0.6	<0.6
^{14}C in soil extract[a]	68.4	64.6	86.8	78.3[b]
^{14}C in soil residues[a]	27.1	27.4	13.7	11.2
^{14}C Total	95.5	92.0	100.5	89.5

[a]All the radioactivity in soil extracts and residues was recovered in the top 0-5 cm section of the column.
[b]2.3% of applied ^{14}C was detected in the lowest section of the column (25-30 cm) and was considered to be anomalous

The leachate from both soils contained no detectable ^{14}C-radioactivity (<0.9% applied radioactivity in the total leachate). Most of the applied ^{14}C-radioactivity (approximately 90%) was retained in the top 5 cm section of the columns with radioactivity in the lower sections being below the limit of reliable measurement (<3% applied) except for Thoresby soil, column B, which is considered an anomoly.

Results suggest that amendment of soils with manure or litter from treated animals should not result in contamination of ground water due to the movement of danofloxacin in soils.

Report Summary: Soil Column Leaching of Danofloxacin

Study Number: PFZ-671

Test System: ^{14}C-danofloxacin mesylate admixed with two low pH soils at a rate equivalent to 0.6 kg/ha (183 ppb) in 30 cm glass columns.

Summary of Experimental Design:

Characteristics of 2 soils employed in the study are as follows:

	Cation Exchange Capacity (meq/100g)	Organic Matter (%)	pH	Bulk density (g/cm^3)
Warwickshire sandy loam	10.8	1.7	5.2	1.09
Warwickshire clay loam	33.6	6.2	4.8	0.83

A leaching study was conducted to estimate the mobility of danofloxacin mesylate in two low pH soils. Two glass columns per soil, 5 cm in diameter and 30 cm in height, were packed with dried, sieved soil. Soil containing ^{14}C-danofloxacin mesylate at a rate equivalent to 0.6 kg/ha soil (183 ppb) formed the top 20 g of air dried soil in the column. After formation, the columns were saturated with 0.01M calcium chloride solution and the void volume determined by addition of ^{36}Cl-sodium chloride. Leachate was then collected in fractions following elution with 1 L of calcium chloride solution at a flow rate of about 50 mL/hr. ^{14}C-danofloxacin mesylate and ^{36}Cl-sodium chloride content in leachate was quantitated by liquid scintillation counting; radioactivity in soil was determined by combustion analysis after dismantling the column into 5 cm sections.

Summary of Results: No appreciable leaching of danofloxacin mesylate was observed in either of the two soils evaluated. Total mean recoveries of ^{14}C-radioactivity and ^{36}Cl-radioactivity were 95% and 101%, respectively, of applied amounts (table).

Recovery of radioactivity from soil columns after elution following application of ^{14}C-danofloxacin mesylate

Recovery expressed as % applied ^{36}Cl or ^{14}C

Fraction	Warwickshire sandy loam		Warwickshire clay loam	
	Column A	Column B	Column A	Column B
^{36}Cl in leachate	99.9	98.0	96.1	108.5
^{14}C in leachate	<0.9	<0.8	<0.6	<0.8
^{14}C in soil extract[a]	82.9	74.1	70.6	75.2
^{14}C in soil residues[a]	12.7	18.7	22.4	22.2
^{14}C Total	95.6	92.8	93.0	97.4

[a]All the radioactivity in soil extracts and residues was recovered in the top 0-5 cm section of the column.

The leachate from both soils contained no detectable ^{14}C-radioactivity (<0.9% applied radioactivity in the total leachate). Most of the applied ^{14}C-radioactivity (92.8-97.4%) was retained in the top 5 cm section of the columns with radioactivity in lower sections being below the limit of reliable measurement (<2% applied).

These results indicate that danofloxacin mesylate would have a low potential for mobility in soils. The lack of vertical movement is an indication of the absence of leaching potential and hence minimal potential for contamination of ground water.

Report Summary: Hydrolysis of Danofloxacin Mesylate

Study Number: 2438-0588-6123-715

Test System: Solutions in aqueous buffers.

Summary of Experimental Design: Sterile solutions containing approximately 430 ppm of danofloxacin mesylate in pH 5, 7, and 9 buffers were prepared. Three replicates of each solution were placed in centrifuge tubes, covered with aluminum foil to protect them from light, and heated for 5 days in a water bath maintained at 50°C. At the beginning and end of the five-day period, an aliquot was removed from each replicate and analyzed for concentration of danofloxacin mesylate by a specific high pressure liquid chromatography assay.

Summary of Results: There was no significant difference between initial and final concentration in any of the replicates, i.e. no hydrolysis was detected at pH 5, 7, or 9 after 5 days at 50°C.

		Concentration (mg/L)		
Interval	Replicate	pH 5	pH7	pH9
Day 0	A	419	437	427
	B	423	442	438
	C	423	440	434
Mean		422	440	433
Day 5	A	426	440	440
	B	419	443	440
	C	431	442	440
Mean		425	442	440
% of initial concentration remaining		101	100	102

The results indicate that danofloxacin mesylate is hydrolytically stable, and is not likely to be removed from the environment at a meaningful rate by hydrolysis.

Report Summary: Aquatic Photolysis Study of Danofloxacin

Study Number: 4470-N-001-93

Test System: Exposure of Aqueous Solutions to Natural Sunlight

Summary of Experimental Design: For definitive photodegradation kinetics, filter-sterilized solutions containing one ppm danofloxacin mesylate in pH 5, 7 and 9 buffers were prepared. Samples of each buffered solution and an actinometer solution of 0.01 mM p-nitroanisole (PNA) and 24.8 mM pyridine were distributed into 8 ml quartz tubes and equilibrated to 25± 1°C. Samples used for dark controls were covered with aluminum foil. Tubes were arrayed in a rack slanted at 30° from the vertical and exposed to mid-day summer sunlight at 38°N latitude. One tube of danofloxacin at each pH and one actinometer were taken at each of six time points over two half-lives. Triplicate sample aliquots were analyzed by high performance liquid chromatography (HPLC) to quantitate unchanged danofloxacin or PNA. A photolysis study with 12 ppm [2-^{14}C]-danofloxacin in pH 7 buffer was conducted to profile and quantitate photolysis products. Two quartz tubes containing [2-^{14}C]-danofloxacin solution were used, one for photolysis and one for a dark control. The irradiated tube was exposed to mid-day November sunlight at 38°N latitude. Duplicate aliquots of the control and photolyzed sample solutions were injected into the HPLC and monitored by diode array and radiochemical detectors. HPLC fractions from the photolyzed samples were collected and samples of each fraction were counted with a liquid scintillation counter to quantitate radiolabeled products.

Calculations: The photolytic rate constant, k_p, at a given pH was obtained by plotting ln C/C_o versus time according the first order equation:

$$\ln C/C_0 = -k_p t$$

where C = concentration of danofloxacin at time t

and C_0 = initial concentration of danofloxacin

The slope of the line, k_p, was obtained from the graph and used to calculate the half-life, $t_{1/2}$, according to the relation

$$t_{1/2} = \ln 2/k_p$$

Photolytic rate constants were corrected to flat surface water conditions by dividing the calculated values by 2.2 to account for the faster rate of degradation observed in 10 mm tubes (Leifer, A. 1988. The Kinetics of Environmental Aquatic Photochemistry, Appendix A. American Chemical Society, Washington, D.C.).

The quantum yield of the photolysis was estimated according to the relation

$$\phi_c = jk_{pc}/2.303\ell\Sigma I_\lambda \varepsilon_\lambda$$

where
- ϕ_c is the quantum yield at a given pH
- k_{PC} is the calculated rate constant in flat surface water in s^{-1}
- ε_λ is the UV extinction coefficient at wavelength λ in $M^{-1}cm^{-1}$
- j = 6.02×10^{20} and converts photons to Einsteins
- ℓ is the pathlength in cm
- I_λ is the sunlight intensity at wavelength λ in units of photons $cm^{-2}sec^{-1}$

Summary of Results: Danofloxacin underwent very rapid photolysis in dilute aqueous solution upon exposure to natural sunlight. Minimum calculated rate constants and corresponding maximum half-lives under clear skies at 38°N latitude close to solar noon are as follows:

	Rate Constant (minutes $^{-1}$)		Half-life (minutes)			
pH	Round Tube	Surface Water	Round Tube	Surface Water	Quantum Yield	PNA Rate Constant (minutes^{-1})
5.3	0.064	0.029	11	24	0.0023	0.054
7.0	0.36	0.16	1.9	4.3	0.012	0.041
9.1	0.59	0.27	1.2	2.6	0.018	0.041

Product profile studies with 12 ppm [2-^{14}C]-danofloxacin, photolyzed to >96% coversion, showed that the ^{14}C-label recovered after chromatography was distributed in more than 20 products, none of which accounted for more than 10% of the starting material. No product identification on these minor photodegradates was carried out.

Report Summary: Aerobic Biodegradation of Danofloxacin Mesylate in Soil

Study Number: SC910201

Test System: ^{14}C danofloxacin mesylate admixed with soils at 35 ppm (27 ppm danofloxacin zwitterion).

Summary of Experimental Design:

Characteristics of 3 soils employed in the study are as follows:

Soil Identification (Location)	Cation Exchange Capacity (meq/100g)	Organic Matter (%)	pH	Field Moisture Capacity %	Texture (%) Sand	Silt	Clay
Ohio Sandy Loam	9.8	1.0	7.1	18.3	63	23	14
Ohio Clay Loam	11.3	2.2	5.0	28.0	25	47	28
Ohio Loam	31.8	5.9	7.7	33.3	43	39	18

Three treatments were employed: 1) ^{14}C danofloxacin at a final zwitterion concentration of 27 ppm in soil (2.07×10^7 DPM activity), 2) glucose (a combination of ^{14}C and unlabeled) at a final concentration of 10 mg C/50 g soil (2.07×10^7 DPM activity), 3) untreated control. Each treatment was evaluated in triplicate for each of the 3 soils. A series of 27 incubation flasks, each containing 50 g of soil, were arranged in a system modified from Marinucci and Bartha (Apparatus for monitoring the mineralization of volatile ^{14}C -labelled compounds. Appl. Environ. Microbiol. **38**: 1020-1022) for trapping $^{14}CO_2$ and where appropriate, organic volatiles. Flasks were incubated in the dark at 22±3°C. The amount of radiolabeled carbon dioxide in the traps was measured periodically by liquid scintillation counting. All treatments were monitored for 64 days.

The glucose treatment demonstrated rapid mineralization to CO_2 in all three soils with measured time to 50% mineralization of approximately 14, 7 and 59 days, respectively, for sandy loam, clay loam and loam soils. Under conditions of the study, mineralization of danofloxacin to CO_2 did not occur to any appreciable extent (0.05%-0.12% in 64 days).

At the termination of the experiment, material balance achieved for the glucose treatment was 99.7 (65.8% mineralized to $^{14}CO_2$, 33.9% bound to soil), 102.4% (66.4% mineralized to $^{14}CO_2$, 36.0% bound to soil), and 101.8% (51.9% mineralized to $^{14}CO_2$, 49.9% bound to soil), respectively, for sandy loam, clay loam and loam soils. For the danofloxacin treatment, it was 96.3% (0.08% mineralization to $^{14}CO_2$, 92.0% extracted by tributyl ammonium hydroxide (TBAH), 4.3% bound to soil); 99.4% (0.05% mineralized to $^{14}CO_2$, 93.0% extracted by TBAH and 6.3% bound to soil), and 102.6% (0.12% mineralized to

$^{14}CO_2$, 86.7% extracted by TBAH, and 15.8% bound to soil), respectively, for the same three soils.

HPLC analysis of TBAH extracts demonstrated danofloxacin as the major component with individual minor components accounting for less than 10% of the applied radioactivity in each of the soils. The amount of danofloxacin transformed to minor metabolites was estimated as 22.3%, 35.2%, and 29.0% for sandy loam, clay loam and loam soils respectively. The estimated time to 50% transformation for sandy loam, clay loam and loam soils was 143, 91, and 110 days, respectively.

Report Summary: Sorption/Desorption of ^{14}C-Danofloxacin in Chicken and Cattle Manure

Study Number: SC930011

Test System: Cattle and chicken feces in contact with aqueous solution

Summary of Experimental Design: The same general procedure was used to conduct a screening test, a kinetics test and an isotherm determination. All tests were conducted in triplicate. Cattle feces were collected from four crossbred beef calves fed a nonmedicated ration of concentrate and corn silage. Fecal organic carbon was 52.2%. Chicken feces were collected from three-week old Hubbard-Peterson cockerel chicks who had been fed a 22% protein nonmedicated starter diet. Chicken feces organic carbon content was 42.7%. Feces were air dried and sieved through a 2.0 mm sieve prior to use.

For sorption studies, fecal samples were agitated in centrifuge bottles with solutions of radiolabeled ^{14}C-danofloxacin in 0.01 M aqueous calcium chloride or water. The ratio of feces to solution was 1:100. The concentration of danofloxacin remaining in the aqueous phase (C_e) at each sampling interval was determined by radioassay and the amount sorbed to feces (X) was calculated from the difference between the initial concentration in the aqueous phase and the measured concentration at the sampling interval. Sorption kinetics were measured at 2, 4, 8 and 24 hour intervals for the kinetics test. The agitation time for the isotherm test using four concentrations of ^{14}C-danofloxacin was 24 hours for cattle feces and 48 hours for chicken feces.

Desorption was measured by equilibrating fecal samples containing sorbed ^{14}C-danofloxacin with 0.01 M aqueous calcium chloride solution. For the screening test, fecal samples were equilibrated for 24 hours, the desorption supernatant decanted from the centrifuged samples, and feces equilibrated with fresh solution for another 24 hours. For desorption kinetics, concentrations of ^{14}C-danofloxacin in supernatants were determined at 4, 8, 24 and 48 hour intervals. A desorption equilibrium time of 48 hours was used in the isotherm test. ^{14}C-danofloxacin concentrations in all supernatants were determined by radioassay.

The sorption and desorption supernatants from the isotherm test were analyzed by HPLC to determine the stability of the test materials under the conditions of the test. A radioactivity mass balance was determined.

Calculations: The percent of ^{14}C-danofloxacin sorbed (%ADS) to the feces is calculated as:

$$\%\ ADS = \%\ Control\ (100) - \frac{DPM\ in\ Supernatant}{DPM\ in\ Control\ Solution} \times 100$$

where: DPM = disintegrations per minute ^{14}C-danofloxacin
Control = test article solution without manure

The percent ^{14}C-danofloxacin desorbed (% DES) is calculated as:

$$\%\ DES = \frac{DPM\ in\ Supernatant}{DPM\ in\ Dose\ Solution} \times 100$$

The percent of sorbed dose desorbed =

$$\frac{\%\ DES}{\%\ ADS} \times 100$$

Estimated distribution adsorption coefficients (K_d) were calculated as follows:

$$K_d = \frac{X/m}{C_e}$$

The Freundlich sorption constant was determined using the following equation:

$$X/m = KC_e^{1/n}$$

where X/m = the concentration of danofloxacin (µg) in feces (g) at equilibrium
C_e = the concentration of danofloxacin in solution at equilibrium (µg/g)
m = dry weight of feces (g)
K = Freundlich adsorption coefficient
n = degree of non-linearity of isotherm

The distribution coefficient based on organic carbon content (K_{oc}) is determined from the following equation:

$$K_{oc} = \frac{K}{\%\ \text{organic carbon}} \times 100$$

Summary of Results: In a screening test conducted to provide a semi-quantitative measure of sorption and desorption, danofloxacin readily sorbed to chicken and cattle feces, with 45.9% and 75.4% sorbed, respectively, at 1:100 feces:solution (danofloxacin in 0.01 M $CaCl_2$ solution) ratio. In the desorption step, 49.2% of the sorbed danofloxacin desorbed from the chicken feces into 0.01 M $CaCl_2$ and 19.8% desorbed from the cattle feces. Estimated distribution sorption ($K_{d\text{-}ads}$) and desorption ($K_{d\text{-}des}$) coefficients and the distribution coefficient relative to organic carbon (K_{oc}) in 0.01M $CaCl_2$ were as follows for chicken and cattle feces, respectively; $K_{d\text{-}ads}$: 80.02 and 291.40; $K_{d\text{-}des}$: 588.92 and 1166.39; $K_{oc\text{-}ads}$: 187.43 and 558.23; $K_{oc\text{-}des}$: 1379.53 and 2234.45.

The advanced test, conducted at a feces:solution ratio of 1:100 in 0.01 M $CaCl_2$, established sorption/desorption equilibrium kinetics and sorption isotherms, from which the Freundlich adsorption coefficient (K) and K_{oc} values were determined. Sorption equilibrium was reached in 24 hours and desorption equilibrium reached in 48 hours for both chicken and cattle feces. The sorption isotherms presented as Freundlich plots exhibited good linearity. The Freundlich adsorption coefficients for chicken and cattle feces were 138 and 541, respectively, with corresponding K_{oc} values of 323 and 1036 (Table 1). Compounds with K_{oc} values approaching 1000 are quite tightly bound to organic matter and are considered immobile, whereas those with K_{oc} values below 100 are moderately to highly mobile. The chicken and cattle feces K_{oc} values for danofloxacin would indicate a potential for low mobility and immobility, respectively.

The percent of radioactivity recovered was 92% of that applied for chicken feces and 93% for cattle (Table 1). HPLC profiles of sorption and desorption supernatants indicated that danofloxacin was stable in both feces during the course of the advanced test.

Table 1. Summary of experimentally determined value from the advanced test.

Feces Type	% ADS*	% DES*	ADS K	DES K	ADS 1/n	ADS r^2	ADS K_{oc}	% Recovery
Chicken	47	15	137.7	315.1	0.88	0.9995	323	92
Cattle	76	9	540.7	1072.0	0.86	0.9979	1036	93

* At feces:solution ratio of 1:100

Report Summary: Microbial Transformations of Danofloxacin

Test Species: Soil bacteria, fungi and yeasts

Summary of Experimental Design: Cultures of 72 typical soil microorganisms representing a diverse panel of bacteria, fungi and yeasts were incubated with 0.1-0.2 mg/ml danofloxacin mesylate substrate and samples withdrawn at 24, 72 and 144 or 168 hours. Control cultures lacking substrate were incubated and sampled in an identical fashion. Samples were centrifuged and aliquots of supernatants analyzed by HPLC for loss of danofloxacin and formation of metabolites. Ring labeled [2-^{14}C]-danofloxacin was used to assess degradation by one culture which rapidly depleted the substrate but which formed no metabolites detectable by the HPLC methods employed. [2-^{14}C]-danofloxacin was added to duplicate cultures of the fungus *Curvularia lunata*; flasks were connected by vacuum to KOH traps to capture $^{14}CO_2$. After 24 hours incubation, cultures were centrifuged and supernatants collected for HPLC analysis. Cell pellets were washed and sequentially extracted with acetone, acidified ethanol and 6 N HCl. Radioactivity of filtered extracts, culture supernatants and KOH trapping solutions was measured by liquid scintillation counting. Extracts were concentrated and examined for presence of metabolites by HPLC separation and fraction collections/^{14}C-liquid scintillation counting.

Summary of Results: Twelve organisms, representing eight different genera, biotransformed danofloxacin to metabolites detectable by the HPLC methods employed (Table 1). Two *Mycobacterium* species, two *Pseudomonas*, and a *Nocardia*, a *Rhizopus* and a *Streptomyces griseus* all formed a metabolite presumptively identified as N-desmethyldanofloxacin. The formation of the 7-amino danofloxacin derivative, 1-cyclopropyl-6-fluoro-7-amino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid by one *Candida*, one *Pseudomonas*, two *Mycobacterium* species and three *Penicillium* species demonstrates the propensities of these cultures to completely degrade the piperazine ring. At least two additional and unidentified metabolite peaks were observed in HPLC chromatograms of *Aspergillus nidulans* and *Penicillium sp.* extracts. Radiolabeled [2-^{14}C]-danofloxacin was apparently mineralized by *Curvularia lunata,* thus indicating the susceptibility of the quinolone ring to microbial metabolic degradation. After only 24 hours incubation, approximately 31% of the radiolabel was mineralized to $^{14}CO_2$ and was recovered in KOH trapping solutions, with the remainder of the radiolabel distributed among cell extracts (Table 2); total recovery of radiolabel was 99.6%. HPLC analysis suggested that the material extracted from cell pellets was undegraded danofloxacin. Results demonstrate the potential for danofloxacin to be transformed or degraded by normal, indigenous soil microorganisms.

Table 1. Cultures metabolizing Danofloxacin

Culture Number	Culture Name	Metabolite N-desmethyl danofloxacin	Metabolite 7-amino danofloxacin	Other
ATCC 24528	*Aspergillus nidulans*	-	-	+
NRRL 5699	*Candida lipolytica*	-	+	-
UI-AM-463	*Mycobacterium bisrymcum*	+	+	-
UI-AM-563	*Mycobacterium smegmatis*	+	+	-
NRRL 5646	*Nocardia sp.*	+	-	-
UI-X-251	*Penicillium chrysogenum*	-	+	-
UI-MR-70	*Penicillium frequentans*	-	+	-
ATCC 12556	*Penicillium sp.*	-	+	+
UI 60690	*Pseudomonas aeruginosa-var.*	+	-	-
UI AM-670	*Pseudomonas fluorescens*	+	+	-
ATCC 11145	*Rhizopus arrhizus*	+	-	-
ATCC 10137	*Streptomyces griseus*	+	-	-

Table 2. Radioactivity from *Curvularia lunata* NRRL 2178 cell extracts and KOH traps

FRACTION	RADIOACTIVITY (%)[a]
KOH-Trap ($^{14}CO_2$)	31.2
Acetone	12.6
EtOH-HCl	29.3
HCl	26.5

[a]Approximately 1 μCi of [2-^{14}C]-danofloxacin was added to each of two culture flasks. The values shown are the averages for each extract or fraction.

Report Summary: Effect of Danofloxacin Mesylate on Soil Microbes

Study Number: 2438-0189-6144-790

Test Species: Soil-dwelling microbes

Summary of Experimental Design: The lowest concentrations of danofloxacin mesylate that will inhibit the growth of pure cultures of representative soil bacteria, ascomycetes, fungi, and blue-green algae were determined by the agar plate dilution technique. The following organisms were used.

Clostridium perfringens, a free-living nitrogen-fixing bacterium

Nostoc, a blue-green alga

Pseudomonas aeruginosa, a soil bacterium

Chaetomium globosum, an ascomycete

Aspergillus flavus, a mold

Each of the above organisms was maintained in pure culture under conditions appropriate for the species. The following testing procedure was followed separately, in duplicate, for each of the five microbial species. A preliminary range-finding study was conducted at widely spaced concentrations, approximately 1,000, 100, 10, and 1 ppm. The results were used to select a geometric series of four closely spaced concentrations, for instance 0, 2, 4, 6, and 8 ppm. Each concentration was obtained by mixing 2 ml of a standard stock solution containing ten times the desired final concentration with 18 ml of molten agar, except that 2 ml of distilled water was mixed with agar to prepare the negative controls. The agar was then poured into a Petri dish, allowed to cool and solidify, inoculated with the organism, and incubated at an appropriate temperature. When colony growth was well developed on the plates which did not contain any drug, the plates containing danofloxacin mesylate were examined visually for microbial growth. The lowest concentration that completely inhibited growth was recorded as the minimum inhibitory concentration (MIC).

The entire set of experiments was repeated in the presence of a small amount of soil. The stock solution was first added to 2 g of sterilized California Sandy Loam Soil (see report on Soil Sorption for characterization) which was then mixed with the agar.

The drug sample tested in this study contained 94.03% danofloxacin mesylate. The balance consisted mostly of water (3.8%) and a small amount of ethanol (0.68%).

The following concentrations were tested:

Species	CP-76,136-27 concentration (ppm)	
	Without soil	With 0.1g soil/ml
Clostridium perfringens	0.2, 0.4, 0.6, 0.8	2, 4, 6, 8
Aspergillus flavus	20, 40, 60, 80	200, 400, 600, 800
Pseudomonas aeruginosa	2, 4, 6, 8	20, 40, 60, 80
Nostoc	0.2, 0.4, 0.6, 0.8	0.2, 0.4, 0.6, 0.8
Chaetomium globosum	2, 4, 6, 8	2, 4, 6, 8

Summary of Results:

The following MIC's were obtained:

	Growth Conditions			MIC (ppm)	
	Medium	Temperature	Other	Without soil	With soil 0.1 g/ml
C. perfringens	AC broth	36±1°C	Under Nitrogen	1.0[a]	6.0
A. flavus	Potato dextrose broth	24±1°C	--	80	600
P. aeruginosa	Difco nutrient broth	36±1°C	--	6.0	100[a]
Nostoc	Medium B6-11	25°C	2,000-3,000 foot candles	0.8	1.0[a]
C. globosum	Mineral salt broth	24±1°C	--	6.0	8.0

[a] These values are based on inhibition observed in the preliminary test.

Thus, relatively high concentrations of danofloxacin mesylate were required to inhibit soil microbes, and substantially higher concentrations were required to inhibit three of the five representative organisms in the presence of even a small amount of soil.

Report Summary: Danofloxacin: Anaerobic Digester Inhibition Test

Study Number: 260E-101

Test System: Anaerobic digester sludge

Summary of Experimental Design: Anaerobic digester sludge and primary sludge collected from a wastewater treatment plant were allocated to nitrogen-flushed test chambers. Stock solutions of danofloxacin mesylate in water were added to give nominal concentrations of 0.1, 1.0, 10 and 100 mg/L. Phenol was used as a reference substance and was added to reference test chambers at 250 and 1500 mg/L to demonstrate a no observed effect concentration and an observed effect concentration, respectively. A blank control group and a pressure control group lacking test substance were used to determine the background level of gas production and to calibrate the pressure meter, respectively. Each group contained three replicates. After dosing, test chambers were flushed with nitrogen, sealed with rubber septa, and incubated in the dark at 33±2°C. Gas production was measured with a pressure meter over 28 days. Cumulative gas production in the treatment vessels was compared with the gas production in the blank control vessels.

Summary of Results: No inhibition of gas production was observed with danofloxacin at 0.1 and 1.0 mg/L; at 10 and 100 mg/L gas production was inhibited by 5.6 and 10.7%, respectively. The EC_{50} for danofloxacin was greater than 100 mg/L, the highest concentration tested. The reference chemical phenol at 250 mg/L did not inhibit gas production whereas 1500 mg/L resulted in approximately 56% inhibition. An EC_{50} for danofloxacin of > 100 mg/L indicates that danofloxacin residues in manure from treated animals should not impact the metabolic activities of anaerobic microorganisms in manure processing or disposal sites.

Test Substance	Concentration (mg/L)	Mean Total Cumulative Gas Production (mL)	Standard Deviation	Percent Inhibition
Blank Control	N/A	324.7	5.5	N/A
Phenol	250	506.8	10.5	0
Phenol	1500	141.8	4.6	56.3
Danofloxacin	0.1	327.9	5.9	0
Danofloxacin	1.0	326.8	17.4	0
Danofloxacin	10	306.7	6.5	5.6
Danofloxacin	100	290.1	8.1	10.7

Report Summary: Subacute Toxicity Study with Danofloxacin Mesylate in Earthworms

Study Number: 2438-1088-6127-630

Test Species: Earthworms (*Lumbricus terrestris*)

Summary of Experimental Design: A slurry containing radiolabelled danofloxacin mesylate, 400 g of rabbit feces, and 800 ml of deionized water was prepared and mixed thoroughly with 8 kg of an artificial soil consisting of 70% by weight industrial sand, 20% kaolinite clay, and 10% sphagnum peat, to which had been added a commercial worm medium (<20%) and enough distilled water to provide a moisture content of about 25%. The intent, based on the results of a range-finding test, was to obtain a concentration of 1,000 ppm of danofloxacin mesylate in the final mixture. A mixture with the same composition minus the danofloxacin mesylate served as negative control. Each mixture was replicated four times. Ten mature earthworms were placed on the surface of each replicate. All replicates were maintained at 13 ± 2°C under fluorescent lights. Earthworm mortality and health were assessed after 7, 14, 21 and 28 days. The health assessment consisted of noting any abnormal behavior and appearance, such as lethargy, absence of burrowing, and softness. Worms were weighed on days 0 and 28. Triplicate aliquots from each replicate were assayed for danofloxacin mesylate by liquid scintillation counting at initiation and termination of the study.

Summary of Results: The mean measured concentration in the replicates containing danofloxacin mesylate was 1,200 ppm; assay values obtained at initiation and termination of the study were in acceptable agreement with each other:

	Measured Concentration (ppm)	
Replicate	Day 0	Day 28
1	970	1300
2	940	1300
3	1300	1500
4	870	1300
Mean of all assays:	1,200 ± 260	
Control (all aliquots)	<160	<54

Danofloxacin mesylate had no adverse effects on the earthworms even at this high concentration. The table below shows the percentage of the earthworms that survived in each replicate after 7, 14, 21, and 28 days of exposure, the mean live weights recorded on days zero and 28, and the percent weight gain calculated from the live weights. There were no statistically significant differences in survival or weight gain between exposed and control earthworms at the end of the study. Furthermore, no abnormal appearance or behavior was observed among the survivors at any time point. Based on these results, earthworms would have to be exposed to a concentration of danofloxacin mesylate in excess of 1,200 ppm to suffer a consequent 50% reduction in survival.

Report Summary: Effect of Danofloxacin Mesylate on Seed Germination and Root Elongation of Six Plant Species

Study Number: SC920086
PRT-12-5PFF-05-003

Test Species: Seeds of the following species were used:

Monocotyledons:
Lolium perenne - perennial ryegrass
Triticum aestivum- wheat
Zea mays - corn

Dicotyledons:
Cucumis sativus - cucumber
Glycine max - soybean
Phaseolus vulgaris - pinto bean

Summary of Experimental Design: The effects of danofloxacin on the germination and root elongation of six different seed species were evaluated. Corn, wheat, rye grass, cucumber, soybean, and pinto bean seeds (300 seeds [six replicates of 50 seeds each] per species per dose level) were incubated at 24±1°C for 3 to 7 days in the dark in buffered nutrient solutions containing danofloxacin. The No Observed Effect Concentration (NOEC) of danofloxacin was determined by means of a preliminary test and two definitive tests, differing in the concentrations of drug evaluated. The preliminary test employed logarithmically separate dose levels ranging from 1000 to 1 ppm; the definitive tests evaluated the drug over narrower ranges of geometrically spaced levels. The NOEC for seed germination was based on a statistical analysis of the percent germination. The NOEC for root elongation was based in statistical analysis of root length measurements made on 60 randomly selected seedlings (10/replicate) per treatment.

Summary of results: No morphological abnormalities nor adverse effects on germination were observed for any of the seed species, even at the highest concentration tested. Therefore, for all species, the NOEC for germination was determined to be 1000 ppm on the basis of the preliminary test (Table). Drug titrations were useful only in determining the danofloxacin concentrations affecting root elongation. Corn and soybean were the least sensitive species with NOECs for root elongation determined to be 1000 ppm and 400 ppm, respectively. Cucumber and rye grass were the most sensitive species tested relative to root elongation and NOECs were not determined (<0.032 and <0.125 ppm, respectively). For pinto bean and wheat, results from one study showed effects on root elongation at 100 and 2.5 ppm, respectively, whereas results from a second study showed no significant effects at 100 and 4 ppm, respectively, the highest concentrations tested. Based on the combined results of the two studies, the NOEC for pinto bean is therefore reported as 20 ppm and for wheat as 2 ppm (Table).

Species	% Germination NOEC (mg A.I./kg)	Root Elongation NOEC (mg A.I./kg)
Corn	1000	1000
Cucumber	1000	<0.032
Perennial ryegrass	1000	< 0.125
Soybean	1000	400
Pinto bean	1000	20
Wheat	1000	2

Report Summary: Effect of Danofloxacin on Seedling Growth of Six Plant Species

Study Number: PRT-13-2PFF-02-008
PRT-11-5PFF-04-004

Test Species: Seedlings of the following species were used:

Monocotyledons: *Lolium perenne* - Perennial rye grass
Triticum aestivum - wheat
Zea mays - corn

Dicotyledons: *Cucumis sativia* - cucumber
Glycine max - soybean
Lycopersicon esculentum - tomato

Summary of Experimental Design: Seedlings of 3 species of monocotyledons and 3 species of dicotyledons grown in characterized loam soil and/or quartz sand support medium were exposed to varying concentrations of danofloxacin over a 21 day period to determine morphological abnormalities, survival and effects on shoot length, shoot weight and root weight. A preliminary test employed drug concentrations ranging from 1000-1 ppm while the definitive test was conducted within narrower concentrations. Seedlings (5 replicas of 5 plants each, Study 2PFF-02-008; 21 replicas of 5 plants each, Study 5PFF-04-004) were exposed to drug in formulated nutrient solution. Studies were conducted in a greenhouse under regulated environmental conditions of temperature, humidity, CO_2 and photoperiod. Mortality and morphological abnormalities were recorded daily and shoot length was recorded on days 1, 3, 5, 7, 14 and 21. Shoot and root weights were recorded on day 21.

Data (seedling shoot length and weight and root weight) were analyzed for variance as a completely randomized design. Data for each length measurement date were analyzed separately. A separate analysis was conducted for the sand and soil growth media. When significant effects of treatment were detected, treatment means were compared with relevant controls using Dunnetts' comparison. Heterogenicity of error variance was tested using Bartlett's test. If no significant treatment effects were detected, a Pearson-Hartley power statistic for the F test was calculated. No observable effect levels (NOELs) were determined on the basis of the definitive test.

Summary of Results: Mortality or desiccation when present occurred with equal frequency in control and treated groups and reflected transplantation shock. Mottling was observed on the upper leaves of some plants exposed to higher drug concentrations, particularly in the preliminary test. Mottling was observed more frequently among plants grown in quartz sand compared to soil. NOEL values are presented in the table below. A comparison of these values indicated that soil mitigated the concentration effects observed in the corresponding sand treatments for three of the four species tested in both support media.

Definitive Test NOEL Determinations
(ppm Danofloxacin mesylate)

	Sand	Soil
Perennial ryegrass	83	>150
Wheat	46	46
Corn	26	>150
Soybean	2.9	ND[1]
Cucumber	1.6	ND[1]
Tomato	83	>150

[1] Not Determined

Livestock Waste Management

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Chapter 3

LIVESTOCK PRODUCTION UNITS, WASTE COLLECTION, AND WASTE CHARACTERISTICS

I. BEEF PRODUCTION

Beef production in the U.S. is a highly specialized and sophisticated industry in which animals, feed, water, technology, and management are brought together at the location where meat output can be maximized. Although there is a limited amount of vertical integration most of the production operations are conducted by independent producers whose activities are facilitated by separate marketing and transport organizations. Thus, cattle may be born and raised to 600 lb in one state or region, shipped to another for fattening, and again shipped to a third location for slaughter.

Feeder cattle, 500 to 700 lb, are typically purchased by a feedlot operator and placed on a high energy feed for 100 to 180 days in a feedlot. After feeding to 1000 to 1200 lb depending upon the market, they are slaughtered. Feeder cattle production is commonly in a cow-calf operation which is land extensive.

Beef cattle production is regionally distributed, Table 1. The west Northcentral and Southcentral account for over 50% of all cattle produced and reflect the proximity to feed sources. As disease control and mass handling techniques have improved, there has been a trend towards larger feedlot operations, Table 2. This change in production unit size has occurred while total number of beef cattle in production has remained about constant, Table 3.

Waste management for beef cattle production must be planned in response to one of three general production schemes. Cattle are raised on range-pasture in which most or all of the feed requirement is provided by existing vegetation; cattle are also fed and finished on unroofed feedlots in which the feed is all grown outside the animal pens. The third option is roofed confinement in which the space allocated per animal is reduced, a flooring material is provided, and rainfall is not a constituent of the waste requiring management. Confinement systems may be open sided in which no attempt is made to modify the temperature from building, or enclosed to obtain a higher level of temperature modification.

A. Range and Pasture Production

Approximately 75% of the beef cattle in the the U.S. is in unconfined systems, Figure 1. In 1971 this represented approximately one half million production units and 81 million animals. About 40% of the land area of the U.S. is used for grazing livestock. Stocking rates are most generally selected to maintain a sustained yield of forage. Hence stocking rates are dependent upon local conditions including soil type, terrain, and climates. Typically between 0.05 and 5 animals (1 animal/20 acres) can be sustained per acre of pasture. Larger stocking rates are utilized for irrigated pasture and for pastures used in rotational grazing.

B. Feedlot Production

As indicated in Table 3 approximately one half the steers and heifers over 500 lb are on feedlots. Since 1962, there has been a 50% reduction in the number of feedlots even though the number of feedlot finished cattle has increased slightly. The decrease in feedlot numbers has been in smaller lots with a capacity of less than 1000 head. This decrease in production capacity has been offset by the construction of larger lots.