JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Degradation and Binding of Atrazine in Surface and Subsurface Soils

ELENA BLUME,[†,§] MARIANNE BISCHOFF,[†] THOMAS B. MOORMAN,[‡] AND RONALD F. TURCO*,[†]

Agronomy Department, Purdue University, West Lafayette, Indiana 47907-1150, and National Soil Tilth Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50011

Understanding the dissipation rates of chemicals in unsaturated and saturated zones of subsurface soils will help determine if reductions of concentrations to acceptable levels will occur. Chemical properties and microbial biomass and activity were determined for the surface (0−15 cm), lower root (50−105 cm), and vadose (175−220 cm) zones in a Huntington silty clay loam (Fluventic Hapludoll) collected from an agricultural field near Piketon, OH. The rates of sorption, mineralization, and transformation (formation of bound residues and metabolites) of atrazine were determined. Microbial activity was estimated from the mineralization of ^{14}C-benzoate. We observed decreased levels of nutrients (total organic carbon, N, and P) and microbial biomass with depth, while activity as measured with benzoate metabolism was higher in the vadose zone than in either the surface or the root zones. Sorption coefficients (K_f) declined from 8.17 in the surface to 3.31 in the vadose zone. Sorption was positively correlated with organic C content. Rates of atrazine mineralization and bound residues formation were, respectively, 12−2.3-fold lower in the vadose than in the surface soil. Estimated half-lives of atrazine ranged from 77 to 101 days in the surface soil, but increased to over 900 days in the subsurface soils. The decreased dissipation of atrazine with increasing depth in the profile is the result of decreased microbial activity toward atrazine, measured either as total biomass or as populations of atrazine-degrading microorganisms. The combination of reduced dissipation and low sorption indicates that there is potential for atrazine movement in the subsurface soils.

KEYWORDS: Microbial biomass and activity; sorption; subsurface microbiology; mineralization; kinetics

INTRODUCTION

In the north-central United States, high atrazine (2-chloro-4-(ethylamino)-6-isopropylamino-*s*-triazine) usage combined with hydrologic factors (seasonal runoff and subsurface drainage) have resulted in water contamination by atrazine and atrazine metabolites (*1*−*5*). The detection of pesticides in surface water and groundwater has increased the public's concern about the impacts of agriculture on the environment (*1*). Atrazine remains one of the most widely used pesticides for control of broadleaf and grassy weeds in corn.

Atrazine can be degraded by isolated bacteria and fungi or by mixed communities in soils, resulting in the dealkylated metabolites DEA (deethylatrazine), DIA (deisopropylatrazine), and DEDIA (dealkylatrazine). The microbial dechlorination of atrazine can result in the formation of HA (hydroxyatrazine) and the dechlorinated analogues of DEA, DIA, and DEDIA (*6*). Abiotic degradation by hydrolysis to hydroxyatrazine can also occur (*7*). Atrazine transport is governed by the interaction of both degradation processes and sorption, which is controlled by organic C content, pH, and clay content (*7*). Because atrazine must be transported through the vadose zone to reach groundwater, an understanding of the sorption, degradation, and transformation processes in the vadose zone is essential.

Previous studies of atrazine degradation in the subsurface environment display a range of results. Relative to surface soil, atrazine was significantly more persistent in most vadose and saturated zone materials (*8*−*13*). However, in some subsurface materials, atrazine degradation rates were not reduced (*14*, *15*). In some studies with vadose zone soils, mineralization removed between 1% and 10% of the applied atrazine (*11*, *16*). The formation (*4*, *16*) and degradation of metabolites (*17*) seems to be soil type and soil depth dependent. Few of these studies have examined the influence of soil properties on atrazine fate.

The objectives of this study were to (i) estimate the capacity of subsurface soils to degrade, bind, and sorb atrazine at different concentrations, (ii) estimate the rates of these various processes, and (iii) determine the effect of physical, biological, and chemical factors on atrazine transformation processes. To gain increased insight on the biological processes governing atrazine fate, we used two different concentrations of ^{14}C-atrazine and several different measures of microbial biomass and activity.

MATERIALS AND METHODS

Soil Sampling and Characterization. Soil samples were collected from a Huntington silty clay loam (fine-silty, mixed, mesic Fluventic Hapludoll) at the Ohio USDA - Management System Evaluation Area

* Author to whom correspondence should be addressed [telephone (765) 494-8077; e-mail rturco@purdue.edu].
† Purdue University.
‡ National Soil Tilth Laboratory.
§ Present address: Plant Protection Department, Federal University of Santa Maria (UFSM), 97105-900 Santa Maria, RS, Brazil.

10.1021/jf049830c CCC: $27.50
Published on Web 11/05/2004

Table 1. Mean Physical and Chemical Properties of a Huntington Silty Clay Loam near Piketon, OH

						nitrogen		phosphorus	
depth (cm)	sand (%)	silt (%)	clay (%)	pH	TOC (%)	mineral[b] (mg N kg^{-1})	TKN (mg N kg^{-1})	total (mg P kg^{-1})	available (mg P kg^{-1})
0–15	16.0[a]	46.0	38.0	6.9	2.1	18.3	1980.0	291.0	2.3
	(5.3)	(2.0)	(4.0)	(0.3)	(0.1)	(2.4)	(108.2)	(22.5)	(0.4)
50–105	15.3	46.7	38.0	7.0	0.8	7.4	1056.7	186.3	0.8
	(3.1)	(3.1)	(0.0)	(0.2)	(0.02)	(0.4)	(56.9)	(0.6)	(0.2)
175–220	11.3	53.3	35.3	7.5	0.5	6.0	746.3	173.0	1.1
	(3.1)	(3.1)	(1.2)	(0.2)	(0.05)	(0.9)	(56.1)	(10.1)	(0.2)

[a] Values are means over the three sites with the standard deviations beneath the means in parentheses. [b] Sum of nitrate, nitrite, and ammonia forms of N.

(MSEA). The MSEA lies adjacent to the Scioto River, near Piketon, OH. Three field locations at the MSEA field station were chosen within a field cropped to a corn, soybean, and wheat-hairy vetch rotation. Three soil cores (220 depth × 10 cm), 2 m apart from each other, were collected using a truck-mounted GSRP-S (The Giddings Machine Co., Fort Collins, CO) hydraulic soil coring unit. The cores were divided into sections that constitute the three depths used: surface zone (0−15 cm), lower root zone (50−105 cm), and vadose zone (175−220 cm). To control contamination, the exterior 1 cm of each section was removed. The sections were placed in sterile plastic bags and transported to the Purdue Soil Microbiology Laboratory where the soil from each section was mixed, passed through a 4-mm sieve, and stored at 4 °C for no longer than 2 months. Biomass extractions (see below) were started within 1 h of core collection; determination of microbial activity, MPN measurement of atrazine degraders, and atrazine mineralization were begun within 2 months of soil collection.

Table 1 shows the physical and chemical characterization of the materials collected at the different depths. The particle size was analyzed by the pipet method, pH in H_2O (1:1 soil/solution), total organic carbon (TOC) by the Leco total carbon analyzer, after removal of inorganic carbon with a 10% phosphoric acid solution. Total N and total P were determined by acid digestion followed by analysis of soluble N and P by colorimetric flow analysis with a Technicon Auto Analyzer (Technicon Inc., New York, NY). Mineral N and available P were extracted with 2 M KCl and analyzed with the Technicon Auto Analyzer.

Microbiological Analysis. Soil microbial biomass was estimated from phospholipids-PO_4 content of the soils following the extraction of microbial phospholipids (*18*). Biomass extraction was done on five replicates of approximately 5 g of soil and was initiated within 1 h of core collection.

Soil microbial activity was estimated from the mineralization of ^{14}C-benzoate. Three replicates of 25 g of soil (oven-dry equivalent) were treated with an aqueous solution of analytical grade benzoate, 99% purity, fortified with ^{14}C-ring-labeled benzoate (specific activity of 4.9 × 10^8 Bq mmol^{-1}) (Sigma Chemicals, St. Louis, MO). A concentration of 1.0 mg kg^{-1} soil with 420 Bq of ^{14}C-activity was applied per flask. Samples were adjusted with sterile water to a water content equivalent to −33 kPa. A vial containing 10 mL of 0.5 M NaOH as a CO_2 trapping solution was placed inside the incubation jar, and the jars were incubated at 22 °C. The NaOH solution was exchanged periodically, and 1 mL of the solution was mixed with 15 mL of scintillation cocktail (Ecolume, ICN Biochemicals Inc., Costa Mesa, CA). The ^{14}C activity was measured in a liquid scintillation counter (LSC) (Tri-Carb 1600 TR, Packard Instruments, Downers Grove, IL).

Atrazine degrading microorganisms were enumerated by the ^{14}C-most probable number method (*3*, *19*). The culture medium consisted of a basal salt solution, 100-fold diluted nutrient broth, sterile trace elements, and atrazine. The final concentration of atrazine was 1.0 mg L^{-1} (nonlabeled analytical grade atrazine, 99% purity) (ChemService, West Chester, PA), plus ^{14}C-ring-labeled atrazine (specific activity of 4.3 × 10^8 Bq mmol^{-1}) (Sigma Chemicals, St. Louis, MO). ^{14}C-activity of 84 Bq was used per vial. Serial dilutions of the soils were prepared to remove the bacteria from soil particles, and aliquots of the dilutions and the medium were transferred to shell vials in five replicate sets. Each shell vial was placed into a scintillation vial containing 1 mL of 1 M NaOH, capped, and incubated for 50 days. The shell vials were then removed, scintillation cocktail was added, and the $^{14}CO_2$ was trapped by the NaOH determined by LSC. The vials were scored as (+) when the evolved $^{14}CO_2$ exceeded 2 times the background and (−) otherwise.

Atrazine Sorption. Atrazine sorption in the soils collected from the different field locations and depths was determined by batch isotherms. Two replicates of 3 g of air-dried soil were equilibrated for 48 h in 20 mL of 0.01 M $CaCl_2$ containing a mixture of nonlabeled and ^{14}C-ring-labeled atrazine (67 Bq ^{14}C-activity per tube) at total concentrations of 1.4, 7, 14, and 70 μM. After centrifugation (10 000 rpm for 15 min), a 1 mL aliquot was removed from the supernatant for LSC. Values of K_f were calculated from fitting the Freundlich sorption equation: $x/m = K_f C_e^N$, where x/m is the concentration (μmol kg^{-1}) of the herbicide sorbed to the soil, C_e is the equilibrium concentration (μmol L^{-1}) in solution, and K_f and N are the sorption capacity and the sorption intensity empirical indexes, respectively.

Atrazine Mineralization. Atrazine mineralization and transformation were determined using atrazine applied at 0.1 and 1.0 mg kg^{-1} soil. Two replicates of 25 g of soil (oven-dry basis) were treated with the two concentrations of ^{14}C-atrazine (1682 Bq of ^{14}C-activity per flask), and the water content was adjusted to −33 kPa equivalent and incubated under the same conditions as the benzoate. Atrazine mineralization was estimated from $^{14}CO_2$ evolution over a period of 205 days. The vials with the NaOH trapping solution were exchanged weekly for the first month and biweekly thereafter.

Atrazine Transformation. To determine atrazine transformation, jars from the mineralization experiment were sacrificed at seven different times over a period of 205 days. After removal of the NaOH vials, the jars were stored frozen (−20 °C) until extraction. Frozen samples were thawed, and the soils were immediately extracted with a 4:1 (v/v) methanol:water solution at a 3:1 solvent:soil ratio, by shaking for 2 h, sitting overnight, and then shaking for another 30 min. The soils were then centrifuged (5000 rpm for 30 min), the supernatant was removed, and the soils were reextracted with the methanol:water solution by shaking for 1 h. After centrifugation, the extracts were combined, and 1 mL was sampled to measure the total extractable ^{14}C. The methanol was evaporated with a rotary evaporator (50 °C), and the aqueous phase was centrifuged (10000 rpm for 15 min) to remove suspended sediments. After acidification with 1 mL of 30% HCl, atrazine and metabolites were separated from the suspension by partitioning three times with 50 mL of chloroform. The solvent extracts were combined and evaporated in a rotary evaporator (30 °C). The extracts were resuspended in 5 mL of acetonitrile, filtered through 0.45 μm nylon membrane filters, and concentrated to a final volume of 2 mL under a stream of nitrogen. The samples were stored at −20 °C until analysis by high performance liquid chromatography (HPLC). The methanol−water extractions removed approximately 95% of added ^{14}C atrazine, but recovery after the evaporation and filtration steps declined to 55%. This loss represents the formation of nonextractable residues, polar metabolites, and nonpolar metabolites in samples treated with atrazine and frozen immediately after treatment and losses in extraction process. The data showing atrazine remaining over time are corrected for this recovery.

Atrazine and the formation of the metabolites deethylatrazine (DEA), deisopropylatrazine (DIA), and dealkylatrazine (DEDIA) was deter-

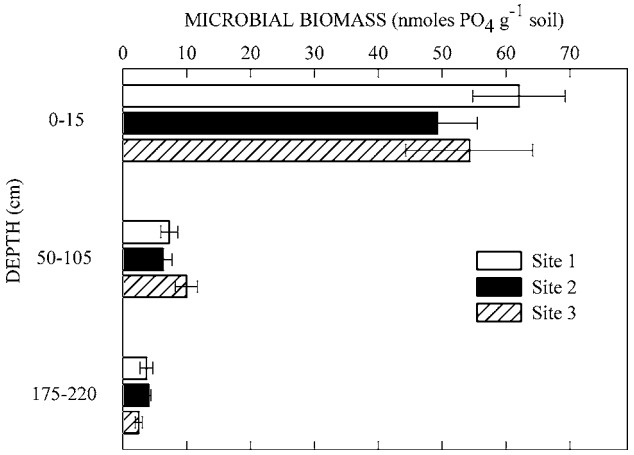

Figure 1. Soil microbial biomass from phospholipids-PO_4 for three soil depths at three field locations. Bars indicate standard deviation of the mean.

mined with a Waters 510 HPLC (Waters Corp., Canberra, Australia). The HPLC was equipped with a Nova-Pak C18, 4 μm, 10 cm column, a Waters 490E programmable multiwavelength detector, and a Radiomatic Flo-one/Beta Series A280 radio-chromatography detector. The mobile phase consisted of deionized water (unmodified pH 5.5) and acetonitrile (75:25), with a flow rate of 1.0 mL min^{-1}. Unlabeled standard mixes of atrazine and metabolites were run to define retention times with UV detection at 220 nm. After HPLC separation of the components, the effluent was passed from the UV detector to the radiochromatography detector and was mixed with a Packard formulated continuous flow scintillation cocktail (Flo-Scint A) at a ratio of 1:3 mobile phase:cocktail (3.0 mL min^{-1} flow rate) and counted in a 2500 μL LSC flow cell.

The bound residues of atrazine were defined as the radioactivity remaining in the soil following extraction. The extracted soils were air-dried and finely ground, and 0.5 g was mixed with 0.3 g of cellulose acetate. This mixture was combusted in an oxidizer (Packard Instrument Co., Downers Grove, IL), and the ^{14}C-carbon was trapped in Carbosorb E, mixed to Permafluor E^+, and counted in LSC.

The degradation processes were quantified by fitting zero and first-order kinetic models to the data and choosing the model with the best fit. The linear and nonlinear procedures (*20*) were used to fit the model parameters to the observed data.

RESULTS AND DISCUSSION

Soil Properties. Table 1 shows selected soil physical and chemical properties of the field sites and depths studied. The percentage of sand, silt, and clay varied from 8 to 22, 44 to 56, and 34 to 42, respectively, with little variation among sites and depths. As expected, a general increase in pH was observed with depth. pH values ranged from 6.58 to 7.76. TOC, N, and P decreased with depth. The decrease was greater from the surface to the lower root than from the lower root to the vadose zone.

Microbiological Analysis. Microbial biomass (**Figure 1**) was estimated by extracting and measuring soil microbial phospholipids-PO_4. There was a significant decline in biomass with depth, but there were no differences among sites within a depth. Biomass was highly correlated with TOC, N, and P ($r > 0.95$) but not with sand, silt, and clay contents or pH. The magnitude of the decrease in microbial biomass was greater from the surface to the lower root zone (5−9-fold) than from the lower root to the vadose zone (1.5−4-fold). This pattern of decline was similar to the pattern of decline in TOC, N, and P in the profile. Other studies show similar depth-related trends in phospholipid biomass in the shallow subsurface (*10*, *21*, *22*).

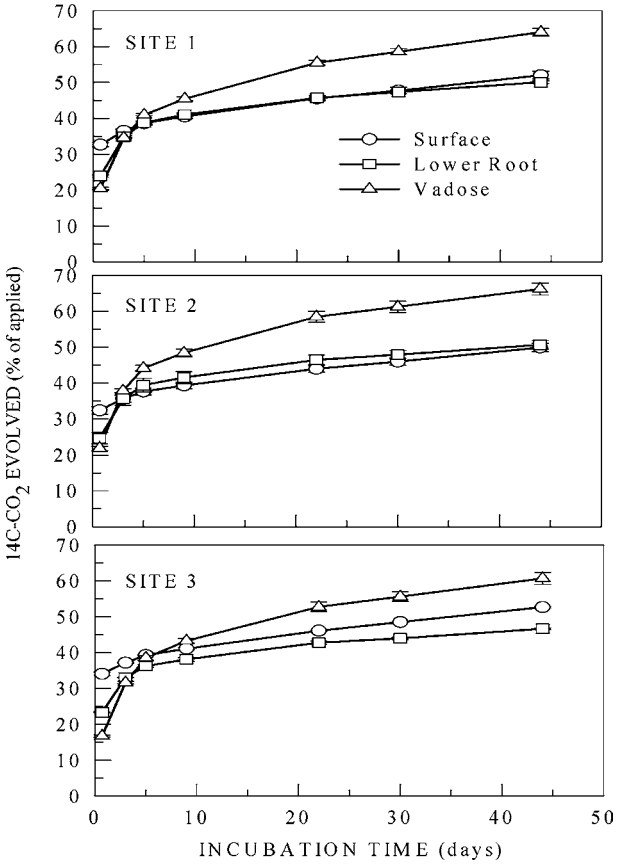

Figure 2. Microbial activity from ^{14}C-benzoate mineralization for three soil depths at three field sites. Bars indicate standard deviation of the mean.

Assuming 50 nmol of phospholipids phosphate for 2.0×10^9 cells (*23*), the average number of bacteria was 2.2×10^9, 3.5×10^8, and 1.5×10^8 cells g^{-1} dry soil for the surface, lower root, and vadose zone, respectively. Estimates of the number of bacteria are higher than the ranges reported of 10^5-10^8 (*24*, *25*) for surface soils and 10^3-10^6 bacteria g^{-1} dry soil (*25*−*27*) for subsurface soils. However, these estimates are based on plate counts that rely on the ability of the organisms to grow on the selected media and tend to underestimate the actual population size.

General microbial activity was estimated from the mineralization of ^{14}C-benzoate (**Figure 2**). Benzoate was chosen as a model compound representative of the general aromatic constituents of soil organic matter (*28*). At the initial sampling time, mineralization was higher in the surface than in the subsurface soil and there was little difference between the lower root and the vadose zone samples. Mineralization of benzoate was greatest in vadose zone soils at the end of the incubation. Total benzoate mineralization was highly correlated to microbial biomass ($r = 0.97$), TOC ($r = 1.00$), mineral N ($r = 1.00$), and total P ($r = 0.95$). Initial benzoate mineralization, estimated by the amount mineralized at the first sampling point, is likely the best indicator of in situ activity. Like microbial biomass (**Figure 1**), initial microbial activity (**Figure 2**) decreased with increasing depth. However, the decline in activity was less than the decline in microbial biomass size. The same was observed for other soils (*21*) and led to the conclusion that each soil has a unique distribution of biomass and activity throughout the profile. Normalizing benzoate mineralization at the first sampling timepoint by the number of cells, estimated from the phospholipid-PO_4 analysis, an estimate of the in situ specific activity was

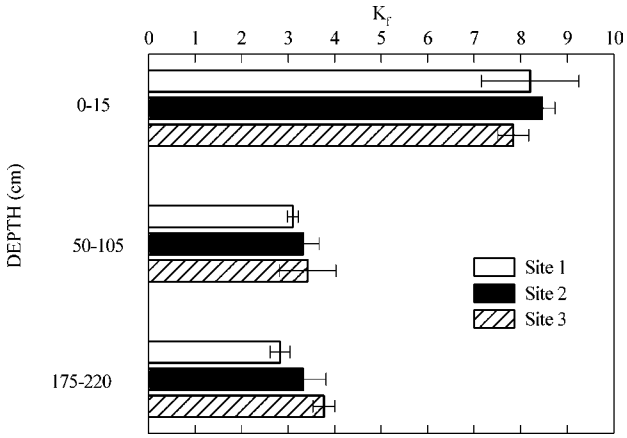

Figure 3. Sorption coefficients (K_f) of atrazine for three soil depths at three field sites. Bars indicate standard deviation of the coefficients.

calculated. The average specific activities obtained were 1.5×10^{-4}, 6.5×10^{-5}, and 1.3×10^{-3} ng benzoate cell^{-1} mineralization for the surface, lower root, and vadose zone, respectively, thus increasing with depth. The subsurface populations were an order of magnitude more active toward benzoate than the surface population, agreeing with another report (*29*) that microbial activity toward succinate, acetate, and salicylate increased with increasing depth although the total number of bacteria decreased. This implies that many of the microbes detected in the surface soil were inactive toward the compounds tested, have other substrates available, or are unable to take up the chemical because it reacts with soil organic matter (*30*). Differences in degradation were a function of the size of the biomass and imply that the subsurface microbial populations were not metabolically hampered or suppressed. Additionally, surface organisms may have incorporated more of the compound into biomass, whereas vadose zone organisms used it as a maintenance energy source.

Atrazine Sorption. As would be expected, atrazine sorption (**Figure 3**) was significantly greater in the surface zone than in the lower root and vadose zones. In general, for any depth increment, there was no difference in sorption among sites. Sorption coefficients (K_f) ranged from 7.84 to 8.47 for the surface, from 3.1 to 3.42 for the lower root, and from 2.82 to 3.77 for the vadose zone. The n values ranged from 0.71 to 0.81. The K_f values in the subsoil are generally larger than those reported at other midwestern USA sites (*10*, *31*, *32*), but this appears to be due to the greater organic C content in the Huntington subsoil. The K_f values were highly correlated with TOC ($r = 0.98$), but poorly correlated with clay content ($r = 0.49$). Some studies show the same correlations (*33*), but others found a poor correlation between adsorption and organic C but a reasonable correlation ($r = 0.64$) with clay (*27*).

Atrazine Mineralization. Overall, mineralization of atrazine was low, especially in the root zone and vadose soils (**Figure 4**). The decline in mineralization paralled the decrease in TOC, N, and P (**Table 1**) and the reduction in overall biomass size (**Figure 1**) with increasing depth. The atrazine mineralized after 205 days of incubation averaged 25.6% for the surface, 3.3% for the lower root, and 2.5% for the vadose. The amounts and pattern of mineralization are similar to other atrazine mineralization results obtained at this site (*34*). Mineralization values twice as high (*11*) and approximately 10 times lower (*16*) have been reported for other sites. Soil physical factors affecting the availability of atrazine for degradation as well as microbial populations with differing sizes and activities may account for these differences.

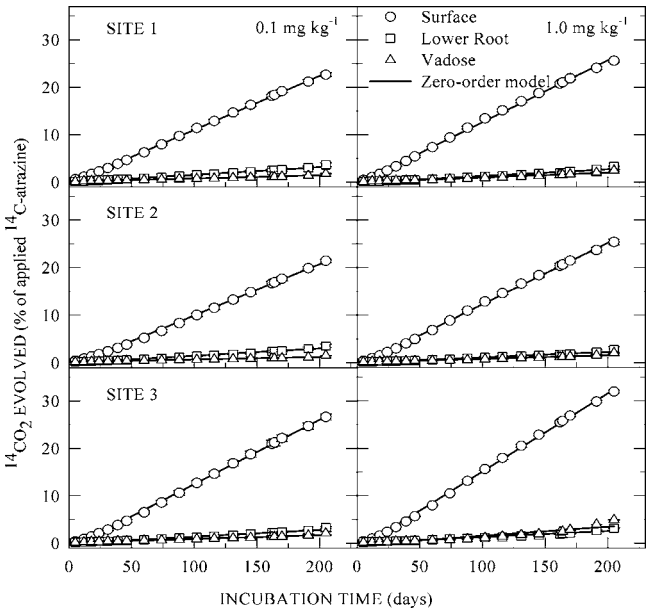

Figure 4. Mineralization of atrazine at two concentrations for three soil depths at three field sites. Bars indicate standard deviation of the mean.

Table 2. Mineralization Rates of Atrazine at 0.1 and 1.0 mg kg^{-1} Soil Application Rates for Three Soil Depths at Three Field Locations[a]

site	application rate (mg kg^{-1})	mineralization rate (ng kg^{-1} day^{-1}) 0–15 (cm)	50–105 (cm)	175–220 (cm)
1	0.1	111 (110–112)	16 (15.2–16.3)	8 (7.10–8.10)
	1.0	1270 (1260–1290)	130 (125–141)	100 (95–106)
2	0.1	102 (99–104)	150 (144–157)	7 (6.0–7.1)
	1.0	1240 (1220–1270)	120 (110–121)	80 (76–87)
3	0.1	127 (124–131)	14 (13.5–14.6)	9 (8.4–9.4)
	1.0	1550 (1510–1580)	130 (127–141)	160 (139–176)

[a] Numbers in parentheses are the confidence intervals for rates.

Linear models gave a strong fit (r^2 generally higher than 0.98) to the mineralization data and were used to estimate the rates of atrazine mineralization (**Table 2**). Increasing the rate of applied atrazine from 0.1 to 1.0 mg atrazine kg^{-1} soil increased the rates (calculated on a mass basis) at the different depths (**Table 2**). Linear mineralization rates can be indicative of zero-order kinetics (*35*, *36*) and indicate that pesticide concentration in soil is in excess of the capacity of the nongrowing microbial community to degrade the pesticide. However, the increase in mineralization rates (**Table 2**) with increasing concentrations of atrazine is inconsistent with zero-order kinetics. The increase in mineralization rates with increasing concentration is consistent with first-order degradation kinetics. We suggest that the apparent linear mineralization rates observed represent the early phase of a first-order process in which degradation will occur over a very long time period. If we use the first-order equation $C/C_0 = e^{-kt}$ with C_0 defined as the initial atrazine concentration and C as the applied atrazine minus the fraction mineralized over time (t), we estimate atrazine mineralization half-lives (time required to produce 50% mineralization) that are greater than 1000 days. These estimates should be considered provisional because first-order mineralization rate constants cannot be accurately estimated without the plateau phase of the

Table 3. Freundlich Sorption Coefficients and *n* Values for Each Site and Depth

site	depth (cm)	K_f	*n*
1	0–15	8.2	0.72
	50–105	3.1	0.81
	175–220	2.8	0.80
2	0–15	8.4	0.75
	50–105	3.3	0.75
	175–220	3.3	0.77
3	0–15	7.8	0.75
	50–105	3.4	0.74
	175–220	3.7	0.79

mineralization curve. In contrast, others observed a generally higher $^{14}CO_2$ evolution when subsurface soils were treated with atrazine at 0.02 instead of 0.1 mg kg^{-1} (*11*). Other chemicals, such as metribuzin, have shown an increase in the percentage mineralization observed when surface soil was treated with 0.1 instead of 1.0 mg kg^{-1}, but not in subsurface soils (*25*). These results may be indicative of different kinetic constraints affecting the mineralization process. Therefore, rates presented in **Table 2** must be regarded as empirically descriptive and not indicative of a defined kinetic model.

Significantly different mineralization rates were obtained for all depths, decreasing about 10-fold from the surface to the lower root and about 2-fold from the lower root to the vadose zone. Different rates of mineralization were also observed among sites, with site 3 having the greatest rate of mineralization in surface soil followed by sites 1 and 2. Atrazine mineralization rates were highly correlated to microbial biomass ($r = 1.00$) and activity ($r = 0.96$), as well as to TOC ($r = 0.99$), N ($r = 1.00$), and P ($r = 0.97$), but not to soil particle size or pH. The correlation of atrazine mineralization with TOC probably reflects the dependence of the microbial biomass on TOC because the K_f values (**Table 3**) show that atrazine would be less sorbed in the subsurface soils than the surface soils. However, the Freundlich isotherms show that as the total atrazine concentration decreases, the fraction of atrazine adsorbed to the soil would increase.

The ^{14}C-MPN procedure detected ^{14}C-chain-labeled atrazine degraders only in the surface and lower root zones at densities of 2212 and 1253 cells g^{-1} dry soil, respectively. The procedure failed to detect any ring-labeled atrazine degraders, although mineralization of ^{14}C-ring-labeled atrazine was observed in the soils (**Figure 4**). This difference may reflect the fact that atrazine may be degraded by a consortia of organisms and that consortium was disrupted by the MPN procedure. Consortium degradation of atrazine has been reported (*37*). It could also indicate the MPN medium was unsuitable for growth of atrazine-degrading microorganisms in these soils. A positive correlation ($r^2 = 0.88$) between atrazine ethyl side-chain degraders and mineralization of ^{14}C-ethyl-atrazine has been observed (*3*), but the population of organisms able to degrade ^{14}C-ring-labeled-atrazine was generally 10-fold less than the population's ability to degrade ^{14}C-ethyl-atrazine and were nondetectable in two of the six soils examined.

Atrazine Transformation. After 205 days, the formation of bound residues (**Figure 5**), expressed as a percentage of the applied atrazine, averaged 39.3 for the surface, 23.4 for the lower root, and 16.5 for the vadose zone. Bound residue formation decreased with depth but increased with time at all three depths. In a soil with chemical characteristics similar to those used in this study, 58.2% of the applied atrazine was recovered as bound residues at the surface and 19.8% at a 120 cm depth (*16*). In another study, surface soils that were more acidic and with less organic carbon also showed similar results (*38*). Increased formation of bound residues with time and decreasing depth was also observed with atrazine metabolites (*17*, *39*). However, the rate of formation was greater, implying that metabolites react faster with soil particles than does the parent compound. Increased bound residue concentration with the loss of one or two alkyl groups from atrazine has been attributed to the higher adsorption coefficients of the metabolites (*40*). In general, a slightly greater, but not statistically significant, amount of bound residue formation was observed for site 3 at the lower concentration than for the other sites and the higher concentration of atrazine. Increased bound residue formation at lower concentrations of atrazine has been observed (*41*).

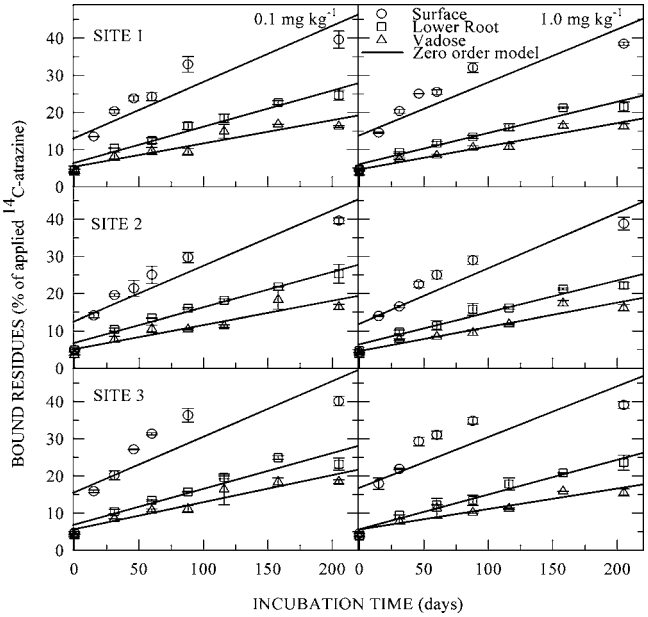

Figure 5. Bound residues of atrazine at two concentrations for three soil depths at three field sites. Bars indicate standard deviation of the mean.

Kinetics of bound residue formation were explained with the use of a zero-order model for the lower root and vadose zones ($r^2 > 0.91$). Although bound residue formation in the surface soils is better described by a first-order regression model, we used the zero-order model for purposes of comparison to the subsurface soils. The zero-order model fit the surface soil data reasonably well with r^2 between 0.76 and 0.85. Significant differences were not observed between the rates of bound residues formation for the different sites, but increasing the atrazine application rate from 0.1 to 1.0 mg kg^{-1} increased the rate of formation of bound residues. At 0.1 mg kg^{-1}, the rates of formation of bound residue were 1.48×10^{-4} mg kg^{-1} day^{-1} for the surface, 9.57×10^{-5} mg kg^{-1} day^{-1} for the lower root, and 6.63×10^{-5} mg kg^{-1} day^{-1} for the vadose zone. At the higher application rate (1.0 mg kg^{-1}), the rates of formation of bound residue were 1.45×10^{-3} mg kg^{-1} day^{-1} for the surface, 8.70×10^{-4} mg kg^{-1} day^{-1} for the lower root, and 6.10×10^{-4} mg kg^{-1} day^{-1} for the vadose zone. The rates of bound residues formation were highly correlated to atrazine mineralization rates ($r = 0.96$), atrazine sorption ($r = 0.95$), microbial biomass ($r = 0.97$) and activity ($r = 1.00$), and TOC ($r = 0.99$), indicating a strong interrelation. To our knowledge, kinetics of bound residues formation have not been previously reported.

Atrazine was less persistent in the surface soil (**Figure 6**) and increasingly more persistent in the lower root and vadose zones. Estimated half-lives of atrazine (1.0 mg kg^{-1}) in surface soils were 87 days for site 1, 77 days for site 2, and 101 days

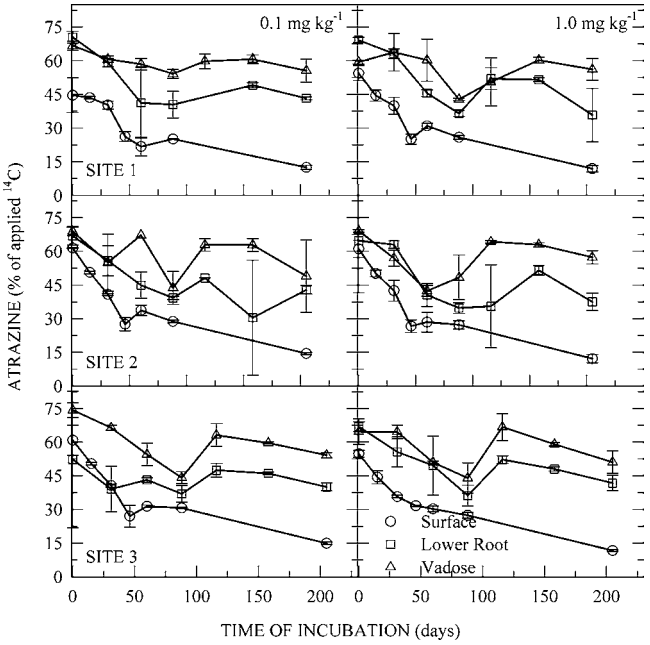

Figure 6. Recovery of atrazine (determined by HPLC) applied at two concentrations for three soil depths at three field sites. Bars indicate standard deviation of the mean.

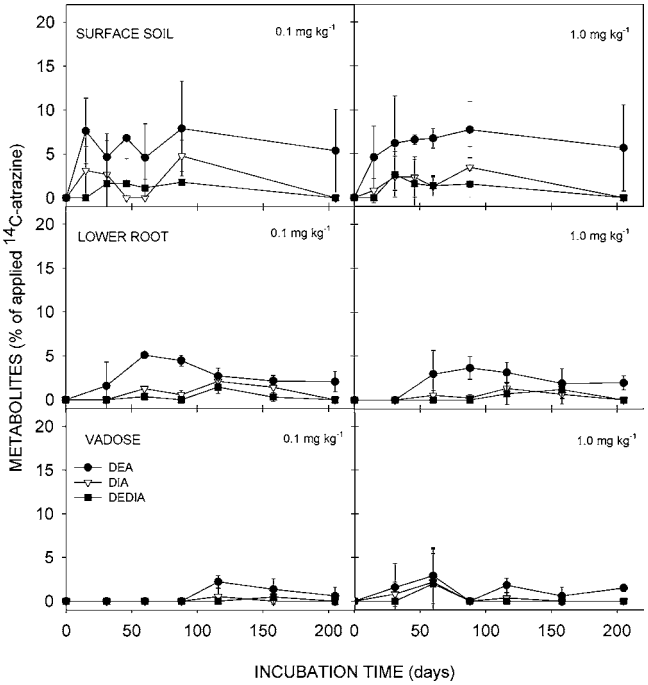

Figure 7. Formation of metabolites DEA (deethylatrazine), DIA (deisopropylatrazine), and DEDIA (dealkylatrazine) from atrazine applied at two concentrations for three soil depths. Bars indicate standard deviation of the mean for the three sites.

for site 3. The increase in atrazine persistence with depth has been observed in several other studies (*12*, *13*, *16*). Estimated atrazine (1.0 mg kg^{-1}) half-lives for the vadose zone were 1700 days for sites 1 and 2 and 990 days for site 3. The increased persistence in the lower root and vadose zones is attributed to the decreased microbial biomass or activity, expressed either as total biomass, benzoate mineralization, or as atrazine-degrading microbial populations. Sorption does not appear to be a factor in limiting subsurface atrazine degradation; K_f values in the subsurface soils are less than half of those found in the surface.

Metabolites accounted for less than 15% of the applied atrazine in all of the surface soils and less than 5% in the subsurface soils (**Figure 7**). Percentages recovered as atrazine and metabolites did not account for all of the radioactivity in the nonpolar fraction. This could indicate that part of the activity was composed of residues not identified in this study. Concentrations of DEA, DIA, and DEDIA tended to increase as metabolites were formed and then decreased over time as they were degraded. A similar degradation pattern, but little difference between the amount of metabolites formed in surface and subsurface soils, has been shown with metribuzin metabolites, as well as more rapid formation of metabolites at the surface as compared to the subsurface soil (*25*). Greater amounts of DEA were recovered from all depths, followed by DIA and DEDIA for the surface soils only. Virtually no DIA and DEDIA were recovered from the lower root and vadose zones. The general rank of metabolite concentrations HA ≥ DEA > DIA > DEDIA has been observed for surface and subsurface soils and tile drainwaters in other studies (*4*, *16*, *38*, *42*−*44*). HA was not determined in this study.

Our data show that atrazine is extremely persistent in the subsurface vadose and saturated zones. This observation appears to be due to decreased biodegradation, evidenced by the decreased $^{14}CO_2$ production, the absence of detectable atrazine ring degrader populations, and decreased metabolite production in the subsurface zones relative to the surface soil. Despite the decreased microbial biomass in the subsurface soils, there are sufficient populations to metabolize the simple aromatic substrate, benzoate, which suggests that the rarity of atrazine-degrading microorganisms is the key factor governing atrazine degradation in the subsurface.

We also show that bound (nonextractable ^{14}C) residues are an important dissipation mechanism in the subsurface. Bound residue formation accounted for approximately 20% of the applied ^{14}C, which was as great an amount as the sum of metabolites and the $^{14}CO_2$ production. Similar patterns of atrazine dissipation (percentages present as $^{14}CO_2$, bound residues, remaining atrazine, and metabolites) were found when atrazine was applied at either 1.0 or 0.1 mg kg^{-1} soil. Some differences in rates of atrazine mineralization and degradation were found between the three sites, indicating that there is a small-scale variation in these processes within the field. The increased persistence of atrazine in the vadose and shallow saturated zones in combination with the low sorption of atrazine to these subsoils indicates that any atrazine leaching from the surface would be largely available for further leaching in these subsurface zones.

LITERATURE CITED

(1) Hallberg, G. R. Pesticide pollution of groundwater in the humid United States. *Agric., Ecosyst. Environ.* **1989**, *26*, 299−367.

(2) Kolpin, D. W.; Goolsby, D. A.; Thurman, E. M. Pesticides in near-surface aquifers: An assessment using highly sensitive analytical methods and tritium. *J. Environ. Qual.* **1995**, *24*, 1125−1132.

(3) Jayachandran, K.; Stolpe, N. B.; Moorman, T. B.; Shea, P. J. Application of ^{14}C-most-probable-number technique to enumerate atrazine-degrading microorganisms in soil. *Soil Biol. Biochem.* **1998**, *30*, 523−529.

(4) Adams, C. D.; Thurman, E. M. Formation and transport of deethylatrazine in the soil and vadose zone. *J. Environ. Qual.* **1991**, *20*, 540−547.

(5) Spalding, R. F.; Burbach, M. E.; Exner, M. E. Pesticides in Nebraska's groundwater. *Ground Water Monit. Rev.* **1989**, *9*, 126−133.

(6) Wackett, L. P.; Sadowsky, M. J.; Martinez, B.; Sapir, N. Biodegradation of atrazine and related *s*-triazine compounds: from enzymes to field studies. *Appl. Microbiol. Biotechnol.* **2002**, *58*, 39−45.

(7) Koskinen, W. C.; Clay, S. A. Factors affecting atrazine fate in north central U. S. soils. *Rev. Environ. Contam. Toxicol.* **1997**, *151*, 117−165.

(8) Jenks, B. M.; Roeth, F. W.; Martin, A. R.; McCallister, D. L. Influence of surface and subsurface soil properties on atrazine sorption and degradation. *Weed Sci.* **1998**, *46*, 132−138.

(9) Miller, J. L.; Wollum, A. G.; Weber, J. B. Degradation of carbon-14-atrazine and carbon-14-metolachlor in soil from four depths. *J. Environ. Qual.* **1997**, *26*, 633−638.

(10) Stolpe, N. B.; Shea, P. J. Alachlor and atrazine degradation in a Nebraska soil and underlying sediments. *Soil Sci*. **1995**, *160*, 359−370.

(11) Helweg, A. Degradation of pesticides in subsurface soil. In: *Proceedings of the International Symposium on Environmental Aspects of Pesticide Microbiology*; Sigtuna: Sweden, 1992; pp 249−265.

(12) Accinelli, C.; Giovanni, D.; Vicari, A.; Catizone, P. Atrazine and metolachlor degradation in subsoils. *Biol. Fertil. Soils* **2001**, *33*, 495−500.

(13) Jacobsen, C. S.; Shapir, N.; Jensen, L. S.; Jensen, E. H.; Juhler, R. K.; Streibig, J. C.; Mandelbaum, R. T.; Helweg, A. Bioavailability of triazine herbicides in a sandy soil profile. *Biol. Fertil. Soils* **2001**, *33*, 501−506.

(14) Radosevich, M.; Traina, S. J.; Hao, Y. L.; Tuovinen, O. H. Degradation and mineralization of atrazine by a soil bacterial isolate. *Appl. Environ. Microbiol.* **1995**, *49*, 711−713.

(15) Vanderheyden, V.; Debongnie, P.; Pussemier, L. Accelerated degradation and mineralization of atrazine in surface and subsurface soil materials. *Pestic. Sci.* **1997**, *49*, 237−242.

(16) Kruger, E. L.; Somasundaram, L.; Kanwar, R. S.; Coats, J. L. Persistence and degradation of [^{14}C]atrazine and [^{14}C]deisopropylatrazine as affected by soil depth and moisture conditions. *Environ. Toxicol. Chem.* **1993**, *12*, 1959−1967.

(17) Baluch, H. U.; Somasundaram, L.; Kanwar, R. S.; Coats, J. R. Fate of major degradation products of atrazine in Iowa soils. *J. Environ. Sci. Health, Part B* **1993**, *28*, 127−149.

(18) Findlay, R. H.; King, G.; Watling, L. Efficacy of phospholipids analysis in determining microbial biomass in sediments. *Appl. Environ. Microbiol.* **1989**, *55*, 2888−2893.

(19) Lehmicke, L. G.; Williams, R. T.; Crawford, R. C. ^{14}C-most-probable-number method for enumeration of active heterotrophic microorganisms in natural waters. *Appl. Environ. Microbiol.* **1979**, *38*, 644−649.

(20) SAS Institute. *SAS/STAT Users Guide*, Version 6.03; SAS Institute: Cary, NC, 1988.

(21) Federle, T. W.; Dobbins, D. C.; Thornton-Manning, J. R.; Jones, D. D. Microbial biomass, activity, and community structure in subsurface soil. *Ground Water* **1986**, *24*, 365−374.

(22) Blume, E.; Bischoff, M.; Reichert, J. M.; Moorman, T. B.; Konopka, A.; Turco, R. F. Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Appl. Soil Ecol*. **2002**, *20*, 171−181.

(23) Langworthy, D. E.; Stapleton, R. D.; Sayler, G. S.; Findlay, R. H. Genotypic and phenotypic responses of a riverine microbial community to polycyclic aromatic hydrocarbon contamination. *Appl. Environ. Microbiol.* **1998**, *64*, 3422−3428.

(24) Alexander, M. *Introduction to Soil Microbiology*, 2nd ed.; John Wiley: New York, 1977.

(25) Moorman, T. B.; Harper, S. S. Transformation and mineralization of metribuzin in surface and subsurface horizons of a Mississippi delta soil. *J. Environ. Qual.* **1989**, *18*, 302−306.

(26) Wilson, J. T.; McNabb, J. F.; Wilson, B. H.; Noonan, M. J. Biotransformation of selected organic pollutants in ground water. *Rev. Ind. Microbiol.* **1983**, *24*, 225−233.

(27) Konopka, A.; Turco, R. F. Biodegradation of organic compounds in vadose zone and aquifer sediments. *Appl. Environ. Microbiol.* **1991**, *57*, 2260−2268.

(28) Ghiorse, W. C.; Wilson, J. T. Microbial ecology of the terrestrial subsurface. *Adv. Appl. Microbiol.* **1988**, *33*, 107−172.

(29) Tate, R. L., III. Microbial activity in organic soils as affected by soil depth and crop. *Appl. Environ. Microbiol.* **1979**, *37*, 1085−1090.

(30) Weber, J. B.; Best, J. A.; Gonese, J. U. Bioavailability and bioactivity of sorbed organic chemicals. In *Sorption and Degradation of Pesticides and Organic Chemicals in Soils*; Linn, D. M., Ed.; Soil Sci. Soc. Am.: Madison, WI, 1993; pp 153−196.

(31) Moorman, T. B.; Jayachandran, K.; Reungsang, A. Adsorption and desorption of atrazine in soils and subsurface sediments. *Soil Sci.* **2001**, *166*, 921−929.

(32) Seybold, C. A.; McSweeney, K.; Lowery, B. Atrazine adsorption in sandy soils of Wisconsin. *J. Environ. Qual*. **1994**, *23*, 1291−1297.

(33) Stehouver, R. C.; Dick, W. A.; Traina, S. J. Characteristics of earthworm burrow lining affecting atrazine sorption. *J. Environ. Qual.* **1993**, *22*, 181−185.

(34) Ostrofsky, E. B.; Traina, S. J.; Tuovinen, O. H. Variation in atrazine mineralization rates in relation to agricultural management practice. *J. Environ. Qual.* **1997**, *26*, 647−657.

(35) Fomsgaard, I. Modelling the mineralization kinetics for low concentrations of pesticides in surface and subsurface soil. *Ecol. Modell.* **1997**, *102*, 175−208.

(36) Alexander, M.; Scow, K. M. Kinetics of biodegradation in soil. In *Reactions and Movement of Organic Chemicals in Soils*; Sawhney, B. L., Brown, K., Eds.; Soil Sci. Soc. Am.: Madison, WI, 1989; pp 243−269.

(37) Assaf, N. A.; Turco, R. F. Accelerated biodegradation of atrazine by a microbial consortium is possible in culture and soil. *Biodegradation* **1994**, *5*, 29−35.

(38) Winkelmann, D. A.; Klaine, S. J. Degradation and bound residue formation of atrazine in a western Tennessee soil. *Environ. Toxicol. Chem.* **1991**, *10*, 335−345.

(39) Winkelmann, D. A.; Klaine, S. J. Degradation and bound residue formation of four atrazine metabolites, deethylatrazine, deisopropylatrazine, dealkylatrazine, and hydroxyatrazine, in a western Tennessee soil. *Environ. Toxicol. Chem.* **1991**, *10*, 347−354.

(40) Schiavon, M. Studies of the movement and the formation of bound residues of atrazine, of its chlorinated derivatives, and of hydroxyatrazine in soil using ^{14}C ring-labeled compounds under outdoor conditions. *Ecotoxicol. Environ. Saf.* **1988**, *15*, 55−61.

(41) Smith, W. N.; Prasher, S. O.; Khan, S. U.; Barthakur, N. N. Leaching of ^{14}C-labelled atrazine in long, intact soil columns. *Trans. Am. Soc. Agric. Eng.* **1992**, *35*, 1213−1220.

(42) Qiao, X.; Hummel, H. E. Atrazine degradation, displacement and occurrence of atrazine metabolites in variously cultivated soils in central Hesse. *Meded. Fac. Landbouwwet*. **1992**, *57*, 1147−1156.

(43) Jayachandran, K.; Steinheimer, T. R.; Somasundaram, L.; Moorman, T. B.; Kanwar, R. S.; Coats, J. R. Occurrence of atrazine and degradates as contaminants of subsurface drainage and shallow groundwater. *J. Environ. Qual.* **1994**, *23*, 311−319.

(44) Assaf, N. A.; Turco, R. F. Influence of carbon and nitrogen application on the mineralization of atrazine and its metabolites in soil. *Pestic. Sci.* **1994**, *41*, 41−47.

Received for review February 1, 2004. Revised manuscript received September 10, 2004. Accepted September 13, 2004. This work was supported by the USEPA and Conselho Nacional de Desenvolvimento Técnico Científico-CNPq, Brazil. This is Paper 17,292 of the Purdue Agricultural Experiment Station Series.

JF049830C