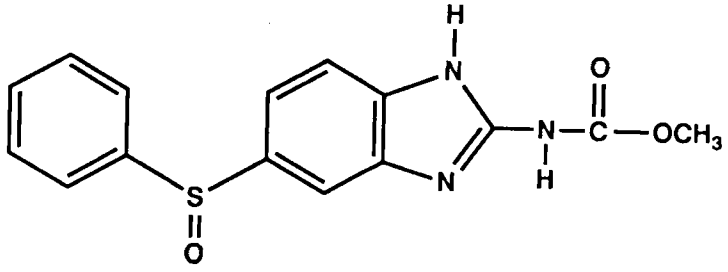
OXFENDAZOLE

IDENTITY

Chemical: Methyl (5-phenylsufinyl)-2-benzimidazole carbamate

Synonyms: CAS 53716-50-0

Structural formula:



Molecular formula: $C_{15}H_{13}O_3N_3S$

Molecular weight: 315.34

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Oxfendazole active ingredient contains not less than 97.0% of $C_{15}H_{13}O_3N_3S$, calculated on a dried basis, not more than 2.0% of methyl(5-phenylsulfonyl)-2-benzimidazole carbamate, and not more than 1.0% of any other individual foreign related substance.

Appearance: White-gray powder. May have a slight color.

Melting point: 245-265 ° (with decomposition)

Solubility: Solubility in water is 3.11 - 4.63 mg/L

Octanol/Water Partition Coefficient: Log_{ow} 1.953 ± 0.162

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Oxfendazole is an anthelmintic used for the treatment of gastrointestinal parasitism in cattle, sheep and horses. Oxfendazole has a broad spectrum of activity against all stages of gastrointestinal nematodes including larval stages, cestodes and lungworms.

The recommended therapeutic doses for cattle, sheep and horses are 4.5, 5.0 and 10.0 mg/kg, respectively. Treatments are seldom repeated at intervals less than about eight weeks in length. Sheep tend to be treated somewhat more frequently than cattle due to grazing pattern. Treatment of sheep varies from one curative treatment per year to about 3 or 4 treatments per year where pasture contamination is high and climate is favorable. Only in rare circumstances are sheep treated more than four times per year. Cattle and horses receive even fewer treatments per year.

Dosages

Several formulations of oxfendazole have been developed for the treatment of food producing animals. These formulations include suspensions (22.5%, 9.06% 4.53% and 1.812%), a paste (185 g/kg) and pellets (5 gm/kg). All the formulations are for oral administration and the 22.5 % suspension is also for intraruminal administration.

METABOLISM

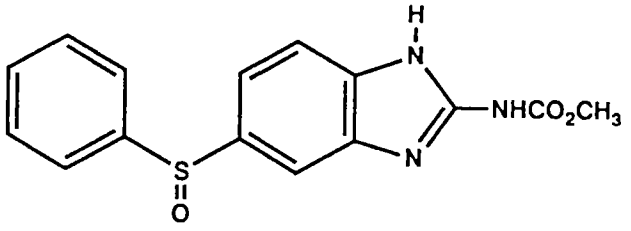
General

The absorption, distribution, metabolism, excretion and tissue residues of oxfendazole in cattle, sheep, and horses have been extensively studied using ^{14}C-oxfendazole and are qualitatively similar in all three species. Oxfendazole is primarily excreted in the feces following oral and intravenous administration. Orally administered oxfendazole is well-absorbed by cattle and sheep. The extent of oral absorption by horses has not been studied but is expected to be high. The parent drug (1) is the major component present in the plasma following oral administration of oxfendazole and the thioether (2) and the sulfone (3) are the only other metabolites of any significance. Urine contains little unchanged oxfendazole and no thioether or sulfone are present. The major metabolites in the urine are the free amine (4) and the 4'-hydroxylated compounds present as free phenols or as glucuronides (5). (See Figure 1)

For all species, liver is the tissue with the highest drug-related residue and is the tissue from which the residue depletes most slowly. The extractable portion of the residue present in liver consists of oxfendazole, the thioether (fenbendazole), and the sulfone. A large portion of the residue present in liver is non-extractable and the proportion of the non-extractable residue increases with the time post dose. Studies have demonstrated that the bound residue has low bioavailability.

Figure 1. Structure of oxfendazole and its metabolites

1



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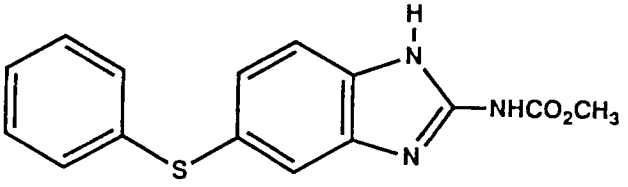

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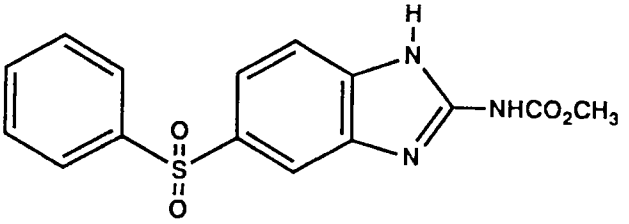

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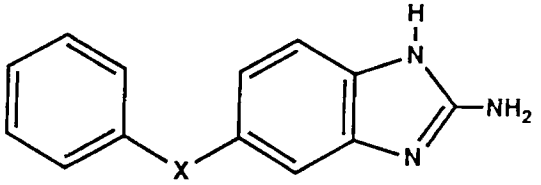
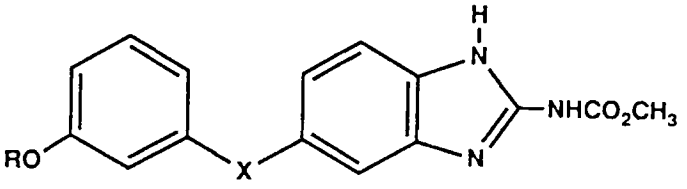


X = S, SO, OR, SO_2

5



R = H, SO_3H or glucuronide
X = S, SO, or SO_2

Studies of the metabolism of oxfendazole in the rat demonstrate that the rat is exposed to the same metabolites that are present in the edible tissues of cattle, sheep, and horses treated with oxfendazole.

Rat

Following IV administration of ^{3}H-oxfendazole (6 mg/kg) to rats, 50% of the radioactivity was excreted in the urine and 38% in the feces. Following oral administration of ^{3}H-oxfendazole to rats, 52% of the radioactivity was excreted in the urine and 41% in the feces. Based on urinary excretion data and on the area under the plasma concentration versus time curve following oral and IV administration, orally administered oxfendazole is completely absorbed. (Tomlinson, 1974a)

The radioactivity present in plasma up to 24 hours following oral administration of ^{14}C-oxfendazole (6 mg/kg) could be extracted completely into organic solvents . Oxfendazole, the sulfone, and the thioether together accounted for all the radioactivity present in the plasma. Based on the area under the 0-24 hour plasma concentration versus time curves, oxfendazole, the sulfone, and the thioether constituted 29%, 71%, and less than 1% respectively of the radioactivity present. (Tomlinson et al, 1986)

Sheep

Following IV administration of ^{14}C-oxfendazole at a dose of 6 mg/kg to sheep, radioactivity depleted from the plasma with a half life of approximately 14 hours. Approximately 26% of the radioactivity was recovered in the urine and 63% was recovered in the feces. Following oral administration of a solution of ^{14}C-oxfendazole at a dose of 6 mg/kg the concentration of radioactivity in the plasma reached a maximum at 8 hours after dosing and depleted from the plasma with a half life of 28 hours. Approximately 22% of the orally administered radioactivity was recovered in the urine and 64% was recovered in the feces. Based upon urinary excretion data an average of 85% of the oxfendazole administered as a solution was absorbed. (Tomlinson, 1974b).

Following oral administration of ^{14}C-oxfendazole to sheep, up to 24 hours after dosing the organic extractable portion of the radioactivity present in the plasma varied from 75% to 90% . During the first 24 hours after dosing the organic extractable portion of the radioactivity consisted exclusively of oxfendazole, the thioether (figure 1, 2), and the sulfone (figure 1, 3). The relative amounts of these components varied with time. Two hours after oral administration of oxfendazole 70% of the extractable material was oxfendazole, 8% was the sulfone, and 22% was the thioether. Twenty-four hours after dosing 56% of the extractable material was oxfendazole, 21% was the sulfone and 23% was the thioether. (Tomlinson, 1976a)

Approximately 8% of the radioactivity in the urine was unchanged oxfendazole. The thioether and sulfone metabolites of oxfendazole were not present. Approximately 16% of the urinary radioactivity was free amine material (figure 1, 4). Approximately 70% of the urinary metabolites of oxfendazole consisted of 4'-hydroxylated compounds present either as free phenols or as glucuronide or sulfate conjugates (figure 1, 5). (Tomlinson, 1976a)

Cattle

Following IV administration of ^{14}C-oxfendazole at a dose of 6 mg/kg to cattle, radioactivity depleted from the plasma with a half life of approximately 20 hours. On average, 27 ± 4.5% of the radioactivity administered IV as ^{14}C-oxfendazole was recovered in the urine and 78 ± 3% was recovered in the feces. Following oral administration of a solution of ^{14}C-oxfendazole at a dose of 6 mg/kg the concentration of radioactivity in the plasma reached a maximum approximately 24 hours after dosing and depleted from the plasma with a half life of approximately 22 hours. On average 21 ± 4% of the radioactivity administered orally as ^{14}C-oxfendazole was recovered in the urine and 65 ± 4.5% was recovered in the feces. Based upon urinary excretion data an average of 77% of the oxfendazole administered orally as a solution was absorbed. (Tomlinson and Bidlack, 1976)

Studies in calves comparing a solution to a drench suspension of oxfendazole showed that the vehicle affects the absorption. The peak plasma concentration post drench formulation was 66% of that achieved after the solution; both peaks occurred 24-hours post-administration. (Tomlinson and Bidlack, 1974)

The composition of the radioactivity present in plasma and urine of cattle following oral administration of ^{14}C-oxfendazole has been examined . The organic extractable portion of the plasma contained 89% of the total drug equivalents present in the plasma during the 0-24 hour period. During this period the organic extractable portion of the radioactivity consisted exclusively of oxfendazole, the thioether (figure 1, 2), and the sulfone (figure 1, 3). The relative amounts of these components varied with time. Unchanged oxfendazole was the predominant species circulating in the plasma 2 hours after oral administration although the thioether and the sulfone were also present. Twenty-four hours after dosing 43% of the extractable material was oxfendazole, 34% was the sulfone and 23% was the thioether. (Tomlinson, 1977a)

Urinary metabolite profiles showed that very little oxfendazole was excreted unchanged. The sulfone and thioether metabolites of oxfendazole have not been detected in urine. Approximately 22% of the urinary radioactivity was free amine material (figure 1, 4). The remaining 68% of the urinary metabolites of oxfendazole consisted of 4'-hydroxylated compounds present either as free phenols or as glucuronide or sulfate conjugates (figure 1, 5). (Tomlinson, 1977a)

Approximately 12 hours following oral administration of ^{14}C-oxfendazole to lactating cattle at a dose of 2.5 mg/kg, the ^{14}C-residue in milk reached a maximum concentration of approximately 5 ppm . The residue depleted from milk with a half-life of 18 hours. Of the 1% of the dose that was excreted in the milk, approximately 93% was excreted during the first 72 hours. On the average 50% of the residue present in the milk collected 0-72 hours after dosing could be extracted into chloroform and of this 70% was unchanged oxfendazole and the remainder consisted of the thioether and the sulfone. (Tomlinson et al, 1977)

A study was conducted comparing the tissue and blood residue levels from cattle treated with either oxfendazole or fenbendazole. The study involved two groups of six calves each which were in the weight range of 280-310 kg bw. One group was treated with

oxfendazole at 4.5 mg/kg by intraruminal injection. The other group recieved fenbendazole (Panacur) at 5.0 mg/kg by oral administration. Two animals (one for each sex) from each treatment group were sacrificed at 8, 14, or 21 days after dosing. Samples of liver, kidney, muscle, and fat were collected from each animal, and blood was taken prior to dosing and at 24 hours post dosing. Concentrations of oxfendazole and the two metabolites were measured in the plasma samples. The quantification limit of the assay was stated to be 10 ppb. The assay results are summarized in Table I. (Spires, 1988a)

Table I. Average concentrations (in ppb) of oxfendazole, oxfendazole sulfone, and fenbendazole in plasma obtained from cattle 24 hours after: a) a single intraruminal injection of oxfendazole suspension at a dose of 4.5 mg/kg, and b) a single oral 5.0 mg/kg dose of fenbendazole.

Treatment	Oxfendazole	Oxfendazole Sulfone	Fenbendazole
Oxfendazole	241 ± 69	76 ± 13	97 ± 37
Fenbendazole	175 ± 39	41 ± 7	109 ± 23

Another study involved three groups of cattle which were arranged and treated as follows:

Group 1. Five calves (180-220 kg, both sexes) were treated with oxfendazole 9.6% suspension at the rate of 4.5 mg/kg as an oral drench.

Group 2. Five calves (180-220 kg, both sexes) were treated with oxfendazole 9.6% suspension at the rated of 4.5 mg/kg as an intraruminal injection.

Group 3. Six calves (160-220 kg, both sexes) were treated with fenbendazole at the rate of 4.5 mg/kg (dosage form unspecified).

Blood samples from each animal were collected prior to treatment and at 4, 8, 12, 24,36,48, 72, 120 and 168 hours post dosing. The results of the assays for oxfendazole, fenbendazole and the sulfone metabolite are given in Table II and also as graphs in Figure 2. (Nguomo et al, 1984)

Table II. The mean concentration (μg/ml) in plasma of oxfendazole (OXF), fenbendazole (FBZ) and oxfendazole sulfone (OXFS) after administration of fenbendazole and oxfendazole orally and oxfendazole intra-ruminally to cattle.

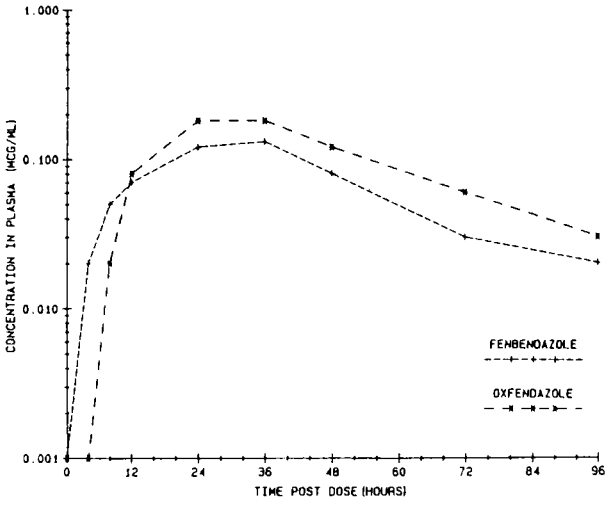
	Fenbendazole orally (n=6)			Oxfendazole orally (n=5)			Oxfendazole intra-ruminally (n=5)		
Hours	FBZ	OXF	OXFS	FBZ	OXF	OXFS	FBZ	OXF	OXFS
0	-	-	-	-	-	-	-	-	-
4	0.01	0.02	0.01	0.01	0.02	-	0.01	-	-
8	0.04	0.05	0.03	0.04	0.06	0.01	0.03	0.02	-
12	0.08	0.07	0.05	0.06	0.07	0.03	0.05	0.08	0.03
24	0.11	0.12	0.10	0.80	0.17	0.07	0.11	0.18	0.06
36	0.08	0.13	0.11	0.10	0.20	0.12	0.09	0.18	0.09
48	0.05	0.08	0.05	0.07	0.11	0.12	0.08	0.12	0.10
72	0.02	0.03	0.02	0.02	0.06	0.13	0.03	0.06	0.13
96	0.01	0.02	0.01	0.01	0.02	0.07	0.01	0.03	0.07
120	-	0.01	-	0.03	0.01	0.03	0.01	0.02	0.02

Horse

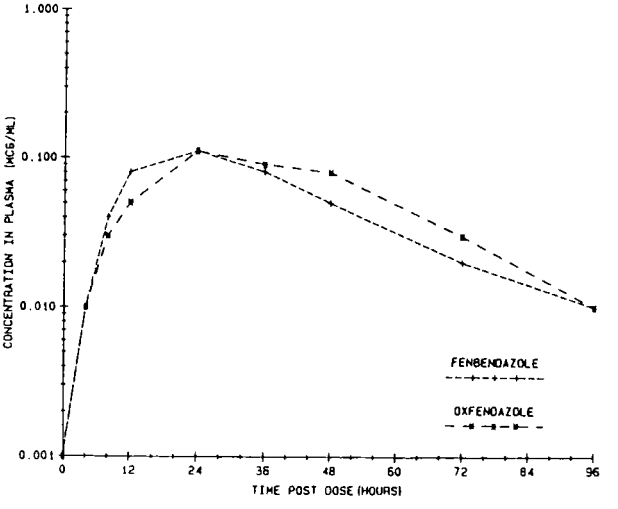
Twelve hours following oral administration of ^{14}C-oxfendazole to horses at a dose of 10 mg/kg the concentration of ^{14}C in the plasma reaches a maximum of 1 microgram equivalent per ml. Total radioactivity depletes from the plasma with a half life of 26 hours. Of the dose administered, 84.4% is excreted in the feces and 6.8% is excreted in the urine. (Tomlinson et al, 1981)

Following oral administration of oxfendazole to horses, oxfendazole, fenbendazole (2, figure 1), and oxfendazole sulfone (3, figure 1) are present in the plasma. The concentrations of oxfendazole, fenbendazole, and oxfendazole sulfone in horse plasma following oral administration of oxfendazole are much lower than those in bovine or sheep plasma following administration of a similar dose. Based on data for the area under the plasma concentration vs time curves the relative amounts of oxfendazole, fenbendazole, and oxfendazole sulfone in horse plasma are 1.4, 1, and 7.7 respectively. Therefore, in the horse, the concentration of oxfendazole sulfone in the plasma relative to that of oxfendazole and fenbendazole is much higher than in cattle or sheep. In view of this, the extractable residue in horse liver is expected to consist primarily of oxfendazole sulfone rather than of oxfendazole. (Marriner and Bogan, 1985)

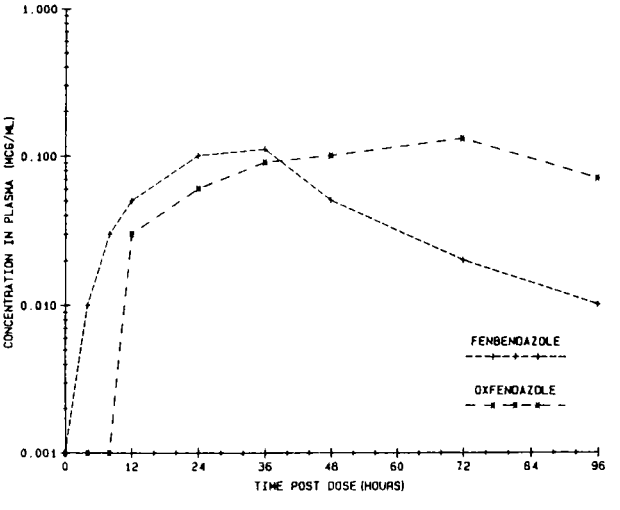
Figure 2. Plasma levels of oxfendazole (a), fenbendazole (b) and the sulfone (c) in cattle treated with fenbendazole (4.5 mg/kg) or with oxfendazole (4.5 mg/kg). Measurements are by an HPLC assay.



a. Plasma levels of oxfendazole.



b. Plasma levels of fenbendazole.



c. Plasma levels of sulfone.

TISSUE RESIDUE DEPLETION DATA

Radiolabeled Residue Depletion Studies

Rat

Following oral administration of ^{14}C-oxfendazole to rats, liver was the tissue with the highest and slowest depleting residue. Of the residue present in liver a significant portion could not be extracted into organic solvents. At 0.5 hours and 24 hours post dose, 41% and 93% respectively of the residue was protein-bound. Although the exact composition of the residues present in liver varied with time, oxfendazole, the thioether, and the sulfone together comprised approximately 98% of the extractable portion. Since studies in cattle showed that liver and milk from treated animals contain oxfendazole, the thioether, the sulfone, and protein-bound materials (liver only), and since the same components are found in the circulatory system and/or liver of rats, it is clear that the rat is a good model for evaluation of the toxicological potential of the residues to which humans will be exposed. (Tomlinson et al, 1986)

Sheep

Two studies have been conducted to determine the total drug-related residue present in tissues following oral administration of ^{14}C-oxfendazole to sheep. In study DM 76-Sh-2778, twelve sheep each received a single 6 mg/kg dose of ^{14}C-oxfendazole through a rumen tube. One animal was slaughtered on days 10, 13, 23, 25, 30, and 38 days post dosing and three animals were slaughtered 58 and 70 days post dosing. Radioassay of the tissues gave the results shown in Table III. (Tomlinson, 1976b)

Table III. Total radioactivity (ppm) in tissues of sheep following oral administration of a single 6 mg/kg dose of ^{14}C-oxfendazole.

Time	Total Residue			
(Days)	Muscle	Fat	Kidney	Liver
10	0.030	0.195	1.085	2.479
13	0.080	0.070	0.011	2.580
23	0.006	0.041	0.044	0.218
25	0.002	0.020	0.019	0.194
30	0.002	0.009	0.023	0.223
38	0.003	0.013	0.017	0.134
58	0.002	0.005	0.007	0.065
70	0.001	0.004	0.006	0.048

In a second study, four sheep each received a single 6 mg/kg dose of ^{14}C-oxfendazole through a rumen tube. One animal was slaughtered on days 3, 7, 14, and 21 post dosing. Radioassay of tissues are summarized in Table IV. (Runkel et al, 1978)

Table IV. Concentrations of total drug-related residue in tissues from sheep following oral administration of oxfendazole at a dose of 6 mg/kg of body weight

Time (Days)	Total Residue (ppm) Muscle	Fat	Kidney	Liver
3	0.35	0.68	1.55	17.1
7	0.02	0.02	0.14	7.34
14	0.01	0.01	0.04	1.31
21	0.01	0.01	0.02	0.27

These studies showed that at all times after dosing, liver is the tissue with the highest drug-related residue and the tissue from which the residue depletes most slowly. Based on data obtained from study table IV, the total residues present in the liver during the period of 3 to 21 days after dosing deplete with a half life of approximately 3 days.

Examination of the residues present in the liver of treated animals showed that 3, 7, 14, and 21 days after administration of oxfendazole approximately 76%, 61%, 41%, and 7% respectively of the total residue was extractable. At these times, unchanged oxfendazole represented 49%, 82%, 57%, and approximately 70% respectively of the extractable residue. (Massey, 1988a)

Studies of the non-extractable (bound) residues present in the liver indicate that they are are only 8% as bioavailable as oxfendazole. (Tomlinson, 1976c).

Following oral administration of ^{14}C-oxfendazole to ewes at a dose of 5 mg/kg, $<1\%$ of the dose was excreted in the milk. The composition of the drug related material in the milk has not been examined. (Brebion et al, no date)

Cattle

Twenty-four feeder calves approximately 6 months of age and 160-210 kg in weight were dosed orally once with a suspension of ^{14}C-oxfendazole at the rate of 5 mg/kg. The doses were calculated on the basis of individual animal weights and were administered directly into the rumen using a 50 ml syringe and a piece of Tygon tubing. The animals were held in metabolism cages for the first seven days and then in pens for the duration of the study.

Oxfendazole was labeled in the C-2 position of the imidazole ring. Formulations with three different levels of radioactivity were used. The activities were 0.0193, 0.05176, and 0.4634 mCi/ml, and the higher two activity formulations were used in the animals at the two longer withdrawal times.

The calves were sacrificed in groups of 6 (half males and half females) at 7, 14, 21, and 28 days post dosing. Samples of muscle, liver, kidney and fat were collected and frozen until assayed. All tissues were assayed for total radioactivity and liver was assayed for combined oxfendazole, fenbendazole and oxfendazole sulfone. The later assays are by an HPLC method which includes an oxidation step and actually measures oxfendazole

sulfone. The average total residue levels are shown below in Table V. (Matin and Tsina, 1979)

Table V. Total radioactivity (ppm) in tissues of calves treated with a single 5.O mg/kg oral dose of ^{14}C-oxfendazole

Days Post Dosing	Muscle	Liver	Kidney	Fat
7	0.041 (±0.011)	5.348 (±1.243)	0.954 (±0.183)	0.040 (±0.013)
14	0.010 (±0.004)	2.372 (±0.562)	0.249 (±0.059)	0.009 (±0.005)
21	0.005 (±0.002)	1.245 (±0.196)	0.097 (±0.025)	0.011 (±0.002)
28	0.004 (±0.001)	0.723 (±0.160)	0.056 (±0.014)	0.010 (±0.004)

The liver tissues were assayed for oxfendazole sulfone by HPLC. The average residue levels were 1.116, 0.026, 0.005, and 0.003 ppm at 7, 14, 21, and 28 days post dosing. The HPLC assay has a claimed level of reliable measurement of 0.002 ppm. (Matin and Tsina, 1978a)

Examination of the residue present in the liver of treated animals (6) showed that 3 days after administration of oxfendazole 76% of the total residue was extractable and that of the extractable residue approximately 68% was oxfendazole, 12% was the thioether, and 15% was the sulfone. For the residue present 7 days after dosing 23% was extractable and of the extractable residue 55% was oxfendazole, 6% thioether, and 8% sulfone. (Matin et al, 1977)

Cattle (Dairy)

A suspension of ^{14}C-oxfendazole, labeled at the 2-position of the imidazole ring was administered by gavage (2.5 mg/kg) to three mature, lactating cows. Absorption and excretion were monitored by measuring the isotope concentrations in milk, plasma, urine and feces. At 22 days post-administration one of the animals was sacrificed and the drug residues in liver, kidney, muscle and fat were determined. The concentrations of drug equivalents in the milk are presented in Table VI. Aliquots of the 0-24, 24-48, and 48-72 hours milk pools were extracted and their metabolite contents characterized by TLC. The extractable fraction constituted about 50% of the total radioactivity through the 72 hour period. The composition of the extractable fraction is 70% oxfendazole and 30% reduced or oxidized metabolites in the 0-24 hour pool. The principal metabolite in the 48-72 hour pool is the oxidized species, 2 (methoxycarbonylamino)-5-phenylsulfonyl benzimidazole. (Tomlinson, 1977b)

Table VI. Total ^{14}C-oxfendazole residues in milk following a single oral dose (2.5 mg/kg) to lactating cows.

Time Period (days)	Time Period (hrs)	Milk Residue Concentration (ppm) Average	±SD
0	0	0	0
1	0-12	0.407	0.084
1	12-24	0.490	0.139
2	24-48	0.363	0.121
3	48-72	0.171	0.083
4	72-96	0.067	0.043
5	96-120	0.024	0.017
6	120-144	0.011	0.008
7	144-168	0.005	0.003
8	168-192	0.002	0.001
9	192-216	0.001	0.001
10	216-240	0.001	0.001
11	240-264	0.002	0.001
12	264-288	0.002	0.000
13	288-313	0.001	0.001

Horse

The total drug related residue present in the edible tissue of horses 10, 20 and 41 days after administration of ^{14}C-oxfendazole at a dose of 10 mg/kg was determined . Based on the data obtained (Table VII) liver is the tissue with the highest concentration of residue and from which the residue depletes most slowly. Ten days after dosing less than 1% of the residue in the liver is unchanged oxfendazole. (Tomlinson et al, 1981)

Table VII. Residues in tissues (ppm) obtained from horses treated with oxfendazole (10 mg/kg)

Days Post-Dose	Liver	Kidney	Muscle	Fat
10	4.461	0.104	0.097	0.048
20	0.843	0.012	0.002	0.009
30	0.464	0.014	0.001	0.009

Other Residue Depletion Studies (with Unlabeled Drug)

Sheep

A study was conducted to obtain residue data to allow a withdrawal period to be established for sheep treated with oxfendazole. In this study 20 sheep were given a single 6 mg/kg oral dose of oxfendazole. Groups of four sheep (two males and two females) were slaughtered at 3, 7, 14, 21, and 30 days after dosing. The concentration

of oxfendazole in the liver collected from each animal at slaughter was determined by an HPLC method that has a sensitivity of 0.05 ppm. The mean levels of oxfendazole at 3, 7, 14, 21, and 30 days in liver were 7.86, 3.15, 0.26, 0.08 and <0.05 ppm. In the same study four sheep were dosed with ^{14}C-oxfendazole at 6 mg/kg. Both total residue and unchanged oxfendazole were measured. The results are summarized in Table VIII. (Runkel, et al, 1978)

Table VIII. Total radioactivity and unchanged oxfendazole in liver (ppm) after oral administration of ^{14}C-oxfendazole to sheep at the dose of 6 mg/kg body weight.

Days Post-Dosing	Sheep ID Number	Total Residue	Oxfendazole
3	37	17.1	6.38
7	36	7.34	3.67
14	35	1.31	0.31
21	38	0.26	ND

ND = not detected

Cattle

A study was conducted to obtain residue data to allow a withdrawal period to be established for cattle treated intraruminally with a 22.5% suspension of oxfendazole at a dose of 4.5 mg/kg. In this study 36 cattle were dosed with oxfendazole. Groups of six cattle (three males and three females) were slaughtered at 7, 8, 9, 10, 12, and 14 days after dosing. The individual concentrations of oxfendazole, the thioether (fenbendazole), and the sulfone in the liver collected from each animal at slaughter were determined by a high performance liquid chromatography-assay which has a quantification limit of 0.005 ppm for each analyte. The results of this study are summarized in Table IX. (Spires, 1988b)

Table IX. Concentrations (ppm) of oxfendazole, fenbendazole, oxfendazole sulfone and total metabolites in liver collected from cattle following intraruminal administration of a 22.5% suspension of oxfendazole at a dose of 4.5 mg/kg.

Days Post Dosing	Concentration (ppm) Oxfendazole	Fenbendazole	Sulfone	Sum of Metab.
7	0.042	0.077	0.006	0.125
8	0.032	0.071	0.005[a]	0.108
9	0.009[b]	0.023	0.005[b]	0.037
10	0.036[b]	0.017[a]	0.005[b]	0.058
12	0.005[b]	0.009[a]	0.006[b]	0.020
14	[c]	0.006[b]	0.005[b]	0.016

[a] Some values below the quantification limit of the assay.
[b] Most values below the quantification limit.
[c] All values below the quantification limit of the assay.

Cattle (Dairy)

In one study, six lactating cows were administered oxfendazole at a dose of 5.0 mg/kg. The concentration of oxfendazole in the milk collected over various intervals was determined by an HPLC method which has a sensitivity of 0.005 ppm. Mean concentrations of oxfendazole are summarized in Table X. (Matin and Tsina, 1978b)

Table X. Concentration (ppm) of oxfendazole in milk of lactating cows following oral administration of 5 mg/kg of oxfendazole.

Time Post-Dose (hours)	Mean ± SD
4	0.109 ± 0.032
8	0.208 ± 0.068
12	0.324 ± 0.109
24	0.426 ± 0.155
36	0.290 ± 0.110
48	0.186 ± 0.086
60	0.071 ± 0.037
72	0.022 ± 0.015
84	[a]
96	[a]

[a] All values below the quantification limit of the assay.

Bioavailability

Cattle

A study was conducted to compare the bioavailability of the 22.5% suspension, the 9.06% suspension, the paste, and the pellets (20). The results of this study showed that the 22.5% suspension administered orally, the 9.06% suspension and the pellets are no more bioavailable than the 22.5% suspension administered intraruminally. However, the paste appeared to be more bioavailable than the 22.5% suspension. No significant difference in the bioavailability of the 9.06%, the 2.265%, and the 1.812% is expected. (Massey, 1988b)

A study was conducted with the bound residue fraction of the oxfendazole total residue in cattle liver using the Gallo-Torres bioavailability model. The study was designed to evaluate the bioavailability of the bound residue fraction of liver (Formulation A) relative to the bioavailability of ^{14}C-oxfendazole as a spike in control liver tissue (Formulation B). The study was divided into two parts. The first involves the dosing of a steer with ^{14}C-oxfendazole in order to obtain liver tissue containing bound residues of oxfendazole and the extraction of the liver tissue to remove non-protein bound residues. The second

part consists of the actual conduct of the Gallo-Torres bioavailability experiment with the bile duct-cannulated rats.

Part 1. The "dosed" liver tissue evaluated in the study (Formulation A) was obtained from a beef-breed steer 209 kg in weight. The animal was administered a single 4.5 mg/kg dose of l4C-oxfendazole by injection through the paralumbar fossa on the left side of the animal using a 15 ga x 3.5 inch needle and a syringe. A total of 58 cc of the dosing solution were injected. The tracer had a specific activity of 18.69 μCi/mg and was 98% radiochemically pure. The concentration of the dosing solution was 13.45 mg/ml and the vehicle was PEG 400: 0.5 N HCl v/v. A blood sample was collected 24 hours after dosing and assayed as verification of dosing. Seven days post dosing the calf was killed. The entire liver was collected, cut up, homogenized, and stored frozen. The level of total radioactivity present in the liver was 2.94 ppm.

The liver tissue was prepared for the Gallo-Torres experiment in 50 g aliquots by placing it in a blender and partitioning it between pH 8 phosphate buffer and three portions of ethyl acetate (ETOAc). The organic extracts were discarded, the aqueous liver suspension centrifuged, and the supernatant discarded. The liver pellet was then resuspended in water and centrifuged a second tine. The radioactivity in each of the fractions is shown below as the percent of total DPM. Total DPM was determined by adding the DPM in the ethyl acetate extractions to that for the aqueous homogenate.

Fraction	% of Total DPM
First ETOAc extraction	4.32
Second ETOAc extraction	2.6
Third ETOAC extraction	0.89
First aqueous wash	7.86
Second aqueous wash	1.0
Total extractables	16.7%
Total nonextractable residue	83.3%

The liver pellet was resuspended in water then freeze-dried. The dried liver was then ground to a fine powder. The dose (Formulation A) was prepared by suspending the powdered liver in water (1:4).

Formulation B (liver spiked with l4C-oxfendazole) was prepared with liver tissue obtained from a control animal. The control liver was subjected to the same extraction and lyophilization procedure as done for Formulation A. The control liver powder that was obtained was suspended in water (1:4) and frozen. On the morning of the experiment the suspension was thawed and spiked with ^{14}C-oxfendazole to yield a formulation with approximately the same DPM (90,000) per gram as that of Formulation A.

Part 2. Charles River rats weighing between 200 and 400 grams were prepared for the experiment in groups of two or four. The rats were anesthetized and cannulas were surgically inserted into the bile duct and the stomach to allow the collection of bile and reinfusion of 0.052 M sodium taurocholate. They were housed in individual metabolism cages and were dosed with either Formulation A or B after regaining

consciousness. The doses were administered by intubation into the stomach using a syringe. Each rat was dosed twice with approximately four grams of Formulation A or B with an interval of two hours between doses. Food was offered to the rats after the second dose.

The urine, feces and bile produced by each rat was collected during the dosing period and in 24 hr periods thereafter. Forty-eight hours after the second dose the rats were killed, and the liver and GI tract were removed. Total radioactivity was determined in urine, feces, bile, liver, intestinal tissue, carcass, and intestinal contents. The measurements for urine and bile were done by direct scintillation counting while the other samples were combusted and carcase and intestinal tissues were solubilized followed by scintillation counting.

A total of 14 rats were prepared and dosed as described. Of these, ten survived for 48 hours after dosing, and the data from nine of the rats are tabulated in the results. Poor accountability of radioactivity was obtained with one of the rats, and it was not included in the study report. The results of the experiment are summarized in tables XI and XII.

Table XI. Absorption and Recovery of Radioactivity Present in Protein-Bound ^{14}C-Oxfendazole Derived Residue (Formulation A)

	Rat Number					
	1	6	11	12	Average	% CV
% Dose Absorbed	3.95	10.79	13.45	7.16	8.84	47.0
% Not Absorbed	93.22	91.82	85.59	94.61	91.31	4.4
% Dose Recovered	97.17	102.6	99.04	101.77	100.15	2.5

Table XII. Absorption and Recovery of Radioactivity Present in Liver Spiked with ^{14}C-Oxfendazole (Formulation B)

	Rat Number						
	7	9	10	13	14	Average	% CV
% Dose Absorbed	89.47	58.57	62.90	63.79	74.53	69.85	17.8
% Not Absorbed	8.18	35.79	28.25	27.95	18.13	23.66	45.2
% Dose Recovered	97.65	94.36	91.15	91.74	92.66	93.51	2.8

These results show that the average absorption of of the protein bound residue was 8.84% whereas the average absorption of ^{14}C-oxfendazole was 69.85%. These values yield a relative bioavailability of 12.6% (8.84/69.85). In other words, the oral bioavailability in rats of the protein-bound residue present in the liver of cattle treated with oxfendazole is only about 13% of that of oxfendazole. (Massey, 1988c)

Sheep

One group of rats was gavaged with a suspension of sheep liver flour containing bound ^{14}C-oxfendazole residue, a second group was gavaged with a suspension of liver flour spiked with free ^{14}C-oxfendazole. The ^{14}C concentrations in plasma, bile, urine, feces and tissues were examined and comparisons of isotope bioavailability were drawn between the test dose and the control dose. These comparisons indicated that the bound ^{14}C-oxfendazole administered to the rats is only 8% as bioavailable as oxfendazole. (Tomlinson, 1976c).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The oxfendazole residue in calf liver can be measured by an HPLC method combined with fluorescence detection. The residue measured by this method represents a combined level of the parent drug and its thioether and sulfone metabolites. Oxfendazole and its two metabolites are extracted from liver homogenate with ethyl acetate. Oxfendazole and oxfendazole thioether are oxidized to oxfendazole sulfone with 40% peracetic acid. The HPLC assay has a claimed level of reliable measurement of 0.002 ppm. (Matin and Tsina, 1978a)

Oxfendazole, oxfendazole sulfone, and fenbendazole in liver tissue of cattle can be simultaneously quantitated by HPLC. The method involves the ethyl acetate extraction of a 20 g sample to which an internal standard has been added. Hexane is added to an aliquot of the ethyl acetate extract and the mixture is partitioned with 3N HCl. The aqueous layer is then separated, made basic with 10 N NaOH, and extracted with a mixture of ethyl acetate and hexane (6:1). The organic extract is evaporated to dryness, and the residue is prepared for chromatography by dissolving it in methanol-water (3:1). A reverse-phase column is used for the chromatography with 18-40% acetonitrile in 0.01 M NaH_2P0_4 as the mobile phase. UV detection at 295 nm is used, and the method is stated to have a sensitivity of 5 or 10 ppb for each compound. Recoveries determined by the spiking of control liver tissue at the 5 ppb level averaged 84% for oxfendazole, 104% for oxfendazole sulfone and 108% for fenbendazole. (Fass et al, 1988)

A HPLC method utilizing UV detection for quantitation measures oxfendazole in milk to levels as low as 0.005 ppm. (Matin and Tsina, 1978c)

A HPLC method utilizing UV detection measures residual oxfendazole in sheep liver, kidney, muscle and fat tissues. The initial step involves extraction of the compound from liver, kidney and muscle homogenates with ethylacetate, and from fat with acetone. With this technique oxfendazole can be measured to levels as low as 0.05 ppm in sheep tissues. (Matin and Tsina, 1978d)

APPRAISAL

(See Annex 2 for a combined appraisal of Febantel, Fenbendazole and Oxfendazole).

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