

## Influence of Soil Aging on Sorption and Bioavailability of Simazine

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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. However, pesticide bioavailability in aged soils has been characterized by a variety of methods with limited success, due in part to methodological limitations. The objective of this study was to use solvent extraction methods to correlate simazine residue bioavailability in aged soils to simazine mineralization using a simazine-mineralizing bacterium. Soils from Brazil, Hawaii, and the midwestern United States were treated with UL-ring-labeled [<sup>14</sup>C]simazine and incubated for up to 8 weeks. At the end of each incubation period, soils were either incubated further, extracted with 0.01 M CaCl<sub>2</sub>, or extracted with aqueous methanol (80:20 v/v methanol/water). In a parallel experiment, after each incubation period, soils were inoculated with the bacterium *Pseudomonas* sp. strain ADP, which is capable of rapidly mineralizing simazine, and <sup>14</sup>CO<sub>2</sub> was determined. The inoculated soil samples were then extracted with 0.01 N CaCl<sub>2</sub> and with aqueous methanol. This allowed for the evaluation of the bioavailability of aged simazine residues, without the contribution of simazine desorption from soil. Results of these studies indicated that simazine sorption to soil increased with aging and that amounts of simazine in aged soils extracted by 0.01 M CaCl<sub>2</sub> and aqueous methanol were highly correlated to amounts of simazine mineralized by *Pseudomonas* sp. strain ADP. Consequently, 0.01 M CaCl<sub>2</sub>/methanol-extractable simazine in aged soils can be used to estimate bioavailable residues. This technique may be useful in determining the bioavailability of other *s*-triazine compounds in soils.

**KEYWORDS:** Simazine; bioavailability; aged residues; sorption–desorption

### INTRODUCTION

Numerous classes of pesticides and their metabolites have been found in surface waters and shallow wells sampled in U.S. agricultural areas (1). This has raised concerns regarding their impact on human health and the functioning and health of aquatic and terrestrial ecosystems. To protect ground and surface waters from pesticide contamination, and ameliorate their impact, broad knowledge is required concerning biodegradation and sorption–desorption processes influencing their persistence in the environment. Sorption–desorption processes are arguably the most important factors influencing pesticide fate, because they control the amount of chemical available for pest control, transport, and degradation.

Sorption–desorption reactions are affected by both the physical and chemical properties of the soils and pesticides and are strongly influenced by soil–pesticide contact time, or aging.

Increased sorption with aging has been observed for a variety of pesticide classes using a variety of methods (2–14). The mechanisms by which these compounds become sorbed, or sequestered, in soils as they age are poorly understood. Slow diffusion within small pores of soil aggregates, hydrophobic partitioning into solid humic materials (15, 16), entrapment into the hydrophobic surface nanopores (17), and sorption into nondesorbable sites of soil organic matter (7) have all been proposed as possible mechanisms involved in the aging process. However, regardless of the mechanism involved, the net effect is that the use of simplistic equilibrium partitioning coefficients, based on freshly treated samples under slurry conditions, predicts much greater availability for movement of these chemicals than the use of sorption coefficients determined on aged residues.

To improve models describing pesticide availability for transport in soil, a better understanding of the complex interactions between sorption–desorption and degradation processes, particularly for aged pesticide residues, is needed. A variety of studies have suggested that only pesticide in solution, or pesticide is readily desorbable from soil, is available for either transport or degradation (11). Recent studies also suggest that

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**Table 1.** Physical–Chemical Properties and Simazine Sorption in Selected Glacial (US) and Tropical (BR, HW) Region Soils

soil	clay (%)	silt (%)	sand (%)	OC <sup>a</sup> (%)	pH	$K_d^b$ (L kg <sup>-1</sup> )	$K_{oc}^c$ (L kg <sup>-1</sup> )	sorbed (%)	SFC <sup>d</sup> (G g <sup>-1</sup> )	textural class
BR-1	22	11	67	0.98	5.6	3.6	367	64	0.23	sandy clay loam
BR-2	40	8	52	1.34	5.5	4.0	299	66	0.34	sandy clay
US-1	4	3	93	0.95	6.3	0.7	74	26	0.11	sand
US-2	19	56	25	2.26	6.3	3.6	159	64	0.34	silt loam
HW-1	15	66	19	2.62	8.0	6.2	237	75	0.35	silt loam
HW-2	74	18	8	1.43	5.5	1.0	70	32	0.55	clay

<sup>a</sup> Organic carbon content. <sup>b</sup> Sorption partition coefficient obtained by batch methods. <sup>c</sup>  $K_{oc} = K_d \times 100/OC$ . <sup>d</sup> Soil moisture at field capacity (−33 kPa).

bacteria can access specific regions where the herbicide is sorbed, which is supported by higher atrazine mineralization rates than would be predicted by solution concentrations (6).

A mathematical desorption model has been developed that assumes that desorption rates can be influenced by three types of processes: rapid desorption (equilibria), rate-limited desorption (nonequilibria), and nondesorption phenomena (6, 7). Availability would be directly related to the pesticide's ability to be desorbed from soil and therefore weakly sorbed and easily desorbed pesticides would be readily available for transport and biodegradation. In contrast, pesticides that are strongly sorbed and hysteretic during desorption would be slowly available over time, and extremely strongly sorbed pesticides would be unavailable, because they tend to form bound residues.

Pesticides that persist in soils often become increasingly less bioavailable, as indicated by markedly declining rates of biodegradation with aging (6, 14, 18–20). In some instances, the sorbed fraction of the pesticide is totally resistant to microbial attack, whereas, in others, sorption only reduces its release rate, but does not eliminate biodegradation.

Bioavailability has also been characterized, or predicted, by using mild solvent extraction as opposed to more vigorous harsh solvent extraction (19, 21). However, results on the efficacy of using mild organic solvent extractants to estimate pesticide bioavailability in soils are still conflicting. For example, Chung and Alexander (20) showed that the extent of the sorption process differed among soils with different properties, but they were not capable of establishing a strong correlation between extractability and direct characterization of bioavailability. In contrast, Barriuso et al. (14) found that 0.01 M CaCl<sub>2</sub>/methanol-extractable atrazine in aged soils could be used to estimate bioavailable residues ( $r^2 > 0.93$ ). The establishment of such a correlation would be useful for predicting the quantities available for uptake, allowing the assessment of actual risks resulting from pesticide application to soil.

In the studies reported here, we determined the effects of aging on the sorption–desorption and biodegradation of simazine (6-chloro-*N*<sup>2</sup>,*N*<sup>4</sup>-diethyl-1,3,5-triazine-2,4-diamine) in both glacial and tropical soils. We also developed a solvent extraction procedure, which allowed prediction of simazine bioavailability in aged soils. Results reported here show that bioavailability corresponded to the amounts of simazine mineralized by *Pseudomonas* sp. strain ADP, an atrazine- and simazine-degrading bacterium, and was correlated to the amounts of simazine extracted by a sequential solvent extraction procedure. Such studies are very scarce in the literature, particularly for tropical soils. Simazine was selected because it is the second most commonly detected pesticide in surface and ground waters in the United States, Europe, and Australia (22), presumably because of the combination of its solubility (6.2 mg L<sup>-1</sup>), low sorption ( $K_{oc} = 20–180$  L kg<sup>-1</sup>), and relatively high persistence in both soil and water (DT<sub>50</sub> = 27–102 days) (23).

## MATERIALS AND METHODS

**Soils and Chemicals.** Six representative soils from glacial and tropical regions were used in this study: two from Brazil (BR-1 and BR-2), two from the midwestern United States (US-1 and US-2), and two from Hawaii (HW-1 and HW-2). The soils were collected from the surface layer (0–0.2 m depth), and part was air-dried and passed through a 2-mm sieve to determine soil properties. The remaining soils were also passed through a 2-mm sieve, but kept in the dark at room temperature. Soil texture was determined according to the hydrometer method. Soil pH was measured using a 1:2 (w/v) soil/deionized water mixture. The organic carbon (OC) content was determined by dichromate oxidation. Results of soil analysis are presented in **Table 1**.

Unlabeled simazine (98.1% purity) was mixed with uniformly ring-labeled [<sup>14</sup>C]simazine (0.57 GBq mmol<sup>-1</sup>, >98% radiochemical purity) to give final solution concentrations of 2.14 μg mL<sup>-1</sup> and 0.14 kBq mL<sup>-1</sup> in 0.01 N CaCl<sub>2</sub> for batch sorption studies and 240.7 μg mL<sup>-1</sup> and 27.16 kBq mL<sup>-1</sup> in methanol for incubation studies.

**Batch Sorption.** Aliquots (10 mL) of aqueous [<sup>14</sup>C]simazine solution were added to 5 g of air-dried soils. Soil slurries were horizontally shaken for 24 h at 25 ± 1 °C and centrifuged at 3000 rpm (1076g) for 15 min. One-milliliter aliquots of the supernatants were removed and added to 6 mL of EcoLite scintillation cocktail, and the solutions were kept in the dark overnight prior to measurement. Radioactivity in the solution was measured ( $C_e$ , dpm mL<sup>-1</sup>) by liquid scintillation counting (LCS) for 5 min using a Tri-Carb1500 Packard liquid scintillation analyzer. Sorbed concentrations ( $S$ , dpm g<sup>-1</sup>) of simazine were calculated by the difference between its initial concentration ( $C_i$ , dpm mL<sup>-1</sup>) and  $C_e$ . Sorption coefficients ( $K_d$ , mL g<sup>-1</sup>) were calculated from  $K_d = S/C_e$ . Sorption coefficients normalized to organic carbon (OC) content ( $K_{oc}$ , mL g<sup>-1</sup>) were calculated by  $K_{oc} = K_d/OC \times 100$ .

**[<sup>14</sup>C]Simazine Dissipation and Sorption in Aged Soils.** Aliquots (0.2 mL) of methanolic [<sup>14</sup>C]simazine solution were added dropwise to 25 g of air-dried soil samples in 250-mL Erlenmeyer flasks. Soil moisture contents were adjusted to 75% of field capacity (−33 kPa), and the soil samples were thoroughly mixed. Vials containing 1 mL of 1 N NaOH were placed inside the Erlenmeyer flasks by attachment to the inside of stoppers. Soils were incubated in the dark at 25 ± 1 °C for 0, 7, 14, 28, 55, and 102 days. To determine the simazine mineralization rate, trapped <sup>14</sup>CO<sub>2</sub> in NaOH solutions was measured weekly. The NaOH solutions were replaced each time, which also aerated the incubated soils. One-milliliter aliquots of NaOH solutions were added to 6 mL of EcoLite cocktail and analyzed for <sup>14</sup>C as described for the sorption experiment.

After each incubation period, replicate soil samples were first extracted with 50 mL of 0.01 N CaCl<sub>2</sub> by shaking on a horizontal shaker for 20 h. The soil slurries were centrifuged at 3000 rpm (1076g) for 15 min and the supernatants removed. One-milliliter aliquots of the supernatant were analyzed for <sup>14</sup>C as previously described. The remaining soil was then extracted with 50 mL of aqueous methanol (80:20 v/v methanol/water) by shaking on a horizontal shaker for 20 h. The soil slurries were again centrifuged at 3000 rpm (1076g) for 15 min and the supernatants removed. One-milliliter aliquots of the supernatant were analyzed for <sup>14</sup>C as previously described. The remaining soil was extracted a second time with 50 mL of aqueous methanol. The individual methanol extracts were rotary evaporated at 35 °C. The <sup>14</sup>C in 0.01 N CaCl<sub>2</sub> extract and remaining aqueous extracts from the two aqueous methanol extractions was partitioned into 10 mL of dichloromethane (DCM) twice, and the two DCM extracts were

combined. The DCM solutions were rotary evaporated to dryness, and the simazine residue was redissolved in 1 mL of acetonitrile/water (50:50 v/v).

Extracts were analyzed by high-performance liquid chromatography (HPLC) using a 1090 Hewlett-Packard high-performance liquid chromatograph and a 250 mm × 4.6 mm i.d. Alltech Inertsil ODS-2 (5 μm) column operating at room temperature (~25 °C). The mobile phase used was a gradient of acetonitrile (ACN) and filtered distilled water. The flow rate was 0.75 mL min<sup>-1</sup>. Injection volumes were 100 μL. On the basis of the retention time of authentic simazine, the HPLC fraction corresponding to parent chemical was collected and mixed with liquid scintillation cocktail, and <sup>14</sup>C was quantified by LSC. Fractions containing <sup>14</sup>C peaks that were more and less mobile than the parent chemical were also collected and quantified. The percentage of <sup>14</sup>C that was represented by the parent herbicide in the supernatant was calculated and used to determine total parent chemical in each supernatant solution.

To calculate apparent sorption coefficients,  $K_{d,app}$ , after different aging periods, it was assumed that water-extractable simazine represented the solution phase concentration ( $C_e'$ ), that methanol-extractable simazine represented the sorbed phase concentration ( $S'$ ), and that  $K_{d,app} = S'/C_e'$ . The sum of the amounts of simazine sorbed and in solutions was used to calculate dissipation kinetics.

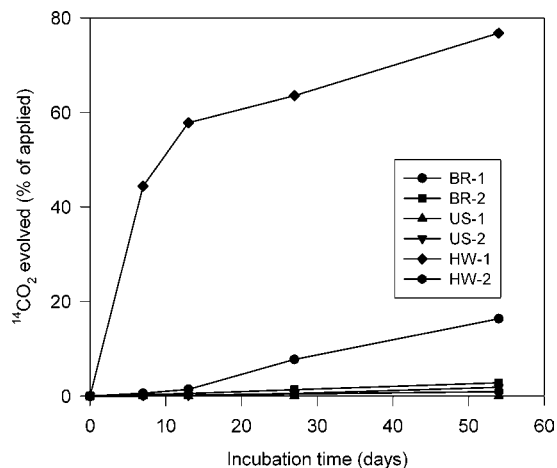
The remaining soil was dried at 40 °C for ~36 h and macerated, and 0.25-g subsamples were combusted using a Packard 307 sample oxidizer (Packard Instrument Co., Downers Grove, IL) to determine nonextractable (bound) [<sup>14</sup>C]simazine residues. The resulting <sup>14</sup>CO<sub>2</sub> was trapped in Carbo-Sorb E mixed with Permafluor V, and the <sup>14</sup>C was quantified by LSC. Oxidizer efficiency was calculated prior to combustion of the samples to correct for any possible error in recovery.

**[<sup>14</sup>C]Simazine Bioavailability to *Pseudomonas* in Aged Soils after Inoculation.** In parallel to the previous solvent extraction experiment, another experiment was performed to determine the bioavailability of aged simazine residues. After each incubation period (0, 7, 14, 28, and 55 days), flasks with soil incubated with [<sup>14</sup>C]simazine were inoculated with a 1.5 mL suspension of *Pseudomonas* sp. strain ADP to a final inoculum density of 1 × 10<sup>8</sup> cells g<sup>-1</sup> of soil. This bacterium was previously isolated for its ability to use atrazine as a sole source of nitrogen for growth and to mineralize this compound to carbon dioxide and ammonia (24). Prior to its use during each incubation period, *Pseudomonas* sp. strain ADP was grown in R minimal medium (25) containing simazine as the sole source of nitrogen for growth, as previously described (26). The soils were moistened to their field capacities and thoroughly mixed, and vials containing NaOH to trap the <sup>14</sup>CO<sub>2</sub> evolved were added and stoppered. Amounts of <sup>14</sup>CO<sub>2</sub> were determined at 6, 18, 30, 42, 66, 90, and 138 h after inoculation. The inoculated soil samples were extracted with 0.01 N CaCl<sub>2</sub> and with 80% aqueous methanol, twice, as described above. Subsamples of the extracted soils were combusted to quantify nonextractable fractions of simazine residues as described above.

## RESULTS AND DISCUSSION

**Sorption.** Sorption of simazine varied from low to moderate in the six soils examined.  $K_d$  values ranged from 0.7 to 6.2 L kg<sup>-1</sup> (Table 1). Sorption was lower in the sandy soil (US-1) with the lowest OC content, in which 26% of the applied simazine was sorbed. In contrast, sorption was higher in the soil (HW-1) with the highest OC content, in which 75% of the applied simazine was sorbed. These results were consistent with previous studies, which showed simazine sorption is affected mainly by the OC content of the soil (27, 28), but can also sorb on hydrophobic microsites of smectites (29). The magnitude of sorption is in agreement with the literature (i.e., refs 27 and 30).

**[<sup>14</sup>C]Simazine Residue Distribution in Aged Soil.** A mass balance of 103 ± 6% of the applied <sup>14</sup>C was obtained in studies performed to evaluate the effect of aging on mineralization and sequential solvent extraction of [<sup>14</sup>C]simazine residues in soils.

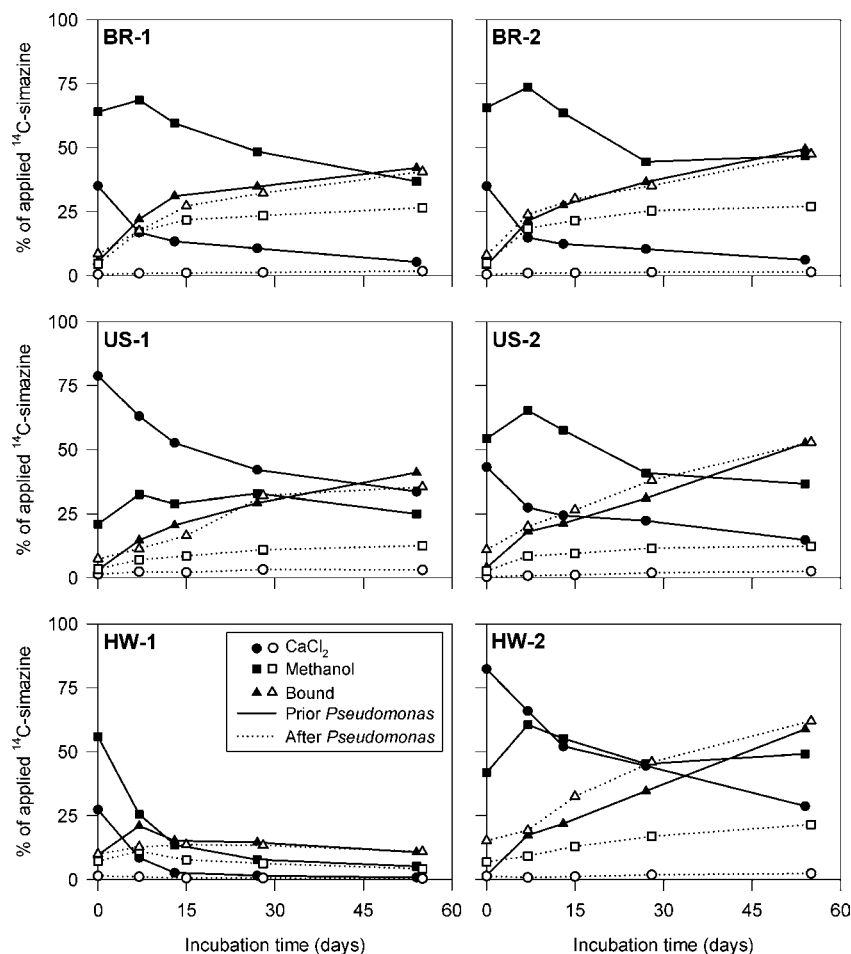


**Figure 1.** [<sup>14</sup>C]Simazine mineralization in Brazilian (BR), midwestern U.S. (US), and Hawaiian (HW) soils. Average coefficients of variation ranged from <2% for HW-1, which had the greatest mineralization, to 31% for US-1, which had negligible mineralization.

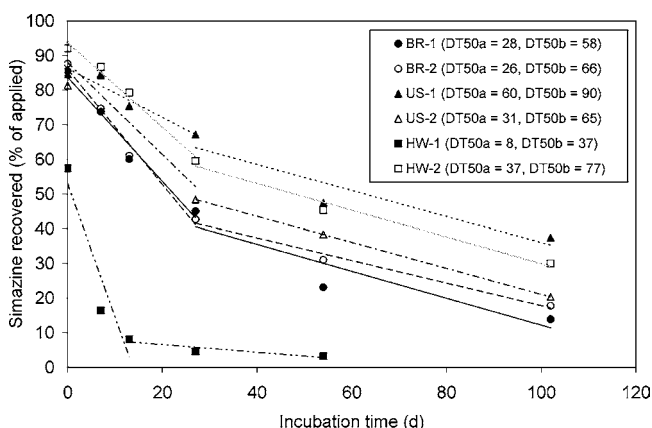
The distribution of the [<sup>14</sup>C]simazine residues between mineralized, water-extractable, methanol-extractable, and unextractable residues varied among the six soils during the 54-day incubation. The amount of [<sup>14</sup>C]simazine mineralized in soils was small (<3% of the applied in the BR-2, US-1, US-2, and HW-2 soils and <16% in the BR-1 soil) in all soils, except HW-1, which had 77% of the applied [<sup>14</sup>C]simazine mineralized (Figure 1). The extent of simazine mineralization in the HW-1 soil was unusual, considering the relatively high sorption potential in this soil (Table 1). This soil must have had an active microbial population efficient in degrading simazine and a desorption rate that was not rate limiting to the mineralization. Additional work on this soil is necessary to identify the degrading organism.

The water-extractable fractions of [<sup>14</sup>C]simazine residues decreased during the 54-day incubation period in all soils: from 35 to 5% in BR-1, from 34 to 6% in BR-2, from 79 to 34% in US-1, from 43 to 15% in US-2, from 27 to 1% in HW-1, and from 66 to 23% in HW-2 (Figure 2, solid circles, solid line). As expected, the soils with the greatest fractions of water-extractable [<sup>14</sup>C]simazine residues (US-1 and HW-2) also had the lowest sorption  $K_d$  values (Table 1). Methanol-extractable fractions of [<sup>14</sup>C]simazine residues also tended to decrease with incubation time, from 69 to 37% in BR-1, from 74 to 47% in BR-2, from 33 to 25% in US-1, from 65 to 37% in US-2, from 56 to 5% in HW-1, and from 48 to 33% in HW-2 (Figure 2, solid squares, solid line). In four of the six soils, the amount of methanol-extracted [<sup>14</sup>C]simazine residues at day 7 of the incubation was greater than that at day 0. This increase could in part be due to the formation of dealkylated metabolites of simazine, which were not water extractable but were methanol extractable. These metabolites could then further degrade or bind to soils; the hydroxyl metabolites are strongly and rapidly sorbed and difficult to remove with mild extractants. In contrast, the formation of nonextractable <sup>14</sup>C-bound residues significantly increased with incubation time in five of the soils: from 6 to 42% in BR-1, from 4 to 50% in BR-2, from 3 to 41% in US-1, from 4 to 53% in US-2, and from 1 to 47% in HW-2 (Figure 2, solid triangles, solid line). The exception was HW-1, the soil that had the highest rate of mineralization.

**[<sup>14</sup>C]Simazine Dissipation and Sorption in Aged Soils.** The main route of simazine dissipation was the formation of bound residues: >60% of the applied [<sup>14</sup>C]simazine was bound to soils at the end of the incubation period (102 days). In the HW-1 soil, however, the main route of dissipation was by the complete



**Figure 2.**  $^{14}\text{C}$ Simazine residues distribution between water-extractable (0.01 N  $\text{CaCl}_2$ ), methanol-extractable (80% methanol), and nonextractable fractions (soil bound), prior to (solid symbols, solid line) and after (open symbols, dotted line) inoculation with *Pseudomonas* sp. strain ADP in aged soils. Average coefficients of variation (CV) for water-extractable data for all incubation periods varied between soils and ranged from 8 to 17%, whereas the average CVs ranged from 2 to 7% for the methanol-extractable data.



**Figure 3.** Biphasic  $^{14}\text{C}$ simazine dissipation in Brazilian (BR), midwestern U.S. (US), and Hawaiian (HW) soils.  $\text{DT}_{50}$  values for the initial ( $\text{DT}_{50a}$ ) and subsequent ( $\text{DT}_{50b}$ ) dissipation are shown.

mineralization of simazine to  $\text{CO}_2$ ; 77% of the applied  $^{14}\text{C}$ -simazine was mineralized in 55 days.

$^{14}\text{C}$ Simazine in extractable  $^{14}\text{C}$  residues was characterized by HPLC during the 102-day incubation period. Dissipation was biphasic; the calculated simazine 50% dissipation times ( $\text{DT}_{50}$ ) for the rapid initial phase ranged from 8 to 60 days and then increased to 37–90 days for the slower phase (Figure 3). The order for the rates of dissipation was  $\text{HW-1} > \text{BR-1} \sim \text{BR-2} \sim \text{US-2}$ ,  $\text{HW-2} > \text{US-1}$ . In general, except for the HW-1, these

**Table 2.** Aging Effects on Simazine Apparent Sorption Coefficients ( $K_{d,\text{app}}$ )<sup>a</sup> in Selected Glacial (US) and Tropical (BR, HI) Region Soils

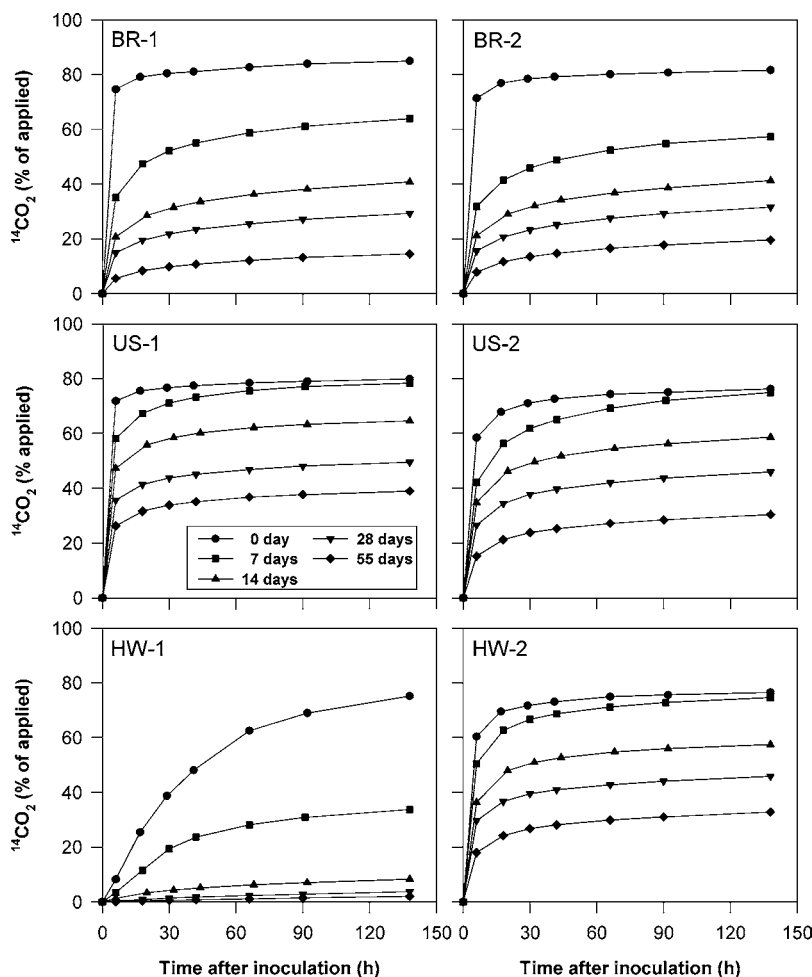
aging time (days)	$K_{d,\text{app}}$ ( $\text{L kg}^{-1}$ )					
	BR-1	BR-2	US-1	US-2	HW-1	HW-2
0	3.7	4.0	0.5	3.0	7.0	1.9
7	8.1	10	1.0	5.2		1.9
13	8.4	9.5	0.9	4.7		2.0
27	9.3	8.2	1.2	3.4		2.0
54	15	15	1.1	6.7		3.4
102	26	17	1.3	8.1		3.5

<sup>a</sup>  $K_{d,\text{app}}$  = methanol-extractable simazine/g of soil/aqueous-extractable simazine/mL.

results were in agreement with those found in the literature for laboratory conditions, with  $\text{DT}_{50}$  values of 14–27 days for two Brazilian tropical soils (31) and 27–102 days for temperate soils (23). Under field conditions,  $\text{DT}_{50}$  values of 4–17 days were found for the same Brazilian soils (32). The US-1 soil, which had the slowest initial  $\text{DT}_{50}$ , was a sandy soil with the lowest OC and clay contents.

Calculated  $K_{d,\text{app}}$  values are shown in Table 2. It was not possible to calculate this value for HW-1 after day 0 due to its rapid mineralization rate. It was observed that simazine sorption increased by a factor of 2–3 after a 7-day incubation, as compared to the initial  $K_{d,\text{app}}$ .  $K_{d,\text{app}}$  then remained relatively constant up to a 27-day incubation and then increased again at the 102-day incubation. A similar magnitude of enhancement





**Figure 4.**  $^{14}\text{CO}_2$  evolution from aged  $^{14}\text{C}$ simazine-treated Brazilian (BR), midwestern U.S. (US), and Hawaiian (HW) soils after inoculation with *Pseudomonas* sp. strain ADP. Coefficients of variation (CV) for all data points were  $<10\%$ ; average CV = 5%.

has been previously found for other compounds such as imidacloprid and its metabolites (9, 11), sulfonylaminocarbonyltriazolinone and its metabolites (12, 13), dicamba (33), isoxaflutole and its diketonitrile degradate (34), and atrazine (14).

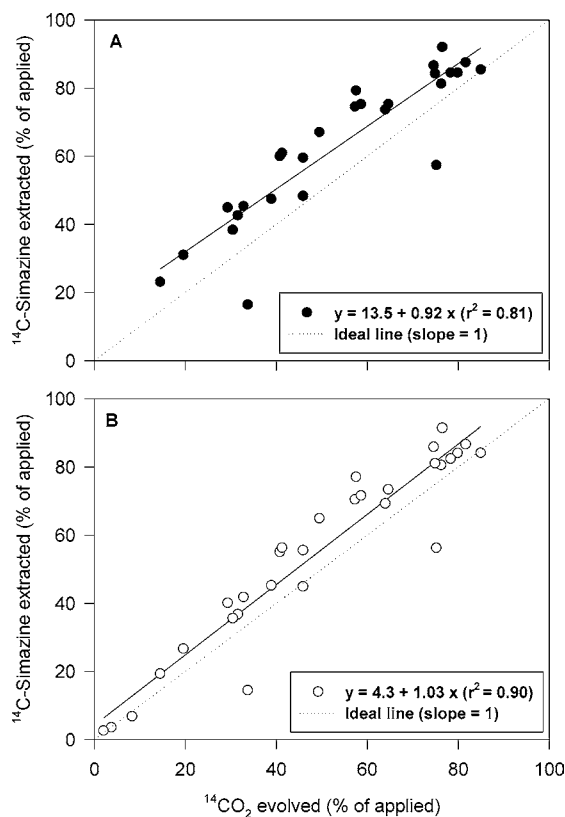
Increases in  $K_{d,app}$  values with aging may explain, in part, why batch  $K_d$  values used in transport and screening models overestimate the potential for herbicides to leach through soils as compared to what is observed under field conditions. Although batch sorption  $K_d$  values (Table 1) were very similar to the day 0  $K_{d,app}$  values (Table 2), batch  $K_d$  values would be useful in expressing simazine sorption only at application time, not for aged residues. For instance, on the basis of criteria in a screening model,  $K_{oc} < 300\text{--}500\text{ L kg}^{-1}$  and  $t_{1/2} > 14\text{--}21$  days, to indicate a potential leaching problem (35); simazine's leaching potential would change in three of these soils depending on whether  $K_d$  or  $K_{d,app}$  was used in the model. In these soils, an increase in  $K_{d,app}$  by a factor of 2–3, as in the aged studies, would result in  $K_{oc}$  values of  $>500\text{ L kg}^{-1}$  and simazine would not be a potential leaching problem.

**$^{14}\text{C}$ Simazine Bioavailability to *Pseudomonas* in Aged Soils.** To determine the bioavailability  $^{14}\text{C}$ simazine, the aged soils were inoculated with *Pseudomonas* sp. strain ADP after each incubation period. This microorganism mineralizes simazine very rapidly. Atrazine mineralization in *Pseudomonas* ADP is initiated by atrazine chlorohydrolase (AtzA) (26), which has been shown to also catalyze the dechlorination of simazine (26, 36). In general, the majority of the applied  $^{14}\text{C}$ simazine

that was mineralized was mineralized in the first 6 h after inoculation (Figure 4). After mineralization, the amounts of  $^{14}\text{C}$ simazine residues distributed between the aqueous-extractable, methanol-extractable, and bound fractions in soils were determined (Figure 2, dotted lines). In this experiment, the average mass balance of  $^{14}\text{C}$  residues was  $95 \pm 5\%$  of the applied  $^{14}\text{C}$ simazine.

The accumulative amounts of  $^{14}\text{CO}_2$  decreased during the 55-day aging period [from 85 to 14% in BR-1, from 82 to 20% in BR-2, from 80 to 39% in US-1, from 76 to 30% in US-2, from 75 to 2% in HW-1, and from 77 to 33% in the HW-2 (Figure 4)]. Previously, it was noted that sorption increased with aging (19, 37–39), which should decrease simazine bioavailability in the soils. This in turn would decrease the extent and rate of mineralization even by such a highly specialized microorganism. Consequently, this bacterium would not be capable of degrading the sorbed fraction of this herbicide, which was continuously increasing with time. This same trend has been observed for the bioavailability of atrazine (6, 14, 20) and carbaryl (40) to soil microorganisms, and for various pesticides, that is, atrazine (38, 41), isoproturon, and dicamba (41), to earthworms.

Regardless of incubation time, the inoculated microorganisms mineralized all of the readily available (water-extractable)  $^{14}\text{C}$ simazine in all soils after different incubation periods ( $<3\%$  of the applied  $^{14}\text{C}$ simazine remained in this fraction) (Figure 2, open circles/dotted line). The microorganisms also mineralized the majority of the less readily available (methanol-extractable) fraction. Because this fraction was decreased with time in



**Figure 5.** Amounts of [ $^{14}\text{C}$ ]simazine extracted by aqueous methanol (twice) (A) or by aqueous methanol (once) (B) from aged [ $^{14}\text{C}$ ]simazine-treated Brazilian (BR), midwestern U.S. (US), and Hawaiian (HW) soils versus amounts of  $^{14}\text{CO}_2$  evolved from aged [ $^{14}\text{C}$ ]simazine-treated Brazilian (BR), midwestern U.S. (US), and Hawaiian (HW) soils after inoculation with *Pseudomonas* sp. strain ADP.

noninoculated soils, but increased with time after soils were inoculated with ADP, it is suggested that part of this fraction is diffusing to even less accessible sites in soil with contact time, where desorption is even slower (15, 16, 42). It has been suggested that sequestration and reduced bioavailability can occur when organic compounds enter into nanopores, which have hydrophobic surfaces (17, 19, 37).

The  $^{14}\text{C}$ -bound residue fraction after bacterial inoculation was practically the same as before inoculation in all soils (Figure 2, dotted line). Therefore, this nonextractable fraction, if it contains [ $^{14}\text{C}$ ]simazine, may be considered to be very recalcitrant and consequently unavailable in soils containing highly efficient microbes.

Despite differences in origin (glacial and tropical) and physical–chemical properties of soils, the amounts of [ $^{14}\text{C}$ ]simazine mineralized by *Pseudomonas* sp. strain ADP were similar to the total amounts of [ $^{14}\text{C}$ ]simazine sequentially extracted by aqueous methanol (twice) solution [% extracted =  $13.5 + 0.92$  (% mineralized),  $r^2 = 0.81$ ] (Figure 5A). If only the first methanol extraction was considered, the correlation between these parameters would be improved [% extracted =  $4.3 + 1.03$  (% mineralized),  $r^2 = 0.90$ ] (Figure 5B). Barriuso et al. (14) obtained a similar slope (1.33) for atrazine using the same extractants. The correlation and comparison of the slope of the data (slope = 1.03, Figure 5B) to the ideal line emphasized that the total amounts of [ $^{14}\text{C}$ ]simazine extracted by 0.01 N  $\text{CaCl}_2$  and aqueous methanol (80%) solutions adequately represented its bioavailability in soils from both glacial and tropical regions, even taking into account aging effects. Therefore, simazine bioavailability can be predicted by

an indirect mild method of extraction, and the establishment of such a correlation is very useful, because it allows the prediction of simazine bioavailability.

**Conclusions.** In this study, simazine showed low to moderate sorption and a relatively slow dissipation rate, emphasizing its potential for mobility. However, the nonextractable fraction increased with soil–pesticide contact time (aging), which resulted in enhancement of sorption and consequently in higher  $K_{d,app}$  values. The nonextractable simazine residue fraction was very recalcitrant, and the  $K_d$  values as estimated for freshly added pesticides may overestimate real molecule leaching potential. The increase in simazine sorption with aging was very likely due to its faster degradation rate in solution and on labile sites in relation to its rate of desorption from soil and simultaneously to its diffusion into less accessible sorption sites or “stronger” binding sites. Regardless of the mechanism involved in sorption, simazine bioavailability to *Pseudomonas* sp. strain ADP was closely correlated to the total amounts of [ $^{14}\text{C}$ ]simazine sequentially extracted by aqueous and methanol solutions, having a similar magnitude slope value (1.03). This correlation allows better prediction of simazine bioavailability and may be useful for regulatory agencies because it supports better the real chemical exposure to humans, animals, or plants, which is often overestimated using harsher extraction procedures.

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