Uptake of Emamectin Benzoate Residues from Soil by Rotational Crops

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Radiocombustion analyses of barley, carrots, and lettuce planted in 30, 120, 141, and 365-DAT plots after applications of the test compound [^{14}C]4″-deoxy-4″-epi-methylaminoavermectin B1a ([^{14}C]-MAB1a; 0.1008 kg ai ha^{-1}) demonstrated that total residues in edible lettuce and carrots were below the limits of quantitation (LOQ; <4 μg/kg). Highest residue levels of 16 and 30 μg/kg were found, respectively, in 30- and 141-DAT plot barley straw. In both straw samples, percentages of extractable and unextractable radioactivity were similar, averaging 54 and 46%, respectively, of total radioactivity. Cellulase/hemicellulase digestions of resulting marcs indicate that some radioactivity was incorporated into plant natural products. Extractable residues in straw and grain were below LOQ. No emamectin or emamectin-like residues were found in extractable residues of straw. Results of this study indicate that significant uptake of emamectin benzoate by rotational crops will not occur. Emamectin benzoate residues in soils were not detected below the top 0−15-cm segment and are, therefore, unlikely to leach into groundwater.

Keywords: *Avermectin; rotational crops; HPLC; emamectin benzoate; uptake.*

INTRODUCTION

Avermectins are macrocyclic lactones produced by the soil antinomycete *Streptomyces avermitilis*. A natural avermectin product, abamectin (avermectin B1), is currently registered in the United States and worldwide as a miticide. Emamectin benzoate or MK-244 (MAB1 benzoate, or *4″-deoxy-4″-epi*-methylaminoavermectin B1 benzoate) is a derivative of abamectin and consists of two avermectin homologs, each with a molecular weight of ~900 Da. By specification, it contains at least 90% of *4″-deoxy-4″-epi*-methylaminoavermectin B1a (MAB1a) benzoate and not more than 10% of *4″-deoxy-4″-epi*-methylaminoavermectin B1b (MAB1b) benzoate. These components differ by only a methylene group on the isobutyl side-chain of the B1a component (Figure 1). Emamectin benzoate is an effective lepidopteran insecticide (Trumble et al., 1987) and is currently under development by Merck Research Laboratories for use on a number of crops, including celery, lettuce, cole crops, and tomatoes.

In practice, the use of a pesticide on crops results in deposition of pesticide residues in soil from which the residues could be taken up by succeeding crops. The minor structural difference between MAB1a and MAB1b coupled with the preponderance of MAB1a in emamectin benzoate and the nearly identical biological activities of the two homologs (Shoop et al., 1995) indicate that [^{14}C]MAB1a can be used as the test substance for emamectin benzoate. Thus, in this study, the soil was treated at the maximum recommended seasonal field use rates (0.1008 kg ai ha^{-1}) with [^{14}C]MAB1a, and rotational crops were planted after aging intervals of 30 days (mimicking crop failure or premature sowing), 120 and 141 days (for seasonal rotations), and 365 days (for yearly rotations). The rotational crops planted were barley, carrots, and lettuce, representing small grains, root crops, and leafy vegetables, respectively.

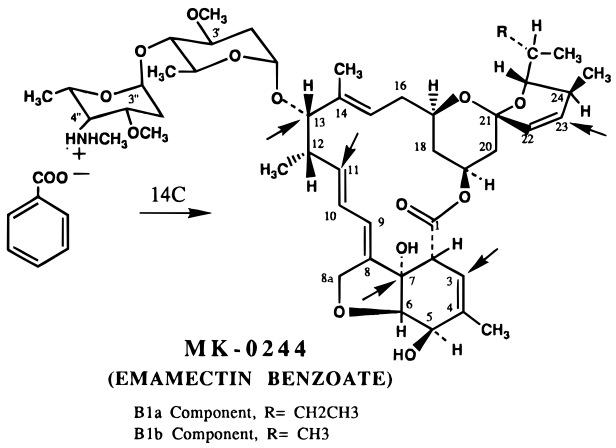

Figure 1. Structure of emamectin benzoate showing major and minor homologues. The major, 4″-deoxy-4″-epi-methylaminoavermectin B1a benzoate, and minor, 4″-deoxy-4″-epi-methylaminoavermectin B1b benzoate, homologues constitute ≥90 and ≤10%, respectively, of emamectin benzoate. Arrows indicate position of ^{14}C radiolabel. The ^{3}H radiolabel is at H-5. The test substance used in this study was 4″-deoxy-4″-epi-methylaminoavermectin B1a.

By determining the nature and levels of emamectin benzoate residues taken up from treated sandy loam soil by representative rotational crops under confined conditions, the potential for the accumulation of these residues in crops used for human and animal consumption can be better understood.

MATERIALS AND METHODS

Chemicals. The test compound, [^{14}C]MAB1a *([^{14}C]4″-deoxy-4″-epi*-methylaminoavermectin B1a), was prepared by the Labeled Compound Synthesis Group at Merck Research

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S0021-8561(96)00517-1 CCC: $12.00 © 1996 American Chemical Society

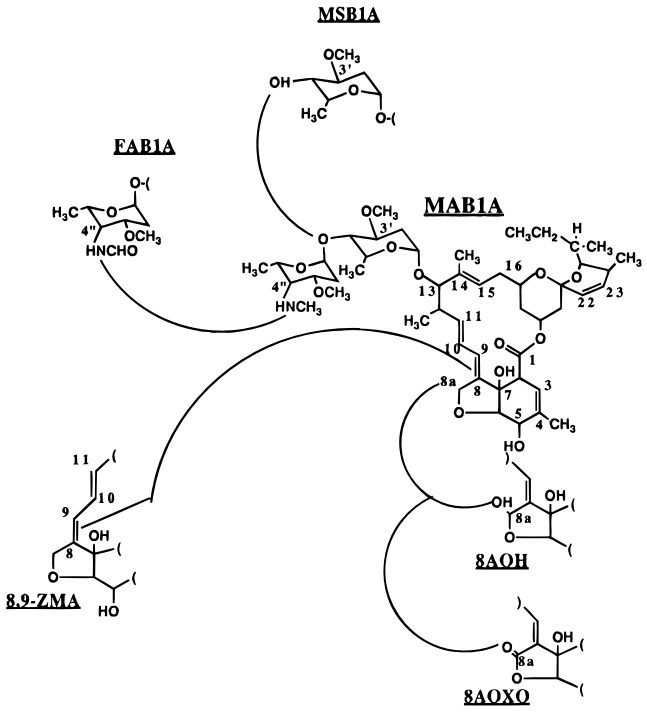

Figure 2. Structures of selected emamectin benzoate-related avermectin standards. Abbreviations are defined in Materials and Methods. Connecting lines illustrate the relationship between MAB1a and each of the related standards (partial structures shown).

Laboratories in Rahway, NJ, and had a specific activity of ~23.9 μCi/mg. The ^{14}C label was located at the C3, C7, C11, C13, or C23 positions. Immediately prior to soil application, the labeled material was dissolved in a formulation blank [emulsifiable concentrate (EC)] and then diluted with water. All stock solutions of the formulated [^{14}C]MAB1a spray solution contained ~44.9 g/L EC and a specific activity of ~1.1 μCi/mL EC. The radiopurity of [^{14}C]MAB1a in the spray solution was ~97.4%. Tritiated MAB1a (^{3}H label at 5 H), which was used as internal standard to monitor the extraction of emamectin benzoate residues from treated crops, was also prepared by the Labeled Compound Synthesis Group at Merck Research Laboratories and had a specific activity of ~11 mCi/mg and a chemical purity of 96%. Reference standards used for the HPLC characterization of extracted residues were unlabeled MAB1, avermectin B1a monosaccharide (MSB1a), *4″-deoxy-4″-epi*-(*N*-formyl)avermectin B1a (FAB1a), *8a-oxo-4″-deoxy-4″-epi*-methylaminoavermectin B1a (8AOXO), *8a-hydroxy-4″-deoxy-4″-epi*-methylaminoavermectin B1a (8AOH), and *8,9-Z-4″-deoxy-4″-epi*-methylaminoavermectin B1a (8,9-ZMA). The structures of the standards are shown in Figure 2.

Emamectin Benzoate Applications. The test soil was sandy loam; its physicochemical properties are shown in Table 1. This soil, in the facilities of Pan-Ag Laboratories in Madera, California, was maintained in six plots (three treated, three control) that were kept outdoors in a fenced-in area without appreciable slope. Ambient temperature and humidity ranges during emamectin benzoate applications were 15−26 °C and 38−76%, respectively. During the months of September and October, the treated plots were sprayed once weekly, for 6 weeks, with a water dilution of [^{14}C]MAB1a in the EC formulation at a rate of 0.0168 kg ai ha^{-1} (total = 0.1008 kg ai ha^{-1}). To ensure confinement of test material within the plot area during spraying, a plastic-wrapped rectangular wooden frame, 5 × 15 cm, projecting to ~5 cm above the soil level was buried into the soil bordering each plot. The total amount of [^{14}C]MAB1a applied to each test plot was 37.6 mg. This amount of material is equivalent to the maximum recommended use rate on an area basis and could result in the maximum expected environmental soil concentration of emamectin benzoate. The spray was evenly applied to the soil in each test plot with a hand-operated, CO_2-pressurized applicator.

Crop Cultivation and Maintenance. After the sixth and final [^{14}C]MAB1a soil application, each plot was divided into three rectangular-shaped areas or subplots (~12 000 cm^2 each). At intervals of 30, 120, and 365 days following the final soil treatment with [^{14}C]MAB1a, barley, carrots, and lettuce were planted in each plot (30-DAT, 120-DAT, and 365-DAT plots, respectively), with one crop for each randomly assigned subplot. Because of barley and carrot germination failure in the 120-DAT plot, these crops were replanted 21 days later in the same plot (referred to now as the 141-DAT plot). Control plots, sprayed with only aqueous EC formulation blank, were similarly divided into subplots and planted with the rotational crops at each of these four DAT intervals. The treated and control plots were separated from each other by a distance of ~0.1 km. The crops were irrigated with on-site well water, using overhead sprinklers, in amounts necessary to ensure normal growth. Manual weeding and fertilization were also conducted on a regular basis.

Soil Sampling and Analysis. Soil cores (~2.2-cm diameter) from the top 30-cm layer were taken from all treated and control plots with a computer-generated random location grid system prior to and immediately after the first spray application, after each additional application, at planting, and at each crop harvesting. A slide hammer with an attached probe containing an acetate liner was used for removing soil cores from the plots. The soil-filled acetate liner was then removed from the probe and capped at both ends after removal of excess liner. A polyethylene pipe was placed in the hole to maintain a stable soil core structure. Each soil core was cut with a separate clean miter saw into 0−15- and 15−30-cm segments. Aliquots (~10−20 g) of each soil segment were removed for moisture determination and radiocombustion analyses (RCA). The remaining segments were immediately packaged in separate polyethylene bags and stored frozen at about −12 °C.

Crop Harvesting. The rotational crops were harvested in the subplots at immature and mature stages. Immature barley forage was clipped just above the soil surface. Mature barley was collected by manually pulling off the grains and cutting the straw just above the soil surface. The grains were threshed and the resulting chaff was combined with the straw samples. Immature and mature carrots were pulled from the ground, after loosening the soil with a shovel, and the tops and roots were separated. Lettuce was cut just above the soil surface at both immature and mature stages. All crop tissues were immediately frozen in dry ice after harvesting and then processed separately into homogenous samples by grinding with the dry ice. The processed samples were placed in polyethylene bags and stored frozen at about −12 °C until analysis.

Soil Moisture Determination. Aliquots of soil (~5 g each) were weighed in tared aluminum weigh boats, and the samples were dried in an oven (115−140 °C) for ~16 h. The dried soil samples were cooled to room temperature, reweighed, and returned to the heated oven for an additional 24 h. After cooling to room temperature, the dried soil samples were weighed again. The weight of the dried soil samples relative to their fresh weight (prior to initial drying) was used to determine soil moisture content.

Radiocombustion Analyses (RCA). The soil and crop samples were combusted on either a Harvey OX-500 Biological Oxidizer (R. J. Harvey Instrument Company) or a Packard model 307 sample oxidizer (Packard Instrument Company). The resulting CO_2 was trapped in Carbosorb and quantified by liquid scintillation counting (LSC) with a Rack-Beta 1209 liquid scintillation counter (LKB-Wallac). Permafluor V (Packard Instrument Company) was used as the scintillation cocktail, and the quench curves, determined by an external standard method, were used for conversion of data from cpm to dpm. The latter were corrected for oxidizer CO_2 recovery by comparing an unoxidized and oxidized [^{14}C]benzoic acid standard. The limits of detection (LOD) and quantitation (LOQ) were estimated according to eqs 1 and 2 (Currie, 1968):

Table 1. Characterization of Test Soil[a]

depth (cm)	OM[b] (%)	pH	CEC[c] (Meq/100 g)	bulk density (g/cm^3)	MHC[d] (%)	sand (%)	silt (%)	clay (%)	textural class
0−15	0.6	6.5	5.6	1.31	11.1	61.3	27.2	11.5	sandy loam
15−30	0.4	7.0	5.5	1.40	10.6	57.3	33.2	9.5	sandy loam

[a] Soil characterization was performed by Agvise Laboratories, Inc., P. O. Box 510, Highway 15, Northwood, ND 58267. [b] Organic matter. [c] Cation-exchange capacity. [d] Moisture holding capacity at 1/3 bar.

$$\text{LOD (dpm)} = \{2.71 + 4.65\,(\mu_B)^{1/2}\}/C_E C_t \quad (1)$$

$$\text{LOQ (dpm)} = 50\,\{1 + (1 + \mu_B/12.5)^{1/2}\}/C_E C_t \quad (2)$$

where μ_B is the total background count (cpm × counting time, C_t in min) and C_E is the counting efficiency. Values of μg/kg for fresh soil were converted to μg/kg dry weight by correcting for moisture content. No correction for moisture content was applied to the μg/kg values obtained for the crop samples. For RCA analysis of crop samples fortified with [^{3}H]MAB1a, the Packard model 307 sample oxidizer was also used. The resulting ^3H_2O was trapped in Monophase S and quantified by LSC on a Packard model 2500 liquid scintillation counter (Packard Instrument Company).

Methanol/Water Extraction of Crop Samples. Crop samples with total radioactive residues at or exceeding 5 μg/kg were fortified with [^{3}H]MAB1a as internal standard. Then, following initial blending with MeOH, the samples were extracted with MeOH and MeOH/H_2O mixtures, each containing ammonium acetate (Figure 3, from Crouch and Feely, 1995). Natural products were precipitated from the extractable residue by chilling for ∼16−48 h at −12 °C, and the precipitate was washed with methanol to yield the methanol/water-extractable residue and the methanol/water-unextractable residue. Aliquots of MeOH/H_2O-extractable residue from barley straw were analyzed for the presence of glucose (Trinder, 1969). The barley straw MeOH/H_2O-extractable residue was further partitioned with CH_2Cl_2 to separate emamectin benzoate residues; the latter (in CH_2Cl_2) were subsequently analyzed by HPLC. The MeOH/H_2O-unextractable residues remaining after the multiple MeOH/H_2O extractions were dried in a reduced pressure oven at 50 °C for ∼60 h. Subsamples were removed and their radioactivity contents analyzed by RCA.

Enzymatic Treatments of MeOH/H_2O Marcs. Aliquots of MeOH/H_2O marcs from barley straw were consecutively treated with commercial cellulase/hemicellulase preparations (Sigma Chemical Company) from four different biological sources that were suspended in sodium acetate buffer (50 mM, pH 5). The enzyme preparations used, sources, specific activities, and order of hydrolytic digestions are as follows: (i) cellulase, *T. viride*, 10 units/mL, and hemicellulase, *A. niger*, 0.1 unit/mL; (ii) cellulase, *P. funiculosum*, 10 units/mL, and hemicellulase, *A. niger*, 0.1 unit/mL; and (iii) cellulase, *T. reesei*, 10 units/mL, and hemicellulase, *A. niger*, 0.1 unit/mL.

Each digestion mixture was centrifuged at 2250 rpm for 20 min, and the pellet was dried in a reduced pressure oven at 50 °C for 48 h; total radioactivity in each pellet was determined by RCA. The resulting supernatant fractions from the cellulase/hemicellulase digestions were pooled together, and aliquots were analyzed by LSC for the determination of total cellulase/hemicellulase-extractable radioactivity. For some samples, total extractable radioactivity from the cellulase/hemicellulase digestions were analyzed by RCA because of color quenching during direct LSC. The pooled cellulase/hemicellulase hydrolysates were also analyzed by HPLC; the presence of released glucose was also determined (Trinder, 1969).

HPLC Characterization of Extractable Residues. Reversed-phase HPLC (RP-HPLC) analyses of the MeOH/H_2O-extractable residues, the cellulase/hemicellulase hydrolysates of the marcs, and avermectin reference standards were conducted with a Spectra Physics 8800 ternary pump, a Hewlett-Packard diode array detector and 1040 data station, and an Axxiom ODS column (5 μm, 4.5 × 250 mm) connected to a Pharmacia Frac-100 fraction collector. A flow rate of 1 mL/min was used, with a mobile phase of MeOH and H_2O, each containing 5 mM NH_4OAc at the following gradient compositions: 0−35 min, 83% MeOH, isocratic; 35−50 min, 83−100% MeOH, linear; and 50−60 min, 100% MeOH, isocratic. For the reference standards, the column eluates were monitored at 245 nm. For MeOH/H_2O-extractable residues and cellulase/hemicellulase hydrolysates, column eluates were monitored at 245 nm, were collected in 1-mL fractions, and were mixed with ∼4 mL of Instagel XF scintillation cocktail; the dpm were determined by LSC.

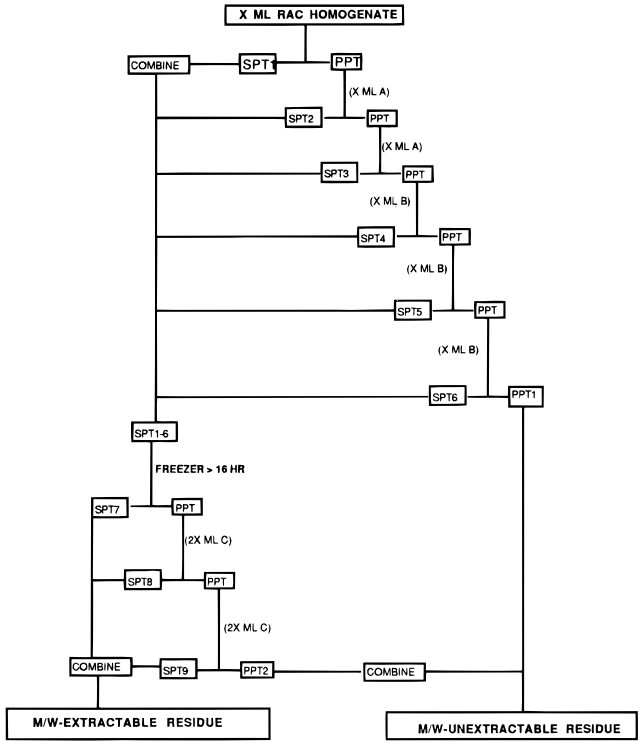

Figure 3. Methanol/water extraction procedure for rotational crop samples (from Crouch and Feely, 1995). MeOH crop homogenates (X mL = volume of homogenate) were extracted as above with 1× or 2× volumes of: A (1 mM NH_4OAc in MeOH), B (5 mM NH_4OAc in 1/1, v/v MeOH/water), and C (MeOH). The supernatants (SPT) and precipitates (PPT) were separated by centrifugation or filtration.

Spectrophotometric Analysis of Glucose. Trinder Reagent (Sigma Chemical Company) was reacted with ∼20−100 μL of MeOH/H_2O supernatant extract, or 10−50 μL of cellulase/hemicellulase hydrolysate, and the absorbance at 505 nm was measured (Trinder, 1969). Appropriate controls were also analyzed.

RESULTS AND DISCUSSION

Residues of Emamectin Benzoate in Soil. Total [^{14}C]MAB1a residues in soil at application, immediately prior to planting of crops, and at each crop harvest are presented in Table 2. All quantifiable radioactive residues were confined to the top 0−15-cm soil layer and, with one exception (120-DAT plot at planting, 2−4 μg/kg), no detectable radioactive residue (i.e., ≥2 μg/kg) was found in the lower 15−30-cm soil layer. This result demonstrates that even at maximum recommended

Table 2. Total Residues of [^{14}C]MAB1a in Soil

soil sample description	soil plot (DAT)[a]	days after last pesticide treatment	total residues, top 0−15 cm (μg/kg)	total residues, top 15−30 cm (μg/kg)
pre-application	Composited	−36	<LOD[b]	<LOD
application #1	Composited	−35	7	<LOD
application #2	Composited	−28	8	<LOD
application #3	Composited	−21	10	<LOD
application #4	Composited	−14	12	<LOD
application #5	Composited	−7	21	<LOD
application #6	Composited	0	17	<LOD
at planting	30 DAT	30	17	<LOD
immature lettuce	30 DAT	180	<LOQ[c]	<LOD
mature lettuce	30 DAT	202	<LOQ	<LOD
immature carrot	30 DAT	231	8	<LOD
mature carrot	30 DAT	278	<LOQ	<LOD
barley forage	30 DAT	155	10	<LOD
barley straw	30 DAT	231	11	<LOD
at planting	120 DAT	120	13	<LOQ
immature lettuce	120 DAT	219	<LOQ	<LOD
mature lettuce	120 DAT	233	<LOQ	<LOD
at planting	141 DAT[d]	141	9	<LOD
immature carrot	141 DAT	252	<LOQ	<LOD
mature carrot	141 DAT	303	6	<LOD
barley forage	141 DAT	191	6	<LOD
barley straw	141 DAT	252	<LOQ	<LOD
at planting	365 DAT	365	5	<LOD
immature lettuce	365 DAT	573	5	<LOD
mature lettuce	365 DAT	614	<LOQ	<LOD
immature carrot	365 DAT	614	4	<LOD
mature carrot	365 DAT	650	<LOQ	<LOD
barley forage	365 DAT	502	<LOQ	<LOD
barley straw	365 DAT	573	<LOQ	<LOD

[a] Days after last treatment; this is the aging period of pesticide on soil before crop planting. [b] LOD, the limit of detection, corresponds to 2 μg/kg. [c] LOQ, the limit of quantification, corresponds to 4 μg/kg. [d] Corresponds to 120-DAT; barley and carrots did not germinate and therefore were replanted at 141 days after the last pesticide treatment.

treatment rates, emamectin benzoate and/or its degradates are not likely to leach below the top 0−15-cm soil layer into groundwater. This finding is also consistent with the soil sorption (Mushtaq et al., 1996) and aged leaching (Merck Research Laboratories, unpublished data) properties of emamectin benzoate. Also, unpublished results of field dissipation studies demonstrate that detectable residues of emamectin benzoate do not leach below the top 15-cm soil layer following six weekly applications at the maximum labeled rate.

As expected, the highest levels (∼17−21 μg/kg) of radioactive residues in soil were found following the fifth and sixth [^{14}C]MAB1a applications, and in the 30-DAT plot at planting. Subsequently, the residues declined progressively to below the LOQ (<0.4 μg/kg, Table 2).

Residues of Emamectin Benzoate in Crops. The concentration of total [^{14}C]MAB1a residues in the rotational crops ranged from below the LOD (<2 μg/kg) to 30 μg/kg in the 141-DAT barley straw (Table 3). In all treated plots, both mature and immature lettuce contained very low levels of radioactive residues, ranging from <LOD to <LOQ. With the exception of the 141-DAT immature and mature tops (9 μg/kg, each), total radioactivity in carrots was also very low, ranging from <LOD to <LOQ. The highest levels of radioactive residues were found in the 30- and 141-DAT barley straw, at levels of 16 and 30 μg/kg, respectively. Residues in other barley matrices were by comparison lower, ranging from <LOD (365-DAT barley matrices) to 9 μg/kg (30- and 141-DAT grains). It thus appears that uptake of emamectin benzoate residues by carrots and barley increased from the 30- to the 141-DAT plot and then declined to nonquantifiable levels in the 365-DAT plot.

The distributions of extractable and unextractable residues in selected rotational crops are shown in Table 4. Release of emamectin benzoate present in crop matrices (if any) was likely quantitative, as evidenced by the 95% recovery of the [^{3}H]MAB1a added prior to extraction. In the 30-DAT barley grains, extractable and unextractable residues were ∼9 and ∼91% respectively, of total radioactivity. In the 141-DAT barley grains, the proportion of extractable and unextractable residues were similar, that is, ∼13 and ∼87%, respectively, of total radioactivity. Thus, a greater proportion of incurred emamectin benzoate residues in grain was unextractable. Neither extractable nor unextractable residues in barley grains attained a concentration equivalent to 10 μg/kg or more. Due to the low radioactivity found in barley grains, no further characterization was performed on these samples.

Total radioactivity values in the 30- and 141-DAT barley straw were 16 and 30 μg/kg, respectively (Tables 3 and 4). Unlike grains, the proportion of MeOH/H_2O-extractable and -unextractable residue levels was similar in the two barley straw samples. In the 30-DAT straw, extractable and unextractable residues were ∼47 and ∼53%, respectively, of total radioactivity. In the 141-DAT straw, the proportions of extractable and unextractable residues were ∼61 and ∼39%, respectively, of total radioactivity. Considering the low levels of total residues involved (16−30 μg/kg), these results indicate that the proportion of MeOH/H_2O-extractable and -unextractable residues are likely similar in both barley straw specimens.

To determine the nature of the MeOH/H_2O-extractable residues, they were first partitioned with CH_2Cl_2. Between 62−68% of the extractable residue of barley

Table 3. Total Residues of [^{14}C]MAB1a in Rotational Crops

	lettuce		carrots				barley		
age[a] (days)	IMM[b]	MAT[c]	IMM tops	IMM roots	MAT tops	MAT roots	forage	grain	straw
				30 DAT[d]					
125							<LOQ[e]		
201								9	16
201			<LOQ	<LOQ					
248					<LOQ	<LOQ			
150	<LOQ								
172		<LOQ							
				120 DAT					
50									
111									
111									
162									
99	<LOQ								
113		<LOQ	113						
				141 DAT					
50							5		
111								9	30
111			9	<LOD[f]					
162					9	<LOD			
99									
113			113						
				365 DAT					
137							<LOD		
208								<LOD	<LOD
249			<LOD	<LOD					
285					<LOQ	<LOQ			
208	<LOD								
249		<LOD							

[a] Age of rotational crop at harvest; crops in the 365-DAT plot experienced delayed growth due to an unusually harsh frost at time of planting, in late 1992. [b] Immature. [c] Mature. [d] Days after treatment, or interval between the last [^{14}C]MAB1a application and crop planting. [e] Limit of quantitation, equivalent to 4 μg/kg. [f] Limit of detection, equivalent to 2 μg/kg.

Table 4. Extractable and Unextractable Residues of [^{14}C]MAB1a in Selected Rotational Crops[a]

		carrot tops		barley		
DAT[b]	residue	immature	mature	forage	grain	straw
30	total (μg/kg)	ND[c]	ND	ND	9	16
	extractable (%)				9	47
	extractable (μg/kg)				1	7.5
	unextractable (%)				91	53
	unextractable (μg/kg)				8	8.5
141	total (μg/kg)	9	9	5	9	30
	extractable (%)	53	50	56	13	61
	extractable (μg/kg)	5	5	3	1	18
	unextractable (%)	47	50	44	87	39
	unextractable (μg/kg)	4	4	2	8	12

[a] See Figure 2 for outline of extraction procedure; ~95% of fortified [^{3}H]MAB1a was extractable with the MeOH/H_2O mixtures. [b] Days after treatment. [c] Not determined; only rotational crop matrices with total residues greater than LOQ, or 4 μg/kg (see Table 3) were extracted.

Table 5. Glucose Content and Distribution of Extractable Residues in Barley Straw between Methanol/Water and Methylene Chloride[a]

sample	MeOH/H_2O extr residues (μg/kg)[b]	glucose (μg/g)[c]	CH_2Cl_2 extractable (%)	CH_2Cl_2 unextractable (%)
30-DAT straw	7.5	718	68	32
141-DAT straw	18	933	62	38

[a] More than 98% of the fortified [^{3}H]MAB1a partitioned into the CH_2Cl_2 phase; therefore, emamectin benzoate is, presumably, not present in the CH_2Cl_2-unextractable radioactivity. [b] From Table 4. [c] Micrograms of glucose extracted per gram of barley straw.

straw and 98% of the added [^{3}H]MAB1a were recovered in the CH_2Cl_2 fraction (Table 5). Thus, the CH_2Cl_2 fraction likely would contain any emamectin residues similar to the parent itself. Additionally, analysis of the

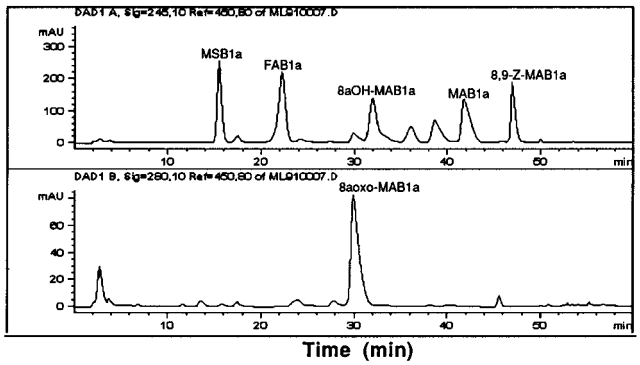

Figure 4. RP-HPLC analyses of nonradioactive avermectin reference standards. The top and bottom UV profiles were at 245 and 280 nm, respectively. Residues eluting before MSB1a (i.e., ~<15 min) are referred to as polar residues or the polar fraction. The structures of these standards are shown in Figure 2.

MeOH/H_2O-extractable residues (Trinder, 1969) also indicated the presence of glucose (718−933 μg/g). This result indicates that radiocarbon incorporated into endogenous free glucose will be extractable with MeOH/H_2O.

The MeOH/H_2O-extractable residues from the barley straw samples were characterized by comparison of their RP-HPLC retention properties (Figure 5) with those of the standards of selected emamectin degradates (Figure 4). Analyses of the CH_2Cl_2 fraction by RP-HPLC, however, showed that essentially all of the residue was a polar fraction eluting close to the column void volume and present at a concentration of <2 μg/kg of straw (Figure 5). The retention time of this polar fraction coincides with that of [^{14}C]glucose extracted from cabbage and lettuce under conditions similar to those employed in this study (L. Crouch, personal

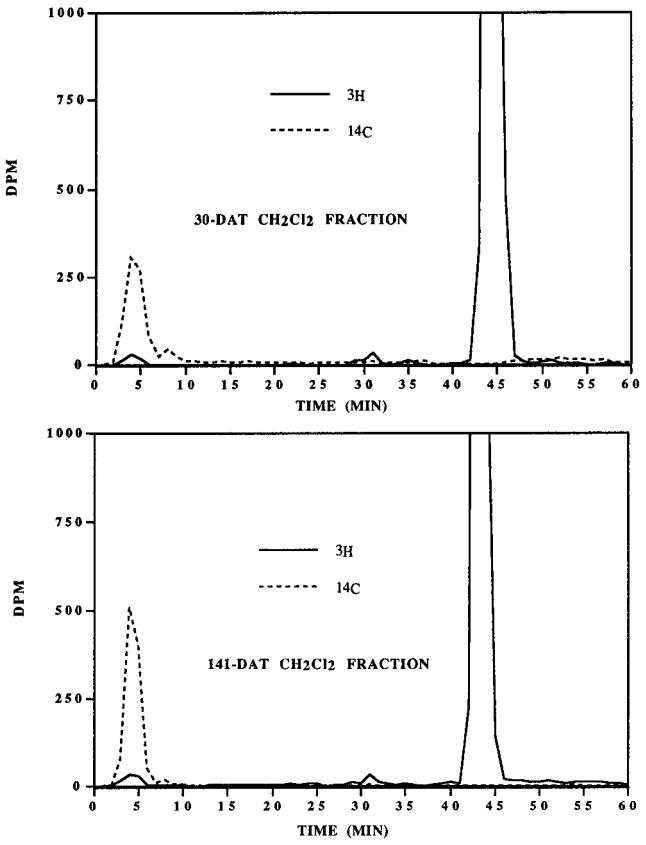

Figure 5. RP-HPLC analyses of CH_2Cl_2 fractions from barley straw. MeOH/H_2O-extractable residues from 30- and 141-DAT straw, fortified with [^{3}H]MAB1a prior to extraction, were partitioned against CH_2Cl_2 and the CH_2Cl_2 fraction assayed by RP-HPLC. Refer to Figure 4 for retention times of avermectin standards. No emamectin-related residue was found (i.e., ≥2 μg/kg).

communication) and further suggests that radioacarbon incorporation into glucose occurred in barley straw. No other emamectin-related residues were detected (i.e., ≥2 μg/kg). Between 32−38% of the MeOH/H_2O supernatant did not partition into the CH_2Cl_2 phase (Table 5). Because this CH_2Cl_2-insoluble radioactive fraction would not contain emamectin-like residues and because glucose and lignins are also insoluble in CH_2Cl_2, the CH_2Cl_2-insoluble radioactive fraction most likely represents residues incorporated into [^{14}C]glucose, lignins, or other natural products with similar solubility characteristics. Alternatively, the CH_2Cl_2-insoluble fraction could also be derived from emamectin-related conjugates of glucose, lignins, or proteins, although no evidence of such conjugates have been found in [^{14}C]MK-244-treated lettuce (Crouch and Feely, 1995), cabbage (L. Crouch, personal communication), or corn (C. Wrzesinski, personal communication).

Serial incubations of the MeOH/H_2O barley straw marc with three cellulase/hemicellulase preparations resulted in release of a total of ∼12% of the total marc radioactivity (Table 6). Analysis of the pooled enzymic hydrolysates (Trinder, 1969) also indicated the presence of glucose (∼72−81 μg/g). This result provides additional evidence that some radioactivity in the straw was incorporated into the glucosyl subunits of cellulose. Incorporation of the ^{14}C fragments arising from emamectin degradation into glucose (or sucrose, lignins, and other natural products) indicates that extensive degradation occurred either in soil or on rotational crops or in both matrices. Extensive light-mediated degradation

Table 6. Release of Unextractable Residues from Barley Straw Marc Using Cellulase/Hemicellulase Treatments[a]

		hydrolysate			remainder	
sample	MeOH/H_2O marc (μg/kg)[b]	(%)[c]	(μg/kg)[c]	glucose (μg/g)[d]	(%)[e]	(μg/kg)
30-DAT straw	8.5	12	2	72	41	6
141-DAT straw	12	12	2	81	28	8

[a] The barley straw MeOH/H_2O marcs were incubated serially with three different cellulase/hemicellulase preparations (see Materials and Methods); the percentages are based on total radioactive residues in barley straw (see Table 4). [b] From Table 4. [c] Refers to [^{14}C]MAB1a equivalents in the cellulase/hemicellulase hydrolysate. [d] Micrograms of glucose released per gram of dried marc. [e] The percentages are based on total radioactive residues in barley straw (from Table 4), which were 16 μg/kg (30-DAT) and 30 μg/kg (141-DAT).

of emamectin benzoate has been previously observed on soil (Chukwudebe, unpublished data) and on lettuce (Crouch and Feely, 1995). Due to the low radioactivity in the cellulase/hemicellulase hydrolysates, no significant residue was detectable by RP-HPLC analyses (not shown).

It must be noted, however, that the barley straw MeOH/H_2O marc was not treated with proteases, lipases, or other hydrolytic enzymes, or with strong acids or bases before the cellulase/hemicellulase treatments. It has been shown, in cob from [^{14}C]MK-244-treated corn, that pretreatment with hydrolytic enzymes and base prior to cellulase digestion greatly facilitated the subsequent cellulase digestion (C. Wrzesinski, unpublished data). Therefore, it is apparent that most of the cellulose in the MeOH/H_2O marc was not digested by the cellulase/hemicellulase mixture. Thus, it is likely that >12% of the radioactivity in barley straw MeOH/H_2O marc are incorporated into cellulose. Due to the low radioactivity in the remaining cellulase/hemicellulase marcs (6−8 μg/kg), further enzymic hydrolytic digestions were not attempted.

In a previous study with emamectin benzoate (Crouch and Feely, 1995) and in studies with the structurally similar abamectin (Bull et al., 1984; Feely and Wislocki, 1991; Maynard et al., 1989; Moye et al., 1990), incorporation of radioactivity into plant natural products, including cellulose, was also indicated. The low levels of this nonhydrolyzable radioactivity (6−8 μg/kg) coupled with nondetectable levels (<LOD) of extractable [^{14}C]-MAB1a in barley straw indicate that significant accumulation of emamectin benzoate residues from soil into representative rotational crops (lettuce, carrots, barley) will not occur.

CONCLUSIONS

After applications to bare soil at maximum recommended treatment rates, emamectin benzoate residues were not found below the top 15-cm soil depth and are, therefore, not likely to leach into groundwater. Total radioactive residues in the rotational crop species were, in general, minimal, and mature lettuce, carrots, and barley grain contained no significant radioactive residues (i.e., ≥10 μg/kg). However, higher radioactive residue levels were detected in barley straw grown in 30- and 141-day aged soil (16 and 30 μg/kg, respectively). No detectable (≥2 μg/kg) emamectin or degradates of similar structure were found in the extractable residue of these straw samples; ∼12%, or more, of the straw radioactive residue could be released with cellulase/hemicellulase treatment, apparently due to incorporation into the glucosyl subunits of cellulose.

The results of this study demonstrate that very little root uptake and translocation of emamectin benzoate residues occurred in rotational crops grown in soils containing aged [^{14}C]MAB1a applied at maximum recommended use rates. Portions of lettuce, carrots, and barley used for human consumption contained no detectable levels of extractable emamectin benzoate residues. Similarly, portions of barley straw that could be consumed by farm animals contained no detectable levels of extractable emamectin benzoate residues. Nearly half of the radioactivity in straw had presumably become incorporated into natural products, including glucose. Therefore, uptake of emamectin benzoate residues from treated soil into rotational crops is not anticipated.

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Received for review July 10, 1996. Revised manuscript received September 24, 1996. Accepted September 30, 1996.[⊗]

JF960517N

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.