Atrazine, Deethylatrazine, and Deisopropylatrazine Persistence Measured in Groundwater in Situ under Low-Oxygen Conditions

Sharon K. Papiernik* and Roy F. Spalding

University of Nebraska Water Sciences Laboratory, Lincoln, Nebraska 68583-0844

The degradation of atrazine [2-chloro-4-(ethylamino)-6-isopropylamino-1,3,5-triazine], deethylatrazine [DEA; 2-amino-4-chloro-6-(isopropylamino)-1,3,5-triazine], and deisopropylatrazine [DIA; 2-amino-4-chloro-6-(ethylamino)-1,3,5-triazine] was assessed under limited oxygen conditions using in situ microcosms. Denitrification was induced in a shallow sand and gravel aquifer to measure the potential for degradation of atrazine, DEA, and DIA under low-O2 conditions. The dissolved oxygen content decreased from 7–8 mg/L to ≤1 mg/L within 4 days and remained ≤3 mg/L for the remainder of the 45-day experiment. Atrazine, DEA, and DIA concentrations (normalized to the chloride concentration at each sampling time to account for dilution) did not show a significant decrease with time, indicating that these compounds are relatively stable under the low-O2 conditions induced in the aquifer. Although removal of one alkyl group has been proposed as the rate-limiting step in atrazine degradation, no transformation of either monodealkylated metabolite (DEA or DIA) was observed in this study.

Keywords: Degradation; triazine; groundwater; denitrification; metabolite

INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-isopropylamino-1,3,5-triazine] and its degradation products are common contaminants of groundwater in agricultural regions of the United States (Kolpin et al., 1996; Ritter, 1990; Spalding et al., 1989). The degradative properties of atrazine have been assessed in both laboratory and field studies. Extensive degradation has been observed in surface soils, with the removal of the alkyl side chains reported as the initial step in the microbial degradation pathway (Behki and Khan, 1986; Giardina et al., 1982; Wolf and Martin, 1975). Others report that the dechlorination of atrazine to form hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine] appears to be the rate-limiting step to further degradation (Assaf and Turco, 1994; Chung et al., 1996; Mandelbaum et al., 1995; Stucki et al., 1995). Formation of hydroxyatrazine may be a biotic process (Mandelbaum et al., 1995; Stucki et al., 1995; Yanze-Kontchou and Gschwind, 1994) as well as an abiotic process; formation of deethylatrazine (DEA) and deisopropylatrazine (DIA) may also be biotic or abiotic (Kruger et al., 1997).

Mineralization of the triazine ring is generally slower and less extensive than dealkylation (McMahon et al., 1992; Nair and Schnoor, 1992, 1994). Mirgain et al. (1993) isolated a bacterium that could degrade atrazine and also identified an apparent synergistic relationship between two bacteria that could degrade atrazine together. Several researchers have isolated bacteria or mixed cultures that could use atrazine as the sole carbon source (Behki and Khan, 1986; Giardina et al., 1980; Mandelbaum et al., 1993; Yanze-Kontchou and Gschwind, 1994) or that could use atrazine or its metabolites as the sole nitrogen source (Alvey and Crowley, 1995; Assaf and Turco, 1994; Giardina et al., 1980; Mandelbaum et al., 1995; Radosевич et al., 1995). The addition of a carbon source has been shown to enhance the degradation of several compounds under various conditions. Examples include the accelerated degradation of atrazine metabolites (Assaf and Turco, 1994) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) under sulfate-reducing or methanogenic conditions (Gibson and Sulfiita, 1990), atrazine and alachlor [2-chloro-2′,6′-diethyl-N-(methoxymethyl)acetanilide] under denitrifying conditions (Wilber and Parkin, 1995), and total triazine in atrazine-spiked sediments under anaerobic conditions (Chung et al., 1996).

Some isolated bacteria capable of degrading atrazine have been classified as facultative anaerobes (J essee et al., 1983) that can reduce nitrate (Mandelbaum et al., 1995; Radosевич et al., 1995; Yanze-Kontchou and Gschwind, 1994). The degradation of atrazine in surface soils has been reported to be slower under anaerobic conditions than under aerobic conditions (Nair and Schnoor, 1992, 1994; Yanze-Kontchou and Gschwind, 1995). Wilber and Parkin (1995) observed no significant differences in the rate of atrazine degradation by aerobic, nitrate-reducing, sulfate-reducing, or methanogenic microbial cultures. The rate of atrazine degradation has been reported to be slower under low-oxygen conditions than under aerobic conditions in estuarine sediments (J ones et al., 1982) and wetland sediments (Chung et al., 1995; Ro and Chung, 1995). Stucki et al. (1995) reported limited atrazine degradation under low-O2 conditions (<2 mg/L) but rapid degradation when nitrate was available as an alternative electron acceptor. Differing results have been obtained for atrazine degradation under anaerobic conditions in wetland soils: Chung et al. (1995) observed ~50% degradation of atrazine (milligrams per liter levels) in 38 weeks under anaerobic conditions in...
slurries of wetland soil and wetland water; Gu et al. (1992) observed no degradation of similar concentrations of atrazine under methanogenic and nitrate-reducing conditions in laboratory microcosms of wetland soil slurries incubated for ≥300 days.

Atrazine degradation rates in groundwater have been estimated by incubating laboratory microcosms containing aquifer material and herbicide solution and monitoring solution concentrations or 14CO2 evolution from 14C-labeled atrazine with time. No dealkylation of atrazine was observed in 70 days in laboratory studies conducted by inoculating cement-grade sand with aquifer microbes at 13 or 28 °C (Wehtje et al., 1983). McMahon et al. (1992) observed no mineralization of the triazine ring in 23 days, with some dealkylation to DIA [2-amino-4-chloro-6-(ethylamino)-1,3,5-triazine] in systems containing shallow aquifer sediments; deeper sediments exhibited less dealkylation. Vanderheyden et al. (1997) observed rapid degradation of atrazine in sediment samples collected from the saturated zone, and the metabolites were unidentified; mineralization of 14C-atrazine began after a lag phase of ~25 days and ~25% of the applied 14C-atrazine was mineralized in 75 days. Agertved et al. (1992) observed no decrease in atrazine concentrations during 74 days in laboratory microcosms of groundwater and aquifer material.

The fate of herbicides in groundwater is difficult to assess, since many physical and chemical processes affect concentrations in situ. Some parameters, such as oxygen content, redox potential, and microbial population, are difficult to simultaneously control in the laboratory and may not be representative of aquifer characteristics. The persistence of contaminants in situ can be determined by monitoring groundwater concentrations in field transport studies. Agertved et al. (1992) observed no degradation of high concentrations of atrazine (~400 μg/L) in 96 days in an aerobic aquifer [dissolved oxygen (DO) ranging from 2.1 to 8.0 mg/L]. Widmer and Spalding (1995) observed no degradation at concentrations more typical of nonpoint source contamination [atrazine, DEA [2-amino-4-chloro-6-(isopropylamino)-1,3,5-triazine], and DIA, each at ~3 μg/L] in 3 months in an aerobic aquifer (DO = 6.9 mg/L).

A method to measure the rate of transformation of contaminants in situ was presented by Gillham et al. (1990), who followed the rate of denitrification and benzene degradation using an in situ microcosm (ISM). ISMs isolate a portion of the aquifer from horizontal advective flow. They also minimize disturbance of the aquifer and allow for measurement of reaction rates under aquifer conditions, since parameters such as pH, Eh, DO, the composition of the groundwater and solid matrix, and the microbial population remain essentially unchanged (Gillham et al., 1990). Agertved et al. (1992) used microcosms of this design to determine the persistence of 400 μg/L atrazine and MCP [potassium salt of 2-(2-methyl-4-chlorophenoxy)propionic acid] in an aerobic aquifer; they observed minimal degradation of both compounds in 47 days.

Information regarding the transformation potential of pesticides in groundwater under low-oxygen conditions is lacking. Much of the research on the rate of triazine herbicide degradation in groundwater has been conducted in the laboratory. Field studies have largely been conducted under aerobic conditions (Agertved et al., 1992; Widmer and Spalding, 1995). The objective of this study was to use ISMs to determine the persis-

tence of atrazine and two of its dealkylated metabolites, DEA and DIA, under oxygen-limited conditions induced in a shallow sand and gravel aquifer contaminated by both atrazine and nitrate.

EXPERIMENTAL PROCEDURES

Experimental Site. The 0.2-ha study site was located in Merrick County in the Central Platte region of Nebraska (Figure 1) and is surrounded by farmland cropped to irrigated corn. The area has a long history of atrazine application; sandy soils and a shallow water table make the area's groundwater vulnerable to agrichemical contamination. Both atrazine and nitrate are commonly detected in the groundwater of Merrick County (University of Nebraska Water Sciences Laboratory, 1994). Groundwater collected from 18 irrigation wells in Merrick County had atrazine concentrations ranging from <0.005 to almost 7 μg/L and nitrate-N concentrations from ~5 to 31 mg/L (Spalding et al., 1979). The aquifer is characterized as Pleistocene Age sands and gravels. The water table is at a depth of 2–3 m.

Microcosms (Figure 2) were constructed and installed as described in Bates and Spalding (1998). Holes were excavated to ~2 m, and steel casings (1.2 m diameter × 2.5 m long) were installed to keep the holes open. A PVC pipe (0.25 m diameter × 1.2 m long) was inserted into the aquifer at the bottom center of each hole. The PVC pipe was voided of sediment so that the microcosms could be installed well below the water table. Microcosms (16 cm diameter × 61 cm long) were vibrated to a depth of ~3 m below land surface with a vibracorer. Replicate ISMs were spaced at ~4.6 m intervals. Each microcosm has a volume of ~11 L when empty; when in place in the aquifer (filled with sediment), they each contain ~3.7 L of pore water. Each ISM is open at the bottom to allow exchange with the aquifer water. The top is sealed with a threaded stainless steel cap. A main screen (Figure 2) was used for the injection or withdrawal of large volumes of water. The ISMs also contained a miniature drive-point (Figure 2), which was used to collect water samples to monitor the processes occurring within the ISM.

Injection and Monitoring of Solutes. At least 3 volumes of stagnant water (~30 L) were removed from duplicate microcosms and discarded. For the injection of solutes, ~10 L of water contained within the microcosm was vacuum-pumped into a glass flask without exposure to the atmosphere. Solutes were injected into the glass flask through a septum
were filtered through 0.2-
membrane filters and collected
precombusted amber glass bottles. Anion samples (20 mL)
DIA analysis were transferred from the syringe to 50 mL
sample collection. Samples (50 mL) for atrazine, DEA, and
of HgCl₂ as a preservative. Samples were kept on ice during
placed in precombusted amber glass vials containing 0.1 mg
mg/L bromide, and atrazine, DEA, and DIA, each at 20

The spiked solution was injected under helium pressure,
spiking the water within the microcosm.
Samples were collected from the drive-point screen using a
60-mL plastic syringe with a Luer tip. One sample was
collected from each microcosm directly following injection;
samples were collected every 2–7 days for 45 days to monitor
solute concentrations. The volume of water held stagnant in
the tubing (~10 mL) was removed and discarded prior to
sample collection. Samples (50 mL) for atrazine, DEA, and
and DIA analysis were transferred from the syringe to 50 mL
precombusted amber glass bottles. Anion samples (20 mL)
were filtered through 0.2-μm membrane filters and collected
in polyethylene vials. Samples for DOC analysis (40 mL) were
placed in precombusted amber glass vials containing 0.1 mg
of HgCl₂ as a preservative. Samples were kept on ice during
transport to the laboratory. Dissolved oxygen samples (30 mL)
were analyzed immediately after collection.

Sample Analyses. Dissolved oxygen was analyzed in the
field using a colorimetric technique calibrated with Winkler
titration (Bates and Spalding, 1998). Anions (Cl⁻, NO₃⁻, Br⁻,
NO₂⁻, and SO₄⁻²) were analyzed by ion chromatography.
Dissolved organic carbon was analyzed using an Oceanographic
International total organic carbon analyzer calibrated with
potassium biphthalate. Since ethanol in the sample
would be volatilized in a sequential C determination, total C
and total inorganic C were determined separately, with the
total organic C determined by difference.

Samples for atrazine, DEA, and DIA analyses were spiked
with known amounts of ring-labeled [¹³C] analogues of atrazine,
DEA, and DIA. The water samples (50 mL) were extracted
by solid-phase extraction using C₁₈ cartridges containing 1 g
of material. The cartridges were eluted with 2 mL of ethyl
acetate, which was dried with anhydrous sodium sulfate and
reduced to ~50 μL under a steady stream of dry nitrogen gas.
A 1.0-μL aliquot was injected onto a gas chromatograph with
a mass selective detector. [¹³C] analogues of each compound
were used as internal standards, and each compound was
analyzed by isotope dilution GC/MS (Cassada et al., 1994).
Isotope dilution accounts for differences in extraction efficiency
and provides for the quantitation and confirmation of each
compound in one analysis (Cassada et al., 1994). Approximate

results observed in this study are similar to but
somewhat slower than those reported by Bates and
Spalding (1998). Repeated measurements of induced
denitrification at this site indicated that from 40 to ~92 h
was required to deplete 50 mg/L nitrate-N and the
nitrite formed during the denitrification reaction (Bates
and Spalding, 1998). Denitrification experiments using
50 mg of C/L as ethanol showed complete removal of 50
mg/L NO₃⁻N in 40 h. Denitrification rates slowed with
the addition of higher concentrations of organic carbon.
The denitrification rates observed in this experiment
may be slower than those reported for other studies at
this site (Bates and Spalding, 1998) because of temporal
and spatial heterogeneity in environmental conditions,
such as temperature, Eh, pH, and microbial population.

Temperature, in particular, may have been a factor
in determining denitrification rates at this site. Tem-
tracer concentration did not show a significant decrease
and DIA concentrations normalized to the conservative
sediments amended with glucose.

declaration rates in laboratory microcosms of an aquifer
concentrations of atrazine (et al. (1994) found that the presence of relatively high
not have affected the rate of denitrification. Bradley
rate cannot be further examined here.
Since no temperature measurements were made in
lower than those measured in the summer (Spalding and Exner, 1980).
Since no temperature measurements were made in

denitrification in soils, with the maximum denitrification
rate observed at 25 °C, and a decreasing rate with
during this study, conducted in mid to late autumn. Average groundwater tem-
teratures in the Central Platte region of Nebraska have
be reported by Spalding and Exner (1980). Temper-
atures at depths similar to those of the installed ISMs
were about 11 ± 3.5 °C, with winter shallow ground-
water temperatures as much as 10 °C lower than those
measured in the summer (Spalding and Exner, 1980).

The atrazine concentrations used in this study should
not have affected the rate of denitrification. Bradley
(1994) found that the presence of relatively high
concentrations of atrazine (~100 µg/L) did not alter
denitrification rates in laboratory microcosms of aquifer
sediments amended with glucose.

Persistence of Injected Triazines. Atrazine, DEA,
and DIA concentrations normalized to the conservative
tracer concentration did not show a significant decrease
with time (α = 0.10) (Figure 4). These results indicate
that these compounds are relatively stable under the
low-oxygen conditions induced in this aquifer. Degra-
dation of atrazine in the presence of additional sources
of carbon and energy has been demonstrated in labora-
tory microcosms (Mandelbaum et al., 1995; Radosevich
et al., 1995; Wolf and Martin, 1975). Degradation in
soils spiked with atrazine and inoculated with atrazine-
degrading bacteria has been documented (Yanze-
Kontchou and Gschwind, 1995; Radosevich et al., 1997)

The change in concentration of atrazine and its
metabolites may be attributed to dilution of the amended
water (injected into the ISM) with aquifer water drawn
in through the open bottom of the ISM. Considering
the ISM to be a cell containing 3.7 L of pore water, the
dilution of the amended water may be approximated by
assuming the same volume of water is drawn in through
the bottom as was withdrawn with each sampling, with
perfect mixing of the water within the ISM. This gives
a "predicted" concentration based only on dilution.
The variability between the two replicate microcosms may reflect the spatial and temporal heterogeneity of conditions. These may include, but are not limited to, the microbial population, the hydrogeological conditions that affect the composition of water available for exchange with the ISMs, and the dynamics of exchange, which is likely more complex than the simple dilution considered here.

It has been proposed that the abiotic hydrolysis of atrazine may be the dominant degradative process occurring in groundwater (Wehtje et al., 1983). The rate of atrazine hydrolysis in aerobic groundwater has been estimated as producing a 3% decrease in atrazine concentration in 70 days (Wehtje et al., 1983). The decrease in relative concentration that would result from a decrease of this magnitude in the ISMs is indicated in Figure 5. Decreases in concentration of this order are too small to be detected in this study, on the basis of the observed variability within and between ISMs (Figure 5).

Atrazine contamination of the groundwater in this area is extensive (Spalding et al., 1989; University of Nebraska Water Sciences Laboratory, 1994); the microbial population has been exposed to these compounds for many years. The time required to partially degrade atrazine in anaerobic laboratory microcosms ranges from a few days [e.g., Jesse et al. (1983), Radosevich et al. (1995), and Stick el et al. (1995)] to months or more (Nair and Schnoor, 1992). In this study, low-O2 conditions could be sustained for only 45 days. It is unknown whether this is sufficient time for the microbial population to acclimate to these conditions. The lack of degradation could be due to unfavorable conditions or the absence of a microbial population capable of degrading atrazine under anaerobic conditions. Atrazine mineralization rates in surface soils have been shown to be reduced by the presence of high concentrations of NO3 (2.5 mg of NO3-N/g of soil) (Alvey and Crowley, 1995); the highest NO3-N concentrations used in this study were =2 orders of magnitude lower than those showing an effect in Alvey and Crowley (1995) and were estimated as 0.01 mg of NO3-N/g of sediment. The rate of naphthalene degradation under denitrifying conditions in surface soils was not affected by the nitrate concentration in a study by Mihelcic and Luthy (1991), the degradation of atrazine's dealkylated metabolites (DEA and DIA) appeared to some evidence for degradation of atrazine and its monodealkylated metabolites in one amended microcosm, these results were not observed in a replicate microcosm (Figure 5). The results of the present study, taken in conjunction with those of a previously conducted field study in an aerobic aquifer (Widmer and Spalding, 1995), indicate that atrazine, DEA, and DIA are relatively persistent in groundwater over periods of several months, regardless of oxidizing conditions. These results support the observation of steady-state concentrations of atrazine, DEA, and DIA in groundwater in this area contaminated by long-term atrazine use (University of Nebraska Water Sciences Laboratory, 1994; R. F. Spalding, 1995, unpublished data).

**ABBREVIATIONS USED**

DEA, deethylatrazine; DIA, desisopropylatrazine; DO, dissolved oxygen; ISM, in situ microcosm; DOC, dissolved organic carbon.

**ACKNOWLEDGMENT**

This research was completed while the principal author was a graduate student at the University of Nebraska. Herbert K. Bates assisted with the injection of solutes and sample collection. We appreciate the cooperation of Jon Efferson in providing a field site. Patrick Shea, Stephen Comfort, J. I. Y. Yates, and two anonymous reviewers provided helpful comments on the manuscript.

**LITERATURE CITED**


University of Nebraska Nebraska Water Sciences Laboratory. Assessment of Pesticide Occurrence in Nebraska Ground Water; University of Nebraska Water Sciences Laboratory, Water Center/Environmental Programs: Lincoln, NE, 1994.


Received for review July 21, 1997. Revised manuscript received November 20, 1997. Accepted November 23, 1997. This research was funded through the U.S. Bureau of Reclamation, the Nebraska Research Initiative, and a fellowship from the University of Nebraska Medical Center’s Environmental Toxicology and Carcinogenesis Graduate Program.