Shimabukuro, R. H. Atrazine metabolism and herbicidal selectivity. *Plant Physiol.* 1967, *42*, 1269–1276.

Shimabukuro, R. H.; Swanson, H. R. Atrazine metabolism, selectivity, and mode of action. *J. Agric. Food Chem.* 1969, *17*, 199–205.

Toth, J. P.; Bardalaye, P. C. Capillary gas chromatographic separation and mass spectrometric detection of cyromazine and its metabolite melamine. *J. Chromatog.* 1987, *408*, 335–340.

Zeiger, E. Carcinogenicity of mutagens: Predictive capability of the *Salmonella* mutagenesis assay for rodent carcinogenicity. *Cancer Res.* 1987, *47*, 1287–1296.

Fate of Avermectin B_{1a} in Rats

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Male and female rats were administered [³H]avermectin B_{1a} or a mixture of [³H]- and [¹⁴C]avermectin B_{1a} as a single oral dosage at 1.4 or 0.14 mg/kg. Most of the dose (69–82%) was recovered in the feces, with 1% or less found in the urine. The total residue levels in liver, kidney, muscle, and fat tissues were <5.3 ppm at 1 day after dosing and essentially depleted within 7 days after dosing. For all tissues analyzed, the depletion half-life of the total radioactive residue was approximately 1.2 days, while the half-life of avermectin B_{1a} was between 0.6 and 1.0 day. The tissue residue was shown to be qualitatively similar between the tissue type, dose, sex, pretreatment with or without unlabeled avermectin B_{1a}, and label (³H or ¹⁴C). A major metabolite (3″-desmethyl) and a minor metabolite (24-hydroxymethyl) isolated and identified from rat liver microsomal incubations of avermectin B_{1a} were identified in the rat tissues. These two metabolites and avermectin B_{1a} accounted for >85% of the tissue residue. The fate of [³H]avermectin B_{1a} was the same as the fate of [¹⁴C]avermectin B_{1a}, demonstrating the stability of the ³H label on avermectin B_{1a} and the validity of its use in animal metabolism studies.

Avermectins, a new class of biological agents that contain a macrocyclic lactone, are produced by *Streptomyces avermitilis* (Burg et al., 1979). Their structures have been elucidated (Albers-Schönberg et al., 1981), and some of the biological activities have been reported (Campbell et al., 1983). Ivermectin (22,23-dihydroavermectin B_1) is a member of the avermectin class that is registered as an anthelmintic for use as an animal health drug. Abamectin (avermectin B_1) is the commercial product that is being developed by Merck & Co. Inc. as an acaricide/insecticide. The major active ingredient in abamectin is avermectin B_{1a} (Figure 1). This report describes the findings from rat metabolism studies with avermectin B_{1a}. Some of these data have been presented previously (Maynard et al., 1985a).

The major objective of this study was to describe the fate of avermectin B_{1a} in rats. The total residue levels in tissues were determined to evaluate the distribution and elimination of the radiolabeled avermectin B_{1a} residues. The amount of avermectin B_{1a} and the identity of the metabolites found in the liver, kidney, muscle, and fat tissues were determined. The effect of dose level, pretreatment with unlabeled avermectin B_{1a}, sex of the rats, and ³H vs ¹⁴C labeling of avermectin B_{1a} on the metabolic fate of avermectin B_{1a} were investigated.

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Male and female rats were administered a single oral dose at 1.4 and 0.14 mg/kg of ³H-labeled avermectin B_{1a} or a mixture of ³H- and ¹⁴C-labeled avermectin B_{1a}. One group of animals received 14 single daily oral doses of unlabeled avermectin B_{1a} at 0.14 mg/kg before receiving a single dose of [³H]avermectin B_{1a} at 0.14 mg/kg. Animals were sacrificed at 1, 2, 4, and 7 days after dosing; 15 tissue and fluid samples were taken, which accounted for the entire animal less skin and tail. Tissue extracts were analyzed for avermectin B_{1a}, and two metabolites were identified by cochromatography with standards.

MATERIALS AND METHODS

Animal Treatment. Male (ca. 295 g) and female (ca. 217 g; nulliparous and nonpregnant) CRCD rats were obtained from Charles River, MA. Animals were indiscriminately selected for grouping and administered a single oral (gavage) dose of [³H]avermectin B_{1a} (labeled at carbon 5) or a mixture of [³H]- and [¹⁴C]avermectin B_{1a} (labeled at carbons 3, 7, 11, 13, and 23). Two dosage levels, 1.4 and 0.14 mg/kg, were employed. At the 1.4 mg/kg dosage level, one group of rats received [³H]avermectin B_{1a} (group 1) and another group received a mixture of [³H]- and [¹⁴C]avermectin B_{1a} (group 4). At the 0.14 mg/kg dosage level, the rats received [³H]avermectin B_{1a} either alone (group 2) or preceded by (group 3) 14 single daily oral doses of unlabeled avermectin B_{1a} at 0.14 mg/kg. The final specific activities of the [³H]avermectin B_{1a} alone and mixed with [¹⁴C]avermectin B_{1a} were 350 and 33 μCi/mg, respectively. The final specific activity of the [¹⁴C]avermectin B_{1a} was 16.4 μCi/mg. The labeled avermectin B_{1a} and unlabeled
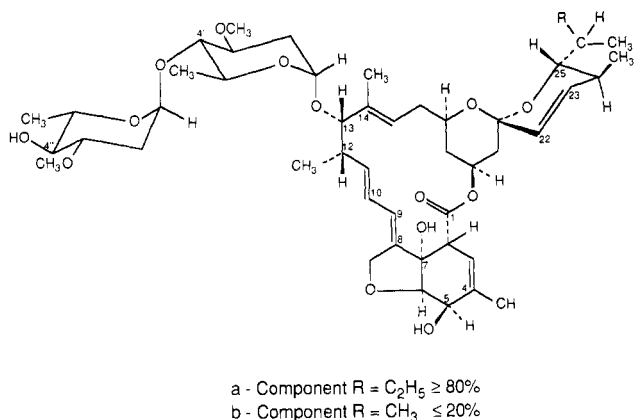
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a - Component R = C_2H_5 ≥ 80%
b - Component R = CH_3 ≤ 20%

Figure 1. Structures of the two major components of abamectin.

Figure 2. Extraction procedure for the isolation of unchanged avermectin B_{1a} from edible tissues.

avermectin B_{1a} were formulated with sesame oil. The concentrations of the dosing solutions were 0.76 and 0.076 mg/mL for the 1.4 and 0.14 mg/kg dosages, respectively. Control animals received the same amount of sesame oil without avermectin B_{1a} as the treated animals. Each treatment group consisted of 12 male and 12 female animals, which were housed separately in metabolism cages as four subgroups (3 animals) for each sex. Daily samples of urine and feces were collected for each subgroup. Respired air was not collected for this study. Animals were given free access to food (Purina rodent chow) and water. Under the experimental conditions of this study, there were no observable toxicological effects in the control and avermectin B_{1a} treated animals.

A subgroup (3 rats) from each treatment group was sacrificed at 1, 2, 4, and 7 days after treatment with the radioactive compound. Animals were anesthetized with ether followed by cardiac puncture for blood samples. The following samples were taken, combined as a composite sample, and frozen before analysis: bone (hind leg), brain, fat (gonadal), gonads, heart, kidney, liver, GI tract (contents not removed), lung, muscle (hind leg), spleen, and residual carcass (less skin and tail).

The [³H]- and [¹⁴C]avermectin B_{1a} standards were supplied by the Labeled Compound Synthesis Group, Department of Animal and Exploratory Drug Metabolism, Merck, Sharp and Dohme Research Laboratories, Rahway, NJ. All other chemicals and solvents were reagent grade, HPLC grade, or better and purchased from commercial sources.

Analysis. The radioactivity in the composite samples for a given treatment, time postdose, and sex was determined by combustion followed by liquid scintillation counting (LSC). For blood and urine samples, ca. 0.5-mL aliquots were weighed and combusted. For brain, lung, bone, spleen, and heart, the tissues for each subgroup of animals were weighed and combusted. For all remaining tissues, ca. 0.5-mL samples of 1/4 (w/v) water homogenates were weighed and combusted. Combustion of the samples was conducted with a Packard 306 sample oxidizer. The ³H₂O was trapped in 18 mL of Monophase 40, while the ¹⁴CO₂ was trapped in 8 mL of Carbosorb and 13 mL of Permafluor V (United Technologies, Packard). All radioactivity measurements were made in a Packard scintillation spectrometer, with corrections for efficiency based on the external standard method.

All samples were combusted in duplicate aliquots except for brain, lung, bone, spleen, and heart. Duplicate aliquots were reassayed if they met all the following criteria: differed by more than ±3σ from their mean, more than ±5% from their mean, and more than 1 ppb from each other. Background counts and detection limits were measured by using appropriate control fluids or tissues. The combustion efficiency during the study averaged 95.7 and 97.9% for ³H and ¹⁴C, respectively. Detection limits were between 0.001 and 3.6 ppb, depending on the tissue. All residue levels below the respective detection limit were reported as zero.

Avermectin B_{1a} Analysis. To quantitate the level of unchanged avermectin B_{1a} in the liver, kidney, muscle, and fat tissues from this study, a reverse isotope dilution assay (RIDA)
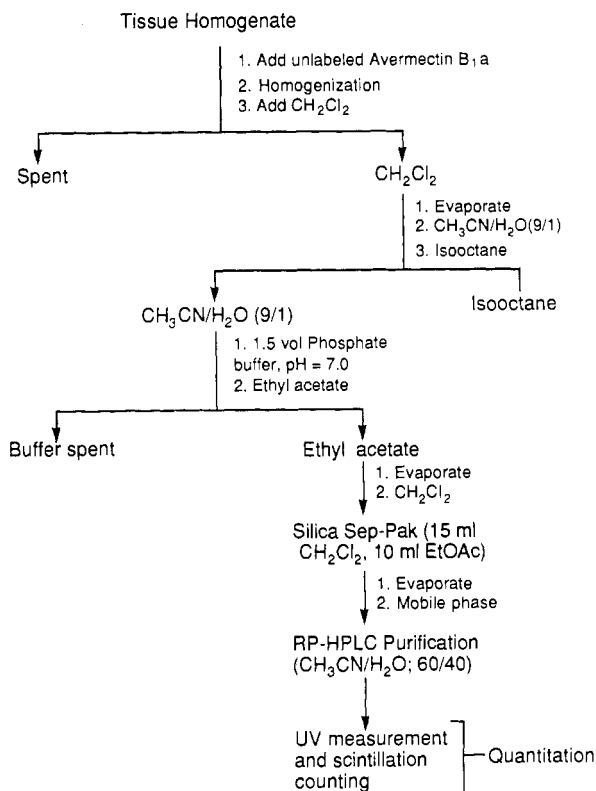
was developed. The procedure shown in Figure 2 was modified from the RIDA procedure reported for ivermectin (Chiu et al., 1985). The amount of unlabeled avermectin B_{1a} added to the tissue homogenate was 84, 192, 188, and 190 μg for liver, kidney, muscle, and fat, respectively. This procedure was used for all samples except where noted below. For the analysis of the liver samples, the silica Sep-Pak step was replaced with a RP-HPLC purification step using a Zorbax ODS column with a mobile phase of $CH_3CN/CH_3OH/H_2O$ (56/18/26) at 1 mL/min. The UV detector was set to monitor the wavelength maxima of avermectin B_{1a} (245 nm). The avermectin B_{1a} was collected from this column and repurified on a second RP-HPLC system. The second system consisted of a Zorbax ODS column with a mobile phase of CH_3OH/H_2O (85/15) at 1 mL/min. For the analysis of the kidney samples, the mobile phase of the RP-HPLC purification step was CH_3CN/H_2O (65/35) at 1 mL/min. For the analysis of the fat samples, the methylene chloride tissue extract was applied to a silica Sep-Pak and sequentially eluted 2 × 5 mL each with CH_2Cl_2, 5% tetrahydrofuran (THF) in CH_2Cl_2, 10% THF in CH_2Cl_2, ethyl acetate, and then methanol. The 10% THF in CH_2Cl_2 and the ethyl acetate eluants were combined, concentrated, and then applied to a RP-HPLC system. The RP-HPLC system consisted of a Zorbax ODS column using a mobile phase of CH_3CN/H_2O (75/25) at 1 mL/min. Avermectin B_{1a} purity and mass amount were determined by a UV spectrophotometer, and the radioactivity was determined by LSC.

Avermectin B_{1a} was quantitated as a RIDA value, which is the initial specific activity divided by the final specific activity of avermectin B_{1a}. The initial specific activity was the total disintegrations per minute (dpm) of the residue in the tissue sample taken divided by the micrograms of unlabeled avermectin B_{1a} added. The mass of the radiolabeled avermectin B_{1a} present in the tissue was negligible compared to the amount of unlabeled avermectin B_{1a} added. The final specific activity of avermectin B_{1a} isolated from the tissue samples was measured as dpm μg⁻¹. The RIDA values for avermectin B_{1a} are expressed as a percentage of the total tissue residue and as a tissue concentration (ppb) value. The RIDA values for control tissues spiked with a known amount and purity of [³H]avermectin B_{1a} were >95%.

Table I. Total Residue Levels (ppm) in Tissues of Rats Administered Avermectin B_{1a}[a]

tissue	days postdose	group 1 M	group 1 F	group 2 M	group 2 F	group 3 M	group 3 F	group 4 [3]H M	group 4 [3]H F	group 4 [14]C M	group 4 [14]C F
liver	1	1.287	1.317	0.092	0.156	0.086	0.094	1.250	1.868	1.513	2.206
	2	0.523	0.484	0.061	0.062	0.041	0.051	0.369	0.585	0.444	0.678
	4	0.090	0.146	0.008	0.020	0.010	0.015	0.067	0.185	0.070	0.215
	7	0.012	0.022	0.001	0.006	0.002	0.002	0.016	0.012	0.013	0.015
kidney	1	1.149	2.142	0.159	0.314	0.095	0.190	1.865	3.566	2.315	3.106
	2	0.996	0.629	0.080	0.057	0.060	0.076	0.668	0.588	0.833	0.738
	4	0.119	0.236	0.017	0.045	0.014	0.025	0.111	0.378	0.121	0.417
	7	0.036	0.059	0.003	0.010	0.003	0.003	0.045	0.029	0.026	0.027
muscle	1	0.477	0.744	0.034	0.066	0.028	0.031	0.449	1.049	0.544	0.909
	2	0.158	0.208	0.018	0.025	0.012	0.015	0.135	0.254	0.174	0.303
	4	0.034	0.069	0.004	0.010	0.003	0.005	0.030	0.074	0.035	0.085
	7	0.014	0.021	0.001	0.003	0.001	0.001	0.010	0.018	0.011	0.023
fat	1	2.887	3.373	0.160	0.315	0.135	0.212	2.454	4.586	2.373	5.304
	2	1.137	0.491	0.099	0.140	0.092	0.128	0.872	1.331	0.775	1.681
	4	0.306	0.215	0.022	0.076	0.032	0.051	0.203	0.559	0.075	0.651
	7	0.079	0.141	0.008	0.023	0.011	0.009	0.074	0.128	0.023	0.146

[a] Group 1, 1.4 mg/kg ^{3}H-B_{1a}, single dose; group 2, 0.14 mg/kg ^{3}H-B_{1a}, single dose; group 3, 0.14 mg/kg ^{3}H-B_{1a}, single dose after 14 daily unlabeled B_{1a} doses; group 4, 1.4 mg/kg ^{3}H- and ^{14}C-B_{1a}, single dose. M = male, F = female.

Residue Characterization. The ethyl acetate eluate (Figure 2), containing 75–95% of the tissue radioactivity, was analyzed by RP-HPLC radioactivity profile. The retention time of the avermectin B_{1a} in the residue was determined by comparative daily retention time to an avermectin B_{1a} standard. The levels of avermectin B_{1a} as determined as a percent of the radioactivity profile were compared to the values determined by RIDA. In all tissues analyzed, a major metabolite and a minor metabolite were observed and designated as metabolite B and metabolite A, respectively. The RP-HPLC column recoveries were >90%. The sum of avermectin B_{1a}, metabolite A, and metabolite B was >85% of the total extractable tissue residue for all tissues analyzed. The remainder of the radioactivity was accounted for as an unknown metabolite (3–15%) and some minor metabolites each containing 1% or less. The tissue residues from group 4 male and female rats were characterized to compare the metabolite profile from the ^{3}H- and ^{14}C-labeled residue.

Microsomal Incubations. Incubations of [^{14}C]- or [^{3}H]avermectin B_{1a} with control rat liver microsomes were conducted to generate metabolite standards. The incubation and isolation procedure has been previously reported (Miwa et al., 1982). In the absence of an NADPH generating system, no metabolites were formed from the microsomal incubations. The 24-hydroxymethyl-B_{1a} (24-OHMe-B_{1a}) metabolite was isolated by RP-HPLC from the microsomal incubations and identified by NMR and mass spectrometry (MS) using previous criteria (Miwa et al., 1982). The 3''-desmethyl-B_{1a} (3''-DM-B_{1a}) metabolite was isolated by RP-HPLC from the microsomal incubations and identified by NMR and MS. Only 2% avermectin B_{1a} was metabolized by these incubations with a formation of 1.5% 3''-DM-B_{1a} and 0.5% 24-OHMe-B_{1a}.

Metabolite Identification. Metabolite identification was accomplished by isolating the metabolite from tissue samples, comparing their chromatographic properties to known standards on several chromatographic systems, and cochromatography with standards. The identification of metabolite A from the liver, kidney, muscle, and fat tissues was accomplished by isolating this metabolite peak from the group 1, 4-day liver tissue sample and cochromatographing it with a ^{14}C-24-OHMe-B_{1a} standard (isolated and identified from the microsomal incubation reaction). The retention time of metabolite A from the above-mentioned four tissues was the same for all tissue samples and identical with that of the 24-OHMe-B_{1a} standard on the same RP-HPLC system (60/40 CH$_3$CN/H$_2$O at 1 mL/min; retention time, 6.4 min; seventh 1-min fraction). The ethyl acetate eluate from the liver tissue sample was injected on an RP-HPLC gradient system (0–25 min, 32.5/35/32.5 CH$_3$CN/H$_2$O/ CH$_3$OH at 1 mL/min; 28–50 min, 0/35/65 CH$_3$CN/H$_2$O/ CH$_3$OH at 1 mL/min; 52–90 min, 0/0/100 CH$_3$CN/H$_2$O/ CH$_3$OH at 1 mL/min). The retention times of metabolite A and the 24-OHMe-B_{1a} standard were the same (16.1 min). The metabolite A peak from the liver sample (purified by RP-

HPLC, mobile phase 85/15 CH$_3$OH/H$_2$O) was isolated, mixed with the ^{14}C-24-OHMe-B_{1a} standard, and injected on another RP-HPLC system (32.5/35/32.5 CH$_3$CN/H$_2$O/CH$_3$OH at 1 mL/ min) and a dual-label radioactivity profile was obtained. The ^{3}H and ^{14}C radioactivity peak shape and retention times were compared.

For all tissues analyzed by RP-HPLC, a major metabolite (metabolite B) was observed at the same retention time. When the tissue residues were analyzed by different RP-HPLC systems, metabolite B always eluted at the retention time of the 3''-DM-B_{1a} standard, the major metabolite isolated from rat liver microsomal incubations of avermectin B_{1a}. Metabolite B was isolated from group 1, 2-day liver tissue by RP-HPLC, mixed with ^{3}H-3''-DM-B_{1a} standard, and injected on another RP-HPLC system (85/15 CH$_3$OH/H$_2$O at 1 mL/min). The retention time of the absorbance peak (245 nm) from the standard and the retention time of the ^{3}H from metabolite B and the standard were compared. Moreover, the sum of the total ^{3}H eluting as a single peak was measured and compared to the combined amount from metabolite B and the ^{3}H-3''-DM-B_{1a} standard.

RESULTS

The total residue levels in the liver, kidney, muscle, and fat tissue at various times after dosing are shown in Table I. After treatment with 1.4 mg/kg avermectin B_{1a}, the total residue levels in all tissues at 1 and 7 days postdose were 0.449–5.304 and 0.010–0.146 ppm, respectively. The total residue levels from rats dosed at 0.14 mg/kg after 1 and 7 days were 0.028–0.315 and 0.001– 0.023 ppm, respectively. The total residue levels in all tissues from rats dosed at 0.14 mg/kg were between $^1/_5$ and $^1/_{20}$ the levels for the corresponding tissues from rats dosed at 1.4 mg/kg. The total residue levels varied among the tissues and were observed in the following order: muscle < liver ≤ kidney < fat, for all treatments. For most samples, the residue levels for the female tissues were higher than for the corresponding male tissues. The tissue residue levels were lower for the rats that were pretreated for 14 days with unlabeled avermectin B_{1a} (group 3) than the rats without pretreatment (group 2). The residue levels determined with the ^{3}H label were similar to the levels determined with the ^{14}C-labeled avermectin B_{1a} (group 4).

The total residue levels in the other tissues at 1 and 7 days after dosing are shown in Table II. Although not shown, the 2- and 4-day values were intermediate to the 1- and 7-day values. The residue levels in the spleen, heart, GI tract, lung, and ovaries were similar to those in the corresponding liver, kidney, muscle, and fat tis-

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Table II. Total Residue Levels (ppm) in Tissues of Rats Administered Avermectin B_{1a}[a]

tissue	days postdose	group 1 M	group 1 F	group 2 M	group 2 F	group 3 M	group 3 F	group 4 [3]H M	group 4 [3]H F	group 4 [14]C M	group 4 [14]C F
GI tract	1	2.869	4.952	0.282	0.410	0.360	0.481	3.498	4.441	3.647	4.621
	7	0.020	0.055	0.002	0.010	0.006	0.004	0.116	0.045	0.022	0.047
blood	1	0.073	0.079	0.005	0.007	0.006	0.007	0.064	0.109	0.072	0.118
	7	0.001	0.001	0.000	0.000	0.000	0.000	0.002	0.000	0.001	0.002
testes/ovaries	1	0.173	1.748	0.009	0.255	0.014	0.124	0.218	2.866	0.227	2.945
	7	0.005	0.117	0.001	0.015	0.000	0.024	0.000	0.082	0.003	0.092
lung[b]	1	1.294	2.121	0.129	0.217	0.078	0.116	2.153	2.867	2.093	2.810
	7	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
brain[b]	1	0.101	0.094	0.007	0.010	0.003	0.005	0.082	0.130	0.075	0.132
	7	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
bone[b]	1	0.328	0.217	0.026	0.026	0.014	0.008 (1)	0.306	0.352	0.336	0.383
	7	0.010	0.011	0.001	0.001	0.001	0.000 (2)	0.009	0.001 (2)	0.016	0.008
spleen[b]	1	1.689	2.016	0.108	0.202	0.097	0.104	1.760	2.589	1.795	2.712
	7	0.020	0.036	0.001	0.007	0.003	0.002	0.010	0.020	0.020	0.032
heart[b]	1	1.205	2.132	0.127	0.205	0.099	0.111	1.810	2.985	1.843	2.999
	7	0.013	0.024	0.001	0.008	0.003	0.001	0.010	0.012	0.016	0.024
carcass	1	0.360	0.128	0.024	0.041	0.023	0.026	0.348	0.002	0.364	0.013
	7	0.008	0.014	0.000	0.001	0.001	0.001	0.011	0.003	0.008	0.013

[a] Same as footnote *a* in Table I. [b] Individual tissue samples were combusted directly and expressed as the mean of 3 rats, except (1) single animal and (2) mean of 2 animals.

Table III. Accountability of the Dose in Tissues Expressed as a Percentage of the Administered Dose[a]

tissue	group 1 M	group 1 F	group 2 M	group 2 F	group 3 M	group 3 F	group 4 [3]H M	group 4 [3]H F	group 4 [14]C M	group 4 [14]C F
edibles[b]	2.22	2.42	1.69	2.64	2.05	2.30	1.73	2.83	1.93	3.23
urine	0.75	0.57	0.81	0.27	1.09	0.32	0.89	0.34	0.94	0.38
feces	72.36	69.73	81.58	71.37	74.25	76.15	70.29	68.72	78.34	76.66
GI tract	8.24	12.12	7.40	9.35	10.33	10.34	8.28	10.68	8.46	11.19
blood	0.03	0.03	0.02	0.16	0.03	0.04	0.02	0.03	0.03	0.04
gonads	0.02	0.01	0.01	0.02	0.03	0.01	0.02	0.01	0.03	0.01
lung	0.09	0.11	0.07	0.12	0.06	0.07	0.11	0.14	0.11	0.13
brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
bone	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
spleen	0.03	0.03	0.02	0.04	0.02	0.02	0.02	0.04	0.02	0.04
heart	0.04	0.05	0.03	0.05	0.03	0.03	0.04	0.06	0.04	0.07
carcass	4.95	5.84	3.46	9.40	4.69	4.86	4.73	2.28	4.96	2.77
% total	88.74	90.93	95.11	93.41	92.59	94.13	86.15	85.14	94.87	94.52

[a] Same as footnote *a* in Table I. Urine and feces values are cumulative (1–7 days). [b] Liver, kidney, muscle, and fat tissues.

sues, while the residue levels in the remaining tissues and fluids were much lower than those of the corresponding liver, kidney, muscle, and fat tissues. The effect of dose, sex, and pretreatment on the residue levels in all tissues was similar. At 7 days after dosing, the total residue levels for all tissues were in the very low ppb range for all rats in this study. The residue levels in these other tissues were very similar when determined by either ^{3}H or ^{14}C radiochemical label. In fact, the [3]H:[14]C residue ratio determined for all group 4 samples from this study was 0.96 ± 0.27 (±SD) (data not shown).

The average tissue depletion half-lives of the total residue for male and female rats from this study as determined by linear regression analysis were 0.94–1.26 and 0.97–1.37 days, respectively (data not shown). In general, the depletion half-lives of the total residue in tissues from males were slightly shorter than the corresponding tissues from females.

The accountability of the dose after 7 days, expressed as a percentage of the dose, is shown in Table III; total recovery was 85–95%. The feces were shown to account for 68.7–81.6% of the administered radioactivity for all rats, with most excreted 2–3 days postdose. The GI tract plus contents accounted for the next highest fraction, containing 7.4–12.1% of the dose. The liver, kidney, muscle, and fat accounted for 1.7–3.2% of the dose, with the residual carcass accounting for an additional 2.3–5.8%. The urine was shown to account for 0.3–1.0% of the dose;

collectively, all other tissues and fluids contained <0.14%. In general, no differences in the accountability of the dose were observed for dosage, pretreatment, or ^{3}H and ^{14}C radiochemical label. The residue levels in the tissues from female rats mostly contained a higher percentage of the dose than did the corresponding tissues from male rats except for the urine and feces, which were lower.

The levels of unchanged avermectin B_{1a} were determined in most liver, kidney, muscle, and fat tissues by RIDA and were expressed as a percentage of the total tissue residue and as ppb (Table IV). The levels of avermectin B_{1a} as a percentage of the total tissue residue were similar for the corresponding liver, kidney, and muscle tissues but higher for fat tissue. For almost all samples, the percent and level of avermectin B_{1a} in the tissues from males were less than the corresponding value for the tissues from females. For example, at 1 and 2 days after dosing the levels of avermectin B_{1a} for male tissues were mostly 30–50% of the total tissue residue, while the levels for female tissues were 40–70%. With regard to levels of avermectin B_{1a}, at 1 day after dosing at 1.4 mg/kg, avermectin B_{1a} levels were 0.212–2.496 and 0.446–3.872 ppm for tissues from male and female rats, respectively. The levels of avermectin B_{1a} at 1 day after dosing at 0.14 mg/kg were 0.006–0.126 and 0.009–0.214 ppm for tissues from male and female rats, respectively. These values demonstrate the sex-linked difference between tissues as well as the dose effect on the levels of avermec-

Table IV. Levels of Unchanged Avermectin B_{1a} in Tissues as a Percentage of the Total Residue[a]

animal	days postdose	liver	kidney	muscle	fat
group 1					
M	1	51 (650)	55 (630)	52 (250)	74 (2100)
M	2	34 (180)	36 (360)	44 (70)	59 (670)
M	4	30 (27)	54 (64)	34 (12)	36 (110)
M	7	6 (1)	2 (1)	20 (3)	3 (2)
F	1	50 (660)	55 (1200)	60 (450)	74 (2500)
F	2	44 (210)	75 (470)	49 (100)	71 (350)
F	4	46 (67)	73 (170)	44 (30)	43 (92)
F	7	28 (6)	28 (17)	24 (5)	16 (23)
group 2					
M	1	40 (37)	37 (75)	45 (15)	77 (120)
M	2	44 (27)	47 (38)	52 (9)	78 (77)
F	1	64 (100)	68 (210)	75 (50)	62 (200)
F	2	63 (39)	67 (38)	65 (16)	68 (95)
group 3					
M	1	35 (30)	38 (38)	50 (14)	81 (130)
M	2	43 (18)	35 (22)	51 (6)	80 (74)
F	1	61 (57)	62 (130)	70 (22)	90 (190)
F	2	48 (24)	53 (43)	62 (9)	81 (100)
group 4					
[3]H M	1	45 (560)	45 (1400)	49 (220)	44 (1100)
[14]C M	1	41 (610)	45 (1500)	39 (210)	50 (1200)
[3]H M	2	31 (110)	34 (300)	48 (65)	42 (370)
[14]C M	2	28 (120)	34 (345)	36 (63)	40 (300)
[3]H F	1	68 (1300)	81 (2600)	89 (930)	72 (3300)
[14]C F	1	63 (1400)	84 (2800)	69 (840)	73 (3900)
[3]H F	2	58 (340)	61 (400)	77 (200)	40 (530)
[14]C F	2	55 (370)	63 (450)	55 (180)	35 (590)

[a] Same as footnote a in Table I. Values in parentheses are tissue levels of avermectin B_{1a} (ppb).

Table V. Avermectin B_{1a} Depletion Half-Lives in Tissues of Group 1 Rats

	males		females	
tissue	$t_{1/2}$, days	correln coeff	$t_{1/2}$, days	correln coeff
kidney	0.64	−0.988	1.01	−0.996
liver	0.65	−0.999	0.92	−0.966
muscle	0.97	−0.978	0.95	−0.977
fat	0.60	−0.993	0.96	−0.909

tin B_{1a}. The levels of avermectin B_{1a} were 5–20 times greater in these tissues from rats administered the 1.4 mg/kg dose than from rats administered the 0.14 mg/kg dose (Table IV and presented above). With dosing at 1.4 mg/kg, the levels of avermectin B_{1a} were nearly depleted at 7 days (avermectin B_{1a} levels were 0.001–0.003 and 0.005–0.023 ppm for male and female tissues, respectively). The percent and levels of avermectin B_{1a} were similar for the same tissue when determined with either the ^{3}H or the ^{14}C radiochemical label.

The depletion half-lives of avermectin B_{1a} in the liver, kidney, muscle, and fat from the group 1 animals (1.4 mg/kg [^{3}H]avermectin B_{1a}) were determined by linear regression analysis (Table V). The half-life of avermectin B_{1a} was 0.60–0.97 and 0.92–1.01 days for tissues from male and female rats, respectively. For all tissues, except muscle, the half-life of avermectin B_{1a} was shorter in tissues from males than females. The depletion half-life of avermectin B_{1a} was shorter than the corresponding depletion half-life of the total residue in these tissues.

The characterization of the residue from selected tissues was obtained by RP-HPLC analysis of the ethyl acetate eluate from the Sep-Pak (Figure 2). The avermectin B_{1a} level as a percent of the tissue residue by RP-HPLC was similar to the level of avermectin B_{1a} determined by RIDA for the same tissue. In all tissues
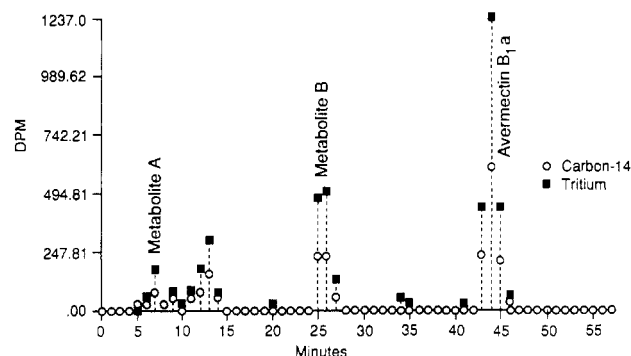
Figure 3. RP-HPLC radioactivity profile of male kidney tissue extract (group 4, 2 days postdose).
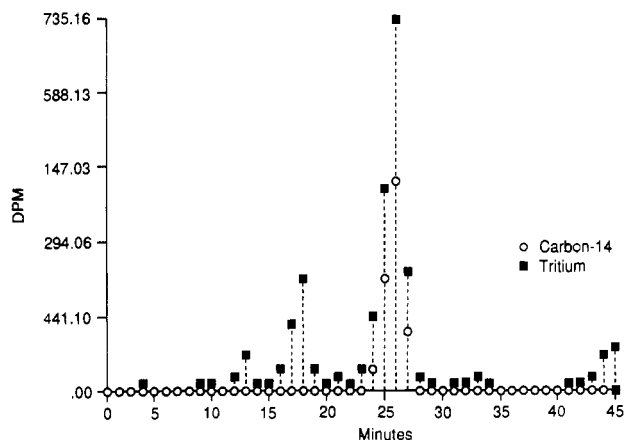
Figure 4. RP-HPLC radioactivity profile of the cochromatography of rat liver metabolite A and carbon-14-labeled 24-OHMe-B_{1a} standard.

analyzed, the residue profiles were qualitatively similar, indicating characteristic major (metabolite B) and minor (metabolite A) metabolites. Aside from avermectin B_{1a}, metabolite A, and metabolite B, the remaining residue was accounted for as a minor unknown metabolite (3–15%) and several other metabolites each containing 1% or less. In general for these tissues analyzed, the level of metabolite B was 20–40% and the level of metabolite A was 3–12%. A representative profile for the group 4, 2-day male kidney sample is shown in Figure 3.

On the basis of retention time comparisons using several HPLC systems, metabolite A appeared to be 24-OHMe-B_{1a}, whereas metabolite B appeared to be 3″-DM-B_{1a}. The identification of metabolites A and B was further accomplished by isolating the metabolites from tissue samples, mixing them with reference standards isolated from the microsomal incubation of avermectin B_{1a}, and cochromatographing the mixture on HPLC. Metabolite A isolated from group 1, 4-day liver was cochromatographed with [14]C-24-OHMe-B_{1a} standard. The RP-HPLC cochromatography profile is shown in Figure 4. Metabolite B isolated from group 1, 2-day liver was cochromatographed with [3]H-3″-DM-B_{1a}. The RP-HPLC cochromatography profile is shown in Figure 5. These profiles show that the chromatographic properties of metabolites A and B are identical with those of [14]C-24-OHMe-B_{1a} and [3]H-3″-DM-B_{1a}, thus demonstrating that these metabolites are 24-OHMe-B_{1a} and 3″-DM-B_{1a}.

DISCUSSION

Male and female rats were administered avermectin B_{1a} at 1.4 and 0.14 mg/kg. The residue levels in all the tissues were dose dependent. The percentage of the dose
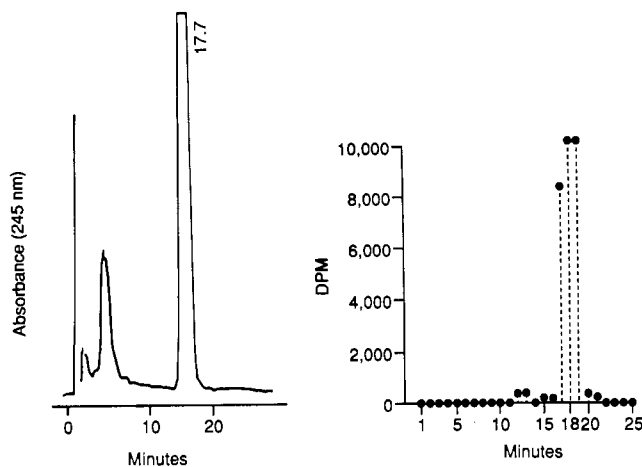
Figure 5. RP-HPLC absorbance and radioactivity profile of the cochromatography of the rat liver metabolite B and ^{3}H-3''-desmethyl-B$_{1a}$ standard.

in the various tissues, fluids, and excreta was dose independent. The half-lives of the total residue in all the tissues, fluids, and excreta were very similar and ca. 1.2 days for both dose rates. At 7 days postdose, all tissue residue levels were in the low-ppb range, with almost all the residue depleted. With regards to excretion, most of the dose was excreted in the feces, with 1% or less in the urine. The residues in the urine and feces as a percentage of dose were the same for both dosage levels.

The levels of avermectin B$_{1a}$ in tissues were dose dependent. Since the residue levels were very low in tissues from rats dosed at 0.14 mg/kg, the RIDA analysis for avermectin B$_{1a}$ was conducted and compared at only 1 and 2 days after dosing. The levels of avermectin B$_{1a}$, expressed as a percentage of the total tissue residue, were similar in the corresponding tissue, indicating a dose-independent metabolism of avermectin B$_{1a}$.

The pretreatment of rats with avermectin B$_{1a}$ was observed to produce small but consistent changes in the levels of the total residue and unchanged avermectin B$_{1a}$. In general, the residue levels in most of the tissues were decreased following pretreatment. The accountability of the dose in the urine, feces, and GI tract was increased following pretreatment. Furthermore, the levels of avermectin B$_{1a}$ were decreased in the liver, kidney, and muscle tissues following pretreatment. In fat tissue, the levels and percentage of the total tissue residue were mostly unchanged with pretreatment. The HPLC radioactivity profiles of the tissue extracts from pretreated animals were qualitatively similar to those without pretreatment (Figure 3). These results suggest that pretreatment of rats with avermectin B$_{1a}$ resulted in a slight increase in the elimination of avermectin B$_{1a}$ residues (which was more pronounced in the female rats).

The total residue levels in the tissue and fluid samples at all time points were higher for females than males. The residue levels in the urine and feces were lower for the females than the males. The depletion half-lives for the total residue in all tissues and avermectin B$_{1a}$ in liver, kidney, muscle, and fat tissues were longer for the females than the males. Avermectin B$_{1a}$ levels in the tissues were higher for the females than the males. These differences, attributed to a more extensive metabolism of avermectin B$_{1a}$ by males than females, were consistent with the tissue residue profiles where the same metabolites (metabolites A and B) were observed but in greater amounts in the male than the corresponding female tissue. A sex difference in the metabolism of xenobiotics

by rats is generally accepted for many compounds (Kato, 1979). Aside from the extent of metabolism, the fate of avermectin B$_{1a}$ and the total residue appeared similar in both male and female rats.

Since a group of rats was dosed with a mixture of [^{3}H]avermectin B$_{1a}$ and [^{14}C]avermectin B$_{1a}$, a direct comparison of the fate of each radiochemical label was conducted. The total residue levels in all tissue, fluid, and excreta samples were the same when measured by either label. Even though small variations were observed, the ratio of the ^{3}H to ^{14}C residue levels for all samples from these rats was very close to 1 (0.96). Furthermore, the results for ^{3}H and ^{14}C were the same for the half-lives of the total residue, accountability of the dose, and elimination levels. The levels of avermectin B$_{1a}$ as a percentage of the total tissue residue and as a concentration (ppb) were very similar for both ^{3}H and ^{14}C. Moreover, the residue profiles from all tissues analyzed demonstrated an identical ^{3}H and ^{14}C radioactivity profile (Figure 3). That is, all metabolites contained the same initial ^{3}H:^{14}C ratio, and no metabolite was observed to contain just one label. To further investigate the stability of the ^{3}H label, the amount of tritiated water was measured in the 1- and 7-day rat urine samples from this study; tritiated water in the rat urine was only 0.01% of the dose. These results demonstrate the stability of the ^{3}H label on avermectin B$_{1a}$ and its validity for use in animal metabolism studies. The stability of the ^{3}H label of avermectin B$_{1a}$ was also indicated in plant metabolism studies (Bull et al., 1984; Maynard et al., 1989a), goat metabolism studies (Maynard et al., 1985b, 1989b), and environmental studies (Ku et al., 1983).

In conclusion, the total tissue residue levels from male and female rats administered avermectin B$_{1a}$ were very low at 1 day after dosing and essentially depleted at 7 days after dosing. Almost all of the dose was recovered in the feces, and no tissue storage of the radiolabeled residue or avermectin B$_{1a}$ was observed. In fact, the total residue depletion was very similar in all tissues ($t_{1/2}$ of 1.2 days) with an even faster depletion of avermectin B$_{1a}$ (0.6–1.0 day). The fate of avermectin B$_{1a}$ was qualitatively similar in male and female rats, with a more extensive metabolism and faster elimination in males than females. Avermectin B$_{1a}$ and the two identified metabolites 3''-DM-B$_{1a}$ and 24-OHMe-B$_{1a}$ accounted for >85% of the total extractable tissue residue. These two metabolites were also formed by microsomal incubations of avermectin B$_{1a}$ presented here and previously (Maynard et al., 1985a). The formation of 3''-DM-B$_{1a}$ and 24-OHMe-B$_{1a}$ has been reported in goats administered avermectin B$_{1a}$ (Maynard et al., 1985b, 1989b). Incubation of avermectin B$_{1a}$ with steer liver microsomes resulted in the formation of 24-OHMe-B$_{1a}$ (Kline et al., 1987). Furthermore, 24-OHMe-H$_2$B$_{1a}$ was identified in rats, cattle, and sheep administered ivermectin (22,23-dihydroavermectin B$_{1a}$) (Chiu et al., 1986). Lastly, 8,9-Z-3''-DM-B$_{1a}$ and 8,9-Z-24-OHMe-B$_{1a}$ were identified in rats administered the 8,9-Z isomer of avermectin B$_{1a}$ (Maynard et al., 1989c), further demonstrating a similar fate of avermectin analogues in rats and other animals.

LITERATURE CITED

Albers-Schönberg, G.; Arison, B. H.; Chabala, J. C.; Douglas, A. W.; Eskola, P.; Fisher, M. H.; Lusi, A.; Mrozik, H.; Smith, J. L.; Tolman, R. L. Avermectins: Structure Determination. J. Am. Chem. Soc. 1981, 103, 4216.

Bull, D. L.; Ivie, G. W.; MacConnell, J. G.; Gruber, V. F.; Ku, C. C.; Arison, B. H.; Stevenson, J. M.; VandenHeuvel, W. J. A. Fate of Avermectin B$_{1a}$ in Soil and Plants. J. Agric. Food Chem. 1984, 32, 94.

Burg, R. W.; Miller, B. M.; Baker, E. E.; Birnbaum, J.; Currie, S. A.; Hartman, R.; Kong; Y.-L.; Monaghan, R. L.; Olson, G.; Putter, I.; Tunac, J. B.; Wallick, H.; Stapley, E. O.; Oiwa, R.; Omura, S. Avermectin, a New Family of Potent Anthelmintic Agents; Producing Organism and Fermentation. *Antimicrob. Agents Chemother.* **1979**, *15*, 361.

Campbell, W. C.; Fisher, M. H.; Stapley, E. O.; Albers-Schönberg, G.; Jacob, T. A. Ivermectin: a Potent new Antiparasitic Agent. *Science (Washington, D.C.)* **1983**, *221*, 823.

Chiu, S. H. L.; Buhs, R. P.; Sestokas, E.; Taub, R.; Jacob, T. A. Determination of Ivermectin Residues in Animal Tissues by High-Performance Liquid Chromatography–Reverse Isotope Dilution Assay. *J. Agric. Food Chem.* **1985**, *33*, 99.

Chiu, S. H. L.; Sestokas, E.; Taub, R.; Buhs, R. P.; Green, M.; Sestokas, R.; VandenHeuvel, W. J. A.; Arison, B. H.; Jacob, T. A. Metabolism of Ivermectin in Tissues of Cattle, Sheep, and Rats. *Drug Metab. Dispos.* **1986**, *14*, 590.

Kato, R. Characteristics and Differences in the Hepatic Mixed Function Oxidases of Different Species. *Pharmacol. Ther.* **1979**, *6*, 41.

Kline, W. F.; Maynard, M. S.; Feely, W. F.; Wislocki, P. G. The Isolation and Identification of Metabolites of Avermectin B_{1a} Utilizing High Performance Liquid Chromatography. *Abstracts of the Meeting*, 38th Pittsburgh Conference Exposition on Analytical Chemistry and Applied Spectroscopy, Atlantic City, NJ; The Pittsburgh Conference: Pittsburgh, PA, 1987; No. 875.

Ku, C. C.; Jacob, T. A.; Maynard, M. S.; Scimone, A. Photodegradation of Avermectin B_{1a} in Water and Soil. 185th National Meeting of the American Chemical Society, St. Louis, MO; American Chemical Society: Washington, DC, 1983; PEST 79.

Maynard, M. S.; Halley, B.; Green, M.; Hwang, S.; Alvaro, R.; Bennett, B.; Gruber, V. F.; Wislocki, P. G.; Jacob, T. A. The Fate of Avermectin B_{1a} in Rats. *Abstracts of Papers*, 190th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1985a; AGRO 59.

Maynard, M. S.; Gruber, V.; Ku, C. C.; Wislocki, P. G.; Jacob, T. A. The Fate of Avermectin B_{1a} in Lactating Goats. *Abstracts of Papers*, 190th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1985b; AGRO 60.

Maynard, M. S.; Iwata, Y.; Ku, C. C.; Wislocki, P. G.; Jacob, T. A. Fate of Avermectin B_{1a} on Citrus Fruit. 1. Distribution and Magnitude of the Avermectin B_{1a} and ^{14}C Residue on Citrus Fruits from a Field Study. *J. Agric. Food Chem.* **1989a**, *37*, 178.

Maynard, M. S.; Wislocki, P. G.; Ku, C. C. The Fate of Avermectin B_{1a} in Lactating Goats. *J. Agric. Food Chem.* **1989b**, *37*, 1491.

Maynard, M. S.; Gruber, V.; Feely, W. F.; Alvaro, R.; Wislocki, P. G. The Fate of the 8,9-*Z* Isomer of Avermectin B_{1a} in Rats. *J. Agric. Food Chem.* **1989c**, *37*, 1487.

Miwa, G. T.; Walsh, J. S.; VandenHeuvel, W. J. A.; Arison, B. H.; Sestokas, E.; Buhs, R.; Rosegay, A.; Avermitilis, S.; Lu, A. Y. H.; Walsh, M. A. R.; Walker, R. W.; Taub, R.; Jacob, T. A. The Metabolism of Avermectins B_{1a}, H_2B_{1a}, and H_2B_{1b} by Liver Microsomes. *Drug Metab. Dispos.* **1982**, *10*, 268.

Studies into the Mode of Action of Herbicides Derived from 4-[(Benzyloxy)methyl]-1,3-dioxolanes and Benzyl Methyl Ethers of Poly(ethylene glycols)

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The known herbicidal activity of 4-[(benzyloxy)methyl]-2,2,4-trimethyl-1,3-dioxolanes and the similar activity of certain benzyl methyl ethers of some poly(ethylene glycols), $ArCH_2O(CH_2CH_2O)_nMe$ (n = 1–3), described in this paper, cannot be explained in terms of efficient complexation of the herbicide with the alkali metal ions lithium, sodium, or potassium or with the alkaline-earth metal ions magnesium and calcium. A study of the extraction of metal picrates from aqueous solution into dichloromethane solutions of the herbicides gave little evidence for effective complexation of the type observed with cyclic polyethers, and there was no correlation between metal ion induced chemical shifts in the ^1H NMR spectra of members of the two series of compounds and their herbicidal activity.

The potent herbicidal activity of the 2,6-dichlorobenzyl ether of 4-(hydroxymethyl)-2,2,4-trimethyl-1,3-diox-

olane (1) (Barker et al., 1975), of the 2-methylbenzyl ether of *r*-2-ethyl-5-methyl-*cis*-5-hydroxy-1,3-dioxane (3) (Young and Hill, 1973), and of related compounds in controlling grass species invites speculation on how such structurally simple compounds, with seemingly benign functionality, can exert their influence on plant growth. Biolog-

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