DANOFLOXACIN

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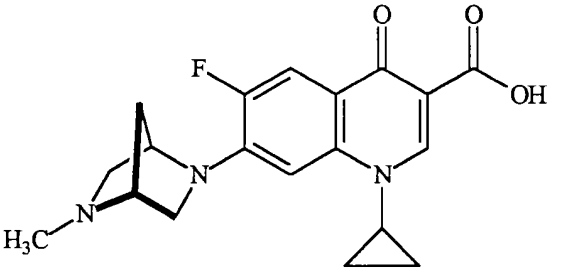
IDENTITY

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-quinoline carboxylic acid methanesulphonate.

Synonyms: Danofloxacin mesylate, Advocin ®, CP-76,136-27

Structural formula:



Molecular formula: Danofloxacin - $C_{19}H_{20}FN_3O_3$

Danofloxacin mesylate - $C_{19}H_{20}FN_3O_3 \cdot CH_4O_3S$

Molecular weight: Danofloxacin 357.39; Danofloxacin mesylate 453.49

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Danofloxacin

Appearance: White to off-white crytalline powder

Melting point: Danofloxacin 263°C
Danofloxacin mesylate 328°C

Solubility (g/l) of Danofloxacin:

water (172-205)	hexane (<0.1)
acetic acid (90-500)	methanol (1-10)
acetone (<0.1)	methylene chloride (<0.1)
DMSO (10-33)	tetrahydrofuran (<0.1)
ethanol (<0.1)	trifluoroacetic acid (90-500)

Optical Rotation: Danofloxacin 1S,4S (desired) enantiomer: +197°

UVmax: 282 nm (acid), 278 nm (base)

Acid-base stability: No significant degradation following four weeks storage at 50°C in either 1.0M HCl or 1.0M NaOH.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Danofloxacin is a synthetic fluoroquinolone with broad spectrum antibacterial activity. It is used in the treatment of respiratory disease in chickens, cattle and pigs. Danofloxacin is not intended for use in dairy cattle producing milk for human consumption and not in laying hens.

Dosage

In chickens, the drug is administered dissolved in the drinking water, with the recommended therapeutic regimens being 5 mg/kg BW for 3 days for treatment of *E. coli* respiratory disease and Chronic Complicated Respiratory Disease and 50 mg/kg BW for 3 days in treating mycoplasmosis of chicks less than 1 week of age. For the treatment of respiratory disease in cattle and swine, the drug is administered by intramuscular (im) or subcutaneous (sc) injection at 1.25 mg/kg BW for 3-5 days.

METABOLISM

Pharmacokinetics

Cattle

Plasma: Danofloxacin was administered at 1.25 mg/kg BW for 5 days by either im or sc routes and for one day only by the iv route to calves at an average starting weight of 112 kg. Plasma danofloxacin concentrations were measured over 24 hours after treatment on days 1, 3 and 5. There was bioequivalence between the routes of administration. The half lives in the plasma ranged from 3.9 to 4.4 h. After im and sc administration the peak plasma concentration was reached at 0.8 - 1.3 h with the maximum concentration of 0.39 - 0.48 mg/l (Pfizer Inc, 1990b, 1991b).

Excreta: Five steers and four heifers (mean BW 186 kg) were each injected once daily for five consecutive days with a 2.5% aqueous solution of ^{3}H-danofloxacin at the recommended dose of 1.25 mg/kg BW. The total concentration of drug-related material in the excreta reached a plateau by the third day of treatment; it declined to a low level (ca. 6 µg/kg) by the second day after the last treatment.

Approximately equal amounts of drug-related material were excreted in urine and faeces. About 54-58% of drug-related material in faeces collected during days 4 and 5 consisted of unchanged danofloxacin. The desmethyl metabolite was also detected, but its concentration was too low to be measured quantitatively. The balance consisted of trace amounts of polar materials. In urine, unchanged drug accounted for 88-94% of the radioactivity, while the desmethyl metabolite accounted for the remainder (Pfizer Inc, 1989a).

Pigs

Male and female pigs (ca. 40 kg) were administered im a single dose of 1.25 mg/kg BW. Peak danofloxacin concentrations in plasma and lung at one hour post-dosing were 0.40 and 1.68 mg/l, respectively with a half-life of 7 h in plasma (Pfizer Inc, 1991c). During the treatment period the excretion into urine was 2 - 3 times higher than that in faeces.

Chickens

Plasma and Lung: Danofloxacin was administered to 18-day old broilers in the drinking water at a dose equivalent to 5 mg/kg BW for 3 days. Twelve hours after treatment initiation, steady-state concentrations of 0.21 mg/l and 0.43 mg/kg Danofloxacin were attained in plasma and lung tissue, respectively. Desmethyldanofloxacin was not detected. After treatment withdrawal, the concentration of danofloxacin in both plasma and lung tissue declined with half lives of ca. 5-6 hours (Pfizer Inc, 1990a).

Excreta: Broilers were given ^{3}H-danofloxacin (5 mg/kg BW) for five days in their drinking water. Overall mean radioactivity ranged from 35 to 46.5 mg/kg for the five days of treatment, and attained an apparent steady state by the first day of treatment. The concentration dropped to 20 mg/kg on the first withdrawal day and to 6.6 mg/kg on the second day. Assays of samples on treatment days 2 to 5 and withdrawal day 2 indicated that the levels of unchanged danofloxacin in excreta were approximately 85% of the total. Excretion was not different between the males and females (Pfizer Inc, 1988a, Pfizer Inc, 1991a).

Metabolism in Animals

The biotransformation of danofloxacin was investigated in rats, dogs, chickens, cattle and pigs. A tritium label was introduced into the danofloxacin molecule at the C-1 position of the cyclopropyl ring. The radiochemical purity of the material used was >99% and it was shown to be metabolically stable as less than 0.1% of radioactivity excreted in the urine of dosed rats was tritiated water.

Danofloxacin was found to be metabolized in a quantitatively similar manner in rats, dogs, and swine along pathways predictable from studies reported for other quinolones. Metabolism in cattle and chickens is also similar to the other species examined. The metabolic pathways are shown in Figure 1. The major component in the excreta of each species was unchanged drug, and in rats and dogs at least, elimination appeared to be by both renal and hepatic routes. Very small quantities of N-desmethyldanofloxacin are detected in the excreta of all species. The N-oxide was detected as a urinary metabolite in dogs (ca. 26%) and swine (ca. 12%) and to a lesser extent in rats (ca. 5%). A glucuronide conjugate was also present in small quantities in the urine of dogs (ca. 6%), rats (2-3%) and swine (2-3%).

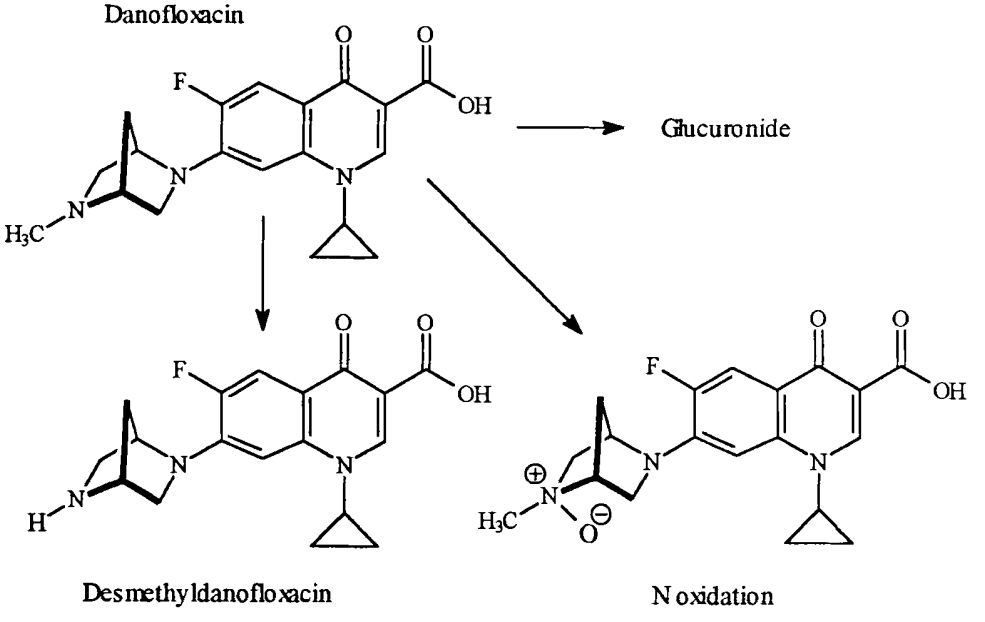


Figure 1. Metabolism of Danofloxacin

In cattle, pigs and chickens the tissue which was shown to have the highest residue was the liver. The liver was shown to contain danofloxacin as a major drug-related component and the only metabolite detected at more than 10% of the total residue was desmethyldanofloxacin (see Table 1). In swine, the desmethyl metabolite comprised the majority of drug-related residues (>90%) by 48 hours post-dose and essentially all of the detectable residues by 6 or more days post-dose. Traces of other components could not be identified or quantitated (Pfizer Inc, 1990c, 1991d, 1991e, 1990d and 1990e).

A comparative excretion study in mice, in which animals received oral doses of either danofloxacin or desmethyl-danofloxacin, showed that mice treated with danofloxacin were also systemically exposed to the major metabolite (Pfizer Inc, 1990f).

Table 1. Comparative metabolism profiles in chickens, cattle, swine, dogs, and rats obtained from liver samples, including recovery data and relative percent of metabolites expressed as Danofloxacin or Desmethyldanofloxacin

	Chicken		Cattle		Swine		Dog		Rat	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
TR (µg/kg)	NA	NA	0.82	1.1	0.95	0.96	6.6	5.3	2.3	2.5
% ^{3}H extracted[a]	81	100	100	90	82	80	100	93	78	81
HPLC recovery[b]	99	95	90	94	100	100	100	99	93	95
% Df[c]	78	74	24	29	58	68	66	58	67	70
% Des[c]	16	12	43	42	23	29	28	37	18	11

a Represents % of total ^{3}H-containing residues; b Represents % radiolabel recovered in HPLC eluent vs radiolabel injected; c Represents % radiolabel relative to radioactivity recovered from HPLC column

Dose: Chicken: 5 mg/kg BW/day in drinking water to broiler chickens for five days.
Cattle: 1.25 mg/kg BW/day by daily im injections over a five day period.
Pigs: 1.25 mg/kg BW/day by daily im injections over a five day period.
Dog: 2.4 mg/kg BW/day orally over a five day period.
Rat: 6.25 mg/kg BW/day orally over a five day period.

All animals were sacrificed 3 - 12 h after the last dose.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Cattle

^{3}H-Danofloxacin was administered to mature cattle by once daily im injection at 1.25 mg/kg BW for 5 successive days. Total, unchanged danofloxacin and desmethyldanofloxacin residues were determined in edible tissues collected at 12, 24, 36, (1st study, Pfizer Inc, 1989c & 1990j) 48, 60 and 72 hours (2nd study, Pfizer Inc, 1990g) after the last treatment. Concentrations of total radiolabeled residues are given in Table 2.

The liver samples were extracted under acidic conditions and further analysed by using HPLC for the content of danofloxacin and desmethyldanofloxacin. The results are shown in Table 3.

Table 2. Concentration ranges and half lives of total residues in various tissues of cattle at specified periods following im injection with Danofloxacin (1.25 mg/kg BW) for five consecutive days

WT (hours)	Total Residues as Danofloxacin Equivalents (µg/kg)				
	Muscle	Liver	Kidney	Fat	Injection site
12	99-125	652-1047	410-555	11-12	361-690
24	20-55	385-635	98-437	4-26	61-85
36	10-14	155-275	48-62	3-11	26-71
48	9-11	149-220	31-40	2-3	10-15
60	7-10	165-199	24-31	1-2	9-13
72	8-9	171-201	22-32	2-2	10-12
Half life (hours)	17	26	14	-	11

Each range is for 3 animals; WT = Withdrawal time

Table 3. Concentrations (µg/kg) of Danofloxacin (Df) and Desmethyldanofloxacin (Des) in livers of cattle at specific periods following intramuscular treatment with Danofloxacin (1.25 mg/kg) for five consecutive days

WT (hours)	Residues			% of Total Residues		
	Total	Df	Des	Df	Des	Df + Des
12	892	220 (288)	360 (470)	25	40	65
24	499	104 (171)	138 (175)	21	28	49
36	218	47 (52)	49 (65)	22	22	44
48	182	50 (77)	36 (48)	26	19	45
60	182	59 (68)	30 (34)	32	16	48
72	186	46 (50)	26 (29)	25	14	39

Values in parentheses are the maximum value for 3 animals per time point; WT = Withdrawal time.

The highest concentrations of residues, as measured by radioactivity, were consistently found in the liver. Total residues in kidney, muscle, fat and at the injection site (IS) declined faster than in liver. Analysis of the liver for danofloxacin and its major metabolite showed that whereas the parent drug comprised about 21-32% of the labeled residue, the metabolite proportion declined gradually from 40% at 12 hours to 14% at 72 hours. At 48 h after dosing the residue concentrations in the injection site muscle were similar to those in muscle (Pfizer Inc, 1989c and 1990g).

The depletion of danofloxacin residues at higher dose levels in cattle was investigated by administering danofloxacin as a single subcutaneous (sc) injection at 4, 6 and 8 mg/kg BW. Total, unchanged danofloxacin and desmethyl metabolite residues were determined in the livers and injection sites collected at 8 and 12 hours after treatment. The highest concentrations were at 8 h with the higher dose (mean total residues were 8768 µg/kg in liver and 138183 µg/kg at injection site) and the lowest concentrations at 12 h with the lower dose (mean total residues were 2146 µg/kg in liver and 1765 µg/kg at injection site) (Pfizer Inc, 1996a).

Pigs

^{3}H-Danofloxacin was administered to mature pigs by once daily im injection at 1.25 mg/kg for 5 successive days. Total, unchanged danofloxacin and desmethyl metabolite residues were determined in edible tissues collected at 12, 24, 48 and 168 hours after the last treatment. The results for the total residues are shown in Table 4.

The highest concentrations of radioactive substances were found in the liver at 12 hours, and this tissue continued to have the highest radioactive content for up to 7 days post-dosing (Pfizer Inc, 1989d). The rate of decline of radioactivity in tissues including the injection site was characterised by half lives of 22-24 h for muscle, fat and injection site, 41 h for kidney, and 72 h for liver.

Table 4. **The range of concentrations (µg/kg) of total residues in various tissues of pigs following intramuscular treatment with Danofloxacin (1.25 mg/kg) for five consecutive days**

WT (hours)	Mean Total Residues as Danofloxacin Equivalents (µg/kg)				
	Muscle	Liver	Kidney	Fat	Injection site
12	324-366	960-1021	824-991	35-125	261-326
24	112-125	406-744	246-394	19-81	96-106
48	23-45	325-462	64-135	11-27	19-43
168	<2	70-338	4-6	<4	<2

Each range is for 3 animals; WT = Withdrawal time.

The content of danofloxacin and desmethyldanofloxacin in the pooled liver samples were measured by an HPLC assay which used acetonitrile as the extractant. The results (see Table 5) show that at 12 h both compounds were present in equal amounts but at later times the desmethyldanofloxacin was the more abundant residue. Although no acidification was used in this assay the values for the summed percentages of both compounds are close to 100% because the standard curve was made in similar liver homogenates.

Table 5. **The residues of Danofloxacin (Df) and Desmethyldanofloxacin (Des) as percentages of total ^{3}H-residues in liver of pigs following intramuscular treatment with Danofloxacin (1.25 mg/kg) for five consecutive days**

Withdrawal time (hours)	% of Total Residues		
	Df	Des	DF + Des
12	51	49	100
24	27	67	94
48	18	89	107
168	NC	92	>92

NC = Not calculated as danofloxacin concentrations were below the limit of quantification

Chickens

^{3}H-Danofloxacin was administered in drinking water to three-week-old chickens to achieve a targeted dose of 5.0 mg danofloxacin/kg BW/day for 5 days. Birds were sacrificed at 6, 12, 24 and 48 hours after withdrawal of the treatment and the total residues in their edible tissues were measured. Results are presented in Table 6.

Table 6. **Ranges of concentrations (µg/kg) of total residues in tissues of chickens following oral administration of Danofloxacin (5 mg/kg BW) in drinking water for five consecutive days**

WT (hours)	Number of birds	Mean Total Residues as Danofloxacin Equivalents (µg/kg)			
		Muscle	Liver	Kidney	Skin/Fat
6	6	61-155	457-850	291-641	32-78
12	6	24-48	174-428	108-182	24-81
24	6	9-14	84-148	38-74	18-47
48	5	2-4	29-81	16-24	9-12
Half life (hours)		9	13	11	18

WT = Withdrawal time

These findings demonstrated that the highest concentration of total residues was consistently found in the liver and that total residues in kidney and muscle declined at the same rate as in liver.

Pooled liver homogenates prepared from birds in the above study were also assayed by an HPLC method for unchanged danofloxacin and desmethyldanofloxacin. The results are shown as percentages of the total residues in Table 7. Danofloxacin was the residue of greatest concentration and desmethyldanofloxacin was the major metabolite (Pfizer Inc, 1988b).

Table 7. Concentrations (µg/kg) of Danofloxacin (Df) and Desmethyldanofloxacin (Des) in liver of chickens at specified periods following oral administration of Danofloxacin (5 mg/kg) in drinking water for five consecutive days

Withdrawal time (hours)	Number of birds	Total Residues (µg/kg)	% of Total Residues in Pooled Liver Homogenates		
			Df	Des	Df + Des
6	6	602	61	17	78
12	6	277	60	20	80
24	6	102	52	14	66
48	5	75	47	<13	<60

Value preceded by " < " indicates values below the limit of assay sensitivity.

Total residues were also determined in one-day-old chickens medicated for 3 days with ^{3}H-danofloxacin at 44 mg/l in the drinking water which was equivalent to approximately 28-48 mg/kg BW. This corresponds to an anti-mycoplasma therapeutic programme of 500 mg/l in the drinking water, which is limited to birds of less than one week of age. Mean total residues in liver, kidney, muscle and fat/skin declined from 2260, 1380, 600 and 430 µg/kg at 6 hours to 430, 19, 5 and 10 µg/kg by 14 days (Pfizer Inc, 1990i).

Other Depletion Studies (with Unlabeled Drug)

Cattle

The depletion of danofloxacin from cattle tissues was evaluated where five groups of six cattle each received a single daily im dose of danofloxacin at 1.25 mg/kg for five successive days. Muscle, liver, kidney, fat and injection site samples were obtained at 12, 36, 60, 84 and 120 hours after the last injection and were analyzed after an acid hydrolysis step by HPLC with fluorescence detection for the presence of danofloxacin and its major metabolite, desmethyldanofloxacin. These findings demonstrate that the residues deplete at rates comparable to those of the total residues. At 5 days withdrawal, danofloxacin was measurable only in the liver and the metabolite was below the assay sensitivity (10 µg/kg) in all tissues (Pfizer Inc, 1991f). The results are shown in Table 8.

Table 8. Concentrations (µg/kg) of danofloxacin and desmethyldanofloxacin in various tissues of cattle at specified periods following intramuscular treatment with danofloxacin (1.25 mg/kg) for 5 consecutive days

WT (h)	Mean Residue Concentrations (µg/kg)									
	Danofloxacin					Desmethyldanofloxacin				
	Muscle	Liver	Kidney	Fat	IS	Muscle	Liver	Kidney	Fat	IS
12	121	372	426	90	669	18	498	96	<5	30
36	24	67	32	<13	31	<10	59	<10	<5	<10
60	<10	30	22	<13	<10	<10	59	<10	<5	<10
84	<10	22	13	<13	<10	<10	12	<5	<13	<10
120	<10	13	5	<13	<10	<10	<10	<5	<13	<10
Half life (h)	-	24	19	-	-	-	20	-	-	-

WT = Withdrawal time; IS = Injection Site

Pigs

The depletion of danofloxacin from swine tissues was evaluated where five groups of four pigs each received a single daily intramuscular dose of danofloxacin at 1.25 mg/kg BW for three successive days. Muscle, liver, kidney, fat and injection site samples were obtained at 2, 6, 10, 14 and 18 days after the last injection and were analyzed after an acid hydrolysis step by HPLC with fluorescence detection for the presence of danofloxacin and its major metabolite, desmethyldanofloxacin. The concentrations of danofloxacin (see Table 9) declined rapidly in all tissues, so that by the first withdrawal interval (2 days) danofloxacin was present in only very low concentrations (<40 µg/kg). By 6 days post-treatment, danofloxacin was not detectable in any tissue. The desmethyldanofloxacin was present only in the liver and the concentrations declined from a maximum at 1065 µg/kg at 2 days to <80 µg/kg at 18 days post-treatment (Pfizer Inc, 1991g).

Table 9. Concentrations (µg/kg) of Danofloxacin and Desmethyldanofloxacin in tissues of swine following intramuscular treatment with Danofloxacin (1.25 mg/kg BW) for 3 consecutive days

WT (days)	Mean Residue Concentrations (µg/kg)									
	Danofloxacin					Desmethyldanofloxacin				
	Muscle	Liver	Kidney	Fat	IS	Muscle	Liver	Kidney	Fat	IS
2	15	27	36	<5	17	<10	622	<10	<5	<10
6	<10	<10	<5	<5	<10	<10	221	<5	<5	<10
10	<10	<10	<5	<5	<10	<10	184	<5	<5	<10
14	<10	<10	<5	<5	<10	<10	156	<5	<5	<10
18	<10	<10	<5	<5	<10	<10	79	<5	<5	<10

WT = Withdrawal time; Four (4) swines at each time points

Chickens

The depletion of danofloxacin from chicken tissues was evaluated where danofloxacin mesylate soluble powder was administered in drinking water to three-week-old chickens at 5.0 mg danofloxacin/kg BW/day for 3 days, according to label directions. Muscle, liver and skin/fat samples were collected at 6, 12, 18, 24 and 36 hours after withdrawal of the treatment from 3 birds/sex and were analyzed after acid hydrolysis by HPLC with fluorometric detection for the presence of danofloxacin and its major metabolite, desmethyldanofloxacin. The findings (see Table 10) show that danofloxacin and desmethyldanofloxacin depleted rapidly from chicken tissues following drug withdrawal with liver always containing the highest concentrations. Both components declined to less than 50 µg/kg at 36 hours withdrawal. All other tissues contained lower concentrations of danofloxacin than did the liver, while the metabolite was undetected (Pfizer Inc, 1990h).

Table 10. Concentrations (µg/kg) of Danofloxacin and Desmethyldanofloxacin in tissues of chickens following oral administration with Danofloxacin (5 mg/kg bw) in drinking water for five consecutive days

WT (hours)	Mean Residue Concentrations (µg/kg)					
	Danofloxacin			Desmethyldanofloxacin		
	Muscle	Liver	Skin/Fat	Muscle	Liver	Skin/Fat
6	63	221	134	<25	68	<25
12	31	150	85	<25	22	<25
18	<25	98	56	<25	28	<25
24	<25	98	<25	<25	34	<25
36	<25	41	<25	<25	<10	<25

Values preceded by " < " indicate the limit of assay sensitivity

Another residue study was reported in which approximate 30-day-old chickens were given danofloxacin in the drinking water at either 5.2 mg/kg bw for 3 days or 5.1 mg/kg bw for 5 days (Stracciari, 1991). The compounds were assayed with HPLC after an acid hydrolysis and solvent extraction procedure. The results are shown in Table 11.

Table 11. Concentrations (µg/kg) of Danofloxacin and Desmethyldanofloxacin in tissues of chickens following oral administration with Danofloxacin (5 mg/kg) in drinking water

WT (hours)	Mean Residue Concentrations (µg/kg)							
	Danofloxacin				Desmethyldanofloxacin			
	Muscle	Liver	Kidney	Skin/Fat	Muscle	Liver	Kidney	Skin/Fat
0	96	952	773	52	<10	195	62	<10
24	11	231	100	12	<10	29	<10	<10
48	<10	38	16	<10	<10	<10	<10	<10
72	<10	<10	<10	<10	<10	<10	<10	<10

WT = Withdawal time; Each value is the mean for 6 birds.

Bound Residues/Bioavailability

The extraction of ^{3}H-labeled residues from the tissues of cattle, chickens and pigs administered ^{3}H-danofloxacin was carried out using two differing sets of conditions. There was an initial extraction using only organic solvents (mild extraction) and a second stage where the tissues were subjected to either acid or alkaline conditions for further organic solvent extraction. After solvent extraction only part of the total radioactivity was extracted indicating there was evidence of binding of a considerable fraction of the radiolabeled residues. However after acid hydrolysis (or alkaline) most of the radioactivity was released (see Table 12). The percentage of the residues which were not liberated by the mild extraction procedure increased with increasing withdrawal time, however, the amount of total residues were declining with time (Pfizer Inc, 1988b, Pfizer Inc, 1989c, Pfizer Inc 1989d).

Table 12. The extraction of ^{3}H-labeled residues from animal liver after administration of ^{3}H-Danofloxacin

Species	WT (hours)	TR (µg/kg)	% of TR Extracted			
			With organic solvents		After hydrolysis	
			Free	Bound	Free	Bound
Cattle	12	892	43	47	85	9
	24	499	35	61	99	13
	36	218	31	66	103	20
Chickens	6	612	65	32	96	6
	48	56	28	50	99	19
Pigs	12	987	67	23	82	6
	48	408	58	34	75	8
	168	178	36-43*	47-55*	72	8-19*

Organic solvents were sequentially; methanol, acetonitrile, ethylacetate, n-hexane
Hydrolysis with 1N HCl at 50°C for 1 hour
*Range for 3 pigs

The specific residues of danofloxacin and desmethyldanofloxacin were measured in the radiolabeled studies (see above) and indicate that after hydrolysis the sum of these two compounds represented about half the free residues in bovine liver and 50-80% in chicken liver. The results for the pig study (see Table 5) are not appropriate.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General. The methods outlined below are those submitted by the sponsor as candidate regulatory methods. For routine monitoring purposes danofloxacin concentration at the µg/kg level in cattle and poultry tissue was determined by a liquid chromatography with fluorescence detection following an extraction using acid hydrolysis and solvent partition from tissue homogenates or fat. The method is a refinement of the methods used in the studies to obtain the data for the content of residues in the target animals. In those studies, they were either carried out without recourse to an acid hydrolysis step (e.g Pfizer Inc, 1989c) or in subsequent studies there were minor variations e.g. the acid hydrolysis was done at 50°C for 1 hour (e.g. Pfizer Inc, 1990g). A method to confirm the presence of danofloxacin in liver tissues used HPLC combined with mass-spectrometry.

Liquid Chromatography-Fluorescence Detection. Liver, kidney, muscle, fat, and injection site muscle samples were extracted following homogenization in the presence of extraction solvent, then incubated at 55°C for 90 minutes. The samples were cooled to room temperature, centrifuged, and a sample of supernate taken for analysis. Separation was achieved on a Inertsil C8 reverse phase LC system using a combination of phosphate buffer (0.05M, pH 3.5) and acetonitrile (12%). Detection was achieved with fluorescence excitation at 280 nm, while monitoring emission at 440 nm. The assay was quantitated using external standards. The assay has a limit of quantification (LOQ) of 10 µg/kg and was linear over the range 10-500 µg/kg in liver, muscle, kidney, fat, and injection site muscle. Recovery of danofloxacin from all tissues was near 100%. The accuracy of the method was between 99-103% with the precision, expressed as the coefficient of variation, 2% or less. The method demonstrated chromatographic resolution from other fluoroquinolones including, desmethyldanofloxacin, enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, sarafloxacin, marbofloxacin, and sparfloxacin (Pfizer Inc, 1995a and 1996b).

Liquid Chromatography-Mass Spectrometry of Liver Tissues. Liver samples were extracted following homogenization in the presence of extraction solvent, then incubated at 55°C for 90 minutes. The samples were cooled to room temperature, centrifuged, and a sample of supernate was subjected to liquid-liquid extraction. The residue from the liquid-liquid extraction was dissolved in the mobile phase at a concentration of 20 µg/L. Separation was achieved on a Hypersil BDS C18 reverse phase LC system using a combination of acetonitrile and 0.1% trifluoroacetic acid in a 8/2 (v/v) mixture. Detection was achieved with a triple quadrupole mass spectrometer equipped with an ion spray interface. Daughter ions (m/z 225 and 340) of the danofloxacin generated by collision induced dissociation (CID) of the parent ion, m/z 358, were monitored for quantitation (LOQ 50 µg/kg). No interferences were found when enrofloxacin and ciprofloxacin were analysed by this method (Schneider et al, 1993).

APPRAISAL

Danofloxacin is a fluoroquinolone with wide-spectrum antibacterial activity. It is used in the therapeutic treatment of respiratory disease in chickens, cattle and pigs.

The drug is well absorbed when administered parentally or orally. Both the parent drug as the major component and desmethyldanofloxacin in very small quantities were excreted in the faeces and the urine.

Danofloxacin was found to be metabolized in a quantitatively similar manner in rats, dogs, cattle, chickens and pigs along pathways predictable from studies reported for other quinolones. The parent drug, danofloxacin comprised a majority of the residues found in edible tissues following treatment of chickens, cattle and swine. The metabolite desmethyldanofloxacin was a major residue in the livers of the three species. No other metabolite was present in significant quantities.

Residue depletion studies using ^{3}H-labeled drug and unlabelled drug were carried out in cattle, pigs and chickens using the doses recommended for normal therapeutic treatment.

Cattle. ^{3}H-Danofloxacin was administered to mature cattle once daily by i.m. injection at 1.25 mg/kg body.weight (BW) for 5 successive days. Total residues, unchanged danofloxacin and desmethyl metabolite were determined in edible tissues collected at 12, 24, 36, 48, 60 and 72 h after the last treatment. The highest concentrations of total residues (ca. 1000 µg/kg at 12 h after treatment and decreasing to ca. 200 µg/kg at 72 h with a half life $t_{1/2}$ of approximately 26 h) as measured by radioactivity, were found in the liver. Total residues depleted more rapidly in the other tissues where the half lives were; kidney, 14 h, muscle,17 h and at the injection site (IS), 11 h. Analysis of

the liver for danofloxacin and desmethyldanofloxacin showed that whereas the parent drug comprised about 21-32% of the labeled residue, the metabolite proportion declined gradually from 40% at 12 hours to 14% at 72 h. At 48 h after dosing the total residue concentrations in the injection site muscle were similar to those in muscle.

The depletion of unlabeled danofloxacin from cattle tissues was evaluated where five groups of six cattle each received a single daily im dose of danofloxacin at 1.25 mg/kg for five successive days. Muscle, liver, kidney, fat and injection site samples were obtained at 12, 36, 60, 84 and 120 h after the last injection and were analyzed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. The residues depleted at rates comparable to those of the total residues. At 5 days withdrawal, danofloxacin was measurable only in the liver (13 µg/kg) and kidney (5 µg/kg), and the level of the metabolite was below the assay quantification limit (10 µg/kg) in all tissues.

Pigs. The depletion of ^{3}H-danofloxacin from pig tissues was evaluated where five groups of four pigs each received a single daily intramuscular dose of danofloxacin at 1.25 mg/kg BW for five successive days. Muscle, liver, kidney, fat and injection site samples were obtained at 12, 24, 48, and 168 hours after the last injection and were analyzed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. The highest concentrations of radioactive substances were found in the liver (960 - 1021 µg/kg) and kidney (824 - 991 µg/kg) at 12 h, and these tissues continued to have the highest radioactive content for up to 7 days post-dosing. The depletion of radioactivity in tissues including the injection site was characterised by half lives of 22-24 h for muscle, fat and injection site, 41 h for kidney, and 72 h for liver. The contents of danofloxacin and desmethyldanofloxacin in the pooled liver samples were measured by an HPLC assay. At 12 h both compounds were present in equal amounts but at later times (>48 h) the desmethyldanofloxacin was the more abundant (about 90%) residue.

The depletion of unlabeled danofloxacin was evaluated where five groups of four pigs each received a single daily intramuscular dose of danofloxacin at 1.25 mg/kg bw for three successive days. Muscle, liver, kidney, fat and injection site samples were obtained at 2, 6, 10, 14 and 18 days after the last injection and were analyzed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. The concentrations of danofloxacin depleted rapidly in all tissues, so that by the first sampling period at 2 days post dosing, danofloxacin was present in only very low concentrations (<40 µg/kg) and by 6 days post treatment, danofloxacin was not detectable in any tissue. The desmethyl metabolite was present in the liver (408 - 1065 µg/kg at 2 d and 192 - 255 µg/kg at 6 d) and concentrations in liver continued to decline over time and were still detectable at 18 days post-treatment (34 - 147 µg/kg).

Chickens. ^{3}H-Danofloxacin was administered in drinking water to 23 three-week-old chickens to achieve a targeted dose of 5.0 mg Danofloxacin/kg bw/day for 5 days. Birds were sacrificed at 6, 12, 24 and 48 h after withdrawal of the treatment and the total residues in their edible tissues were measured. The highest concentrations of total residues were at 6 h and were consistently found in both the liver (457 - 850 µg/kg) and the kidney (291 - 641 µg/kg). The half lives were similar (9 - 11 h) for the total residues in liver, kidney and muscle and slightly longer (18 h) in skin/fat tissue. In pooled liver homogenates danofloxacin was the most abundant residue (47-61% of the total residues) at all withdrawal times and desmethyldanofloxacin was the major metabolite (13-20% of the total residues).

The depletion of unlabeled danofloxacin from chicken tissues was evaluated where danofloxacin mesylate soluble powder was administered in drinking water to 30 three-week-old chickens at 5.0 mg danofloxacin/kg bw/day for 3 days. Muscle, liver and skin/fat samples were collected at 6, 12, 18, 24 and 36 hours after withdrawal and were analyzed by HPLC for the presence of danofloxacin and desmethyldanofloxacin. Danofloxacin and desmethyl-danofloxacin depleted rapidly from chicken tissue following drug withdrawal with liver always containing the highest concentrations of both danofloxacin and Desmethyldanofloxacin, 157-319 µg/kg and 35-193 µg/kg at 6 hours, respectively. All other tissues contained lower concentrations of danofloxacin than did the liver, while the metabolite, desmethyl-danofloxacin, was not found in tissues other than liver. The concentrations of danofloxacin declined in muscle and fat to <25 µg/kg at 36 h withdrawal. In muscle the maximum concentration of danofloxacin was 91 µg/kg at 6h in one bird but by 18 h the residue was not measurable (<25 µg/kg). Skin/fat residues of parent drug were 61-235 µg/kg at 6 h which decreased to <25-41 µg/kg by 36 h. The concentrations of both parent drug and the metabolite in liver decreased rapidly so that at 36 h the concentration of the parent drug was 41 µg/kg and that of the metabolite < 10 µg/kg.

The analytical methods submitted by the sponsor are suitable for their purpose. For routine monitoring purposes danofloxacin concentrations at the µg/kg level in cattle and poultry tissue can be determined by liquid chromatography with fluorescence detection following an extraction using acid hydrolysis and solvent partition from tissue homogenates or fat. The assay has a limit of quantification (LOQ) of 10 µg/kg and was linear between 10-500 µg/kg in liver, muscle, kidney, fat, and injection site muscle. Recovery of danofloxacin from all fortified tissues

was near 100%. The accuracy of the method was between 99-103% with the precision, expressed as the coefficient of variation, 2% or less. The method demonstrated chromatographic resolution from other fluoroquinolones including, desmethyldanofloxacin, enrofloxacin, ciprofloxacin, norfloxacin, ofloxacin, sarafloxacin, marbofloxacin, and sparfloxacin. A method to confirm the presence of danofloxacin in liver tissues used HPLC combined with mass spectrometry.

Maximum Residue Limits

The following factors were used in establishing the MRLs.

1. The ADI is 0-1200 µg/60-kg person/day.

2. The marker residue is danofloxacin.

3. Desmethyldanofloxacin is ten times more toxic than the parent drug.

4. Only the toxicological activity of the parent drug and desmethyldanofloxacin are known. Therefore all the residues including the bound residues are assumed to be of toxicological concern and to have the same toxicity as parent drug

5. The target tissues are liver and muscle.

6. Desmethyldanofloxacin occurs only in the liver.

7. The danofloxacin and desmethyldanofloxacin content as a fraction (%) of total residues varies between tissues and species.

	Cattle				Pigs				Chickens			
	M	L	K	F	M	L	K	F	M	L	K	F
Danofloxacin	100	25	50	100	40	10	25	100	90	50	100	100
Desmethyldanofloxacin	0	25	0	0	0	90	0	0	0	15	0	0
Unknown residues	0	50	50	0	60	0	75	0	10	35	0	0

Muscle (M), liver (L), kidney (K), and fat (F)

8. The sum of all the residues covered by the ADI expressed as danofloxacin equivalents is given by the formula;

Total residues = Residues of Df + Unidentified residues as Df + Residues of Des as Df equivalents

where (Df is danofloxacin, Des is desmethyldanofloxacin).

The Committee recommended MRLs for cattle and chickens of 200 µg/kg in muscle, 400 µg/kg in liver, 400 µg/kg in kidney, and of 100 µg/kg for fat (fat/skin for chickens), expressed as danofloxacin.

The Committee recommended MRLs for pigs of 100 µg/kg in muscle, 50 µg/kg in liver, 200 µg/kg in kidney, and of 100 µg/kg for fat, expressed as danofloxacin.

The total residues were calculated as danofloxacin using the above formula to give the theoretical maximum daily intake (TMDI) for the tissues for each species (Table 13).

Table 13. Theoretical maximum daily intake (TMDI) of danofloxacin residues (µg)

Tissue	MRL (µg/kg)	Standard daily food basket (g)	Df in basket (µg)	Des in basket (µg)	UR in basket (µg)	TMDI (µg)
Cattle						
Muscle	200	300	60	-	-	60
Liver	400	100	40	400	80	520
Kidney	400	50	20	-	20	40
Fat	100	50	5	-	-	5
Total						625
Pigs						
Muscle	100	300	30	-	45	75
Liver	50	100	5	450	-	455
Kidney	200	50	10	-	30	40
Fat	100	50	5	-	-	5
Total						575
Chickens						
Muscle	200	300	60	-	7	67
Liver	400	100	40	120	28	188
Kidney	400	50	20	-	-	20
Fat	100	50	5	-	-	5
Total						280

Df = danofloxacin, Des = desmethyldanofloxacin and UR = unknown residues as micrograms of Df equivalents

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