IVERMECTIN

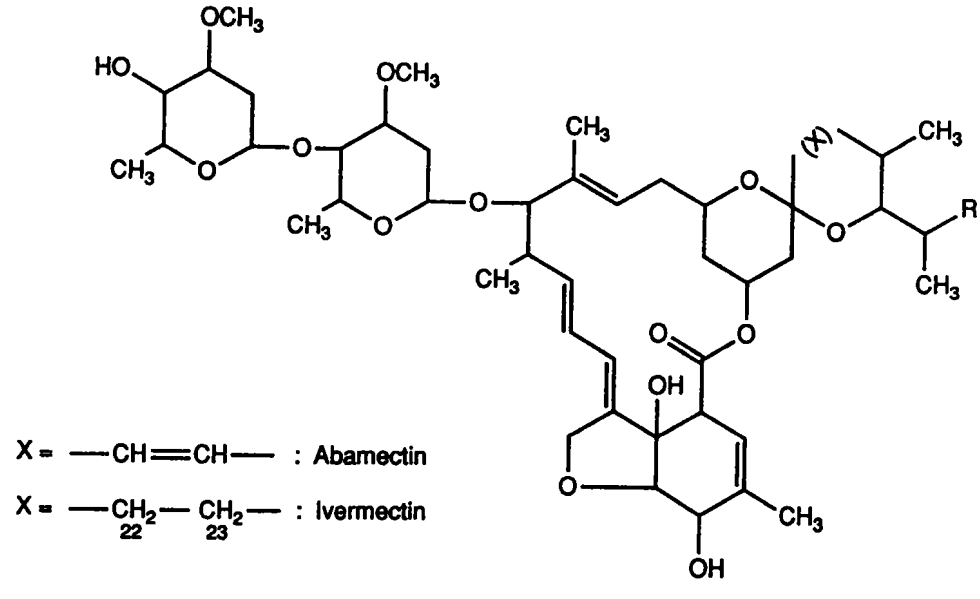
IDENTITY

Chemical composition:

Ivermectin is a chemically modified fermentation product consisting of two compounds differing from each other by only one methylene (-CH_2-) group. The two compounds are designated as 22,23-dihydroavermectin-B1a (or H2B1a) and 22,23-dihydro-avermectin-B1b (or H2B1b). Ivermectin contains no less than 80% H2B1a and no more than 20% H2B1b.

Chemical name: 22,23-dihydroavermectin B1a
22,23-dihydroavermectin B1b

Structural formula:



H2B1a : R = C2H5 H2B1b : R = CH3

Molecular formulae: H2B1a: $C_{48}H_{74}O_{14}$
H2B1b: $C_{47}H_{72}O_{14}$

Molecular weights: H2B1a: 875.1
H2B1b: 861.1

OTHER INFORMATION ON IDENTITY AND PROPERTIES.

Appearance: Off-white powder. $[a]_D$ +71.5°
UV maximum absorbance wavelength in methanol is 238, 245 nm. (E 27, 100, 30, 100)

CONDITIONS OF USE

Ivermectin is a potent endo- and ectoparasitic agent with a broad spectrum of activity in several animal species including cattle, sheep, pigs, horses and humans. Ivermectin may be administered orally, parentally or as a pour-on preparation. The dose is usually in the range 0.2 - 0.6 mg/kg body weight (B.W.).

METABOLISM STUDIES

PHARMACOKINETICS

The most effective antiparasitic agents are those which maintain a high concentration of active ingredient at the site of the parasite for as long a period as possible. This of course is in opposition to the need to have low residues in the edible tissues. The concentrations of Ivermectin and or it's residues in tissues and fluids is influenced by the route of administration. On the other hand the route of administration does not greatly affect the persistence, as measured by the terminal half lives (t1/2), of the residues.

Residue half lives in tissue

The half lives of the total residues and the two components of the parent drug were determined from the data on residues in the animal tissues for muscle, liver, kidney and fat in the cow, sheep and pig. There were insufficient time points or data in the experiments on rats (1, 3 and 7 days post-dosing) and the horse (28 days post-dosing) to perform the calculations for all the tissues and plasma.

The total residues were calculated as Ivermectin equivalents following dosing with tritium labelled Ivermectin. The concentrations of 3H-H2B1a and 3H-H2B1b were measured after purification of the tissues with solvent/solvent partition, Sep-Pak chromatography and high performance liquid chromatography (HPLC).

The half lives were obtained by fitting the best line using least squares regression analysis. They are presented as the terminal half lives and the results are shown in table I.

Table I. Half Lives (days) of Residues in Edible Tissues

	Residue	Muscle	Liver	Kidney	Fat
Steer:	Total	4.6	4.8	5.4	7.6
	H2B1a	4.8	4.7	4.1	4.3
	H2B1b	2.3	4.0	2.2	3.2
Sheep:	Total	1.5	1.2	1.7	1.8
	H2B1a	1.3	1.2	0.9	1.1
	H2B1b	0.7	1.0	0.6	0.9
Pig:	Total	112(a)	5.2	7.0	5.1
	H2B1a	13	3.8	5.4	3.8
	H2B1b	5.3	3.2	4.6	4.0

(a) the correlation coefficient (r) was 0.31 because the concentrations were near the limit of measurement and this result should be disregarded.

Steers were dosed intraruminally with 0.3 mg/kg B.W. and slaughtered over 28 days post-dosing (Baylis et al., 1979)

Sheep were dosed as steers but slaughtered over 7 day period post-dosing (Arison et al., 1981a).

Pigs were dosed subcutaneously with 0.4 mg/kg B.W and slaughtered over a 28 day post-dosing period (Argenbright et al., 1982)

The total residues have longer half lives than those of the parent drug. This is more noticeable in kidney and fat tissues. The long half life of total residues in fat may in part be explained by the deposition of lipophillic acyl esters of metabolites. The half life of the H2B1b moiety is shorter than that for H2B1a suggesting that the metabolism or clearance of H2B1b is faster.

The half lives of the residues in the tissues is several days shorter in the sheep than either the pig or the steer. The reason for this is not established.

Residue Half Lives in Plasma

The concentrations of total residues or Ivermectin residues were measured in plasma samples from cattle, sheep, pigs and horses in experiments long enough to determine the terminal half lives (t1/2) in plasma. The total residues were measured radiometrically and the concentration of Ivermectin determined either radiometrically or by HPLC/UV or Reverse Isotope Dilution Assay (RIDA). The results for farm animals are given in table II.

Table II. Plasma half lives of Ivermectin or total residues

Species	Dose route (mg/kg)	t1/2 (days)	Area under curve (µg.l/h)	Ref.
Cattle:	0.3 i.rumen	5.2		1
	0.3 subcut	5.0		2
Sheep:	0.2 i.venous	ca. 8	9 + 2	3
	0.2 i.abomasal		11 + 5	3
	0.2 i.rumen		2.3	3
	0.2 oral	2.5	2.0 + 0.2	4
	0.2 subcut	3.7	5.7 + 1.2	4
Pig:	0.4 subcut	ca. 4		5
Horse:	0.2 oral	2.8	4.8 + 0.5	4
	0.2 subcut	3.7	13.2 + 0.6	4
	0.6 paste	7.5		6
	0.2 subcut	6.5		6

References: 1. Baylis et al. (1979a); 2. Baylis et al. (1979b); 3. Prichard R.K. et al.(1985); 4. Marriner, S.E., McKinnon, I. and Bogan, J.A., (1987); 5. Baylis et al. (1979c); 6. McKissick et al.

Effect of dose route on plasma levels

Rats were dosed with 3H-H2B1a at a level of 0.3 mg/kg B.W. by one of five routes: gavage (oral), subcutaneous, intramuscular, intravenous or intraperitoneal. Blood (plasma) samples were collected by orbital bleeding at 10, 15 and 30 minutes, 1, 2, 4, 8, 12, 16 and 24 hours after dosing. The animals were sacrificed at 1, 2 and 3 days after dosing and plasma samples collected and stored deep frozen until assayed. The total radioactive residues in the plasma samples was measured and the best fit straight lines were obtained by using least squares regression analysis.

Four hours post dose the residues are roughly the same for all routes of administration, even though the early time values are as expected quite different. The depletion rate for the period 8-24 hours is roughly similar for all routes. After three days the residues levels (0.05 - 0.07 ppm) are generally similar except for the intraperitoneal route where the value is 0.02 ppm and much lower. (Arison et al.,1981b).

Effect of Dose Level on Blood and Tissue Levels

Rats were dosed orally with 3H-H2B1a at four dose levels, namely; -0.06, 0.3, 1.5 and 7.5 mg/kg B.W. The rats were sacrificed 24 hours after dosing and the total radioactivity in the plasma and tissues determined.

There was good agreement between the ratio of the concentration of the dose and the concentration of the total residues in blood and the tissues, see table III. (Arison et al., 1981b)

Table III. Dose Level and Total Residues in Rats

Dose (mg/kg)	Ratio	Plasma	Muscle	Liver	Kidney	Fat
0.06	1	1.2	0.7	0.6	1	2.6
0.3	5	6	5	4	5	5
1.5	25	31	24	19	26	26
7.5	125	125	125	125	125	125

(All ratios calculated by assigning the value of 125 to the highest residue).

Further groups of rats were sacrificed at 3 and 7 days post dosing and the half lives in plasma and liver were determined as,

Dose (mg/kg B.W.)	t1/2 PLASMA (days)	t1/2 LIVER (days)
0.06	-	1.0
0.3	0.86	0.82
1.5	0.98	0.91
7.5	0.98	0.95

The agreement between the ratios and the similar half lives at different doses might be concluded that the metabolism of Ivermectin in the rat is similar over a wide range of dosing.

Excretion of Ivermectin

In the studies reported the excretion pattern is similar for farm animals and rats. The main route of excretion is by the faeces with only about 1% of the dose excreted in the urine.

In a study using three pigs (barrows) dosed subcutaneously with 3H-Ivermectin at 0.4 mg/kg B.W. faeces and urine were collected daily for 7 days post dosing. After 7 days the cumulative amount as a percentage of the dose which was excreted in the faeces was 39.12%, 39.18% and 28.8% (mean 35.7%) and in the urine 0.56%, 0.36% and 0.37% (mean 0.43%). The drug remaining at the injection site in the three pigs was 2.6%, 4.4% and 6.8% of the dose. (Baylis et al., 1979c).

Studies in cattle and sheep where the drug was given intraruminally showed that 64-81% dose was excreted in the faeces within a similar time period to the pig study but again <1% was excreted in the urine. (Baylis et al., 1979a).

METABOLISM IN FOOD AND LABORATORY ANIMALS

Metabolism studies were carried out in four species, the rat, cow, sheep and pig. The metabolism of Ivermectin was studied in liver and fat by dosing the animals with tritium-labelled Ivermectin and separating and identifying most of the residues using modern methods of chromatography and spectroscopy. The metabolism found in vivo was compared with the metabolism of Ivermectin in in vitro studies using microsomal preparations.

The number of rats, steers, sheep and pigs and their treatment with tritium-labelled IVM (3H-Ivermectin) are shown in table IV. The animals were slaughtered at intervals after dosing, the tissues were collected and the total residues of tritium measured.

After examination of the total residue concentrations, liver and fat tissues which were assumed the most appropriate for determining the metabolic profiles of the animals were analysed.

Tavle IV. Total Residue of Tritium (ppb) from 3H-Ivermectin in liver and fat at various withdrawal times

Species	number	Dose mg/kg B.W.	ROUTE	Withdrawal Time (days)	Total Residue(ppb) Liver	Fat
Rat	24	0.3	Gavage	1	152	232
Cow	1	0.3	Intraruminal	14	55	
Cow	1	0.3	Intraruminal	28		40.8
Sheep	1	0.3	Intraruminal	5	34	95
Sheep	1	0.3	Intraruminal	7		32
Pig	1	0.4	subcutaneous	14		21
Pig	5	0.4	subcutaneous	7,14	(a)	

(a) The composite liver contained approx. 1 kg of liver from 3 pigs slaughtered at 7 days withdrawal time and 1 kg liver from 2 pigs slaughtered at 14 days withdrawal time. The liver contained approx. 10 μCi tritium.

Purification and Idenfification of Metabolites

The tissues were stored at -20°C until analysed. The metabolites were extracted from 30-2000 g tissue by a series of solvent solvent partitions and Sep-Pak™ column chromatography. A general outline of the scheme is:

1. Homogenise tissue in polar solvent, usually aqueous acetone.
2. Extract metabolites with polar solvent, e.g. heptane, iso-octane or dichloromethane.

3. Evaporate to dryness and redisolve in acetonitrile/methanol or acetonitrile/water.

4. Wash with isooctane or heptane.

5. Either a) evaporate to dryness and dissolve in heptane (liver) or, b) add phosphate buffer and ethyl acetate and collect ethyl acetate phase (fat).

6. Apply to Sep-Pak™ column and elute sequentially with; dichloromethane, ethyl acetate, methanol/acetonitrile, methanol.

The fraction eluted with ethyl acetate was analysed exhaustively by reversed phase HPLC. (see Chiu et al., 1980a; Chiu et al., 1981).

Where possible the metabolites were further identified by co-chromatography with standards or with in vitro products prepared from the incubation of Ivermectin with steer and rat liver microsome preparations. Also some of the major metabolites were identified by mass-spectroscopy, FAB-MS, and NMR, either with or without hydrolysis to known derivatives. These latter methods were used to identify the 24-hydroxy-methyl-derivatives in ruminant tissues and the 3"-O-Desmethyl-derivatives in pig tissues. (Sestokas et al., 1980; Miwa et al., 1980, Carlin et al.,1980; Chiu et al., 1982a, 1982b, 1982c, 1982d)

Liver Microsomal Preparations

Microsomes from either rat, steer or pig livers were prepared by homogenising 10 g freshly obtained liver samples in Tris, pH 7.5, KCl buffer. The microsomal pellet was obtained after centifugation and washing. The pellet was resuspended in 0.25M sucrose and stored at -80° until needed.

In a typical incubation 10 mg of either 3H-B1a, 3H-H2B1a, 3H-H2B1b or H2B1a in 0.5 ml methanol were mixed with microsomes (equivalent to 200 mg protien), phosphate buffer, NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase in a total volume of 90 ml. The sample was aerobically incubated with shaking at 37° for 30 min.,another addition of the same amount of microsomes was made and the incubation continued for 30 min. The reaction was stopped by the addition of acetone.The samples were purified by solvent/solvent partition and the metabolites eluted from a Silica-gel Sep-Pak™ column as outlined above. The ethyl acetate fraction was further purified on reverse phase HPLC and the metabolites identified by MS and NMR. (Miwa et al., 1980; Chiu et al., 1982e,1982f).

Metabolism of Ivermectin in the Rumen

Ivermectin (40 μg) was incubated with filtered rumen fluid from sheep at 38° under nitrogen/carbon dioxide for two hours. Samples were collected at 30 min intervals and analysed for Ivermectin content (Prichard et al, 1985).

RESULTS

The major metabolites formed in the microsomal incubations were similar for the rat and steer. The major metabolite was the 24-Hydroxy-methyl derivative of the parent

compound with a smaller amount of the monosaccharide of the C24 methyl alcohols of H2b1b (in the rat) and H2B1a (in the steer).

While all of the metabolites have some bioactivity, none were as potent as Ivermectin against C.Elegans.

The C24 alcohols were not formed by the pig microsomes. In fact only about 3% of the parent drug was converted to metabolites. This was sufficient to identify the 3"-O-Desmethyl derivatives of H2B1a and H2B1b as the major metabolites.

The metabolic profiles in liver and fat tissues are shown in tables V and VI. In all 4 species the parent drug was found as the major (45-71%) metabolite in the liver. The metabolism in the rat and the two ruminants was similar in that the same major metabolites were found and there was not a great difference in the percentage distribution of the metabolites.

The metabolism in the pig was different from the other species. Whereas the rat and the ruminants formed 24-hydroxy-methyl derivatives the pig did not, but the pig uniquely produced 3"-O-Desmethyl derivatives in significant quantities (at least 24% of total residues).

There was a linear depletion of the concentration of Ivermectin in the in vitro incubation with sheep rumen fluid. 50% Ivermectin was metabolised in 1.5 hours.

Table V. Metabolites in Liver as Percentage of Total Residue

Metabolite		Rat	Cow	Sheep	Pig
I	Polar	0.06	1.1	-	2
II	Polar	0.4	2.8	8.9	3
III	Polar	3.3	4.2	1.6	?
IVA	24OH-Me derivatives	8	22(a)	20	?
IVB	3"-O-Des-Me derivat.	0	0	0	24(b)
V	Drug-like metabolites	10	5.1	13	14
VI	Parent Ivermectin	71	60	48	45
VII	Non-polar	7.3	1.4	8.1	12
VIII	Non-polar	1.9	3.8	1.4	

(a) Consists of 24-hydroxy-Methyl-H2B1a (17%) with the remainder (5%) a mixture of 24-OH-Me-H2B1b and the monosaccharide of 24-OH-Me-H2B1a.

(b) The residue is divided equally between the derivatives of H2B1a and H2B1b.

Rats were sampled 1 day after dosing with 0.3 mg/kg B.W. (Green et al., 1980)
Steers were sampled 14 days after dosing with 0.3 mg/kg B.W. (Chiu et al., 1980b)
Sheep were sampled 5 days after dosing with 0.3 mg/kg B.W. (Arison et al., 1981).
Composite liver sample from pigs slaughtered 7 and 14 days after dosing with 0.4 mg/kg B.W. (Argenbright et al., 1982).

Table VI. Metabolites in Fat as Percentage of Total Residue

Metabolite	Cow	Sheep		Pig
Days post-dose	28	5	7	14
Polar metabolites	7.3	18.6	3.9	16
Drug like metabolites:				
3"-O-Des-Me-H2B1a	0	0	0	12
3"-O-Des-Me-H2B1b	0	0	0	6
H2B1a-2-epimer	5.5	0	0	0
Others unidentified	0	2.7	0	3
Ivermectin (H2B1a)	12.7	24.0	25.2	28
Ivermectin (H2B1b)	0.9	5.9	5.3	9
Non-polar fraction:				
acyl-24OH-H2B1a	32	+	+	0
acyl-24OH-H2B1b	4	+	+	0
Unidentified	28	40	55	26
Unknown	10	11.6	10.6	0
TOTAL %	100.4	102.8	100	100
Residue (ppb)	44	95	32	28

(+ is identified but not quantitated)

The doses in mg Ivermectin per kg body weight were: Cow, 0.3; Sheep, 0.3; Pig, 0.4.

RESIDUE DEPLETION STUDIES

The concentrations of the total residues and of the parent drug in the edible tissues have been determined in cattle, sheep and pigs. Values are also reported for residues in bovine milk, rats and horses.

The concentrations of residues were highest in liver and fat with much lower values in kidney and muscle.

The parent drug, a mixture of H2B1a and H2B1b, accounted for a major proportion of the total residues and H2B1a is probably a satisfactory marker substance for monitoring residues. The percentage of the total residues found as H2B1a were roughly the same

in sheep and cattle tissues but less H2B1a as a percentage of total residues was found in pigs compared to ruminants.

The radioactive residue in the edible tissues is essentially all extractable in organic solvent such as dichloromethane or toluene. Thus there is very little, if any, intractable covalently bound residues in these tissues.

In sheep, cattle and rats the 24OH-methyl derivatives formed significant amounts of residues. In pigs these derivatives were not found but significant amounts of the 3"-O-Desmethyl derivatives were present.

A non-polar fraction more polar than the parent drug accounted for a main fraction of the residues in fat. The residues were probably the acyl-esters of the metabolites identified above. This formation of lipophilic esters and their deposition in fat most probably explains the high concentrations of and the persistence of residues in fat.

RADIOLABELLED RESIDUE DEPLETION STUDIES

Cattle

In a radiometric study steers were dosed intraruminally or subcutaneously with Ivermectin labelled with tritium in the C22-23 positions at 0.3 mg/kg B.W. Animals were slaughtered at 7, 14, 21 and 28 days after dosing and the total residues and the concentrations of H2B1a and H2B1b measured in the edible tissues. The results are shown in table VII. (Arison et al., 1981).

Table VII. Residues (in ppm equivalent Ivermectin) in tissues of cattle

	Muscle			Liver			Kidney			Fat		
WT (days)	T	1a	1b	T	1a	1b	T	1a	1b	T	1a	1b
Subcutaneous Dose (0.3 mg/kg B.W.)												
7	22	66%	15%	622	56%	8%	66	52%	10%	220	61%	6%
14	5	78%	8%	104	49%	3%	15	45%	5%	88	49%	2%
21	4	50%	?	48	36%	4%	5	50%	?	45	35%	1%
28	1	?	?	32	37%	2%	4	?	?	34	18%	1%
Intrarumenal Dose (0.3 mg/kg B.W.)												
7	8	68%	?	119	31%	8%	19	42%	12%	84	58%	12%
14	3	48%	?	49	43%	6%	5	?	?	26	47%	8%
21	0			14			0			10		
28	0			4			0			8		

WT is the withdrawal time or days post dose; T is Total residues in ppb equivalents of Ivermectin; 1a is H2B1a; 1b is H2B1b. The values for 1a and 1b are expressed as percentages of the total residues.

Pigs

Radiometric residue studies were carried out in pigs dosed subcutaneously with Ivermectin labelled with tritium at the C22-23 positions and at a level of 0.4 mg/kg B.W. The pigs were slaughtered at 1, 7, 14 and 28 days after dosing. Edible tissues were assayed for total radiolabelled residues and both H2B1a and H2B1b using the methods described for residues The results are shown in table VIII. (Argenbright et al., 1982).

Table VIII. Residues in Pigs after dosing subcutaneously with 3H-Ivermectin at 0.4 mg/kg B.W.

	Muscle			Liver			Kidney			Fat		
WT (days)	T	1a	1b	T	1a	1b	T	1a	1b	T	1a	1b
1	43	53%	23%	194	37%	17%	117	36%	13%	288	43%	18%
7	24	39%	11%	110	31%	20%	62	32%	9%	141	50%	13%
14	4	?	?	22	20%	7%	13	24%	7%	22	23%	10%
28	0			3			0			6		

WT is withdrawal time in days post dose. T is total radiolabelld residue expressed as ppb equivalents of Ivermectin. 1a is H2B1a and 1b is H2B1b and the values are the amount of each component as a percentage of the total residue. Each value for the total residues is the mean value for 3 pigs.

Sheep

In two radiometric studies sheep were administered into the rumen tritium labelled Ivermectin at a dose of 0.3 mg/kg B.W. The sheep were slaughtered and the total radioactive residues as Ivermectin equivalents and the concentrations of H2B1a and H2B1b were determined as for the bovine tissues.The results are shown in table IX. (Arison et al., 1981a)

Table IX. Radiolabelled Residues (ppb) in Sheep after Intraruminal Administration of 3H-Ivermectin at 0.3 mg/kg B.W.

	Muscle			Liver			Kidney			Fat		
WT (days)	T	1a	1b	T	1a	1b	T	1a	1b	T	1a	1b
1	43	67%	22%	212	54%	21%	75	51%	22%	245	71%	24%
3	37	52%	22%	105	51%	27%	37	44%	26%	153	57%	8%
5	7	58%	?	23	54%	11%	8	?	?	63	25%	1%
7	10	?	?	44	?	?	13	?	?	73	19%	3%
7	0			11			4			32		
14	0			5			2			24		
21	0			1			0			13		
28	0			2			2			10		

T is total residues; 1a is H2B1a; 1b is H2B1b; ? is either not measured or the value is below the sensitivity of the method. The values for 1a and 1b are expressed as percentages of the total residue.

Horses

The tissue residues of tritium radiolabelled Ivermectin were studied in female horses after a single intramuscular dose of Ivermectin (0.3 mg/kg B.W.) to one horse and single oral doses (0.3 mg/kg B.W.) to two horses. At 28 days post dosing the horses were slaughtered and the total radiolabelled residue concentrations and the concentration of H2B1a (by fluorescent method) were determined and are recorded in table X. (Wood et al., 1981a).

Table X. Residues (ppb) in Horses at 28 days post dosing

Horse	Dose	Muscle	Liver	Kidney	Fat	Inj. site
1	oral	4	3	4	5	-
2	oral	2	3	2	2	-
3	i.m.	14 (13%)	43 (22%)	17 (21%)	36	64

The three values in parenthes is are the amount of H2B1a as a percentage of the total residue. In all the other samples the concentration of H2B1a was below the limit of detection of the fluorescent method.

Rats

Rats were dosed by gavage with tritium labelled Ivermectin at 0.3 mg/kg B.W. The rats were sacrificed at 1 and 3 days post dosing and the total radioactive residues and the

concentrations of H2B1a and H2B1b (by RIDA method) were measured. The results are shown in table XI. (Green et al.,1980).

Table XI. Residues in Rats after oral 3H-Ivermectin.

WT	Muscle			Liver			Kidney			Fat		
(days)	T	1a	1b	T	1a	1b	T	1a	1b	T	1a	1b
1	44	53%	10%	152	66%	6%	140	28%	21%	282	50%	13%
3	18	51%	11%	47	56%	9%	46	34%	27%	137	68%	12%

T is total residues expressed as ppb equivalents Ivermectin. 1a is H2B1a and 1b is H2B1b and the amounts of 1a and 1b are expressed as apercentage of the total residue.

RESIDUE DEPLETION STUDIES

Cattle

In a study using non-radiolabelled Ivermectin, cattle were administered 0.3mg/kg B.W. either injected subcutaneously or given orally. The fluorescent method was used to measure the concentration of H2B1a in edible tissues and the subcutaneous injection site. The results shown in table XII are uncorrected for recovery which was about 80% in spiked tissues. (Wood et al., 1980a)

Table XII. H2B1a (ppb) in Cattle Tissues after Subcutaneous Administration of 0.3 mg/kg B.W.

WT (days)	Muscle	Liver	Kidney	Fat	Inj. Site
2	47	454	92	257	46800
7	37	244	62	201	3550
14	7	84	15	69	83
21	2	38	5	19	27
28	0	11	1	4	1
42	0	0	0	0	0

WT is withdrawal time. Each value is the mean of 5 animals.

Bovine milk

Three dairy cows were dosed parentally (subcutaneously?) with 0.3 mg/kg B.W. Ivermectin. Milk samples were collected and stored deep frozen. Immediately before analysis the sample was heated at 45° with continuous ultrasound for 30 minutes. An aliquot of the mixture was extracted and analysed by the fluorescent assay method for

H2B1a and H2B1b. The residues are shown in table XIII. (Downing, Tait and Wood, 1984)

Table XIII. Residues (ppb) of H2B1a and H2B1b in Bovine Milk

WT (days post dose)	cow 34		cow 199		cow 214		Mean	
	1a	1b	1a	1b	1a	1b	1a	1b
1	9	<3	27	5	15	5	17	3
2	26	7	32	5	23	6	27	6
3	20	6	29	4	36	6	28	5
5	12	<3	17	6	21	5	17	4
7	11	<3	18	6	18	6	16	4
10	11	<3	14	5	11	<3	12	2
14	3	<3	6	<3	4	<3	4	0
21	2	<3	<2	<3	3	<3	2	0
28	<2	<3	<2	<3	<2	<3	0	0

The sensitivity of the method is 2 ppb for H2B1A (1a) and 3 ppb for H2B1b (1b). All values for control milk samples were below the limit of sensitivity for the method.

Residues after external (pour on) application of Ivermectin to cattle

An Ivermectin preparation can be applied to cattle externally by pouring it on the backs of cattle at a dose of 0.5 mg/kg B.W. Steers and heifers were treated with a pour on preparation and the residues of H2B1a in edible tissues determined using the fluorescent assay method. The results are shown in table XIV. (Wehner et al., 1986)

Table XIV. Residues (ppb) of H2B1a in Cattle after External Application of Ivermectin at 0.5 mg/kg B.W.

WT (days)	Muscle	Liver	Kidney	Fat	Dose Site
7	4	48	8	29	13
14	2	27	8	29	14
21	2	19	3	11	4
28	0	12	3	9	5
35	-	9	-	3	2
42	-	3	-	3	2
56	-	0	-	1	2

Pigs

A study using non-radiolabelled Ivermectin was carried out in pigs dosed subcutaneously with 0.4 mg/kg B.W. A total of 35 pigs were dosed and the animals were slaughtered in groups of five at 1, 3, 5, 7, 10, 14 and 28 days post dosing. The concentration of H2B1a was measured in the edible tissues and the injection site using the fluorescent method. The results are shown in table XV and are uncorrected for

recovery. The recovery of spikes of H2B1a from edible tissues was about 80%. (Wood et al., 1981b)

Table XV. Residues (ppb) of H2B1a in Pigs dosed subcutaneously with Ivermectin at 0.4 mg/kg B.W.

WT (days post dose)	Muscle	Liver	Kidney	Fat	Inj. Site
1	24	67	47	74	12500
3	32	69	48	110	5100
5	20	53	32	91	1110
7	13	41	23	73	2300
10	9	23	14	47	2500
14	4	13	5	24	230
28	0	0	0	0	0

Sheep

Sheep were administered subcutaneously non-radiolabelled Ivermectin at 0.3 mg/kg B.W. at weekly intervals for three weeks. Another group of sheep were given Ivermectin orally at a dose of 0.3 mg/kg B.W. The residue concentrations of H2B1a were measured in tissues and the subcutaneous injection site using the fluorescent method. The results are shown in table XVI and not corrected for recovery. The recoveries from tissues spiked with H2B1a were about 80% in all tissues. (Wood et al., 1984; Wood et al., 1980b).

Table XVI. H2B1A (ppb) in Sheep tissues

WT (days post dose)	Muscle	Liver	Kidney	Fat	Inj. Site
Dose: 3 X 0.3 mg/kg subcutaneous					
3	64	160	45	2360	17000
7	75	190	60	310	2900
10	48	97	29	180	2300
14	30	55	20	99	460
28	3	7	2	13	220
Dose: 0.3 mg/kg B.W. oral					
1	20	72	30	145	-
3	4	12	5	32	-
5	2	11	2	11	-
7	2	8	1	9	-
10 & 14	0	0	0	0	-

Each value is the mean value for 4 or 5 sheep per group.

Bioavailability

The sponsors indicate that all the residues are extractable into organic solvents and that none of the residues are covalently bound to tissues. Thus it is assumed that all the residues are bioavailable to the consumer.

METHODS OF RESIDUE ANALYSIS

Tissues were collected from animals dosed with 3H-Ivermectin and stored deep frozen until assayed.

Total residues

Tissues were homogenised in water and an aliquot of the homogenate burned in an oxidiser. The radiolabelled water was collected in Monophase-40 and the radioactivity measured in a scintillation counter in the normal procedure making corrections for quenching. The residue was calculated as Ivermectin equivalents.

Reverse Isotope Dilution Analysis (RIDA)

A microgram RIDA method was developed to measure the amounts of both components of Ivermectin. The method consists of (1) multistep extraction of tissue radioactivity by solvent/solvent partition with the addition of unlabelled Ivermectin as carrier (2) HPLC of tissue extract to resolve H2B1a and H2B1b and (3) determination of the specific activity of chromatographically-recovered H2B1a and H2B1b. The method is appropriate at concentrations of Ivermectin >60 ppb. (Chiu et al., 1980a).

Fluorescence analysis method

A fluorescent assay method was developed for the quantitation of the residues of the two components of Ivermectin and can be applied to tissues in the range 5-60 ppb. This method is more sensitive than the RIDA method. The method consists of 5 steps: (1) Tissue extraction with the addition of H2B1a-monosaccharide as internal standard (2) Fluorogenic derivatization of final tissue extract with aceticanhydride / 1-methylimidazole / DMF at 135° for 1 hour (3) Reversed phase HPLC (4) Construction of standard curve (5) Measurement of Ivermectin by iteration. The sensitivity of the method is about 3 ppb for 10 g tissue. (Chiu et al., 1980c).

H2B1a as a Marker Residue

Where Ivermectin is used in animal production it is essential to have a possible means of monitoring the residues in the animal and the meat products. The measurement of total residues is not possible but this may be overcome by measuring the residue of the parent drug or metabolite if it can be related to the amount of residues. H2B1a is a good candidate as a reference compound (so-called marker residue) because it is the main constituent of the total residues. The general relationship between the amount of H2B1a and the total residues is shown in table XVII.

Table XVII. H2B1a as a percentage range of total residues

	DAYS POST DOSING	MUSCLE %	LIVER %	KIDNEY %	FAT %
Cow	7 - 14	48 - 78	31 - 56	42 - 52	47 - 61
Sheep	1 - 5	52 - 67	51 - 54	44 - 51	25 - 71
Pig	7 - 10	39 - 53	20 - 37	24 - 36	25 - 50
Rats	1 - 3	51 - 53	55 - 56	28 - 34	50 - 66

Whereas the percentage of H2B1a was roughly the same in the ruminant tissues it tended to be less in the pig tissues. Also, but not shown in the table, the percentage of H2B1a tended to decrease in fat with increasing time after dosing.

H2B1a is a reasonably satisfactory indicator of residues of Ivermectin. The method for measuring H2B1a is the fluorescent method which is an accurate and precise method with a good lower limit of sensitivity. However it is a long and expensive method and is not an ideal method for routine screening. There are studies to develop rapid routineimmunoassay methods but no method is yet available.

APPRAISAL

The slow excretion rate together with the long half lives in the peripheral tissues, especially fat, and the short residing time of the drug at the site of administration suggest that the drug is rapidly absorbed into the peripheral circulation from whence it is taken up into secondary depots. Ivermectin and it's metabolites are probably released gradually into the periheral circulation, metabolised in the liver and excreted into the bile and then pass to the faeces.

The parent drug, a mixture of H2B1a and H2B1b, accounted for the major proportion of the total residues and H2B1a was considered a satisfactory marker compound for measuring residues.

A non-polar fraction, more polar than the parent drug, accounted for a main fraction (26-55%) of the residues in fat. The residues in these fractions were tentatively identified as acyl-esters of the major metabolites and their deposition in fat most probably explains the the high concentrations of and the persistence of residues in fat.

There are very little if any bound residues and no further consideration of the bound residues is necessary.

A satisfactory chemical analytical method for measuring the marker compound, H2B1a, is available. The method uses HPLC purification of fluorogenic derivatives and the end point detection by fluorescence of the fluorogenic derivative of H2B1a. The lowest limit of detection is 3 μg/kg.

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