ABAMECTIN

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IDENTITY

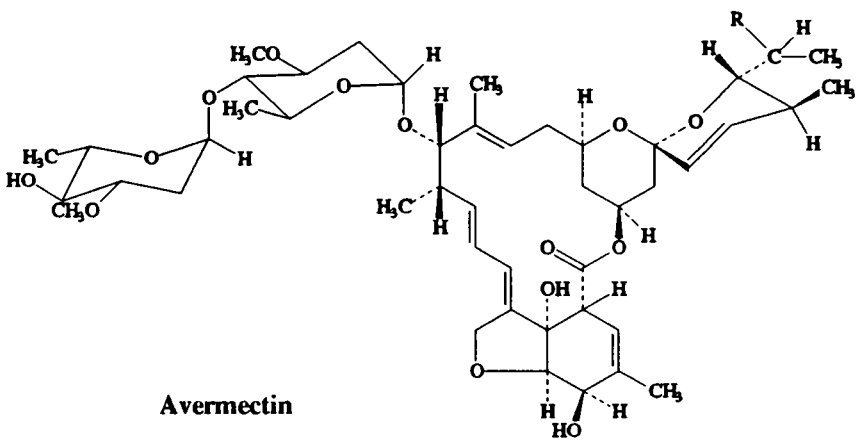
Abamectin consists of two compounds differing from each other by only one methylene ($-CH_2-$) group. The two compounds are designated as avermectin-B_{1a} (or B_{1a}) and avermectin-B_{1b} (or B_{1b}). Abamectin contains no less than 80 % B_{1a} and no more than 20 % B_{1b}.

Chemical name:

Component B_{1a}: (2aE,4E,8E)-(5'S,6S,6'R,7S,11R,13S,15S,17aR,20R,20aR,20bS)-6'-[(S)-sec-butyl]-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-7-yl 2,6-dideoxy-4-O-(2,6-dideoxy-3-O-methyl-α-L-arabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoside

Component B_{1b}: (2aE,4E,8E)-(5'S,6S,6'R,7S,11R,13S,15S,17aR,20R,20aR,20bS)-5',6,6',7,10,11,14,15,17a,20,20a,20b-dodecahydro-20,20b-dihydroxy-6'-isopropyl-5',6,8,19-tetramethyl-17-oxospiro[11,15-methano-2H,13H,17H-furo[4,3,2-pq][2,6]benzodioxacyclooctadecin-13,2'-[2H]pyran]-7-yl 2,6-dideoxy-4-O-(2-6-dideoxy-3-O-methyl-α-L-arabino-hexopyranosyl)-3-O-methyl-α-L-arabino-hexopyranoside

Structural formula:



Avermectin

B_{1a}: R = $-CH_2-CH_3$
B_{1b}: R = $-CH_3$

CAS name and number:

5-O-Demethylavermectin A_{1a}, 65195-55-3

5-O-Demethyl-25-de(1-methylpropyl)-25(1-methylethyl) avermectin A_{1a}, 65195-56-4

Molecular formula:	$C_{48}H_{72}O_{14}$ (B_{1a} component); and $C_{47}H_{78}O_{14}$ (B_{1b} component)
Molecular Weight:	873.09 (B_{1a} component); and 859.06 (B_{1b} component)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Degree of impurity:	Avermectin B_{1a}, min. 80 % Avermectin B_{1a} + B_{1b}, min. 95 %
Appearance:	Off-white to slightly yellow, nonhygroscopic crystalline solid
Melting point:	150-155°C
Optical rotation:	$[\alpha]_D^{25^\circ} = 60°$
Solubility:	Insoluble in water; freely soluble in acetone, chloroform or toluene; soluble in isopropanol; sparingly soluble in n-butanol, ethanol or methanol; slightly soluble in cyclohexane.
Partition (Distribution) coefficient:	9.9×10^3 determined in a n-octanol-aqueous buffer system, pH 7.
Ultraviolet absorbance spectrum:	Absorbance maxima at 237 nm, 245 nm, and a «shoulder» at 253 nm with respective log ϵ values of 4.48, 4.53 and 4.34 (in methanol).

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Avermectin has been developed for use in controlling various phytophagous pests in field crops and citrus (Putter et al., 1981) and fire ants (Lofgren et al., 1982).

Avermectin is also a potent endo- and ectoparasitic agent with a broad spectrum of activity in several animal species. It is a fermentation product produced by the actinomycete *Streptomycetes avermitilis*. (Burg et al., 1979).

PHARMACOKINETICS AND METABOLISM STUDIES

Rat

A study was carried out to evaluate the tissue distribution and elimination of tritium- and carbon-14-labeled avermectin B_{1a} after oral administration to male and female rats (Maynard et al., 1986). Sixty-two male and sixty-two female rats were divided into six groups each. The dosing of the groups were as follows : Group 1, a single high dose (1.4 mg/kg bw) of tritium-labeled avermectin B_{1a}; Group 2, a single low dose (0.14 mg/kg bw) of tritium-labeled avermectin B_{1a}; Group 3, fourteen daily low doses (0.14 mg/kg) of unlabeled avermectin B_{1a} followed by a single low dose of tritium-labeled avermectin B_{1a}; Group 4, a single high dose (1.4 mg/kg) of a mixture of tritium- and carbon-14 labeled avermectin B_{1a}; Group 5, a single dose of vehicle only to serve as control for groups 1, 2, and 4; and Group 6, fifteen daily doses of vehicle only to serve as control for Group 3. The control groups were sacrificed seven days after the last (or single) dose, while rats from the treated groups were sacrificed on days 1, 2, 4 and 7 after dosing. Samples were assayed for total radioactivity and also measured by reverse isotope dilution assay (RIDA) and reverse phase high performance liquid chromatography

(RP-HPLC).

Total Radioactivity

Since plasma or bile data were not available, no absolute measure of absorption is provided. High concentrations of radioactivity in faeces suggest either poor absorption or elimination through bile. 90% of the dose was recovered of which 70% in faeces.

The residue levels measured as total radioactivity were dose dependent as the residues in Groups 1 and 4 (1.4 mg/kg dose) were roughly ten-fold greater than those in Groups 2 and 3 (0.14 mg/kg dose). In contrast, the depletion rates were dose-independent, as to the half lives of Groups 1 and 4 compared to those of Groups 2 and 3 in the same tissues. There was no great difference in depletion half-lives among the tissues as most of the calculated half-lives were within the range of 1.2 ± 0.3 days.

Among liver, kidney, muscle, and fat, the fat had the highest residue levels, though kidney levels at one and two days in some groups were comparable to the fat levels. In most cases, kidney residue levels were higher than the levels in liver.

The tritiated avermectin B_{1a} residue levels and depletion rates in Groups 1 and 4 were of comparable value to the same parameters of the carbon-14 labeled avermectin B_{1a} in Group 4.

There was no effect on either the residue levels or depletion rates from fourteen daily 0.14 mg/kg doses of unlabeled avermectin B_{1a} prior to one 0.14 mg/kg dose of tritiated avermectin B_{1a} (Group 3) relative to a single 0.14 mg/kg dose of tritiated avermectin B_{1a} (Group 2).

The total residue levels in liver, kidney, muscle, and fat of the females of each group were generally higher than residue levels in the corresponding tissues of the males. However, the depletion rates were similar for residues in the tissues of the male and female rats.

The female rats eliminated 0.3 to 0.6% of the dose in urine, as compared to 0.8 to 1.1% of the males. The females excreted 68.7 to 76.7% of the dose in feces, as compared to 70.1 to 81.6% of the males.

RIDA

From data on the levels of avermectin in tissues as measured by RIDA the following results were observed: (a) the amount avermectin B_{1a} of the total residues was not dose dependent; (b) the half-life for avermectin B_{1a} in male tissues (0.6-1 day) was slightly less than in female tissues (0.9-1 day); (c) the amount of avermectin B_{1a} of the total residues was similar for both tritium and carbon-14 labeled avermectin B_{1a}; (d) the amount of avermectin B_{1a} of the total residues was lower in male than female tissues; (e) the amount of avermectin B_{1a} of the total residues was only slightly affected by the pre-treatment with avermectin B_{1a}.

RP-HPLC

The tissue residues were characterized by RP-HPLC radio-activity profiles. In addition to unchanged avermectin B_{1a}, two metabolites were observed. Identification of these two metabolites was accomplished by retention time comparison to standard compounds and by co-chromatography with these standard compounds. These metabolites that were also isolated from a rat liver microsomal incubation of avermectin B_{1a} and were identified as 24-hydroxymethyl-avermectin B_{1a} (24-OHMe-B_{1a}) and 3"-desmethyl avermectin B_{1a} (3"-DM-B_{1a}). Most of the residues in muscle, fat and organ tissues of rat were identified as avermectin B_{1a}, 24-OHMe-B_{1a}, and 3"-DM-B_{1a}.

The RP-HPLC radioactivity profiles of tissue extracts from rats (Group 4) dosed with both tritium and carbon-14 labeled avermectin B_{1a} were investigated. Both ^{3}H-b_{1a} and ^{14}C-B_{1a} had identical profiles with all radio-active peaks having a constant $^{3}H/^{14}C$ ratio and the same percentage of recovered radioactivity. Therefore, the metabolism of both ^{3}H- and ^{14}C-avermectin B_{1a} by rats was identical demonstrating that the use of tritium labeled avermectin B_{1a} is appropriate for animal metabolism studies.

In males, avermectin B_{1a} accounts for 50% of the total residues in kidney, liver and muscle at one day post dosing. At 4 days, it represents 35%, and at 7 days less than 10%. In females, the average values were respectively 55-74% at one day, 45% at 4 days, and 16-28% at 7 days. These results indicate that the metabolism of avermectin B_{1a} was higher in males than in females. A non-polar fraction has been investigated in fat as it accounts for 5-8 % of the total residues at one day post dosing and 41-64% at 7 days. The results obtained indicated that this fraction contains conjugates of 24-OHMe-B_{1a} and 3"-DM-B_{1a} metabolites.

TISSUES RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies in cattle

Cattle

Twelve Angus steers were given a subcutaneous injection of 0.3 mg tritium-labeled abamectin /kg body weight. The animals were slaughtered at 7, 14, 21, and 35 days post-dose for collection of tissues. Tissues were assayed for total radioactive residues and also by RIDA and RP-HPLC.

Total radioactivity

The mean plasma concentration of radioactivity peaked at 95 μg abamectin equivalents/l (range 34 to 162 μg/l) at 1-2 days post administration and declined with a half life of 4.7 days. The mean plasma concentration declined to 1 μg/l at day 35.

Abamectin is eliminated mainly in faeces. About 50% of the dose was recovered within 7 days in faeces and 1-2% in urine.

The average residue levels for the tissues are summarized in Table 1.

The limit of detection was respectively 0.7 μg/kg for muscle, 4 μg/kg for injection site, 0.3 μg/kg for liver, 1 μg/kg for kidney and 3 μg/kg for fat. (Lu et al., 1984).

Table 1. Total residues as abamectin equivalents ± SD (μg/kg) found in cattle injected subcutaneously with abamectin at 0.3 mg/kg bw

Days Post Dose	Muscle	Injection Site Muscle	Liver	Kidney	Fat
7	42 ± 10	2022±1720	619 ± 190	143 ± 37	444±110
14	10 ± 8	537 ± 61	168 ± 95	36 ± 8	130±76
21	4 ± 1	59 ± 87	61 ± 33	11 ± 5	63±22
35	1 ± 1	< 4	9 ± 6	2 ± 1	35±10

The depletion rates of residues in kidney, liver, and muscle between 7 and 35 days were all similar. Of the edible tissues, the liver had the highest residue levels at 7 and 14 days. By 21 days, the average liver residue levels had dropped to less than the average fat residue levels. The corresponding half-lives for residue depletion in kidney, liver and muscle were 5.7, 4.6, and 5.6 days, respectively.

The residue half-life in fat, 8.1 day, was longer than that in the other tissues. The muscle injection site had the highest residue level at 7 days but had also the highest depletion rate, with a half-life of 3.3 days.

RIDA

Liver and fat tissues from steers slaughtered 7, 14, and 21 days post dosing were analyzed for unaltered drug

by RIDA. Results indicate that B_{1a} accounted for 34-61% of the total tissue residues in all liver samples studied. The minor drug component, B_{1b}, contributed to only 0.9-4.8% of the total residues in these tissue samples. The unaltered B_{1a} in the fat tissue accounted for 52, 40 and 25% of the total tissue residues at 7, 14, and 21 days post dose, respectively (Table 2). Similar to that in the liver tissue the B_{1b} component contributed to only 0.5-5.2% of the total residues in these fat samples. The RIDA results indicate that the unaltered drug should be a satisfactory marker substance in both the liver and fat tissue and the former should be a satisfactory target tissue for metabolism studies. (Lu et al., 1985).

Table 2. Total residue concentrations (μg/kg) and % of B_{1a} in liver and fat of cattle dosed subcutanously with 5-^{3}H-abamectin at 0.3 mg/kg bw

Days Post Dose	Liver		Fat	
	TR	% B_{1a}	TR	% B_{1a}
7	616 811	61 34	462 544	47 58
14	275 139	41 48	216 105	41 39
21	62 94	44 41	62 85	24 26

RP - HPLC

In addition to the assay of the unaltered drug, the profile and composition of the total radioactive residues from the liver tissue of a 14 days post dose steer was studied by solvent fractionation and HPLC. The liver residues were classified on the basis of polarity by RP-HPLC elution. A group of polar metabolites accounting for 22% of the total residue is present in this sample among which the major metabolite was identified as 24-hydroxymethyl-B_{1a} by chromatographic comparison with an *in vitro* metabolite sample generated by rat liver microsomal incubation of B_{1a}.

Similar residue profile studies were carried out with a fat tissue sample from a steer slaughtered 21 days after dosing. Other than the unaltered drug, the major portion of the radioactivity resided in two groups of nonpolar metabolites of which the major one consisted of at least 7 compounds and accounted for 51% of the total tissue residues. Upon treatment with cholesterol esterase, these nonpolar metabolites gave one major polar product which was identified as 24-hydroxymethyl-B_{1a} by mix-sample chromatography with unlabeled *in vitro* metabolite samples.

For the purpose of comparison, residues from composite liver and fat tissue of rats dosed orally with 5-^{3}H-B_{1a} were studied similarly to those from the steer. Examination of the residue profile and composition shows that essentially the same pattern of metabolism exists in the two species. (Lu et al., 1985).

Other Residue Depletion Studies (with Unlabeled Drug)

Cattle

Thirty nine male castrates and 39 female cattle, aged approximately 18 months and weighing between 292 and 388 kg, were allocated to one of seven treatment groups: Group 1 untreated controls (3 males and 3 females), and Groups 2 to 7 each consisting of six males and six females treated subcutaneously with 0,2 mg abamectin/kg bw. The animals were sacrificed on days 21, 28, 35, 42, 49, and 56, respectively, and assays were carried out using an HPLC/fluorescence method having a detection limit of 1 μg/kg.

By 42 days post-treatment the mean tissue residues of avermectin B_{1a} had depleted to 2 μg/kg or less, for injection site from 5,200 μg/kg (range 11 to 33,000) at 21 days to 2 μg/kg (range 0 to 22), for liver from

53 μg/kg (14 to 88) to 2 μg/kg (0 to 6), for fat from 78 μg/kg (17 to 200) to 2 μg/kg (0 to 6), for muscle from 6 μg/kg (1 to 9) to below the limit of detection-1 μg/kg (0 to 1), and for kidney, from 13 μg/kg (3 to 27) to 1 μg/kg (0 to 2). Mean residues in liver and injection site had depleted to below the detection limit by 49 days post-treatment. Other tissues at this time point were not assayed. The results are summarized in Table 3. (D.G. Baggot et al,. 1992).

Table 3. Avermectin B_{1a} residues (μg/kg) found in cattle injected subcutaneously with abamectin at 0.2 mg/kg bw

Days Post Dose	Muscle	Injection Site	Liver	Kidney	Fat
21	6	5200	53	13	78
28	2	2000	14	4	13
35	1	550	9	2	5
42	<1	2	2	1	2
49	NA	<1	<1	NA	NA

NA: Not Assayed

Six groups of 5 cattle each were dosed subcutaneously at approximately 0.3 mg/kg bw. Selected liver, fat, muscle, injection site and kidney tissues were assayed from animals sacrificed at 5, 10, 15, 20, 25 and 35 days post injection. The assays were carried out with an HPLC fluorescence method having a detection limit of 1 μg/kg. The injection site tissue residues averaged 21 μg/kg avermectin B_{1a} for animals sacrificed at 5 days post dose and depleted to an average of 0.57 μg/kg at 35 days post dose. The liver tissue residues depleted from a mean of 310 μg/kg at 5 days post dose to a mean of 16 μg/kg at day 35. The fat tissue residues paralleled the liver residues and averaged 320 and 12 μg/kg at 5 and 35 days, respectively. The muscle tissue contained residues averaging 27 μg/kg at 5 days post dose and depleted to an average of 2 μg/kg at 35 days post dosing. The kidney tissue residues depleted from an average of 36 μg/kg at 15 days to an average of 6 μg/kg avermectin B_{1a} by 35 days post dose. These results are summarized in Table 4. (T.A. Wehner et al., 1991).

Table 4. Avermectin B_{1a} residues (μg/kg) found in cattle injected subcutaneously with abamectin at 0.3 mg/kg bw

Days Post Dose	Muscle	Inj. Site Muscle	Liver	Kidney	Fat
5	27	21000	310	NA	320
10	NA	3300	210	NA	130
15	8	5400	110	36	73
20	3	800	30	9	30
25	< 1	1600	7	2	4
35	2	570	16	6	12

NA: Not Assayed

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The method proposed to control abamectin residues is an HPLC fluorescence assay of avermectin B_{1a}.

(Wehner et al., 1991b).

The basis of the method is the isolation of the marker residue by solvent extraction followed by preparation of a fluorescent derivative. The method is applicable to muscle, liver, kidney and fat. Its limit of quantification is 5 μg/kg and limit of detection 1-2 μg/kg.

At about 2.5 μg/kg level, improvement in the method and greater separation from control tissue constituents can be achieved by adding an additional clean up step. At the 1 μg/kg level satisfactory assay values can only be achieved by either major revisions of the method or, slight modification of the HPLC system used. Below 1 μg/kg the HPLC system is not sensitive enough when 5-gram samples are used.

The average recoveries of avermectin B_{1a} for the different tissues were in the range of 70-90% and the coefficient of variation below 15%. This method is specific to avermectin B_{1a}.

APPRAISAL

In reaching its decision on the MRLs for abamectin, the Committee took into account the following:

- An ADI of 0-0.2 μg per kg of body weight has been established by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1994. This would result in a maximum daily intake of residues of 12 μg for a 60-kg person;

- Liver and fat are considered as the appropriate target tissues;

- Abamectin, used as veterinary drug, is only intended for use in beef cattle;

- In fat tissue, abamectin does not lead to bound residues and less than 15% in liver;

- Avermectin B_{1a} is considered as an appropriate marker residue;

- Avermectin B_{1a} accounts for 42% of the total residues in liver and 25% for fat at 21 days post-dosing; and

- The limit of quantification of the proposed analytical method is 5 μg/kg.

The Committee noted that the 1992 JMPR evaluated abamectin residues resulting from pesticide use. The ADI of 0-0.2 μg/kg of body weight was established by JMPR using a safety factor of 500 to account for the concern of the delta-8,9 isomer of B_{1a} which does not occur with use as a veterinary drug.

MRLs have been recommended by JMPR for some vegetables and fruits, and for cattle and goat meat and milk to take into account possible contamination of these food commodities resulting from the ingestion of vegetables and fruits treated with abamectin by these animals.

According to the food basket that the Joint FAO/WHO Expert Committee on Food Additives (JECFA) uses, the daily intake of residues, resulting from the following MRLs recommended by JMPR for these two animal species, 10 μg/kg for muscle, 50 and 100 μg/kg for offal in cattle and goat, respectively, and 5 μg/kg for milk, would significantly exceed the ADI of 12 μg/person.

In order to comply with the ADI, the Joint FAO/WHO Expert Committee on Food Additives would have to recommend MRLs for cattle muscle, liver, kidney and fat different from those recommended by the Joint FAO/WHO Meeting on Pesticide Residues.

Therefore, the Committee did not recommend MRLs for abamectin used as veterinary drug and recommended that consultations be held between representatives of JECFA and JMPR to resolve the ADI and MRL issues.

(Editor's note: Consultations were held 15 September 1995. Subsequently, JMPR at its 1995 meeting increased the ADI for abamectin to 0-1 μg per kg of body weight for uses in animal health. MRLs for abamectin will be reconsidered by JECFA at its 47th meeting, scheduled to be held in Rome, June 1996.)

REFERENCE

Baggot, D.G. et al. (1992). A study to determine avermectin B_{1a} residues in liver, kidney, fat, muscle and injection site tissue from cattle dosed subcutaneously with abamectin (MK 0936) at 1 ml 1 % formulation per 50 kg body weight. Merck Sharp and Dohme Research Laboratories report

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Wehner, T.A. et al. (1991b). HPLC fluorescence assay method for avermectin B_{1a} (MK 0936) in bovine tissue. Merck Sharp and Dohme Research Laboratories report