Immobility of Emamectin Benzoate in Soils

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The soil mobility of 4″-deoxy-4″-(*epi*-methylamino)avermectin B_{1a} (MAB_{1a}) benzoate, the major (>90%) component of emamectin benzoate (MK-0244), was determined by a batch equilibrium technique and also by thin layer chromatography (TLC). Four concentrations of [^{3}H]MAB_{1a} benzoate (10−650 ng/5 mL) in calcium chloride solution were equilibrated with 1 g of sandy loam, sand, clay loam, and silt loam soil types. The test compound was very tightly bound (≥99%) to each of the four soils and did not significantly desorb (<1.3%). The values of the Freundlich distribution constant extrapolated to 100% carbon content (sorption K_{oc}) were 2.5×10^4 to 7.3×10^5. The high K_{oc} values indicate that MAB_{1a} benzoate is immobile in soils. The mobility of [^{14}C]MAB_{1a} benzoate was also assessed in six different soils by using the soils as adsorbent phases in TLC. Mobile and immobile pesticide controls were also assayed in these systems for comparison. [^{14}C]MAB_{1a} benzoate was classified as immobile in all soils. The results of batch equilibrium and of soil TLC experiments demonstrate that MAB_{1a} benzoate will bind tightly to soil and not move readily in the environment.

Keywords: *Emamectin; MK-0244; soil; sorption; desorption; TLC*

INTRODUCTION

Avermectins are naturally occurring disaccharide derivatives of a pentacyclic, 16-membered lactone ring produced by the soil microorganism *Streptomyces avermitilis* (Burg et al., 1979). The natural avermectin pesticide, abamectin (avermectin B_{1a}), is a potent miticide (Campbell et al., 1984), but it is not active against some insect families (Putter et al., 1981). A semisynthetic avermectin, emamectin benzoate (Figure 1), was subsequently developed which has high activity against a broad range of lepidopterous larvae and reduces feeding damage on vegetables and sweet corn (Dybas et al., 1989). Thus, the compound has great potential for application to control lepidopterous larvae in vegetables, cotton, and other crops.

Emamectin benzoate (MK-0244) is chemically synthesized from abamectin by modification of the terminal disaccharide by substituting an *epi*-aminomethyl (−$NHCH_3$) group for a hydroxyl (−OH) group at the 4″-position and 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 homologs differ only by a methylene unit on the C-25 side chain (Figure 1).

As soil will be exposed to emamectin benzoate through agricultural use, possible contamination of surface and ground water may occur. The present study was therefore conducted to assess the potential for leaching of MAB_{1a} benzoate from soil. The mobility of [^{3}H]MAB_{1a} benzoate was determined by measuring its sorption onto and subsequent desorption from four soil types. The potential for [^{14}C]MAB_{1a} benzoate to leach through the soils was also determined by soil thin layer chromatography (TLC) comparisons with selected pesticides of known mobility.

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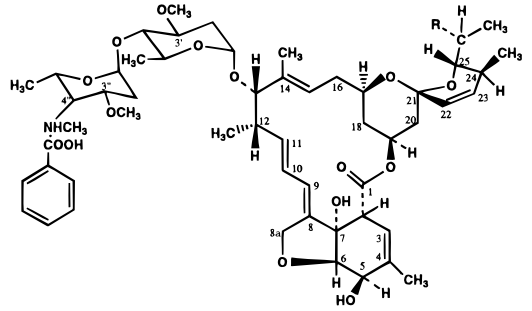

Figure 1. Structure of emamectin benzoate (MK-0244): MAB_{1a} component (>90%), R = CH_2CH_3; MAB_{1b} component (<10%), R = CH_3. [5-^{3}H]MAB_{1a} benzoate was used for sorption/desorption equilibrium study. [3, 7, 11, 13, or 23-^{14}C]MAB_{1a} benzoate was applied to soil TLC plates.

MATERIALS AND METHODS

Chemicals. The test chemicals [5-^{3}H]MAB_{1a} benzoate (11.5 mCi/mg) and [3, 7, 11, 13, or 23-^{14}C]MAB_{1a} benzoate (29 μCi/mg) were prepared by the Labeled Compound Synthesis Group, Department of Drug Metabolism II, Merck Research Laboratories, Rahway, NJ. The radiolabeled pesticide standards were purchased from Sigma Chemical Co., St. Louis, MO. The test chemicals and control pesticide standards were analyzed for radiopurity by HPLC before use. Four stock solutions in 0.01 M calcium chloride (11, 51, 158, and 646 ng/5 mL) of [5-^{3}H]MAB_{1a} benzoate (>98% radiopurity) were prepared for the batch equilibrium study. The specific activities of the [5-^{3}H]MAB_{1a} benzoate in these solutions were 11.04, 2.58, 0.78, and 0.21 μCi/mg, respectively. Four pesticide standards, [*ring*-UL-^{14}C] atrazine (84.3 μCi/mg, 96% radiopurity), [*ring*-UL-^{14}C] parathion (42.9 μCi/mg, 95% radiopurity), [*carboxy*-^{14}C] 2,4-dichlorophenoxyacetic acid (2,4-D, 43.1 μCi/mg, 99% radiopurity), and [^{14}C]trifluralin (57.4 μCi/mg, 98% radiopurity), and [^{14}C]MAB_{1a} benzoate (29.0 μCi/mg, 98% radiopurity) were used for the soil TLC study.

Soils. Six different soils were selected on the basis of texture and organic matter content: sandy loam from College Station, TX; sand from Lakeland, FL; clay/clay loam from Waco, TX; silt loam from Three Bridges, NJ; loam/sandy loam from Riverside, CA, and sand from Sanford, FL. The properties of the soils are presented in Table 1. Prior to use, the soils were air-dried and were passed either through a 35 mesh screen (0.5 mm) for the preparation of soil TLC plates or

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Table 1. Properties of Test Soils[a]

soil properties	College Station, TX[b]	Lakeland, FL[b]	Waco, TX[b]	Three Bridges, NJ[b]	Riverside, CA	Sanford, FL
organic carbon (% w/w)	0.73	0.03	2.62	1.04	1.07	0.47
organic matter[c] (% w/w)	1.26	0.05	4.52	1.79	1.84	0.81
pH in $CaCl_2$ (standard units)	6.6	6.5	6.2	6.4	6.3	4.9
cation exchange capacity (mequiv of M^+/100 g)	8	0.4	31.7	10.9	10.8	1.1
water holding capacity						
field capacity, 0.33 bar (% w/w)	14.2	1.2	31.5	31.3	13.8	2.1
dry basis (% w/w)	5.3	1.4	21.7	8.4	5.8	2.9
disturbed bulk density (g/cm^3)						
field capacity	1.21	1.75	1.64	1.54	1.45	1.83
dry basis	1.06	1.73	1.25	1.17	1.28	1.79
sand	65	100	31	19	52	98
silt	24	0	29	64	35	0
clay	11	0	40	16	13	2
textural class (USDA)	sandy loam	sand	clay/clay loam	silt loam	loam/sandy loam	sand

[a] Soils analyses were conducted by PTRL East, Inc., Richmond, KY. [b] Four soils were used to determine sorption/desorption of [^{3}H]MAB_{1a} benzoate. TLC plates were prepared from all of the above-described test soils. [c] The organic carbon was multiplied by a factor of 1.724 to estimate the organic matter (Hamaker, 1975).

through a 10 mesh screen (2 mm) for the batch equilibrium study. The screened soils were stored in a desiccator before use.

Equipment. Radioactivity in liquid samples was determined by liquid scintillation counting (LSC) on a Model 4530 or 460 counter from the Packard Instrument Company, Inc., Downers Grove, IL. The ^{3}H or ^{14}C radioactivity in soil samples was converted into ^3H_2O or $^{14}CO_2$, respectively, by combustion on a Packard sample oxidizer (Model 306 or 307). The resultant ^3H_2O or $^{14}CO_2$ was trapped and the radioactivity determined by LSC. A Perkin-Elmer (UV−visible) spectrophotometer (Model 320) was used to determine the concentration of test compound solutions. For all RP-HPLC analyses, an Axxiom Axxi-Chrom ODS column (4.6 mm × 25 cm, 5 μm particle size, Analytical Sales and Services, Inc. Mahwah, NJ), a Spectra Physics (SP) 8700 or 8800 solvent delivery system, a SP UV2000 or a SP 8480 UV−visible detector, a Rheodyne 7125 injector, a SP 4400 Chromjet integrator or a SP 4200 integrator, and a Pharmacia Frac-100 collector were used. The eluant was monitored at 245 nm. One minute fractions of eluate were collected, and the radioactivity was determined by LSC.

RP-HPLC Analyses. The purity and stability of emamectin benzoate solutions were determined using an eluant of 90% (v/v) methanol in water containing 5 mM ammonium acetate at a flow rate of 1 mL/min. The pesticide standards, atrazine, 2,4-D, parathion, and trifluralin, were analyzed using eluants of 50%, 60%, 70%, and 70% (v/v) acetonitrile in water, respectively, each containing 0.1% phosphoric acid at a flow rate of 1 (atrazine and parathion) or 2 mL/min (2,4-D and trifluralin). No degradation of any test or control standard occurred during the experimental period.

Batch Equilibrium. The soils used were sandy loam from College Station, TX, sand from Lakeland, FL, clay/clay loam from Waco, TX, and silt loam from Three Bridges, NJ. The procedure for soil sorption and subsequent desorption was similar to the method used by Murray et al. (1975). A probe study indicated that MAB_{1a} benzoate sorption/desorption equilibrium was reached within 2 h and that no binding of [^{3}H]MAB_{1a} benzoate to the surfaces of glassware occurred in the presence of soil. For the present study, periods of 5 h for the sorption phase and 14 h for the desorption phase were used for all soil types. Four concentrations of [^{3}H]MAB_{1a} benzoate (2−130 ng/mL) for sorption/desorption equilibrium were chosen on the basis of emamectin benzoate water solubility (24 ppm at pH 7) and the expected application rate of 0.075−0.15 lb of active ingredient/acre.

Test samples (triplicate) contained 5 mL of each concentration of [^{3}H]MAB_{1a} benzoate in 0.01 M calcium chloride solution and 1 g of soil in 40 mL conical test tubes. Control samples contained 1 g of each soil type and 5 mL of 0.01 M calcium chloride solution or 5 mL of [^{3}H]MAB_{1a} benzoate in 0.01 M calcium chloride solution and no soils (duplicate). All tubes were covered with aluminum foil, capped, and shaken by a Burrell wrist-action shaker for sorption equilibration at 23−25 °C. Following equilibration and subsequent centrifugation, the supernatant from each sample tube was withdrawn and replaced by 5 mL of freshly prepared 0.01 M calcium chloride solution for the desorption phase. The above-described procedure was repeated, and the supernatants were withdrawn. The radioactivity in each control and test sample supernatant was determined by LSC.

Isotherm Determination. The binding constants for MAB_{1a} benzoate to soil were determined by using the Freundlich equation (Bailey and White, 1970)

$$x/m = KC^{1/n} \quad (1)$$

where x/m equals the micrograms of [^{3}H]MAB_{1a} sorbed per gram of soil, C equals the [^{3}H]MAB_{1a} concentration in the supernatant (micrograms per milliliter), and K and n are constants. The equation can be expressed as

$$\log[\text{sorbed } (\mu g/g)] = \log[K] + (1/n) \log[\text{solution } (\mu g/mL)] \quad (2)$$

The log[sorbed (μg/g)] was plotted versus log[solution (μg/mL)] to a best fit line using a Cricket Graph III software program. The Freundlich constants, K and n, were calculated from the intercept (at 1 μg/mL) and from the slope of the best fit line. The distribution constant extrapolated to 100% organic carbon content (K_{oc}) was determined from the equation (Hamaker, 1975)

$$K_{oc} = (100K)/\%\ \text{organic carbon content} \quad (3)$$

The distribution constant (K_d) at a given MAB_{1a} concentration was determined by dividing the MAB_{1a} concentration in the soil (micrograms per gram) by the MAB_{1a} benzoate concentration (micrograms per milliliter), in the solution (Swann et al., 1983).

Mass Balance. After the desorption phase, the soils were extracted with 7.5 mL of ethyl acetate saturated with ammonium hydroxide, centrifuged, and then reextracted with 5 mL of methanol containing 100 mM ammonium acetate. The radioactivity of the combined organic extracts was determined. The radioactivity remaining in the organic extracted soils was determined by oxidative combustion.

Soil TLC. The soil TLC technique used was similar to that of Helling (1971). Soil TLC plates were prepared by spreading a slurry of soil and water onto clean 20 × 20 cm glass plates to an even thickness. One plate was made from each of the six soils listed in Table 1. For each plate, the slurry contained approximately 60 g of soil and between 15 and 35 mL of water, depending on the soil type. It was necessary to add calcium

Table 2. R_f Values for Soil TLC

test and control substances	sandy loam[a] (College Station, TX)	sand (Lakeland, FL)	clay/clay loam[a] (Waco, TX)	silt loam[a] (Three Bridges, NJ)	loam/sandy loam (Riverside, CA)	sand (Sanford, FL)	mean
MAB_{1a}	0	0	0	0	0	0	0
trifluralin	0	0	0.09	0	0	0.21	0.05
parathion	0.12	0.48	0.11	0	0	0.29	0.17
atrazine	0.50	0.95	0.31	0.44	0.44	0.96	0.60
2,4-D	0.99	0.98	0.60	0.84	0.77	1.00	0.86

[a] Mean R_f values from three soils for trifluralin, atrazine, and 2,4-D were calculated for comparison to the reference studies and the values are 0.03, 0.42, and 0.81, respectively.

Table 3. Percent Sorption of [^{3}H]MAB_{1a} Benzoate onto Soils and Percent Desorption of Sorbed [^{3}H]MAB_{1a} from Soils (Mean ± SD Calculated from Triplicate Samples)

MAB_{1a} benzoate per g of soil (ppb)	sandy loam (College Station, TX)	sand (Lakeland, FL)	clay loam (Waco, TX)	silt loam (Three Bridges, NJ)
sorption[a]				
11	99.88 ± 0.02	99.45 ± 0.11	99.78 ± 0.04	99.28 ± 0.36
51	99.89 ± 0.02	99.18 ± 0.15	99.66 ± 0.05	99.43 ± 0.13
158	99.88 ± 0.04	99.19 ± 0.11	99.72 ± 0.05	99.40 ± 0.13
646	99.85 ± 0.05	99.04 ± 0.06	99.66 ± 0.03	99.00 ± 0.16
desorption[b]				
11	0.10 ± 0.02	0.54 ± 0.16	0.15 ± 0.04	0.33 ± 0.03
51	0.09 ± 0.01	0.61 ± 0.13	0.13 ± 0.03	0.67 ± 0.39
158	0.12 ± 0.02	0.84 ± 0.13	0.15 ± 0.04	0.33 ± 0.08
646	0.10 ± 0.03	1.28 ± 0.20	0.16 ± 0.03	0.75 ± 0.39

[a] Five milliliters of [^{3}H]MAB_{1a} benzoate in 0.01 M calcium chloride solution per gram of soil was used for sorption. [b] Percent of sorbed [^{3}H]MAB_{1a} benzoate which was desorbed.

sulfate (2 g) to the slurry of Lakeland sand soil as a binder. The plates were air-dried at room temperature. A total of about 40 000 dpm each of ^{14}C-labeled MAB_{1a} benzoate, atrazine, 2,4-D, parathion, and trifluralin was applied side by side to each plate approximately 1.5 cm from the bottom. Each compound was applied in several 20 μL aliquots to a single spot, and drying between aliquots minimized spreading during application. The plates were placed in glass tanks containing a 0.5 cm depth of HPLC grade water. After development, the plates were air-dried for 3−7 days. Autoradiography was then performed at −60 °C, for 3−7 days, with Kodak SB-5 medical X-ray film. R_f values were calculated by measuring the distance from the origin to the leading edge of a spot or streak.

After film development, the radioactive soils were scraped from each area containing emamectin and serially extracted up to 11 times using the following solvents in order: ethyl acetate saturated with ammonium hydroxide, methanol containing 100 or 500 mM ammonium acetate, 60/40 (v/v) methanol/water containing 500 mM ammonium acetate, and 50/50 (v/v) methanol/water saturated with ammonium hydroxide. The extracted soils and soil extracts were analyzed for total radioactivity.

RESULTS AND DISCUSSION

Soil TLC. The R_f values for [^{14}C]MAB_{1a} versus other pesticides in the six different soils are presented in Table 2. The results indicate that [^{14}C]MAB_{1a} did not move from the origin in any soil; even the low organic matter content sand soils tightly bound MAB_{1a}. Therefore, from the initial work by Helling and Turner (1968), MAB_{1a} is classified as immobile (class 1, R_f 0.0−0.09). Furthermore, it was difficult to remove all of the radioactivity from the soils by serial organic extractions. The recoveries of [^{14}C]MAB_{1a} after serial extractions and subsequent oxidative combustion of the soil areas containing [^{14}C]MAB_{1a} from TLC plates were about 80%. The behavior of the control pesticides was consistent with previously reported observations. Helling and Turner (1968) reported the movement of atrazine, 2,4-D, and trifluralin on three different soils: Chillum silt loam (26.3% clay, 3.1% organic matter), Hagerstown silty clay loam (39.5% clay, 2.5% organic matter), and Lakeland sandy loam (12.0% clay, 0.9% organic matter). In the present study, Three Bridges, NJ, silt loam (16% clay, 1.79% organic matter), Waco, TX, clay/clay loam (40% clay, 4.52% organic matter), and College Station, TX, sandy loam (11% clay, 1.26% organic matter) are the soils (Table 1) that most closely match the properties of the soils used by Helling and Turner (1968). The mean R_f values and classifications for atrazine, 2,4-D, and trifluralin for the three soils in the referenced study and for the three soils in the present study (Table 2), respectively, are as follows: trifluralin, 0 and 0.03 (immobile, class 1 in both cases); atrazine, 0.57 and 0.42, (intermediate, class 3 in both cases); 2,4-D, 0.73 and 0.81 (mobile, class 4 in both cases). [^{14}C]MAB_{1a} benzoate was immobile on all of the soil TLC plates tested and can be classified as an immobile pesticide.

Batch Equilibrium. The percent sorption onto and desorption from each soil type were determined. More than 99% of the MAB_{1a} at each of the four concentrations of MAB_{1a} benzoate (11−646 ng/5 mL) was sorbed onto each of the four soil types (Table 3). The sorption of MAB_{1a} onto the soils was apparently independent of MAB_{1a} benzoate concentration, soil organic matter, or soil texture (Tables 1 and 3). Although the subsequent desorption into fresh calcium chloride solution was minimal (0.1−1.28%), it was dependent on MAB_{1a} concentration and soil type (Table 3). The desorption of MAB_{1a} from sand and silt loam increased from 0.54% to 1.28% and from 0.33% to 0.75%, respectively, with increasing MAB_{1a} concentration, although it remained constant for sandy loam (0.10%) and clay loam (0.15%) soils at all concentrations. The desorption of MAB_{1a} benzoate from soils exposed to 646 ng/5 mL calcium chloride solutions was in the following order (Table 3): sand (1.28%) > silt loam (0.75%) > clay loam (0.16%) > sandy loam (0.10%). The Lakeland sand soil, which had the lowest organic matter (Table 1), exhibited higher desorption than the other soil types at all MAB_{1a} benzoate concentrations (Table 3). However, the overall results indicated no relationship between desorption (Table 3) and organic matter content (Table 1).

Table 4. Sorption/Desorption Distribution and Freundlich Constants[a]

soils		K ($\times 10^2$)	K_{oc} ($\times 10^4$)	K_d[b] ($\times 10^2$)
sandy loam (College Station, TX)	sorption	20.4	27.9	38.4 ± 4.0
	desorption	40.9	56.0	52.1 ± 6.5
sand (Lakeland, FL)	sorption	2.2	72.9	6.4 ± 1.8
	desorption	1.3	42.4	7.1 ± 2.7
clay loam (Waco, TX)	sorption	6.7	2.5	14.9 ± 3.2
	desorption	25.9	9.9	35.5 ± 2.5
silt loam (Three Bridges, NJ)	sorption	3.0	2.8	6.8 ± 1.6
	desorption	2.5	2.4	12.2 ± 4.0

[a] n values were between 1.0 and 1.2, indicating linear isotherms for all soils. [b] The mean and standard deviation of K_d values (μg of MAB_{1a} per g of soil divided by μg of MAB_{1a} per mL of calcium chloride solution) were calculated from four concentrations of [^{3}H]MAB_{1a} benzoate.

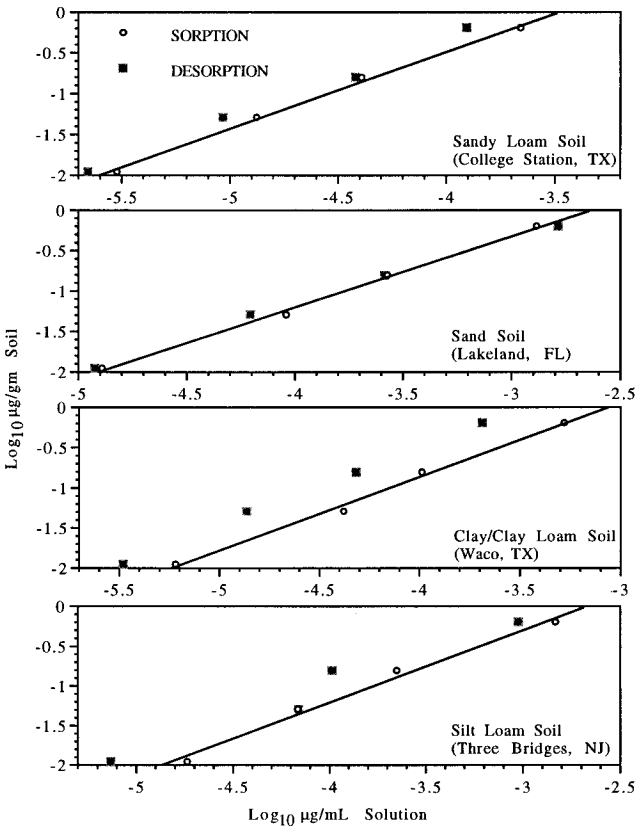

Figure 2. Batch equilibrium plots of sorption (○) and desorption (■) of MAB_{1a} with sandy loam, sand, clay loam, and silt loam soils.

Table 4 lists MAB_{1a} benzoate sorption and desorption distribution constants (K_d) of four soil types. Figure 2 shows graphs of log [^{3}H]MAB_{1a} bound to soil (x/m) versus log [^{3}H]MAB_{1a} benzoate concentration in calcium chloride solution (C) for both sorption and desorption isotherms. The regression parameters from these plots fitted to the Freundlich equation are shown in Table 4. Although the values for n are very near to unity, the values of the mean distribution constant (K_d) are generally higher (Table 4) than the values of the Freundlich distribution constant (K) for each of the four soil types. The values of the mean distribution constant (K_d) or the Freundlich distribution constant (K) vary considerably for the four types of soil tested, as would be expected on the basis of the varying soil properties. However, K values did not correlate with the organic matter concentration (Table 1), as demonstrated by the large range of K_{oc} values (Table 4). If organic carbon was the sole factor for [^{3}H]MAB_{1a} binding, then K_{oc} would be expected to be similar for all of the soils. Further, there was no apparent correlation between distribution constant and other characteristics of soils such as texture or cation exchange capacity. Therefore, [^{3}H]MAB_{1a} binds tightly to all soils irrespective of their organic content or texture.

The dissociation constants of emamectin benzoate (K. Anderson, Merck Research Laboratories, personal communication, 1993) as determined by potentiometric titration are 4.2 (pK_a, benzoic acid) and 7.6 (pK_b, emamectin). The solubility of emamectin benzoate is pH dependent (320, 24, and 0.1 ppm at pH 5, 7, and 9, respectively). At pH 6, emamectin will be mainly cationic. The effect of pH on soil binding of the ionizable emamectin molecule was not explored. However, the high affinity of the neutral emamectin analog avermectin B_{1a} for soils (Gruber et al., 1990) suggests that high affinity of emamectin for soil is largely by nonionic mechanisms.

The index of binding capacity for each soil (Hamaker, 1975) is related to its corresponding distribution constant (K) value. These values for the soils were in the following order: sandy loam > clay loam > silt loam > sand (Table 4). Among the four soils, the desorption of the test compound from soils was in the following order: sand > silt loam > clay loam > sandy loam (Table 4). When the sorption and desorption isotherm graphs were compared (Figure 2), hysteresis (existence of nonsingularity) of desorption data was observed for sandy loam, silt loam, and clay/clay loam soils. Thus, overestimates of pesticide desorption could be made if predictions are based only on the sorption isotherm parameters (Bowman and Sans, 1985). The sorption and desorption isotherm graphs for sand soil were approximately the same, and no hysteresis effect was observed. The results of soil TLC and batch equilibrium experiments demonstrate that the binding of MAB_{1a} benzoate with each soil tested is very strong and only partially reversible by water.

The mobility of chemicals in soils has been categorized on the basis of their K_{oc} values. The K_{oc} values of MAB_{1a} benzoate [$(2.5-7.3) \times 10^4$] can be compared with those of other immobile environmental chemicals (Hamaker and Thomson, 1972; Gruber et al., 1990), including chloroxuron (5×10^3), paraquat (20×10^3), DDT (243×10^3), and abamectin [$(5.3-30) \times 10^3$]. The observed K_{oc} values for MAB_{1a} benzoate categorize it as an immobile chemical.

A minimum of 90% and 1% of the radioactivity sorbed onto the soils was recovered by organic extractions and combustion of the extracted soil samples, respectively. The total recoveries of MAB_{1a} benzoate from all of the soil samples ranged between 97% and 107%. The organic extracts of the soils with bound [^{3}H]MAB_{1a} were analyzed by RP-HPLC. Most of the radioactivity (96–98%) was accounted for as MAB_{1a}. The results indicate that no metabolism or degradation of MAB_{1a} benzoate occurred during the sorption and desorption phases.

Emamectin is a mixture of two components, MAB_{1a} (>90%) and MAB_{1b} (<10%), which differ only by the presence of an additional methylene unit on the C-25 side chain (Figure 1). This minor difference in the components of emamectin would not significantly alter the soil binding properties. Emamectin can be classified as an immobile pesticide that will bind tightly to soil and not move readily in the environment.

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