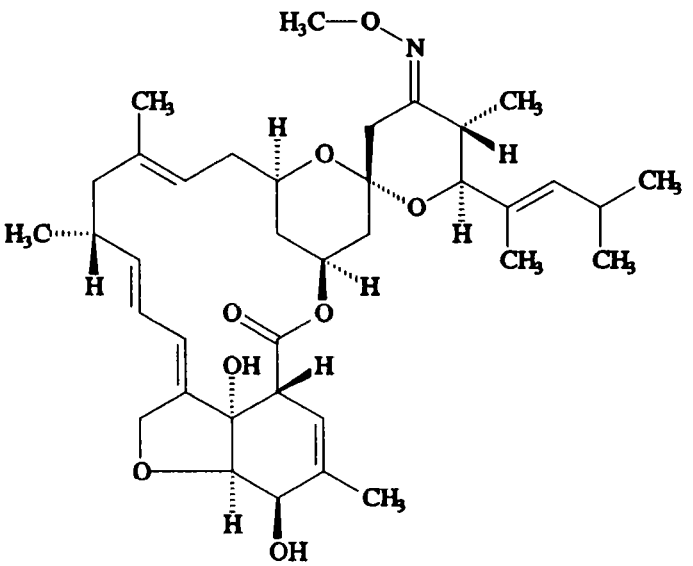
MOXIDECTIN

First draft prepared by
Dr. Raymond J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

Chemical Name: Spiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]-benzodioxacyclo-octadecin-13,2'-[2H]pyran-17-one]-6'-[1,3-dimethyl-1-butenyl]-3',4',5',6,6',7,10,11,14,15,17a,20,20a,20b-dihydro-4'-[methoxyimino]-5',6,8,19-tetramethyl-[6R-[2aE,4E,4'E,5'S*,6R*,6'S*(E),8E,11R*,13R*,15S*,17aR*,20R*,20aR*,20bS*]]-

Structural formula:



Moxidectin

CAS number: 113507-06-5

Molecular formula: $C_{37}H_{53}NO_8$

Molecular weight: 639.84

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: White to pale yellow crystalline powder.

Melting point: Glass transition at 110°C, 145-154°C.

Solubility:	0.51 mg/l water; very soluble in polar organic solvents
Optical rotation:	$+104 \pm 2.7°$
UV maxima:	242 nm in acetonitrile
Fluorescence:	Excitation 380 nm, emission 464 nm
Stability:	Can be stored at 4-25°C for 12 months.
	Moxidectin is a very weak base with a pKa<2
Purity of veterinary preparation:	>90% with <9% Moxidectin related minor components

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Moxidectin is used to control a broad range of internal and external parasites in food producing and companion animals.

Dosage

1. As a subcutaneous injection of 0.2 mg/kg body weight (b.w.) to cattle and sheep
2. As an oral drench of 0.2 mg/kg body weight to sheep
3. As a pour-on dosage of 0.5 mg/kg body weight for cattle and deer

METABOLISM

Pharmacokinetics

Radiolabeled Moxidectin

The radiolabeled materials used were either ^{14}C or ^{3}H-labelled Moxidectin. The ^{3}H-Moxidectin was used only for a preliminary study in cattle. ^{14}C-Moxidectin with a radiochemical purity of 94-98% was used in studies on rats, cattle and sheep.

Excretion into Faeces and Urine

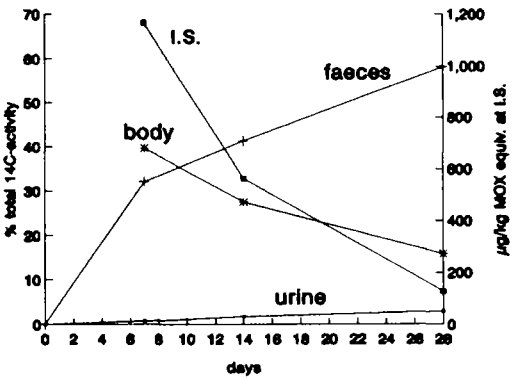
Rats

Male and female rats were administered a single oral dose (1.5 mg/kg b.w. or 12 mg/kg b.w.) or as a daily dose (1.5 mg/kg) repeated for 7 days. The excretion into urine and faeces was measured for 7 days after dosing. Total ^{14}C-recovery averaged 86 to 90% for both sexes and dose levels. The primary route of excretion was through the faeces, 81% for the low dose and 75% for the higher dose. Less than 1% of the radioactivity was excreted via the urine.

Cattle

Three steers averaging 224 kg b.w. were administered a single s.c. injection of ^{14}C-Moxidectin providing 0.2 mg/kg b.w. (MR19). The distribution of the radioactivity is shown in Figure 1.

Figure 1. Radioactivity accumulated in excreta and remaining in the body of steers after s.c. injection of ^{14}C-Moxidectin at 0.2 mg/kg b.w.



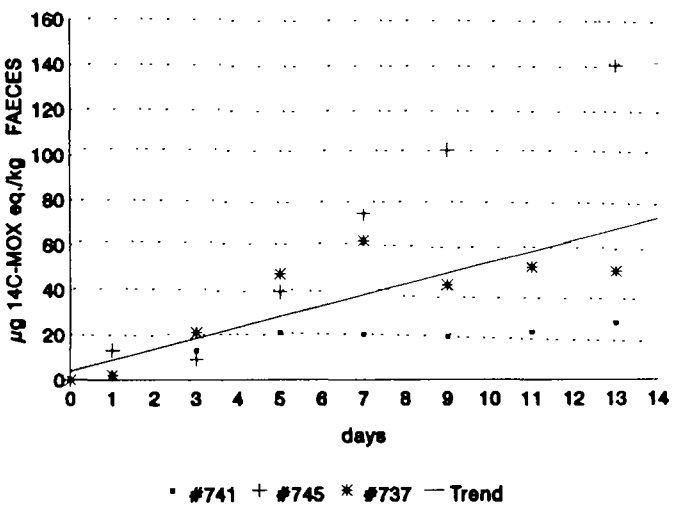
Each time point represents data for a separate steer.

The results for the body include all tissues and body fluids except the head, feet, tail and some large bones which could not be ground. The residues at the injection site (I.S.) are in µg/kg of ^{14}C-Moxidectin equivalents. 96.9 to 98.0% of the residue at the injection site are unchanged drug.

Moxidectin was readily absorbed from the injection site with a half life of about 6.55 days (r = 1.000). In a preliminary study (MR9) using s.c. 0.4 mg/kg b.w. ^{3}H-Moxidectin, the half life at the injection site was 6.4 days. The faeces were the primary route of excretion with <3% of the radioactivity excreted into the urine. This suggests that the large molecule of Moxidectin is not broken down into much smaller molecules which would be cleared into the urine via the kidneys but rather that the molecule and any metabolites are excreted via the bile. Also the residues remaining in the body are clearing gradually indicating that the radioactive residues have a long half life (see below).

Six steers were administered with a pour-on preparation of ^{14}C-Moxidectin at a dose of 0.5 mg/kg b.w. Three steers were slaughtered two days after treatment and three steers at 14 days after treatment. The concentrations of total residues in the urine and faeces were measured daily. The results are shown in Figure 2 for the steers slaughtered at 14 days.

Figure 2. Excretion of radioactivity as ^{14}C-Moxidectin equivalents into faeces of steers treated with a pour-on preparation of Moxidectin at a dose of 0.5 mg/kg b.w.



The excretion into the urine was very low compared to that into the faeces. No radioactivity above the limit of detection (2 μg/l) was detected in the urine of the three steers up to slaughter at 2 days post dosing and in the remaining three steers for the period up to 9 days. Thereafter residues were detected in steers # 745 and #737 between days 10 and 14. The concentrations (μg/l) of residues were:

Steer Days after dosing	0-9	10	11	12	13	14
#741	<2	<2	<2	<2	<2	<2
#745	<2	18	11	12	7	5
#737	<2	2	2	<2	2	<2

Blood Pharmacokinetics

Cattle, sheep and rats were dosed with ^{14}C-Moxidectin (see Table 1 for details) and the radioactivity in blood was monitored with respect to time. The percentage of the dose absorbed, the maximum concentration and the time to peak concentration were calculated and the results are shown in Table 1.

Table 1. Pharmacokinetic Parameters (mean ± 1 SD) for Moxidectin in whole blood following oral or subcutaneous administration in rats, cattle and sheep

Parameter Number	Rats[1] (oral) n=9	Sheep[2] (oral) n=2[5]	Sheep[3] (s.c.) n=3	Cattle[4] (s.c.) n=3
Absorption (%)	18.6 ± 4.6	24.4, 21.0	75.9 ± 18.3	103.3 ± 12.0
C_{max}(μg/l)	13.1 ± 2.3	8, 9	12.3 ± 1.2	47.7 ± 9.3
T_{max} (h)	4.8 ± 1.2	10, 8	8.0 ± 2.0	7.3 ± 4.2
$t_{1/2}$(h)	23[m], 45[f]	18, 21	88	75 ± 19
Reference	MR17	MR2	MR2	MR20

[1]Rats (5m, 4f) were administered a single oral dose of 0.2 mg/kg b.w. (m = male, f = female)
[2]Sheep (wethers) were administered a single oral dose of 0.2 mg/kg b.w.
[3]Sheep (wethers) were administered a single s.c. dose of 0.2 mg/kg b.w.
[4]Steers were administered a single s.c. dose of 0.2 mg/kg b.w.
[5]Individual values for two sheep.

Following subcutaneous administration, ^{14}C-Moxidectin was completely absorbed by cattle and slightly less well absorbed (76% of dose) by sheep. The drug was much less absorbed when administered orally to sheep and rats. The maximum concentration in the blood occurs in less than ten hours, however, the elimination half lives are long in cattle and sheep dosed subcutaneously. When the subcutaneous dose of Moxidectin (as ^{3}H-Moxidectin) was doubled for cattle, the elimination half life was even longer (140 h). (MR9). Comparative analysis of the total ^{14}C in the whole blood, serum and clot of cattle demonstrated that essentially all of the radioactivity was associated with the serum fraction.

Metabolism in food animals and rats

The metabolism of Moxidectin was studied in cattle, sheep and rats. The results are shown in Table 2.

The identity of the codes is:

- 189,056	C-29/C-30 hydroxymethyl	189,021	C-14 hydroxymethyl
- 189,023	C-4 hydroxymethyl	301,310	23-keto

The residues were extracted from both the edible tissues and faeces with mild organic solvents (acetonitrile, methanol) and water. In all cases the majority (86-95%) of the total radiolabeled residues were extracted, indicating that only a low fraction of the residues could be bound residues.

Table 2. Metabolism of Moxidectin in rats, cattle and sheep expressed as a percentage of the total residues

Metabolite Tissue	Rat (oral)[1]	Cattle (s.c.)[2]	Cattle (pour-on)[3]	Sheep (drench)[4]
Moxidectin				
M	63.9	50.0	39	92
L	55.9	40.3	39	51
K	37.2	71.1	55	52
F	86.4	76.4	76[of], 81[bf]	91
189,021				
M	1.4	7.7	11	<1
L	7.5	11.7	17	6
K	2.6	5.3	7	4
F	1.0	1.7	2[of], 2[bf]	1
301,310				
M	<0.1	nd	nd	nd
L	0.7	nd	nd	nd
K	<0.1	nd	nd	nd
F	0.15	nd	nd	nd
189,023				
M	4.2	nd	nd	nd
L	7.5	nd	nd	nd
K	2.9	nd	nd	nd
F	6.9	nd	nd	nd
189,056				
M	<0.1	4.9	10	<1
L	<0.1	9.1	11	12
K	<0.1	2.6	5	12
F	<0.1	1.7	2[of], 3[bf]	2

[1]Rats were administered a single oral dose of 1.5 mg/kg b.w. 7 d previously.
[2]Cattle were administered a single subcutaneous dose of 0.2 mg/kg b.w. 14 d previously.
[3]Cattle were administered a single topical application of 0.5 mg/kg b.w. 14 days previously.
[4]Sheep were administered a single oral drench dose of 0.2 mg/kg b.w. 7 days previously.
[of] is omental fat, [bf] is back fat, nd is less than limit of detection.
M = muscle; L = liver; K = kidney; F = fat

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

The studies carried out using ruminants are listed in Table 3.

Table 3. Studies using radiolabeled Moxidectin.

Radiolabel	Species	Dose/route (mg/kg b.w.)	Sampling time (days)	Reference
^{3}H-Moxidectin	Cattle	0.4 / s.c.	7, 14, 28, 49	MR9
^{14}C-Moxidectin	Cattle	0.2 / s.c.	7, 14, 28	MR19
^{14}C-Moxidectin	Cattle	0.5 / pour-on	2, 14	MR18
^{14}C-Moxidectin	Sheep	0.4 / s.c.	7, 14, 28, 36	MR14
^{14}C-Moxidectin	Sheep	0.2 / oral drench	1, 7, 28	MR1
^{14}C-Moxidectin	Sheep	0.4 / oral drench	7, 14, 28, 36	MR15

Cattle

The total residues in edible tissues was determined using ^{3}H- or ^{14}C-Moxidectin administered either as a s.c. injection or as a pour-on preparation.

Subcutaneous injection administration

Twelve steers were administered a single s.c. injection of ^{3}H-Moxidectin to provide a dose of approximately 0.4 mg/kg b.w. (twice normal dose). Three animals each were sacrificed at 7, 14, 28 and 49 days after injection and samples of edible tissues and the injection site (I.S.) were analyzed for total tritium residues measured as Moxidectin equivalents (MR19). The results are shown in Table 4.

Table 4. Total Residues of ^{3}H-Moxidectin (μg Moxidectin eq/kg) in steers administered a single s.c. injection of ^{3}H-Moxidectin to provide a dose of approximately 0.4 mg/kg b.w.

Tissue	7 days	14 days	28 days	49 days
Muscle	29 ± 4.2	39 ± 8.4	<10	<4
Liver	148 ± 43.2	97 ± 4.2	47 ± 11.1	17 ± 2.9
Kidney	92 ± 39.5	46 ± 2.3	21 ± 4.4	<10
Fat (back)	920 ± 403	685 ± 126	359 ± 84.5	182 ± 14.1
Fat (omental)	974 ± 306	778 ± 60.4	350 ± 82.8	181 ± 12.2
Inj. Site	6220 ± 4530	570 ± 182	667 ± 882	34 ± 35.3

Three steers were administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.2 mg/kg b.w. (the recommended dose). One animal each was sacrificed at 7, 14 and 28 days after injection and samples of edible tissues were analyzed for total ^{14}C-residues expressed as Moxidectin equivalents (MR9). The results are shown in Table 5.

Table 5. **Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in steers administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.2 mg/kg b.w.**

Tissue	7 days	14 days	28 days
Muscle (loin)	21	10	4
Liver	109	77	31
Kidney	42	38	13
Fat (back)	495	424	186
Fat (omental)	898	636	275

Pour-on administration

Six steers weighing 163 to 167 kg were administered a single topical dose of ^{14}C-Moxidectin providing 0.5 mg/kg b.w. Three animals each were sacrificed at 2 and 14 days after treatment. Total residues were determined in the edible tissues (MR18). The results are shown in Table 6.

Table 6. **Total Residues of ^{14}C-Moxidectin (μg Moxidectin equiv./kg) in steers administered a single topical pour-on treatment of ^{14}C-Moxidectin to provide a dose of 0.5 mg/kg b.w.**

Tissue	2 Days	μg/kg	14 Days	μg/kg
	Range (n=3)	Mean	Range (n=3)	Mean
Muscle	<2-<2	<2	<2-3	<3
Liver	2-4	3	5-26	12
Kidney	<2-<2	<2	3-18	8
Fat (back)	<2-7	4	12-129	55
Fat (omental)	7-10	8	33-259	113

The residues were much lower when the pour-on formulation was used compared with s.c. injection treatments. No residue data was provided for the skin or subcutaneous tissues close to the place of application.

Sheep

Subcutaneous injection administration

Four wethers were administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w. (twice the recommended dose). One animal each was sacrificed at 7, 14, 28 and 36 days after injection and samples of edible tissues were analyzed for total ^{14}C-residues measured as Moxidectin equivalents (MR14). The results are shown in Table 7.

Table 7. Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in sheep administered a single s.c. injection of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w.

Tissue	7 days	14 days	28 days	36 days
Muscle (loin)	27	23	<10	<10
Liver	118	83	16	12
Kidney	54	24	<10	<10
Fat (back)	819	363	44	79
Fat (omental)	934	448	49	87

The residues were highest in fat and lowest in muscle. Why the values in fat were higher on day 36 than day 28 was not clear but similar results were observed when an oral drench was administered (see Table 8).

Oral drench administration

Twelve wethers averaging 34 kg b.w. were administered a single oral dose of ^{14}C-Moxidectin providing 0.4 mg/kg b.w. Three animals were sacrificed at 7, 14, 28 and 36 days after treatment. Total residues were determined in the edible tissues (MR15). The results are shown in Table 8.

Table 8. Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in sheep administered a single oral drench of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w.

Tissue	7 days	14 days	28 days	36 days
Muscle (loin)	12	<10-11	<10	<10
Liver	79	45	<10-17	23
Kidney	22	18	<10	<10
Fat (back)	345	284	62[a]	171[a]
Fat (omental)	411	351	70[a]	183[a]

[a] These tissues were reassayed and the high results at day 36 were confirmed.

The residues were highest in fat and lowest in muscle. Why the values in fat were higher on day 36 than day 28 was not clear but similar results were observed when a subcutaneous dose was administered (see Table 7).

In another study eight wethers averaging 36 kg b.w. were administered a single oral dose of ^{14}C-Moxidectin providing 0.2 mg/kg b.w. Three animals were sacrificed at 1 and 7 days and two animals at 28 days after treatment. Total residues were determined in the edible tissues and blood (MR1). The results are shown in Table 9.

The residues were highest in fat. Residues were detected in the blood on day 1 but radioactivity was not measurable by day 7. This might suggest that the drug was rapidly absorbed from the gut.

Table 9. **Total Residues of ^{14}C-Moxidectin (μg Moxidectin eq/kg) in sheep administered a single oral drench of ^{14}C-Moxidectin to provide a dose of 0.4 mg/kg b.w.**

Tissue	1 day n=3	7 days n=3	28 days n=2
Muscle (loin)	25	12	< 4
Liver	135	50	17
Kidney	41	22	< 4
Fat (back)	220	287	113
Fat (omental)	277	322	123
Blood	5	< 2	< 2

Elimination half-lives for cattle and sheep

The half lives were calculated for each tissue using simple semi-logarithmic linear regression analysis of data recorded for residues found at day 7 and later. The results are shown in Table 10 for both cattle and sheep for all the formulations.

Table 10. **Half-lives (days) and regression coefficients of total residues in cattle and sheep with various formulations of Moxidectin**

Tissue / Dose	Cattle s.c. 0.2 mg/kg	Cattle s.c. 0.4 mg/kg	Sheep s.c. 0.4 mg/kg	Sheep drench 0.4 mg/kg
	$t_{1/2}$ (days) r	$t_{1/2}$ (days) r	$t_{1/2}$ (days) r	$t_{1/2}$ (days) r
Muscle	9.0 -0.992	N/A	N/A	N/A
Liver	11.4 -1.000	13.7 -0.978	8.1 -0.984	N/A
Kidney	11.8 -0.967	10.5 -0.917	N/A	N/A
Fat (back)	14.3 -0.983	18.4 -0.943	7.4 -0.909	17.5 -0.677 8.1[a] -0.974
Fat (omental)	12.2 -0.999	17.0 -0.963	7.3 -0.912	15.8 -0.717 7.8[a] -0.968
Inj. Site		6.4 -0.854		
Reference	MR19	MR9	MR14	MR15

[a] The values were calculated after omitting the results for day 36. However the results for day 36 which were much higher values than for day 28 were confirmed after reanalysis (MR15).
N/A indicates that the concentration of the residues were not quantifiable at a sufficient number of time points, usually because they were not detectable at later times.

Ratio of total residues to unchanged drug

Unchanged Moxidectin was the major residue in all edible tissues. The ratio of unchanged drug (parent compound) to total residues was calculated for some of the radiolabeled studies and the results are shown in Table 11.

Table 11. **Percentage of total residues as unchanged parent drug in ruminant tissues.**

Species Dose (mg/kg b.w.)	Days	Muscle	Liver	Kidney	Back Fat	Omental Fat
Cattle s.c. 0.2 mg/kg	7 14 28	62 50 50	48 40 36	74 71 77	83 76 86	95 88 91
pour-on 0.5 mg/kg	14	39	39	55	76	81
Sheep oral 0.2 mg/kg	7	92	51	52	91	
Mean percentage		59	43	66	82	87

Main points from Radiolabeled studies

The residue patterns were similar in cattle and sheep.

1. The residues were lowest when the drug was administered as a pour-on preparation.

2. The residues were always highest in fat with little difference between the residues in omental and back fat.

3. Parent drug was always the major component of the residues, representing about 40-70% of the total residues in muscle, liver and kidney and 75-95% in fat.

4. The residues persisted in fat tissues and the percentage of unchanged drug in the total residues did not alter significantly with time.

5. Fat is recommended as the marker tissue and unchanged drug as the marker compound for all tissues.

6. There is only limited information on residues at injection sites or the tissue in the immediate vicinity of the pour-on application.

Other Residue Depletion Studies (with unlabelled drug)

The list of studies submitted for evaluation are shown in Table 12.

Table 12. Residue studies using unlabelled Moxidectin

Species Tissue	Dose (mg/kg b.w.)	Route	Sampling time (days)	Reference
CATTLE				
M,L,K,F,I.S.	0.2	s.c.	14, 21, 28, 35, 42, 49	MR12
I.S.	2 x 0.2	s.c.	28	MR11
M,L,K,F	0.5	pour-on	7, 14, 21, 28, 35	MR5
M,L,K,F	0.5	pour-on	14, 21, 28, 35, 42	MR8
milk	0.2	s.c.	daily 1-38	MR13
milk	0.2	s.c. 14-70 days prepartum	daily 2-7 postpartum	MR7
SHEEP				
M,L,K,F	0.2	oral drench	7, 14, 20, 28	MR4
M,L,K,F	0.2	oral drench	14, 21, 28, 35, 42	MR3
Fat	0.2 then 0.2 at day 10	s.c. s.c.	10 20, 30, 40, 50	MR21
DEER				
M,L,K,F	0.5	pour-on	7, 14, 21, 28	MR6

M = muscle; L = liver; K = kidney; F = fat; I.S. = injection site

The concentration of residues of the unchanged parent drug, Moxidectin, were determined by an HPLC method. The limit of quantitation (LOQ) was 10 μg/kg tissue and the limit of detection (LOD) 4 μg/kg tissue. In all of the studies residues of Moxidectin in muscle tissue distant from the injection site were below the LOQ. Residues of Moxidectin in liver and kidney were < 10 μg/kg in the studies where either the oral drench or pour-on preparations were used. In the studies using the s.c. dosing, residues were sometimes above the LOQ in liver and kidney during the first month after treatment, however they rarely exceeded 50 μg/kg.

Cattle

Subcutaneous administration

Eighteen steers and eighteen heifers weighing 191-298 kg received a single s.c. injection of 0.2 mg/kg b.w. The animals were sacrificed in groups of six at 7 day intervals. The residues were determined in the edible tissues and at the injection site. The results are shown in Table 13. The 99% upper confidence limits (CL) for the results for fat also were calculated and are shown in Table 13.

Table 13. Residues of Moxidectin (μg/kg) in cattle after s.c. injection of 0.2 mg/kg b.w.

Withdrawal time	Injection Site	Liver	Kidney	Back Fat	99% upper CL for fat
Control	<10	<10	<10	<10	
14	3269	14	27	275	438
21	3848	15	29	243	402
28	4019	<10	22	225	367
35	2332	<10	19	153	332
42	1326	<10	<10	77	296
49	1178	<10	11	141	261

Pour-on application

Two studies were carried out using a pour-on application of Moxidectin at a dose of 0.5 mg/kg b.w. (MR5, MR8). The animals were sacrificed in groups at 7 day intervals over either a 35 or a 42 day period. The residues were determined in the edible tissues and the results for residues in fat are shown in Table 14. The 99% upper confidence limits (CL) for the results for fat also were calculated and are shown in Table 14. The residues in muscle, liver and kidney were less than the LOQ of 10 μg/kg except in one liver sample at 7 days when the concentration was 11 μg/kg.

Table 14. Residues (μg/kg) in bovine fat after administration of a pour-on dose of 0.5 mg/kg b.w.

Withdrawal time (days)	Australian study (MR5)		USA study (MR8)	
	Mean	upper 99% CL	Mean	upper 99% CL
7	21	70	N/A	N/A
14	36	67	92	201
21	31	63	106	192
28	10	59	77	183
35	<10		65	174
42			67	165

N/A = not applicable

Milk

Moxidectin, as with other abermectins, would not normally be recommended for use in lactating animals. Nevertheless studies of residues in milk were submitted (MR7, MR13). In the first study four lactating cows were administered a s.c. injection of 0.2 mg/kg b.w. and residues monitored in the milk for the following 25 days. The concentration of Moxidectin was at a maximum on day 1 (range 60-201 μg/kg) and declined to <20 μg/kg by day 14 and were not detectable (LOQ 10 μg/kg) after day 23.

The second study used thirty three non-lactating late pregnant cows which were given a s.c. dose of 0.2 mg/kg b.w. of Moxidectin at differing times, 1 to 67 days, before parturition. The residues in the milk for the first 7 days after calving were measured. The residues in milk on days 2, 3 and 4 were similar and significantly higher than those on days 5, 6 and 7. The latter values were close to the LOQ of 10 μg/kg or below it. The

residues in the former samples were used to calculate the upper 99% CL for residues in milk with respect to the time of dosing. The 99% CL were:

Time dosed before parturition (days)	14	21	28	35	42	49	56	63	70
Upper 99% CL conc (μg/kg) in milk 2-4 days post-partum	32	30	27	24	21	19	16	13	10

The calves were sacrificed within 24 hours of birth and the residues measured in the edible tissues. No residues were detected in muscle, liver and kidney but were present in fat. Statistical analysis of the data for fat indicated that the 99% upper CL ranged from 122 μg/kg for calves born from cows treated within 14 days of calving to 62 μg/kg for cows receiving Moxidectin 70 days prior to calving.

Sheep

Oral drench administration

Two studies were carried out administering Moxidectin as an oral drench at a dose of 0.2 mg/kg b.w. (MR3, MR4). The animals were sacrificed in groups at 7 day intervals over either a 35 or a 42 day period. The residues were determined in the edible tissues and the results for residues in fat are shown in Table 15. No residues were detected in muscle, liver or kidney.

Table 15. Residues (μg/kg) in sheep fat after administration of an oral drench dose of 0.2 mg/kg b.w.

Withdrawal time (days)	Australian study (MR4)		USA study (MR3)
	Omental fat Mean	upper 99% CL	Back fat range
7	66	131	N/A
14	80	116	25 - 58
21	44	104	< 10 - 23
28	29	88	< 10 - 26
35	N/A		< 10
42	N/A		< 10

N/A = not applicable

Subcutaneous administration

Thirty lambs received a subcutaneous dose of 0.2 mg/kg b.w. After 10 days six sheep were sacrificed and the remainder received a second injection at the same dose of 0.2 mg/kg b.w. (MR21). The lambs were sacrificed in groups of six at 10 day intervals and the residues of Moxidectin in back fat samples were measured (see Table 16). No residues were measured at the sites of injection or in the other edible tissues. Control lambs were also monitored and no residues were detected.

Table 16. **Residues (μg/kg) of Moxidectin in back fat of sheep receiving one or two subcutaneous doses of Moxidectin at 0.2 mg/kg b.w. The second dose was given 10 days after the first dose.**

Withdrawal time (days) 1st	2nd	Mean	Range
10	N/A	222	167 - 314
	10	324	197 - 433
	20	234	183 - 284
	30	139	91 - 223
	40	164	91 - 290

Statistical analysis of the above data indicated that the 99% upper CL on residues in fat were 527, 487, 446 and 406 μg/kg at 7, 14, 21 and 28 days after the second injection, respectively.

Deer

Twenty red deer, 15-16 months old, were treated with Moxidectin pour-on at a dose of 0.5 mg/kg b.w. (MR6). Five animals were sacrificed at 7, 14, 21 and 28 days after treatment. Edible tissues were collected and the Moxidectin content assayed. All residues were below the LOQ in muscle (<24 μg/kg), liver (<6 μg/kg) and kidney (<11 μg/kg). The residues in fat are shown in Table 17.

Table 17. **Residues (μg/kg) in fat of Red Deer after administration of a pour-on dose of 0.5 mg/kg b.w.**

Withdrawal time (days)	Mean conc in fat	Calculated 99% upper CL
7	126	266
14	155	226
21	57	185
28	31	144

Residues at the Injection Site

The residues at the site of subcutaneous injection were measured using ^{3}H-Moxidectin (MR9), ^{14}C-Moxidectin (MR19) and unlabelled Moxidectin (MR12). The samples of injection site were either by size, 15 cm diameter x 2.5 cm depth or by weight about 500 g. The results were very different between animals and also showed relatively low concentrations in the radiolabeled study and high concentrations in the unlabelled Moxidectin study even though the dose of unlabelled drug was a half that of the labeled dose. A further study was carried out using unlabelled Moxidectin at the same dose, 0.2 mg/kg b.w. by s.c. injection at two separate sites, with the purpose of checking the residues at the injection sites at 28 days after injection. About 500 g of tissue was sampled from each site and after separation the amount of muscle tissue was 433-477 g and the 21-66 g were subcutaneous tissue (MR11). The results for each of the three studies are shown in Table 18.

Table 18. Residues (μg/kg) of Moxidectin (MOX) at the injection site in steers.

Withdrawal time (days)	^{14}C-MOX (0.2 mg/kg b.w.) Total residues n=1	^{3}H-MOX (0.4 mg/kg b.w.) Total residues n = 3		Unlabelled MOX (0.2 mg/kg b.w.) n = 6 Moxidectin	
		Range	Mean	Range	Mean
7	1168	517-8685	6220		
14	563	359-712	570	932-8664	3269
21	nm	nm		1479-11988	3848
28	127	90-1484	667	821-11376 19-292[m] 2723-11770[st]	4019 99 6709
35	nm	nm		206-9042	2332
42	nm	nm		78-7020	1326
49	nm	<10-79	34	234-3190	1178

[m] and [st] are the results from study MR11.

There was a wide variation in the results between animals. High concentrations of residues were present in samples throughout the withdrawal period after treatment with the recommended dose of the unlabelled preparation. The majority of the residues may be assumed to be in the fatty tissue of the samples. No explanation was offered as to why the total residues in the radiolabeled study using a two times higher dose should be lower than those for parent drug residues in the unlabelled study. Perhaps there was extensive tritium exchange although this will remain an enigma since no measurements were made of the Moxidectin concentration using the HPLC analytical method.

Bound Residues/Bioavailability

The majority of the radiolabeled residues were extractable with mild solvents. The amount of bound residues is small and insufficient to be taken into account in the calculation of MRLs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

A practical HPLC with fluorescence detection was developed for the determination of the unchanged drug, Moxidectin, in the edible tissues of sheep and cattle (MR26). Samples were extracted with acetonitrile. The extract was partitioned into hexane and concentrated. The compound was reacted with acetic anhydride, 1-methylimidazole and dimethylformamide to form a fluorescent derivative. Further clean-up was done on a fluorosil Sep-Pak column and the hexane fraction was dried and redissolved in methanol. An aliquot of the methanol mixture was applied to an HPLC column (Zorbax ODS 5 μm, 25 cm x 4.6 mm ID) using aqueous methanol as the eluent. The retention time was approximately 8.6 min. A standard curve was prepared and the concentration of Moxidectin determined by interpolation. The LOQ was 10 μg/kg and the limit of detection was 1-4 μg/kg. The average recoveries were measured at three different laboratories and were 96 ± 13%, 94 ± 14% and 96 ± 16%. The method applied to fat was specific for Moxidectin at the 250 μg/kg level in the presence of several of the most commonly used animal health products, namely, Ivermectin, Monensin, Levamisole, Oxytetracycline, Procaine Penicillin G, Rafoxanide, Sulphamethazine and Trenbolone, at fortified levels of 50 - 200 μg/kg and 20 mg/kg for Fenbendazole (MR29). No other abermectins were tested. A similar method was also applied to deer tissues (MR6). This method used ivermectin as the internal standard, recoveries were 70-95%, LOD and LOQ were 1 and 2 μg/kg respectively. An LC-MS method was described for the

confirmation of Moxidectin at the 250 μg/kg level in fat including other macrocyclic lactones (MR31).

APPRAISAL

Pharmacokinetics

^{14}C-Moxidectin was completely absorbed, with peak blood concentrations at 7 - 8 hours, by cattle and slightly less well absorbed (76% of dose) by sheep following sucutaneous administration. When administered orally to sheep and rats the drug was about 20% absorbed. The maximum concentration in the blood occurs in less than ten hours and the half life of Moxidectin is about 80 hours in cattle and sheep.

The excretion of Moxidectin was determined in cattle and rats using radiolabeled drug. The faeces were the primary route of excretion with <3% in cattle and <1% in rats of the radioactivity excreted into the urine.

Metabolism

The metabolism of Moxidectin was similar for cattle and sheep where the principle component of the residues was parent drug, accounting for 75-95% of the residues in fat and 40-90% in other edible tissues at 7 to 28 days after dosing cattle and at 7 days after dosing sheep. In rats parent drug was also the major residue. Two minor residues were found in the ruminants and there was one similar minor metabolite in rats and two different minor metabolites. In all studies the majority (86-95%) of the total radiolabeled residues were extracted, indicating that only a low fraction of the residues was bound.

Residues

The total residues were determined in the edible tissues using radiolabeled drug at one or two times the recommended doses for the s.c. and pour-on preparations for cattle and using the s.c. or oral drench preparations for sheep. The main points from the radiolabeled studies were;

1. The depletion of residues were similar in cattle and sheep and independent of the route of administration or formulation.

2. The residues were lowest when the drug was administered as a pour-on preparation.

3. The residues were always highest in fat and lowest in muscle (for example, in steers given a s.c. dose of 0.2 mg/kg b.w. the levels in fat were 424 and 186 μg/kg compared with 10 and 4 μg/kg in muscle for 14 and 28 days respectively). There was little difference between the concentrations of residues in omental and back fat.

Fat and liver are recommended as the target tissues and unchanged drug as the marker compound for all tissues.

Ten residue studies were carried out using the commercial preparations of Moxidectin. Cattle and sheep were treated with the s.c. preparation, cattle and deer with the pour-on preparation and sheep with the oral drench. The residues of unchanged Moxidectin, at time periods ranging from 7 -50 days, were measured using an HPLC method in the edible tissues and milk. In all cases the residues were low, usually <50 μg/kg, in muscle, liver, kidney and milk and much higher in omental and back fat. The sponsors calculated the upper 99% confidence limits (CL) for the concentration of Moxidectin in fat with respect to time. Over a 14 to 28 day withdrawal period the upper 99% CL were in the range 88-438 μg/kg and declined slowly.

The residues at the site of subcutaneous injection were measured using ^{3}H-Moxidectin and unlabelled Moxidectin. The samples of injection site were either by size, 15 cm diameter x 2.5 cm depth, for the radiolabeled treatment or by weight about 500 g in the unlabelled drug studies. The results were very different between animals and also showed relatively low concentrations (< 10-79 μg/kg at 49 days) in the radiolabeled study and high concentrations (e.g. 234-3190 μg/kg at 49 days) in the unlabelled Moxidectin study even though the dose of unlabelled drug was a half that of the labeled dose. No studies were made to measure the residues

in the tissue in the immediate vicinity of the pour-on application.

Analytical Methods

An HPLC method with fluorescence detection was developed for the determination of Moxidectin in the edible tissues of sheep and cattle. Samples were extracted with organic solvents. The Moxidectin residues were derivatised and after further clean-up on small disposable chromatography columns, quantitated on an HPLC column. The limit of quantitation (LOQ) was 10 μg/kg and the limit of detection (LOD) was 1-4 μg/kg. The average recoveries were measured at three different laboratories and were 94-96%. The method applied to fat was specific for Moxidectin at the 250 μg/kg level in the presence of several of the most commonly used animal health products, namely, Ivermectin, Monensin, Levamisole, Oxytetracycline, Procaine Penicillin G, Rafoxanide, Sulphamethazine and Trenbolone, at fortified levels of 50-200 μg/kg and 20 mg/kg for Fenbendazole. A similar method was also applied to deer tissues. Ivermectin was used as the internal standard, recoveries were 70-95% and the LOQ was 2 μg/kg. An LC-MS method was described for the confirmation of Moxidectin at the 250 μg/kg level in fat.

Maximum Residue Limits

In reaching its decision on MRLs the Committee took into account the following factors:

- the ADI 0-2 μg/kg of body weight is equivalent to 120 μg per day for a 60-kg person

- fat and liver are the target tissues

- the marker compound is parent drug

- 40% of the total residues in muscle, liver and kidney are unchanged drug and 75% of the total residues in fat are unchanged drug

- bound residues are 5-15% of the total residues and information is not available to discard them from the calculation of MRL

- the LOQ of the analytical method is 10 μg/kg

- the sponsors are not proposing to make the drug available for use in lactating cows and late pregnant cows. Thus residues in milk will not have to be taken into account.

The Committee recommends MRLs for cattle, sheep and deer of 500 μg/kg in fat, 100 μg/kg in liver, 20 μg/kg in muscle and of 50 μg/kg for kidney, expressed as parent drug. Using these values for the MRLs then the maximum theoretical intake of residues could be 79 μg:

	MRL (μg/kg)	Factor TR/UD	Total residues (μg/kg)	Daily Food Intake (g)	Residue consumed (μg UD equivalents)
Muscle	20	100/40	50	300	15
Liver	100	100/40	250	100	25
Kidney	50	100/40	125	50	6
Fat	500	100/75	665	50	33
Total					**79**

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

The above MRLs would be compatible with a maximum ADI of 120 μg/person/day.

The Committee recommends a temporary MRL for deer until further information on the marker compound for deer tissues is available. This information was requested for review in 1998.

The Committee noted the very high concentrations and the great variation of residues at the injection site over a 49-day period after dosing cattle.

REFERENCES

All of the references are confidential documents submitted by the sponsors. They are listed in the text by their MR number assigned by the sponsor.