Sorption and Aerobic Biodegradation of Strychnine Alkaloid in Various Soil Systems

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Strychnine alkaloid was demonstrated to sorb strongly to several western soils that varied in organic matter and ranged in texture from a loamy sand to a sandy clay loam. Freundlich sorption coefficients (K) for the test soils varied from 40 to 169. A direct relationship was observed between strychnine sorption and cation exchange capacity but not between sorption and organic matter content. Strychnine sorption was only partially reversible (12−44%). Degradation of 10 μg/g strychnine in sandy loam and sandy clay loam soils occurred in three distinct phases, which included a lag phase, a rapid loss phase, and a leveling off or soil binding phase. Approximately 50% of the strychnine was lost from the two soils within 24−27 days, with the appearance of a degradation product occurring early in the study. The initial breakdown products of strychnine are believed to be polar compounds with strong sorption characteristics.

Keywords: *Strychnine; soil sorption; degradation; breakdown products*

INTRODUCTION

Limited data are available regarding the fate of the plant alkaloid strychnine ($C_{21}H_{22}N_2O_2$) in soil, when used as a vertebrate damage control minor-use pesticide. Thus, to satisfy various environmental concerns and to comply with EPA reregistration data requirements, soil sorption and biodegradation research was initiated at our laboratory to evaluate the effects of these important processes upon the mobility and persistence of strychnine in soils.

Smith (1982) and Miller et al. (1983) reported strong sorption of the alkaloid to soils of different texture. Moreover, strychnine was not absorbed and translocated in young alfalfa plants in detectable quantities.

Few data are available concerning the degradation of strychnine in soil or aqueous systems. Howard et al. (1991) provided both low (1 week) and high (4 weeks) "half-life" estimates for strychnine in aqueous media. In addition, biotransformation data for strychnine are available in other biological systems, i.e., in vitro and in vivo metabolism in rats and rabbits and in cultures of isolated bacteria and fungi. The in vitro metabolism of strychnine was studied in supernatant fractions from rabbit and rat liver tissues (Mishima et al., 1985). During a 1-h incubation period, about 71% of the applied strychnine disappeared from the incubation mixture at an optimum pH of 8.4. The major metabolite identified was strychnine *N*-oxide, which accounted for about 15% of the metabolized strychnine. Another metabolic pathway may have involved the epoxidation of the double bond between C-21 and C-22, followed by hydrolysis to 21α,22α-dihydroxy-22-hydrostrychnine. It was proposed that an 11,12-dehydrostrychnine metabolite was derived from a hydroxyl metabolite. An in vivo rat study using [^{3}H]strychnine was conducted by Oguri et al. (1989). Most metabolites were more polar than strychnine, with radiochromatograms indicating the presence of metabolites corresponding to strychnine *N*-oxide, 21α,22α-dihydroxy-22-hydrostrychnine, 2-hydroxystrychnine, and 16-hydroxystrychnine. The major metabolite of strychnine was a stable product identified as strychnine 21,22-epoxide. As suggested by these authors, the metabolism of strychnine appeared to be associated with detoxification, with the 2-hydroxystrychnine, 16-hydroxystrychnine, and strychnine *N*-oxide products indicated to be of relatively low toxicity. Nguyen-Dang and Bisset (1968), using a strychnine-treated fungal culture (*Helicostylum piriforme*), reported the presence of the *N*-oxide product within 2 days, followed by the appearance of 16-hydroxystrychnine at the 7-day sampling period. Bucherer (1965), using the bacterium *Arthrobacter* incubated in a medium containing no auxiliary source of nitrogen, indicated the presence of mainly low molecular weight degradation products of strychnine. Niemer and Bucherer (1962) also studied the metabolism of strychnine by *Arthrobacter*, but added an inorganic nitrogen source to the strychnine-containing incubation medium; they demonstrated the presence of C_{16} hanssenic acid, which resulted from the indole ring splitting from the remainder of the strychnine molecule.

The two studies presented in this paper describe the sorption and breakdown of nonradiolabeled strychnine alkaloid in several different soils. Degradation of the plant alkaloid was evaluated by determining residual concentrations of the parent compound in soil during a 2-month incubation period, as an indirect measurement of biodegradation. Also, a two-step extraction procedure and HPLC/UV analysis were used to extract and detect major metabolites. The various metabolites of strychnine that were identified in previous metabolism research, and presented above, are discussed in this paper as to possible initial breakdown products, and the mechanism by which strychnine may degrade in soils.

MATERIALS AND METHODS

Strychnine Alkaloid. Although a method for strychnine synthesis is available (Woodward et al., 1954), commercial quantities of the plant alkaloid are generally obtained by

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Table 1. Selected Chemical and Physical Soil Properties

		%							
soil series	pH (1:1)	organic matter	TOC	sand	silt	clay	texture	CEC (mequiv/100 g)	H_2O at 1/3 bar (%)
Valent[a]	6.7	0.46	0.27	89	2	8	loamy sand	2.3	2.3
Ascalon[a]	8.3	1.22	0.71	76	12	12	sandy loam	11.5	10.6
Table Mountain[a]	7.7	2.76	1.60	51	29	20	loam	15.3	17.7
Kim[a]	8.0	2.00	1.16	54	21	25	sandy clay loam	16.4	18.6
Ascalon[b]	7.6[c]	1.70	0.99	73	10	17	sandy loam	11.4	10.9
Ascalon[b]	7.9[c]	2.30	1.33	60	13	27	sandy clay loam	18.8	16.9

[a] Sorption study. [b] Biodegradation study. [c] pH (paste).

extracting the seeds of *Strychnos nux-vomica L* with various solvents. Radiolabeled strychnine was unavailable for our use because of the expense in obtaining a sample of custom-synthesized [^{14}C]strychnine, due to the complex synthesis procedure.

The strychnine alkaloid (free-base, reagent grade, CAS no. 57-24-9) used in the two studies was purchased from Sigma Chemical Co., St. Louis, MO. Mean purity values for the chemical, assayed at several different time periods at our laboratory using HPLC with UV detection at 254 nm, ranged from 99.0 to 101.5%.

Soil Selection and Preparation. The four soils selected for the sorption research were classified as Ascalon sandy loam, fine loamy, mixed, mesic Aridic Arguistoll; Kim sandy clay loam, fine loamy, mixed (calcareous), mesic Ustic Torriorthent; Table Mountain loam, fine loamy, mixed, mesic Pachic Haplustoll; and Valent loamy sand, mixed, mesic Ustic Torripsamment. These soils were collected in Larimer and Logan Counties, Colorado. The Ascalon sandy loam and sandy clay loam soils used in the biodegradation study (fine loamy, mixed, mesic Aridic Argiustoll) were also collected in Larimer County, Colorado, but at different sites and time periods than the Ascalon sandy loam soil used in the sorption research.

The soil samples for the sorption research were thoroughly mixed by passing each through a sample splitter; the samples were then screened, collecting that fraction which passed through a 2-mm sieve. These samples were stored at ambient laboratory temperature. Collection of the two soils for the biodegradation research involved removal of approximately the upper 10 cm of surface soil. The soils were dried, and plant fragments were removed, and then thoroughly mixed by passing each soil through a sample splitter several times, followed by collecting the fraction that passed through a 2-mm sieve. These samples were stored in polypropylene bags under refrigeration at about 3−4 °C until used (2−4 weeks) to minimize changes in the microbial populations. Characterization data for the test soils are presented in Table 1.

Soil Sorption. The sorption research was conducted using batch equilibrium methodology. Corex glass 25-mL centrifuge tubes with Teflon-lined caps were used to contain 2 g of soil, and the strychnine alkaloid was dissolved in 10 mL of aqueous 0.01 M $CaCl_2$ (1:5 soil:solution ratio). The concentrations of strychnine used to develop the sorption isotherms (4, 8, 16, 32, and 64 μg/mL) were based upon field application rates and the water solubility of strychnine (156 μg/mL; Merck Index, 1989). During sorption equilibration, the centrifuge tubes, containing the soil−strychnine−$CaCl_2$ mixture, were mechanically shaken in an environmental chamber at a constant temperature of 25 ± 1 °C. Following a 24-h equilibration period, the supernatant solutions were withdrawn from the soils after centrifuging each tube at 3000 rpm for 15 min. An aliquot of each solution was analyzed for strychnine using HPLC/UV at 254 nm, to determine the ratio (K) of strychnine sorbed per unit of soil mass (μg/g) to the concentration of strychnine remaining in the supernatant solution (μg/mL) (Timm et al., 1992). During desorption, the residual soil was washed with 10 mL of aqueous 0.01 M $CaCl_2$ and equilibrated for a 24-h period; this procedure was repeated three additional times. Sorption coefficients and constants were derived using the Freundlich empirical equation ($x/m = KC^{1/n}$), where x/m represents the quantity of strychnine per unit mass of soil (μg/g), C is the concentration of strychnine in solution at equilibrium (μg/mL), and K is the distribution coefficient. The same model was used to describe the desorption (K_{des}) rates. The supernatant solutions were filtered before analysis, with the strychnine content determined using calibration curves; the limit of detection for strychnine was 0.01−0.02 μg/mL.

The Hewlett-Packard 1090 M HPLC operating conditions were as follows: flow rate, 1.0 mL/min; column, Keystone C8, 5 μm, 4.6 mm i.d.; injection volume, 100 μL; oven temperature, 40 °C; run time, 8 min; mobile phase, 70%:30% mixture of 5 mM heptanesulfonic acid/acetonitrile.

Biodegradation. The soil incubation apparatus involved the use of 250-mL Erlenmeyer flasks with solid glass stoppers modified to contain full-length slots (about 2 mm width × 2 mm depth) to assure that aerobic conditions were maintained during the incubation period. Strychnine alkaloid was dissolved in methanol at a concentration of 487 μg/mL, as verified using HPLC/UV. Twenty-gram quantities of the test soils were weighed into 250-mL Erlenmeyer flasks. The soil samples were uniformly surface-treated with 1 mL (about 487 μg) of the strychnine solution; control soil samples were treated with 1 mL of methanol. After evaporation of the methanol solvent from the soils for about 16−17 h under a fume hood, an additional 30 g of each respective soil type was weighed into each Erlenmeyer flask; the soil was then mixed thoroughly by stirring and rotating the flask. This procedure resulted in about 10 ppm of strychnine (487 μg/50 g soil), based upon uniform distribution of the chemical within the soil. The methanol solvent was evaporated from the soils prior to reactivation of the microorganisms to avoid possible toxic effects of the methanol upon the microbial populations (Starr et al., 1992).

After soil treatment, deionized water (pH 6.6) was added dropwise to each of the soil samples, until a final moisture content approximating 75% of field capacity was obtained. After a thorough mixing of the soil by stirring, the flasks were maintained in an environmental chamber under dark conditions at a temperature of 25 ± 1 °C during the 2-month incubation period. Every 6 or 7 days deionized water was added to each soil sample to maintain the proper soil moisture content. The mean moisture evaporation rate during the 2-month incubation period for both the sandy loam and sandy clay loam soils was about 0.01 g per day. Eight sampling periods were chosen, based upon results of previous biodegradation research (Starr et al., 1992): time of treatment (t = 0) and 7, 14, 21, 28, 35, 42, and 56 days following treatment. At each time interval, three treated samples and a control were selected according to a prearranged random sampling schedule for strychnine extraction and analysis.

To verify the microbial viability of the two test soils, and to evaluate possible effects of 10 μg/g strychnine upon microbial populations, soil microbial growth was evaluated 7 and 56 days following treatment. At each sampling period, triplicate-treated and control soil samples were analyzed. Five replicate petri plates containing the proper nutrient media were inoculated with 0.1-mL aliquots of an appropriate dilution of each replicate soil sample. The plates were then incubated at 28 °C for 5 and 10 days for the counts of fungal/bacterial and actinomycete colonies, respectively, in accordance with the methods of Wollum (1982).

The use of *n*-butyl chloride as a soil extractant has been demonstrated in our laboratory to be effective in removing strychnine residues from the soil matrix (Hurlbut et al., 1992; Starr et al., 1992; Timm et al., 1992) with a high extraction efficiency observed for the parent compound from a moist

Table 2. Sorption of Strychnine to Four Different Soils

	Freundlich sorption/desorption constants								selected soil characterization parameters			
	sorption phase			desorption phase		% sorption	% desorption	mass balance				
soil series and texture	K	$1/n$	log K_{oc}	K_{des}	range	(64 μg/mL)	(64 μg/mL; 4 washes)	(% recovery)	pH	% TOC	% clay	CEC (mequiv/100 g)
Valent loamy sand	40	0.75	4.20	55	28−84	79.1	43.7	92.8	6.7	0.25	4	2.3
Ascalon sandy loam	95	0.97	4.14	89	61−117	94.7	17.4	89.3	8.3	0.68	18	11.5
Table Mountain sandy clay loam	119	0.82	3.97	115	32−172	94.9	16.7	97.4	7.7	1.26	12	15.3
Kim loam	169	0.93	4.20	146	60−288	97.2	11.5	99.4	8.0	1.05	23	16.4

sandy loam soil (microorganisms suppressed) throughout 2 months of incubation; furthermore, the parent compound did not appear to bind irreversibly to the soil colloids during this period. However, since strychnine metabolites were suggested to be more polar than the parent compound, a two-step extraction procedure was developed.

Methanol Extraction. The incubated soil samples (50 g), each contained in a 250-mL Erlenmeyer flask, were first extracted with 200 mL of HPLC-grade methanol. The flask, after being sealed with a solid glass stopper, was placed on a mechanical shaker for 15 min; after the particulate matter had settled, the methanol solution was decanted into a stainless-steel parabolic filtering bell. Using vacuum, the liquid was filtered through a 0.2-μm Teflon membrane into a 250-mL Erlenmeyer flask. The solution was then evaporated using a hot water bath (≈68 °C) under nitrogen to a volume less than 25 mL. The residual solution was then transferred to a 25-mL volumetric flask and diluted to volume with methanol. An aliquot of this solution was filtered through a 0.45-μm Teflon filter disk into a glass vial for analysis by HPLC.

n-Butyl Chloride Extraction. After evaporation of residual methanol from the soil, 15 mL of a 1.5 N NaOH aqueous solution was added to the dry sample. The sample container was sealed and shaken by hand to distribute the NaOH throughout the soil. Five milliliters of *n*-butyl alcohol was added to the sample to reduce the possibility of emulsion formation during the soil extraction. To extract strychnine and other weakly polar compounds from the soil, 100 mL of *n*-butyl chloride was added to the sample. The container was sealed and placed on a mechanical shaker for 10 min. After the extractant was decanted into a 250-mL Erlenmeyer flask, the solution was evaporated on a hot plate (≈100 °C) under nitrogen. The soil sample was then extracted 2 additional times using *n*-butyl chloride as described above. After evaporation of the combined extracts to dryness, 2 mL of *n*-butyl alcohol was added to the flask to dissolve any residual material present; to this solution was added 50 mL of a diluent [5 mM aqueous heptanesulfonic acid solution and acetonitrile (70%:30%)] to the flask. After the flask was sealed, the solution was sonicated for 15 min. An aliquot of this solution was withdrawn and filtered through a 0.45-μm nylon filter into a glass vial for strychnine analysis by HPLC. The extraction efficiency observed during method validation was about 89% for 50-g sandy loam and sandy clay loam samples that had been spiked with 728 μg of strychnine, extracted with the *n*-butyl chloride solvent, and then analyzed using HPLC/UV at 254 nm.

The methanol extracts were analyzed for potential polar degradates using a Hewlett-Packard (HP) 1050 HPLC and UV detection at 210, 254, and 300 nm. Strychnine residues in the *n*-butyl chloride extracts were determined using an HP 1090 HPLC with UV detection at 254 nm. The HPLC operating conditions were the following: for methanol extracts, flow rate, 0.75 mL/min; column, CN, 150 mm × 4.6 mm i.d., 7 μm packing, C8 guard column; oven temperature, ambient; run time, 10 min (standards), 60 min (samples); mobile phase, 60%:40% 0.05 M KH_2PO_4/acetonitrile; for *n*-butyl chloride extracts, flow rate, 1.0 mL/min; column, C8, 250 mm × 4.6 mm i.d., 5 μm packing, C8 guard column; oven temperature, ambient; run time, 9 min (standards), 25 min (samples); mobile phase, 70%:30% 5 mM heptanesulfonic acid/acetonitrile.

Standard solutions of strychnine, prepared in both methanol and 5 mM heptanesulfonic acid and acetonitrile (70%:30%), were used to generate calibration curves for strychnine quantitation. A linear relationship was observed between chromatographic peak response and strychnine concentration (0.05−1.01 μg/mL) for standard solutions prepared in methanol; also, this same relationship was observed for standard solutions of strychnine prepared in 70%:30% 5 mM heptanesulfonic acid solution/acetonitrile, over the concentration ranges of 0.10−5.06 μg/mL and 5.01−15.2 μg/mL; linearity was determined by inputting the peak response data into a linear regression analysis program (SAS Institute, Cary, NC). The smallest quantity of strychnine that could be detected and definitely attributed to a strychnine chromatographic response was determined to be 0.9 μg of the alkaloid, as applied to 50 g of either the sandy loam or the sandy clay loam soil, and extracted using the *n*-butyl chloride solvent system.

RESULTS AND DISCUSSION

Soil Sorption. Strychnine sorbed strongly to the four different soils (Table 2). The Ascalon sandy loam, Table Mountain sandy clay loam, and Kim loam soils demonstrated a high capacity for strychnine sorption, with 95−97% of the parent compound sorbed to these soils after a 24-h equilibration period at the 64 μg/mL application level; moreover, the coarse Valent loamy sand sorbed a relatively high percentage of the applied strychnine (79%), considering the low clay and total organic carbon (TOC) contents of this soil (Table 2). The mean K and K_{oc} values for the soils were 106 ± 54 and 13 823 ± 3091, respectively; the mean desorption value (K_{des}) was 101 ± 39. The strychnine mass balance values for the soils ranged from 89 to 99% (Table 2). Strychnine sorption was only partially reversible (12−44%). A direct relationship was observed between strychnine sorption and cation exchange capacity (Table 2), but not between strychnine sorption and organic carbon, as has been demonstrated previously with various nonionic pesticides in soil−aqueous systems (Chiou et al., 1979, 1983; Chiou, 1989).

Strychnine, as with other alkaloids, is alkaline in reaction (pK_a = 8.3 and 2.3; Perrin et al., 1981; Merck Index, 1989). Thus, at the pH range of the test soils (pH 6.7−8.3), one would expect the strychnine to be in a partially protonated form, allowing ion-exchange processes to occur at negatively-charged clay micelles, and/or at sites within the colloidal organic fraction containing ionizable carboxylic acid and hydroxyl groups. Moreover, a direct relationship between soil pH and curvature ($1/n$) of the sorption isotherms was observed (Table 2, Figure 1) with a greater degree of linearity occurring at the more alkaline soil pH. This may suggest that a greater degree of chemical partitioning of strychnine into the organic matter fraction occurred as the alkalinity of the soils increased and as the strychnine molecule became more nonionic (Table 2).

Thus, strychnine soil sorption is suggested to involve a combination of mechanisms, including surface adsorption and partitioning into the organic matter fraction, which appears to be pH-dependent.

Strychnine Biodegradation. Both test soils were biologically active, as determined by microbial plate

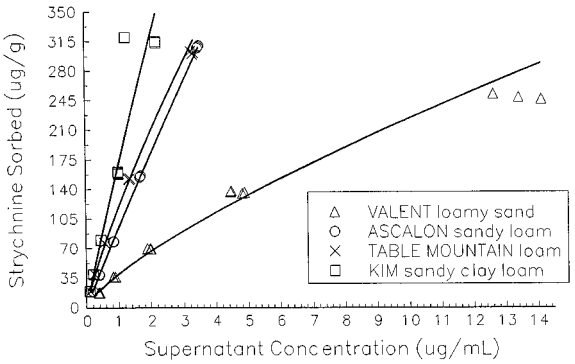

Figure 1. Strychnine sorption isotherms for the four soil series.

counts (Tables 3 and 4). These population determinations were made on triplicate soil samples that had been reactivated for 7 days at a moisture content of 75% of field capacity. In general, strychnine did not appear to affect microbial populations of either test soil, when applied at a concentration of 10 μg/g (Tables 3 and 4).

The mean recovery of strychnine from triplicate samples at each sampling point is presented in Table 5; as may be noted, strychnine applied at a concentration of 10 μg/g was found to dissipate (degrade) over a 2-month incubation period.

Degradation of pesticides and other organic chemicals in soil is dependent upon the chemical concentration (C) and the microbial population (X). If the microbial population is assumed to remain constant over the entire study incubation period, a pseudo-first-order rate expression can be developed as illustrated in eq 1, where k is the pseudo-first-order rate constant.

$$-\mathrm{d}C/\mathrm{d}t = k[\mathrm{C}] \quad (1)$$

Integration of eq 1 yields eq 2, where C_0 and C_t are the concentration of test chemical at time zero and time t.

$$\ln (C_t/C_0) = -kt \quad (2)$$

Thus, a plot of ln (C_t/C_0) versus t will yield a straight line with a slope of k, which is equal to the degradation rate constant. Once the slope is determined, the half-life ($t_{1/2}$) can be calculated from eq 3.

$$t_{1/2} = \ln (2/k) \quad (3)$$

When the strychnine recovery data for both soil types were plotted in this manner [i.e., ln (C_t/C_0) versus incubation time], sigmoidal-like recovery curves were obtained, with the strychnine loss from the treated soils occurring in three distinct phases (Table 5; Figure 2).

Lag Phase. During the initial lag phase, about 93% of the strychnine application was recovered from the sandy clay loam soil at the end of this 21-day period (Table 5). Although a lag period was observed with the treated sandy loam soil before a rapid loss of the alkaloid occurred (Figure 3; Table 5), the lag period was not as apparent, with about 71% of the strychnine application recovered at day 21.

The lag phase may be the result of a combined microbial adaptation period and soil sorption. Thus, with both soils, which had not received any prior applications of strychnine, the degradation is suggested to have followed kinetics typical of microbial metabolism, in which the slow or lag phase was followed by a rapid or exponential loss of strychnine (Figure 2). During the initial slow phase, an increase in the microbial populations to a suitable size, and/or changes in group dominance, may have been required before substantial breakdown of strychnine took place. The fungal and bacterial populations differed between the two soils 7 days following reactivation of the microbes (Tables 3 and 4). The sandy loam soil, which was obtained from an area that had been planted with beans, contained larger initial bacterial and fungal populations than did the sandy clay loam soil that had been cropped in corn. Thus, the larger fungal and bacterial populations in the sandy loam soil could have contributed to a less-defined strychnine lag phase in this soil. Lag phases prior to the degradation of various pesticides have been previously reported to vary from a few days or weeks, depending upon the compound, microorganisms, and soil medium (Parker and Doxtader, 1983; Starr and Cunningham, 1975; Starr et al., 1992; Burns and Gibson, 1980; Sandmann et al., 1988).

Rapid Loss Phase. During this period, beginning at about day 21 and extending to about day 35, loss of strychnine from the two different soils occurred in essentially an exponential manner (Figure 2). At the end of this rapid loss phase (day 35), about 5 and 20% of the strychnine remained in the sandy loam and sandy clay loam soils, respectively (Table 5).

Soil Binding Phase. The apparent decline in the rate of strychnine loss (days 35−56) from both soils (Figure 2) is suggested to be due to soil binding, whereby residual chemical is bound within the soil colloidal matrix. At day 56 of the study, only between 2 and 8% of the initial strychnine application was extractable from the two test soils (Table 5).

For a complex system such as soil, it is reasonable to expect that a single rate law would not apply to the degradation of a large alkaloid molecule such as strychnine, since the reaction rate would undoubtedly be modified and complicated by the onging biological processes, combined with the sorption and diffusion processes (Hamaker, 1972). Although the kinetics of strychnine loss from the Ascalon sandy loam and sandy clay loam soils cannot be expressed using a single pseudo-first-order rate constant, the degradation rate can be described in terms of time required for 50 and 90% dissipation of the parent chemical (Table 6).

Analysis of methanol extracts of the treated and control sandy loam and sandy clay loam soils, using HPLC/UV, revealed the presence of a secondary peak in treated sample extracts early in the study (day 7), which reached a maximum height at either day 14 (sandy loam) or day 21 (sandy clay loam); the peak then decreased in height with increased incubation time. The peak was not detected in any of the sandy loam methanol extracts after day 21; although the peak height decreased with time in extracts of the sandy clay loam soil, the peak was still detected in one of the sandy clay loam replicate samples at days 42 and 56. No attempt was made to isolate and identify the metabolite. Considering the large size of the strychnine alkaloid molecule, it is surprising that additional peaks, representing a variety of possible nonvolatile metabolites, were not detected in soil extracts over time, especially during the rapid strychnine loss period. The fact that other breakdown products were not detected could be explained if the polar products that formed were rapidly and tenaciously sorbed to the soil colloids and not extracted with either of the solvent systems, or were rapidly degraded by soil microorganisms.

Table 3. Microbial Abundance in a Moist Sandy Loam Soil[a] following Treatment with 10 μg/g Strychnine

	soil microbial abundance, no. of colony-forming units/g of dry soil (mean ± 1 SD, $n = 5$)					
	bacteria ($\times 10^6$)		actinomycetes ($\times 10^5$)		fungi ($\times 10^3$)	
days after treatment	control	treated	control	treated	control	treated
7	16.8 ± 2.75	15.6 ± 2.83	20.0 ± 2.42	18.6 ± 2.85	14.8 ± 1.3	19.2 ± 2.93
	15.7 ± 2.84	16.9 ± 2.24	18.6 ± 2.86	21.5 ± 2.54	16.7 ± 2.74	16.7 ± 2.52
	17.0 ± 3.10	16.3 ± 2.44	20.0 ± 4.72	20.0 ± 2.37	17.0 ± 3.1	16.9 ± 2.70
56	16.8 ± 2.57	16.0 ± 1.73	88.0 ± 15.9	86.1 ± 13.7	12.8 ± 1.87	14.1 ± 2.05
	15.4 ± 2.89	14.8 ± 1.36	89.5 ± 18.9	81.3 ± 13.6	9.99 ± 2.03	14.4 ± 2.59
	13.6 ± 2.23	15.9 ± 3.09	98.6 ± 12.0	73.4 ± 12.8	10.9 ± 2.41	12.4 ± 1.87

[a] Soil previously cropped in beans.

Table 4. Microbial Abundance in a Moist Sandy Clay Loam Soil[a] following Treatment with 10 μg/g Strychnine

	soil microbial abundance, no. of colony-forming units/g of dry soil (mean ± 1 SD, $n = 5$)					
	bacteria ($\times 10^6$)		actinomycetes ($\times 10^5$)		fungi ($\times 10^3$)	
days after treatment	control	treated	control	treated	control	treated
7	2.46 ± 0.94	4.26 ± 1.46	17.9 ± 2.86	29.3 ± 4.44	5.82 ± 0.94	5.38 ± 0.50
	6.38 ± 2.12	3.58 ± 1.23	19.8 ± 3.01	28.7 ± 3.03	4.84 ± 1.48	4.93 ± 0.61
	5.06 ± 1.67	2.89 ± 0.99	19.1 ± 1.84	28.0 ± 4.33	4.62 ± 1.43	5.99 ± 0.61
56	23.0 ± 2.12	24.5 ± 1.81	216 ± 26.6	207 ± 26.3	8.39 ± 1.26	10.8 ± 0.49
	21.1 ± 1.80	24.4 ± 2.84	213 ± 28.6	204 ± 31.7	8.34 ± 1.25	11.9 ± 1.81
	19.8 ± 2.33	24.7 ± 3.97	207 ± 23.8	209 ± 23.3	10.1 ± 1.20	13.2 ± 2.06

[a] Soil previously cropped in corn.

Table 5. Recovery of Strychnine from Two Soils Treated with about 10 μg/g[a] of the Alkaloid and Sampled at Various Intervals over a 2-Month Period

	strychnine recovery			
	sandy loam soil		sandy clay loam soil	
period of incubation (days)	mean concn (μg ± 1 SD)	%[b]	mean concn (μg ± 1 SD)	%[b]
7	410 ± 6	89.1	422 ± 1	98.4
14	389 ± 15	84.6	411 ± 13	95.8
21	326 ± 20	70.9	397 ± 2	92.5
28	116 ± 60	25.2	217 ± 29	50.6
35	25 ± 4	5.4	86 ± 16	20.1
42	14 ± 11	3.0	28 ± 28	6.5
56	11 ± 2	2.4	33 ± 15	7.7

[a] Strychnine treatment rate about 487 μg/50 g of soil. [b] Values based upon the mean recovery at time of treatment of 460 and 429 μg of strychnine from replicate samples of sandy loam and sandy clay loam soil, respectively.

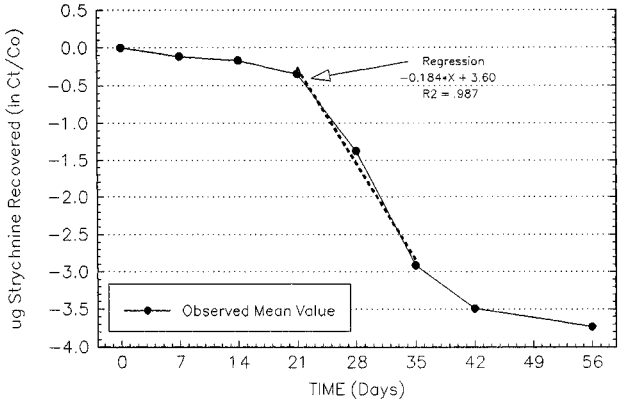

Figure 2. Natural log plot of strychnine recovery versus incubation time for a sandy loam soil treated with 10 μg/g of the chemical.

Possible Mechanism by Which Strychnine Degraded in the Two Soils. Upon application of strychnine to the soils, the chemical was rapidly sorbed to the soil colloids. Following remoistening of the soils to about 75% of field capacity, and during a 21-day lag phase, strychnine sorption/desorption processes were likely ongoing, combined with microbial adaptation—

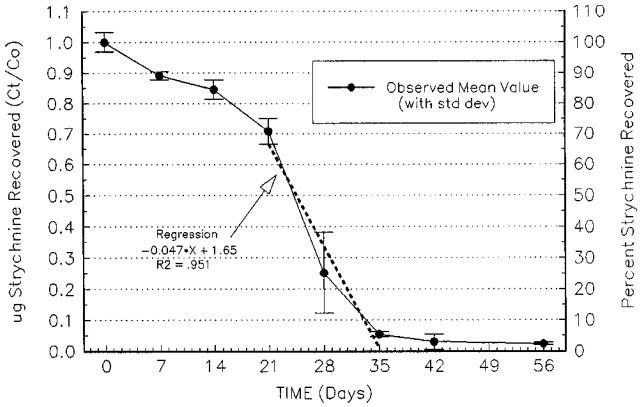

Figure 3. Strychnine recovery from a sandy loam soil treated with 10 μg/g of the chemical and incubated for 2 months.

Table 6. Dissipation Times for 50 and 90% Loss of 10 μg/g Strychnine from Two Different Soils

soil type	DT_{50}[a] (days)	DT_{90}[a] (days)
sandy loam	24	33
sandy clay loam	27	40

[a] Taken from sigmoidal plots of strychnine recovery versus incubation time.

evidence of a breakdown product was suggested to have occurred in both soil types during this period. This adaptative phase may have involved one or more of the following: (1) induction of enzymes specific for degradation of the alkaloid; (2) random mutation by which new metabolic capabilities were produced that allowed breakdown of the chemical to occur; and/or (3) increase in the number of strychnine-degrading organisms. Random microbial mutations of this type would serve to help explain the variability in strychnine recovery results observed between sample replicates in the previous soil biodegradation research that we have conducted at this laboratory (Starr et al., 1992).

During the exponential dissipation phase (days 21−35), a rapid loss of strychnine occurred, after a sufficient quantity of the compound desorbed from the soil and became available in the soil solution, and as microbial activity became sufficiently great. If one assumes that

the initial breakdown product(s) of strychnine was (were) similar to that (those) observed in previous metabolism studies, i.e., strychnine *N*-oxide, 16-hydroxystrychnine, and/or 2-hydroxystrychnine, plus other possible products, these polar metabolites were likely bound rapidly and strongly to the soil colloidal fractions, which resulted in the exponential loss of strychnine from the soil solution during the rapid loss phase (Figure 2). These bound product residues would be anticipated to be degraded by microorganisms, and eventually to be completely mineralized to carbon dioxide, together with the organic matter fraction. Prior to further breakdown of the initial degradation products, movement of these bound compounds within either soil would likely be low.

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