ENROFLOXACIN

First draft prepared by
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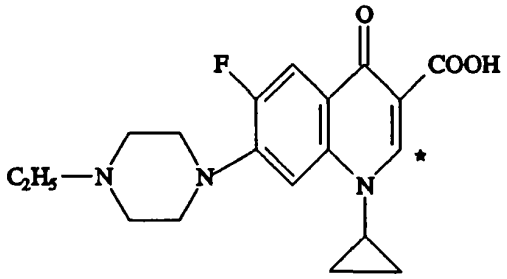
IDENTITY

Chemical name: 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid (hydrochloride)

Synonym: BAY Vp 2674

Structural formula:



[Note: **Ciprofloxacin** is formed by the loss of the ethyl group in the piperazine ring;
* C-2 position of ^{14}C radiolabel]

Molecular formula: $C_{19}H_{22}FN_3O_3$

Molecular weight: 359.4

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Assay min. 98%

Appearance: Pale yellowish to light yellow crystalline substance

Melting point: decomposes

Solubility (g/100 ml): Water pH 7 0.01; ethanol 0.20;
0.1M HCl 0.17; 0.1M NaOH 1.19

UV_{max}: 277 nm in 0.1M HCl

Stability: 3 years

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Enrofloxacin is a drug with a wide spectrum of activity against bacteria. The major metabolite formed in farm species is ciprofloxacin, an antibacterial drug used in human medicine.

Dosages

Enrofloxacin may be administered orally or parentally to calves and pigs at a dose of 5 mg/kg bodyweight (BW) and orally to poultry at a dose of 10-12 mg/kg BW. There is some use in lactating dairy cows and laying hens. The normal treatment period is 3 to 5 days.

METABOLISM

The radiolabeled compound used for the pharmacokinetic, metabolism and residue depletion studies was 2-^{14}C-enrofloxacin except in two studies, one in rats (Bayer report PF 3784) and another in broiler chickens (Bayer Report PF 3963) in which piperazine-2,3-^{14}C-enrofloxacin was used. The radiochemical purity of the batches was between 96.4% and 99.2%

Pharmacokinetics

Calves

Radiolabeled enrofloxacin was administered as a single oral dose of 5 mg/kg BW. The concentrations of radiolabel in blood cells and plasma reached peak values of about 1 mg drug equivalents per l or kg eight hours after administration. Biological activity (bioassay method) in blood peaked at 6-8 h (Bayer Report 73208).

Poultry

After administration of 2-^{14}C-enrofloxacin in multiple oral doses of 12 mg/kg body weight daily for 7 days to chickens peak blood levels of radioactivity were reached in less than 6 h (Bayer Report 73185 Table 4). The elimination half-life in plasma was about 1.4 days.

Piperazine-2,3-^{14}C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to broiler chickens at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). 87.4% of the totally administered dose was excreted within 150 hours of the first administration, i.e. 6 hours after last administration. The excretion during the 24 hour period after the last dosage was 12-15% of the final dose.

Rats

Enrofloxacin was rapidly absorbed following oral administration of 2-^{14}C-enrofloxacin to rats and about 75% of the compound was bioavailable. The peak concentration in blood was reached in under 30 minutes with an elimination half-life of 11.7 $\pm$ 2.45 h. After intravenous (i.v.) injection of enrofloxacin the elimination half-life was 7.91 $\pm$ 0.65 h. There was a rapid elimination of the radiolabel into the bile reaching a plateau value of 40% of the oral dose by 24 h (Bayer Report 73217).

Piperazine-2,3-^{14}C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to 15 male Wistar rats at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). The excretion was fast and mainly faecal (ca. 68% of the dose within 24 hours after the first dosage).

Metabolism

Rats

Rats (Bayer Reports 73217, 73349 and PF 3784) and farm animals (73296, 73288, 73413, 73380 and PF 3963) were administered orally ^{14}C-enrofloxacin. The results are summarised in Table 1. In all the samples parent drug and ciprofloxacin were the major residues except in poultry muscle and skin in which only parent drug but not ciprofloxacin was present. This is in contrast to the metabolism in the bovine where ciprofloxacin is the most abundant residue. In rat urine enrofloxacin glucuronide was a major metabolite. In the edible tissues several other metabolites were detected. These minor metabolites were not identified and usually each

metabolite represented <2% of the total residue. The non-extractable residues formed a significant proportion of the residues in muscle, liver, kidney, fat and poultry skin. The antimicrobial activity of the residues other than parent drug and ciprofloxacin were not determined.

Metabolism in Food Animals

Cattle

Two calves aged 2-4 weeks, were administered by oral gavage ^{14}C-enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. The calves were sacrificed at twelve hours and 72 hours after the final dose and muscle, liver, kidney and fat samples were examined for the characterisation of the radiolabeled residues (Bayer Report, 73296). Enrofloxacin and ciprofloxacin were the major residues. Unlike the other species examined in which enrofloxacin is the major residue, in these calves ciprofloxacin was present in similar amounts to the parent drug. The results are shown in Table 2. Similar results were obtained in the residue depletion studies using unlabeled drug (see Table 7.)

Pigs

One pig, body weight 20-25 kg, was administered by oral gavage ^{14}C-enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. Twelve hours after the final dose the pig was sacrificed and muscle, liver, kidney and fat were examined for the characterisation of the radiolabeled residues using either HPLC or TLC separation. There was a good agreement between the chromatography methods and enrofloxacin and ciprofloxacin accounted for more than 80% of the total residues in the four tissues (see Table 2). No other metabolites were identified (Bayer Report, 73288).

Table 1. Major metabolites of enrofloxacin found in farm animals.

Metabolite		Rat	Cow	Pig	Poultry
Parent (Bay Vp 2674)	Urine Bile Liver	+++ +++ +++	++	+++	+++
Ciprofloxacin (Bay O 9867)	Urine Bile Liver	+++ + +	+++	+	++
M2 (Parent glucuronide ?)	Urine Bile Liver	++ ++	?		?
M6	Urine Bile Liver	+			
Bay p 9357	Urine Bile Liver				++
Non-extractable	Liver	n.m.	++	+	++ (6h) ++[C1](3-10d) +++[T] (3d) 0[C2] (6h)

Total residues;- +++ >25%, ++ 10-25%, + 2 - 10%, n.m. is not measured
Result for [C1] chickens (Bayer report 73185), [C2] chickens (Bayer report PF 3963), [T] turkeys, other poultry results are for both species.

Poultry

The study on chickens was done with 30 day-olds and the study for turkeys with 21 day-olds. For each species

three birds per group in four groups of birds were administered orally 2-^{14}C-enrofloxacin at a rate of 12 mg/kg BW for 3, 5, 7 and 10 consecutive days, respectively. The birds were sacrificed at six hours after the final dose. The samples for birds dosed 3 and 5 days were combined as also were the samples for 7 and 10 days dosing and samples of muscle, liver and skin with adhering fat were examined for the characterisation of the radiolabeled residues. In the first two studies (Bayer Reports, 73179 and 73292) enrofloxacin and ciprofloxacin were the major residues in liver but only parent drug was present in muscle and skin. However there was incomplete extraction of the residues and the studies were repeated in which >90% of the residues were extracted (Bayer Reports, 73413 and 73380).

The results from the repeated studies for the combined 7 and 10 days dosed birds are shown in Table 2. In all tissues enrofloxacin was the major residue and ciprofloxacin was the major metabolite. Most of the remaining residue not accounted for was either insoluble in the HPLC mobile phase or not detected in the HPLC due to interfering lipophilic substances.

Piperazine-2,3-^{14}C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to broiler chickens at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). The birds were sacrificed 6 hours after the last dose and metabolism was investigated in excreta and body tissues. The concentrations of residues were 1.24 mg/kg in liver, 0.50 mg/kg in kidney, 0.25 - 0.30 mg/kg in muscles and 0.13 - 0.15 mg/kg in fat. The radioactivity in the edible tissues was readily extractable with acetonitrile and water with recoveries ranging from 86 - 104%. In liver, fat, kidney and muscle enrofloxacin accounted for more than half the total residues. Ciprofloxacin was a major residue in liver (24%) and kidney (24%) and a minor residue in muscle and fat (3 - 6%). The presence (11%) of a unique metabolite, the N-hydroxy derivative formed after N-dealkylation was found in the liver. Another metabolite (5%) was found in fat and was formed by the opening of the piperazine ring.

Table 2. Characterisation of residues of ^{14}C-enrofloxacin in farm species.

Tissue Species	WT (days)	Total equivalents(μg /kg)	ENX (% total)	CIPX (% total)	Non-extractable (% total)
Liver					
Calf	0.5	9170	24	39	12
Calf	3.0	3860	c.7	14	17
Pig	0.5	2975	73	8.3	-
Chicken	0.25	2060	66	13	2
Turkey	0.25	5220	40	5	7
Kidney					
Calf	0.5	4870	33	41	1
Calf	3.0	370	?	27	11
Pig	0.5	2943	82	5.7	-
Muscle					
Calf	0.5	3680	48	41	2
Calf	3.0	120	17	42	33
Pig	0.5	1564	81	3.4	-
Chicken	0.25	2610	79	3	9
Turkey	0.25	1600	54	0	3
Fat					
Calf	0.5	3050	47	35	(?)
Calf	3.0	300	43	20	17
Pig	0.5	293	75	3.6	-
Skin					
Chicken[a]	0.25	1330	50	4	4
Turkey[a]	0.25	1000	70	0	9

[a] See also Table 4 for more values; ENX is enrofloxacin; CIPX is ciprofloxacin

Metabolism in Toxicological Test Species

Rats were administered a single oral dose of 2-^{14}C-enrofloxacin of 5 mg/kg BW (Bayer report 73217) or daily oral doses of 165 mg/kg BW for 3 days (Bayer reports, 73349, 73379 and 73474). Urine and bile were examined and the major residues were parent drug, ciprofloxacin and enrofloxacin glucuronide and M6 (6%) an unidentified metabolite. Other minor metabolites, (each <2%) were not identified.

Piperazine-2,3-^{14}C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to 15 male Wistar rats at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). The rats were sacrificed 6 hours after the last dose. Metabolism was investigated in faeces, urine and body tissues. Enrofloxacin and ciprofloxacin were identified as the major metabolites as shown in Table 3. The highest concentrations of residues were observed in the liver (1.2 mg/kg) with lower amounts in kidney (0.68 mg/kg) and muscle (0.24 mg/kg) and very low concentrations in fat, plasma and erythrocytes.

Table 3. Metabolites of Piperazine-2,3-^{14}C-enrofloxacin as a percentage of total residues in rat tissues.

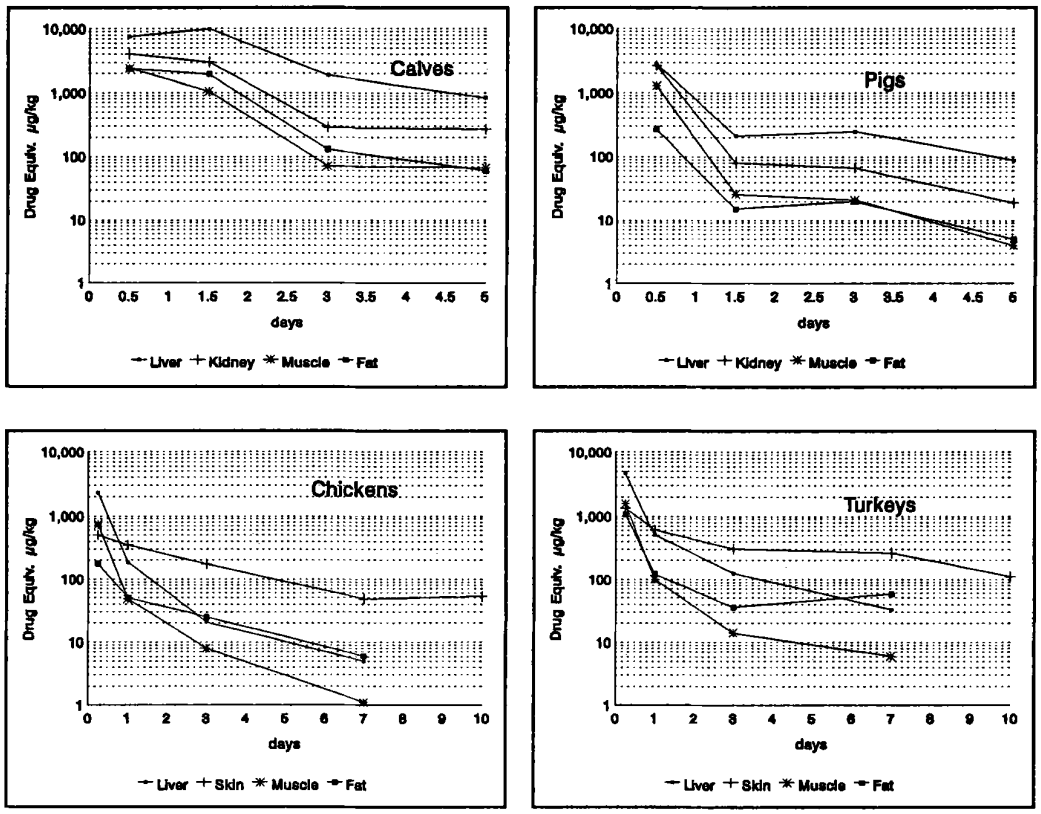
Metabolite	Liver	Kidney	Muscle
Enrofloxacin	20.2	46.6	48.5
Ciprofloxacin	4.9	13.3	17.7
Total	**25.1**	**59.9**	**66.2**

TISSUE RESIDUE DEPLETION STUDIES

Overview

The depletion of the total residues of 2-^{14}C-enrofloxacin was measured in calves, pigs, chickens and turkeys. The results for edible tissues are illustrated in Figure 1 and Table 4. There were large standard deviations of the means for the residue concentration values for most tissues. The semi-logarithmic depletion was not linear during the elimination phase. However, except in the case of bovine liver the residues were below 500 μg/kg in all edible tissues by the third day after the last drug administration. The residues were most persistent in poultry skin and bovine liver and kidney tissues.

Figure 1. Total residues of ^{14}C-enrofloxacin in farm species.



Cattle

Twelve calves, body weight 31-52 kg and aged 2-4 weeks, were administered by oral gavage ^{14}C-enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. The calves were sacrificed in groups of three at 0.5, 1, 3 and 5 days after the final dose and muscle, liver, kidney and fat samples were assayed for the total radiolabeled residues (Bayer Report, 73208), see Table 4. Enrofloxacin and ciprofloxacin were the major residues.

Pigs

Ten pigs, body weight 20-25 kg, were administered by oral gavage ^{14}C-enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. The pigs were sacrificed in pairs at 0.5, 1.5, 3 and 5 days after the last dose. Muscle, liver, kidney and fat were assayed for total radiolabeled residues and the results are shown in Table 4 (Bayer Report, 73313).

Poultry

The study on chickens was done with 14 day-olds weighing 134-217 g and the study for turkeys with 21 day-olds. For each species four birds per group in five groups of birds were administered orally ^{14}C-enrofloxacin at a rate of 12 mg/kg BW for 7 consecutive days. The birds were sacrificed at 0.25, 1, 3, 7 and 10 days after the final dose. Samples of muscle, liver, fat and skin were assayed for the total radiolabeled residues (Bayer Reports, 73184 and 73185) and the results are shown in Table 4.

The skin samples were reanalysed for total radiolabeled residues and for enrofloxacin by an HPLC method (Bayer Report 73220) and the results are shown in Table 4.

Table 4. Total residues (average mean ± SD)(μg/kg) in farm species after multiple oral dosing with ^{14}C-enrofloxacin.

Species & Report Nº Tissue	0.5 days	1.5 days	Withdrawal time 3 days	5 days	
CALF 73208					
Liver	7545 ± 1631	10020 ± 4749	1908 ± 1260	838 ± 152	
Kidney	4047 ± 559	3082 ± 2476	288 ± 40	272 ± 90	
Muscle	2425 ± 769	1055 ± 751	72 ± 12	67 ± 28	
Fat	2413 ± 794	1953 ± 1690	131 ± 82	61 ± 34	
PIG 73313					
Liver	2841 ± 119	215 ± 34	250 ± 20	88 ± 10	
Kidney	2668 ± 448	81 ± 18	68 ± 35	19 ± 2	
Muscle	1295 ± 233	26 ± 8	21 ± 5	4 ± 1	
Fat	<300	<20	<20	<6	
	0.25 days	1 day	Withdrawal time 3 days	7 days	10 days
CHICKEN 73185					
Liver	2335 ± 474	194 ± 82	29 ± 12	12 ± 4	n.m.
Muscle	748 ± 178	52 ± 16	11 ± 3	3 ± 2	n.m.
Fat	181 ± 58	56 ± 31	30 ± 33	12 ± 12	n.m.
Skin	547 ± 59	391 ± 39	214 ± 47	90 ± 37	94 ± 37
Skin 73220	466 (89%)	378 (68%)	119 (61%)	44 (50%)	
TURKEY 73184					
Liver	4713 ± 1384	513 ± 181	125 ± 49	33 ± 11	n.m.
Muscle	1573 ± 472	101 ± 16	14 ± 4	6 ± 2	n.m.
Fat	1093 ± 264	121 ± 66	36 ± 30	58 ± 37	n.m.
Skin	1360 ± 369	610 ± 298	303 ± 74	260 ± 74	111 ± 63
Skin 73220	874 (94%)	407 (85%)	210 (63%)	92 (83%)	

Each value represents the average mean of duplicate assays for 4 birds (2M, 2F). LOD 25 μg/kg for liver and muscle, 85 μg/kg for skin and fat based on 76 dpm (twice background).

The values for total residues in study 73220 using poultry skin are for composite samples; the values in parenthesis are the percentage of the total residues measured as enrofloxacin by HPLC.

Table 5. Residue Studies with Unlabeled Enrofloxacin.

Species	Report Nº	Nº animals	Tissues	Dose (mg/kg/day) [w](mg/l water) [days]	Withdrawal Time (days)	LOD/LOQ (μg/kg)
Calves	12459	12	M,L,K,F	5[o] [3]	3, 7, 14, 21	5/?
	12459	3	M,L,K,F	2.5[o] [3]	7	5/?
	12459	5	M,L,K,F	5[s.c.] [3]	7, 14	5/?
	13176	12	L	5[s.c] [5]	1, 3, 7	2/10E 5/10C
	12462	5	IS	5[s.c.] [3]	7, 14	- /10
Pigs	1148	9	IS	5[o] [3]	5, 7, 10	?/10
	12456	12	M,L,K,F	2.5[o] [3]	1, 3, 7, 14	5/20
	12456	12	M,L,K,F	2.5[i.m.] [3]	1, 3, 7, 14	5/20
	12456	12	M,L,K,F	50[f] [3]	3, 7, 14	5/20
Chickens	73616	6	L,Skin	25[ow] [7]	0.25, 1, 2	10/50
	73616	80	L	41[ow] [7]	0.25, 1, 1.5, 1.75, 2, 2.5, 3	10/50
	13016	16	Egg	10[o] [5]	1, 2, 4, 7, 10	?/10
Turkeys	None	-	-	-	-	-

[o] is oral dose; [ow] is dose in drinking water; [s.c.] is subcutaneous dose; [f] is concentration in feed, [i.m.] is intramuscular injection; M is muscle, L is liver, K is kidney, F is fat, IS is injection site; E is enrofloxacin and C is ciprofloxacin

Other Residue Depletion Studies (with unlabeled drug)

The studies carried out by the sponsors are listed in Table 5.

Calves

Following the administration of enrofloxacin by either the oral route or as a subcutaneous (s.c.) injection, no residues of the parent compound were detected at 3 - 21 days after administration (Bayer Report, 12459). Ciprofloxacin was the major residue and was measured in the edible tissues by HPLC. The results of the studies outlined in Table 5 are shown in Table 6.

Table 6. Residues (μg/kg) of ciprofloxacin in calves after multiple administration of enrofloxacin

Route WT (days)	Muscle	Liver	Kidney	Fat
Oral				
3	<5 - 5	14 - 39	5	<5 - 5
7	<5	5 - 17	<5 - 5	<5
14	<5 - 5	5	<5 - 5	<5
21	<5 - 5	<5 - 5		<5
Subcutaneous				
7	<5	13 - 36	14 - 21	<5
14	<5	10 - 12	13 - 18	<5

WT is the withdrawal time; ENX is enrofloxacin; CIPX is ciprofloxacin

In a second study (Bayer Report, 13176) residues of enrofloxacin and ciprofloxacin were both found in livers of calves administered subcutaneous doses. In a third study (Bayer Report, 12462) residues were measured in

the neck of calves after three daily subcutaneous injections. The results of both studies are shown in Table 7.

Table 7. Residues if enrofloxacin and ciprofloxacin (μg/kg) in livers and injection sites of calves after multiple subcutaneous injection

WT (days)	Liver ENX	Liver CIPX	IS ENX	IS CIPX
1	58 - 395	325 - 614	nm	nm
3	7 - 30	37 - 68	nm	nm
7	<2 - 4	6 - 16	<10 - 12	12 - 24
14	nm	nm	<10	13 - 17

WT is the withdrawal time; ENX is enrofloxacin; CIPX is ciprofloxacin; IS is injection site; nm is not measured

Lactating dairy cows

Lactating dairy cows were administered enrofloxacin by intravenous injection daily for five days. One group of twelve cows were administered a dose of 2.5 mg/kg BW (Bayer report, V93-001) and a second group of five cows were dosed with 5 mg/kg BW (Bayer report, RA - 982/88). The morning and evening milk samples were combined to produce a daily sample for the lower dose group and were kept separate as morning and evening samples for the high dose group. The concentrations of enrofloxacin and ciprofloxacin were determined in the samples in the lower dosed cows and ciprofloxacin was measured for the higher dosed cows. The analysis was carried out using an HPLC method with an LOQ of 5 μg/l. The concentration of ciprofloxacin was higher than enrofloxacin in all the samples. The number of animals testing positive and the range of milk concentrations for both analytes is shown in Table 8.

Table 8. Residues of enrofloxacin and ciprofloxacin in milk of dairy cows following intravenous injection of enrofloxacin daily for five days

Dose/Analyte	Day 1	Day 2	Day 3	Day 4	Day 5
2.5 mg/kg bw					
Enrofloxacin (range μg/kg)	<5 - 14	<5 - 7	<5	<5	<5
Positive out of 12	10	3	0	0	0
Ciprofloxacin (range μg/kg)	7 - 49	<5 - 18	<5 - 12	<5 - 7	<5
Positive out of 12	12	6	4	2	0
5 mg/kg bw					
Enrofloxacin (range μg/l, am/pm)	<2	<2	<2	<2	nm
Ciprofloxacin (range μg/l, am)	30 - 132	5 - 23	2 - 4	<2 - 2	nm
Positive out of 5	5	5	5	3	-
Ciprofloxacin (range μg/l, pm)	8 - 39	3 - 7	<2 - 5	nm	nm
Positive out of 5	5	5	3	-	-

nm is not measured. The values between 2 and 5 are based on the LOD of 2 μg/l and are therefore imprecise as the LOQ is 5 μg/l.

Pigs

Residues of enrofloxacin and ciprofloxacin in edible tissues were measured by HPLC after oral dosing, intramuscular injection and inclusion in the feed of enrofloxacin (see Table 5). The results are shown in table 9. The residues are low within one day of dosing and deplete rapidly so that at three days ciprofloxacin is not detected and enrofloxacin is only found in the liver and kidney tissues. No residues were detected at 7 or 14 days withdrawal time nor were any residues detected following in-feed administration (Bayer Reports 12456 & RA-1148/88).

Residues of enrofloxacin and ciprofloxacin were measured in the neck tissue (sample size between 240 and 850 g) of nine pigs after 3 daily injections in the same site (see Table 5). The results are shown in table 10. The drug was almost completely absorbed from the injection site by day 10 withdrawal time. Residues of ciprofloxacin (20 μg/kg) were detected in one pig on day 5 and one animal on day 7 (Bayer Report RA-1148/88).

Chickens

The study, Bayer Report 73616 (see Table 5), measuring residues of enrofloxacin was divided into two parts. The first part was a pilot study in which measurements for residues in liver and skin were made where the dose in the drinking water was 25 mg/l. The residues in μg/kg in skin were 220 and 140 at 6 h post dosing, 45 and 49 at 24 h and 21 and 16 at 48 h. In the main study the dose was unintentionally high at 41 mg/l water and residues were only measured in liver samples (see Table 11). There were no significant differences in the residues of males and females, thus the results are combined in the Table. The residues depleted rapidly and were <500 μg/kg two days after cessation of administration of the drug.

Table 9. Residues of enrofloxacin and ciprofloxacin (μg/kg) in pigs after multiple administration of enrofloxacin

Route WT (days)	Muscle ENX	 CIPX	Liver ENX	 CIPX	Kidney ENX	 CIPX	Fat ENX	 CIPX
Oral								
1	240	30	190	40	290	100	60	nd
3	nd	nd	20	nd	20	nd	nd	nd
7 & 14	nd	nd	nd	nd	nd	nd	nd	nd
Intramuscular								
1	170	30	180	40	240	70	80	nd
3	20	nd	20	nd	40	nd	nd	nd
3 & 14	nd	nd	nd	nd	nd	nd	nd	nd
Feed								
3, 7 & 14	nd	nd	nd	nd	nd	nd	nd	nd

WT is the withdrawal time; ENX is enrofloxacin; CIPX is ciprofloxacin; nd is <10 μg/kg

Table 10. Residues (μg/kg) of enrofloxacin at injection site of pigs

Withdrawal time 5 days	 7 days	 10 days
68400	430[c]	10
10900	180	nd
430[c]	40	nd

[c] these samples contained 20 μg/kg ciprofloxacin.

Table 11. Residues of enrofloxacin in livers of chickens administered 41 mg/l in drinking water

Withdrawal Time (days)	Mean n = 10 (μg/kg)	Range (μg/kg)
0.25	2610	1850 - 4860
1	870	410 - 1350
1.5	420	180 - 620
1.75	240	160 - 320
2	190	60 - 310
2.5	130	60 - 200
3	90	50 - 210

When the recommended dose of 50 mg/l in the water was administered the residues were higher and persisted for a longer period, e.g. in skin at day 4 enrofloxacin was 120 μg/kg and ciprofloxacin was 20 μg/kg, at day 7 parent drug was 100 μg/kg and ciprofloxacin 20 μg/kg, at day 10 enrofloxacin was 50 μg/kg and ciprofloxacin was not detectable. Also residues of enrofloxacin but not ciprofloxacin were detected in liver of broilers for at least 17 days after dosing.

Eggs

Laying hens were administered enrofloxacin (see study 13016 in Table 5) and the residues of enrofloxacin and ciprofloxacin were measured by HPLC in egg yolk and egg white. The results are shown in Table 12. Enrofloxacin was the major residue and the levels of both compounds dropped to below the LOQ (10 μg/kg) on the tenth day after the last dose.

Table 12. Residues (μg/kg) in eggs after administration of enrofloxacin.

WT (days)	Egg White Enrofloxacin	Egg white Ciprofloxacin	Yolk Enrofloxacin	Yolk Ciprofloxacin
1	3630	200	3140	210
2	440	20	1960	250
4	40	<10	490	100
7	10	<10	70	30
10	<10	<10	<10	<10

Each value is the mean of 12-16 eggs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES.

HPLC Methods

All of the methods submitted from the sponsor are basically the same. They are based on homogenisation and extraction of both enrofloxacin and ciprofloxacin into an organic phase. The extract may be further purified and then dissolved in the HPLC mobile phase. After resolution by HPLC, detection is by either UV or fluorescence. The earlier methods were used to measure the levels of residues in treated animals. More

recently the methods were improved by selecting better extraction solvents and changing the HPLC conditions (Bayer Reports, 93/14231-4). Samples of muscle, fat, skin or blood were extracted by homogenisation with cold ethanol/acetic acid. The extract was evaporated to dryness and the residue dissolved in the HPLC mobile phase. After clarification by centrifugation an aliquot of the solution was assayed by HPLC using fluorescence detection. The analysis of liver and kidney required a clean up step of the homogenate extract by passing it through an XAD-4 polystyrene resin. Milk samples were directly extracted into acetonitrile.

The new methods appear to meet the contemporary criteria (EC Decision 93/256). The limits of detection (LOD) are down to 1 μg/kg tissue and the limits of determination (quantification) (LOQ) are now 10 μg/kg for most tissues. Recoveries of spikes of enrofloxacin and ciprofloxacin equivalent to 10-210 μg/kg from muscle, fat, skin, liver, kidney and blood were mostly >70% except for ciprofloxacin in liver (57-59%) and kidney (68-69%) with intraassay precision well below 10% except for enrofloxacin from pig liver (10-12%) and for ciprofloxacin in fat (13-16%).

A method for the simultaneous determination of enrofloxacin and ciprofloxacin in pig muscle, bacon and bovine muscle with extraction into 1% acetic acid/ethanol and clean up on a Bond-Elut SCX column followed by HPLC with fluorescence detection is published (see Heitzman, 1994). The LOQ was 10 μg/kg.

APPRAISAL

Enrofloxacin is a member of the new group of fluoroquinolone antibiotics. The major metabolite in farm animals is ciprofloxacin which is a widely used drug in human medicine.

The drug is rapidly absorbed from the gut of calves, poultry and rats with plasma concentrations reaching peak values in less than 8 hours. 40% of an oral dose was excreted in the bile of rats. The route of excretion in farm animals was not investigated.

Metabolism was studied in farm animals and rats using ^{14}C-radiolabeled enrofloxacin. In all the samples parent drug and ciprofloxacin were major residues except in poultry muscle and skin in which only parent drug but not ciprofloxacin was present. This is in contrast to the metabolism in the bovine where ciprofloxacin is the most identified abundant residue.

The depletion of the total residues of 2-^{14}C-enrofloxacin was measured in calves, pigs, chickens and turkeys. There were large standard deviations of the means for the values for most tissues, also the depletion was not linear during the elimination phase. However, except in the case of bovine liver the residues were below 500 μg/kg in all tissues by the third day after the last drug administration. The residues were most persistent in poultry skin and bovine liver and kidney tissues.

The successful measurements of residues of enrofloxacin and ciprofloxacin were achieved in a single analyses by a validated HPLC method. The LOQs for edible tissues were about 10 μg/kg and 5 μg/l for milk. Residues of enrofloxacin and ciprofloxacin were measured by an HPLC method in the edible tissues, eggs and injection sites of farm animals which had been administered either orally or parentally multiple doses of the sponsors recommended doses of enrofloxacin. The residues in all tissues depleted rapidly and were either not detectable or at very low concentrations at 7 days after administration of drug.

Maximum Residue Limits

Based on the temporary ADI of 0 - 0.6 μg/kg for parent drug established by the Committee, the permitted daily intake of parent drug and/or its equivalents of antimicrobial activity is 36 μg for a 60 kg person per day.

The following factors were considered in estimating the MRLs.

1. The temporary ADI is set on a microbiological end point.
2. The limit of quantification of the method (10 μg/kg for enrofloxacin and ciprofloxacin in tissues and 5 μg/kg or l in milk).
3. The drug is for use in meat animals, poultry, lactating cows and laying hens.
4. The marker residue is the sum of enrofloxacin and ciprofloxacin for tissues. Ciprofloxacin is the marker residue for bovine milk.
5. There is an uncertain ratio of the marker residues to the total residues. Also the percentage of the total residues that possess antimicrobial activity is not known. A factor of up to 5 is probable.

The Committee noted that assuming theoretical MRLs equal to two times the LOQs for the analytical methods, the amount of marker residues in the total food basket (muscle, liver, kidney, fat, milk and eggs) would be 39 μg and just in excess of the ADI of 36 μg. However this would not take into account any antimicrobial activity of the significant fraction (up to 80% in tissues and percentage not known for milk) forming the remaining residues. It was therefore not possible to allocate MRLs which would guarantee an observance of the ADI. The Committee requests information concerning the antimicrobial activity of the remaining residues.

The following information is required for evaluation in 1997.

- studies to determine the antimicrobial activity of the residues other than enrofloxacin and ciprofloxacin.

REFERENCES

Bayer Reports

RA-1148/88. Riegner, K and Scheer, M. (1988). Liquid chromatographic assay of BAY Vp 2674 (Enrofloxacin) and BAY O 9867 (Ciprofloxacin) at the site of injection in pigs after intramuscular administration of Baytril.

12456. Riegner, K. (1986). HPLC determination of BAY Vp 2674 and BAY O 9867 (Ciprofloxacin), in muscle, fat, liver and kidney of swine after oral and parental treatment with Baytril formulations.

12459. Riegner, K. (1986). Analytical determination of residue of BAY Vp 2674 (parent substance) and BAY O 9867 (metabolite; Ciprofloxacin), in tissues of cattle after oral and subcutaneous administration of Baytril formulation.

12462. Riegner, K. (1986). Analytical determination of residues of BAY Vp 2674 (enrofloxacin) and its main metabolite BAY O 9867 (ciprofloxacin), in tissue of the injection site after subcutaneous administration of BAY Vp 2674 to calves.

13016. Riegner, K. (1988). Liquid chromatographic assay of BAY Vp 2674 and BAY O 9867 in eggs of hens after treatment.

13176. Riegner, K. (1988). HPLC determination of BAY R 3964, BAY Vp 2674 (enrofloxacin) and BAY O 9867 (Ciprofloxacin), in calve livers following subcutaneous administration of Baytril.

14231. Heukamp, U. (1993). Analytical method for the determination of Baytril (a.i.:Enrofloxacin) and its metabolite Ciprofloxacin in muscle, fat, skin and blood of pigs.

14232. Heukamp, U. (1993). Analytical method for the determination of Baytril (a.i.:Enrofloxacin) and its metabolite Ciprofloxacin in liver and kidney of pigs.

14233. Heukamp, U. (1993). Analytical method for the determination of Baytril (a.i.:Enrofloxacin) and its metabolite Ciprofloxacin in muscle, fat and skin of poultry.

14234. Heukamp, U. (1993). Analytical method for the determination of Baytril (a.i.:Enrofloxacin) and its metabolite Ciprofloxacin in muscle, fat and milk of cattle.

73179. Waggoner, T.B. (1986). Total residue levels of BAY Vp 2674 in livers of chicken and turkeys from multiple oral dosing.

73184. Waggoner, T.B. (1985). Total residue levels of BAY Vp 2674 in tissues of turkeys from multiple oral dosing.

73185. Waggoner, T.B. (1985). Total residue levels of BAY Vp 2674 in tissues of chicken from multiple oral dosing.

73208. Waggoner, T.B. (1985). Total residue levels of BAY Vp 2674 in tissues of non-ruminating calves from multiple oral dosing.

73217. Hamann, S.R., Ewing, A.D. and Lawrence, L.J. (1985). Disposition and metabolism of BAY Vp 2674 in male rats.

73220. Waggoner, T.B. (1985). Comparative analyses of chicken and turkey skin for levels of BAY Vp 2674 and metabolites.

73288. Waggoner, T.B. (1986). Metabolism of BAY Vp 2674[2-^{14}C] in the cow.

73292. Waggoner, T.B. (1986). Metabolism of BAY Vp 2674[2-^{14}C] in chicken and turkeys.

73296. Waggoner, T.B. (1986). Metabolism of BAY Vp 2674[2-^{14}C] in swine.

73313. Waggoner, T.B. (1986). Total residue levels of BAY Vp 2674[2-^{14}C] in tissues of swine from multiple oral dosing.

73349. Waggoner, T.B. (1986). Biotransformation of BAY Vp 2674 in Sprague-Dawley rats.

73379. Waggoner, T.B. (1986). Characterisation of a rat urinary polar metabolite of BAY Vp 2674.

73380. Waggoner, T.B. (1986). Metabolic profile of [2-^{14}C] BAY Vp 2674 in turkey tissue after multiple oral administration.

73413. Waggoner, T.B. (1986). Metabolic profile of [2-^{14}C] BAY Vp 2674 in chickens.

73474. Waggoner, T.B. (1986). Confirmatory identification BAY Vp 2674 glucuronide from rat urine.

PF 3784 Klein, O. and Brauner, A. [Piperazine-2,3-^{14}C]Enrofloxacin: Investigation of the biokinetic behaviour and the metabolism in the rat after multiple oral dosing.

PF 3963 Weber, H., Ecker, W. and Anderson, C. [Piperazine-2,3-^{14}C]Enrofloxacin: Metabolism in broiler chicken.

RA - 982/88 Riegner, K. (1988). Determination of Bay R 3964, Bay Vp (enrofloxacin) and Bay O 9867 (ciprofloxacin) in cow's milk by HPLC after intravenous administration of Baytril.

V93-001. Mencke, N. and Krebber, R. (1993). Baytril (Enrofloxacin) 2.5 mg/kg body weight, i.v. dairy cattle study.

Commission Decision, 93/256/EEC, laying down the methods for detecting residues of substances having a hormonal or thyrostatic action. [OJ. N°L118, 14.5.93, pp 75-79].

Heitzman, R.J. (1994). 2nd Edition, Veterinary Drug Residues. Residues in food-producing animals and their products: Reference Materials and Methods. Ed. R.J.Heitzman. CEC Report EUR 15127-EN. Publ. Blackwells, Oxford, UK, ISBN 0-632-03786-5, Ch. 6; Method Sg 3.5