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Sorption kinetics of atrazine and hydroxyatrazine in freshwater wetlands

by

Michael Patrick Matter

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INTRODUCTION

The s-triazine herbicide atrazine (2 chloro-4-[ethylamino]-1,3,5 triazine) is one of the most heavily used agricultural pesticides in North America. Each year, 110 million pounds are applied to over 62 million acres in the United States for broadleaf and grass control in corn (Eisler 1989). As might be expected, atrazine is one of the most frequently detected pesticides in surface waters and groundwater in the midwestern cornbelt. Its occurrence in groundwater is 10 to 20 times as frequent as the next most commonly detected pesticides (Hallberg 1989). Atrazine has been detected in streams in the region at concentrations as high as 108 ug/L during the spring (Thurman et al. 1992). In runoff water immediately adjacent to treated fields, atrazine concentrations have been reported ranging from 27 to 69 ug/L and may reach 1000 ug/L (DeNoyelles et al. 1982). This suggests that prairie pothole wetlands commonly surrounded by cropland may be receiving significant atrazine loads.

The presence of atrazine in surface water and groundwater (Eisler 1989, Leonard et al. 1988, Barrett and Williams 1989, Hallberg et al. 1984) has sparked an interest in research concerning atrazine behavior in aquatic systems, yet there is surprisingly little literature available characterizing the fate of atrazine in freshwater wetlands.

Recent attention to the water quality functions of wetlands has led to an interest in the establishment of created and restored wetlands for the treatment of agricultural drainage water with respect to nutrients and pesticides. Potential exists in the Midwestern corn belt for the improvement of water quality through wetland restoration based on both wetland drainage and surface water quality criteria (van der Valk and Jolly 1992). In Iowa 99% of the native wetlands have been drained primarily for agricultural production, and over 90% of the total land area is used for agricultural production (Isenhart 1992).
Despite the interest in using restored wetlands to treat agricultural drainage water, very little is known about the fate of pesticides in freshwater wetlands (van der Valk and Jolly 1992, Olson 1992). More information is needed to evaluate the capacity of restored wetlands to effectively act as sinks for pesticides and pesticide degradation products through degradation and sorption processes.

**Atrazine Degradation**

Hydroxyatrazine is the major atrazine degradation product in both soil and aquatic systems (Eisler 1989, Schiavon 1988a,b, Winkelmann and Klaine 1991, Jones et al. 1982). It is the result of the removal of the chlorine from the number two position on the triazine ring and its subsequent replacement with a hydroxy group (Figure 1). Although hydrolysis is generally considered to be a physical process (Paris and Lewis 1973, Erickson and Lee 1989), Couch et al. (1965) reported rapid hydrolysis of atrazine by *Fusarium roseum*.

Winkelmann and Klaine (1991c) detected hydroxyatrazine at higher levels than any of the chlorinated, monoalkylated atrazine metabolites in intact soil microcosm experiments using incubation periods as long as 180 days. Khan and Saidak (1981) found hydroxyatrazine to be the primary atrazine degradation product present in soil after 20 consecutive annual field applications of atrazine.

Jones et al. (1982) reported a fairly rapid conversion of atrazine to hydroxyatrazine in estuarine sediment/water systems. Hydroxyatrazine was by far the most significant by-product found in both the sediment and water extracts. After 180 days, hydroxyatrazine comprised over 95% of the extracted activity from the water fraction and 70% to 90% of the extracted activity from the sediment fraction.

Hydroxyatrazine resulting from the non-microbial hydrolysis of atrazine has been found to be quite vulnerable to microbial degradation. It has been shown that microbes degrade hydroxyatrazine 3 times faster than unhydrolized atrazine (Skipper et al.
Figure 1. Diagram of atrazine degradation pathways in the soil environment
Kaufman and Kearney (1970) reported the dealkylation of hydroxyatrazine in higher plants. Breakdown products resulting from the degradation of hydroxyatrazine have not been clearly identified. This is thought to be due to the rapid degradation of the metabolites produced after the initial dealkylation (Erickson and Lee 1989).

The primary mechanism of the microbial degradation of atrazine is the dealkylation of either of the two alkylamino groups (Paris and Lewis 1972). It is the first step in the microbial degradation of all chloro-s-triazine herbicides (Erickson and Lee 1989). Atrazine can be dealkylated to deethylatrazine or deisopropylatrazine by removal of the ethyl or isopropyl groups respectively (Kaufman and Kearney 1970). These ethyl and isopropyl side chains contain the only available sources of energy that can be obtained by microorganisms through oxidative phosphorylation of atrazine (Erickson and Lee 1989). Giardi et al. (1985) observed the subsequent biodegradation of deethylatrazine and deisopropylatrazine with the chloro group remaining intact.

Adsorption of Atrazine and Hydroxyatrazine

Atrazine sorption dynamics in field soils are very well characterized. Hance (1974) found that atrazine adsorption decreased as successive amounts of organic matter were removed from a soil. Rao and Davidson (1979) found that atrazine adsorption was correlated to soil organic matter and that adsorption was described by the Freundlich equation even at very high concentrations, thus indicating that adsorption sites did not become saturated. Harris (1966) found atrazine to adsorb to a greater extent to silt loam soils than to a sandy loam soil.

Tallbert and Fletchall (1965) reported that atrazine adsorption decreased with increasing temperature and increased with time. They also reported that adsorption increased linearly with atrazine concentration in an equilibrium solution. Harris and Warren (1964) found that atrazine adsorption by bentonite was greater at 0°C than at 50°C.
Colbert et al. (1975) reported atrazine to be adsorbed to a greater extent at lower pH values than at higher. Adams and Pritchard (1977) found a decrease in the phytotoxicity of atrazine as soil pH decreased and soil organic matter increased. Adsorption and hydrolysis were considered to be responsible for this decrease in phytotoxicity. Clay and Koskinen (1990) also found atrazine adsorption to be greater on low pH soils than on high. They also reported that as the initial concentration of atrazine was increased in a slurry system, the percentage of atrazine adsorbed decreased.

There is a general agreement in the literature that hydroxyatrazine tends to adsorb rather readily to soil and that this binding, along with the relative insolubility of hydroxyatrazine, inhibits its mobility (Schiavon 1988a, Schiavon 1988b, Somasundaram et al. 1991, Brouwer et al. 1990, Erickson and Lee 1989).

Clay and Koskinen (1990) found that in soil/water slurry systems more hydroxyatrazine was adsorbed than atrazine at both high and low pH levels. They also reported that more hydroxyatrazine was adsorbed at low pH than at high pH levels and that as the initial concentration of hydroxyatrazine increased, the percentage adsorbed to the soil decreased.

Using the soil thin-layer technique, Somasundaram et al. (1991) found hydroxyatrazine to be much less mobile than the parent atrazine. Schiavon (1988a, 1988b) found that hydroxyatrazine was the least mobile of atrazine and its metabolites through undisturbed soil columns and that more hydroxyatrazine remained in the top 24 cm of the columns than the parent atrazine or any other chlorinated metabolite. Brouwer et al. (1990) used the traditional batch equilibrium technique for determining sorption characteristics of atrazine and its degradation products. They reported equilibrium constants for hydroxyatrazine that were consistently higher than those for atrazine and its other metabolites for all soils involved.
There is a discrepancy in the literature regarding the strength of binding of hydroxyatrazine as determined by the formation of bound residue. Bound residues are residues which remain unextractable after exhaustive organic solvent extractions of the soil or sediment. Studies by Schiavon (1988a), Winkelmann and Klaine (1991c) and Baluch (1992) would suggest that although hydroxyatrazine does bind readily to soil it is still, for the most part, recoverable with traditional extraction techniques. However, Khan and Saidak (1981) and Erickson and Lee (1989) have done work which would indicate that hydroxyatrazine and other hydroxy analogs are strongly bound and comprise the unextractable portion of the mass balance.

**Atrazine Behavior in Aquatic Systems**

The degradation pathways and sorption kinetics of atrazine in wetlands are not well characterized. The work that has been done appears to deal primarily with fate and effects of atrazine loading in constructed laboratory microcosms (Isensee 1987, Brockway et al. 1984, Huckins et al. 1986). There is no literature that deals with factors affecting atrazine loss kinetics using intact wetland sediment cores.

Huckins et al. (1986), using mixed systems consisting of terrestrial soil, wetland sediment and water, observed a 50% loss of atrazine from the water column after one day. Another 25% was lost over the remaining 41 days of incubation. Jones et al. (1982) found the loss of activity from the overlying water of an estuary sediment/water system dosed with $^{14}$C-atrazine to follow first order kinetics. They reported a 50% loss within 3 to 12 days and a loss of 85% to 90% after 60 days. Loss was more rapid in the aerobic treatment than the low oxygen treatment. After 60 to 80 days of incubation, nearly all of the atrazine had been converted to hydroxyatrazine.

Wauchope and Myers (1985), using lake and stream sediment/water slurries, reported a 50% loss of atrazine from the water fraction in the first minute of stirring and a 75% loss after 20 minutes. They found that 90% of the atrazine that would adsorb in the
slurry system was adsorbed in the first 8 minutes of stirring. Desorption was also rapid and equilibrium was reached in approximately 20 minutes.

Wolf and Jackson (1982) found atrazine adsorption in a horizontally-shaken Arkansas River sediment to increase with organic matter content. Adsorption was also shown to be greater at lower pH values than at neutral pH. Brown and Flagg (1981), using sediment from a northern Georgia pond, determined an empirical relationship between octanol/water partition coefficients and sediment sorption coefficients from adsorption isotherms.

One of the major shortcomings to date with atrazine studies in aquatic systems is that they have failed to use intact sediment systems. Work in the literature has been done with either slurry systems or with highly disturbed sediment. Another problem is that studies have not used environmentally realistic atrazine concentrations. Most work has been done in the parts per million range, while realistic concentrations tend to be below 100 ug/L. This study will examine atrazine behavior in microcosms using intact sediment cores and environmentally relevant atrazine concentrations.

**Thesis Objectives**

The overall objective of this thesis is to examine the long-term and short-term fate of atrazine in freshwater wetlands using intact wetland sediment microcosms. In the long-term, atrazine degradation is examined under aerobic and anaerobic conditions. In the short-term, the sorption kinetics of atrazine and hydroxyatrazine are examined. Specific objectives in the short-term study include:

1. Evaluate the effect of litter, suspended sediment, microcosm sterilization and initial herbicide concentration on the loss of atrazine and hydroxyatrazine from overlying water.

2. Determine the potential for desorption of atrazine and hydroxyatrazine from wetland sediments, and examine their distributions within the sediment profile.
MATERIALS AND METHODS

Radiolabeled chemicals were obtained from the Ciba-Geigy Corporation, Greensboro, NC. The [U-ring-\(^{14}\text{C}\)] atrazine used in the long-term degradation study had a radiochemical purity of 97.0% and a specific activity of 20.9 uCi/mg. The radiolabeled atrazine and hydroxyatrazine used in the short-term adsorption study had the following radiopurities and specific activities: [U-ring-\(^{14}\text{C}\)] atrazine (98.8% pure) 14.6 uCi/mg; [U-ring-\(^{14}\text{C}\)] hydroxyatrazine (98.6% pure) 44.2 uCi/mg.

Sediment cores for both studies were taken from Cooper Prairie Marsh located 3 miles northeast of Ames, IA in Story county. The coring devices used in the degradation study, which also served as the incubation microcosms, consisted of 12 cm long (5.1 cm ID) polycarbonate cylinders which were beveled and machined on the bottom ends to easily penetrate sediment and to accept O-ring fitted end caps. The top end of each was machined to accept an airtight plug, which was fitted with a stoppered, perforated centrifuge tube containing 1.0N NaOH to serve as a CO\(_2\) trap (Figure 2). Cores were obtained by pressing the polycarbonate cylinder into the sediment, sliding the end cap underneath the cylinder and removing the unit as an intact sediment core. In the case of the adsorption study, sediment cores were obtained in a similar fashion as in the degradation study. However, microcosms used in the adsorption study were sealed on both ends with rubber stoppers.

Degradation Study

Upon return from the field, the overlying water was carefully drawn off each microcosm and 35 ml of wetland water was slowly added to each. The overlying water of each microcosm was then dosed with 0.42 ug (12 ug/L) of \(^{14}\text{C}\)-labeled atrazine. Immediately following dosing, the CO\(_2\) traps were emplaced and the microcosms were then allowed to incubate for ten months. The pH of the traps was monitored regularly with pH paper.
Figure 2. Diagram of experimental wetland microcosm
When it was observed that NaOH saturation was approaching, the traps were removed, assayed for $^{14}\text{C}$ using a Beckman LS3801 liquid scintillation counter and replaced.

All samples counted on the Beckman LS3801 were corrected for quench using a quench curve generated with Beckman quenched standards, and calibration was performed with Beckman calibration standards. Duplicate blanks were counted with each sampling.

Upon the completion of incubation, the microcosms were sacrificed. The overlying water was assayed for $^{14}\text{C}$ and the sediment was extracted four times with a 9:1 methanol:water solvent system. Repeated spike and recovery extraction efficiency studies involving methanol:water, acetonitrile:water and HCL:acetonitrile:water combinations with both $^{14}\text{C}$-atrazine and $^{14}\text{C}$-hydroxyatrazine indicated that the 9:1 methanol:water system would be the most effective.

After final extraction, sediments were oxidized by combusting pellets made up of 0.5 g of sediment and 0.1 g of hydrolyzed starch in a Packard Sample Oxidizer (Packard Instrument Co., Downer's Grove, IL). $^{14}\text{CO}_2$ was trapped in Carbo-Sorb$^\text{R}$ E and Permafluor$^\text{R}$ V (Packard Instrument Co., Downer's Grove, IL). Trapping efficiency was determined using Spec-Chec$^\text{TM}$ $^{14}\text{C}$ standard ($9.12 \times 10^5$ dpm/ml) and Spec-Chec$^\text{TM}$ nonactive standard. Triplicate pellets were burned for each sample and radioactivity was determined by using a Beckman LS3801 liquid scintillation counter (Beckman Instrument Co., Irvine, CA).

Experimental design for the degradation study consisted of four replicates per treatment for both aerobic and anaerobic treatments of atrazine-dosed cores and non-treated controls. Anaerobic microcosms were evacuated with nitrogen prior to dosing and their $\text{CO}_2$ traps were exchanged in a nitrogen environment using a glove bag.
Adsorption Study

Upon returning from the field, the overlying water was carefully removed from each microcosm and slowly replaced with 100 ml of wetland water obtained from Cooper Prairie Marsh. In determining the effect of concentration on the loss of atrazine, treatment microcosms consisted of water overlying intact sediment which was dosed at atrazine concentrations of 50, 100 and 300 μg/L (each treatment in all experiments in the adsorption study contained three replicates). The role of a biological component in the loss of atrazine and hydroxyatrazine was examined by autoclaving treatment microcosms, including both intact sediment and overlying water, for 40 minutes. Following treatment preparation, microcosms were dosed at 100 μg/L with either labeled atrazine or hydroxyatrazine. The effect of sediment suspension on the loss of atrazine and hydroxyatrazine was studied by stirring treatment microcosms with a stainless-steel canula which bubbled compressed air. In the study examining the role of litter in the loss of atrazine and hydroxyatrazine, treatments included microcosms containing water overlying intact sediment, microcosms containing only decaying cattail litter within the water column and microcosms containing both intact sediment and cattail litter. Controls consisted of microcosms which contained only water. Those microcosms containing litter were prepared by adding 200 cm² of litter to the overlying water. This 200 cm² is based on an average yearly production of 10 m² of litter for 1 m² of wetland surface area and a 20 cm² surface area in each microcosm. Litter treatments were prepared by covering a 10 cm by 10 cm area with 10 cm segments (approximately 1 cm wide) of intact, partially decomposed cattail litter collected from the wetland sediment surface. These 10 cm segments were then broken in half and placed in the overlying water of the microsoms prior to dosing.

Following treatment preparation, microcosms were dosed at 100 μg/L with either 14C-labeled atrazine or hydroxyatrazine. With the exception of the concentration
experiment, all microcosms associated with the adsorption study were dosed to an initial concentration of 100 ug/L of either atrazine or hydroxyatrazine in an attempt to mimic environmentally relevant concentrations. The overlying water was sampled (1 ml) periodically and assayed for $^{14}$C using 15 ml of Scintiverse™ II scintillation cocktail (Fisher Scientific, Chicago, IL) and a Beckman LS3801 liquid scintillation counter.

Upon completion of the concentration experiment, the microcosms were again dosed with the amount of activity initially added. Overlying water was sampled and assayed as previously described. Following completion of the suspended sediment experiment, the overlying water was removed and replaced with clean water on two separate occasions. Again, overlying water was sampled and assayed for $^{14}$C activity.

Upon completion of the litter effects experiment, microcosms involved were sacrificed for mass balance purposes. The overlying water was drawn off and assayed for $^{14}$C activity as previously described. The sediment and litter fractions were separated and dried. In the case of the sediment fraction, sediment was dried, finely ground with a mortar and pestle and homogenized. Subsamples were oxidized as previously described for the long-term study. All of the litter was combusted to avoid subsampling errors due to the very low mass of the litter fraction.

Following the completion of the concentration experiment, microcosms involved were sectioned and sacrificed. Each core was divided into three sections. The first section was the top 1 cm of the sediment core, the second was the second 1 cm of the core and the third section included all of the core below the 2 cm mark (Figure 3). The sections were separated, dried, ground, homogenized and combusted with a Packard oxidizer as previously described.

**Statistical Analysis**

All statistical analyses were performed using CSS Statistica produced by Statsoft. Analysis of variance was performed on percent recovery data in the activity distribution
study with three replicate cores sacrificed for atrazine and hydroxyatrazine separately. A two-way analysis of variance was performed at two data points in the litter effects experiment (75.5 and 412.5 hours). A t-test was also performed on the control and litter only treatments at 75.5 and 412.5 hours in the litter effects experiment. T-tests were performed at 83 hours in the autoclaving effects experiment and 83 and 240.5 hours in the suspended sediment study. Error bars in all figures represent one standard error above and below the treatment mean and there were three replicates per treatment in all cases.
Figure 3. Diagram of sediment core sections within experimental microcosm
RESULTS AND DISCUSSION

Degradation Study

There was no evidence of atrazine ring degradation under either aerobic or anaerobic conditions during the long-term degradation study. At no time during the 10-month incubation was $^{14}$C recovered in any of the CO$_2$ traps. This agrees with the very low mineralization rates reported by Goswami and Green (1971), Kruger (1992), and Huckins et al. (1986) and suggests that ring cleavage is not a significant atrazine degradation pathway in wetland systems, at least in the time frame dealt with in this study. Goswami and Green (1971) reported that 0.005% of the originally applied atrazine-$^{14}$C was recovered as $^{14}$CO$_2$ after 60 days. Kruger (1992) recovered less than 1% of applied atrazine-$^{14}$C activity as $^{14}$CO$_2$ after 180 days. Huckins et al. (1986) found that 0.2% of the applied atrazine-$^{14}$C activity was recovered as $^{14}$CO$_2$ after a six-week incubation in a constructed wetland microcosm. These recoveries are lower than the amounts of radiochemical impurities in the atrazine, so they cannot be positively attributed to actual atrazine mineralization.

The sacrificing of microcosms following incubation revealed that no $^{14}$C activity was associated with the overlying water. Extraction of the highly organic sediment resulted in an extract that was very rich in organic substances. It was thus difficult to assay the extract by liquid scintillation counting due to its dark color, which had a quenching effect during the counting process.

The high organic content of the sediment, along with the relatively long incubation period, also resulted in a very high percentage of the activity being unextractable. Nearly all (88%) of the originally applied $^{14}$C activity was tied up as unextractable residue. As previously discussed, this is the fraction that is referred to in the literature as bound residue, or that portion of the applied pesticide or its degradation products
that is not extractable with traditional solvent extractions techniques. Bound residue formation has been shown to be enhanced by exposure time (Jones et al. 1982) and organic matter (Schiavon 1988a,b), both of which were high in this study.

There are a number of hypotheses dealing with the composition of bound residues of atrazine (Khan and Saidak 1981, Erickson and Lee 1989 and Schiavon 1988a). These hypotheses are, for the most part, the result of dosing constructed systems with atrazine and atrazine degradation products and then comparing their extraction recoveries. There is disagreement among authors as to which products have a greater tendency to form bound residues. The problem in characterizing this bound fraction centers on the lack of a technique capable of analyzing the residue in the bound state and the lack of extraction techniques capable of breaking these bonds without altering the residue structure.

The environmental significance of bound residue is not well understood (Sethunathan et al. 1991) and there is still a question in the literature as to whether or not these residues will remain permanently bound or be released and available for further degradation (Bollag 1991). There is no meaningful way to relate residue extractability with organic solvents to bioavailability.

The significance of the bound residue fraction and the absence of mineralization in the degradation study indicated that adsorption was playing a major role in the removal of atrazine and atrazine residue from the overlying water of the wetland microcosms. This brought up the questions concerning factors influencing activity loss from the water column that were explored in the adsorption study. Here the focus shifted so as to concentrate on the behavior of atrazine and hydroxyatrazine in wetlands strictly from a water quality point of view.
Adsorption Study

There are a number of possible routes that atrazine and hydroxyatrazine could take as they leave the overlying water. In the short-term, it may be possible that the loss of activity from the water column could simply be a reflection of diffusion into interstitial water. Slurry studies (Wauchope and Myers 1985, Wolf and Jackson 1982) that report rapid adsorption of atrazine to sediment would suggest that this is not a realistic scenario. However, this has not been demonstrated on undisturbed wetland sediment. The alternative to this hypothesis is that there is adsorption of activity to the sediment. The degradation study showed this to be the case in the long-term, but in order for restored wetlands to be effective sinks for pesticides this adsorption would need to take place in a relatively short period of time.

Degradation in the water column is another possibility in terms of atrazine fate in wetlands. Jones et al. (1982) reported very rapid degradation of atrazine, particularly conversion to hydroxyatrazine, in the overlying water of microcosms. These degradation products can then also be subject to adsorption (Winkelmann and Klaine 1991c, Schiavon 1988a,b).

Activity loss from the overlying water column was rapid in all experiments. Loss rates for the first 3 days in the case of the atrazine-dosed cores ranged from 6.2%/day to 15.8%/day. These rates are much more rapid than those observed by Jones et al. (1982) in their study using constructed estuary microcosms. They reported activity loss rates from overlying water in the range of 1.04%/day to 1.10%/day over the first 21 days of incubation. Activity loss in the case of the hydroxyatrazine-dosed cores was also rapid with loss rates ranging from 6.4%/day to 23.9%/day for the first 3 days of incubation.

Activity loss kinetics resembled those observed by Huckins et al. (1986). They found loss to be initially quite rapid. However, this initial phase was followed by a
slower loss phase that they believed to be controlled by diffusion in the interstitial water. Karickhoff (1980) described this type of loss as kinetic factoring, whereby chemical concentration in the aqueous phase initially decreases rapidly and then approaches equilibrium at a much slower rate. Karickhoff also described desorption in terms of kinetic factoring.

In all experiments, activity loss resembled first-order decay kinetics even at relatively high concentrations. Figure 4 illustrates the activity loss curves for microcosms dosed with atrazine at concentrations of 50, 100 and 300 ug/L. The re-dosing of this series of microcosms resulted in activity loss curves that again resemble first-order decay (Figure 4). This indicates that repeated inputs of high concentrations of atrazine would seem not to saturate binding sites in the sediment. This has implications for the use of restored wetlands as remediation sites for agricultural drainage water. It is conceivable that these wetlands would receive high doses of agricultural chemicals, including atrazine.

In an effort to determine the potential of sorbed activity to be desorbed from the sediment, microcosm overlying water was removed and replaced with clean water. Replacement of the overlying water in all cases resulted in the desorption of activity from the sediment back into the water column. Desorption curves for the atrazine-dosed cores appear in Figure 5 and curves for the hydroxyatrazine-dosed cores appear in Figure 6. In the case of each chemical, activity moved from the sediment into the water column upon each of two water replacements.

In the case of atrazine, 35.8% of the initial activity sorbed to the sediment and was potentially available for desorption. Of this 35.8%, 17.7% of the initial activity (49.4% of the activity sorbed to the sediment) desorbed back into the overlying water with two subsequent water replacements. In the hydroxyatrazine-dosed cores, 58.0% of the initial activity sorbed to the sediment. Of this 58.0%, 16.3% of the initial
Figure 4. Atrazine + atrazine residue loss in microcosms following initial experimental additions 50, 100 and 300 ug/l and subsequent redosing. Error bars indicate ± one standard error (n=3)
Figure 5. $^{14}$C activity loss and the effect of overlying water replacement in atrazine-dosed microcosms. Error bars indicate ± one standard error (n=3)
Figure 6. $^{14}$C loss and the effect of overlying water replacement in hydroxyatrazine-dosed microcosms. Error bars indicate ± one standard error (n=3)
activity (28.1% of the activity sorbed to the sediment) desorbed back into the water column as a result of the two water replacements. More activity associated with the atrazine-dosed microcosms desorbed from the sediment than that in the hydroxyatrazine-dosed microcosms. These results indicate that the hydroxyatrazine-associated activity is more strongly bound than the atrazine-associated activity.

**Distribution of Activity in Sediment Cores**

Relatively equal amounts of activity were associated with each of the three core sections in the atrazine-dosed cores (Figure 7). Analysis of variance indicated no statistical difference in the amount of activity recovered between the sections (p=0.44). It should be pointed out again that the bottom section of each core contained more sediment than either of the top two, therefore, strict comparisons of total activity recovered in each of the top two sections with that recovered in the bottom section are not realistic.

In the case of the hydroxyatrazine-dosed cores, there was a striking decrease in activity with depth in the cores (p=0.003). The top section accounted for 53.8% of the recovered activity, while 34.8% was recovered in the second section and 11.5% recovered in the bottom section (Figure 7). It is not surprising to find that hydroxyatrazine did not migrate as deeply in the sediment profile as the atrazine. It is widely held that hydroxyatrazine is more prone to binding than atrazine given its marginal solubility, relative lack of mobility in soil systems and polarity (Schiavon 1988a,b, Somasudaram et al. 1991, Simmons 1993).

**Effect of Autoclaving on Activity Loss**

The importance of a biological component in the removal of activity from the overlying water of the microcosms was examined by autoclaving treatment microcosms. Activity loss curves for the atrazine-dosed microcosms appear in Figure 8 and the loss curves for the hydroxyatrazine-dosed microcosms appear in Figure 9.
Figure 7. Distribution of activity in microcosm sediment cores for microcosms dosed with atrazine and hydroxyatrazine. Error bars indicate ± one standard error (n=3)
Figure 8. Effect of sterilization on $^{14}$C loss from microcosms dosed with atrazine. Error bars indicate ± one standard error (n=3)
Figure 9. Effect of sterilization on $^{14}$C loss from microcosms dosed with hydroxyatrazine. Error bars indicate ± one standard error (n=3)
tests performed at 83 hours indicated that loss was more rapid in the microcosms that were not autoclaved than in those that were autoclaved for both the atrazine- and hydroxyatrazine-dosed cores ($p < 0.001$ and $p=0.0005$ respectively). It is possible that the autoclaving process may have altered some of the sediment characteristics, so the data from this particular experiment should be examined with that in mind. It is also possible that contamination, or reinnoculation of the microcosms by microorganisms, may have occurred during the sampling of the overlying water. As it stands, however, there does appear to be a biotic factor involved in the loss process.

**Effect of Suspended Sediment on Activity Loss**

Activity loss curves for the atrazine-dosed microcosms in the suspended sediment effects experiment are shown in Figure 10. Although it would appear from the graph that loss is more rapid in the stirred cores than in the clear cores, this is unsubstantiated statistically at 83 hours and 240.5 hours ($p=0.21$ and $p=0.19$ respectively).

Activity loss curves for microcosms dosed with hydroxyatrazine appear in Figure 11. As was the case with atrazine, the apparent accelerated loss of activity in the stirred cores is not statistically significant at 83 hours and 240.5 hours ($p=0.19$ and $p=0.17$ respectively).

One might expect to see more rapid loss of activity in the cores that had been stirred due to the binding of the dosed atrazine and hydroxyatrazine to the sediment particles in suspension. As the sediment settles from the overlying water column, the activity associated with that sediment would also be removed. Thus, activity loss would not only be a function of the diffusive movement of activity through the water column, but also a function of the sedimentation rate of the suspended particles. However, this was not statistically substantiated.
Figure 10. Effect of suspended sediment on 14C loss from microcosms dosed with atrazine. Error bars indicate ± one standard error (n=3).
Figure 11. Effect of suspended sediment on $^{14}$C loss from microcosms dosed with hydroxyatrazine. Error bars indicate ± one standard error (n=3)
Effect of Litter on Activity Loss

The activity loss curves for the microcosms dosed with atrazine in the litter experiment appear in Figure 12. Analyses of variance (ANOVA) were performed on the data at sampling points of 75.5 hours and 412.5 hours. At 75.5 hours, results indicated that both litter and sediment had a statistically significant effect on activity loss from overlying water (p=0.0003 and p=0.00015 respectively). Interaction of litter and sediment was significant at p=0.014. Litter and sediment also proved to have a significant effect on loss at 412.5 hours (p=0.0004 and p=0.00007 respectively) while interaction was significant at p=0.004. It is apparent from Figure 12 that the presence of litter accelerated the loss of activity from the sediment + litter treatment in comparison to the sediment-only treatment.

Mass balance results for the atrazine-dosed cores appear in Table 1. Final oxidation of the cores revealed that more activity was associated with the sediment fraction than with the litter fraction in the litter + sediment treatment, and more activity was associated with the sediment in the sediment-only treatment than with the litter in the litter-only treatment.

Activity loss curves for microcosms dosed with hydroxyatrazine appear in Figure 13. As in the case of atrazine, ANOVAs were performed on the data at the sampling points of 75.5 hours and 412.5 hours. The effect of sediment on activity loss was significant at the p=0.000001 level at 75.5 hours, and at p=0.000001 at 412.5 hours. In the case of the litter effect, ANOVAs at 75.5 and 412.5 hours produced F-values that suggested significance at p=0.13 and p=0.28 respectively. These values do not seem to be consistent with the appearance of the loss curves. They also disagree with t-tests between the litter-only treatment and the control at 75.5 and 412.5 hours, which gave significant differences at p=0.001 and p=0.0004 respectively. This discrepancy is due to the negative interaction between litter and sediment, which was significant at
Figure 12. Effect of litter on $^{14}$C loss from microcosms dosed with atrazine. Error bars indicate ± one standard error (n=3)
Table 1. Mass balance results of microcosms dosed with $^{14}$C-atrazine

**Effect of Litter on the Loss of Atrazine from Cores**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Water only</th>
<th>Sediment</th>
<th>Litter+Sed</th>
<th>Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100.5 ± 0.4</td>
<td>59.7 ± 4.0</td>
<td>44.4 ± 1.7</td>
<td>51.7 ± 6.1</td>
</tr>
<tr>
<td>Sediment</td>
<td>45.8 ± 2.9</td>
<td></td>
<td>36.5 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>Litter</td>
<td>17.8 ± 2.4</td>
<td>37.8 ± 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100.5 ± 0.4</td>
<td>105.4 ± 1.8</td>
<td>98.9 ± 1.4</td>
<td>89.5 ± 2.4</td>
</tr>
</tbody>
</table>

Mass Balance as % of Applied $^{14}$C
Figure 13. Effect of litter on $^{14}$C loss from microcosms dosed with hydroxyatrazine. Error bars indicate $\pm$ one standard error ($n=3$)
p = 0.0001 and p = 0.0000007 at 75.5 and 412.5 hours respectively. The main effect of litter in this case is buried in the negative interaction between sediment and litter. Activity loss was accelerated, to a point, by the presence of litter in the absence of sediment, and was retarded by litter in the presence of sediment. This is illustrated by the mean plot shown in Figure 14. The loss curves in Figure 13 show the dramatic loss of activity in the presence of sediment without litter as compared to the litter-only and sediment + litter treatments. This may be due to a conversion process occurring at the litter/water interface where hydroxyatrazine may be converted to a chemical form that is not particularly susceptible to binding. This may also help to explain the rapid equilibration of activity loss in the litter-only treatment. However, the methodology used in this study does not allow for the substantiation of this hypothesis.

Mass balance results for the hydroxyatrazine dosed cores appear in Table 2. As in the case of atrazine, more activity was associated with the sediment fraction than with the litter fraction in the litter + sediment treatment upon final core oxidation, and more activity was associated with the sediment in the sediment-only treatment than with the litter in the litter-only treatment.
Figure 14. Plot of treatment means in litter effects study for microcosms dosed with hydroxyatrazine. Along the x-axis, 0 indicates the absence of litter and 1 indicates the presence of litter.
Table 2. Mass balance results of microcosms dosed with $^{14}$C-hydroxyatrazine

Effect of Litter on the Loss of Hydroxyatrazine from Cores

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water only</th>
<th>Sediment</th>
<th>Litter+Sed</th>
<th>Litter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
<td>99.0 ± 0.4</td>
<td>26.8 ± 3.7</td>
<td>43.5 ± 2.3</td>
<td>77.9 ± 0.6</td>
</tr>
<tr>
<td><strong>Sediment</strong></td>
<td>71.2 ± 2.4</td>
<td>43.6 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Litter</strong></td>
<td>5.5 ± 1.8</td>
<td>13.3 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>99.0 ± 0.4</td>
<td>98.0 ± 1.3</td>
<td>92.6 ± 1.8</td>
<td>91.1 ± 1.6</td>
</tr>
</tbody>
</table>
SUMMARY

The major fate of atrazine in wetlands seems to be adsorption to sediment. Long-term degradation studies provided no evidence of ring degradation. After a 10-month incubation period nearly all (88%) of the applied activity was unextractable and none was recovered in the overlying water.

Activity loss from the overlying water of microcosms for both atrazine- and hydroxyatrazine-dosed cores approximated first-order decay kinetics even at high concentrations. The replacement of overlying water with fresh water resulted in the desorption of activity from the sediment in all cases. Atrazine residue activity was evenly distributed in the top two centimeters of the sediment profile. Hydroxyatrazine residue activity decreased rapidly with depth in the top two centimeters of the sediment profile. The presence of litter accelerated the loss of activity in atrazine-dosed cores and retarded loss in the hydroxyatrazine-dosed cores in the presence of sediment. In both cases, more activity was associated with the sediment fraction than the litter fraction.
LITERATURE CITED


