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Solvent Extraction Characterization of Bioavailability of Atrazine Residues in Soils

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Characterization of pesticide bioavailability, particularly in aged soils, is of continued interest because this information is necessary for environmental risk assessment. The objective of this study was to correlate atrazine residue bioavailability in aged soils, as determined by solvent extraction methods, to atrazine mineralization by an atrazine-degrading bacterium. Webster clay loam and Zimmerman fine sand soils were treated with UL-ring-labeled [^{14}C]atrazine and incubated for up to 8 weeks. At the end of each incubation period, soils were either not extracted, extracted with 0.01 M $CaCl_2$, or extracted with 0.01 M $CaCl_2$/aqueous methanol. Soils were then inoculated with the bacterium *Pseudomonas* sp. strain ADP, which is capable of rapidly mineralizing the atrazine ring. This allowed for the evaluation of the bioavailability of aged atrazine residues without the contribution of atrazine desorption from soil. Results of these studies indicated that the amounts of atrazine residues in aged soils extracted by 0.01 M $CaCl_2$ and aqueous methanol were correlated to amounts of atrazine mineralized by *Pseudomonas* sp. strain ADP. Consequently, 0.01 M $CaCl_2$/methanol extractable atrazine in aged soils may be used to estimate bioavailable residues, and this technique may be useful to determine the bioavailability of other compounds in soils, especially other triazine herbicides.

KEYWORDS: Bioavailability; atrazine; *Pseudomonas* sp. strain ADP; aged residues

INTRODUCTION

Characterization of pesticide availability for transport, plant uptake, and microbial degradation, particularly in aged soils, is of continued interest because this information is necessary for environmental risk assessment. Pesticide availability in soils has been evaluated using a variety of indirect and direct methods with various degrees of success. The most common indirect method to characterize pesticide availability is by using a simplistic sorption coefficient (K_d), which is the ratio of the amount of chemical sorbed to that in solution, as determined using batch slurry techniques. Many transport and degradation models have traditionally used sorption K_d values to predict the amount of chemical that is, or can be, available in solution at a given time. However, sorption−desorption processes are complex and cannot be adequately determined using a single value (*1*). For instance, the desorption of many pesticides cannot be predicted from their sorption isotherms due to a hysteresis effect; less chemical is desorbed than would be predicted by the sorption isotherm.

Increased pesticide−soil contact time, that is, aging, has been shown to affect sorption−desorption processes in soil. Aging effects on sorption−desorption processes have been characterized by the calculation of apparent sorption coefficients, $K_{d,app}$, for the pesticide remaining after a given incubation period (*2*−*9*). In these studies, after each aging period, the parent chemical was first extracted from soil with aqueous $CaCl_2$ to give the solution phase concentration and subsequently by organic solvents to obtain the sorbed phase concentration. $K_{d,app}$ can be calculated from these concentrations as is done in traditional batch sorption studies. Increases in apparent sorption coefficients with incubation time have generally been observed using this method with a variety of classes of pesticides and their metabolites, including triazines (*2*, *3*), imidazolinones (*4*, *5*), acetanilides (*2*), nitroguanidines (*6*, *7*), and sulfonylaminocarbonyltriazolinones (*8*, *9*). It is generally accepted that the increase in sorption resulting from aging decreases the availability of the pesticide for transport, plant uptake, and microbial degradation, resulting in the pesticide becoming increasingly recalcitrant.

Indirect methods have also used other solvent extraction techniques. In some cases water-extractable residues were assumed to be available (*10*), whereas in other cases residues that were not extractable (i.e., "bound residues") were assumed to not be available (*11*). However, there are few data to support these contentions.

Direct methods to characterize bioavailability include measurements of pesticide residues taken up from soil by plants, earthworms, and microorganisms. Plants have been shown to

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take up small amounts of ^{14}C-labeled pesticide residues from soils, which had been exhaustively extracted with a variety of solvents (see, for example, refs *12* and *13*); these unidentifiable residues would be considered to be available. Earthworms have also been shown to take up small amounts of ^{14}C-labeled pesticide residues from soils that had been exhaustively extracted with a variety of solvents (*12*, *14*). These authors concluded that ^{14}C-labeled pesticide residues that were not available to organic solvents were available to earthworms.

Pesticide-degrading microorganisms have been recently used to initially characterize the bioavailability of aged pesticide residues. For instance, the bioavailability of aged atrazine residues to *Pseudomonas* sp. strain ADP (*15*), *Ralstonia* sp. strain M91-3 (*16*), and *Agrobacterium radiobacter* strain J14a (*17*) has been shown to decrease with increased aging (*18*−*21*). In addition, aging of isoproturon and its monodesmethyl-isoproturon metabolite in agricultural soil reduced the availability to *Sphingomonas* sp. strain SRS2 (*22*).

Direct characterization of pesticide bioavailability in soil using plants or microorganisms can be expensive and time-consuming. More importantly, it requires the identification of a plant or microorganism that can rapidly take up and/or degrade the pesticide and will provide an easily measured factor that can be correlated to the amount of pesticide that was available. To overcome these difficulties, attempts have been made to characterize bioavailability using solvent extraction techniques (*23*, *24*). These techniques have considerable potential, but have had limited success. The objective of this study was to develop a solvent extraction procedure that could characterize aged *s*-triazine bioavailability in dissimilar soils. Amounts of atrazine extracted by sequential solvent extraction from aged soils were correlated to amounts mineralizable by a specific degrading microorganism.

MATERIALS AND METHODS

Chemicals and Soils. Uniformly ring-labeled [^{14}C]atrazine (0.38 GBq mmol^{-1}, >98% radiopurity) was mixed with unlabeled atrazine (98.7% purity) to give final solution concentrations of 10.63 μg mL^{-1} atrazine and 9.05 MBq mL^{-1}. Two soils from Minnesota were used in this study, a Webster clay loam (Typic Haploquoll) (4.1% OC, 35% clay, pH 6.7) and a Zimmerman fine sand (Alfic Udipsamment) (0.5% OC, 3% clay, pH 5.8). Surface soils (0−20 cm depth) were collected, air-dried, and passed through a 2 mm diameter sieve. Soil texture was determined according to the hydrometer method. Soil pH was measured using a 1:2 (w/w) soil/deionized water mixture. The organic carbon content of the soil samples was determined by dichromate oxidation.

Soil Treatment and Incubation. Air-dry soils (10 g) were spiked with 1 mL of the aqueous [^{14}C]atrazine solution in large-mouth centrifuge bottles. The moisture content of the soil was adjusted to −33 kPa, which corresponds to 0.34 and 0.10 g g^{-1} of water for the clay loam and sand, respectively. A vial containing 5 mL of 1 N NaOH was placed in the centrifuge bottles, which were then sealed. Soils were incubated at 25 ± 0.5 °C for 0, 2, 4, and 8 weeks.

To determine atrazine residue mineralization, $^{14}CO_2$ evolution was measured after trapping in NaOH. NaOH was replaced weekly, thereby allowing aeration of the soil mixture. To determine $^{14}CO_2$ released, a 1 mL aliquot of NaOH solution was mixed with 6 mL of EcoLite scintillation cocktail, and the amount of radioactivity was determined by liquid scintillation counting (LSC) for 5 min using a 1500 Tri-Carb Packard liquid scintillation analyzer.

After each incubation period, soils were either not extracted (treatment 1), extracted with 0.01 M $CaCl_2$ (treatment 2), or extracted with 0.01 M $CaCl_2$ followed by aqueous methanol (80:20 v/v methanol/water) (treatment 3). For treatment 1, the soil was dried at 40 °C for 24 h after the incubation. Treatment 2 consisted of adding 50 mL of 0.01 M $CaCl_2$ to the soil in centrifuge bottles and shaking for 16 h. Soil suspensions were centrifuged at 3000*g* for 15 min, and the supernatant was removed and analyzed for ^{14}C. The soil in the pellet was dried at 40 °C for 24 h. For treatment 3, the soils were first extracted as in treatment 2, and the soil remaining after centrifugation was extracted twice by shaking for 4 h each time with 50 mL of aqueous methanol. The methanol extracts recovered after centrifugation (15 min, 3000*g*) were combined and analyzed for ^{14}C. The soil remaining after centrifugation was dried at 40 °C for 24 h.

The dried soil in centrifuge bottles from each treatment was macerated, and the soil was thoroughly mixed and inoculated with 1 mL of *Pseudomonas* sp. strain ADP that was prepared just before use at each incubation time. *Pseudomonas* sp. strain ADP (*15*) was grown in MSB medium (*25*) containing 100 μg mL^{-1} atrazine. Cultures were centrifuged at 10000*g* for 10 min and resuspended in 0.85% NaCl to a final concentration of 1×10^9 cells mL^{-1}. The final inoculum density was 1×10^8 cells g^{-1} of soil. The moisture content of the soil was adjusted to −33 kPa, and the inoculated soils were incubated for 20 days at 25 ± 0.5 °C with monitoring of the $^{14}CO_2$ evolved as previously described. At the end of the incubation period, soils were extracted with aqueous methanol as described above. All experiments were done in triplicate.

RESULTS AND DISCUSSION

[^{14}C]Atrazine Residue Distribution. Distribution of the applied [^{14}C]atrazine residues during incubation in the clay loam soil was significantly different from that in fine sand soil. Whereas 46% of the applied atrazine was mineralized during the 8 week incubation in clay loam soil, <4% was mineralized in fine sand soil. The results for the clay loam were similar to those previously reported (*26*). Water-extractable fractions of [^{14}C]atrazine residues in both soils decreased with incubation time, from 36 to 6% in clay loam soil (**Figure 1a**, EX) (bars labeled EX) and from 72 to 34% in fine sand soil (**Figure 2a**, EX). In the clay loam soil, methanol-extractable fractions of [^{14}C]atrazine residues decreased from 53 to 17% after a 4 week incubation and then remained constant to the end of the incubation period (**Figure 1a**,**b**, EX). In contrast, the methanol-extractable fraction remained relatively constant, decreasing only from 23 to 16% during the 8 week incubation in fine sand soil (**Figure 2a**,**b**, EX). The nonextractable fraction (bound residues) of [^{14}C]atrazine residues significantly increased with incubation time in both soils (**Figure 1**, EX, and **Figure 2**, EX).

Data in **Figures 1** and **2** were used to calculate effects of aging on apparent sorption coefficients ($K_{d,app}$) in clay loam and fine sand soils, respectively. $K_{d,app}$ values calculated from aqueous $CaCl_2$-extractable amounts of atrazine residues (solution phase) and methanol-extractable amounts (sorbed phase) at time 0 (without incubation) were 7.4 mL g^{-1} for the clay loam soil and 1.6 mL g^{-1} for the fine sand soil. These values were similar to those previously obtained by batch equilibration with similar initial concentration ranges (*27*, *28*). $K_{d,app}$ values increased to a greater extent in the clay loam soil during the 8 week incubation period than in the fine sand soil (**Figure 3**). Values of $K_{d,app}$ after the 8 week incubation period increased by factors of 2.0 for the clay loam soil ($K_{d,app}$ = 14.7 mL g^{-1}) and 1.4 for the sand ($K_{d,app}$ = 2.3 mL g^{-1}), as compared to values determined in freshly treated soil. $K_{oc,app}$ increased from 180 to 359 mL g^{-1} for the clay loam and from 320 to 460 mL g^{-1} for the sand during the 8 week incubation.

The increase in $K_{d,app}$ was similar to that observed for other pesticides under similar conditions (see, for example, refs *4*−*7*) and could indicate a decrease in availability. The increase in calculated sorption coefficients for atrazine residues with increasing incubation time may be attributed to an increased rate of degradation in solution and on labile sites relative to the rate of desorption from soil (*29*, *30*). Thus, degradation of labile chemical would leave more strongly sorbed residues on the soil

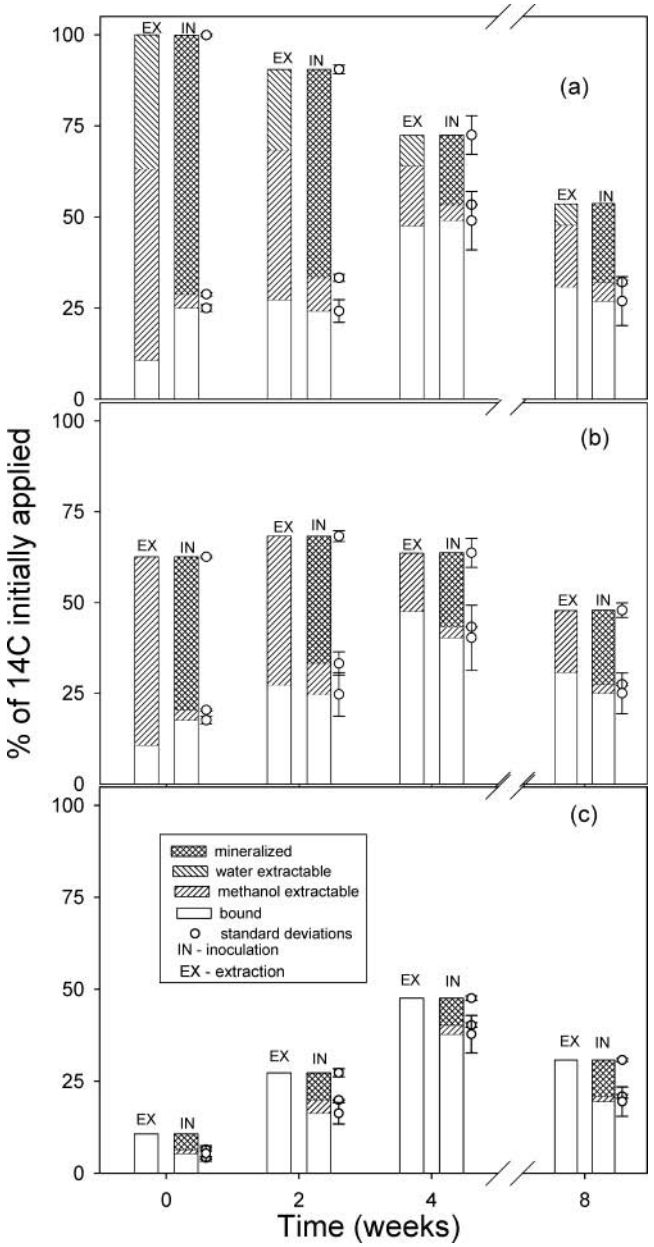

Figure 1. [^{14}C]Atrazine residue distribution among aqueous extractable (**a**, EX), aqueous methanol (**b**, EX) extractable, and unextractable (**c**, EX) fractions in clay loam soil as a function of incubation time. Mineralizable [^{14}C]atrazine residue fractions as a function of incubation time are the differences between 100% and the totals in **a**, EX. Effects of aging on mineralization of [^{14}C]atrazine residues by *Pseudomonas* sp. strain ADP are shown for unextracted [^{14}C]atrazine-treated clay loam soil (**a**, IN), [^{14}C]atrazine-treated soil previously extracted with 0.01 M $CaCl_2$ (**b**, IN), and [^{14}C]atrazine-treated soil previously extracted with 0.01 M $CaCl_2$/methanol (**c**, IN).

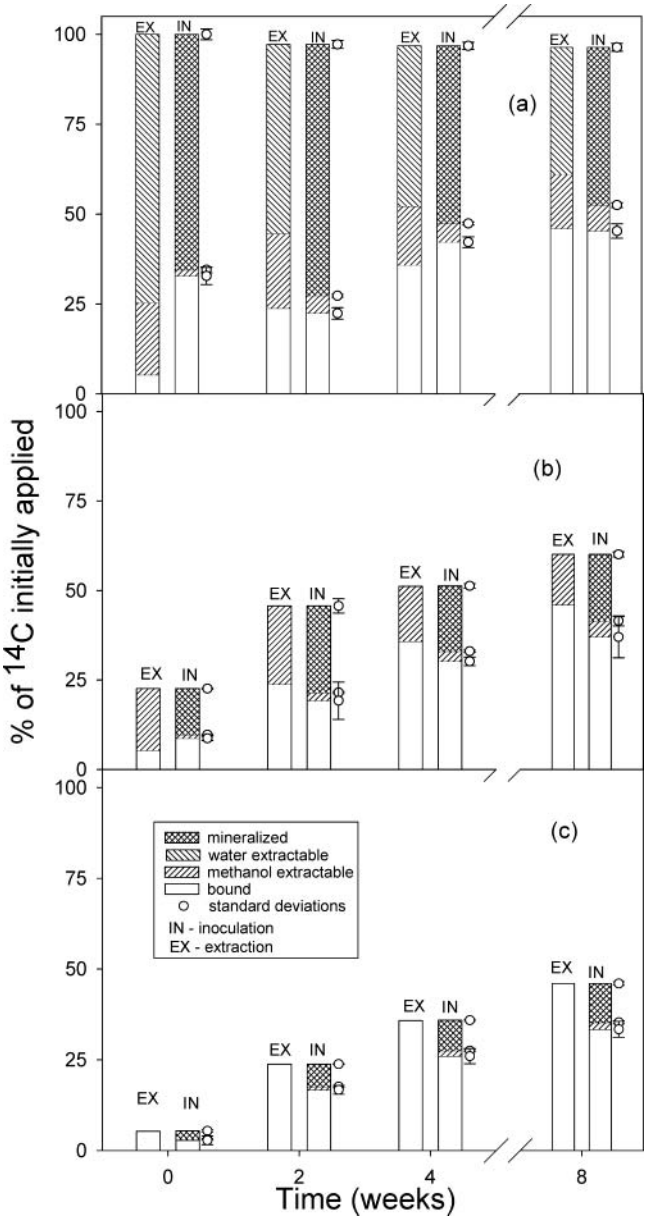

Figure 2. Distribution of [^{14}C]atrazine residues among aqueous extractable (**a**, EX), aqueous methanol (**b**, EX) extractable, and unextractable (**c**, EX) fractions in fine sand soil as a function of incubation time. Mineralizable [^{14}C]atrazine residues fractions as a function of incubation time are the differences between 100% and the totals in **a**, EX. Effects of aging on mineralization of [^{14}C]atrazine residues by *Pseudomonas* sp. strain ADP are shown for unextracted [^{14}C]atrazine-treated clay loam soil (**a**, IN), [^{14}C]atrazine-treated soil previously extracted with 0.01 M $CaCl_2$ (**b**, IN), and [^{14}C]atrazine-treated soil previously extracted with 0.01 M $CaCl_2$/methanol (**c**, IN).

surface, resulting in a net increase in $K_{d,app}$ values. The increase in calculated sorption coefficients can also be attributed to diffusion of the residues to less accessible sorption sites or "stronger" binding sites. We hypothesize that if diffusion processes were coupled to degradation of readily available chemical, it would also result in the net effect observed, decreased solution phase and increased sorbed phase concentrations.

Characterization of Bioavailability. *Pseudomonas* sp. strain ADP rapidly mineralized significant amounts of [^{14}C]atrazine immediately after inoculation into both soils. In nonincubated soil, 71% of applied [^{14}C]atrazine was mineralized immediately after [^{14}C]atrazine application (the majority of which was mineralized in <2 days) in the clay loam soil (**Figure 1a**, IN) (bar labeled IN). When [^{14}C]atrazine-treated clay loam soil, which had been incubated for 4 weeks, was inoculated with *Pseudomonas* sp. strain ADP, the amount of [^{14}C]atrazine mineralized decreased to 20% of that applied. A similar amount of [^{14}C]atrazine was mineralized after inoculation of soil that had been incubated for 8 weeks.

In fine sand soil, 66% of applied [^{14}C]atrazine was mineralized by *Pseudomonas* sp. strain ADP immediately after [^{14}C]-atrazine application to nonincubated soil (**Figure 2a**, IN). The

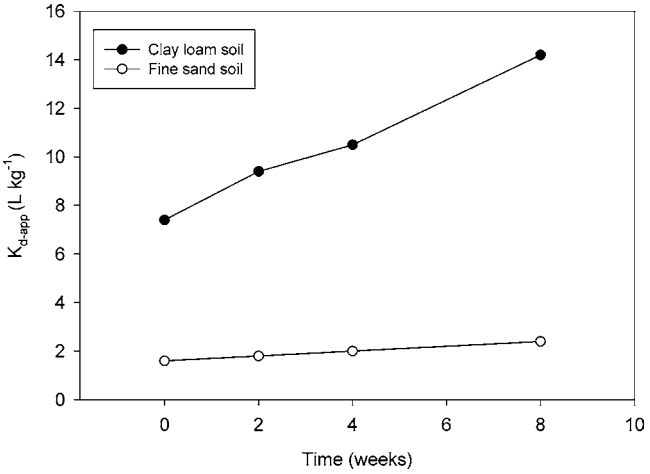

Figure 3. Effects of aging on apparent sorption coefficients, $K_{d,app}$, for atrazine residues in clay loam and fine sand soils.

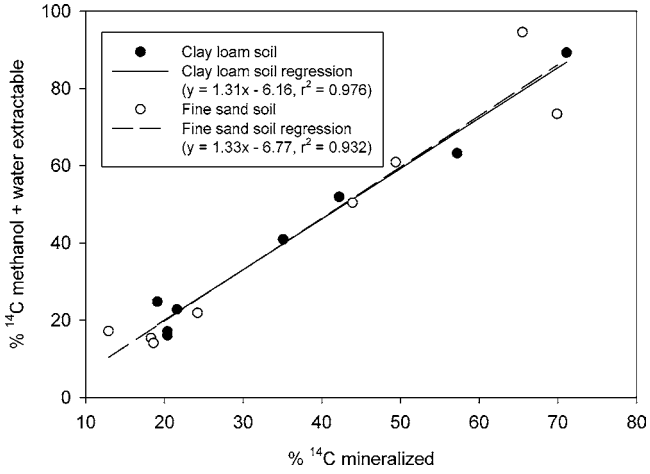

Figure 4. Correlation of aqueous methanol extractable and mineralizable [^{14}C]atrazine residues in clay loam and fine sand soils.

amount of [^{14}C]atrazine mineralized decreased to 49% when soil was inoculated after a 4 week incubation period and remained the same throughout the rest of the incubation.

After the water-extractable [^{14}C]atrazine residues were removed from incubated soil samples, [^{14}C]atrazine was still bioavailable as evidenced by *Pseudomonas* sp. strain ADP being able to mineralize significant amounts of [^{14}C]atrazine at each sampling time. For instance, the amount of atrazine residues mineralized by *Pseudomonas* sp. strain ADP immediately after water-extractable [^{14}C]atrazine residues were removed from the clay loam soil at time 0 was 42% and decreased to 20% after removal of the water-extractable [^{14}C]atrazine residues after an 8 week incubation (**Figure 1b**, IN). In contrast, during the 8 week incubation of the fine sand soil, an average of 19% of [^{14}C]atrazine was mineralized by *Pseudomonas* sp. strain ADP after removal of water-extractable [^{14}C]atrazine residues from the samples (**Figure 2b**, IN). There were still small amounts (<10%) of [^{14}C]atrazine that were bioavailable after sequential extraction with water and methanol solutions in both clay loam (**Figure 1c**, IN) and sand (**Figure 2c**, IN) soils. From these data it appears that small amounts of residues desorbed from the soils during the 2 day incubation with *Pseudomonas* sp. strain ADP, which were subsequently mineralized.

The amounts of [^{14}C]atrazine mineralized by *Pseudomonas* sp. strain ADP in the clay loam soil at each sampling time were similar to the total amounts of [^{14}C]atrazine residues sequentially extracted by water and methanol solutions: % extracted = 1.31 × % mineralized − 6.16, r^2 = 0.976 (**Figure 4**). Soil type did not affect the relationship between bioavailability and extractability; a similar relationship was found for the fine sand soil (% extracted = 1.33 × % mineralized − 6.77, r^2 = 0.932).

The slightly greater amounts of [^{14}C]atrazine residues extracted from soils as compared to the amounts being mineralized may be attributed to a number of factors including differences in desorption/extraction kinetics and diffusion of residues. Some of the differences between the amounts of solvent extractable and the mineralizable residues may be reduced or eliminated by decreasing the shaking time for the solvent extraction or decreasing the solvent strength (using a less efficient solvent), both of which would decrease the amount of extractable residues. Moreover, improving the distribution of the microorganism would provide more opportunities for exposure of the residues to microorganisms, which would increase mineralization within the time frame of the experiment.

Nevertheless, regardless of the mechanisms involved in the observed differences, which we characterized as being minor, the 0.01 M $CaCl_2$/methanol solvent extraction procedure accurately determined atrazine bioavailability in dissimilar aged soils and may be useful to determine the bioavailability of other compounds in soils, particularly other *s*-triazine herbicides. Although it might be suggested that the greatest amount of recoverable pesticide would be the best indicator for exposure and risk assessment for a pesticide, amounts extractable by harsh, exhaustive treatments have not been shown to correlate to amounts available for degradation, transport, or uptake. It may have been fortuitous, but it should be noted that the most commonly used solvent for the past 25 years for *s*-triazine extraction from soil in field dissipation studies has been aqueous methanol, an extraction procedure that has now been correlated to bioavailability. More work is needed in this area, particularly with the newer classes of chemistries currently being used in agriculture

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