Aged Soil Column Leaching of Emamectin Benzoate (MAB_{1a})

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The mobility of [^{14}C]emamectin benzoate (MAB_{1a}) and its photochemically and metabolically produced soil degradates was examined in four different agricultural soils. Microbially active soils were treated with [^{14}C]MAB_{1a} and irradiated in a photolysis unit. Irradiated soil samples were applied to soil columns, which were eluted with calcium chloride solution. The majority of the radioactivity (77−93%) remained in the irradiated soil placed on the surface of the top of the column. The 6 cm soil segments immediately below this soil contained 5.1−8.8% of the initially applied radioactivity. Extracts of soil segments below the applied soil plug did not show the presence of MAB_{1a} or degradates having structures with the intact avermectin ring. The total amount of applied radioactivity found in the leachate ranged from 3.5 to 5.4%. In addition, 0.9−1.4% of the initially applied radiolabel present in the leachates could be partitioned into ethyl acetate. The leachate extracts were separated into four degradate fractions, none of which exceeded 0.001 ppm (1.5% of the initial radioactivity). The leachate did not contain any MAB_{1a} or materials having the avermectin ring intact.

Keywords: *Emamectin benzoate; MK-0244; irradiated aged leaching*

INTRODUCTION

Emamectin benzoate (MK-0244, MAB_1) is a semisynthetic avermectin developed for its activity against a broad range of lepidopterous larvae, thus reducing feeding damage on vegetables (Dybas et al., 1989). Emamectin benzoate is chemically synthesized from abamectin by modification of the terminal disaccharide. An *epi*-aminomethyl group is substituted for the hydroxyl group at the 4″-position, and the product is formulated as a benzoate salt (Figure 1). Emamectin benzoate is composed of a mixture of two homologous compounds: a major constituent (≥90%), 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB_{1a}) benzoate, and a minor constituent (≤10%), 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1b} (MAB_{1b}) benzoate. The homologues differ only by a methylene unit on the C-25 side chain (Figure 1).

Adsorption/desorption and soil thin-layer chromatography (TLC) studies with [^{14}C]MAB_{1a} benzoate have been reported (Mushtaq et al., 1996). These studies have shown that parent MAB_{1a} is very tightly bound to soil, with high K_{oc} values (2.5×10^4 to 7.3×10^5), and indicate that MAB_{1a} is immobile in soils. Furthermore, it is known that the primary route of emamectin benzoate degradation, applied to soil, is via photolysis (unpublished data). Emamectin benzoate demonstrated a laboratory photolytic DT_{50} of 5 days on Maryland soil thin films and an average field DT_{50} of 0.28 days (unpublished data). Initially, at least eight primary photodegradates having the intact avermectin ring were formed, but none individually amounts to >2% of the initial radioactivity. Further degradation rapidly occurred to form a multitude of more polar components, which consisted of at least 12 discrete fractions with no single component containing >5% of the initial radioactivity (unpublished data).

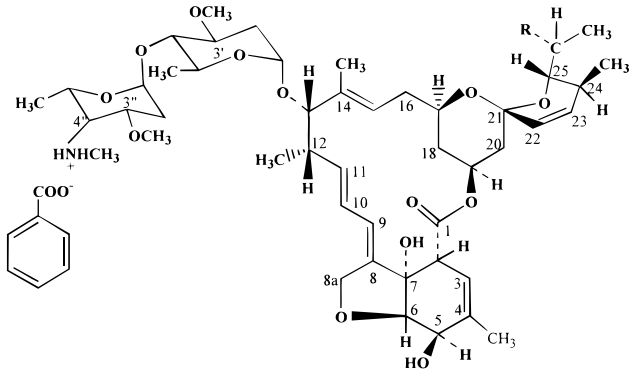

Figure 1. Structure of emamectin benzoate (MK-0244): MAB_{1a} component (≥90%), R = CH_2CH_3; MAB_{1b} component (≤10%), R = CH_3.

Aged column leaching studies typically employ soil microbial degradation processes to generate the aged residues (U.S. EPA, 1982). However, emamectin benzoate degrades in soil primarily via photolysis, as discussed above. Thus, to assess the risk to surface water and groundwater contamination by emamectin degradation products, the mobility of this complex mixture of soil photodegradates was determined on soil columns comprising four soil types. Since the photodegradation was conducted with microbially active soils, both photodegradation and biodegradation processes could be involved in the formation of secondary and tertiary degradates.

MATERIALS AND METHODS

Chemicals. The test chemical [3-, 7-, 11-, 13-, or 23-^{14}C]-MAB_{1a} benzoate (22.26 μCi/mg, >96% radiopurity) was pre-

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Table 1. Physical and Chemical Properties of Soils[a]

soil characteristic	Fayette, KY	Lakeland, FL	Three Bridges, NJ	Yuma, AZ
sand (%)	67	100	19	64
silt (%)	23	0	64	22
clay (%)	10	0	16	14
organic matter (%)	1.9	0.06	1.98	0.6
organic carbon (%)	1.0	0.03	1.04	0.32
CEC[b]	5.5	0.4	10.9	20.9
pH	6.8	6.5	6.4	8.1
bulk density[c]	1.2	1.75	1.54	1.18
% moisture at 0.33 bar	15.1	1.2	31.3	11.2
classification	sandy loam	sand	silt loam	sandy loam

[a] Analyses on the KY, FL, and NJ soils were performed by PTRL, East, Inc., Richmond, KY. The soil from AZ was analyzed by AGVISE Laboratories, Northwood, ND. [b] Cation exchange capacity (CEC) is expressed as mequiv/100 g. [c] Bulk density is expressed as g/cm^3. Bulk density for KY, FL, and NJ soils was determined on undisturbed soil by the University of Kentucky, College of Agriculture, Lexington, KY. Analyses were not conducted under GLP.

pared by the Labeled Compound Synthesis Group, Department of Drug Metabolism, Merck Research Laboratories, Rahway, NJ. Reference materials were MAB_1 (purity > 96%), 4″-deoxy-4″-(*epi*-amino)avermectin B_1 (AB_1) (purity > 94.9%), and 8,9-*Z*-4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (8,9-Z MAB_{1a}) (purity > 92%).

Soils. For the soil columns, four soils were selected on the basis of texture and organic matter content: sand from Lakeland, FL; sandy loam from Fayette, KY; sandy loam from Yuma, AZ; and a silt loam from Three Bridges, NJ. The properties of these soils are presented in Table 1. KY sandy loam soil was employed for the photoirradiation of [^{14}C]MAB_{1a} benzoate, because it had been employed for the aerobic and anaerobic degradation studies. Prior to use, the soils were first passed through a 2-mm sieve. To ensure that the KY, NJ, and FL soils were microbially active, each soil was periodically misted with water and maintained for several weeks at ambient temperature before the start of the definitive study. The freshly collected AZ soil did not require this preactivation.

Photolysis Equipment. A Heraeus SUNTEST CPS xenon arc lamp equipped with a UV filter was used in this study. The spectral distribution and output of the xenon lamp was shown to correspond to average natural sunlight in New Jersey. The average light flux for the xenon lamp over the 330−800 nm spectral region was 142.6 W/m^2 (measured at the same distance as the samples). The average light flux of natural sunlight (full sunlight) in the same spectral range was 138.1 W/m^2, as measured on June 13 and August 7, 1990. Irradiation occurred in a stainless steel chamber equipped with a Pyrex glass lid and coolant insulation. The temperature of the chamber monitored hourly was 25 ± 1 °C throughout the photolysis duration.

Soil Treatment and Irradiation. To Petri dishes measuring 100 mm in diameter was added ≈57 g of KY sandy loam soil (50 g dry weight basis) per dish. The moisture content was adjusted to ∼65% with water. The soil was then tamped into a uniform layer (∼5 mm thick). The [^{14}C]MAB_{1a} benzoate solution in water was applied dropwise to cover as much of the soil surface as possible. The nominal dose rate was 0.09 ppm in the soil (approximately equivalent to a total of six applications at 0.015 lb of active ingredient/acre at a 3-in. depth). The water content in the dosing solution was sufficient to raise the moisture content to ∼75%. Immediately after dosing, the dishes were covered with a piece of quartz glass that was secured in place. The prepared dishes were placed into the photolysis chamber and irradiated continuously for 34 days (≈1 half-life under conditions of this experiment, that is, 5-mm-thick KY soil and artificial light). Following irradiation, the dishes were removed from the chamber, and four homogeneous composite samples (one for each soil column type) of the irradiated material were made for analysis.

Soil Column Leaching. Heavy-walled glass tubes (6-cm length by 3.6-cm i.d.) were connected with Teflon tape and sealed with vinyl plastic electric tape to form 42-cm-long columns. The bottom piece of the assembly was equipped with a fritted glass disk of coarse porosity and a Teflon stopcock to control the flow rate of the leachate.

Subsamples of four different agricultural soils were air-dried and passed through a 2-mm sieve. Duplicate columns of each soil type were prepared. The packing of the columns with KY sandy loam, FL sand, and AZ sandy loam soils was conducted using the slurry technique. Small amounts of the 0.01 M $CaCl_2$ solution were poured first into the column, and then weighed portions of soil were added slowly to 0.01 M $CaCl_2$ until the soil-column length reached 30 cm. Because of the nature of the soil, the column preparation of NJ silt loam was conducted in a different manner. Weighed amounts of the untreated dried soil were applied to duplicate columns to give a soil column length of 30 cm. The soil was hand-packed by gently tapping the columns. The bulk density of each column was measured, and the columns were saturated with 0.01 M $CaCl_2$. This saturation process was conducted from the bottom up to remove the entrapped air among soil products, to avoid channeling in the column. The void volume of the soil column was determined by measuring the amount of $CaCl_2$ solution required to saturate only the soil.

A weighed amount of the irradiated KY aged soil (treated plug) was applied to the top of each soil column. The soil columns were then leached with a volume of 0.01 M $CaCl_2$ solution equal to 50.8 cm (20 acre in.) times the cross-sectional area of the column (equivalent to ∼529 mL). The leachate was collected as a composite fraction. After the leachate was collected, each column was disassembled into the treated plug and the 6-cm-length segments and the soil were air-dried and homogenized. Subsamples of leachate and soil segments were assayed for total radioactivity.

Extraction and Fractionation of Samples. Approximately 50 g of irradiated soil composite (prior to column leaching), or soil segment samples (containing >0.005 ppm of radiolabeled material), and irradiated plugs (after column leaching) were extracted by twice blending with ∼150 mL of methanol containing 100 mM ammonium acetate (AA), followed by filtration. The combined organic soluble fractions were subjected to chromatographic analysis. The column leachate was directly partitioned into ethyl acetate. The remaining aqueous fraction was freeze-dried and redissolved in methanol/water.

RP-HPLC Analyses. Emamectin benzoate, the organic extracts of the soils, and leachate samples were analyzed using a linear step gradient elution system with mobile phases A, 5 mM AA in methanol, and B, 5 mM AA in water, on a Supelcosil LC-18 column (4.6 × 250 mm) at a flow rate of 1 mL/min. At 0 time, % phase A/% phase B was 85:15. After 25 min, a step gradient was initiated to 90:10 (A/B) and after 35 min was again changed to 100%. After 10 min, the gradient was returned to the initial gradient. A Waters 484 UV tunable absorbance detector and a Raytest Ramona-5 flow-through radioactivity monitor with a solid glass cell were employed as detectors. The eluant was monitored at 254 nm. Fractions of eluant were collected at 2-min intervals, and the radioactivity was determined using a liquid scintillation counter. A Waters WISP model 712 autoinjector was used for application of the sample to the column.

Electrospray Ionization (ESI) Liquid Chromatography/Mass Spectrometry (LC/MS). Organic extracts from irradiated composite soil samples and treated soil plugs following leaching were analyzed by LC/MS using the electrospray mode in order to confirm the presence of MAB_{1a} in irradiated samples. For reference compounds, no column was used. For sample extracts, a Phenomenex Prodigy 5 ODS-2 column, 4.6 × 250 mm, was used with a gradient mobile phase of A, 0.4% formic acid, and B, methanol. At 0 time, the mobile phase consisted of % phase A/% phase B (50:50). At 12 min, the step gradient system was changed to 10:90 (A/B).

Table 2. Distribution (Percent)[a] of Radioactivity in Irradiated Aged Emamectin Benzoate Soil Column

sample analyzed	KY sandy loam	FL sand	NJ silt loam	AZ sandy loam
irradiated soil plug	81.1	93.3	77.5	82.0
0−6 cm (segment 1)	6.8	5.1	8.8	7.2
6−12 cm (segment 2)	4.4	1.8	2.0	2.0
12−18 cm (segment 3)	2.9	1.9	1.3	1.8
18−24 cm (segment 4)	3.1	2.7	1.1	1.1
24−30 cm (segment 5)	2.7	2.8	0.7	0.8
leachate	5.4	5.2	4.0	3.5
total recovery[a]	106.4	112.6	95.4	98.4

[a] Total recovery based on the applied radioactivity.

After 23 min, the step gradient was changed to 100% B for 5 min. The step gradient was then adjusted to the original gradient. Flow rate was 0.5 mL/min. A Finnigan MAT TSQ 7000 mass spectrometer was used in the positive ion ESI mode.

Radiochemical Assays. The ^{14}C radioactivity in soil samples was converted to $^{14}CO_2$ in a Harvey biological sample oxidizer (model OX-300 or OX-500). The resultant trapped $^{14}CO_2$, as well as radioactivity in organic extracts, was determined using a liquid scintillation counter (Beckman model LS 3801, LS 5000TD, LS 6000IC, or 6000TA).

RESULTS AND DISCUSSION

Distribution of ^{14}C Radioactivity in Soil Columns and Leachate. The average distribution of applied radioactivity for each of the soil types examined is summarized in Table 2. The majority of the applied radioactivity (77−93%) remained in the irradiated soil plug segment. The highest averaged percentage of applied radioactivity detected in any segment, except the irradiated soil plug segment, was found in segment 1 (immediately underneath the irradiated soil plug). Radioactivity in segment 1 ranged from 5.1 to 8.8% of the total applied radioactivity. The leachate from the soil columns contained 3.5−5.4% of the initially applied radioactivity. The overall recoveries ranged from 95 to 113%, with an average recovery of 103% for the eight soil columns.

Distribution of Extractable ^{14}C Radioactivity in Irradiated Aged Soil Samples. The amount of radioactivity that could be extracted from the KY soil after 34 days of continuous irradiation ranged from 71 to 81% (Table 3). The radioactivity remaining in the soil after extraction ranged from 20 to 30%. Analyses of the organic soil extracts by RP-HPLC (extracts were co-chromatographed with a reference chemical mixture) showed that the parent compound (MAB_{1a}) accounted for 48−53% of the radioactivity applied to the KY soil (Table 4). No significant amounts of 8,9-Z-MAB_{1a} (>0.5%) were observed. Five degradate fractions were detected and amounted to 2.8−11.1% of the total applied radioactivity. Significant amounts (7−11%) of radioactivity could also be found in all cases at a retention time (t_R) of 4−7 min; these polar degradates (D-1) have been shown to be a complex mixture (at least 12 discrete peaks) of non-emamectin-like materials (unpublished data). Significant amounts (7−10%) of radioactivity in all cases could also be found at a retention time of 21−23 min (D-6). This HPLC fraction corresponds to the retention time of several degradates having the intact avermectin ring, for example, AB_1, 8a-OH-MAB_1, and 8a-oxo-MAB_1. As many as eight individual components have been characterized in this region using refined HPLC systems (unpublished data).

Analyses of Extractable ^{14}C Radioactivity in Irradiated Aged Soil after Elution and Column Segments. The irradiated plugs, after column elution, from all four soil types and segment 1 from KY, NJ, and AZ soils were extracted. Since levels of radioactivity were <0.005 ppm in the remainder of the soil column segments, these segments were not extracted. Levels of radioactivity that were extracted from the irradiated plugs ranged from 48 to 66% of the total radioactivity placed on the column (Table 3). In addition to the parent compound (34−57%), six other degradates were detected, but none individually exceeded 7.6% (0.007 ppm) of the initial radioactivity (Table 4). Approximately 27−31% of the radioactivity was in the postextracted solids (PES).

The averaged amount of radioactivity extracted from the top soil horizon (0−6 cm, segment 1) under the treated plug ranged from 3.9% (0.004 ppm) to 4.8% (0.005 ppm) of the total applied radioactivity. The

Table 3. Distribution (Percent)[a] of Radioactivity in Irradiated Aged Emamectin Benzoate Soil Column Fractions

	KY sandy loam		FL sand		NJ silt loam		AZ sandy loam	
sample analyzed	EXT[b]	UNEXT[c]	EXT	UNEXT	EXT	UNEXT	EXT	UNEXT
irradiated aged soil	79.5	25.2	71.3	23.4	80.9	19.5	74.2	29.7
irradiated soil plug after elution	51.5	29.7	65.9	27.4	48.3	29.1	50.9	31.1
segment 1, 0−6 cm	3.9	2.9	NA[d]	NA	3.9	5.0	4.8	2.4
leachate	1.1	4.3	1.4	3.8	1.2	2.9	0.9	2.6

[a] Percent of initially applied radioactivity to the KY soil. KY sandy loam was treated with MAB_{1a}, aged, and used as the irradiated plug for all soil columns. [b] For soils, extractable into methanol/ammonium acetate. For leachate. extractable into ethyl acetate. [c] For soils, postextraction solids. For leachate, aqueous fraction. [d] NA, not analyzed.

Table 4. RP-HPLC Distribution (Percent)[a] of Radioactivity in Irradiated Aged Emamectin Benzoate Soil Column Fractions

	KY sandy loam			FL sand			NJ silt loam			AZ sandy loam		
sample analyzed	MAB_{1a}	D-1[b]	D-6[c]	MAB_{1a}	D-1	D-6	MAB_{1a}	D-1	D-6	MAB_{1a}	D-1	D-6
irradiated aged KY soil before elution	53.3	11.1	10.2	51.6	7.2	6.5	51.5	8.7	8.1	48.2	10.2	9.7
treated soil plug after elution	37.6	6.5	2.3	56.6	3.4	5.0	35.8	4.4	4.7	33.9	3.0	7.6
segment 1, 0−6 cm	ND[d]	3.9	ND		NA		ND	3.3	ND	ND	4.8	ND
leachate												
ethyl acetate	ND	1.1	ND	ND	1.3	ND	ND	1.2	ND	ND	0.9	ND
aqueous		NA		ND	3.9	ND		NA			NA	

[a] Percent of initially applied radioactivity to the KY soil. [b] D-1 retention time 4−7 min (polar fraction). [c] D-6 retention time (21−23 min) corresponds to the HPLC fraction in which emamectin-like degradates such as AB_{1a} and 8a-OH- and 8a-oxo-MAB_{1a} elute. [d] ND, not detected; NA, not analyzed.

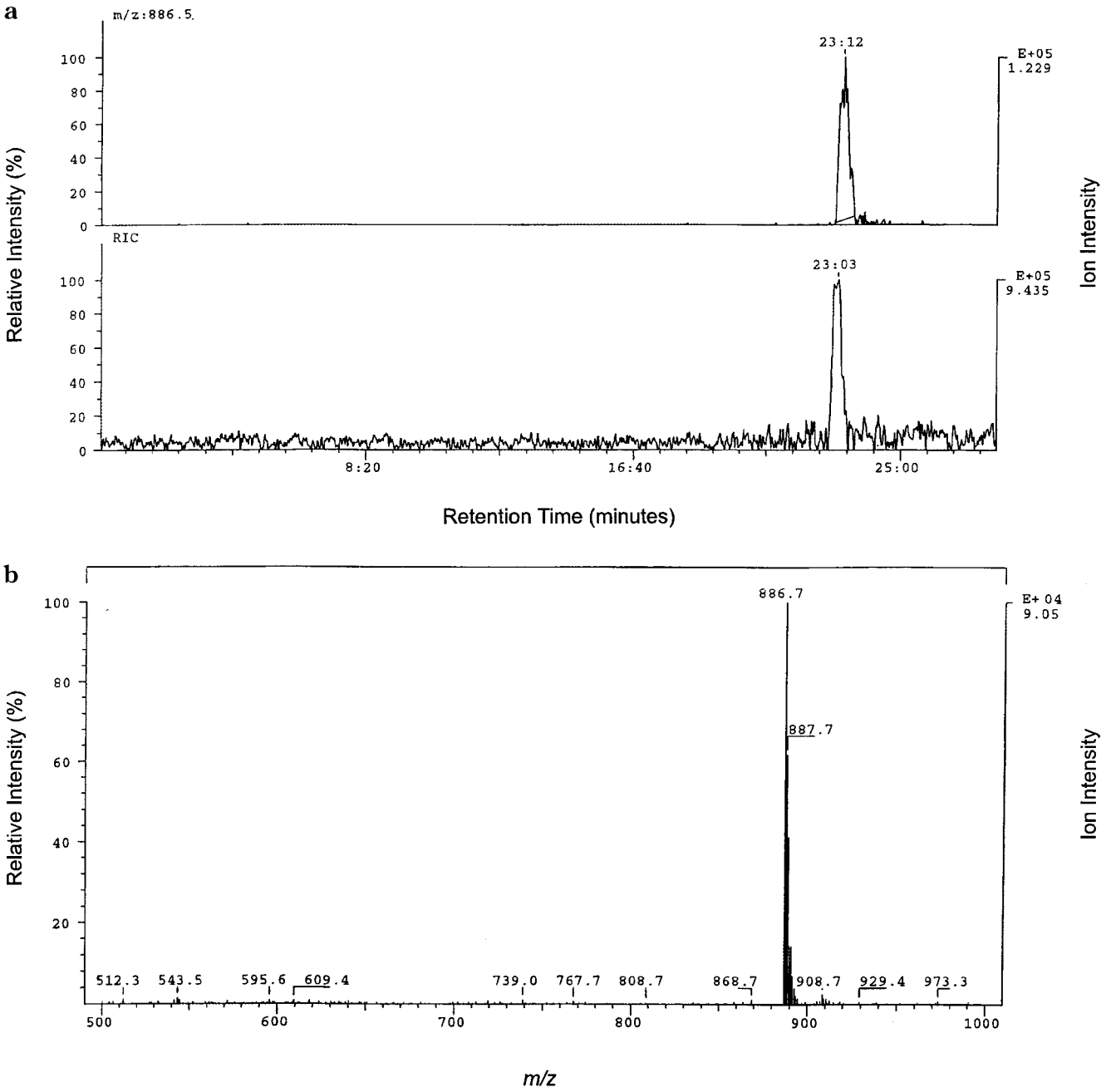

Figure 2. (a) LC/MS/ESI(+) reconstructed ion chromatogram and ion chromatogram at *m/z* 886 from $CH_3OH/NH_4OAc/H_2O$ extract-irradiated plug NJ silt loam soil. (b) LC/MS/ESI(+) mass spectrum at HPLC RT 23 min of MAB_{1a} isolated from $CH_3OH/NH_4OAc/H_2O$ extract-irradiated plug NJ silt loam soil.

remaining radioactivity was present in the PES. Only two degradates were observed in segment 1 (0−6 cm) extracts. The major one, D-1 (t_R = 4−7 min) did not exceed 4.8% (0.005 ppm) of the applied radioactivity and was detected in all columns. Previous studies have demonstrated that this degradate fraction is a complex mixture of at least 12 discrete fractions (unpublished data). A second degradate, D-2 (t_R =of 8−10 min) was detected only in the NJ silt loam columns. This degradate amounted to 0.6−1.1% (0.001 ppm) of the total radioactivity applied to the columns. No significant amount of radioactivity was found at the D-6 region, where several emamectin-like degradates (AB_{1a} and 8a-OH-/8a-oxo-MAB_{1a}) elute.

Analyses of ^{14}C Radioactivity in Column Leachate. The amount of radioactivity that could be extracted from the column leachate into ethyl acetate ranged from 0.9 to 1.4% of the applied radioactivity (Table 3). The ethyl acetate extracts contained one major fraction—D-1 (t_R = 4−7 min)—which amounted to 0.9−1.3% of the initially applied radioactivity (Table 4). The majority of the radioactivity in the leachate (2.6−4.3%, 0.002−0.004 ppm of the applied radioactivity) could not be partitioned into ethyl acetate but remained in the aqueous fraction.

The aqueous fraction from the FL sand column was freeze-dried and dissolved in methanol/water and analyzed by RP-HPLC. RP-HPLC analysis showed one major region of radioactivity with a t_R of 3−9 min, which was similar to the chromatographic profile seen from the ethyl acetate extract. Because of this very polar behavior, both the ethyl acetate extractable and aqueous portions of the leachates were considered to contain a

mixture of degradates not containing the intact avermectin ring.

LC/MS Confirmation of MAB_{1a}. During all of the HPLC analyses, the parent chemical and/or reference chemicals were injected on the same day. The HPLC properties of the parent chemical were consistent with those of the reference chemical. To confirm the presence of MAB_{1a} in the composite sample following irradiation, and in the treated plugs from the various soil columns, a standard of [^{14}C]MAB_{1a} was prepared and analyzed by LC/MS using ESI (positive ion mode). RP-HPLC chromatograms from each extract showed a t_R of ≈23 min that corresponds to the parent chemical. Figure 2a shows both the reconstructed (total) ion chromatogram and the ion chromatogram at *m*/*z* 886 obtained by LC/MS. Figure 2b depicts the LC/MS/ESI mass spectrum of MAB_{1a} isolated from the treated plug of NJ silt loam soil, which was identical to that seen for authentic MAB_{1a}.

CONCLUSIONS

The parent material, MAB_{1a} benzoate, exhibited no mobility in any of the four soils examined. One major degradate fraction was observed in the 6-cm segment immediately below the irradiated plugs, which did not exceed 4.8% (0.004 ppm) of the applied radioactivity. The degradate fraction having RP-HPLC chromatographic properties similar to those of AB_1 and 8 a-oxo-MAB_1 (and other degradates having the intact avermectin ring) was present in the irradiated aged soil plugs but did not leach into segment 1, or below, indicating that these primary photodegradates of emamectin benzoate do not leach. Thus, it can be concluded that degradates having the intact avermectin ring either remain in the irradiated plugs or migrate only to the 6-cm segment immediately below the irradiated plug. The mobility of avermectin B_{1a} (Gruber et al., 1990) under aged soil column conditions was very similar to that of emamectin benzoate shown here. In the avermectin B_{1a} study, only a small portion (1.4−6.4%) of the total applied radioactivity was present in the leachate, and most of the radioactivity remained in the top 6-cm segment of the soil column. The emamectin benzoate results are also consistent with those seen in an emamectin benzoate outdoor rotational crop study, wherein no ^{14}C radioactive residues (<0.002 ppm) were found below the 0−15-cm soil horizon (Chukwudebe et al., 1996).

Analyses of the ethyl acetate extract of the leachate showed up to four degradate fractions. The major degradate (t_R = 4−7 min) was fairly polar and did not exceed 1.28% of the initially applied radioactivity (0.001 ppm). The other three degradates (not amounting to >0.17% of the total applied radioactivity to the columns) were seen occasionally. The aqueous fraction from the FL soil column after ethyl acetate extraction (having 3.92%, 0.004 ppm, of the initial radioactivity) also exhibited a chromatographic profile similar to that of this major degradate found in the ethyl acetate extracts of the leachate.

Because of its chromatographic behavior, it is unlikely that the leachate contains material having the intact avermectin ring.

The lack of mobility of emamectin and degradates having the intact avermectin ring in soil columns having low clay and organic carbon content demonstrates the immobility of irradiated aged emamectin benzoate residues. Therefore, applications of emamectin benzoate, which have been shown to have field DT_{50} values of ∼0.28 days, should not pose a threat to surface water or groundwater (Kenaga, 1980).

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