Metabolic Fate of Emamectin Benzoate in Soil

Amechi C. Chukwudebe*

Department of Drug Metabolism, Merck Research Laboratories, P.O. Box 450, Hillsborough Road, Three Bridges, New Jersey 08887

Rebecca H. Atkins

PTRL East, Inc., 3945 Simpson Lane, Richmond, Kentucky 40475

Peter G. Wislocki

Department of Drug Metabolism, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Aerobic and anaerobic incubation of microbially active soil with [^{14}C]MAB$_{1a}$ resulted in measurable evolution of $^{14}CO_2$. Chromatographic and mass spectral analyses of extractable residues demonstrated the degradation of [^{14}C]MAB$_{1a}$ to 8aOH-MAB$_{1a}$ and 8aoxo-MAB$_{1a}$. The highest levels of 8aOH-MAB$_{1a}$ and 8aoxo-MAB$_{1a}$ were found at the 60-day incubation interval, comprising about 7 and 3%, respectively, of applied radioactivity. At least eight other residues, more polar than MAB$_{1a}$, were also formed at low levels (each <5%). By base extraction, HPLC, and gel chromatography, the unextractable residues were found to be non-avermectin components associated with soil humic substances. The majority (76−85%) of these unextractable residues were found in fulvic acid fraction while relatively minor amounts remained in humins (9−14%) and high molecular weight humic acids (4−11%). Quantitatively, soil microbial counts were not affected by MAB$_{1a}$ treatment. These results demonstrate that emamectin benzoate is biodegradable in soil and will eventually be mineralized to CO_2.

Keywords: *Avermectin; soil metabolism; metabolic fate; gel chromatography; HPLC; emamectin benzoate*

INTRODUCTION

Avermectins are macrocyclic lactones produced by the soil actinomycete *Streptomyces avermitilis*. A natural avermectin product, abamectin (avermectin B$_1$), is currently registered in the United States and worldwide as a miticide. Emamectin benzoate (MK-244, MAB$_1$ benzoate, 4″-deoxy-4″-epimethylaminoavermectin B$_1$ benzoate), is a derivative of abamectin and consists of two avermectin homologs, each with a molecular weight of approximately 900. By specification it consists of at least 90% of 4″-deoxy-4″-epimethylaminoavermectin B$_{1a}$ (MAB$_{1a}$) benzoate and not more than 10% of 4″-deoxy-4″-epimethylaminoavermectin B$_{1b}$ (MAB$_{1b}$) benzoate. These components differ by only a methylene group on the C-25 side chain (Figure 1). Emamectin benzoate is an effective insecticide against lepidopteran larvae (Trumble et al., 1987) and is currently under development for use on a number of crops including celery, lettuce, cole crops, and tomatoes. The minor structural difference between MAB$_{1a}$ and MAB$_{1b}$ coupled with the preponderance of MAB$_{1a}$ in emamectin benzoate and the nearly identical biological activities of the two homologs (Shoop et al., 1995) indicate that [^{14}C]MAB$_{1a}$ can be used as the test substance for emamectin benzoate. In addition, both homologs are metabolized in similar fashion by rat liver slices (Mushtaq, unpublished data).

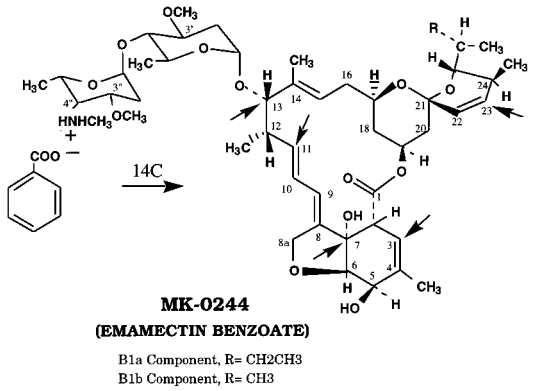

Figure 1. Structure of emamectin benzoate showing major and minor homologues. The major, 4″-deoxy-4″-epimethylaminoavermectin B1a benzoate, and minor, 4″-deoxy-4″-epimethylaminoavermectin B1b benzoate, homologues constitute ≥90 and ≤10%, respectively, of emamectin benzoate. Arrows indicate position of ^{14}C radiolabel. The test substance used was 4″-deoxy-4″-epimethylaminoavermectin B1a.

In practice, the application of a pesticide to crops often results in pesticide deposits on soil where metabolic transformations, usually mediated by microorganisms, can take place (Vink et al., 1994). In previous studies (Chukwudebe et al., 1996b; Mushtaq et al., 1996), it was found that emamectin benzoate binds very tightly to topsoil and is, hence, immobile. Herein we report on the metabolic fate of emamectin benzoate in soil in the absence of light, under both aerobic and anaerobic conditions.

* Address correspondence to this author at the Department of Toxicology, Novartis, Inc., P.O. Box 18300, Greensboro, NC 27419-8300 [telephone (910) 632-2870; fax (910) 632-2997; e-mail amechi_chukwudebe@cp.novartis.com].

S0021-8561(97)00589-X CCC: $14.00

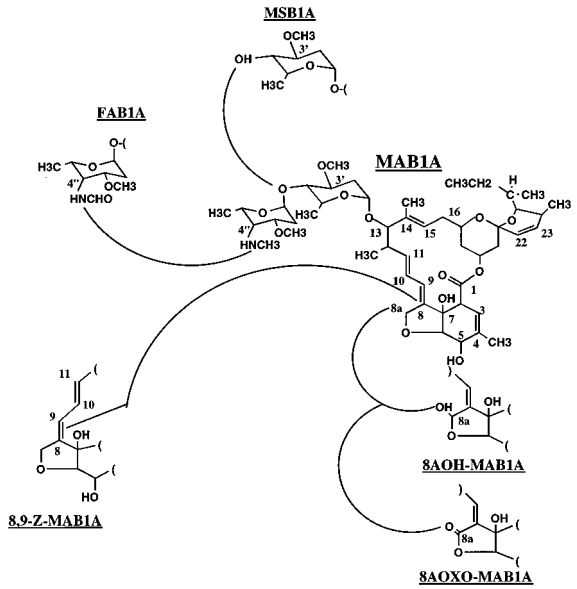

Figure 2. Structures of selected emamectin benzoate-related standards. Abbreviations are defined in the Materials and Methods section. Connecting lines illustrate the relationship between MAB_{1a} and each of the related standards (partial structures shown).

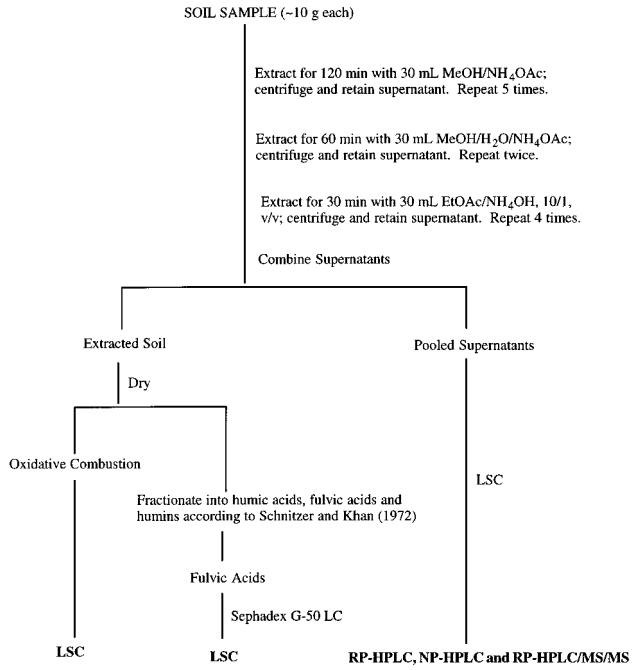

Figure 3. Flow diagram for the extraction and characterization of $[^{14}C]MAB_{1a}$ residues in soil.

MATERIALS AND METHODS

Chemicals. The test substance, $[^{14}C]MAB_{1a}$, was prepared by the Labeled Compound Synthesis Group at Merck Research Laboratories in Rahway, NJ, and purified to a specific activity and radiochemical purity of about 23.9 μCi/mg and 99.8%, respectively. The ^{14}C label was incorporated by fermentation at the C3, C7, C11, C13, or C23 positions of MAB_{1a}. For soil treatment, the labeled material was diluted to a concentration of about 0.1 μCi/μL in MeOH. Authentic standards used for residue characterizations were available from previous studies (Chukwudebe et al., 1996a,b); these standards were unlabeled MAB_1, avermectin B_{1a} aglycone, avermectin B_{1a} monosaccharide (MSB_{1a}), 4″-deoxy-4″-epiaminoavermectin B_{1a} (AB_{1a}), 4″-deoxy-4″-epi-(*N*-formyl)avermectin B_{1a} (FAB_{1a}), 8a-oxo-4″-deoxy-4″-epimethylaminoavermectin B_{1a} (8aoxo-MAB_{1a}), 8a-hydroxy-4″-deoxy-4″-epimethylaminoavermectin B_{1a} (8aOH-MAB_{1a}), and 8,9-*Z*-4″-deoxy-4″-epimethylaminoavermectin B_{1a} (8,9-ZMAB_{1a}). The structures of some of these standards are shown in Figure 2. The polydextran gel, Sephadex G-50, and its calibration standards (Blue Dextran 2000, Yellow Dextran, Dextran T-10, vitamin B12) were obtained from Pharmacia Biotech Inc., in Piscataway, NJ.

Incubation of $[^{14}C]MAB_{1a}$ in Soil. The test soil was sandy loam, maintained for more than 5 years without pesticide use and sieved through a 2-mm screen; its physicochemical properties are shown in Table 1. Fifty-gram aliquots of the sandy loam soil were placed in 500-mL biometer flasks, and 6 μCi of $[^{14}C]MAB_{1a}$, in about 56 μL of MeOH, was added to each flask, to give a concentration of about 5 μg of $[^{14}C]$-MAB_{1a} per gram of soil. This concentration, about 50 times the maximum recommended rate, was chosen to ensure sufficient test material and/or radiolabel for the characterization and quantification of low-level degradates. The soil was stirred for about 20 min after which water was added to moisten the soil to 75% of field moisture capacity at $^1/_3$ bar. The soil was stirred again for about 2 min and the biometer flasks fitted with air inlets and outlets connected to a vacuum manifold system. The outlet of each flask was connected to a series of two traps containing 50 mL each of ethylene glycol (to trap volatile organic materials) and 10% KOH_{aq} (to trap $^{14}CO_2$). The soil-containing biometer flasks, totaling 24 in number, and the gas dispersion traps were sealed and incubated at 25 °C.

At intervals of approximately every 14 days and at the time of sampling, the biometer flasks were flushed with air or oxygen such that air removed from the flasks were passed through the series of ethylene glycol and KOH traps. Thus, soil, ethylene glycol, and KOH samples were removed, in duplicate, at intervals of approximately 0, 1, 3, 7, 14, 30, 60, 90, 120, 180, 270, and 366 days, following the passage of air or oxygen through the sealed system. The soil samples and their associated ethylene glycol and KOH from the traps were analyzed for the presence of radioactive residues. Immediately after analysis by liquid scintillation counting (LSC), aliquots of the KOH were also diluted with about 10 mL of 0.5 M $BaCl_2$ and the mixture centrifuged for about 10 min at 1000*g*. Any precipitated $Ba^{14}CO_3$ was resuspended in ethanol, dried on a filter paper, and weighed before oxidative combustion and LSC analyses.

For anaerobic metabolism, the pesticide-free soils were initially incubated under aerobic conditions at 25 °C for 30 days. At intervals of approximately every 14 days and at each sampling time, the biometer flasks were flushed with air or oxygen such that the effluent air was passed through the series of ethylene glycol and KOH traps. Anaerobic conditions were then introduced by passing N_2 through the biometer flasks. At successive 14-day intervals following the establishment of anaerobic conditions, and at the time of sampling, the biometer flasks were again flushed with N_2 such that air removed from the flasks were passed through the series of ethylene glycol and KOH traps. Soil samples and their associated ethylene

Table 1. Characterization of Test Soil[a]

study conditions	OC[b] (%)	pH	CEC[c] (mequiv/100 g)	bulk density (g/cm^3)	MHC[d] (%)	sand (%)	silt (%)	clay (%)	textural class
aerobic	1.17	6.6	7.5	1.24	17.1	68	23	9	sandy loam
anaerobic	1.37	7.0	8.2	1.24	14.2	71	21	8	sandy loam

[a] Soil characterization was performed by the College of Agriculture, University of Kentucky, Lexington, KY. [b] Organic carbon. [c] Cation exchange capacity. [d] Moisture holding capacity at $^1/_3$ bar.

Table 2. HPLC Methods

method	gradient	conditions
1	75% A to 80% A in 40 min, linearly to 100% A in 20 min, hold at 100% A for 10 min	A = MeOH, B = H_2O, Axxiom ODS analytical column (5 μm × 4.6 mm × 250 mm); 1 mL/min flow rate; 5 mM NH_4OAc used as a modifier in A and B; eluates monitored at 245 and 280 nm, or by LSC
2	55% A to 80% A in 25 min, linearly to 100% A in 10 min, hold at 100% A for 10 min	A = MeCN, B = H_2O, Shiseido ODS analytical column (5 μm × 4.6 mm × 250 mm); 1 mL/min flow rate; 0.4 mM TEA used as a modifier in A and B; eluates monitored at 245 and 280 nm, or by LSC
3	hold at 4% A for 25 min, linearly to 7% A in 1 min, hold at 7% A for 15 min, linearly to 20% A in 1 min, hold at 20% A for 10 min	A = EtOH, B = Isooctane, Lichrospher Diol analytical column (5 μm × 4.6 mm × 250 mm); 1 mL/min flow rate; 0.4 mM TEA used as a modifier in A and B; eluates monitored at 245 and 280 nm, or by LSC
4	15% A to 20% A in 55 min, linearly to 35% A in 65 min, linearly to 55% A in 60 min, linearly to 70% A in 40 min, linearly to 85% A in 10 min, linearly to 90% A in 10 min, linearly to 100% A in 14 min, hold at 100% A for 6 min	A = MeOH, B = H_2O, Axxiom ODS analytical column (5 μm × 4.6 mm × 250 mm); 1 mL/min flow rate; 5 mM NH4OAc used as a modifier in A and B; eluates monitored at 245 and 280 nm, or by LSC

Table 3. Recovery and Extraction of Radioactivity from Soil

days after application	conditions	extracted radioactivity (%)[a]	unextracted radioactivity (%)	CO_2 (%)	volatiles (%)	recovery (%)
0	aerobic	97.05	3.60	ND[b]	ND	100.65
1	aerobic	93.20	5.40	ND	ND	98.60
3	aerobic	93.50	6.90	ND	ND	100.40
7	aerobic	96.90	4.70	0.2	ND	101.80
14	aerobic	94.60	5.90	0.9	ND	101.40
30	aerobic	93.70	9.10	2.2	ND	105.00
60	aerobic	79.70	14.90	3.1	ND	97.70
90	aerobic	73.80	18.10	5.1	0.2	97.20
120	aerobic	68.60	19.90	8.2	ND	96.70
180	aerobic	64.90	22.00	10.2	0.3	97.40
270	aerobic	55.80	27.40	14.3	0.1	97.60
366	aerobic	52.60	25.20	16.3	0.1	94.20
0	aerobic	94.80	2.10	ND	ND	96.90
30	aerobic	80.70	10.00	0.5	ND	91.20
59	anaerobic	81.10	16.60	2.0	ND	99.70
90	anaerobic	75.00	15.50	3.0	ND	93.50

[a] Percent of applied radioactivity. [b] Nondetectable, or <0.1% of applied radioactivity.

glycol and KOH traps were removed at intervals of approximately 0 and 30 days after treatment with [^{14}C]MAB_{1a}, and at 30 and 60 days after the establishment of anaerobic conditions, for analysis of residues.

Microbiology. For both aerobic and anaerobic incubations, the soil microbial counts were determined at the beginning and end of the studies. Total microorganisms were evaluated by enumerating the total colony forming units (CFU) of bacteria, actinomycetes, and fungi, as described by Smith et al. (1995). Bacteria, actinomycetes, and fungi were enumerated on tripticase soy agar, actinomycetes isolation agar, and potato dextrose agar, respectively. The culture plates were incubated at 35 °C for 24 and 48 h, respectively, to detect bacteria and actinomycetes. Fungi were detected by incubating culture plates for 48 h at 25 °C.

Extraction and Characterization of Residues. This method is summarized in Figure 3. Using about 10 g of composited soil as starting material, samples were extracted at room temperature on a wrist shaker for 120 min with about 30 mL of MeOH containing 100 mM NH_4OAc. The mixture was centrifuged for about 5 min to separate supernatant extracts from extracted soil solids. Extraction was repeated for 60 min with additional 30 mL of MeOH/H_2O (1/1, v/v) containing 100 mM NH_4OAc. The resulting soil matrix was further extracted at room temperature with EtOAc/NH_4OH (10/1, v/v). For each soil sample, supernatant extracts were combined and adjusted to known volumes in measuring cylinders and 0.5−1.0 mL aliquots were analyzed by LSC to determine the total extractable radioactivity. The extractable radioactivity was analyzed by a combination of RP-HPLC, NP-HPLC, and RP-HPLC/MS/MS.

Unextractable residues were fractionated into humic acids, fulvic acids, and humin fraction using the method described by Schnitzer and Khan (1972). The fulvic acid fraction was taken up in 0.025 M sodium borate buffer, pH 9.1 (Weast et al., 1984), for gel chromatographic characterization.

HPLC. The HPLC conditions are presented in Table 2 (methods 1−4). These conditions were established by monitoring unlabeled avermectin standards using UV absorbance detection at 245 and 280 nm. The column eluates from soil extracts were monitored by both UV (245 and 280 nm) and LSC. For LSC detection, eluates were collected in 1-mL fractions, mixed with about 4 mL of Packard Insta-gel XF cocktail and the radioactivity determined using the Packard 4530, 460, or 460M liquid scintillation counting system. Radiochromatograms were generated from these counts with Microsoft Excel (ver. 4.0) and Cricket graph III softwares.

RP-HPLC eluate fractions (method 1) containing [^{14}C]-MAB_{1a}, 8aOH-MAB_{1a}, 8aoxo-MAB_{1a}, and an early-eluting polar fraction were reanalyzed by NP-HPLC (method 3) and a RP-HPLC method with different selectivity (method 2) from method 1. In addition, the polar fraction was also analyzed by a 260-min RP-HPLC (method 4). The identity of residues in the RP-HPLC eluates (from method 1) was confirmed by RP-HPLC/MS/MS.

RP-HPLC/MS/MS. Residues isolated by RP-HPLC from soil extracts (method 1, Table 2), were characterized by RP-HPLC/MS/MS. The RP-HPLC separation for MS/MS was conducted on a Zorbax ODS column (5 μm × 4.6 mm × 250 mm) using an isocratic mobile phase consisting of 10 mM NH_4OAc in MeOH/H_2O (85/15, v/v). The flow rate was 1 mL/min with a split ratio of 20:1, and N_2 was used as the collision gas (71 eV) for the MS/MS experiments. Mass and daughter ion spectra were obtained on a SCIEX API III mass spectrometer using the ionspray interface in the positive ion mode.

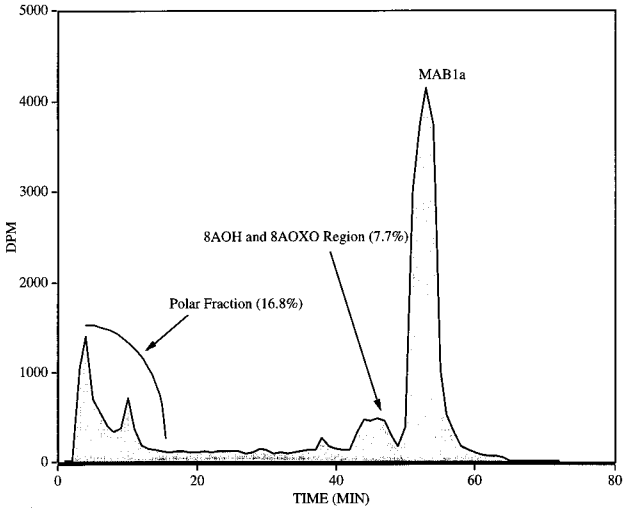

Figure 4. RP-HPLC chromatogram of extractable radioactivity from soil sample obtained after incubating for 30 days under aerobic conditions. Analyzed by HPLC method 1 (see Table 2).

Gel Chromatography. A 2.6 × 70-cm Sephadex G-50 column was prepared as recommended by the manufacturer (Pharmacia, 1993) and calibrated for MW using solutions of Yellow Dextran (MW 20 000), Dextran T-10 (MW 10 000), and cyanocobalamin (MW 1350) in sodium borate buffer. Void volume (V_0) was determined using Blue Dextran 2000 and total bed volume (V_t) was calculated to be approximately 319 mL. An aliquot (1.2 mL) of the fulvic acid fraction in buffer was loaded on the column and a steady mobile phase (sodium borate buffer) flow at 4 mL/min was maintained by gravity. Eluates (1 mL each) were collected at 0.25-min intervals, mixed with Instagel cocktail, and quantified by LSC.

Over a wide range, the elution volume (V_e) of noninteracting compounds from a dextran gel approximates to a linear relationship with the logarithm of MW (Andrews, 1965; Carnegie, 1965a,b; Laurent and Killander, 1964). Yellow Dextran and cyanocobalamin were determined by absorbance detection at 380 and 360 nm, respectively. Dextran T-10 was determined using a modification of the phenol−sulfuric acid reaction followed by absorbance detection at 490 nm (Dubois et al., 1956). A linear regression of ln MW vs gel affinity constant (K_{av}) was used to calculate apparent MW of the fulvic acids from their elution volume, V_e. The gel affinity constant, K_{av}, is independent of column geometry and packing density (Laurent and Killander, 1964) and its magnitude bears a direct relationship to the affinity of the eluted molecule for the gel. K_{av} is related to V_e and is determined as follows:

$$K_{av} = (V_e - V_0)/(V_t - V_0)$$

By first-order regression analysis, the apparent molecular weight of the fulvic acid fraction was determined according to the relationship:

$$K_{av} = (m \ln \mathrm{MW}) + c$$

where m and c are constants. Based on these relationships, the apparent molecular weights, nature, and distribution of unextractable residues in soil fulvic acid fraction were determined.

RESULTS AND DISCUSSION

Recovery and Extraction of Radioactivity. Recovery data from soil at all sampling intervals, as percent of applied radioactivity, is shown in Table 3. Total recoveries averaged 91.2−105% of applied radioactivity. During this soil incubation time course, solvent extractability decreased from 97% (day 0) to about 53% (day 366) of applied radioactivity. Conversely, unextractable residues increased from 3.6 to 25.2%.

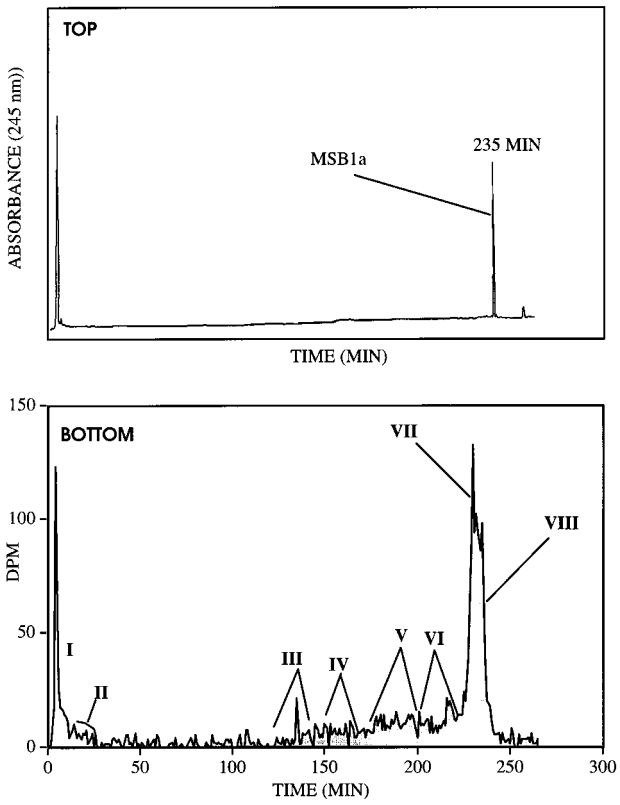

Figure 5. RP-HPLC chromatogram of MSB1a standard and polar fraction isolate. The polar fraction (bottom) was isolated from a day 90 soil (30-day aerobic followed by 60-day anaerobic incubation). Isolate was fortified with MSB_{1a} standard (top); analyses by HPLC method 4 (see Table 2).

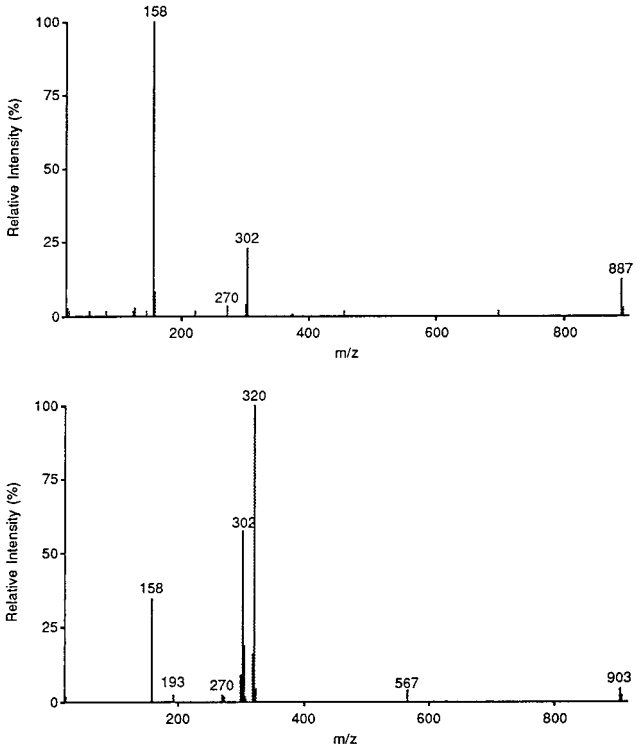

Figure 6. Daughter ion spectra of MAB_{1a} and 8aOH-MAB_{1a} standards: (top) MAB_{1a} standard; (bottom) 8aOH-MAB_{1a} standard.

Microbial Populations. The microbial profile of the soil, measured by the number of colony forming units,

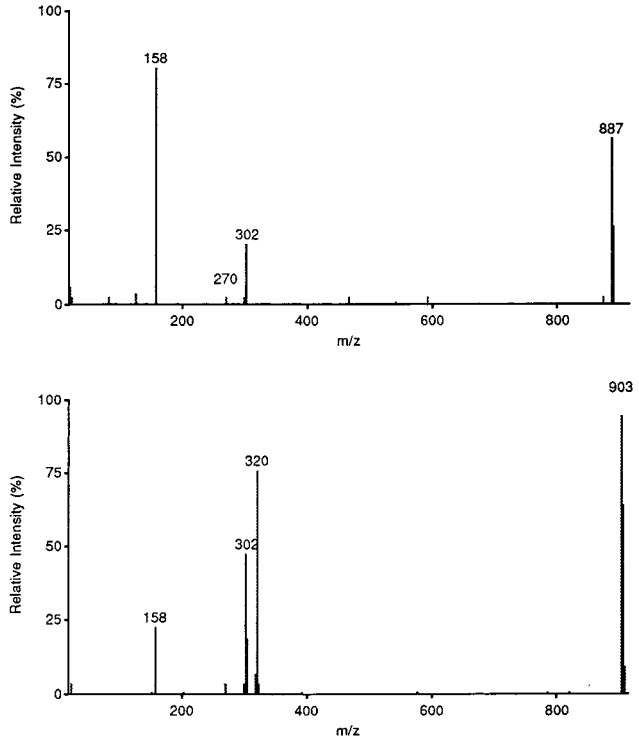

Figure 7. Daughter ion spectra of extractable soil residues. Day 180 aerobic soil sample; the presence of MAB_{1a} (top) and 8aOH-MAB_{1a} (bottom) was also demonstrated by similarities in daughter ion spectra with the external standards. Refer also to Figure 6.

was not affected by treatment with [^{14}C]MAB_{1a}. Total microbial counts (bacteria, actinomycetes, and fungi) in soil at the beginning and conclusion of the 366-day aerobic incubation with [^{14}C]MAB_{1a} was relatively unchanged, and averaged about 1.9×10^6 CFU/g. Similarly, total microbial counts in soil at the beginning and conclusion of the 60-day anaerobic incubation was also relatively unchanged, averaging about 3.9×10^6 CFU/g. These values are indicative of microbially active soils (Brock, 1979). Thus, emamectin benzoate is not likely to have an adverse impact on the population of soil microorganisms.

Mineralization to $^{14}CO_2$. With three minor exceptions, volatile organic materials collected in ethylene glycol traps did not attain or exceed 0.1% of applied radioactivity. Nearly all of the volatile materials released from the treated soils were recovered in KOH traps, suggestive of $^{14}CO_2$. Under aerobic conditions, a low level of $^{14}CO_2$ (0.1%) was found after 3 days and increased steadily thereafter, attaining a maximum (16.3%) at the 366-day sampling interval (Table 3). Similarly, a low level of $^{14}CO_2$ was found at initiation of anaerobic conditions (0.5%, 30-day interval), and increased to about 3% after 60 days of anaerobic incubation. At all intervals examined, $BaCl_2$ precipitation (to $BaCO_3$) confirmed that these KOH-trapped volatiles were $^{14}CO_2$. Thus, under both aerobic and anaerobic incubation conditions, measurable mineralization of emamectin benzoate to $^{14}CO_2$ occurred.

Nature of the Extractable Residues. Based on RP-HPLC (method 1), residues found in soil following aerobic and anaerobic incubations included the parent MAB_{1a}, 8aOH-MAB_{1a}, 8aoxo-MAB_{1a}, and a polar fraction eluting between MSB_{1a} and void volume under RP-HPLC conditions. The RP-HPLC radiochromatogram of a selected soil extract is shown in Figure 4; eluate fractions containing MAB_{1a}, 8aOH-MAB_{1a}, and 8aoxo-MAB_{1a} were collected and analyzed by NP-HPLC (method 3) followed by a second RP-HPLC system (method 2) with different surface selectivity (not shown). Under these conditions, the presence of MAB_{1a}, 8aOH-MAB_{1a}, and 8aoxo-MAB_{1a} was again demonstrated based on coincidence in retention time with authentic internal and external standards. Residues of 8aOH-MAB_{1a} and 8aoxo-MAB_{1a} were relatively low and highest levels were found at a 60-day (30-day aerobic followed by 30-day anaerobic) incubation interval, comprising about 7 and 3%, respectively, of applied radioactivity. Thus, neither 8aOH-MAB_{1a} nor 8aoxo-MAB_{1a} was individually present at 10% or more of initially applied MAB_{1a}.

At all sampling intervals, the polar fraction was a major component of total residues. The RP-HPLC polar fraction isolate (by method 1) from a selected sampling interval (30-day aerobic followed by 60-day anaerobic) was further characterized by a 260-min RP-HPLC (method 4). The resulting radiochromatogram indicates that the polar fraction is a complex mixture consisting of at least eight different compounds, with retention

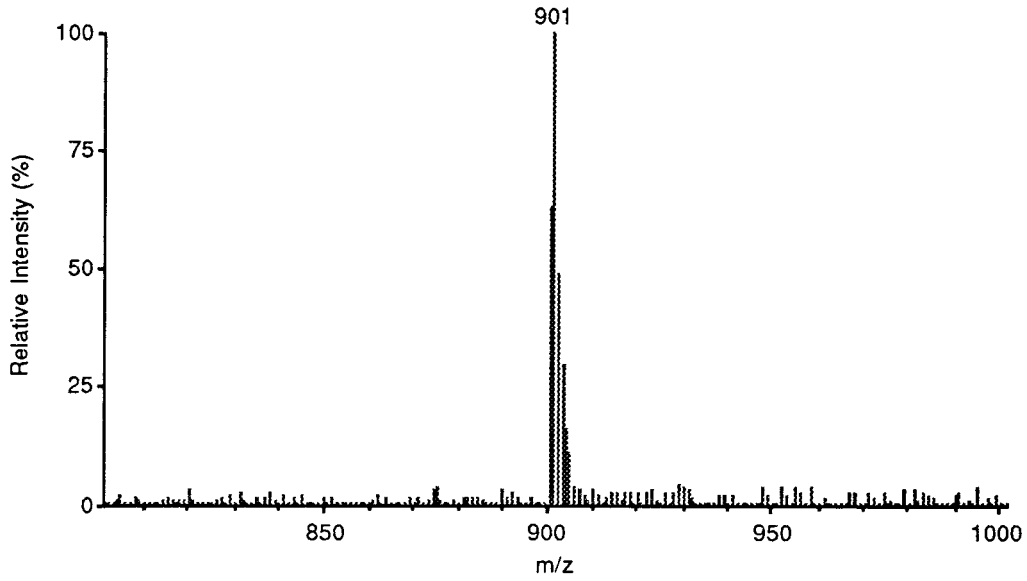

Figure 8. Mass spectrum of extractable soil residue showing the presence of 8aoxo-MAB_{1a}. The M + H^+ ion at 901 is consistent with the presence of 8aoxo-MAB_{1a}.

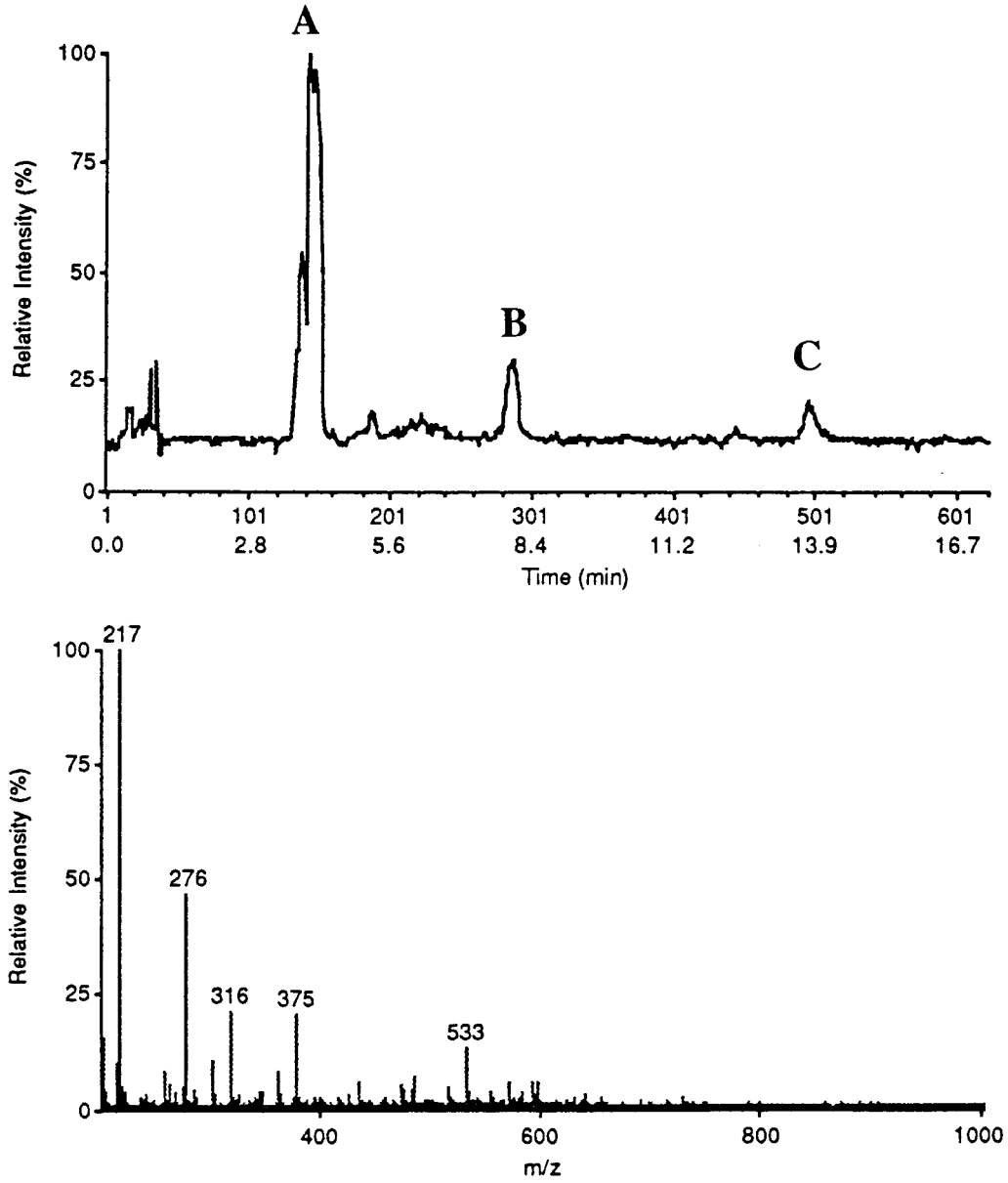

Figure 9. (Top) total ion chromatogram of polar fraction and (bottom) mass spectrum of major component (**A**) in polar fraction. The polar fraction was isolated from 180-day extractable soil residues by HPLC method 1. Indicates the presence of at least three residual components.

times ranging from about 3 to 260 min (Figure 5). The major polar fraction component (**VIII**) had a retention time of about 232−250 min and constituted about 3% of the initially applied radioactivity. In this 260-min HPLC run (i.e., method 4), the proportion of methanol in mobile phase increased from 90 to 100% after the 240-min solvent program interval. Therefore, it is likely that polar component **VIII** was unresolved and is actually a mixture consisting of several coeluting, but discrete, residues. Similar chromatographic profiles were also observed in isolated polar fractions from the 7-, 30-, 60-, and 360-day incubated soil samples (not shown). These results therefore indicate that, under aerobic and anaerobic conditions, the polar fraction is a multicomponent mixture. For this 260-min RP-HPLC, the polar fraction isolate was fortified with unlabeled MSB_{1a} standard. The retention time of MSB_{1a}, monitored at 245 nm, was about 235 min (Figure 5). A relatively major polar fraction component (**VII**, ~2% of initially applied radioactivity) also had a similar retention time (226−231 min). However, since the polar fraction for this chromatogram was collected based on its RP-HPLC elution time earlier than that of MSB_{1a}, this coincidence in retention time does not demonstrate the formation of MSB_{1a} as a soil residue.

RP-HPLC/MS/MS. In general, 8aOH-MAB_{1a} and 8aoxo-MAB_{1a} coelute under most reversed phase HPLC conditions but are well resolved under normal phase conditions. Under the RP-HPLC/MS/MS conditions of this study, authentic standards of 8aOH-MAB_{1a} and 8aoxo-MAB_{1a} coeluted at about 12 min while MAB_{1a} had a retention time of about 15 min. The presence of MAB_{1a}, 8aOH-MAB_{1a}, and 8aoxo-MAB_{1a} in a selected (180 day) soil isolate was confirmed by a match in retention times and molecular mass ($M + H^+ = 887$, 903, and 901, respectively) with the authentic external standards. The identity of MAB_{1a} and 8aOH-MAB_{1a} in the isolate was further established by MS/MS based on similarities in daughter ion spectra between the respective authentic external standards (Figure 6) and the soil HPLC isolate (Figure 7). The mass spectrum of 8aoxo-MAB_{1a}, isolated from soil, is shown in Figure 8; however, its daughter ion spectra were not obtained.

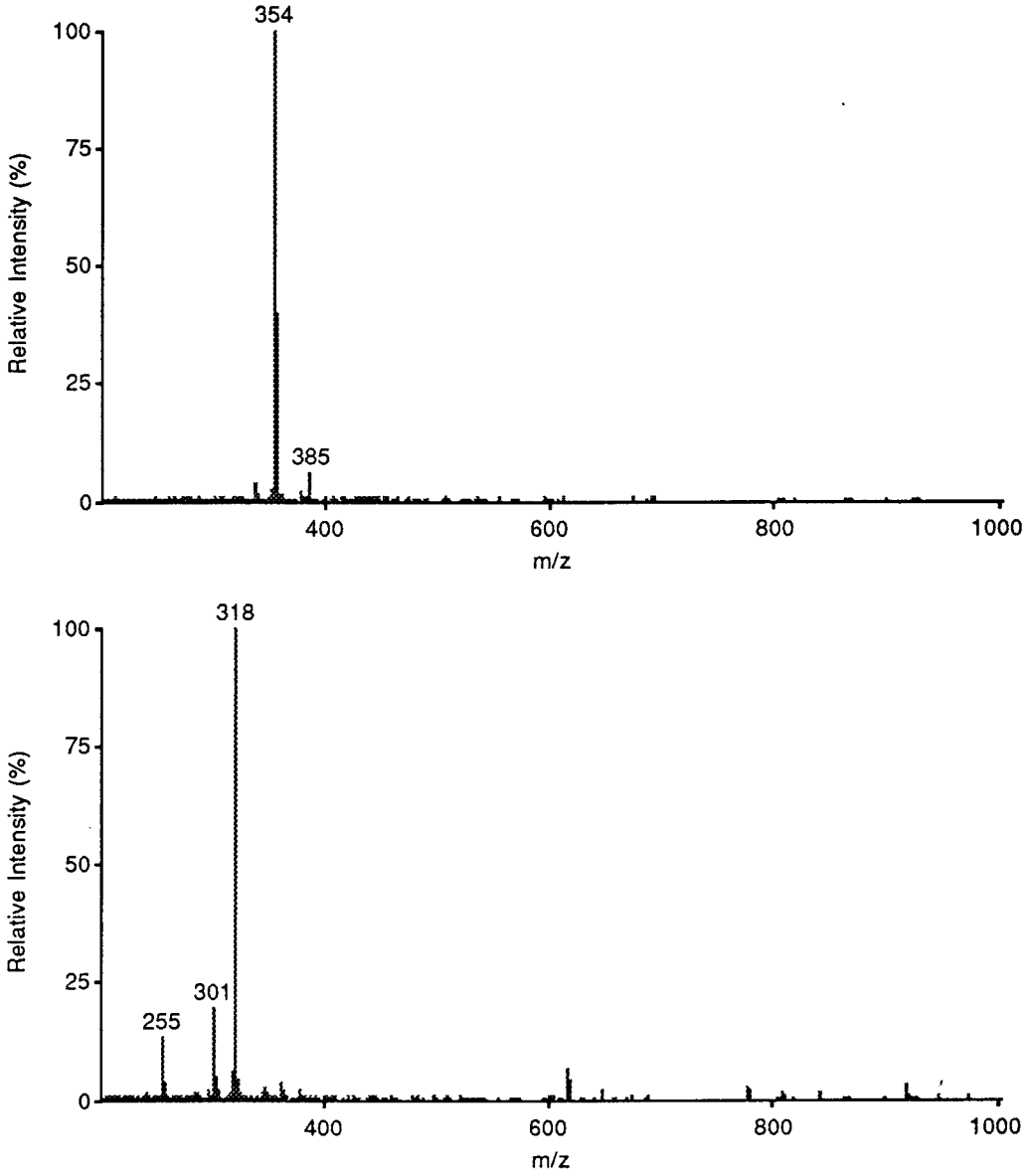

Figure 10. Mass spectrum of minor components (**B, C**) in polar fraction: (top) **B**; (bottom) **C**; see also Figure 9.

The RP-HPLC polar fraction isolate was also characterized under the same RP-HPLC/MS/MS conditions. The reconstructed total ion chromatogram (Figure 9) indicates the presence of at least three residual components (**A**, **B**, **C**) eluting between about 3 and 14 min. The major component of the polar fraction, component **A**, eluted at about 3 min and the mass spectra (Figure 9) is consistent with the presence of lower molecular weight (<600) material(s). A minor component of the polar fraction, component **B**, eluted at about 8 min and the mass spectra (Figure 10) are again consistent with the presence of still lower (<400) molecular weight material(s). The most minor component of the polar fraction, component **C**, eluted at about 14 min and the mass spectra, with the presumed $(M + H)^+$ ion at *m/z* 919, are consistent with a dihydroxylated derivative of MAB_{1a} (Figure 10). Thus, due to the complexity of the polar fraction spectra, no discrete individual compound was identified. However, the mass spectra demonstrate the presence of multiple polar components with either lower molecular weights or higher polarity than parent MAB_{1a}.

Degradation Half-Life of [^{14}C]MAB_{1a} in Soil. The quantitative degradation profile of MAB_{1a} in soil is shown in Table 4. The aerobic degradation rate was biphasic, being relatively rapid between 0 and 60 days ($DT_{50} \sim 74$ days, $R = -0.970$). Between 60 and 366 days, however, the degradation rate was less rapid ($DT_{50} \sim 349$ days, $R = -0.968$). It is possible that this biphasic DT_{50} profile for MAB_{1a} is related to its rapid and strong initial sorption to soil (Mushtaq et al., 1996) and, subsequently, reduced availability of the sorbed residue to soil microorganisms. This competitive abiotic influence on degradation rates has also been observed in other pesticides that have amino groups (Spanggord et al., 1996).

The cumulative aerobic and anaerobic DT_{50} for MAB_{1a} was determined to be about 174 days ($R = -0.90$). However, MAB_{1a} was not rapidly degraded under obligatory anaerobic conditions.

Fractionation of the Unextractable Residues. The proportion of unextractable residues in soil ranged from about 2 to 27% of applied radioactivity (Table 3). After exhaustive extraction of selected samples with NaOH, the majority (~86−90%) of these unextractable residues were released into the total (or base soluble) humic acid fraction while relatively minor amounts (~9−14%) remained in insoluble humin (Table 5).

Table 4. Degradation Rates of MAB_{1a} in Soil Under Aerobic and Anaerobic Conditions[a]

		average % dose		
days after application	conditions	MAB_{1a}	combined 8aoxo-MAB_{1a} and 8aOH-MAB_{1a} fraction	av polar fraction
0	aerobic	96.6	2.72	6.6
1	aerobic	90.1	1.52	10.27
3	aerobic	92.7	ND[b]	ND
7	aerobic	83.2	4.50	9.07
14	aerobic	78.3	1.0	12.5
30	aerobic	75.3	8.30	10.63
60	aerobic	51.0	6.8	13.16
90	aerobic	46.8	8.4	15.0
120	aerobic	38.7	10.8	14.4
180	aerobic	35.7	8.1	16.3
270	aerobic	30.9	6.6	18.5
366	aerobic	27.1	4.04	13.18
0	aerobic	88.6	2.1	5.3
30	aerobic	66.8	8.9	9.9
59	anaerobic	63.4	11.5	13.4
90	anaerobic	60.6	11.0	10.6

[a] DT_{50} of MAB_{1a} in soil under aerobic (fast and slow phase) and anaerobic conditions was calculated by assuming pseudo-first-order kinetics: $\ln C_0/C_t = -kt$. Plot of $\ln C_0/C_t$ vs t yielded a straight line, using linear regression, with a slope of $-k$. From the slope, $-k$, DT_{50} was calculated according to the following relationship: $DT_{50} = \ln 2/k = 0.693/k$. [b] Nondetectable, or <0.1% of applied radioactivity.

Among the total humic acids, the majority (~76−85%) was acid soluble (i.e., fulvic acids) while relatively minor amounts (~4−10%) were acid insoluble (i.e., high molecular weight humic acids). Thus, the majority of the unextractable residues in soil were found in the fulvic acid fraction.

Characterization of Fulvic Acid Fraction. Following characterization of a selected (366 day) fulvic acid fraction by gel chromatographic analysis, the result shown in Figure 11 was obtained, with a column radioactivity recovery of about 97%. About 3.1% of the fulvic acids consisted of components with apparent molecular weights of 8000−20 000. The majority of the fulvic acids, about 66.5%, had apparent molecular weights of about 500−8000. Another 27.8% were made up of components with apparent molecular weights of 500 or less. These molecular weights are consistent with humic acids literature values which have been reported to range from about 300 to 700 000 (Gjessing, 1965; Meikle et al., 1976).

Contrary to the reported strong adsorption of humic substances to Sephadex (Gelotte, 1960; Gjessing, 1965), nearly all (97%) of the column-applied total humic acids eluent was recovered. This is probably due to the low molecular weight fulvic acids involved, since molecular asymmetry associated with higher molecular weights would be expected to result in stronger adsorption to Sephadex (Meikle et al., 1976). Therefore, this high radiobalance recovery provides another indication that the majority of the unextractable residues was associated with the low molecular weight fulvic acids.

In spite of the expected instability of MAB_{1a}, or its structurally related (i.e., emamectin-like) metabolites, under strong basic and acidic extraction conditions, attempts were made to determine if emamectin-like residues would have been unextractable and be found in the low molecular weight fulvic acid fraction. When an aliquot of the fulvic acid fraction was concentrated and dissolved in MeOH, about 90% of the radioactivity precipitated out of solution, contrary to what would be expected of an emamectin-like residue. Under similar conditions, 46% of the total humic acids in radioactivity was also precipitated. The RP-HPLC radiochromatograms of MeOH-soluble radioactivity from the fulvic and total humic acid solutions are shown in Figures 12 and 13, respectively. In both cases, only a single component, more polar than MSB_{1a}, was found. It is probable that these single polar components, due to their early elution under reversed phase chromatographic conditions, are mixtures of several coeluting, but discrete, polar and/or low molecular weight residues. These results further indicate that the components in the fulvic acid fraction are not structurally related to extractable [^{14}C]MAB_{1a}

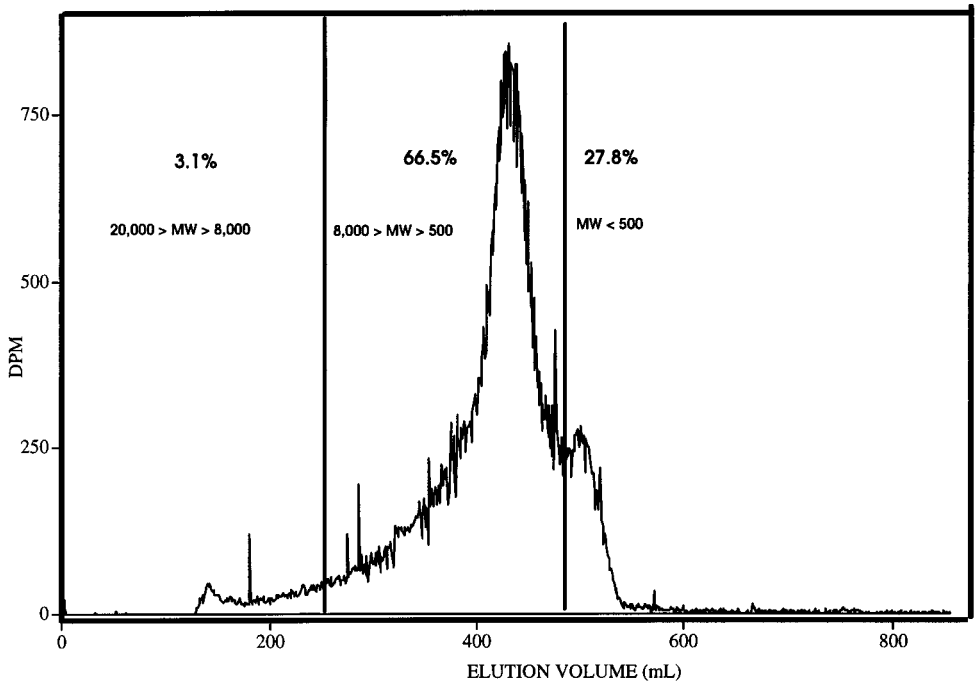

Figure 11. Sephadex G-50 radiochromatogram of fulvic acid fraction from [^{14}C]MAB_{1a}-treated soil incubated for 366 days under aerobic conditions.

Table 5. Percentage Distribution of Unextractable Radioactive Residues in Soil Fractions at Selected Times after Application[a]

days after application	unextractable residues (ppm)[b]	fulvic acids (%)[c]	insoluble humin (%)	humic acids (acid insoluble) (%)	total humic acids (%)
control	ND	0	0	0	0
7	0.22	82.9	12.8	4.3	87.2
30	0.47	80.8	14.3	4.9	85.7
60	0.51	76.4	13.1	10.5	86.9
180	0.9	81.8	12.1	6.1	87.9
366	1.3	84.9	9.5	5.6	90.5

[a] The soils were incubated entirely under aerobic conditions. [b] Determined by oxidative combustion followed by LSC. [c] Percent of total unextractable radioactive residues in soil fraction.

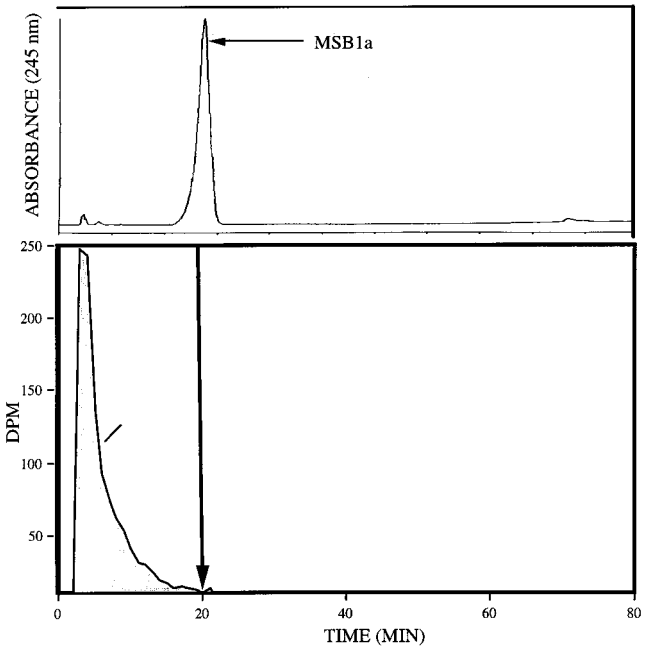

Figure 12. RP-HPLC chromatogram of MSB_{1a} standard and fulvic acid fraction from soil. (Top) RP-HPLC-UV chromatogram of MSB_{1a} standard (Table 2, method 1). The retention time of MSB_{1a} was about 20 min. (Bottom) RP-HPLC of fulvic acid fraction from the 366-day soil extract. The fulvic acid fraction was partitioned into methanol. No emamectin-like residue, including MSB_{1a}, was found. The retention time of MAB_{1a} under these conditions (Table 2, method 1) is about 50 min.

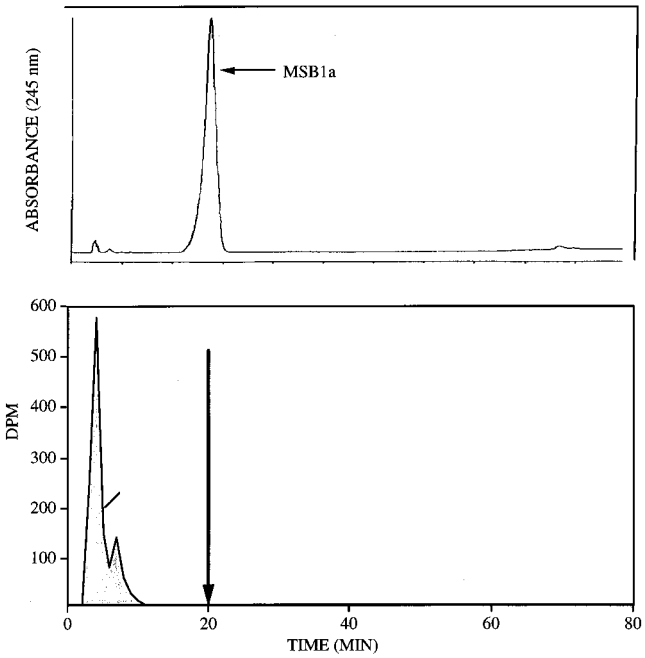

Figure 13. RP-HPLC chromatogram of MSB_{1a} standard and humic acid fraction from soil. (Top) RP-HPLC-UV chromatogram of MSB_{1a} standard (Table 2, method 1). The retention time of MSB_{1a} was about 20 min. (Bottom) RP-HPLC of total humic acids from the 366-day soil extract. The total humic acids fraction was partitioned into methanol. No emamectin-like residue, including MSB_{1a}, was found. The retention time of MAB_{1a} under these conditions (Table 2, method 1) is about 50 min.

but rather are residual fragments incorporated into the fulvic acid macromolecule. These fragments have, therefore, lost all recognizable relationship to the parent [^{14}C]MAB_{1a} molecule. Previous investigators have also reported on similar formation of humic substances from degraded organic materials (Chekalar and Illyuvieva, 1962; Sinha, 1972).

CONCLUSIONS

The degradation of MAB_{1a} in soil follows biphasic kinetics, being relatively rapid between 0 and 60 days and becoming less rapid between 60 and 366 days. This biphasic degradation profile has also been observed in other amine pesticides. Under anaerobic conditions, MAB_{1a} also degrades in microbially active soil, albeit more slowly. Results from HPLC and HPLC/MS/MS confirm the formation of 8aOH-MAB_{1a} and 8aoxo-MAB_{1a} following the incubation of MAB_{1a} in soil under aerobic and anaerobic conditions. The majority of the residual degradates, however, are a complex polar fraction consisting of at least eight low-level components. Based on HPLC and MS/MS, the polar fraction is a multicomponent mixture with constituents which are more polar than MSB_{1a}, a known polar emamectin-like residue.

Therefore, the constituents of the polar fraction will likely include more polar, or lower molecular weight, degradation products arising from biotic transformations of [^{14}C]MAB_{1a}. Each polar or lower molecular weight (i.e., relative to MAB_{1a}) degradation product could also be a substrate for additional biotic transformations. Based on the residue profiles found in this study, a proposed route for [^{14}C]MAB_{1a} degradation in soil is shown in Figure 14. It is converted to low levels of 8aOH-MAB_{1a}, 8aoxo-MAB_{1a}, a dihydroxylated MAB_{1a} derivative, and other unknown residues, all of which are more polar than MAB_{1a}. Due to the presence of multiple labile bonds on the glycone and macrolide moieties, these residues could be subject to additional transformations to form other secondary polar components. It is highly likely that a combination of these secondary polar residues constitutes the multicomponent polar fraction, as observed under typical reversed phase chromatographic conditions. The results of this study, therefore, demonstrate that emamectin benzoate is biodegradable in soil to multiple residual products and will eventually be incorporated into soil components and mineralized to CO_2.

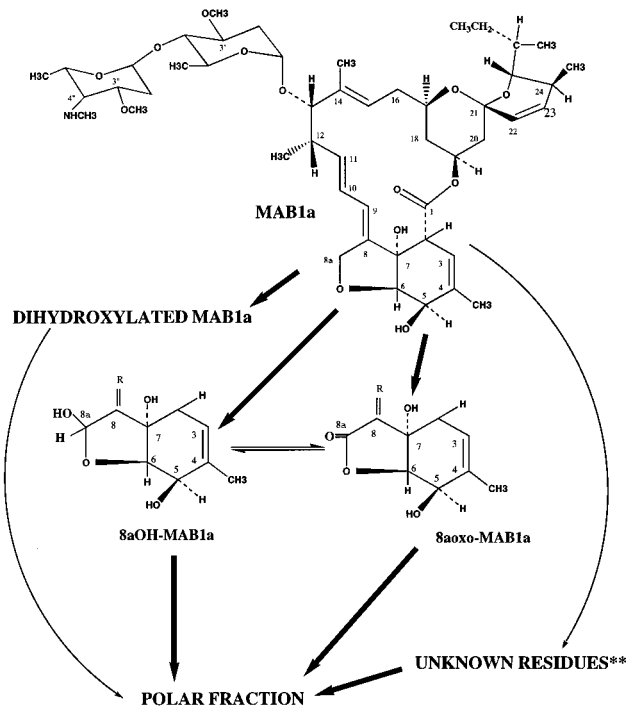

Figure 14. Proposed pathway for MAB_{1a} degradation in soil. Neither unknown nor any degradate individually accounted for 10%, or more, of applied radioactivity.

ACKNOWLEDGMENT

We are grateful to Mr. Randy Miller and Dr. Ralph Stearns (Department of Drug Metabolism, Rahway) for the mass spectral analyses and to Mr. Bill Feely and Ms. Loretta Allen (Department of Drug Metabolism, Three Bridges) for assistance in the HPLC assays. We are also grateful to Dr. Dennis Dean for the synthesis of [^{14}C]MAB_{1a} and to Dr. Allen Jones for establishing the radiochemical purity of this compound. The critical and insightful questions and advice from Dr. Anthony Lu (Executive Director, Department of Drug Metabolism) throughout the design, conduct, and reporting of this research investigation are highly appreciated.

LITERATURE CITED

Andrews, P. The Gel-Filtration Behaviour of Proteins Related to their Molecular Weights over a Wide Range. *Biochem. J.* **1965**, *96*, 595−606.

Brock, T. D. *Biology of Microorganisms*; Prentice-Hall: Englewood Cliffs, NJ, 1979; 410 pp.

Carnegie, P. R. Estimation of Molecular Size of Peptides by Gel Filtration. *Biochem. J.* **1965a**, *95*, 9.

Carnegie, P. R. A Peptide-Mapping Technique for the Estimation of Molecular Size. *Nature* **1965b**, *206*, 1128−1130.

Chekalar, K. I.; Illyuvieva, V. P. Application of the Radioactive C^{14} in the Study of Organic Matter Decomposition in the Soil. *Pochvovedenie* **1962**, *No. 5*, 40−50.

Chukwudebe, A. C.; Andrew, N.; Drottar, K.; Swigert, J. and Wislocki, P. G. Bioaccumulation Potential of 4″-epi-(Methylamino)-4″-deoxyavermectin B_{1a} Benzoate (Emamectin Benzoate) in Bluegill Sunfish. *J. Agric. Food Chem.* **1996a**, *44*, 2894−2899.

Chukwudebe, A. C.; Feely, W. F.; Burnett, T. J.; Crouch, L. S.; Wislocki, P. G. Uptake of Emamectin Benzoate Residues from Soil by Rotational Crops. *J. Agric. Food Chem.* **1996b**, *44*, 4015−4021.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.* **1956**, *28*, 350−356.

Gelotte, B. Studies on Gel Filtration Sorption Properties of the Bed Material Sephadex. *J. Chromatogr.* **1960** *3*, 330−342.

Gjessing, E. T. Use of "Sephadex Gel" for the Estimation of Molecular Weight of Humic Substances in Natural Water. *Nature* **1965**, *208*, 1091−1092.

Laurent, T. C.; Killander, J. A Theory of Gel Filtration and its Experimental Verification. *J. Chromatogr.* **1964**, *14*, 317−330.

Maynard, M. S.; Iwata, Y.; Wislocki, P. G.; Ku, C. K.; Jacob, T. A. Fate of Avermectin B1a on Citrus Fruits: 1. Distribution and Magnitude of the Avermectin B1a and ^{14}C Residue on Citrus Fruits from a Field Study. *J. Agric. Food Chem.* **1989**, *37*, 178−183.

Meikle, R. W.; Regoli, A. J.; Kurihara, N. H. Classification of Bound Residues Soil Organic Matter: Polymeric Nature of Residues in Humic Substance. In *Bound and Conjugated Pesticide Residues*; Kaufman, D. D., Still, G. G., Paulson, G. D., Bandal, S. K., Eds.; ACS Symposium Series 29; American Chemical Society: Washington, DC, 1976; pp 272−284.

Mushtaq, M.; Feely, W. F.; Syintsakos, L. S.; Wislocki, P. G. Immobility of Emamectin Benzoate in Soils. *J. Agric. Food Chem.* **1996**, *44*, 940−944.

Pharmacia. *Gel Filtration Principles and Methods*; Pharmacia Biotech.: Rahms i Lund, Sweden, 1993; pp 6−88.

Schnitzer, M.; Khan, S. U. *Humic Substances in the Environment*; Dekker: New York, 1972; p 17.

Shoop, W. L.; Mrozik, H.; Fisher, M. H. Structure and Activity of Avermectins and Milbemycins in Animal Health. *Vet. Parasitol.* **1995**, *59*, 139−156.

Sinha, M. K. Organic Matter Transformation in Soils I. Humification of C^{14}-Tagged Oat Roots. *Plant Soil* **1972**, *36*, 283−293.

Smith, A. M.; Mao, J.; Doane, R. A.; Kovacs, M. F. Metabolic Fate of [^{14}C]Acrolein under Aerobic and Anaerobic Conditions. *J. Agric. Food Chem.* **1995**, *43*, 2497−2503.

Spanggord, R. J., Gordon, G. R., Starr, R. I. and Elias, D. J. Aerobic Biodegradation of [^{14}C]3-Chloro-p-Toluidine Hydrochloride in a Loam Soil. *Environ. Toxicol. Chem.* **1996**, *15*, 1664−1670.

Trumble, J. T.; Moar, W. J.; Babu, J. R. and Dybas, R. Laboratory Bioassays of the Acute and Antifeedant Effects of Avermectin B1 and a Related Analogue on *Spodoptera exigua* (Hubner). *J. Agric. Entomol.* **1987**, *4*, 21−28.

Vink, J. P. M.; Nortersheuser, P.; Richter, O.; Diekkruger, B.; Groen, K. P. Modelling the Microbial Breakdown of Pesticides in Soil Using a Parameter Estimation Technique. *Pestic. Sci.* **1994**, *40*, 285−292.

Weast, R. C., Astle, M. J., Beyer, W. H., Eds.; *CRC Handbook of Chemistry and Physics*; CRC Press, Inc.: Boca Raton, FL, 1984; p D-149.

Received for review July 10, 1997. Revised manuscript received August 8, 1997. Accepted August 8, 1997.[⊗]

JF970589U

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.