Fate of ^{3}H- and ^{14}C-Labeled Emamectin Benzoate in Lactating Goats†

Mohammad Mushtaq,* Loretta R. S. Allen, Louis S. Crouch, and Peter G. Wislocki

Pesticide Metabolism and Environmental Safety, Department of Drug Metabolism, Merck Research Laboratories, P.O. Box 450, Hillsborough Road, Three Bridges, New Jersey 08887-0450

Radiolabeled 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB_{1a}) benzoate was administered orally to lactating goats for 7 consecutive days at a dose equivalent to 10 ppm in the diet. The total radioactive residues at 10 h after the last dose of [^{3}H]MAB_{1a} benzoate were in the following order: liver (1002 ppb) > kidney (499 ppb) > renal and omental fat (283 ppb) > *biceps femoris* (leg) muscle (118 ppb) and *longissimus dorsi* (loin) muscle (96 ppb). The plasma (8−26 ppb) and milk (13−43 ppb) residue concentrations were much lower than tissue residues. More than 98% of the total administered radioactivity was accounted for in the sum of feces (>86%) and gastrointestinal tract contents (>12%). The residues in feces, milk, and tissues generally consisted of the parent compound, MAB_{1a} (>89%), and its N-demethylated metabolite (<6%). The concentrations of ^{3}H and ^{14}C residues and their composition in the specimens of goat which received dual-labeled MAB_{1a} benzoate were approximately the same and followed the same order as indicated above.

Keywords: *Goat; emamectin; abamectin; 4″-deoxy-4″-(epi-methylamino)avermectin B_{1a}; MK-0244; pesticide; residues; HPLC; metabolism*

INTRODUCTION

The avermectins are a class of natural products produced by the soil microorganism *Streptomyces avermitilis*, containing a macrocyclic lactone linked to an oleandrosyl disaccharide (Burg et al., 1979). Avermectin B_1 or abamectin, a crop pesticide and anthelmintic, is a potent miticide (Campbell et al., 1984) but is less active against other crop pests (Putter et al., 1981). Emamectin is synthesized from abamectin (avermectin B_{1a} and B_{1b}) by substitution of an *epi*-methylamino ($-NHCH_3$) group for a hydroxyl ($-OH$) group at the 4″-position on the disaccharide and is isolated as a benzoate salt (Figure 1). Emamectin benzoate is active against a broad range of lepidopterous larvae and is being developed for use on cole crops and leafy and fruiting vegetables (Dybas et al., 1989; Lasota and Dybas, 1991). Like abamectin, emamectin benzoate (MK-0244) is composed of a mixture of two homologous compounds, a major (≥90%) constituent 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB_{1a}) benzoate and a 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 difference in a compound of molecular weight of 886 (MAB_{1a}). The metabolism of abamectin in rats and goats has not been shown to occur in this side chain (Maynard et al., 1989b, 1990). Thus, it is likely that the fate of the minor B_{1b} homologue in goats would be analogous to that of the major B_{1a} homologue. This is further substantiated by the fact that both homologues are indeed metabolized in a similar fashion by rat liver slices (Mushtaq, unpublished results). Potential residues of emamectin benzoate in the processed commodities of some crops may be consumed by ruminants and consequently could result residues in milk or meat from such animals. It is therefore necessary to determine the nature of residues which occur when ruminants (e.g., goats) are treated with emamectin benzoate. A dose equivalent to 10 ppm in the diet was selected to ensure that sufficient material would be available for metabolite identification. In the present study, the metabolic stability of the tritium label at C-5 position of MAB_{1a} was compared to that of carbon-14 at C-25 (Figure 1).

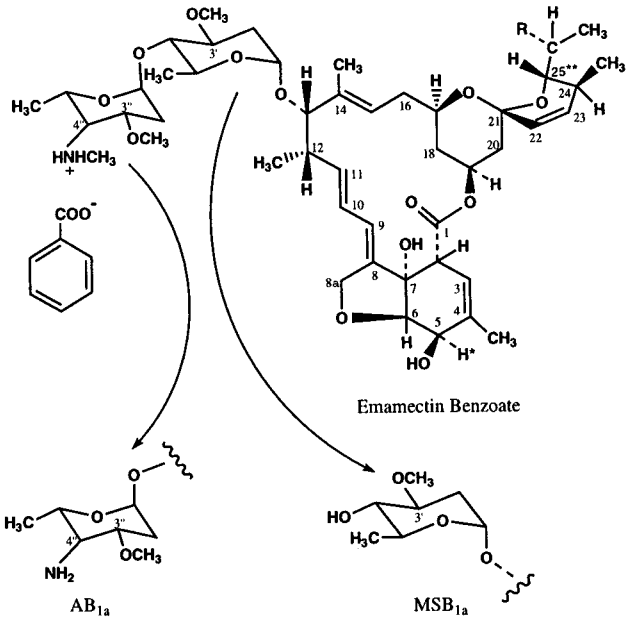


Figure 1. Structures of test and reference compounds. Emamectin benzoate (MK-0244): MAB_{1a} component (≥90%), R = CH_2CH_3; MAB_{1b} component (≤10%), R = CH_3. *[5-^{3}H]-MAB_{1a} benzoate and **[25-^{14}C]MAB_{1a} benzoate were administered to goats. Nonradioactive emamectin benzoate (MK-0244), 4″-deoxy-4″-(*epi*-amino)avermectin B_{1a} (AB_{1a}), and avermectin B_{1a}-monosaccharide (MSB_{1a}) were used as reference standards.

† Part of the data were presented as a poster (AGRO Abstract #054) at the ACS National Meeting, Chicago, August, 20−24, 1995.

* To whom correspondence should be addressed [e-mail, mohammad_mushtaq@merck.com; FAX, (908) 369-8811].

S0021-8561(96)00408-6 CCC: $14.00

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 eluates and combusted samples were obtained from Packard Instrument Co., Downers Grove, IL. The clear gelatin capsules used for dosing were received as a gift from Eli Lilly and Co., Indianapolis, IN. Cellulose powder was purchased from Sigma Chemical Co., St. Louis, MO.

Chemicals. The test chemicals [5-^{3}H]MAB_{1a} benzoate (16.022 mCi/mg) and [25-^{14}C]MAB_{1a} benzoate (18.24 μCi/mg) were prepared by the Labeled Compound Synthesis Group, Department of Drug Metabolism, Merck Research Laboratories, Rahway, NJ. Nonradioactive emamectin benzoate was used to dilute the radiolabeled MAB_{1a} benzoate to an appropriate specific activity. Two solutions of radiolabeled MAB_{1a} benzoate (^{3}H or ^{3}H + ^{14}C) were prepared in ethanol. The radiochemical purity of MAB_{1a} in each dosing solution was determined by reversed-phase HPLC (RP-HPLC), and no impurity more than 1% was observed. For the dual-labeled compound, the specific activities of [^{3}H]MAB_{1a} benzoate and [^{14}C]MAB_{1a} benzoate were 29.1 μCi/mg (97% radiopurity) and 12.9 μCi/mg (98% radiopurity), respectively. For the single-labeled compound, the specific activity of [^{3}H]MAB_{1a} benzoate was 45.8 μCi/mg (97% radiopurity). The radiolabeled MAB_{1a} benzoate was homogeneously distributed in the dosing solution. Unlabeled emamectin benzoate, 4″-deoxy-4″-(*epi*-amino)-avermectin B_1 (AB_1), and avermectin B_{1a} monosaccharide (MSB_{1a}) were used as analytical standards (Figure 1). Also, MAB_{1a} and AB_{1a} were isolated by RP-HPLC from emamectin benzoate and AB_1 standards, respectively, for use as analytical standards.

Equipment. Radioactivity in liquid samples (extracts and HPLC eluates) was determined by liquid scintillation counting (LSC) on a Model 2500 TR counter from Packard Instrument Co., Inc., Downers Grove, IL. The [^{3}H] or [^{14}C] residues in goat tissues and excreta were converted to ^3H_2O or $^{14}CO_2$, respectively, by radiocombustion assay (RCA) using a Packard sample oxidizer (Model 307). The resultant ^3H_2O and $^{14}CO_2$ were 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 solution. For HPLC analysis, a Spectra Physics (SP) 8700 or SP P4000 solvent delivery system, a SP UV2000 UV/visible detector, a SP 4400 Chromjet or a PE Nelson 1020x integrator, a Rheodyne 7125 injector, and a Pharmacia Frac-100 collector were used. An Axxiom AxxiChrom ODS, Advantage Plus ODS (4.6 mm i.d. × 25 cm, 5 μm particle size, Axxiom Chromatography, Analytical Sales and Services, Mahwah, NJ), Shiseido Capacell Pak C18 (4.6 mm i.d. × 25 cm, 5 μm particle size, Type 120, Dychrom, Santa Clara, CA), or LiChrospher 100 Diol (4 mm i.d. × 25 cm, 5 μm particle size, E. Merck, Germany) column was used. The eluate was monitored at 245 nm. Brownlee C_{18} and silica guard columns were used to protect the analytical C_{18} and diol columns, respectively. HPLC eluates were collected in 1 min fractions for radioactivity determination.

Animal Procurement, Housing, and Treatment. The in-life phase of the study was conducted at the Metabolic Laboratory, Colorado State University (CSU), Fort Collins, CO. Eight nonpregnant lactating Alpine goats (age 1−5 years) were obtained from the Bull Mountain Ranch, Billings, MT. All goats were examined by a veterinarian and were found to be in good health. Tap water and feed were provided ad libitum. The diet consisted of ∼2 kg of grain-based milking ration (Ranch-Way Klassie Goat: 16.0% minimum crude protein, 2.0% minimum crude fat, 8.0% maximum crude fiber) and ∼2 kg of roughage (chopped alfalfa hay) per day. All goats were acclimated for approximately 1 week before the start of the dosing in individual metabolism cages (each 48 in. x 28 in. x 30 in., made of galvanized angle-iron frame). Daily feed consumption was quantitated gravimetrically during acclimation, and dosing periods and average feed consumption per day was calculated.

Dosing capsules were prepared by drying the aliquots of the test compound on cellulose contained in the gelatin capsules. Five goats (40−58 kg) were randomly selected from the eight animals. Three lactating goats (mean weight 54 kg) received an oral dose of 27 mg of [^{3}H]MAB_{1a} benzoate [0.5 mg/kg of body weight (bw)], and one lactating goat (48 kg) received an oral dose of 32 mg of [^{3}H/^{14}C]MAB_{1a} benzoate (0.67 mg/kg of bw) in the prepared gelatin capsules daily for 7 days. A control lactating goat (40 kg) similarly received gelatin capsules containing cellulose only. The gelatin capsules were administered to goats in the morning by the use of a balling gun.

Blood, Milk, and Excreta Collection. Blood was collected daily in heparinized vacutainer tubes from the jugular vein of each goat before the dose administration. The blood sample tubes were then centrifuged and the plasma saved for analysis. The goats were milked twice daily; the milk specimens collected in the morning prior to dosing and in the late afternoon were saved for analysis. Just before the daily dosing, feces and urine were also collected. The final blood, milk, urine, and feces were collected just before slaughter.

Tissue Collection. All goats were euthanatized ∼10 h after the last dose. Immediately after euthanasia, liver, kidney, *biceps femoris* (leg) muscle, *longissimus dorsi* (loin) muscle, omental fat, renal fat, contents of the stomach, large intestine and small intestine, urine from the bladder, and bile from the gall bladder were collected separately from each goat. For the three separate gastrointestinal tract (GIT) specimens, the entire amount of each was mixed well and an aliquot of each was removed for analysis. All the specimens were stored frozen. All animal procedures were reviewed and approved by the Merck Institutional Animal Care and Use Committee.

Specimen Preparation and Assay for Total Radioactivity. Milk samples were centrifuged in a VCR centrifuge at 985 g for 10 min to separate the whole milk into skim milk and cream. Feces were homogenized in a large commercial Waring blender with water. All tissue specimens were chilled and were ground without addition of water. Liver and kidney specimens were ground in a 3.74 L Waring blender and a Presto Mini/Max food processor, respectively. Fat and muscle specimens (≤1 kg) were ground with an Omas meat grinder. If necessary, the ground tissue specimens were homogenized (1:2, w:v) in 0.05 M phosphate buffer (pH 7.5) using a Potter-Elvehjem (Teflon-Glass) homogenizer before analysis. Aliquots of feces, liver, kidney, muscle, and fat specimens (ground or homogenized) were dried prior to RCA. The radioactivity in bile, urine, plasma, and milk specimens was determined directly by LSC. The dpm data were corrected for the radioactive decay of ^{3}H isotope and combustion efficiency (>96%) of the oxidizer. The background radioactivity in specimens from the control goat was used to calculate limits of detection (Chiu et al., 1989) which ranged from 0.1 to 2 ppb.

Extraction of Residues. Figure 2 outlines the organic extraction and subsequent solid-phase extraction (SPE) of residues from milk and the homogenates of feces or nonfat tissues. About 1−5 g of specimen was extracted three times with ∼2 vol of acetone, and the supernatants were collected after centrifugation. The residual precipitate after the third extraction was suspended in methanol (fraction A) and saved. The pooled acetone supernatants were then extracted once with an equal volume of ethyl acetate, and the resultant upper organic phase was removed. The aqueous phase was then twice extracted with approximately 1 vol of ethyl acetate, and the resultant organic phases were combined with the previous organic phase. The organic-extracted aqueous phase was diluted with methanol (fraction B) and saved. A benzenesulfonyl-2 (CUBCX-253) SPE column (Worldwide Monitoring, Horsham, PA) was conditioned with ethyl acetate. The pooled organic extracts (in ethyl acetate) were applied to conditioned SPE columns followed by elution with ∼5 mL of ethyl acetate. The ethyl acetate eluate (EtOAc SPE eluate, fraction C), generally a colored solution, was saved. The column was subsequently eluted with ∼12 mL of ethyl acetate saturated with ammonium hydroxide and the eluate (EtOAc/NH_3 SPE eluate, fraction D) was saved. The radioactivity in the aliquots of each fraction (A, B, C, and D) was determined by LSC. For the fat specimens, homogenates (1:6, w/v) were prepared in

isotonic saline/acetone (1:1, v/v). Each homogenate was then extracted three times with approximately 2 vol of ethyl acetate. The organic extracts were then processed as described for feces and nonfat tissues. Suitable aliquots from the EtOAc/NH_3 SPE eluate (fraction D) were mixed with nonradioactive standards, i.e., AB_1 or AB_{1a}, MK-0244 or MAB_{1a} and/or MSB_{1a}. The mixtures containing radioactive residues and nonradioactive standards were dried under nitrogen, reconstituted in methanol and analyzed by RP-HPLC (methods 1 and 2 as described below) for the quantitation of metabolite(s) and parent MAB_{1a}.

HPLC Methods. The test compounds and residues in the EtOAc/NH_3 SPE eluates were analyzed by one of the following four RP-HPLC methods. For all RP-HPLC methods, a flow rate of 1 mL/min was used. Methanol−water containing 5 mM ammonium acetate or acetonitrile−water containing 0.4 mM triethylamine (TEA) was used as the eluting solvent. For RP-HPLC method 1, an Advantage Plus ODS column was used with a 35-min isocratic elution at 82% methanol, followed by a 10-min linear gradient from 82 to 100% methanol. An Axxiom AxxiChrom ODS column was used for the two other RP-HPLC methods. A 45-min (method 2) or a 50-min (method 3) linear gradient from 80 to 90% methanol was followed by a 5-min linear gradient from 90 to 100% methanol. For RP-HPLC method 4, a Shiseido C18 column was used with a 30-min isocratic elution at 60% acetonitrile, followed by a 10-min linear gradient from 60 to 100% acetonitrile. For confirmation of AB_{1a} metabolite, a normal-phase HPLC (NP-HPLC) method with LiChrospher diol column was used. The eluant was 18% ethanol in isooctane containing 0.4 mM triethylamine at a flow rate of 1 mL/min.

Identification of Metabolites. The metabolite was confirmed by serial RP/NP-cochromatography with a reference standard. Both AB_1 and MSB_{1a} standards were added to each liver and feces EtOAc/NH_3 SPE eluate. The radioactivity coeluting with the added standards was isolated both from liver and feces samples after multiple RP-HPLC analyses (method 3). The RP-HPLC fractions containing AB_{1a} or MSB_{1a} standard as indicated by the UV chromatograms were dried and reconstituted in ethanol/isooctane (1:2, v/v) and were analyzed by NP-HPLC. The NP-HPLC eluates which were associated with the UV peak of AB_{1a} standard were combined, dried, reconstituted in acetonitrile for confirmation by cochromatography. The reconstituted NP-HPLC fractions containing AB_{1a} standard as indicated by UV chromatogram were analyzed by RP-HPLC (method 4). For these serial chromatographic analyses, suitable aliquots from each HPLC eluate were withdrawn for determination of radioactivity and the rest of the eluate fractions were saved for the next HPLC analysis.

Statistical Methods. The results were expressed as mean ± standard deviation. Statistical difference was evaluated by unpaired Student's *t*-test for two-sample comparison. *P* values of <0.05 were considered significant.

RESULTS AND DISCUSSION

Selection of Dose and Condition of Goats. The dose for this study was nominally equivalent to 10 ppm in diet. This dose is >500 times that expected for MAB_{1a} residues in plants or plant parts resulting from the agricultural use of the compound (Prabhu et al., 1991). The dietary equivalent dose was calculated on the basis of the average feed intake during treatment period although the animals were actually dosed orally by capsules once daily. Thus, the goats were treated with 8.5 ± 1.1 ppm of [^{3}H]MAB_{1a} benzoate (0.5 mg/kg of bw) or 9.6 ppm of [^{3}H/^{14}C]MAB_{1a} benzoate (0.67 mg/kg of bw) equivalents in the diet for 7 days. All goats appeared to be normal during the treatment period, and no signs related to emamectin benzoate toxicity were observed. Moreover, in a separate goat toxicity study where emamectin benzoate was administered orally in capsules to goats for 7 consecutive days at doses of 0.26, 0.45, and 0.87 mg/kg of bw or 4, 7, and 14 ppm equivalent in diet, no effects on daily food consumption, milk production, and behavior were observed (Mushtaq, unpublished results).

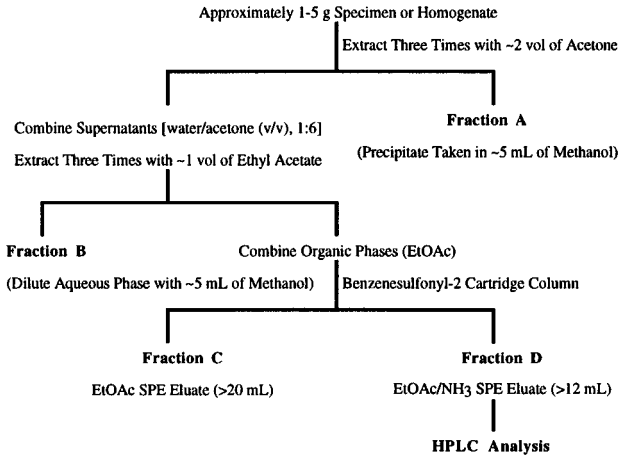


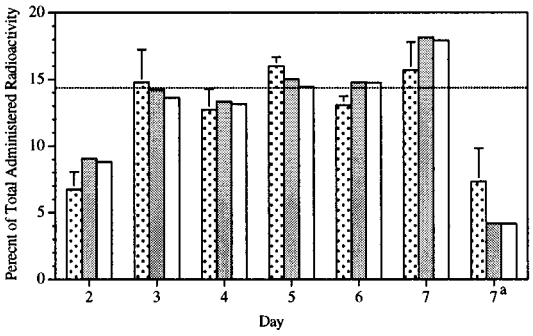
Figure 2. Preparation of extractable residues by benzenesulfonyl-2 (CUBCX) cartridge column before HPLC analysis.

Table 1. Percent Recoveries of Radioactivity in Goat Tissues and Excreta

	percent of total administered radioactivity[a]		
	single labeled ($n = 3$)[b]	dual labeled ($n = 1$)[b]	
specimen	mean ± standard deviation [^{3}H]	[^{3}H]	[^{14}C]
feces[c]	86.32 ± 4.57 (86.30)	88.72 (87.82)	86.84 (87.51)
GIT	12.68 ± 1.19 (12.68)	11.29 (11.17)	11.40 (11.49)
urine[c]	0.15 ± 0.04 (0.15)	0.08 (0.08)	0.04 (0.04)
bile	0.10 ± 0.07 (0.10)	0.11 (0.11)	0.10 (0.10)
liver	0.72 ± 0.13 (0.72)	0.77 (0.76)	0.79 (0.80)
kidney	0.05 ± 0.01 (0.05)	0.05 (0.05)	0.05 (0.05)
total recoveries	100.02 ± 5.76 (100)[d]	101.03 (100)[d]	99.23 (100)[d]

[a] Since the total radioactive residues in milk, muscle, and fat were very low in comparison to kidney and liver, their combined contribution was therefore not included in this table. [b] n = number of goats. [c] Total radioactivity excreted in feces or urine over 7-day treatment period. [d] Percent recovery normalized to 100% is shown in parentheses.

Elimination and Recoveries of Total Radioactivity. Table 1 indicates that essentially all of the administered radioactivity was recovered in the feces (86.3%) and in the GIT contents (12.7%) of the goats treated with [^{3}H]MAB_{1a} benzoate. The combined ^{3}H radioactivity in urine, bile, liver, and kidney was insignificant (1%, Table 1). The equivalent recovery of ^{3}H and ^{14}C radioactivity (Table 1) from the [^{3}H/^{14}C]-MAB_{1a} benzoate-treated goat (101% vs 99%) indicated metabolic stability for both labels. Due to transit time in the GIT, the elimination of radioactivity in feces (Figure 3) on day 2 (after the first dose) was low (7−9% of the total dose administered). However, after that, about 14% of the total administered radioactivity was eliminated on each day between day 3 and day 6 (Figure 3). The elimination of the dose by day 7 (post sixth dose but before the administration of seventh dose) was slightly higher (16−18%) as the regular 24 h sample collection period was extended (2−6 h) to schedule the time intervals for euthanasia. Since each daily dose was approximately 14% of the total administered radioactivity and about 14% of the total administered radioactivity was eliminated daily in feces between day 3 and day 6 (Figure 3), the results indicate that a single orally administered dose would be eliminated in just over 24



Mean and standard deviation of total administered radioactivity in [^{3}H]MAB$_{1a}$ benzoate treated goats (n=3).

[^{3}H] radioactivity recovered from [^{3}H/^{14}C]MAB$_{1a}$ benzoate treated goat.

[^{14}C] radioactivity recovered from [^{3}H/^{14}C]MAB$_{1a}$ benzoate treated goat.

Figure 3. Elimination of radioactivity in feces. The specimens were collected daily in the morning before the administration of the next dose. Thus, after the administration of first, second, third, fourth, fifth, and sixth doses, the feces specimens were collected on days 2, 3, 4, 5, 6, and 7, respectively. [a]After the administration of the seventh dose, feces specimens were collected on day 7 just before the euthanasia. The dotted line corresponds to the calculated average daily excretion (100% dose divided by 7 days is equal to 14.3% of the total administered dose, i.e., the daily dose) assuming complete elimination of the total administered dose in feces by the time of euthanasia.

Table 2. Total Radioactive Residues (ppb equivalent) in Goat Tissues[a]

	single labeled ($n = 3$)	dual labeled ($n = 1$)[b]	
tissues	mean ± standard deviation [^{3}H]	[^{3}H]	[^{14}C]
liver	1002 ± 159	1411	1480
kidney	499 ± 158	722	696
omental fat	283 ± 85	349	318
renal fat	283 ± 91	330	320
leg muscle	118 ± 23	137	131
loin muscle	96 ± 27	114	114

[a] The detection limit of radioactivity in tissues was ≤2 ppb. [b] Due to approximately one-third higher dual-labeled dose (0.67 mg/kg of bw) in comparison to single-labeled dose (0.5 mg/kg of bw), higher TRR contents were observed in the tissues of goat which received dual-labeled compound.

h. Since the goats were euthanatized ~10 h after the seventh dose, the percent elimination in the feces specimens which were collected before euthanasia (day 7) was low (5−8% of the total dose administered) when compared to the regular 24 h sample collection period. However, the remainder of the dose was recovered in the contents of the GIT (11−13% of the total dose administered, Table 1). This rapid elimination in feces was also observed in rats which received single doses of radiolabeled MAB$_{1a}$ benzoate (Mushtaq et al., 1996). Moreover, several other compounds containing a macrocyclic structure similar to that in MAB$_{1a}$ such as abamectin, the 8,9-Z isomer of avermectin B$_{1a}$, ivermectin (dihydroavermectin B$_1$), 4″-deoxy-4″-(*epi*-acetylamino)avermectin B$_1$, and moxidectin were eliminated mainly via feces in rat, goat, and cattle (Chiu et al., 1990; Maynard et al., 1989a,b, 1990; Zeng et al., 1996; Zulalian et al., 1994).

Tissue Radioactivity. The concentrations of total radioactive residues (TRR) (ppb equivalent) in goat tissues are presented in Table 2. The average concentrations of TRR in the tissues of [^{3}H]MAB$_{1a}$ benzoate-treated goats were found to be in the following order: liver > kidney > omental fat ≈ renal fat > leg muscle > loin muscle. The concentrations of TRR for ^{3}H/^{14}C radioactivity in liver (1411/1480 ppb), kidney (722/696 ppb), omental fat (349/318 ppb), renal fat (330/320 ppb), leg muscle (137/131 ppb), and loin muscle (114/114 ppb) from the [^{3}H/^{14}C]MAB$_{1a}$ benzoate-treated goat were nearly identical to each other. Probably due to the ca. one-third higher dose, the concentrations of TRR in the tissues of the [^{3}H/^{14}C]MAB$_{1a}$ benzoate-treated goat were higher than the mean of the corresponding tissue TRR concentrations of the [^{3}H]MAB$_{1a}$ benzoate-treated goats (Table 2). There was apparently no substantial difference in the TRR concentrations within omental fat and renal fat. However, TRR concentrations in leg muscle were somewhat higher than in loin muscle. Maynard et al. (1989b) reported that the amount of edible tissue TRR from abamectin-treated goats followed the order liver > fat > kidney > muscle, which was similar to that obtained in this study except that the concentrations of TRR in kidney were more than in fat (Table 2). The relative total tissue radioactivity in goats was in the same order as that observed in rats which received a single dose of 0.5 mg of [^{14}C]MAB$_{1a}$ benzoate/kg of bw (Mushtaq et al., 1996).

Plasma and Milk Radioactivity. Table 3 indicates that the TRR in plasma (8−38 ppb) and milk (12−56 ppb) were low in comparison to the concentrations of TRR in tissues (Table 2). When compared, the residues in milk were about 1.2−1.6-fold higher than the corresponding residues in plasma (Table 3). After the administration of each dose, the concentrations of TRR in milk were somewhat higher at the afternoon (PM) than the morning (AM) collection periods (Table 3), indicating that the milk residues decreased with time after dosing. As observed in the tissues (Table 2), the concentrations of the corresponding ^{3}H- and ^{14}C-TRR in plasma or milk from the [^{3}H/^{14}C]MAB$_{1a}$ benzoate-treated goat were similar to each other but were somewhat higher than the respective mean ^{3}H-residue concentrations from [^{3}H]MAB$_{1a}$ benzoate-treated goats (Table 3). The difference was due to a ca. one-third higher dose of [^{3}H/^{14}C]MAB$_{1a}$ benzoate than the corresponding dose of [^{3}H]MAB$_{1a}$ benzoate. The mean concentrations of plasma TRR from the three goats treated with the [^{3}H]MAB$_{1a}$ benzoate and from a single goat treated with the [^{3}H/^{14}C]MAB$_{1a}$ benzoate between day 3 and day 7 ranged from 12 to 20 ppb and from 17 to 25 ppb, respectively (Table 3). Similarly, the mean concentrations of milk TRR from the three goats treated with the [^{3}H]MAB$_{1a}$ benzoate and from a single goat treated with the [^{3}H/^{14}C]MAB$_{1a}$ benzoate between day 3 and day 7 ranged from 17 to 43 ppb and from 21 to 56 ppb, respectively (Table 3).

No statistically significant difference was observed when the mean and standard deviation of 15 individual TRR values for day 3 to day 7 from [^{3}H]MAB$_{1a}$ benzoate-treated goats for AM milk, PM milk, and plasma (Table 3) were separately compared with their respective mean and standard deviation of the three TRR values for each day (day 3 to day 7). Similarly, no statistically significant difference was observed when the mean and standard deviation of day 7 TRR values for AM milk, PM milk, and plasma were compared with their respective mean and standard deviation of TRR values for each day (day 3 to day 6). Similarly, no substantial difference was noticed between the mean of 5 TRR values (^{3}H or ^{14}C) and the individual TRR values (day 3 to day 7) for AM milk, PM milk, or plasma from the [^{3}H/^{14}C]MAB$_{1a}$ benzoate-treated goat (Table 3). The

Table 3. Total Radioactive Residues (ppb equivalent) in Goat Milk and Plasma

	milk[a]						plasma[b]		
	single labeled ($n = 3$)		dual labeled ($n = 1$)				single labeled ($n = 3$)	dual labeled ($n = 1$)	
day	[^{3}H] AM	[^{3}H] PM	[^{3}H] AM	[^{14}C] AM	[^{3}H] PM	[^{14}C] PM	[^{3}H] AM	[^{3}H] AM	[^{14}C] AM
1	NA[c]	12 ± 5	NA	NA	19	18	NA	NA	NA
2	13 ± 4	24 ± 5	18	17	42	42	8 ± 2	15	14
3	17 ± 4	29 ± 10	23	21	56	55	12 ± 4	22	19
4	21 ± 6	25 ± 7	27	26	41	40	13 ± 4	20	17
5	22 ± 6	30 ± 4	29	28	43	40	14 ± 3	22	18
6	24 ± 6	35 ± 6	31	27	48	45	17 ± 4	25	21
7	29 ± 13	43 ± 12	28	25	53	50	20 ± 7	24	20
mean ± sd[d]	23 ± 8	33 ± 10	28 ± 3	25 ± 3	48 ± 6	46 ± 6	15 ± 5	23 ± 2	19 ± 1
7[e]							26 ± 8	38	33

[a] The goats were milked twice, just before the daily dosing (AM) and in the late afternoon (PM) approximately 8−10 h after the daily dosing. [b] The blood was collected once just before the daily dosing (AM), and the plasma was separated after centrifugation. [c] NA: not applicable. The specimens which were collected before the administration of the first dose (day 1) had no radioactive residues. [d] Mean ± standard deviation of 15 TRR values between day 3 and day 7 (individual values not shown) from three goats treated with [^{3}H]MAB1a benzoate and of five TRR values from one goat treated with [^{3}H/^{14}C]MAB1a benzoate were calculated. [e] The specimens were collected just before the euthanasia (~10 h after the administration of seventh dose) and were therefore not averaged with the other data. Likewise, day 1 and day 2 data were not averaged since the steady state was not reached.

results suggest that the milk and plasma concentrations plateaued between day 3 and day 7. In comparison to plasma TRR concentrations between day 3 and day 7, the TRR concentrations of both specimens collected before euthanasia (post seventh dose) were comparatively high as the specimens was collected at ~10 h after the administration of the last dose (Table 3).

The concentrations of TRR in skim milk were slightly lower (9−41 ppb), and concentrations of TRR in cream (59−351 ppb) were at least 5 times higher in comparison to the corresponding concentrations in milk specimens collected between day 1 and day 7 (Table 3, data for skim milk and cream not shown). The corresponding concentrations of TRR in skim milk and cream from the [^{3}H/^{14}C]MAB_{1a} benzoate-treated goat were about 1.2- and >1.5-fold higher, respectively, in comparison to the [^{3}H]MAB_{1a} benzoate-treated goats (data not shown). Because goat milk contains about 41 g of lipid/L (Long, 1968) and higher residues in cream were observed, the results suggest that MAB_{1a} residues were partitioned preferentially into the cream. MAB_{1a} is hydrophobic (octanol/water partition coefficient, log P at pH 7.0 is 5.0 ± 0.2) and higher residue concentrations in cream might be expected. But, MAB_{1a} was not biologically lipophilic as its residue concentrations in fat tissue were less in comparison to kidney and liver (Table 2) and were much less in cream than expected on the basis of octanol/water partitioning.

Metabolism of MAB_{1a} Benzoate. The EtOAc/NH_3 SPE eluates (Figure 2) obtained after processing of fat, feces, liver, kidney, milk, or muscle for HPLC analysis of residues contained more than 90% of the total specimen radioactivity, and the combined radioactivity in other fractions (A, B, and C) was <10%. RP-HPLC analyses of feces indicated one detectable metabolite (≤2% AB_{1a}) which eluted just prior to MAB_{1a} (≥91%, Table 4 and Figure 4). In all feces samples analyzed, the HPLC profiles of MAB_{1a} benzoate residues were similar. RP-HPLC analyses of composite milk specimens from all collection periods (Table 4) also indicated the presence of the AB_{1a} metabolite (≤4%). As was observed in the feces and milk specimens, the AB_{1a} metabolite (1−8%) was also detected in the RP-HPLC analyses of liver, kidney, muscle, and fat and the parent MAB_{1a} (77−96%) was the major residue (Table 4 and Figure 4). Between the same type of tissues (Table 4), the amount of AB_{1a} metabolite in omental fat and renal fat was nearly the same (1−3%) whereas it was greater in loin muscle (≥5%) than in leg muscle (1%). The

Table 4. Residue Components (AB_{1a}/MAB_{1a}) of Goat Feces, Milk, and Tissues (Percent of Total Column Radioactivity)

		single labeled[a]		dual labeled	
specimen	isotope	%AB_{1a}	%MAB_{1a}	%AB_{1a}	%MAB_{1a}
feces[b]	[^{3}H]	1.2	90.7	2.1	92.9
	[^{14}C]			2.0	93.0
milk[c]	[^{3}H]	2.7	90.9	4.0	89.2
	[^{14}C]			3.4	94.3
liver	[^{3}H]	4.7	89.8	5.2	91.0
	[^{14}C]			4.8	91.6
kidney	[^{3}H]	5.6	88.7	5.2	91.8
	[^{14}C]			5.0	90.5
leg muscle	[^{3}H]	0.9	93.0	1.0	96.3
	[^{14}C]			0.9	94.9
loin muscle	[^{3}H]	4.5	88.7	7.5	81.8
	[^{14}C]			6.6	76.8
omental fat	[^{3}H]	1.7	91.9	1.4	94.3
	[^{14}C]			1.0	95.3
renal fat	[^{3}H]	1.8	94.3	2.6	89.7
	[^{14}C]			2.4	87.7

[a] Composite feces and tissue specimens from [^{3}H]MAB_{1a} benzoate-treated goats were analyzed. [b] The values of percent AB_{1a} and MAB_{1a} from seven analyses (one/day) were averaged. [c] The composite milk specimens from all collection periods (days 1−7) were analyzed.

reason for this difference is not known. Two very minor unknown metabolites, P1 and P2 (Figure 4, about 1% each), were detected in some but not all goat specimens. When seen, the P1-metabolite (Figure 4) was eluted in the 15−16 min HPLC fractions near the retention time of MSB_{1a}. When seen, the P2-metabolite (Figure 4) was eluted in the 47−49 min HPLC fractions just after the elution of MAB_{1a} and ~2 min prior to the elution of 8,9-Z isomer of MAB_{1a}. The composition of TRR in goat feces and tissues was similar as that reported in the respective rat specimens (Mushtaq et al., 1996). Chiu et al. (1990) and Maynard et al. (1989b, 1990) reported the metabolism of the structurally related compounds abamectin and ivermectin in rats, cattle, and goats. For both abamectin and ivermectin, 24-hydroxymethyl and 3″-desmethyl metabolites were identified, but for emamectin benzoate, the N-demethylated metabolite (AB_{1a}) was detected in goats and rats (Mushtaq et al., 1996). Also, N-deacetylated metabolite (AB_{1a}) of 4″-deoxy-4″-(*epi*-acetylamino)avermectin B_1 in rat tissues was recently reported by Zeng et al. (1996). Thus, N-demethylation or N-deacetylation is the main route of metabolism for avermectin compounds containing a

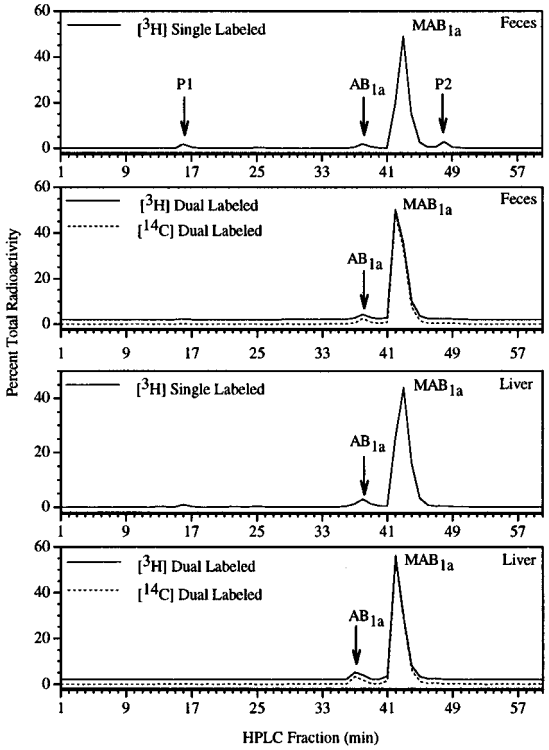


Figure 4. RP-HPLC analyses of extractable residues from goat feces (postdose 7) and liver [single and dual labeled]. The [^{14}C] radiochromatograms of dual-labeled residues were offset by 2 units for clarity. Feces and liver specimens from [^{3}H]-MAB_{1a} benzoate-treated goats were composited. The EtOAc/NH_3 SPE eluates (fraction D, Figure 2) were analyzed by RP-HPLC method 1.

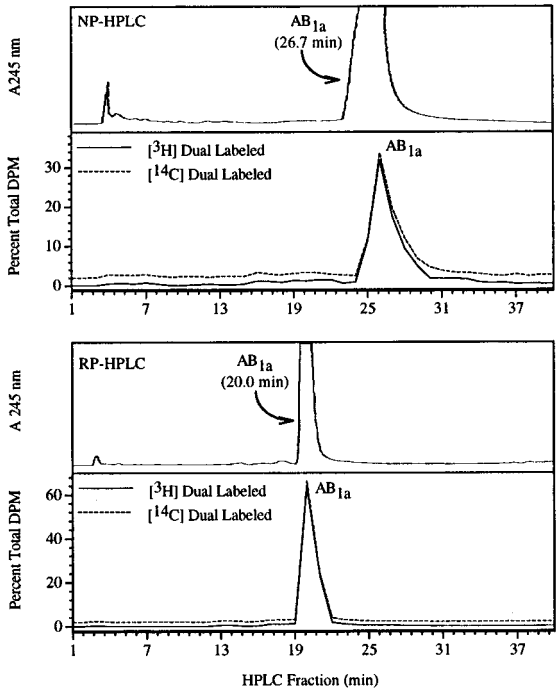


Figure 5. Cochromatography of the AB_{1a} metabolite with AB_{1a} standard by NP- and RP-HPLC systems. The [^{14}C] radiochromatograms were offset by a unit of 2 for clarity. Nonradioactive AB_{1a} standard was added to the EtOAc/NH_3 SPE eluate of liver from [^{3}H/^{14}C]MAB_{1a} benzoate-treated goat. AB_{1a} metabolite eluting under the UV peak of AB_{1a} standard was isolated by RP-HPLC method 3 (chromatograms not shown). The isolated metabolite−standard mixture was rechromatographed by NP-HPLC (top). HPLC fractions associated with the UV peak of the AB_{1a} standard were collected and reanalyzed by RP-HPLC methods 4 (bottom).

methylamino or acetylamino group, respectively, at the 4″-position.

Confirmation of Metabolites. By using serial RP- and NP-HPLC analyses, the identity of the AB_{1a} metabolite found in liver and feces was separately confirmed by coelution with reference standard. The AB_{1a} metabolite from liver coeluting with AB_{1a} standard was isolated by RP-HPLC (method 3). The isolated metabolite was then rechromatographed by NP-HPLC. The analyses indicated that 82% of the column radioactivity was associated with the UV peak of the AB_{1a} standard (R_t 26.7 min, top part of Figure 5). The isolated metabolite was then rechromatographed a second time by RP-HPLC method 4. Figure 5 (bottom part) indicated that 92% of the total eluted radioactivity corresponded to the UV peak of AB_{1a} (R_t 20.0 min). The metabolite was thus identified as AB_{1a}, the N-demethylated product of MAB_{1a} by coelution with standard in three different HPLC systems. Similarly, the identity of AB_{1a} metabolite isolated from goat feces was confirmed (data not shown). The polar metabolite P1 (Figure 4) was also isolated from feces and liver by RP-HPLC. The NP-HPLC analyses of P1 metabolite indicated one main peak of unknown identity both in liver and feces, but the radioactivity did not coelute with the UV peak of MSB_{1a} standard (HPLC results not shown). The results of NP-HPLC analyses indicated that MSB_{1a} was not a metabolite of MAB_{1a} in goats. No further characterization of the unknown P1 and P2 residues detected in some specimens was conducted due to their low levels.

Stability of Tritium Label. When calculated using ^{3}H- or ^{14}C-specific activity, the TRR contents and radiobalance in the tissues and excreta of the [^{3}H/^{14}C]-MAB_{1a} benzoate-treated goat (Tables 1−3) were essentially the same. Further, no differences in ^{3}H and ^{14}C radioprofiles of the extractable residues from [^{3}H/^{14}C]MAB_{1a} benzoate-treated goat were observed (Table 4 and Figures 4 and 5). The TRR contents and their metabolic profile of milk, tissues, and excreta from [^{3}H/^{14}C]]MAB_{1a} benzoate- and [^{3}H]MAB_{1a} benzoate-treated goats were also similar (Tables 1−4, Figure 4). It would be expected that, if the ^{3}H-label was lost, $^{3}H_2O$ would be formed which would be excreted in urine and not in feces. This was not observed (Table 1). The stability of ^{3}H label at the C-5-position of MAB_{1a} and avermectin B_{1a} was also demonstrated in rat metabolism studies (Maynard et al., 1990; Mushtaq et al., 1996). 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 this goat metabolism study indicate that MAB_{1a} benzoate (emamectin benzoate) was eliminated rapidly and almost entirely in feces and did not accumulate in the tissues. The TRR concentrations in milk and plasma plateaued between days 3 and 7. The residues in milk were 1.2−1.6-fold more than in plasma. The residue concentrations in the goat tissues at 10 h after the last dose were in the following order: liver > kidney > fat > muscle. The same order of tissue residue concentrations were seen in cows treated with 30, 90, or 300 ppb of MK-0244 in feed daily for 28 consecutive days (Wehner, unpublished results). Moreover, the residues in cream from cows milk were about 3−10-fold higher than the corresponding milk residues which were also observed in this study. AB_{1a}, the N-demethylated product of MAB_{1a}, was the only metabolite identified in the feces, liver, kidney,

fat, muscle, or milk. The amount of this metabolite in feces, milk, and tissues generally ranged between 1% and 6%, and the parent drug was the major residue (≥89%). Moreover, two minor unknown metabolites (each ~1%) were inconsistently detected. Similar to rats (Mushtaq et al., 1996), when dual-labeled MAB_{1a} benzoate was administered to goats, the residue concentrations and their composition were the same based upon either ^{3}H or ^{14}C radioactivity. The results indicate the stability of the ^{3}H label at the 5-position of MAB_{1a}. As the amount of MAB_{1a} benzoate used in this study was >500 times than the expected residues in diet (Prabhu et al., 1991), negligible residues are expected in milk and edible tissues of cattle which may consume emamectin benzoate-treated crops or crop parts.

ACKNOWLEDGMENT

We thank Drs. Donald E. Johnson and Greg McGinty of Colorado State University, Fort Collins, CO, and Dr. Laurie C. Wilkes of Analytical Development Corp., Colorado Springs, CO, for their advice and conduct of the in-life phase of the study. We are also grateful to Drs. Henry G. Wall and Jennifer E. Drisko of Merck Research Laboratories, Rahway, NJ, for monitoring the in-life phase of the study.

LITERATURE CITED

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. Avermectins, New Family of Potent Anthelmintic Agents: Producing Organism And Fermentation. *Antimicrob. Agents Chemother.* **1979**, *15*, 361−367.

Campbell, W. C.; Burg, R. W.; Fisher, M. H.; Dybas, R. A. The Discovery of Ivermectin and Other Avermectins. In *Pesticide Synthesis Through Rational Approaches*; Magee, P. S., Kohn, G. K., Menn, J. J., Eds.; ACS Symposium Series 255; American Chemical Society: Washington, DC, 1984; pp 5−20.

Chiu, S.-H. L.; Baylis, F. P.; Taub, R.; Green, M.; Halley, B. A.; Bodden, R. M. Depletion of [^{14}C]Clorsulon in Cow's Milk. *J. Agric. Food Chem.* **1989**, *37*, 819−823.

Chiu, S.-H. L.; Green, M. L.; Baylis, F. P.; Eline, D.; Rosegay, A.; Meriwether, H.; Jacob, T. A. Absorption, Tissue Distribution and Excretion of Tritium-Labeled Ivermectin in Cattle, Sheep, and Rats. *J. Agric. Food Chem.* **1990**, *38*, 2072−2078.

Dybas, R. A.; Hilton, N. J.; Babu, J. R.; Preiser, F. A.; Dolce, G. J. Novel Second Generation Avermectin Insecticides and Miticides for Crop Protection. In *Novel Microbial Products for Medicine and Agriculture*; Demain, A. L., Somkuti, G. A., Hunter-Cevera, J. C., Rossmoore, H. W., Eds.; Elsevier Press: New York, 1989; pp 203−212.

Lasota, J. A.; Dybas, R. A. Avermectins, A Novel Class of Compounds: Implications for Use in Arthropod Pest Control. *Annu. Rev. Entomol.* **1991**, **36**, 91−117.

Long, C. The Chemical Composition of Milk, Colostrum and Mammary Gland. *Biochemists' Handbook*: D. Van Nostrand Co. Inc.: Princeton, NJ, 1968; pp 895−896.

Maynard, M. S.; Gruber, V. F.; Feely, W. F.; Alvaro, R.; Wislocki, P. G. Fate of the 8,9-Z Isomer of Avermectin B_{1a} in Rats. *J. Agric. Food Chem.* **1989a**, *37*, 1487−1491.

Maynard, M. S.; Wislocki, P. G.; Ku, C. C. Fate of Avermectin B_{1a} in Lactating Goats. *J. Agric. Food Chem.* **1989b**, *37*, 1491−1497.

Maynard, M. S.; Halley, B. A.; Green-Erwin, M.; Alvaro, R.; Gruber, V. F.; Hwang, S.−C.; Bennett, B. W.; Wislocki, P. G. Fate of Avermectin B_{1a} in Rats. *J. Agric. Food Chem.* **1990**, *38*, 864−870.

Mushtaq, M.; Syintsakos, L. R.; Krieter, P. A.; Colletti, A.; Arison, B.; Crouch, L. S.; Wislocki, P. G. Absorption, Tissue Distribution, Excretion and Metabolism of ^{3}H- and ^{14}C-Labeled Emamectin Benzoate in Rats. *J. Agric. Food Chem.* **1996**, *44*, 3342−3349.

Prabhu, S. V.; Wehner, T. A.; Egan, R. S.; Tway, P. C. Determination of 4″-Deoxy-4″-(epimethylamino)avermectin B_1 Benzoate (MK-0244) and its Delta 8,9-Isomer in Celery and Lettuce by HPLC with Fluorescence Detection. *J. Agric. Food Chem.* **1991**, *39*, 2226−2230.

Putter, I.; MacConnell, J. G.; Preiser, F. A.; Haidri, A. A.; Ristich, S. S.; Dybas, R. A. Avermectins: Novel Insecticides, Acaricides and Nematicides from Soil Organism. *Experientia* **1981**, *37*, 963−964.

Zeng, Z.; Andrew, N. W.; Green-Erwin, M. L.; Halley, B. A. Fate of 4″-Epiacetylamino-4″-deoxyavermectin B_1 in Rats. *Drug Metab. Dispos.* **1996**, *24*, 572−578.

Zulalian, J.; Stout, S. J.; daCunha, A. R.; Garces, T.; Miller. P. Absorption, Tissue Distribution, Metabolism, and Excretion of Moxidectin in Cattle. *J. Agric. Food Chem.* **1994**, *42*, 381−387.

Received for review June 10, 1996. Revised manuscript received October 11, 1996. Accepted October 16, 1996.[⊗]

JF9604089

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1996.