Sorption/Desorption of [^{14}C]Efrotomycin with Soils

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The sorption and subsequent desorption of efrotomycin, which is being developed as a growth promoter in swine, was investigated in five soils of various physical and chemical properties. Sorption equilibration occurred within 7 h. Sorption distribution coefficients ranged from 8 to 290. The Freundlich constant, K, spanned a similar range as n values were near 1. The sorption distribution coefficients, K_d, were not correlated with organic matter content; cation-exchange capacity; sand, silt, or clay content; or soil pH. Hysteresis was observed between the sorption and desorption isotherms for one of the soils. For four soils, only about 50% of the sorbed efrotomycin could be desorbed, even with organic solvents. Efrotomycin is classified as immobile in most soils by comparison of its K_{oc} values (580–11 000) with those of other chemicals.

One of the most important factors involved in the mobility of solutes in the soil environment is the sorption–desorption process. Sorption is a general term that includes adsorption (surface binding) and partitioning. Desorption is the reverse process of sorption (U.S. FDA, 1987). Physical and chemical factors that have been related to soil binding of chemicals are size, shape, solubility, pK, and polarity. Soil properties that play important roles in binding are organic carbon content, cation-exchange capacity, particle size, pH, clay content, water content, and salt concentration (Hamaker, 1975; Chiou et al., 1979). The aqueous environment also is important in the amount of binding to soil. Factors such as pH and ionic strength of the water environment affect binding. The rate of movement of organic chemicals through soil is inversely correlated with sorption (Hamaker, 1975). Thus, chemicals that are strongly sorbed are much less mobile than weakly sorbed chemicals.

Efrotomycin (MK-0621) is a fermentation product isolated from *Nocardia lactamdurans* (formerly *Streptomyces lactamdurans*). It is a member of the kirromycin family of antibiotics and is active against a broad spectrum of infections in animals (Maehr et al., 1980). It is currently under investigation as a growth promoter in swine. Efrotomycin exists as a number of naturally occurring isomers. The A_1 isomer (Figure 1) is the major component. Other isomers exist as internal Michael adducts (Fink and Stong, 1984) and are termed B isomers. This report describes the sorption and desorption of efrotomycin with five soils of varying physical and chemical properties. The methodology follows that outlined by the U.S. FDA (1987) to assess the soil mobility of animal health drugs in the environment.

MATERIALS AND METHODS

Efrotomycin. ^{14}C-Labeled efrotomycin A_1, prepared and purified by the Labeled Compound Synthesis Group, Department of Animal and Exploratory Drug Metabolism, Merck Sharp & Dohme Research Laboratories (MSDRL), was isotopically labeled specifically at C-7 by introduction of [1-^{14}C]propionate into the fermentation mixture (Mertel et al., 1984). Efrotomycin A_1 was >98% radiochemically pure by chromatographic analysis. The stock solution contained 0.206 mg/mL and 2.35 μCi/mL (11.41 μCi/mg) in methanol. Unlabeled efrotomycin was a mixture of A_1 (74.6%) and B isomers (19.9%) (Chemical Data Department, MSDRL), reflecting the distribution of components in the fermentation product.

Soils. Soil A was a Three Bridges, NJ, silt loam, and soil B was a Riverside, CA, loam. Soil C was a Lakeland, FL, sand. Soils D and E were a Lufkin sandy loam from College Station, TX, and a Newton, IA, clay loam, respectively. The properties of each of the test soils are shown in Table I. The Lufkin sandy loam has been previously characterized (Bull and Ivie, 1982). Only the Iowa clay loam (E), a fine, silty, mixed, mesic aquic argiudoll, was classified according to its soil taxonomy.

Reagents. HPLC-grade methanol and anhydrous calcium chloride ($CaCl_2$) were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). HPLC-grade water was deionized distilled water (Hydro Service and Supplies, Inc., Garfield, NJ). Insta-gel scintillation cocktail was purchased from Packard Instruments (Downers Grove, IL). All other chemicals were reagent grade and were used as received.

Apparatus. Radioactivity of solutions was measured on a Beckman Instruments (Nuclear Systems Operations, Irvine, CA) Model LS 3800 liquid scintillation counter. The external standard (H-Number) method was used to correct for quenching. Solid samples were oxidized on a Packard Instruments Model B306 sample oxidizer followed by radioactivity measurements in a Packard Instruments Tri-Carb liquid scintillation spectrometer. The external standard channels ratio (ESCR) method was used to correct for quenching. Samples were equilibrated on a Fisher Scientific (Springfield, NJ) Roto-rack mixer.

Sorption Equilibria. Each equilibration used 0.01 M $CaCl_2$ solution to minimize the suspension of soil particles and to better simulate natural water (U.S. FDA, 1987), despite the fact that efrotomycin may complex calcium ions (D. W. Fink, personal communication). In a 16-h screening test of the five soils, only the Lakeland, FL, sand (soil C) showed little sorption of efrotomycin (less than 17%) and was therefore excluded from further tests. The time required to equilibrate the remaining soils (A, B, D, E) with efrotomycin was estimated with use of the following soil kinetics test. Solutions of efrotomycin, 5 mL each at 25 μg/mL and 7000 dpm/mL in 0.01 M $CaCl_2$, were equilibrated with 1 g of each soil in triplicate. Blanks contained 5 mL of $CaCl_2$ solution and 1 g of each soil, and the control contained 5 mL of the efrotomycin solution in a test tube without soil. Mixing was performed with the exclusion of light. At 1.2, 2, 4, 7, and 24 h, a sample from each soil group was analyzed. The control and blanks were analyzed at 1.2 h. Triplicate 1.0-mL aliquots of the supernatant were removed from each sample for scintillation counting. Equilibration appeared complete by 7 h since the sorption percents were 97–100% of

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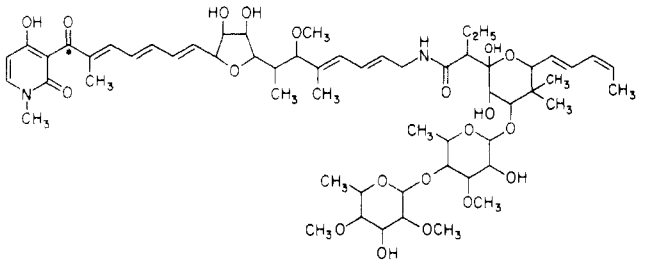

Figure 1. Structure of efrotomycin A_1 isomer. Asterisk denotes the position of the ^{14}C label.

Table I. Composition and Physical Properties of Test Soils

soil source	Three Bridges, NJ	Riverside, CA	Lakeland, FL	College Station, TX	Newton, IA
soil	A	B	C	D	E
pH	7.5	6.7	7.5	7.5	5.0
% OM[a]	2.1	2.5	0.1	1.1	4.6
CEC[b]	12.5	12.3	1.5	8.7	24.3
sand, %	11	50	92	57	26
silt, %	62	35	6	28	46
clay, %	27	15	2	15	28
soil texture	silt loam	loam	sand	sandy loam	clay loam

[a] Percent organic matter. [b] Cation-exchange capacity (mequiv/100 g).

the values at 24 h.

Sorption Isotherms. Four solutions of efrotomycin (0.20–27 μg/mL) were made in $CaCl_2$ solution at concentrations well below the aqueous solubility (1.0 mg/mL, pH 7). Each contained 3200 dpm/mL of [^{14}C]efrotomycin. Triplicate samples containing 1 g of soil and 5 mL of efrotomycin solution were made for each soil at each concentration. A blank ($CaCl_2$ solution plus soil) for each soil and a control (efrotomycin solution without soil) for each concentration were made. Equilibrations were performed for about 16 h, while samples were protected from light, at room temperature (23 °C). Following equilibration, 3 mL from each sample and 1 mL from each control and blank were analyzed by liquid scintillation counting (LSC). A total of 4 mL of solution was removed from each tube, and a slurry was made from the remaining liquid and soil by vortexing. Aliquots (approximately 0.1 g) of each slurry were removed, weighed, allowed to dry, reweighed, and combusted. Quantitation of the samples was by LSC of the $^{14}CO_2$ dissolved in the cocktail delivered by the oxidizer. Corrections were made for the weight and radioactivity of the solution in each slurry aliquot, and the concentration of sorbed efrotomycin was calculated.

Desorption. Duplicate desorptions were performed on each sample, control, and blank. Following sorption analysis, 4 mL of $CaCl_2$ solution was added to each tube and equilibration was performed for at least 16 h as for the sorption process. Analysis, including aliquot combustion, was performed, and the second desorption was done in a manner identical to the first. Because of the small amounts of efrotomycin that desorbed, sufficient material was not available for both scintillation counting and chromatography. Since Tate et al. (1989) showed that sorption of efrotomycin to a mineral soil did not change the isomer distribution, the desorbed material was assumed to be efrotomycin A_1.

Data Analysis. Radioactivity in liquid samples was quantitated by aliquotting the solutions into scintillation vials, adding 15 mL of Instagel, and counting for 5–10 min. Radioactivity in solid samples combusted in the oxidizer was also counted for 5–10 min.

The data were fitted to the logarithmic transform of the Freundlich equation, $\log (x/m) = \log K + (1/n) \log C_e$, where x is the amount of sorbate in micrograms per mass, m, of sorbent (soil) in grams and C_e is the solution concentration in micrograms per milliliter at equilibrium (Felsot and Dahm, 1979; U.S. FDA, 1987). When $n = 1$, the Freundlich constant K equals the distribution constant (K_d). Fitting was performed by a least-squares regression analysis program using an RS/1 statistical package (BBN Research Systems, Bolt Beranek and Newman Inc., Cambridge, MA) on a VAX-11/785 computer (Digital Equipment, Maynard MA). This statistical package was also used to determine the degree of correlation between sorption and soil properties.

Table II. Summary of Soil-Binding Isotherm Parameters[a]

parameter	Three Bridges, NJ	Riverside, CA	College Station, TX	Newton, IA
soil	A	B	D	E
sorption				
K_d	18	8.3	51	290
K_{om}	850	330	4640	6390
K_{oc}	1460	580	8000	11000
Freundlich				
K	13.4	8.0	46.4	336
n	1.3	1.1	1.0	0.96
desorption				
K_d	42	14	100	480
K_{om}	2010	540	9060	10490
K_{oc}	3470	930	15600	18100

[a] K_d, K_{om}, K_{oc}, K, and n described text.

RESULTS AND DISCUSSION

Accountability. The accountabilities of the efrotomycin in solution and on soil after the sorption step were 86, 83, 57, and 82% for soils A, B, D, and E, respectively. At concentrations lower than about 25 μg/mL, a significant amount of efrotomycin adsorbs to glass. The preliminary experiments were performed at or greater than 25 ppm, where adsorption to glass was not significant (less than 5%). The isotherm experiments however required concentrations approximately 125 times lower (as low as 0.02 μg/mL) than those in the preliminary tests. Rather than assume that the remainder of the test chemical, no longer in solution, was all sorbed on soil, it was necessary to measure the amount of material actually on the soil. This was accomplished by combustion of the soil samples. The material not accounted for, presumably adsorbed to the glass tubes, did not affect the calculation of the sorption parameters.

Sorption Isotherms. The sorption distribution constant (K_d) is defined as the ratio of concentration bound to soil divided by the concentration free in solution following equilibration. K_{om} is the distribution coefficient normalized to the percentage of organic matter in the soil and can be expressed as $[K_d/(\%\ \text{organic matter})] \times 100$. K_{oc} is the distribution coefficient normalized to the percent of organic carbon and has been estimated to be $K_{om} \times 1.724$ (Hamaker, 1975). K_{om} and K_{oc} are sometimes useful in comparing the ability of compounds to bind to soil and in determining whether the organic component of soil is involved in the binding. Table II contains a summary of the means of these three distribution coefficients for sorption and desorption of efrotomycin to each of the four soils tested. The range of K_d values was from 8 to 290, and the K_{oc} values ranged from 580 to 11 000. Figure 2 contains the sorption and desorption data (means of triplicate determinations) for efrotomycin binding with the four soils tested, as well as the sorption isotherms. Table II contains the Freundlich values K and n from the linear regression fits of the sorption data in Figure 2. The value of n for each soil is very nearly unity. Reasonable agreement is observed between the Freundlich K values and the K_d values, with the greatest divergence occurring where n is farthest from unity.

Desorption. Isotherms for the data in Figure 2 for two consecutive desorptions of each sample appear to have smaller slopes than the indicated sorption isotherms. Fam-

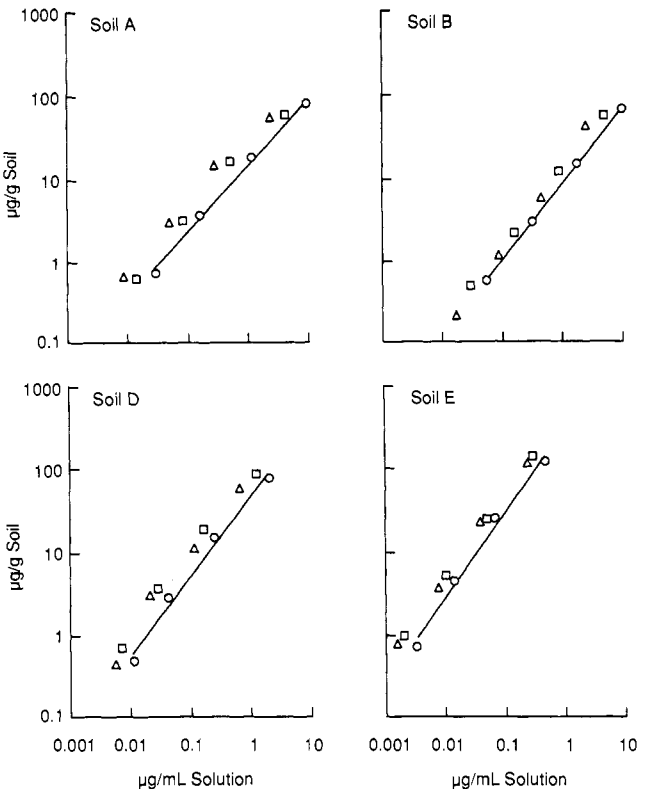

Figure 2. Freundlich isotherm plots of efrotomycin A_1 with soils A, B, D, and E: sorption (○), first desorption (□), and second desorption (△). Solid line is fitted to sorption data.

ilies of roughly parallel curves fitted to the desorption data (not shown) extend from each sorption point (Bowman and Sans, 1985). For soil A, the individual second desorption data points all are above the upper 95% confidence interval curve (not shown) determined from the individual sorption values. For soils B, D, and E, the desorption data fall in the vicinity of the respective upper 95% confidence interval curves. Thus, for soil A, hysteresis (indicating partial irreversibility; Bowman and Sans, 1985) was observed between sorption and desorption. Additional desorptions would have to be done with the other soils to determine whether the desorption curves truly diverge from the sorption lines.

Additional experiments also indicated the sorption of efrotomycin to soil may be partly irreversible. A sample of each soil was extracted in duplicate with methanol following the desorptions. Only 16–20% of the efrotomycin still bound was extracted from the soil, bringing the total removed from soil to about 50%. Similar results were obtained with ethyl acetate, a good solvent for efrotomycin. The sorption to glass appears to be predominantly reversible, since efrotomycin was liberated from controls in the desorption steps by addition of $CaCl_2$ solution.

Overestimation of efrotomycin desorption could result if sorption parameters were used.

Correlation of Binding to Soil Properties. There were no significant correlations ($p < 0.05$) between K_d and percent organic matter; cation-exchange capacity; soil pH; or percent sand, clay, or silt. The sorption of efrotomycin A_1 to soil organic and ionic binding sites should be affected by the protonation state of the pyridone ring. At pH values below the pK_a (about 6; Kaplan et al., 1984) the molecule would be neutral. At pH values above the pK_a, ionization should likely reduce the strength of hydrophobic interactions. Ionic interactions should also be affected, with enhanced binding to cationic sites and weakened interactions with anionic sites. Additional studies on the sorption of efrotomycin to soil organic and clay fractions, and on the influence of aluminum ion (which is chelated by efrotomycin; Kaplan et al., 1984; Fink and Stong, 1984) on binding, have been reported (Tate et al., 1989).

CONCLUSIONS

Efrotomycin was highly sorbed by four soils (60–98%), and little of the sorbed material was desorbed (less than 31% of sorbate). The sorption to sand was about 17% in a preliminary screening test, so sorption/desorption parameters were not determined for this soil. The nature of the sorption to soil could not be determined from this study since sorption did not correlate with any single soil parameter, including pH, percent organic matter (K_{oc} was not similar for all soils), cation-exchange capacity, percent sand, percent silt, or percent clay. Additional studies on the nature of the interactions between efrotomycin and soil indicate that organic matter and clay fractions are both involved in the sorption (Tate et al., 1989).

The mobility of a compound in soil is commonly related to K_{oc} (Hamaker and Thompson, 1972). By this criterion, efrotomycin can be classified as "highly immobile" in the Newton, IA, soil (E) and the College Station, TX, soil (D). Although the K_{oc} values for efrotomycin with the Three Bridges, NJ, and Riverside, CA, soils (A and B, respectively) are considerably lower than with the other two soils studied, they are still 1 order of magnitude higher than those values associated with "mobile" compounds. The mobility of efrotomycin, entering the environment in the excreta of treated pigs, is expected to be low in most soils due to this high amount of sorption. The data will be used in the environmental assessment of this compound.

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Registry No. Efrotomycin A_1, 56592-32-6.

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Mobility of Avermectin B_{1a} in Soil

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Avermectin B_{1a} (AVM) was determined to be immobile in soil by three methods: sorption/desorption using a batch equilibrium technique, soil thin-layer chromatography, and soil column leaching using aged and unaged soil. From the sorption data from batch equilibrations of AVM with three soils, the K_{oc} (distribution constant normalized to the percent organic matter) for AVM was determined to be 4.76×10^3. When ascending chromatography was performed on TLC plates prepared with six different soils, average R_f values for AVM and 2,4-dichlorophenoxyacetic acid, a pesticide of known mobility, were determined to be 0.00 and 0.78, respectively. Also, AVM did not readily leach from four soils in soil column leaching studies.

Avermectin B_{1a} (AVM, Figure 1) is a macrocyclic lactone produced by the actinomycete *Streptomyces avermetilis* (Burg et al., 1979). It is effective in controlling different phytophagous pests of field crops, ornamentals, vegetables, and fruits (Putter et al., 1981) and in controlling fire ants (Lofgren and Williams, 1982), which are a serious problem in some areas of the southern United States. Abamectin (the commercial product containing AVM) is being developed for these purposes.

The contamination of surface water and groundwater by the agricultural use of pesticides and other chemicals is a major national concern. How tightly a compound binds to the soil of the area of use largely determines whether the pesticide is likely to leach into groundwater or be carried in runoff water into lakes and streams. Soil TLC studies performed with three soils show AVM to be immobile (Bull et al., 1984; Bull, 1985). To more firmly establish AVM's mobility characteristics in soil, additional studies were performed with AVM using three commonly used methods: (I) sorption/desorption of the chemical with soil using a batch equilibrium technique for determination of Freundlich and distribution constants, (II) soil thin-layer chromatography (TLC), and (III) soil column leaching of the chemical.

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MATERIALS AND METHODS

Chemicals. Three different preparations of AVM were used for these studies: [5-^{3}H]avermectin B_{1a} ([^{3}H]AVM) with a specific activity of 1.63 mCi/mg, 98+% radiopure; [3,7,11,13,23-^{14}C]avermectin B_{1a} ([^{14}C]AVM) with a specific activity of 16.4 μCi/mg, 99+% radiopure; and [^{3}H]AVM with a specific activity of 118 μCi/mg, 99+% radiopure. The syntheses of both [^{3}H]AVM and [^{14}C]AVM have been published [Chabala et al. (1981) and Ku and Hwang (1985), respectively]. The four ^{14}C-labeled pesticides, 2,4-dichlorophenoxyacetic acid-*carboxy*-^{14}*C* (2,4-D), Temik-*methylthio*-^{14}*C*, [U-^{14}C]mirex, and [ring-U-^{14}C]parathion, used as standards in the TLC study had specific activities of 4.48, 4.91, 7.25, and 7.42 mCi/mmol, respectively, and were obtained from Pathfinder Laboratories Inc., St. Louis, MO.

Soils. The six soils used in these studies (Table I) (silt loam from Three Bridges, NJ; loam from Riverside, CA; sand from Lakeland, FL; sand from Sanford, FL; Houston clay loam from Waco, TX; Lufkin sandy loam from College Station, TX) were nonsterile and were air-dried and sieved to pass a 35-mesh screen prior to use in all three studies. The soils were not classified by their soil taxonomies.

Equipment. Radioactivity contained in a liquid was quantified by direct liquid scintillation counting (LSC) on either a Model 460 or Model 4530 liquid scintillation counter from Packard Instrument Co., Inc., Downers Grove, IL. Radioactivity contained in a solid was quantified by LSC after conversion of the radioactivity to tritiated water with a Packard Tricarb Oxidizer, Model B306.

Autoradiography was performed with "blue-sensitive" medi-

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