DORAMECTIN

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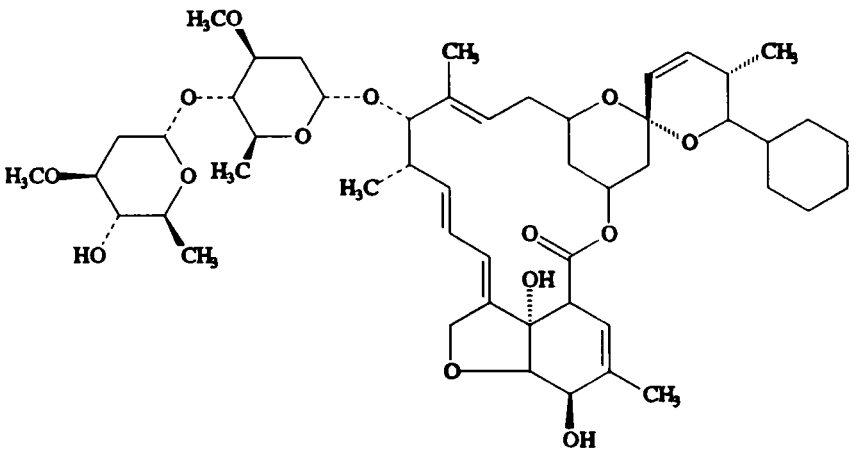
IDENTITY

Chemical name: 25-cyclohexyl-5-O-demethyl-25-de(1-methylpropyl)avermectin A_{1a}

Structural formula:



Doramectin

Synonyms: Doramectin, Dectomax®, UK-67,994

Molecular formula: $C_{50}H_{74}O_{14}$

Molecular weight: 899.14

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Doramectin

Appearance: White to light tan powder

Melting point (average): 160.5°- 162.2°C

Solubility (at 22-25°C): water 0.0003 g/l
acetonitrile 33 g/l
methylene chloride 530 g/l

Optical Rotation: + 12.2° (anhydrous)

UV_{max}: 244 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Doramectin is administered to cattle for the treatment of endo- and ecto-parasitic infections.

Dosage

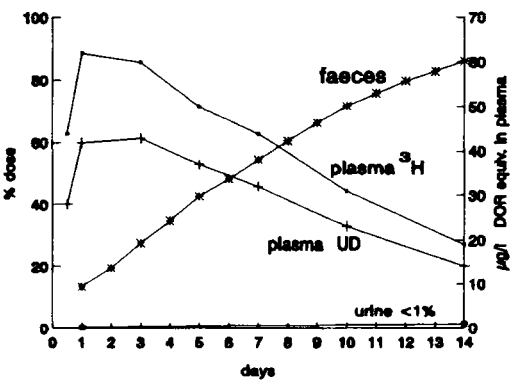
Doramectin is administered to cattle as a single subcutaneous (s.c.) or intramuscular (i.m.) dose of 0.2 mg/kg body weight (b.w.). A repeat dose may be administered eight weeks later.

METABOLISM

Pharmacokinetics

The plasma kinetics of Doramectin were determined in six cattle dosed s.c. with tritiated Doramectin at 0.2 mg/kg b.w. using a prototype commercial formulation (75% sesame oil/25% ethyl oleate) (Pfizer Inc, 1992a). The concentrations of labeled and unlabeled Doramectin and the percentage of the dose excreted in the faeces and urine are shown in Figure 1.

Figure 1. Residues in plasma, faeces and urine of cattle.



Analysis of plasma concentrations used liquid scintillation for quantitation of total residues and chromatographic techniques for the quantitation of unchanged Doramectin (UD).

The apparent terminal half-lives of elimination from plasma of total ^{3}H-labeled materials and unchanged Doramectin were 5.9 and 6.2 days, respectively.

Excretion was almost entirely in the faeces presumably by biliary excretion since significant concentrations of labeled materials were measured in the bile of treated cattle. 86% of the dose was excreted by day 14 and because the curve had not reached a plateau state more drug would be eliminated after day 14. Only about 1% of the label was found in the urine (Pfizer Inc, 1992a).

The drug was well dispersed from the injection site, with less than 1% of the dose remaining at 21 days.

Plasma levels of unchanged Doramectin were also determined in a bioequivalence study comparing s.c. and i.m. administration in cattle. In this study, twenty cattle (10 male castrates and 10 females per group) were treated by either i.m. or s.c. injection with the commercial formulation of the drug at a dose of 0.2 mg/kg b.w. Peak concentration (C_{max}), areas under the concentration curve from 0 to infinity hours post injection ($AUC_{0-\infty}$), and

the K_{el} and elimination half-life ($t_{1/2}$) were determined from plasma concentrations. The half-life of elimination from plasma was determined as 8.0 ± 2.9 days and 6.9 ± 1.6 days following s.c. and i.m. administration, respectively. Animals treated by the s.c. route showed a mean $AUC_{0-\infty}$ of 457 ± 66 ng·day/ml (± 1 SD) and a mean C_{max} of 27.8 ± 7.9 ng/ml. Results from the i.m. treatment group showed a mean $AUC_{0-\infty}$ of 475 ± 82 ng·day/ml and a mean C_{max} of 33.1 ± 9.0 ng/ml. The s.c. and i.m. routes of administration were considered to be bioequivalent since the 90% confidence limits on the difference between the mean $AUC_{0-\infty}$ values fell within ± 20% of the mean value for the s.c. group (Pfizer Inc, 1992b).

Metabolism in Food Animals

The biotransformation of Doramectin was investigated in the rat, dog and cattle (1990a). Tissue distribution studies, discussed in detail below, showed that the highest concentrations of total residues were found in liver and fat of treated cattle, with only traces detectable in muscle and kidney. For this reason, metabolite identification studies were limited to liver and fat. The methods employed included gradient liquid chromatography with radiochemical detection and Fast Atom Bombardment mass spectrometry. A tritium label was introduced into the Doramectin molecule at the 5-position with high specificity (minimum 14.1 mCi/mg). The material used ranged in radiopurity from 95 to ≥97.2%. The label was metabolically stable, since less than 1% was recovered as tritiated water from cattle faeces containing 87% of the dose collected over a 14 day withdrawal period (1992a).

In all species, a large portion of the administered dose remained unchanged drug (component UD). The products of Doramectin metabolism were similar in all species investigated. These metabolites were more polar than Doramectin and were the result of O-demethylation in the distal saccharide ring (component C), of hydroxylation of the 24-methyl group (component B) and a combination of these biotransformations (component A).

Table 1 compares the distribution of metabolites in the liver, fat and faeces of cattle, rats and dogs.

Table 1. Percentage of total radioactivity of the major labeled components in the liver and faeces of cattle, rats and dogs at 2, 3 or 21 days post dose. Rats and dogs were dosed orally with 5 and 3.5 mg/kg b.w. respectively. Cattle received a s.c. injection of 0.2 mg/kg b.w.

Tissue	Species	Day	% ^{3}H recovered	Component[a]			
				A	B	C	UD
Liver	Cattle	3[b]	95	7	ND	9	70
Liver	Cattle	21[c]	82	8	4.4	6.8	57.8
Liver	Rat		37	2	3	12	18
Liver	Dog		51	ND	ND	12	28
Fat	Cattle	21	74-91	ND	ND	ND	91[f]
Faeces	Cattle		75-82	4	5	14	24
Faeces	Rat		NC[e]	16	14	19	22
Faeces	Dog		46	4	5	8	6

[a] As ^{3}H-5-Doramectin
[b] Study CM-92-01, Pfizer Inc, 1992e
[c] Study CM-93-01, Pfizer Inc, 1993c
[d] ND = Not detected
[e] NC = Not calculated since this sample was used as a reference standard.
[f] 4.8-10.9% (mean 7.4%) of total residues were epi-Doramectin.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Overview

Total drug-related residues were measured by the use of tritium labeled Doramectin. The radiochemical purity of ^{3}H-Doramectin used in these studies was ≥ 97.2%. The ^{3}H-label was found to be metabolically stable since less than 1% of faecal radioactivity was recovered as tritiated water (Pfizer Inc, 1990a, 1992a, 1994d).

Two studies were conducted to determine the depletion of Doramectin residues from cattle tissues (see Table 2).

Table 2. Radiolabeled depletion studies using ^{3}H-Doramectin in cattle.

Dose/route mg/kg b.w.	Number cattle per time point	Sampling times (days)	Tissues	Reference
0.2 s.c.	4 (2 MA, 2 FE)	21, 28, 35	M, L, K, F, I.S.	Pfizer, 1990a 1992a, 1993d
0.2 i.m.	4 (2 MA, 2 FE)	7, 14, 21, 28, 35, 42	M, L, K, F, I.S.	Pfizer, 1994d

MA = male, FE = female, M = muscle, L = liver, K = kidney, F = fat, I.S. = injection site.

The first study, 1535N-60-89-009/010 (Pfizer Inc, 1990a and 1992a), suffered from several limitations that were addressed in a second study, 1535N-60-93-016 (Pfizer Inc, 1994d). These limitations, the preparation of the dosing solution under non-GMP conditions and the use of a less robust analytical method for determination of unchanged drug in tissues, particularly liver, do not render the results of this study invalid. However, a more recent study has been completed using, GMP-quality materials, a wider range of time points and the validated regulatory analytical method for the determination of unchanged drug in tissues.

In the first study, 1535N-60-89-009 (Pfizer Inc, 1990a), calves weighing approximately 175 kg received a single s.c. dose of 0.2 mg/kg b.w. ^{3}H-Doramectin in the commercial prototype formulation. Two calves of each sex were euthanized 21, 28 and 35 days after dose administration and samples of muscle, liver, kidney, fat and injection site collected for analysis of total radioactivity and unchanged Doramectin. Subsequent to the completion of the study, deficiencies were noted in the performance of the HPLC analytical method for the quantitation of Doramectin in liver. The improved assay incorporates a more efficient extraction of residues from liver samples. Re-assay of stored samples using the improved method resulted in quantitation of higher concentrations of Doramectin while reassay using the original method indicated that the residues were stable over the three year storage period. Therefore, samples of liver tissue from this study which had been stored frozen at approximately -20°C for up to three years were re-assayed using the proposed analytical method. The results of this study incorporating the reanalysis data for liver tissue using the improved assay are presented in Table 3 (Pfizer Inc, 1993d). There were no differences between the results for males and females.

Table 3. Radiolabeled residues and unchanged drug (μg/kg) in cattle tissues after a single s.c. dose of 0.2 mg/kg ^{3}H-Doramectin[a].

Tissue	Residue Concentration (μg/kg)[b]	Day 21	Day 28	Day 35
Muscle	Total residues (^{3}H)	5	3	2
	Unchanged drug[c]	2.8	0.9	<2.5
	% Unchanged drug	55	41	NE*
Liver	Total residues	86	48	20
	Unchanged drug[d]	41.4	22	7.2
	% Unchanged drug	48	46	36
Kidney	Total residues	14	9	5
	Unchanged drug	6	2	<2.5
	% Unchanged drug	42	30*	NE*
Fat	Total residues	76	42	22
	Unchanged drug	59	19	<2.5
	% Unchanged drug	75	61*	*
Injection Site	Total residues	328	343	7
	Unchanged Drug	128	353	4*
	% Unchanged drug	32	82	82* (N=1)

[a] Study 1535N-60-89-009/010 (Pfizer Inc, 1990a and 1992a); data are means from four animals at each time.

[b] Expressed as Doramectin equivalents.

[c] For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those unchanged drug concentrations falling below the LOQ. Asterisks (*) indicate that some data were below the LOQ. In such cases, the ratio of unchanged drug to total residues is reported as NE (not estimable) or is based on data from three animals or fewer.

[d] Concentrations of unchanged drug determined in the reanalysis of stored samples using proposed determinative method for Doramectin as reported in AHDM-93-02 (Pfizer Inc, 1993d).

A second total residue depletion study was conducted in cattle. This study used i.m. administration of ^{3}H-Doramectin in the commercial formulation (prepared under GMP conditions) at a single dose of 0.2 mg/kg b.w. Calves with a mean weight of 234 kg (2 per sex) at the time of dosing were euthanized at 7, 14, 21, 28, 35 and 42 days after dosing. Samples of muscle, injection site, kidney, fat and liver were collected for analysis of total radioactivity and unchanged Doramectin using a validated HPLC method. The injection site sampled was processed to provide an indication of the concentration of Doramectin residues at the inner 300 g core and the total 500 g sample (Pfizer Inc, 1994d). The results are presented in Table 4.

The results of both total residue depletion studies clearly indicate that the liver and fat are most appropriate for selection as target tissues since the residue concentrations were the highest and were measurable throughout the 42 day withdrawal period. Figures 2 and 3 show the depletion of total and unchanged Doramectin, respectively, over the 42 day withdrawal period in study 1535N-60-93-016. The depletion of total residues and unchanged Doramectin was found to be nearly linear in all tissues over the 7 to 42 day withdrawal period, with the exception of liver which was linear from days 14 to 42, indicating the distribution to the liver was still occurring at day 7. The depletion of the total residues in the edible tissues is shown in Figure 2.

Table 4. Total radiolabeled residues and unchanged drug concentrations (μg/kg) in cattle tissue after a single i.m. dose of 0.2 mg/kg b.w. [^{3}H]-Doramectin[a]

Tissue	Residue Concentration (μg/kg)[b]	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Muscle	Total Residues	40	20	13	10	<3[c]	<3
	Unchanged Drug[c]	33	15.9	10.7	7.78	<2.35	<2.13
	% Unchanged	84	78	80	75	NE*	NE*
Liver	Total Residues	470	415	257	120	42	24
	Unchanged drug %	319	253	154	72.2	22.6	13.2
	Unchanged	68	61	60	61	53	56
Kidney	Total Residues	108	60	35	22	7	4
	Unchanged Drug	96.2	52.1	28.1	16.7	<4.85*	3.11
	% Unchanged	89	87	81	77	71*	77
Fat	Total Residues	551	265	180	115	36	23
	Unchanged Drug	493	230	155	102	25.6	16.7
	% Unchanged	89	87	86	88	74*	73
Injection Site (300 g)	Total Residues	2530	303	206	113	<32	<18
	Unchanged Drug	2230	272	174	93.4	<15.7	12.9
	% Unchanged	90	82	83	84	60*	78
Injection Site (500 g)	Total Residues	2540	672	421	571	<24	18
	Unchanged drug	2300	594	264	331	<12.6	16.4
	% Unchanged	89	80	75	59	64*	91*

[a] Study 1535N-60-93-016 (Pfizer Inc, 1994d), data are means from four animals (2/sex) at each time point.

[b] Expressed as Doramectin equivalents.

[c] For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those unchanged drug concentrations falling below the LOQ. Asterisks (*) indicate that some individual sample values were below the LOQ. In such cases, the ratio of unchanged drug to total residues is reported as NE (not estimable) or is based on data from three animals or fewer.

The ratio of unchanged drug to total residues was found to be essentially constant over the 42-day withdrawal period in all tissues. The ratio of unchanged drug in all tissues was found to range from 53 to 91% with an overall mean of 76%, in liver from 68% on day 7 to 53% on day 35, with an overall mean of 60% and in fat from 89% on day 7 to 73% on day 42, with an overall mean of 83% (Pfizer Inc, 1994d). The total residues and the content of unchanged drug for fat and liver are shown graphically in Figure 3.

Figure 2. **The depletion of the total residues of ^{3}H-Doramectin in cattle tissues after an i.m. dose of 0.2 mg/kg b.w.**

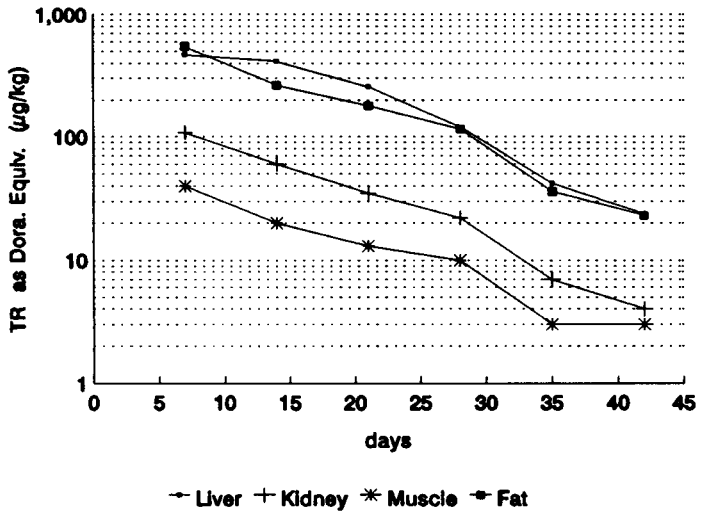
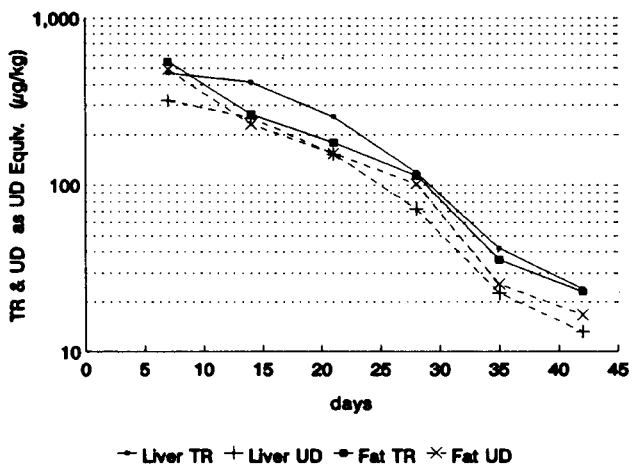


Figure 3. **Residues in fat and liver of cattle treated with an i.m. dose of ^{3}H-Doramectin at 0.2 mg/kg b.w.**



TR is total residues; UD is unchanged drug

Residues at the Injection Site

Concentrations of total residues and unchanged Doramectin in 300 and 500 g injection site samples showed considerable inter animal variability, an important factor in the observed differences in the means shown in Table 5. However, by day 35 post-dose the concentrations of total residues and unchanged drug in edible tissues were found to be less than 100 μg/kg and essentially similar in injection site samples regardless of the size of the injection site sample.

Other Depletion Studies (with Unlabeled Drug)

Cattle

The depletion of Doramectin from cattle tissues was evaluated in three marker residue depletion studies, two of which were conducted by Pfizer in the US and one in Australia by Commonwealth Scientific and Industrial Research Organization (CSIRO) and McMaster Labs. Two of these studies, 1531N-60-90-049 and 2139A-14-92-190 used s.c. administration of Doramectin while the third, 1531N-60-91-050 used i.m. administration of the drug. In all three studies six animals, three per sex, were euthanized at each time point. The results of the three studies are presented in Tables 5, 6 and 7 (Pfizer Inc, 1991a, 1992d, 1994a).

Table 5. Depletion of Doramectin (μg/kg) from tissues of cattle treated with a single s.c. dose of 0.2 mg/kg b.w. of Doramectin[a]

Tissue	Day 14	Day 21	Day 28	Day 35
Muscle	13	<7[b]	<4	<3
Liver[c]	177	107	66	29
Kidney	23	11	8.8	<4.5 ± 2
Fat	288	182	94	57
I.S.	7300	1900	380	930

[a] Study 1531N-60-90-049 (Pfizer Inc, 1991a). Data are means from 6 animals (3/sex) at each time point.
[b] LOQ: 2.5 μg/kg for liver, muscle, kidney and injection site, 5 μg/kg for fat. For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those concentrations falling below the LOQ.
[c] Concentrations in liver were obtained using proposed determinative method as reported in AHDM-93-02 (Pfizer Inc, 1993d)

Table 6. Depletion of Doramectin (μg/kg) from tissues of cattle treated with a single i.m. dose of 0.2 mg/kg b.w. Doramectin[a]

Tissue	Day 14	Day 21	Day 28	Day 35
Muscle	12	7	3	<2[b]
Liver[c]	116	63	30	27
Kidney	24	12	4	3
Fat	182	97	48	37
Inj. Site	838	1033	162	177

[a] Study 1531N-60-91-050 (Pfizer Inc, 1992d). Data are means from 6 animals (3/sex) at each time point.
[b] LOQ: 2.5 μg/kg for liver, muscle, kidney and injection site, 5 μg/kg for fat. For calculation of the means 2.5 or 5 μg/kg, as appropriate, was substituted for those concentrations falling below the LOQ.
[c] Concentrations in liver were obtained using proposed determinative method as reported in AHDM-93-02 (Pfizer Inc, 1993d)

Table 7. Depletion of Doramectin (μg/kg) from tissues of cattle treated with a single s.c. dose of 0.2 mg/kg b.w. Doramectin[a]

Tissue	Day 35	Day 42	Day 49	Day 56
Muscle	<2.9[b]	<2.5	<2.5	NM[c]
Liver	15.9	6.6	3.4	NM
Kidney	<3.6	<2.5	<2.6	NM
Fat	16.6	<10.2	<3.9	NM

[a] Study 2539A-14-92-190 (CSIRO study) (Pfizer Inc, 1994d). Data are means from 6 animals (3/sex) at each time point.
[b] LOQ = 2.5 μg/kg. For calculation of the mean 2.5 μg/kg was substituted for those concentrations falling below the LOQ.
[c] Day 56 samples were not analyzed since day 49 samples were generally below the "practical regulatory limit of analytical determination" defined by the authors as 10 μg/kg.

In their monograph (Pfizer 1994e) the sponsors claim that a statistical analysis of the data obtained in the three studies indicated that the slopes of the three depletion curves were parallel. Furthermore, the variances in the data were similar so that it was appropriate to pool the data to estimate a common depletion curve.

In all three studies the residues of parent drug were highest at the injection site, fat and liver and lowest in muscle and kidney. When all the values for the three studies were plotted for liver and fat the values for the regression line reached 100 μg/kg and 10 μg/kg at 19 days and 40 days for liver and 23 days and 43 days for fat (see Figures 4a and 4b). The elimination half lives in liver and fat were about 7.1 days and 6.7 days respectively.

The elimination half-lives and the regression coefficient of both total residues and unchanged Doramectin from liver and fat of cattle medicated with a single parental dose (i.m. or s.c.) of 0.2 mg/kg b.w. Doramectin were calculated from the individual values (Pfizer 1990a, 1993d, 1994d) and are shown in Table 8. The r values suggest some variance between the individual values, nevertheless the elimination half-lives calculated from the semi-logarithmic regression all lie between 5.6 and 8.1 days and there is no significant difference between the values for either the treatment or the total residues and residues of unchanged drug.

Table 8. Elimination of Doramectin residues from liver and fat after dosing cattle with a parental administration of 0.2 mg/kg b.w. ^{3}H-Doramectin.

Tissue	Route	Residue	Sampling Period (days)	Half-life (days)	r value
Liver	i.m.	Total	14-42	6.44	-0.942
Liver	i.m.	Doramectin	14-42	6.19	-0.940
Liver	s.c.	Total	21-35	6.71	-0.843
Liver	s.c.	Doramectin	21-35	5.59	-0.872
Fat	i.m.	Total	7-42	7.43	-0.964
Fat	i.m.	Doramectin	7-42	6.92	-0.951
Fat	s.c.	Total	21-35	8.06	-0.823
Fat	s.c.	Doramectin	21-35	7.8	-0.812

Bound Residues/Bioavailability

The results of the metabolism and total residue depletion studies conducted with [^{3}H]-Doramectin indicate that Doramectin residues are not tightly bound to tissues. Extraction of Doramectin from all edible tissues of cattle treated with the drug results in quantitation of nearly identical levels of total residues, as quantitated by LSC analysis, and unchanged Doramectin, as quantitated by HPLC. Only in liver are levels of unchanged drug and total Doramectin residues appreciably different; however, this difference is related to the presence of a greater proportion of metabolites rather than tighter binding of unchanged Doramectin to the tissue (Pfizer Inc, 1992e, 1993c, 1994d).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

Doramectin concentration in cattle tissues was determined by a validated HPLC method. The detection and quantitation of Doramectin at the μg/kg level was based on the extraction from tissue homogenate or fat and subsequent conversion to a chemically stable aromatic fluorescent derivative. The method was specific for Doramectin and showed good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin.

High Performance Liquid Chromatography (HPLC)

Liver, kidney, muscle and injection site samples were extracted by incubation at 55°C followed by re-homogenization in the presence of extraction solvent. Fat samples were extracted using incubation at 55°C in hexane followed by homogenization and re-partitioning into acetonitrile. The extracted residues were derivatized using trifluoroacetic anhydride and triethylamine, minimizing exposure to moisture, followed by treatment with methanolic ammonia to yield a fluorescent derivative that was stable to moisture. It was necessary to protect the derivative from light using amber coloured glass vials. Separation was effected using a reverse-phase C18 HPLC-system using acetonitrile, tetrahydrofuran and water in the mobile phase. The amount of Doramectin in each sample was quantitated with or without the use of an internal standard, UK-71,647. The assay showed good sensitivity with a lower LOQ of 2.5 μg/kg and an upper LOQ of 400 μg/kg in liver, kidney, muscle and injection site, and a lower LOQ and upper LOQ of 5 μg/kg and 400 μg/kg, respectively, in fat. Recovery of Doramectin from liver and fat were in excess of 80% and the accuracy of the method was found to be better than 90% and the precision, expressed as the coefficient of variation, was $\leq$ 10% (Pfizer Inc, 1993a, 1993b, 1994b).

High Performance Liquid Chromatography - Mass Spectrometry (HPLC - MS)

The presence of Doramectin in cattle liver and fat may be confirmed at trace (μg/kg) levels using HPLC - MS techniques. Doramectin was extracted from liver or fat using the same methods employed in the determinative HPLC method. Extracts from both tissues were then subjected to clean up by analyte partitioning and re-partitioning into acetonitrile and hexane or elution from a solid phase extraction column. The extract was then analyzed by HPLC-MS/MS, using a triple quadrupole mass spectrometer equipped with an ion spray HPLC interface. Daughter ions (m/z 145, 331, 593, and 899) of the Doramectin ammonium adduct (m/z 916) generated by collision activated dissociation were monitored. The analyses of tissues fortified with 25 to 250 μg/kg Doramectin were successfully carried out as was the analysis of tissues incurred with Doramectin residues at concentrations predetermined to be in the range of 50 and 85 μg/kg. The method was specific for the confirmatory identification of Doramectin in tissue. No interference was found when ivermectin was analyzed by this method (Pfizer Inc, 1992c, 1994c).

APPRAISAL

The assessment of this parasiticide was made easier by the limitations for its use. The drug is only intended for use in non-lactating cattle. There are two applications, a s.c. or an i.m. injection at a dose of 0.2 mg/kg b.w.

Pharmacokinetics

Doramectin was equally well absorbed from the intramuscular (i.m.) and subcutaneous (s.c.) sites of injection. Most of the dose (>86%) was eliminated by day 14 through the faeces with <1% eliminated in the urine.

Metabolism

The metabolism was studied in cattle, rat and dog. The biotransformation was similar in the three species. Unchanged drug was the major component of residues in all tissues and its concentration was linearly related to the concentration of total residues in liver and fat. Three minor metabolites were identified in liver, fat and faeces. These metabolites were more polar than Doramectin and were the result of O-demethylation in the distal saccharide ring, of hydroxylation of the 24-methyl group and a combination of these biotransformations. More than 90% of the residues were readily extracted from the tissues.

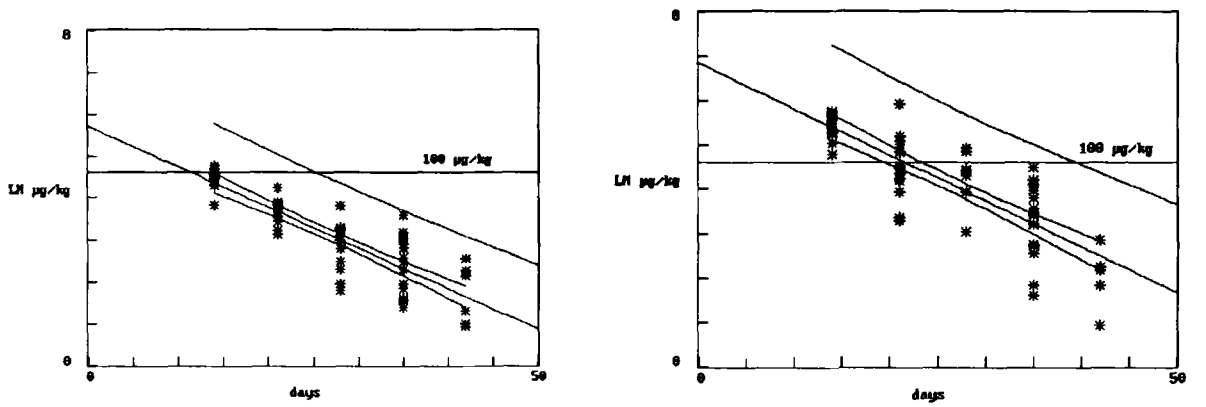
Residues Depletion

Two residue depletion studies were carried out using ^{3}H-labeled Doramectin at the recommended parenteral dose of 0.2 mg/kg b.w. The concentrations of total residues and unchanged drug were measured in the edible tissues and the injection site. In the first study the dose was administered as a s.c. injection and cattle were sampled between days 21 and 35. In the second study the dosing was an i.m. injection and samples were assayed for the period 7 to 42 days post injection. The highest levels of residues in the edible tissues were found in fat and liver. The depletion of total residues and unchanged Doramectin was near linear (semi-logarithmic plot) in all tissues over the sampling periods, with the exception of liver in study two which was only linear from days 14 to 42. The total residues at the injection site were approximately 2.5 mg/kg at day 7 and depleted to values below 100 μg/kg by day 35. The rate of depletion was essentially the same for total residues and unchanged drug in liver and fat with elimination half lives varying between 5.6 and 8.1 days in the studies. Combining the results of both studies the mean percentage of unchanged drug in the total residues was approximately 55% in liver and 80% in fat.

Three studies were reported for measuring the depletion of unlabeled drug in cattle after treatment with either a s.c. or an i.m. injection at the recommended dose of 0.2 mg/kg b.w. The concentrations of the residues appeared slightly higher in the tissues of the cattle given the s.c. dose compared to those in the animals injected i.m. The residues of parent drug were highest at the injection site, fat and liver and lowest in muscle and kidney. For example the mean concentrations in μg/kg at 35 days post dosing by the s.c. and the i.m. routes respectively were 930 and 177 for the injection site, 57 and 37 for fat, 29 and 27 for liver, <3 and <2 in muscle and <5 and 3 μg/kg for kidney. The results from the three studies using parental doses of unlabeled Doramectin were combined for fat (Figure 4a) and liver (Figure 4b). The values for the regression line reached 100 μg/kg and 10 μg/kg at 19 days and 40 days for liver and 23 days and 43 days for fat respectively.

Figure 4. **Residues of Doramectin in fat and liver following parental dosing of 0.2 mg/kg b.w. in cattle.**

4a Fat **4b Liver**



Fat: $t_{1/2}$ = 6.7 days;
r = -0.824

Liver: $t_{1/2}$ 7.1 days;
r = -0.866

The upper curve is the curve to show the limit for the 99th percentile with a 95% confidence that the values would not be greater. The other curves are the regression line bounded by the 95% confidence limits (CL).

The concentration of total residues at the injection site, as measured on day 35, was much lower using radiolabeled drug (7 µg/kg) compared with the concentrations of parent drug of 930 µg/kg and 177 µg/kg for the s.c. and i.m. samples, respectively, in the unlabeled drug studies. There was clearly great variability in the concentration of residues at the injection site.

A analytical method using HPLC was suitable for monitoring residues of parent drug in the edible tissues and injection site. The method was further modified to include mass spectrometry and this provides a confirmatory method. The detection and quantitation of Doramectin at the µg/kg level was based on the extraction from tissue homogenate or fat and subsequent conversion to a chemically stable aromatic fluorescent derivative. The method was specific for Doramectin and showed good chromatographic separation from other avermectins and milbemycins, including ivermectin, abamectin and moxidectin. The assay showed good sensitivity with an LOQ of 2.5 µg/kg in liver, kidney, muscle and injection site and 5 µg/kg for fat and was linear up to 400 µg/kg. Recovery of Doramectin from liver and fat were in excess of 80% and the accuracy of the method was found to be better than 90% and the precision, expressed as the coefficient of variation, was $\leq$ 10%.

Doramectin residues were confirmed by analysis using HPLC-MS/MS. The analyses of tissues fortified with 25 to 250 µg/kg Doramectin were successfully carried out as was the analysis of tissues incurred with Doramectin residues at concentrations predetermined to be in the range of 50 and 85 µg/kg. Ivermectin did not cause interference with the confirmation of the method.

Maximum Residue Limits

The ADI of 0-0.5 µg/kg of body weight established by the Committee is equivalent to 30 µg per day for a 60-kg person. In recommending MRLs the Committee took account of the following factors:

- the drug is only intended for use in non-lactating cattle;

- the target tissues are fat and liver;

- the parent drug is the marker residue and that the percentage of residues of parent drug out of the total residues in each tissue are 55% for liver, 80% for fat, 70% for muscle and 75% for kidney;

- there are < 10% bound residues;

- no multiple or repeat doses are administered; and

- the LOQs of the analytical methods are 2.5 μg/kg for muscle, liver and kidney and 5 μg/kg for fat.

The Committee recommends MRLs for cattle of 150 μg/kg in fat, 100 μg/kg in liver, 10 μg/kg in muscle and of 30 μg/kg for kidney expressed as parent drug. Using these values for the MRLs then the maximum theoretical intake of residues could be 33 μg, expressed as Doramectin equivalents. This would be compatible with a maximum ADI of 30 μg for a 60-kg person.

	MRL (μg/kg)	Factor TR/UD	Total Residues (μg/kg)	Daily food intake (g)	Residue consumed (μg UD equivalents)
Muscle	10	100/70	14	300	4
Liver	100	100/55	182	100	18
Kidney	30	100/75	40	50	2
Fat	150	100/80	188	50	9
Total					**33**

UD is unchanged parent drug; TR is total residues as UD equivalents.

The sponsors have not submitted data for higher doses than 0.2 mg/kg b.w. whether administered as single doses or as multiple spaced repeat doses. The Committee also notes the high concentrations of residues at the injection sites during the 35 day period after parenteral administration of the unlabeled recommended dose.

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