Absorption, Tissue Distribution, Excretion, and Metabolism of ^{3}H- and ^{14}C-Labeled Emamectin Benzoate in Rats

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Male and female rats were administered a single high (20 mg/kg, oral) or low (0.5 mg/kg, oral or intravenous) dose of radiolabeled 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB_{1a}) benzoate. The bioavailability of MAB_{1a} benzoate (low dose) in male and female rats was 43% and 63%, respectively. More than 98% of the total radioactivity was found in feces, with <0.4% in urine and <1.6% in the tissues. The total radioactive residues (TRR) in feces and tissues consisted of MAB_{1a} and a single N-demethylated metabolite. The TRR contents (ppb parent equivalent) of kidney, liver, fat, muscle, spinal cord, and brain at day 1 post low oral dose in male rats were 437, 355, 241, 109, 30, and 8 ppb, respectively, and in female rats 260, 244, 192, 90, 11, and 5 ppb, respectively. The TRR contents of these tissues from both male and female rats declined to very low levels (≤12 ppb) by day 7. The TRR content of tissues at day 7 post high dose was relatively high (8−2033 ppb). Lung and spleen had higher residue contents in comparison to other tissues. Daily pretreatment with the compound for 14 days had no effect on the tissue residue levels and metabolism.

Keywords: *Emamectin; MK-0244; 4″-deoxy-4″-(epi-methylamino)avermectin B_{1a}; 4″-deoxy-4″-epi-aminoavermectin B_{1a}; residues; metabolism; HPLC*

INTRODUCTION

The avermectins, a class of biological agents containing a macrocyclic lactone linked to an oleandrosyl disaccharide, are produced by the soil microorganism *Streptomyces avermitilis* (Burg et al., 1979). Abamectin (avermectin B_{1a} and avermectin B_{1b}) has been developed as an insecticide and anthelmintic (Fisher and Mrozik, 1992). Although abamectin is a potent miticide (Campbell et al., 1984), it is less active against other crop pests (Putter et al., 1981). Emamectin benzoate (MK-0244) has high activity against a broad range of lepidopterous larvae and reduces feeding damage on vegetables and sweet corn (Dybas et al., 1989; Fisher and Mrozik, 1992). Emamectin benzoate is chemically synthesized from abamectin by substitution of an aminomethyl ($-NHCH_3$) group for a hydroxyl ($-OH$) group at the 4″-position on the disaccharide and is isolated as a benzoate salt (Figure 1). Like abamectin, emamectin benzoate is composed of a mixture of two homologous compounds, the major (≥90%) constituent 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB_{1a}) benzoate and the minor (≤10%) constituent 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1b} (MAB_{1b}) benzoate. MAB_{1a} differs from MAB_{1b} only by the presence of an additional methylene unit on the side chain at C-25 (Figure 1). This is a minor alteration in a compound of molecular weight of 886 Da. It is therefore likely that the fate of the B_{1b} homolog in rats will be analogous to the major B_{1a} homolog. This is further substantiated by the fact that both homologs are metabolized *in vitro* in a similar fashion by rat liver slices (Mushtaq, unpublished results).

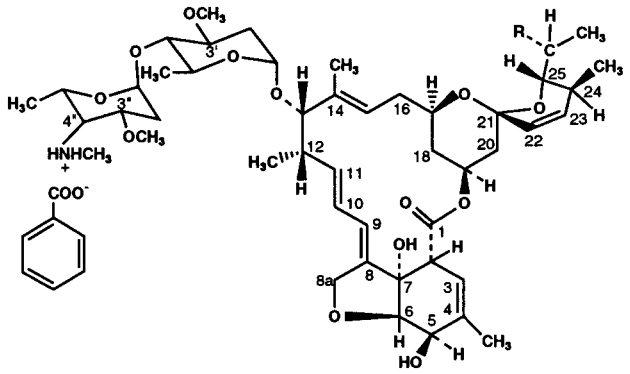

Figure 1. Structure of emamectin benzoate (MK-0244). MAB_{1a} component (>90%), R = CH_2CH_3; MAB_{1b} component (<10%), R = CH_3. [5-^{3}H]MAB_{1a} benzoate and [3-, 7-, 11-, 13-, or 23-^{14}C]MAB_{1a} benzoate were administered to rats.

The toxicity of emamectin benzoate has been extensively studied (Lankas, unpublished results). Since the rat was used as one of the test species to determine the toxicity of emamectin benzoate in a broad range of studies, this species was chosen in the present study to evaluate bioavailability, tissue distribution, metabolism, and excretion of radiolabeled MAB_{1a} benzoate after oral and intravenous administration. The effect of dose level, pretreatment with unlabeled emamectin benzoate, sex, and ^{3}H vs ^{14}C labeling of emamectin benzoate on the metabolic fate of radiolabeled MAB_{1a} were also investigated.

MATERIALS AND METHODS

Solvents and Reagents. All organic solvents and reagents were of analytical grade purity. Insta-Gel XF scintillation cocktail, Carbosorb, Permafluor V, and Monophase S for the determination of radioactivity present in HPLC fractions and

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combusted samples were obtained from Packard Instrument Co., Downers Grove, IL.

Chemicals. The test chemicals [5-^{3}H]MAB_{1a} benzoate (12.265 mCi/mg) and [3-, 7-, 11-, 13-, or 23-^{14}C]MAB_{1a} benzoate (28.82 μCi/mg) were prepared by the Labeled Compound Synthesis Group, Department of Drug Metabolism, Merck Research Laboratories, Rahway, NJ. Unlabeled emamectin benzoate obtained from Chemical Data, Merck Research Laboratories, Rahway, NJ, was used to dose rats in the pretreatment phase (multiple low dose) and to dilute labeled MAB_{1a} benzoate to the appropriate specific activity. Four dosing solutions of radiolabeled MAB_{1a} benzoate were prepared in propylene glycol/saline solution (1:1, v:v). The radiochemical purity of MAB_{1a} in each dosing solution was determined by reversed-phase HPLC (RP-HPLC). There were no impurities greater than 1%. For the high-dose (dual labeled) group, the specific activities of [^{3}H]MAB_{1a} benzoate and [^{14}C]MAB_{1a} benzoate were 34.0 (95% purity) and 5.8 (97% purity) μCi/mg, respectively. The specific activities of [^{14}C]MAB_{1a} benzoate in the multiple low-dose group, low-dose group (male rats), and low-dose group (female rats) were 17.7 (92% purity), 28.1 (98% purity), and 24.1 (98% purity) μCi/mg, respectively. The radiolabeled MAB_{1a} benzoate was homogeneously distributed in all the dosing solutions. Unlabeled MAB_{1a} and 4″-deoxy-4″-*epi*-aminoavermectin B_{1a} (AB_{1a}), the major components of emamectin benzoate and 4″-deoxy-4″-*epi*-aminoavermectin B_1 (AB_1), respectively, were isolated by RP-HPLC for use as analytical standards.

Equipment. Radioactivity in liquid samples (extracts and HPLC fractions) was determined by liquid scintillation counting (LSC) on a model 4530 or 460 counter from Packard Instrument Co., Inc., Downers Grove, IL. The ^{3}H or ^{14}C residues in rat tissues and excreta were converted to ^3H_2O or $^{14}CO_2$, respectively, by combustion on a Packard sample oxidizer (model 306 or 307). The resultant ^3H_2O or $^{14}CO_2$ was trapped separately and mixed with the liquid scintillation fluors, and the radioactivity was determined by LSC. A Perkin Elmer (UV/visible) spectrophotometer (model 320) was used to determine the concentration of test compound in solutions. For HPLC analysis, a HPLC system consisting of a Spectra Physics (SP) 8700 or 8800 solvent delivery system, a SP UV2000 or SP 8480 UV/visible detector, a SP 4400 Chromjet or SP 4200 integrator, a Rheodyne 7125 injector, and a Pharmacia Frac-100 collector with Axxiom AxxiChrom ODS or silica columns (4.6 mm i.d. × 25 cm, 5 μm particle size; Axxiom Chromatography, Analytical Sales and Services, Mahwah, NJ) or a Hibar LiChrosorb RP-18 (ODS) column (4 mm i.d. × 25 cm, 5 μm particle size; E. Merck, Germany) were used. The eluate was monitored at 245 nm. Brownlee C_{18} and silica guard columns were used to protect the analytical C_{18} and silica columns, respectively. The eluates were collected every 1 or every 0.5 min, and the radioactivity was determined by LSC after adding scintillation fluor.

HPLC Methods. The dosing solutions and the ethyl acetate/ammonia ($EtOAc/NH_3$) solid-phase extraction (SPE) eluate were analyzed by either of the three RP-HPLC methods. For each RP-HPLC, a 5 mM concentration of ammonium acetate was present as a modifier in the mobile phase at all times. An Axxiom AxxiChrom ODS column was used at a flow rate of 1 mL/min for two RP-HPLC methods.

Method 1: A 45-min linear gradient from 80% to 90% methanol in water was followed by a 5-min linear gradient from 90% to 100% methanol in water.

Method 2: A 40-min isocratic elution at 85% methanol in water was followed by a 10-min linear gradient from 85% to 100% methanol in water. The column was subsequently washed for another 20 min with methanol for both methods.

Method 3: A LiChrosorb RP-18 column was used at a flow rate of 1.5 mL/min. A 20-min isocratic elution with 88% methanol in water was followed by a 5-min linear gradient from 88% to 100% methanol in water. The column was subsequently washed for another 25 min with methanol.

The normal-phase HPLC (NP-HPLC) method employed an Axxiom AxxiChrom silica column for the isolation and cochromatography of the AB_{1a} metabolite. An isocratic elution with 15% ethanol in isooctane containing 0.4 mM triethylamine at a flow rate of 2 mL/min was used.

Animal Treatment and Sample Collection. Approximately 5-week-old Sprague−Dawley male and nulliparous and nonpregnant female albino rats were received from Charles River Breeding Laboratories, Inc., Wilmington, MA, and acclimated for approximately 1 week before the start of the experiment. The rats were fed Purina rat diet and water *ad libitum* during the acclimation and experimental periods. Some rats were catheterized in the femoral artery and/or vein the day before the administration of dose to collect blood and for intravenous dose administration, respectively. The rats were housed individually in Nalgene metabolism cages. The animal rooms were maintained at ∼23 °C with a 12 h light/dark cycle. Rats were randomly selected before the dose administration. For administration of radiolabeled MAB_{1a} benzoate in rats, each dose was dissolved in 0.4−0.6 mL of the propylene glycol/saline (1:1, v:v). The rats were divided into four groups.

(1) High Oral Dose. Single oral doses of 20 mg of [^{3}H/^{14}C]-MAB_{1a} benzoate/kg of body weight (bw) were administered by gavage to each of six male rats (average weight 211 g) and six female rats (average weight 175 g), respectively.

(2) Multiple Low Oral Dose. A nonradioactive dose of 0.5 mg of emamectin benzoate/kg of bw was administered orally by gavage to male rats (average weight 229 g) and female rats (average weight 177 g), daily for 14 days. On day 15, a dose of 0.5 mg of [^{14}C]MAB_{1a} benzoate/kg of bw was administered orally by gavage to each of six male rats (average weight 266 g) and six female rats (average weight 189 g), respectively.

(3) Low Oral Dose. A single dose of 0.5 mg of [^{14}C]MAB_{1a} benzoate/kg of bw was administered orally by gavage to each of 12 male rats (average weight 233 g) and 12 female rats (average weight 152 g).

(4) Low Intravenous (iv) Dose. A single dose of 0.5 mg of [^{14}C]MAB_{1a} benzoate/kg of bw was administered intravenously into the femoral vein to each of 12 male rats (average weight 233 g) and 12 female rats (average weight 152 g).

Blood and Excreta Collection. Blood (∼0.4 mL) was withdrawn from the femoral artery catheters from half of the male and female rats in the oral and iv low-dose groups at 2, 4, 8, 12, and 18 h, and these rats were sacrificed at day 1 postdose. Approximately the same volume of blood was withdrawn by retro-orbital bleeding from the remaining half of the male and female rats in the oral and iv low-dose groups at 2, 3, 4, 5, and 6 days. At day 7 postradioactive dose, all rats were euthanatized by an overdose of anesthetic ether and the blood was collected by heart puncture. Blood specimens were collected in heparin-coated microfuge tubes and centrifuged using a Brinkmann Eppendorf Centrifuge 5415 for ∼2 min at ∼14 000 rpm. The plasma was removed and saved for radioactivity determination by LSC. Urine including cage rinsate and feces of all rats were collected at 8 h and days 1, 2, 3, 4, 5, 6, and 7 postradioactive dose. All animal procedures were reviewed and approved by the Merck Institutional Animal Care and Use Committee.

Tissue Collection. Immediately after euthanasia, the bone (from hind limbs), brain, fat (from testicular region or abdominal area), gastrointestinal tract (GIT), heart, kidney, liver, lung, muscle (from hind limbs), spinal cord, spleen, and testes or uterus and ovaries were collected from each rat which received MAB_{1a} benzoate orally (euthanatized at day 7 postdose, groups 1−3). The same specimens were collected from untreated control male and female rats. The brain, fat (from testicular region or abdomen area), kidney, liver, muscle (from hind limbs), and spinal cord were collected from each rat which had received MAB_{1a} benzoate orally (euthanatized at day 1 postdose, group 3) or intravenously (euthanatized at days 1 and 7 postdose, group 4). The tail, feet, and skin were removed, and the residual carcass of each rat was collected.

Specimen Preparation and Assay for Total Radioactivity. Homogenates of feces (w:v, 1:5) and tissues (w:v, 1:2−4) were made in 0.05 M sodium phosphate buffer (pH 7.5) by using either a stainless-steel commercial blender (Waring Product Division, New Hartford, CT), a Brinkmann homogenizer (model PT 10/35, generator model PTA 10TS, Brink-

mann Instruments Inc., Westbury, NY), or a Potter-Elvehjem (Teflon−glass) homogenizer with appropriate size probe. Each frozen rat carcass (without skin, tail, and feet) was chopped with a cleaver into small pieces. The small frozen pieces of each rat carcass were ground by the use of a meat grinder (Krefft Fleetwood Typ AL2-3, Nr. 05/1952-0080-120V, WW Lowensten, Inc., Newark, NJ). All the tissues, homogenates, and excreta specimens were stored in a freezer (∼−20 °C) until analysis. The rat specimens except for plasma were combusted by use of a sample oxidizer (model B306 or 307), and the radioactivity was determined by LSC (model 460 or 4530). The dpm data were corrected for the radioactive decay of the ^{3}H isotope and combustion efficiency (>96%) of the oxidizer. The dpm data obtained after the combustion of feces, urine, or tissue replicates from control rats were used to calculate the limit of detection (Chiu et al., 1989).

Radioactive Carbon Dioxide in Exhaled Air. [^{14}C]-MAB_{1a} benzoate was administered by oral gavage to two young adult male and two nulliparous and nonpregnant female rats at 0.38 and 0.45 mg/kg of bw, respectively. The rats were housed in the air-sealed glass metabolism cages (model MC-600M, Crown Glass Co., Somerville, NJ), and the exhaled air was purged from the cages and collected in 1 N sodium hydroxide through the use of an air-inlet tube and vacuum-charged outlet tube connected to the metabolism cages. The radioactivity in alkaline solutions collected at 24 and 48 h postdose was determined by LSC.

Bioavailability and Plasma Half-Life of MAB_{1a} Benzoate. The average plasma residue concentrations at 2, 4, 8, 12, 18, and 24 h (rats sacrificed at day 1 postdose) were computed to calculate the area under the curve (AUC) using the linear trapezoidal rule. The concentration at time 0 for the iv group was estimated by extrapolating the logarithmic concentrations at the first two time points to $t = 0$. The terminal elimination rate constant was estimated by fitting a weighted least-squares to the log−concentration data over time. The AUC from day 1 to infinity was calculated by dividing the concentration at day 1 by the elimination rate constant. The bioavailability was calculated by the following equation:

$$\text{bioavailability} = 100\% \times [AUC_{oral}]/[AUC_{iv}] \qquad (1)$$

Extraction of Residues. The homogenates of either feces or tissue (approximately 1−3 g) were extracted three times with approximately 2 volumes of acetone/g. The supernatants were collected after each centrifugation and pooled. The residual precipitate was suspended in methanol (fraction A) and saved. The pooled acetone supernatants were extracted once with an equal volume of ethyl acetate, and the upper organic phase was removed. The aqueous phase was then extracted with approximately 2 volumes of ethyl acetate, and the resulting organic phase was combined with the other organic phase. The organic-extracted aqueous phase was diluted with methanol (fraction B) and saved. The pooled organic extracts were concentrated by a Buchi rotovapor and reconstituted in ∼6 mL of ethyl acetate. A benzenesulfonyl-2 (Clean UP CUBCX 253, 500 mg/3 mL, Worldwide Monitoring, Horsham, PA) cartridge column was equilibrated with ethyl acetate. The reconstituted organic extracts were applied to the cartridge column followed by elution with ∼4 mL of ethyl acetate. The EtOAc SPE eluate was collected and saved (fraction C). The column was subsequently eluted with ∼12 mL of ethyl acetate saturated with ammonium hydroxide, and the EtOAc/NH_3 SPE eluate was collected (fraction D). The radioactivity in the aliquots of each fraction (A−D) was determined by LSC.

HPLC Identification of AB_{1a} Metabolite. The EtOAc/NH_3 SPE eluates from six feces specimens (days 1, 3, and 7 from male and female rats of the high-dose group) were pooled, dried under nitrogen, and reconstituted in methanol. The AB_{1a} metabolite (t_R ∼39 min) and unmetabolized MAB_{1a} were isolated by RP-HPLC method 1. The peak of unlabeled MAB_{1b}, a minor component of emamectin benzoate, eluted as a shoulder on the AB_{1a} peak. The isolated AB_{1a} metabolite was mixed with the AB_{1a} standard. The mixture was dried for additional HPLC analyses by RP-HPLC methods 2 and 3 and NP-HPLC.

Identification of Metabolite by UV, Mass, and NMR Spectral Analyses. About 25 μg of metabolite was isolated from composited feces of rats treated with [^{3}H/^{14}C]MAB_{1a} benzoate (high dose) after multiple RP-HPLC analyses (method 3). The isolated metabolite was repurified by NP-HPLC, and the UV spectrum of the AB_{1a} metabolite and parent MAB_{1a} was compared. FAB mass spectrometric analysis of the metabolite was performed using a Finnigan-MAT MAT90 mass spectrometer. The metabolite was dissolved in methanol and loaded on a probe tip coated with a 4:1 solution of dithiothreitol/dithioerythritol (magic bullet). The sample was then ionized by a xenon gas FAB gun operated at 6 kV. Proton NMR spectra of the metabolite and standards MK-0244 and AB_1 were determined in deuterated chloroform using a Varian Unity 400 MHz spectrometer with the ambient probe temperature regulated at 25 °C. The spectra were referenced to the residual chloroform peak set at 7.26 ppm.

RESULTS AND DISCUSSION

Selection of Dose and Condition of Rats. A low dose of 0.5 mg of MAB_{1a} benzoate/kg of bw, a presumed no-effect level, was selected for administration to rats based on the acute oral LD_{50} of emamectin hydrochloride (MK-0243), which in this species is 75 mg/kg of bw (Dybas and Babu, 1988). A high dose of 20 mg of MAB_{1a} benzoate/kg of bw was selected for administration to rats in order to produce minimum toxic effects but not preclude meaningful evaluation due to toxicity. After the administration of a high dose, one rat was sluggish for ∼4 h but no other signs related to emamectin benzoate toxicity were observed. Also, no gross abnormalities were observed in other rats. There were no mortalities after single or multiple administration of MAB_{1a} benzoate to rats. A few mortalities unrelated to emamectin benzoate toxicity occurred in the low-dose groups 3 and 4. All the rats appeared to be normal at the time of euthanasia.

Half-Life and Bioavailability of Labeled MAB_{1a} Benzoate. Table 1 shows the TRR levels in plasma after a single oral or intravenous dose of [^{14}C]MAB_{1a} benzoate at 0.5 mg/kg of bw in male and female rats. In the orally treated male and female rats, the maximum plasma concentrations (T_{max}) of MAB_{1a} residues were observed at 12 and 4 h, respectively. The plasma levels then dropped rapidly to 2−4 ppb by 96 h postdose. The plasma depletion curve for TRR followed first-order kinetics from peak values. Since two groups of rats (0−24 and 48−196 h) were used and the TRR in plasma dropped to levels approximately equal to the limit of detection, TRR values up to 24 h (Table 1) were used for the calculation of area under the curve (AUC) and MAB_{1a} elimination half-life. The half-life of plasma TRR was 22.3 and 18.2 h in male and female rats, respectively, after oral administration and 27.9 and 15.1 h in male and female rats, respectively, after intravenous administration. The AUC (ng of residues·h/mL of plasma) was 706 (oral) and 1661 (iv) in male rats. Similarly, the AUC in female rats was 559 (oral) and 882 (iv). Using the ratio of AUC_{oral} and AUC_{iv}, the bioavailability of MAB_{1a} benzoate was 42.5% and 63.3% in male and female rats, respectively (Table 1). Since the plasma TRR values between male and female rats (oral or iv group) at each time point were not statistically different, the observed sex difference of bioavailability and half-life may not be significant.

Table 1. Total Radioactive Residues (ppb Parent Equivalent) in Rat Plasma, Bioavailability, and Plasma Half-Life of MAB_{1a} Benzoate[a]

	residues in male rats		residues in female rats	
postdose (h)	oral	iv	oral	iv
2	8 ± 5	42 ± 10	9 ± 4	35 ± 8
4	14 ± 10	38 ± 7	21 ± 8	34 ± 9[b]
8	16 ± 7	36 ± 6	16 ± 6	30 ± 10[b]
12	17 ± 6	33 ± 5[b]	15 ± 3[b]	26 ± 7[b]
18	15 ± 5	29 ± 6	11 ± 3[b]	16 ± 5[c]
24	12 ± 4	22 ± 8	9 ± 4	13 ± 4[c]
48	6 ± 3	8 ± 2	10 ± 5	11 ± 6
72	ND	6 ± 2	4 ± 1	8 ± 6
96	3 ± 2	4 ± 2	ND	ND
120	3 ± 2	ND	ND	ND
144	ND	3 ± 2[b]	ND	3 ± 3[b]
168	ND	ND	3 ± 1[b]	ND
half-life ($t_{1/2}$)[d] (h)	22.3	27.9	18.2	15.1
AUC[d] (ng of residues·h/mL of plasma)	706	1661	559	882
bioavailability	42.5%		63.3%	

[a] No statistically significant difference was observed when the TRR values of male rats were compared with the respective TRR values of female rats at each time point (oral or iv group). [b,c] All values are expressed as mean ± standard deviation of six replicates ($n = 6$) except as indicated by [b]$n = 5$ or [c]$n = 4$. [d] The rats from the low-oral and iv dose groups were divided into two subgroups. The total radioactive residues in rat plasma from 2 to 24 h and from 48 to 168 h postdose were determined in the first and second subgroups of rats, respectively. AUC of plasma TRR levels between 0 and 24 h postdose was calculated by a software program using the trapezoidal rule. Since the plasma residues in the second subgroup of rats (48−196 h) were low or approximately equal to the limit of detection (not detected, ND), these TRR values were not used. The half-life ($t_{1/2}$) values were calculated by the linear regression analysis from the peak plasma residue levels to the 24 h plasma residue levels. Bioavailability was calculated from the ratio of AUC_{oral} and AUC_{iv} for 0−24 h.

Elimination and Recoveries of MAB_{1a} Benzoate. The recovery of dose was expressed as percent of the administered dose in excreta and tissues of rats. No radioactivity was detected in the exhaled air of male and female rats after oral administration of [^{14}C]MAB_{1a} benzoate. Nearly all of the intravenously or orally administered radioactivity in rats was accounted for in feces (94−104%; Table 2). Moreover, the data presented in Table 2 and Figure 2 show no significant difference in the elimination of MAB_{1a} benzoate residues in feces of male and female rats. More than 90% of the administered radioactivity was eliminated by day 5 postdose (Figure 2). Further, the rate of MAB_{1a} benzoate elimination via the feces was independent of the route of administration (oral or iv) and pretreatment (multiple low dose or single low dose). A very minor fraction of the dose (0.1−0.4%) was accounted for in the urine (Table 2). For all groups of rats, 0.1−1.6% of the total dose was accounted for in the tissues and remain-

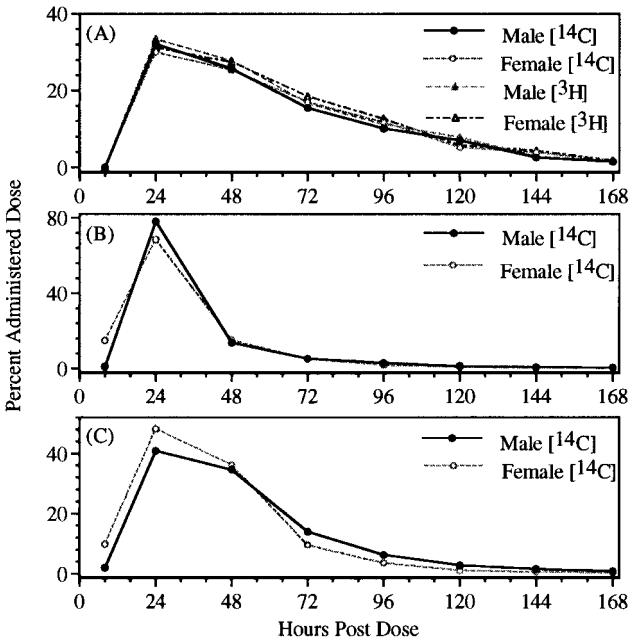

Figure 2. Elimination of radioactivity with time in feces: (A) single oral high dose (20 mg/kg of bw), (B) single oral low dose (0.5 mg/kg of bw), and (C) single intravenous low dose (0.5 mg/kg of bw). The elimination of radioactivity in the multiple oral low-dose group was similar to that in the single oral low-dose group as shown in panel B.

Table 2. Percent Recoveries of Radioactivity in Rat Tissues and Excreta

		recoveries of the administered dose (%)			
rat groups	sex (route)	feces	urine	tissues	total
high dose,[b] ^{14}C (20 mg/kg of bw)	male (oral)	94.43 ± 5.60	0.34 ± 0.15	1.46 ± 0.37	96.23
	$n = 6$	(98.13)[a]	(0.35)[a]	(1.52)[a]	
	female (oral)	94.86 ± 6.16	0.29 ± 0.05	1.24 ± 0.41	96.39
	$n = 6$	(98.41)[a]	(0.30)[a]	(1.29)[a]	
high dose,[b] ^{3}H (20 mg/kg of bw)	male (oral)	101.37 ± 5.98	0.31 ± 0.15	1.59 ± 0.42	103.27
	$n = 6$	(98.16)[a]	(0.30)[a]	(1.54)[a]	
	female (oral)	101.62 ± 6.42	0.26 ± 0.05	1.36 ± 0.47	103.24
	$n = 6$	(98.43)[a]	(0.25)[a]	(1.32)[a]	
multiple low dose,[c] ^{14}C (0.5 mg/kg of bw)	male (oral)	102.19 ± 2.57	0.25 ± 0.02	0.81 ± 0.21	103.25
	$n = 6$	(98.97)[a]	(0.24)[a]	(0.78)[a]	
	female (oral)	103.35 ± 1.58	0.14 ± 0.02	0.25 ± 0.05	103.74
	$n = 6$	(99.62)[a]	(0.13)[a]	(0.24)[a]	
low dose,[d] ^{14}C (0.5 mg/kg of bw)	male (oral)	102.93 ± 3.70	0.12 ± 0.01	0.44 ± 0.13	103.49
	$n = 6$	(99.46)[a]	(0.12)[a]	(0.43)[a]	
	female (oral)	103.90 ± 2.12	0.06 ± 0.01	0.22 ± 0.09	104.18
	$n = 5$	(99.73)[a]	(0.06)[a]	(0.21)[a]	
low dose,[d] ^{14}C (0.5 mg/kg of bw)	male (iv)	101.62 ± 2.43	0.26 ± 0.12	0.12 ± 0.03	102.00
	$n = 5$	(99.63)[a]	(0.25)[a]	(0.12)[a]	
	female (iv)	103.01 ± 1.04	0.17 ± 0.02	0.07 ± 0.02	103.25
	$n = 6$	(99.77)[a]	(0.16)[a]	(0.07)[a]	

[a] All percent recovery values were normalized to 100% and are shown in parentheses (n = number of rats). [b] A single high dose of [^{3}H/^{14}C]MAB_{1a} benzoate was administered. [c] A single low dose of [^{14}C]MAB_{1a} benzoate was administered after pretreatment with emamectin benzoate. [d] A single low dose of [^{14}C]MAB_{1a} benzoate was administered.

Table 3. Total Radioactive Residue Contents (ppb Parent Equivalent) in Rat Tissues (Days 1 and 7 Post Low Dose)[a]

rat groups	route	postdose (day)	brain	fat	kidney	liver	muscle	spinal cord
male ($n = 6$)	oral	1	8 ± 3	241 ± 91	437 ± 191	355 ± 217	109 ± 38	30 ± 20
female ($n = 6$)	oral	1	5 ± 2	192 ± 100	260 ± 105	244 ± 103	90 ± 34	11 ± 6
male ($n = 6$)	iv	1	14 ± 3	409 ± 61	793 ± 265	563 ± 160	196 ± 39	35 ± 8
female ($n = 4$)	iv	1	9 ± 2	300 ± 60	398 ± 63	340 ± 43	189 ± 57	16 ± 5
male ($n = 5$)	iv	7	ND	7 ± 2	12 ± 3	9 ± 2	ND	ND
female ($n = 6$)	iv	7	ND	3 ± 1	5 ± 2	6 ± 2	ND	ND

[a] A single low dose of [^{14}C]MAB_{1a} benzoate/kg of bw was administered orally and intravenously to male and female rats. The rats were euthanatized at 24 h (day 1) and day 7 postdose. ND (not detected): The tissue residues were approximately equal to the limit of detection.

ing carcass of the rats. Recoveries of total radioactivity by 7 days postdose ranged between 96% and 104% (Table 2).

It is expected that as a compound of high molecular weight, MAB_{1a} would be cleared via the hepatic−biliary route. This is evident by the fact that almost all of the intravenously administered radioactivity was excreted in feces. The clearance of orally administered MAB_{1a} benzoate via the hepatic−biliary route was also shown (O'Grodnick, unpublished results). The route of elimination of MAB_{1a} in rats was similar to that of closely related compounds such as 4″-deoxy-4″-(*epi*-acetylamino)avermectin B_1, avermectin B_{1a}, the 8,9-*Z* isomer of avermectin B_{1a}, and ivermectin in rats and other animal species (Chiu and Lu, 1989; Chiu et al., 1990; Halley et al., 1992; Maynard et al., 1989a,b, 1990; Zeng et al., 1996).

Tissue Residues. The total radioactive residue contents (ppb parent equivalent) at days 1 and/or 7 postdose for liver, kidney, fat, muscle, brain, lung, spleen, spinal cord, and other tissues are presented in Tables 3 and 4. The tissue residue contents at day 7 post high dose (20 mg of MAB_{1a} benzoate/kg of bw) ranged between 8 and 2033 ppb and were in the following order: lung > spleen > testis > GI tract ≥ ovary > uterus > kidney ≥ liver > fat > heart > carcass ≥ bone > muscle > spinal cord > blood ≥ brain. The background tissue residue levels as determined by the limit of detection were ≤3 ppb (Table 4). The tissue residue contents at day 7 postdose from either the multiple low-oral dose group or single low-oral and iv dose groups were low (<10 ppb) except for spleen, lung, and kidney tissues which had notable residue contents (12−44 ppb). The total radioactive residue contents (5−793 ppb) in kidney, liver, fat, muscle, spinal cord, and brain detected at day 1 postdose decreased sharply to ≤12 ppb by day 7 post low dose (Tables 3 and 4). The results indicate a short half-life of all tissue residues. Subchronic and chronic toxicity studies of emamectin benzoate have indicated that the compound exerts neurotoxic effects in rats and dogs (Lankas, unpublished results); however, the total radioactive residues in brain and spinal cord were very low in comparison to that in liver, lung, kidney, or spleen (Tables 3 and 4).

Among the major tissues, kidney, liver, lung, and spleen had higher residue contents than those in fat and muscle. In contrast, the residue levels of abamectin and ivermectin in fat were slightly higher than or similar to the corresponding residue levels in liver, kidney, and muscle (Chiu and Lu, 1989; Maynard et al., 1990). There was no difference in the tissue residue distribution pattern between orally and intravenously administered rats; however, the residues in the tissues from intravenously treated rats were somewhat higher than the residues in the analogous tissues from rats treated orally (Tables 3 and 4). The residues in the tissues from male rats were generally higher than those in the respective tissues from female rats of the same treatment group. The tissue residue contents were slightly higher in pretreated rats (multiple low oral dose) in comparison to respective tissue residue contents in rats without pretreatment. Since the rats were treated at different times, and the tissue residue contents were low (1−44 ppb), this difference is probably due to the experimental variation.

Metabolism of MAB_{1a} Benzoate. The EtOAc/NH_3 SPE eluates (fraction D, Figure 3) obtained after processing of feces, liver, kidney, or muscle homogenate generally contained more than 90% of the total radioactivity in the sample. These radioactivity-enriched SPE eluates of feces and tissues were analyzed by RP-HPLC (method 1). The percentages of the total column radioactivity associated with UV peaks of AB_{1a} and MAB_{1a} standards were determined. RP-HPLC analyses of feces indicated one detectable metabolite (AB_{1a}) which eluted prior to MAB_{1a}, whereas the HPLC analysis of untreated rat feces spiked with dosing solution indicated no significant radioactivity under the peak of AB_{1a} (radiochromatograms not shown). In all the feces samples analyzed, the components of MAB_{1a} benzoate residues were similar. Table 5 indicates that the percentage of the metabolite in feces increased with time from 1−6% (day 1) to 11−20% (day 7) postadministration of MAB_{1a} benzoate in all groups of rats.

Similarly as observed in the feces of rats, the RP-HPLC analyses of liver, kidney, muscle, and fat collected at day 7 postdose from the high-dose group of rats indicated a single metabolite, AB_{1a} (4−23%) (Table 5 and Figure 4). RP-HPLC analyses (Table 5) of liver and kidney collected at day 1 from the low-dose group of rats (oral and iv, male and female rats) also indicated the presence of the metabolite AB_{1a} (5−15%). The distribution of radioactivity between the EtOAc (fraction C) and EtOAc/NH_3 (fraction D) SPE eluates from fat was different in comparison to other tissues. The radioactivity was equally distributed between the EtOAc (<50%) and EtOAc/NH_3 (>50%) SPE eluates. Therefore, both SPE eluates obtained from fat were analyzed by RP-HPLC. The EtOAc/NH_3 SPE eluates contained a single metabolite, AB_{1a} (Table 5). RP-HPLC analyses of the EtOAc SPE eluate indicated radioactivity in regions more polar than AB_{1a} (data not shown). Since the fat tissues contained low residue levels (~0.4 ppm; Table 4, high dose), no attempt was made to characterize the components of the EtOAc SPE eluate. The metabolic profile and percent AB_{1a} formed were not related to sex, dose level (20 or 0.5 mg/kg), route of administration (low oral or iv dose), or pretreatment with nonradioactive emamectin benzoate (multiple low oral dose).

A general pattern of an increase in the AB_{1a} metabolite relative to parent MAB_{1a} was observed as the total residue contents declined in feces and tissues (Table 5). For example, the percent AB_{1a} metabolite in feces

Table 4. Total Radioactive Residue Contents (ppb Parent Equivalent) in Rat Tissues after Oral Administration of [^{14}C]MAB$_{1a}$ Benzoate and [^{3}H]MAB$_{1a}$ Benzoate (Day 7 Postdose)

rat groups	label	blood	bone	brain	carcass	fat	GI tract	heart	kidney	liver	lung	muscle	spleen	spinal cord	testis/ovary	uterus
high dose																
male (n = 6)	^{14}C	15 ± 5	228 ± 65	13 ± 3	255 ± 57	386 ± 70	753 ± 175	191 ± 60	675 ± 217	578 ± 193	2033 ± 830	121 ± 25	1281 ± 296	18 ± 8	880 ± 157	NA[a]
	^{3}H	17 ± 5	216 ± 62	13 ± 3	249 ± 58	348 ± 74	771 ± 180	188 ± 55	659 ± 217	590 ± 209	1971 ± 798	122 ± 26	1234 ± 302	21 ± 6	879 ± 161	NA[a]
female (n = 6)	^{14}C	11 ± 95	256 ± 95	8 ± 3	192 ± 49	428 ± 128	1060 ± 471	206 ± 55	501 ± 160	559 ± 213	1445 ± 487	115 ± 44	1440 ± 453	16 ± 4	872 ± 329	603 ± 244
	^{3}H	13 ± 4	244 ± 99	10 ± 3	191 ± 54	368 ± 107	1088 ± 490	202 ± 57	490 ± 168	566 ± 217	1427 ± 469	110 ± 42	1376 ± 442	20 ± 7	787 ± 297	566 ± 241
multiple low dose																
male (n = 6)	^{14}C	ND	5 ± 3	ND	4 ± 1	7 ± 2	13 ± 9	4 ± 2	12 ± 4	9 ± 3	38 ± 15	ND	44 ± 25	ND	8 ± 2	NA[a]
female (n = 6)	^{14}C	ND	3 ± 1	ND	ND	4 ± 2	4 ± 1	ND	ND	ND	9 ± 3	ND	11 ± 5	ND	4 ± 3	3 ± 1
low dose																
male (n = 6)	^{14}C	ND	3 ± 1	ND	ND	ND	6 ± 3	ND	5 ± 2	3 ± 1	15 ± 8	ND	22 ± 9	ND	4 ± 1	NA[a]
female (n = 5)	^{14}C	ND	ND	ND	ND	ND	ND	ND	ND	ND	4 ± 2	ND	8 ± 4	ND	3 ± 1	ND
limit of detection[b]	^{14}C	1	2	1	1	1	3	1	1	1	2	1	3	2	1	1[c]

[a] NA: not applicable for male rats. ND (not detected): The tissue residues were approximately equal to the limit of detection. [b] The combustion dpm data of the same tissue from untreated male and female rats (low dose) were pooled for the determination of the limit of detection. [c] Ovary and uterus combustion dpm data from untreated female rats (low dose) were pooled for the determination of the limit of detection.

Figure 3. Extraction procedure for the isolation of radioactive residues by a benzenesulfonyl-2 (CUBCX) cartridge column. The EtOAc/NH_3 SPE eluate from feces or tissue generally contained more than 90% of the total radioactivity in all fractions. An aliquot of fraction D was mixed with analytical standards (MAB$_{1a}$ and AB$_{1a}$), dried, and reconstituted in methanol for RP-HPLC analysis.

increased from ≥1% to ≤20% from day 1 to 7 postadministration as the percent daily excretion in feces decreased to <4% of the administered dose (Table 5 and Figure 2). The results indicate that the total radioactive residues were comprised only of parent MAB$_{1a}$ and the metabolite AB$_{1a}$ in feces and tissues of rats (Table 5). Zeng et al. (1996) also reported AB$_{1a}$ as a major metabolite of 4″-deoxy-4″-(*epi*-acetylamino)avermectin B$_1$ in rat tissues. Thus, N-demethylation or N-deacetylation is the main route of metabolism for avermectin compounds containing a methylamino or acetylamino group, respectively, at the 4″-position. The 24-hydroxymethyl and 3″-desmethylmetabolites of avermectin B$_{1a}$ and ivermectin, the structurally related compounds but containing a hydroxyl group at the 4″-position, have been reported previously in the rat (Maynard et al., 1990; Halley et al., 1992), but metabolism apparently does not occur at these positions of MAB$_{1a}$.

Confirmation of AB$_{1a}$ Metabolite. The radioactive AB$_{1a}$ metabolite isolated by RP-HPLC was mixed with unlabeled AB$_{1a}$. The mixture was analyzed by two RP-HPLC and one NP-HPLC methods (Figure 5). The results of first RP-HPLC analysis (method 2) indicated a UV peak of AB$_{1a}$ (t_R 33.3 min). Unlabeled MAB$_{1b}$, a minor component present in the sample, coeluted with AB$_{1a}$. Most of the radioactivity (^{3}H, 98%; ^{14}C, 97%) was eluted in HPLC fractions associated with the UV peak of AB$_{1a}$ (chromatograms not shown). The results of the second RP-HPLC analysis (method 3) indicated one major peak of AB$_{1a}$ (t_R 15.9 min) and a very minor peak of MAB$_{1b}$ (t_R ~22 min). Again, most of the radioactivity (^{3}H, 83%; ^{14}C, 87%) was eluted in HPLC fractions associated with the UV peak of AB$_{1a}$ (Figure 5A). The

Table 5. Residue Components (AB_{1a}/MAB_{1a}) of Rat Feces and Tissues (Percent of Total Column Radioactivity)

			feces[a]			day 7			
rat groups and sex	route	label	day 1	day 3	day 7	liver[a]	kidney[a]	muscle[a]	fat[a]
high dose									
male	oral	^{3}H	1.5/90.0	8.8/82.3	18.1/73.0	16.9/72.6	20.2/70.0	17.1/77.2	17.0/61.9
		^{14}C	1.5/88.9	9.0/82.7	18.2/73.2	16.7/71.2	19.1/67.4	17.3/68.4	14.3/67.4
female	oral	^{3}H	2.2/86.7	7.4/80.1	19.4/71.8	22.5/70.9	21.0/70.1	22.3/59.3	4.4/86.5
		^{14}C	2.0/85.4	7.5/80.0	19.8/72.1	19.7/68.7	21.3/69.3	18.3/41.0	6.0/67.9
multiple low dose									
male	oral	^{14}C	1.7/91.3	9.6/82.0	15.2/56.2	NA[b]	NA[b]	NA[b]	NA[b]
female	oral	^{14}C	2.9/92.3	11.6/75.4	12.7/45.8	NA[b]	NA[b]	NA[b]	NA[b]
low dose							day 1		
male	oral	^{14}C	3.3/90.1	10.0/80.8	17.7/67.0	11.9/82.5	12.4/84.4	10.9/86.7	NA[b]
female	oral	^{14}C	2.9/93.7	6.6/84.4	12.6/46.3	13.6/82.7	15.5/77.6	14.3/68.6	NA[b]
low dose									
male	iv	^{14}C	5.8/88.6	10.1/83.6	20.6/75.8	8.6/85.5	8.1/86.0	NA[b]	NA[b]
female	iv	^{14}C	3.5/92.9	7.1/86.0	11.3/51.0	4.7/93.8	7.2/87.6	NA[b]	NA[b]

[a] The feces and tissue specimens from the same dose group, sex, and day of collection were composited. Each composited specimen was subjected to solid-phase extraction (Figure 3), and the resulting EtOAc/NH_3 SPE eluate was analyzed by RP-HPLC. [b] NA: not applicable. The specimens which had low radioactive residues were not analyzed.

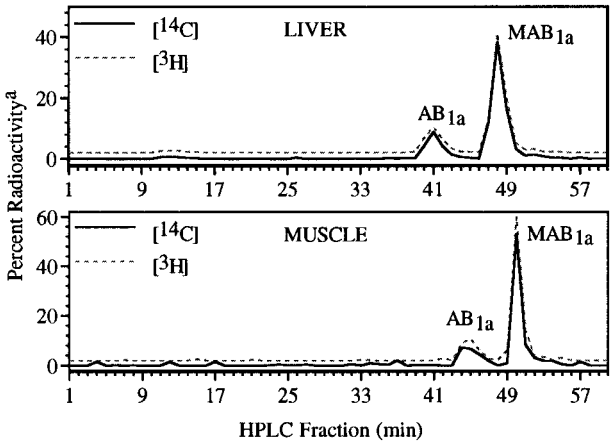

Figure 4. RP-HPLC analyses of extractable residues from rat liver and muscle (high-dose group). The EtOAc/NH_3 SPE eluates (Figure 3) were analyzed by RP-HPLC method 1. The ^{3}H radiochromatograms were offset by a unit of 2. [a]Percent of total column radioactivity.

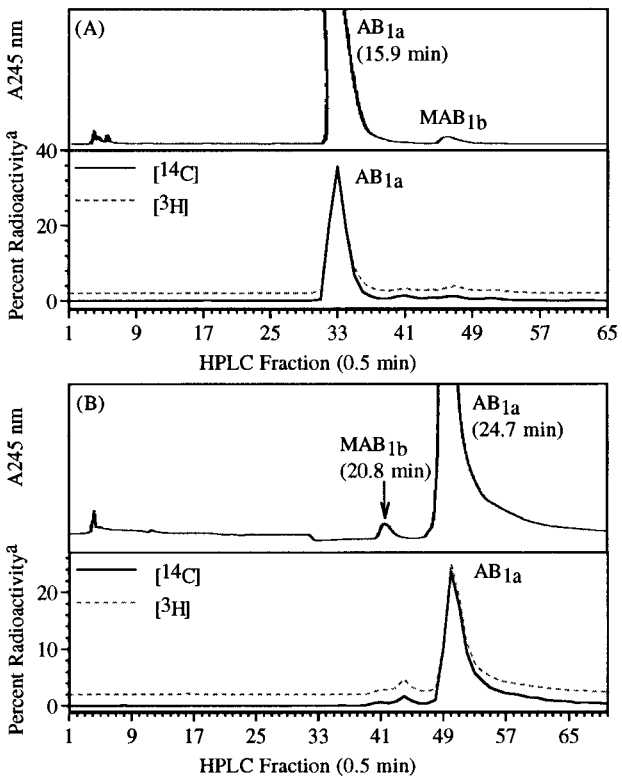

Figure 5. Cochromatography of the AB_{1a} metabolite with AB_{1a} standard by RP- and NP-HPLC. The AB_{1a} metabolite was isolated from the extractable residues of composite feces specimens by RP-HPLC method 1. The isolated metabolite was mixed with unlabeled AB_{1a} standard. Aliquots of the mixture were rechromatographed by RP-HPLC method 2 (chromatograms not shown) and RP-HPLC method 3 (A) and by NP-HPLC (B). Most (83−98%) of the column radioactivity was accounted for in the HPLC fractions collected under the UV peak of AB_{1a}. The ^{3}H radiochromatograms were off set by a unit of 2. [a]Percent of total column radioactivity.

results of the NP-HPLC analysis indicated a minor peak of MAB_{1b} (t_R 20.8 min) and a major peak of AB_{1a} (t_R 24.7 min). Most of the radioactivity (^{3}H, 87%; ^{14}C, 91%) was eluted in HPLC fractions associated with the UV peak of AB_{1a} (Figure 5B). Thus, the RP and NP radiochromatograms indicated one predominant peak of AB_{1a}, and >80% of the total column radioactivity was eluted under the UV peak of AB_{1a} by all three HPLC methods (Figure 5).

About 25 μg of radioactive metabolite was isolated from feces by HPLC for UV, FAB-MS, and NMR spectral analyses. The UV spectra of MAB_{1a} standard and its metabolite AB_{1a} (λ_{max} 245 nm) in methanol were identical (UV spectra not shown), indicating no chromophore change as expected. The FAB-MS indicated an intense pseudomolecular ion $[M + H]^+$ at m/z 872 corresponding to a molecular weight of 871 (mass spectrum not shown). The molecular weight of the metabolite was 14 mass units less than that of MAB_{1a} (885), consistent with demethylation of the parent molecule. The NMR spectral analysis of the metabolite indicated the absence of the *N*-methyl peak at 2.63 ppm and a 0.28 ppm downfield displacement of H-4″ relative to emamectin benzoate (NMR spectra not shown). Both MS and NMR observations are consistent with the 4″-*epi*-amino structure of the metabolite. This conclusion was further reinforced by the close resemblance with the NMR spectrum of a reference standard AB_1. Thus, the results of spectral analyses and cochromatography confirmed the metabolite as the *N*-desmethyl analog of MAB_{1a}.

Stability of Tritium Label. Recoveries of tritium and carbon-14 in feces and tissues of rats treated with a high dose (20 mg of [^{3}H/^{14}C]MAB_{1a} benzoate/kg of bw) and tissue residue levels based on the respective specific activities were essentially identical (Tables 2 and 4 and Figure 2). Further, HPLC analyses of the extractable residues from rats treated with dual labeled compound indicated no difference in the metabolic profile (Figure 4). Therefore, the tritium label at the 5-position of the

MAB_{1a} molecule was stable, and the use of [^{3}H]MAB_{1a} benzoate is appropriate in animal metabolism studies.

In conclusion, the results of the rat metabolism study indicate that MAB_{1a} benzoate was eliminated rapidly and almost entirely in feces. The elimination of MAB_{1a} benzoate was independent of sex and route of administration. The bioavailability of MAB_{1a} benzoate in male and female rats was 42.5% and 63.3%, respectively. The half-life of MAB_{1a} benzoate elimination in male rats after oral or intravenous administration was 22.3 or 27.9 h, respectively. Similarly, the half-life of MAB_{1a} benzoate elimination in female rats after oral or intravenous administration was 18.2 or 15.1 h, respectively. The total radioactive tissue residues were dose dependent and depleted rapidly with time, and no tissue storage of radiolabeled MAB_{1a} was observed. The residues in feces and tissues consisted primarily of MAB_{1a}, and little conversion to the AB_{1a} metabolite was observed. Pretreatment with unlabeled compound for 14 consecutive days prior to the administration of radioactive dose had no effect on tissue residue levels and metabolism. The results also indicate the stability of the ^{3}H label at the 5-position of MAB_{1a} which was previously demonstrated for abamectin (Maynard et al., 1990).

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