Bioavailability of Organoclay Formulations of Atrazine in Soil

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Pesticide formulations based on organoclays have been proposed to prolong the efficacy and reduce the environmental impact of pesticides in soil. This research addressed the question of whether atrazine in organoclay-based formulations is irreversibly sorbed or is bioavailable for bacterial degradation in soil. Different cations of L-carnitine (CAR), tyramine (TYRAM), hexadimethrine (HEXADIM), phenyltrimethyl-ammonium (PTMA), hexadecyltrimethylammonium (HDTMA), and Fe(III) were incorporated into Na-rich Wyoming montmorillonite (SWy-2) and Ca-rich Arizona montmorillonite (SAz-1) at 100% of the cation exchange capacity (CEC) of the clays as a strategy to enhance the affinity of the clay minerals for atrazine. A Buse loam soil from Becker, MN, was treated with three organoclay-based formulations of ^{14}C-atrazine or free herbicide and incubated for 2 weeks. To determine the bioavailability of ^{14}C-atrazine, the soil was inoculated with *Pseudomonas* sp. strain ADP, which rapidly mineralizes atrazine. At day 0, and after a 2 week incubation, mineralization and the amount of ^{14}C-atrazine residues distributed between the aqueous-extractable, methanol-extractable, and bound fractions in the soil were determined to characterize the availability of nonaged and aged atrazine residues. By the end of the 2 week incubation, the microorganisms had mineralized >80% of the initial readily available (water-extractable) and >70% of the less readily available (methanol-extractable) ^{14}C-atrazine in the soil. Bound residues increased from <4% at day 0 to ~17% after the 2 week incubation for both the formulated and free forms of atrazine. The results of these incubation experiments show that the bioavailabilities of atrazine were similar in the case of the organoclay formulations and as free atrazine. This indicated that whereas more atrazine was sorbed and less likely to be transported in soil, when formulated as organoclay complexes, it was ultimately accessible to degrading bacteria, so that the herbicide is likely to be naturally attenuated by soil microorganisms.

KEYWORDS: Bioavailability; organoclays; *Pseudomonas* sp. strain ADP; sorption; triazine herbicides

INTRODUCTION

The increasing use of pesticides has created serious potential human health and environmental problems. In Europe and the United States, numerous cases of pesticide contamination of ground and surface waters have been reported (*1*). This has led authorities to limit the use of these products. For example, atrazine and simazine have been classified as priority substances by the European Parliament and the Council of the European Union in water policy directive 2455/2001/EC.

When pesticides are applied to soil, only a small percentage reaches the target site. Most of the pesticide is subject to various processes such as sorption, degradation, runoff, and leaching (*2*). To compensate for transport and degradation losses, pesticides need to be applied in concentrations greatly exceeding those strictly required for pest control, and the excessive quantities used

increase the risk of environmental pollution (*3*). It has been suggested that the use of slow-release formulations can be an efficient strategy to reduce the environmental impact of soil-applied pesticides (*4*−*6*). These formulations reduce pesticide mobility and the amount of chemical required for pest control, increase safety issues for the pesticide applicator, and display a general decrease in nontarget effects (*3*). Unaltered and modified clay minerals have been shown to be good adsorbents for different classes of pesticides and, therefore, have been proposed as carriers for the design of pesticide slow-release formulations (*7*−*12*).

Different studies have shown that the nature of the interlayer inorganic cation can strongly influence the adsorptive properties of clay minerals. As an example, Celis et al. (*10*) showed that the herbicide hexazinone displayed very high adsorption on Fe-saturated compared to Mg-, Na-, and K-saturated Wyoming montmorillonite (SWy-2). Hexazinone formulations prepared by supporting the herbicide on Fe-montmorillonite showed slow-release properties, reduced herbicide leaching in soil, and retained herbicidal activity as compared with a standard commercial herbicide formulation (*10*).

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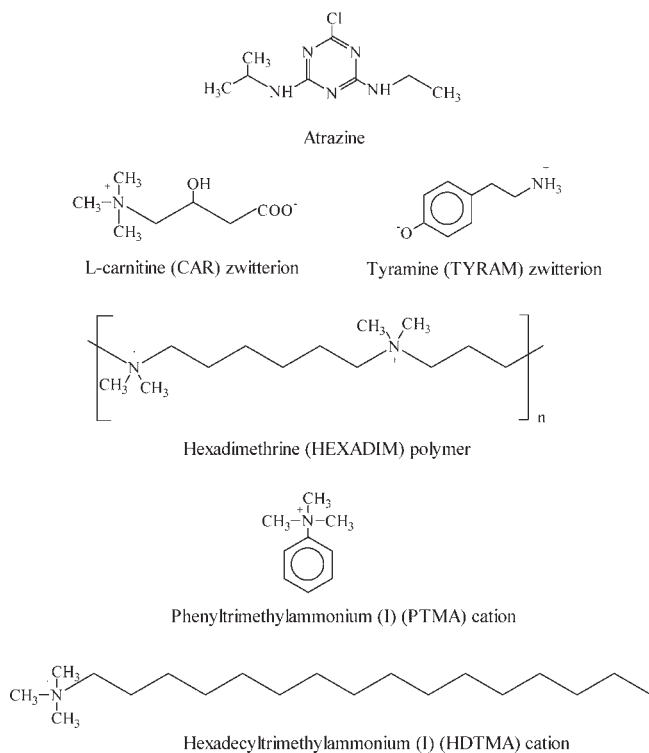
Figure 1. Chemical structures of atrazine and the organic cations used for montmorillonite modification.

Organoclays, that is, natural clay minerals with their original inorganic exchangeable cations replaced with organic cations, have been shown to display improved adsorption properties for many pesticides and, therefore, have also been proposed as carriers in the design of pesticide slow-release formulations (*8, 10, 13–16*).

To protect ground and surface waters from pesticide contamination, broad knowledge is required concerning biodegradation processes influencing pesticide persistence in the environment. Biodegradation by soil microorganisms is a natural attenuation process that often determines the need of larger pesticide doses to be applied to soil. Therefore, the availability of pesticides from formulated materials to soil microorganisms needs to be evaluated from the point of view of the efficacy and the long-term contamination of the pesticide formulations (*17, 18*). Results of previous studies have indicated that sorbed organic compounds are not directly available for microorganisms (*19, 20*), but recently it has been suggested that bacteria can access specific regions where the herbicide is sorbed (*21, 22*).

In this work, two montmorillonites (SWy-2 and SAz-1) modified with different natural and synthetic cations were tested as adsorbents of atrazine, and three organoclays displaying different affinities for the herbicide (SW-CAR, SA-HDTMA, and SW-HDTMA) were selected to prepare ^{14}C-atrazine formulations. We examined the effect of the addition of such formulations on the bioavailability of atrazine for degradation in a soil. Characterization of bioavailability of free atrazine and organoclay formulations of atrazine was facilitated by using *Pseudomonas* sp. strain ADP, a bacterium that can rapidly mineralize this herbicide (*23*).

MATERIALS AND METHODS

Herbicide, Soil, and Montmorillonites. Atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) (**Figure 1**) was purchased from Chem Service (West Chester, PA) (98% purity), and UL-*ring*-^{14}C-labeled atrazine (1.62 MBq mg^{-1} specific activity; 98.7% radiochemical purity) was purchased from Sigma-Aldrich (St. Louis, MO).

The soil used in this study was a Buse loam (fine-loamy, mixed, superactive, and frigid Typic Calciudoll) from Becker, MN, had a pH of 7.7, and contained 49% sand, 27% silt, 24% clay, and 1.30% organic carbon. The soil was collected from the surface layer (0–20 cm depth) and was air-dried and passed through a 2 mm sieve to determine soil properties. Soil texture was determined according to the hydrometer method by density measurements of a soil suspension at intervals calculated to delineate the desired particle sizes. Soil pH was measured using a 1:2 (w/v) soil/deionized water mixture. The organic carbon content was determined by dichromate oxidation.

The synthesis and characteristics of the montmorillonite samples used in this work have previously been described in detail (*10, 24*). Briefly, Na-rich Wyoming montmorillonite (SWy-2) and Ca-rich Arizona montmorillonite (SAz-1) from The Clay Minerals Society (Purdue University, W. Lafayette, IN) were modified with the following cations: L-carnitine (CAR), tyramine (TYRAM), hexadimethrine (HEXADIM), phenyltrimethylammonium (PTMA), hexadecyltrimethylammonium (HDTMA), and Fe(III). It should be noted that although CAR and TYRAM are zwitterionic compounds (**Figure 1**), their cationic forms should have predominated at the pH of the solutions in which they were dissolved (pH 3.0). For this reason, they are referred to herein as "cations". Thus, for the synthesis of the montmorillonites exchanged with L-carnitine, tyramine, and hexadimethrine, the amount of organic cation corresponding to 100% of the cation exchange capacity of SWy-2 (CEC$_{SWy-2}$ = 764 mmol kg^{-1}) or SAz-1 (CEC$_{SAz-1}$ = 1200 mmol kg^{-1}) was dissolved in 50 mL of 1 mM HNO$_3$ (pH 3.0) and added to 1 g of montmorillonite. The suspensions were shaken for 24 h, centrifuged, washed three times with 100 mL of distilled water, and then freeze-dried. Blank clay samples were also prepared by shaking 1 g of montmorillonite in 50 mL of 1 mM HNO$_3$ for 24 h, washing three times with 100 mL of distilled water, and then freeze-drying. These samples served as a control to assess whether the acid treatment of the montmorillonites during the synthesis procedure had any effect on atrazine sorption. For the synthesis of PTMA- and HDTMA-montmorillonites, 10 g of SWy-2 or SAz-1 was treated with 100 mL of an ethanol/water (50:50) solution containing an amount of alkylammonium (chloride salt) equal to the CEC of the clay. The suspensions were shaken for 24 h, centrifuged, washed with distilled water until Cl-free, and then freeze-dried. The Fe(III)-modified montmorillonite (SW-Fe) was prepared by treating 10 g of SWy-2 with 100 mL of a 1 M solution of FeCl$_3$. After treatment (24 h, three times), the sample was washed with distilled water until Cl-free and then freeze-dried.

Adsorption–Desorption Experiments. The adsorption of atrazine by the unmodified and modified montmorillonite samples was measured at a single initial herbicide concentration C_{ini} = 1 mg L^{-1} using the batch equilibration procedure. Duplicate 20 mg adsorbent samples were equilibrated by shaking at 20 ± 2 °C for 24 h with 8 mL of a 1 mg L^{-1} atrazine solution containing 46.9 Bq mL^{-1} of ^{14}C-atrazine. After equilibration, the suspensions were centrifuged, and 1 mL aliquots of the supernatant solutions were removed for analysis. Radioactivity of the 1 mL aliquots of the supernatants was measured by liquid scintillation counting (LSC) to determine the herbicide equilibrium concentration (C_e). Herbicide solutions without adsorbent were also shaken for 24 h and served as controls. The percentage of herbicide adsorbed by the different samples (%Ads) was calculated by using the following formula: %Ads = [(C_{ini} − C_e)/C_{ini}] × 100.

Adsorption–desorption isotherms of atrazine on selected organoclays (SW-CAR, SA-HDTMA, and SW-HDTMA) were also obtained by the batch equilibration procedure. Duplicate 20 mg adsorbent samples were equilibrated with 8 mL of atrazine initial solutions (C_{ini} = 0.1, 0.2, 1, and 2 mg L^{-1}) by shaking mechanically at 20 ± 2 °C for 24 h. Each initial solution contained 46.9 Bq mL^{-1} of ^{14}C-atrazine. After equilibration, the suspensions were centrifuged, and 4 mL of the supernatant solutions was removed for analysis. The equilibrium herbicide concentration in the supernatant solutions (C_e) was determined by LSC. The amount of herbicide adsorbed (C_s) was calculated from the difference between the initial and the equilibrium solution concentrations. Desorption isotherms were obtained immediately after adsorption from the highest equilibrium point of the adsorption isotherm, that is, that corresponding to the initial concentration 2 mg L^{-1}. The 4 mL of supernatant removed for the adsorption analysis was replaced with 4 mL of distilled water. After shaking at 20 ± 2 °C for 24 h, the suspensions were centrifuged, and the herbicide concentration was determined in the supernatant by LSC. This desorption procedure was repeated three times. Atrazine adsorption–desorption isotherms were fit to the logarithmic form of the Freundlich equation: log C_s = log K_f + N_f log C_e, where C_s (mg kg^{-1})

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is the amount of atrazine adsorbed at the equilibrium concentration C_e (mg L^{-1}) and K_f and N_f are the empirical Freundlich constants.

In addition to the above-described conventional adsorption−desorption experiment, successive desorption experiments were conducted using different desorbing solutions. In these experiments, duplicate 20 mg adsorbent samples were equilibrated with 8 mL of atrazine initial solution (C_{ini} = 1 mg L^{-1}) by shaking mechanically at 20 ± 2 °C for 24 h. After equilibration, the suspensions were centrifuged, and 4 mL of the supernatant solutions was removed for analysis. The herbicide concentration in the supernatants was determined by high-performance liquid chromatography (HPLC) as described below. The amount of herbicide adsorbed was calculated from the difference between the initial and the equilibrium solution concentrations. Desorption was conducted immediately after adsorption by replacing the 4 mL of the supernatant removed for the adsorption analysis with 4 mL of differently desorbing solutions (water, 0.01 M NaCl, 0.01 M NaOH, or methanol). After shaking at 20 ± 2 °C for 3 h, the suspensions were centrifuged, and the herbicide concentration was determined in the supernatant by HPLC. For each desorbing solution, this desorption procedure was repeated three times.

Organoclay Formulations of Atrazine. The organoclays used for the preparation of atrazine formulations (SW-CAR, SA-HDTMA, and SW-HDTMA) were selected on the basis of the sorption study that showed different affinities for the herbicide (high affinity for SW-CAR, moderate affinity for SA-HDTMA, and low affinity for SW-HDTMA). Relevant physicochemical characteristics of these organoclays are included in **Table 1**. Elemental analyses were performed using a Perkin-Elmer model 1106 elemental analyzer (Perkin-Elmer Corp., Norwalk, CT), whereas X-ray diffraction patterns were obtained on oriented specimens using a Siemens D-5000 diffractometer (Siemens, Stuttgart, Germany) with Cu Kα radiation.

Organoclay-based formulations of atrazine were prepared by adding 10 mL of a methanolic solution containing 20 mg L^{-1} of ^{14}C-atrazine to 300 mg of each organoclay (SW-CAR, SA-HDTMA, and SW-HDTMA), and the resultant solids were dried using a Zymark Turbo Vap II evaporator at 35 °C. The final concentration of atrazine in the organoclay formulations was 0.67 mg g^{-1}, and the radioactivity was 1.08 kBq mg^{-1} of organoclay. The low herbicide content of the formulations prepared was to ensure that all herbicide present in the formulations was actually sorbed on the organoclays.

^{14}C-Atrazine Bioavailability in Soil Inoculated with *Pseudomonas* sp. Strain ADP. Aliquots containing 0.01 mg of atrazine and 1.682 kBq of ^{14}C, either as organoclay formulations (20 mg) or as free herbicide (0.75 mL of 18 mg L^{-1} methanolic solution), were added to 10 g of air-dried soil that was individually weighed into duplicate glass tubes. The final atrazine concentration in the soil was 1 mg kg^{-1}, and the radioactivity was 168.2 Bq g^{-1} of soil. The soil mixture was inoculated with 1 mL of a suspension of *Pseudomonas* sp. strain ADP to a final inoculum density of 1×10^8 cells g^{-1} of soil. The soil was moistened to its field capacity and thoroughly mixed. Glass wool was placed on the soil surface, and a vial containing 4 mL of 0.5 M NaOH was placed in the tube to trap $^{14}CO_2$ resulting from the mineralization of ^{14}C-atrazine. Tubes were stoppered, and soils were incubated in the dark at 25 ± 1 °C for 2 weeks. The NaOH was replaced periodically, a 1 mL aliquot was sampled, and its radioactivity was measured by LSC.

After the 2 week incubation, the inoculated soil samples were extracted by shaking with 20 mL of 0.01 M $CaCl_2$ for 24 h in a horizontal shaker and centrifuged at 4000 rpm for 10 min. The supernatant volume was removed and measured, and a 1 mL aliquot was sampled to determine its radioactivity by LSC. The soil was subsequently extracted with 20 mL of methanol/water (80:20, v/v) by shaking for 24 h in a horizontal shaker. After the sample was centrifuged at 4000 rpm for 10 min, the supernatant was removed and the methanol was evaporated from the aqueous methanol supernatant using a Zymark Turbo Vap II evaporator at 35 °C. A 1 mL aliquot of the water extract remaining was sampled, and its radioactivity was determined by LSC. The extraction with methanol/water (80:20, v/v) was repeated three times. The two solutions from the 0.01 M $CaCl_2$ extraction (readily available herbicide) and from the aqueous methanol supernatant extractions (less readily available herbicide) were also analyzed by HPLC. Atrazine fractions were collected from time 0 to 3 min and then at each minute until minute 10. One milliliter aliquots of the remaining fractions were counted to enable calculation of the percentage of parent herbicide in the extracted ^{14}C.

After extraction, the remaining soil was dried at 40 °C, and 0.33 g (dry weight) of each sample in triplicate was combusted by using a Packard 307 sample oxidizer. The $^{14}CO_2$ released was trapped in Carbo-Sorb E mixed with Permafluor V, and the ^{14}C was quantified by LSC.

Extraction and combustion processes were also carried out with control soil samples, treated with ^{14}C-atrazine but noninoculated, immediately after addition of the herbicide as organoclay formulations or as free herbicide, in order to compare the distribution of radioactivity in the extracts and that retained in the soil at time zero to the data from the 2 week incubation.

Instrumental Analysis of Atrazine. Radioactivity was measured by LSC using a Tri-Carb 1500 Packard liquid scintillation analyzer. Analysis time was 5 min using vials with 1 mL of sample and 5 mL of scintillation cocktail EcoLite from BP Biomedical (ICN) (Irvine, CA). Vials were kept in the dark overnight prior to counting to reduce chemiluminescence.

HPLC analyses of atrazine were conducted using a Waters 600E chromatograph coupled to a Waters 996 diode array detector. The following chromatographic conditions were used: NovaPak C18 column

Table 1. Some Characteristics of the Unmodified and Modified Montmorillonite Samples Used in This Work

sample	montmorillonite	modifying cation	OCtS[a] (%)	d_{001}[b] (Å)
SWy-2 (blank)	SWy-2	none		15.1
SW-Fe	SWy-2	Fe(III)		12.8
SW-CAR	SWy-2	L-carnitine	61	14.2
SW-TYRAM	SWy-2	tyramine	62	14.4
SW-HEXADIM	SWy-2	hexadimethrine	88	14.0
SW-PTMA	SWy-2	phenyltrimethylammonium	82	14.6
SW-HDTMA	SWy-2	hexadecyltrimethylammonium	93	18.0
SAz-1 (blank)	SAz-1	none		15.2
SA-CAR	SAz-1	L-carnitine	41	14.8
SA-TYRAM	SAz-1	tyramine	50	15.0
SA-HEXADIM	SAz-1	hexadimethrine	88	14.2
SA-PTMA	SAz-1	phenyltrimethylammonium	79	15.0
SA-HDTMA	SAz-1	hexadecyltrimethylammonium	101	24.0

[a] Organic cation saturation: percentage of the cation exchange capacity of SWy-2 (76.4 $cmol_c$ kg^{-1}) or SAz-1 (120 $cmol_c$ kg^{-1}) compensated by organic cations (calculated from the N content of the samples). [b] Basal spacing values for air-dried oriented specimens.
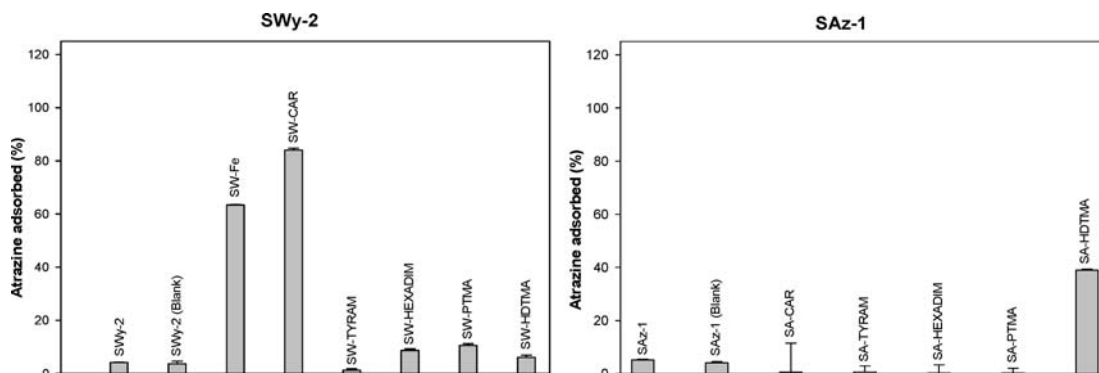

Figure 2. Percentage of atrazine adsorbed by unmodified and modified montmorillonites at an initial herbicide concentration of 1 mg L^{-1}.

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(150 mm length × 3.9 mm i.d.) (Waters), acetonitrile/water (50:50) eluent mixture at a flow rate of 1 mL min^{-1}, 25 μL injection volume, and UV detector at 220 nm.

RESULTS AND DISCUSSION

Adsorption−Desorption Experiments. Figure 2 shows the percentage of atrazine adsorbed by the unmodified and modified montmorillonite samples at a single initial herbicide concentration of 1 mg L^{-1}. Atrazine displayed moderate affinity for SAz-1 exchanged with HDTMA (SA-HDTMA) and low affinity for SAz-1 exchanged with other organic cations (**Figure 2**). The paraffinic ($d_{001} > 22$ Å, **Table 1**) structure resulting from incorporation of HDTMA cations in SAz-1 has previously been proposed to explain the affinity of different pesticides for SA-HDTMA (*12*, *24−26*) because HDTMA creates a wide interlayer organic phase where pesticides are adsorbed through hydrophobic interactions. The small basal spacing (**Table 1**) combined with the proximity of two adjacent cations resulting from the incorporation of the other organic cations in the interlayers of the high-charge montmorillonite SAz-1 results, in general, in little internal space being available for herbicide adsorption (*24*).

For samples prepared from the low-charge montmorillonite SWy-2, modification with CAR and Fe^{3+} cations resulted in materials with high affinities for atrazine, whereas modification with TYRAM, HEXADIM, PTMA, and HDTMA resulted in organoclays with low affinity for this herbicide (**Figure 2**). The high affinity of atrazine for carnitine-exchanged SWy-2 (SW-CAR) can be attributed to the weakly basic character of atrazine (pK_a = 1.7), which allows an acid−base interaction with the carboxylic group of carnitine, as proposed for other *s*-triazines such as simazine and terbuthylazine (*24*, *27*). A similar mechanism operates with Fe^{3+}, which enhances the surface acidity of montmorillonite, promoting the adsorption of atrazine probably in its protonated form (*28*, *29*). Therefore, as observed for other triazine herbicides, atrazine adsorption by the modified montmorillonites is governed by a combination of steric and functionality effects, where the nature of the modifying cation together with its arrangement in the clay mineral interlayer determines the affinity of the herbicide for the modified montmorillonite (*24*, *27*).

Adsorption−desorption isotherms of atrazine on selected adsorbents (SW-CAR, SA-HDTMA, and SW-HDTMA) were also obtained (**Figure 3**). In agreement with the adsorption results obtained at a single initial concentration of 1 mg L^{-1}, adsorption isotherms revealed atrazine adsorption increased in the following order: SW-HDTMA < SA-HDTMA < SW-CAR. In addition, atrazine desorption from the three organoclays was hysteretic. Nevertheless, it should be noted that cases of very high or very low adsorption usually result in poorly defined desorption isotherms (*30*).

Figure 4 further shows the hysteretic behavior of atrazine from the organoclays. Successive desorption equilibration with water and 0.01 M NaCl did not desorb atrazine from SW-CAR. Desorption with methanol removed ~50% of the atrazine with the first equilibration, but no addition in the remaining equilibration. Interestingly, atrazine desorption from SW-CAR was complete with 0.01 M NaOH, probably due to the high pH of the solution, causing the rupture of the interaction of atrazine with carnitine-exchanged SWy-2 for the deprotonation of the carboxylic group of carnitine (*27*). These results are in contrast to those found for SA-HDTMA, in which desorption occurred progressively with water, 0.01 M NaCl, and 0.01 M NaOH, whereas methanol led to complete desorption of the herbicide with the first equilibration. Methanol was therefore very efficient in extracting atrazine from the hydrophobic environment of SA-HDTMA in which the herbicide is
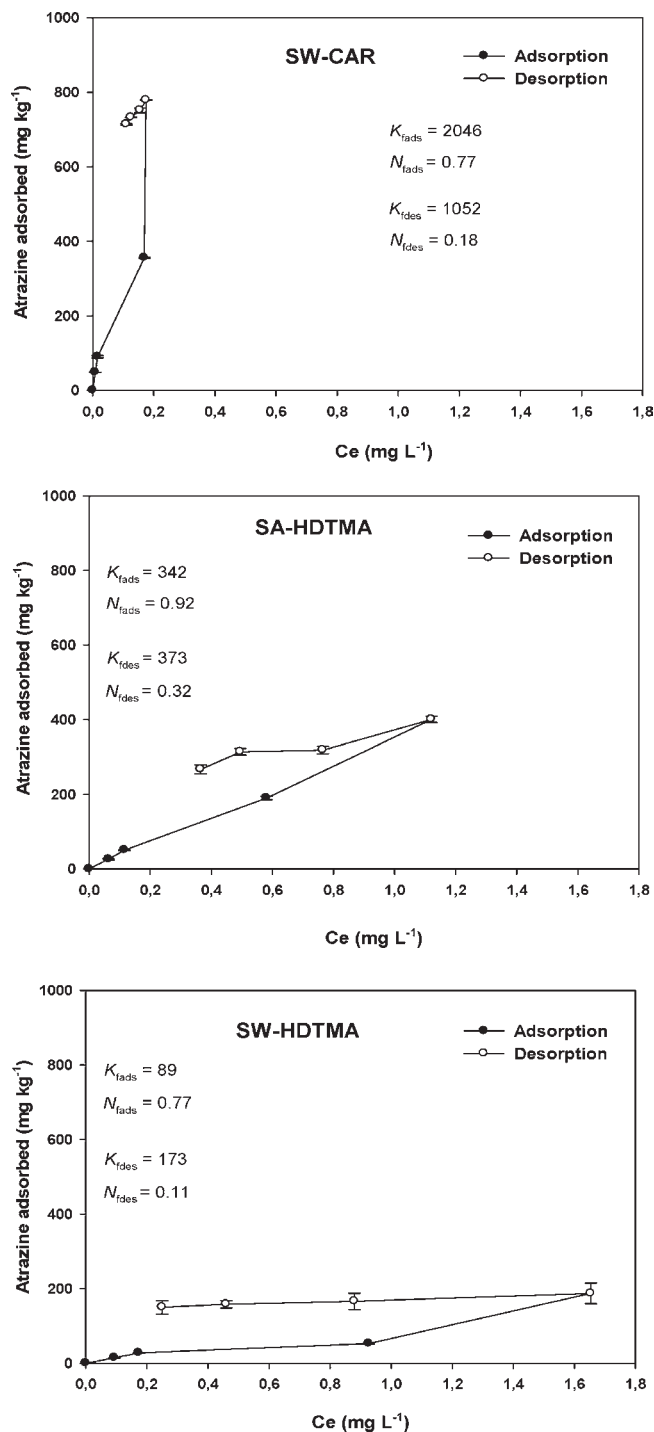

Figure 3. Adsorption−desorption isotherms of atrazine on selected samples. Freundlich coefficients for adsorption (K_{fads}, N_{fads}) and desorption (K_{fdes}, N_{fdes}) isotherms are included.

retained. Desorption of atrazine from SW-HDTMA was not carried out due to the low adsorption of atrazine by this organoclay.

^{14}C-Atrazine Bioavailability in Soil Inoculated with *Pseudomonas* sp. Strain ADP. At day 0, the amounts of ^{14}C-atrazine residues distributed between the aqueous-extractable, methanol-extractable, and bound fractions in the soil were determined to characterize the availability of nonaged atrazine residues (**Figure 5**). The amounts of total extractable and unextractable ^{14}C-atrazine were about the same for the organoclay-based formulations of atrazine and free atrazine at day 0, with >96% extractable and <4% unextractable (bound). The readily available (water-extractable) ^{14}C-atrazine in

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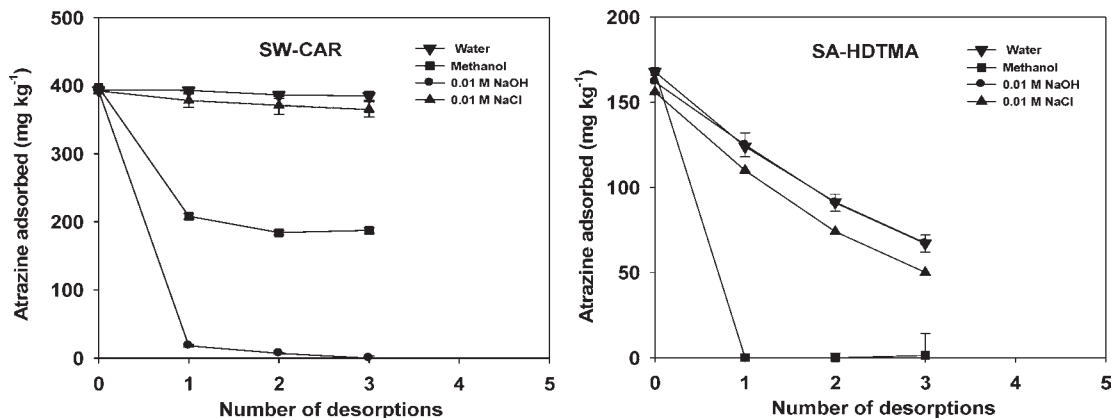*J. Agric. Food Chem.*, Vol. 58, No. 22, 2010   **11861**

Figure 4. Desorption of atrazine from SW-CAR and SA-HDTMA by successive dilutions with different solutions.
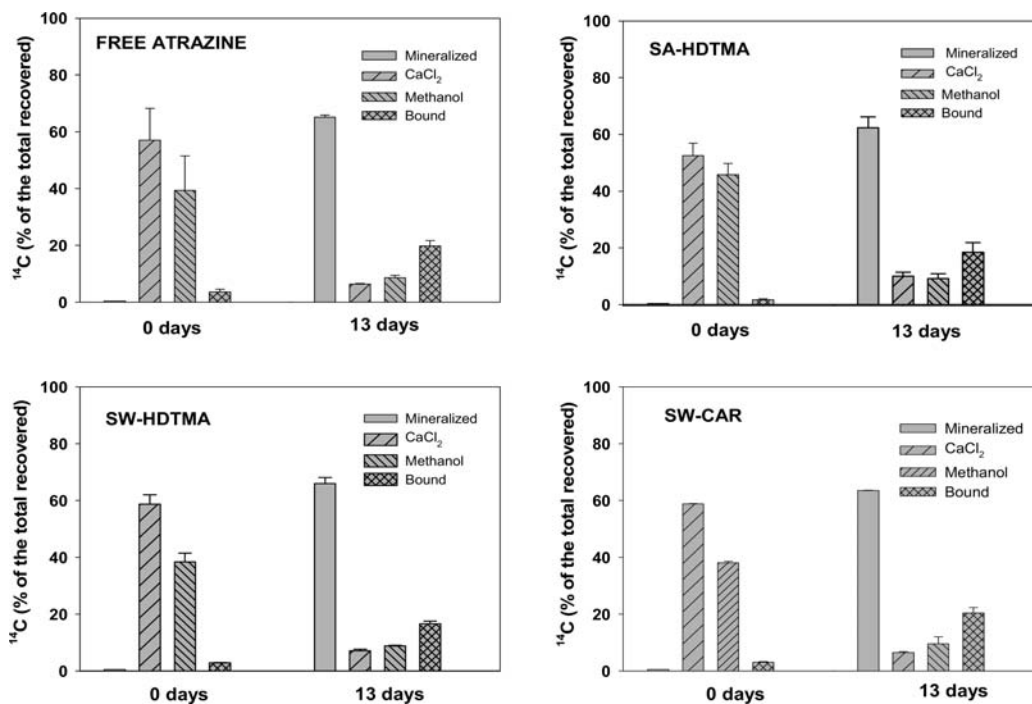

Figure 5. Percentage of atrazine mineralized, aqueous-extractable ($CaCl_2$), methanol-extractable, and nonextractable (bound) in soil at day 0 and after the 13 day incubation.

the soil was ~53% in SA-HDTMA and ~59% in SW-HDTMA, SW-CAR, and free atrazine. Less readily available (methanol-extractable) sorbed atrazine ranged from 46% in SA-HDTMA to ~39% in SW-HDTMA, SW-CAR, and free atrazine. From these data, it appears that atrazine would be less available for degradation and transport when formulated as a SA-HDTMA−atrazine complex as compared to other formulations, because the fraction of atrazine sorbed (methanol-extractable) is greater in SA-HDTMA compared to the other formulations.

To determine the bioavailability of ^{14}C-atrazine sorbed to the organoclays as compared to free atrazine, the soil was inoculated with *Pseudomonas* sp. strain ADP. This microorganism rapidly mineralizes atrazine (*23*, *31*). In general, most of the applied ^{14}C-atrazine was mineralized in the first 6 days after inoculation (46% in SA-HDTMA, 56% in SW-HDTMA, 54% in SW-CAR, and 56% in free atrazine) (**Figure 6**). The cumulative amounts of [14]CO_2 at the end of the experiment were 62% in SA-HDTMA, 66% in SW-HDTMA, 63% in SW-CAR, and 65% in free
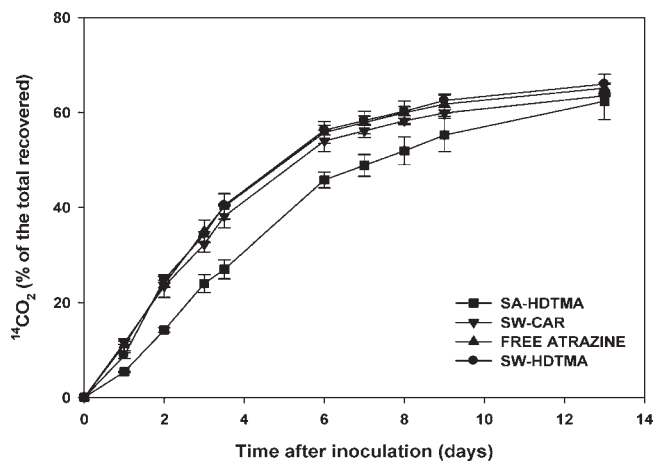

Figure 6. [14]CO_2 evolution from ^{14}C-atrazine-treated soil inoculated with *Pseudomonas* sp. strain ADP.

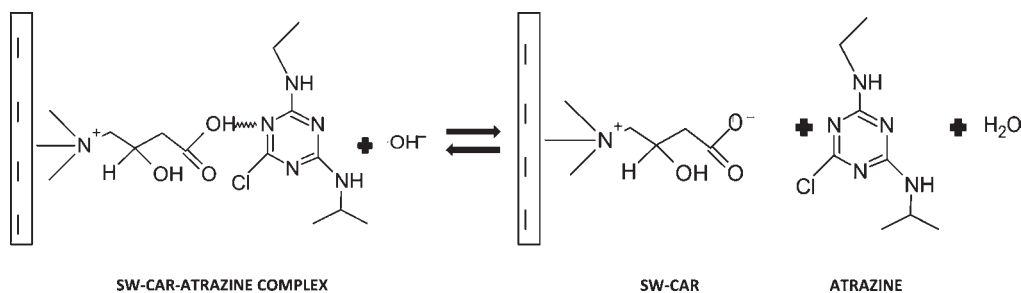**11862** *J. Agric. Food Chem.,* Vol. 58, No. 22, 2010

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Figure 7. Possible mechanism of decomposition of the SW-CAR−atrazine complex at high pH levels.

atrazine. SA-HDTMA formulation of atrazine initially retarded the bioavailability of the herbicide in the soil compared to the atrazine formulated with SW-HDTMA and SW-CAR and the free ^{14}C-atrazine (**Figure 6**). This result is consistent with the adsorption−desorption isotherms, whereby herbicide availability was inversely related to the affinity of the organoclays for the herbicide, except in the case of SW-CAR, the organoclay showing the highest affinity for atrazine. However, by the end of the 2 week incubation, there was no difference in mineralization between the formulations. We believe that the distinct behavior of the SW-CAR formulation could be due to the high pH provided by the soil (pH 7.7). According to the above-discussed successive desorption experiments (**Figure 4**), despite SW-CAR showing the highest affinity for atrazine, the herbicide sorbed to SW-CAR is rapidly and completely desorbed at high pH levels, thus becoming bioavailable (**Figure 7**). It should also be noted that the stability of the organoclays in the presence of soil was not determined, so that differences in the stability of the organoclays with regard to chemical and biological degradation soil processes could have also affected the availability of atrazine formulated with the different organoclays.

By the end of the 2 week incubation, the inoculated micro-organisms mineralized > 80% of the initial readily available (water-extractable) and > 70% of the less readily available (methanol-extractable) ^{14}C-atrazine in the soil (**Figure 5**). There were no significant differences in the amounts of bound atrazine residues among formulations of atrazine and free atrazine at the end of the incubation; independent of the formulation, bound residues increased from < 4% at day 0 to ~17% after the 2 week incubation.

The results of this work show, in summary, that organoclay formulations of a herbicide could potentially affect the bioavail-ability of the herbicide in soil as herbicide bioavailability is inversely related to the affinity of the organoclay for the herbicide. However, in addition to affinity, the mechanism of interaction between the herbicide and the organoclay is also important in determining the bioavailability of a herbicide formulation in soil. For instance, atrazine sorbed on SW-CAR displayed enhanced bioavailability to *Pseudomonas* sp. strain ADP in soil compared to atrazine sorbed on SA-HDTMA, even though adsorption experiments revealed that SW-CAR shows higher affinity for atrazine than SA-HDTMA. This is because in SA-HDTMA, the interaction between the alkyl chains of HDTMA and atrazine is a hydrophobic interaction, whereas in SW-CAR the interaction between the acid group of carnitine and atrazine is an acid−base interaction, the latter being disrupted at alkaline soil pH levels. Nevertheless, the results of our incubation experiments also show that the final bioavailability of atrazine was similar in the case of the organoclay formulations and as free atrazine. This indicated that whereas more atrazine was sorbed and less likely to be transported in soil, when formulated as organoclay complexes, it was ultimately accessible to degrading bacteria. This suggests that

the amount of atrazine applied as organoclay formulations is likely to be naturally attenuated by soil microorganisms.

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; LSC, liquid scintillation counting; CAR, L-carnitine; TYRAM, tyramine; HEXADIM, hexadimethrine; PTMA, phenyltrimethylammo-nium; HDTMA, hexadecyltrimethylammonium; SW-CAR; car-nitine-exchanged Wyoming montmorillonite, SW-HDTMA, hexadecyltrimethylammonium-exchanged Wyoming montmoril-lonite; SA-HDTMA, hexadecyltrimethylammonium-exchanged Arizona montmorillonite.

ACKNOWLEDGMENT

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