Biodegradation of Ozonated Atrazine as a Wastewater Disposal System

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Destruction of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] by ozonation and subsequent soil biodegradation was examined as a low-volume two-step dilute wastewater disposal system. The rate of oxidation was first order for 100 mg/L ozonized solutions of formulated atrazine ($t_{1/2}$ = 16 min). Atrazine oxidation was more rapid at pH 10 than pH 8 or pH 6.5 unbuffered solutions. No loss of ^{14}C occurred from [U-*ring*-^{14}C]atrazine in 1-h studies. Degradation of atrazine proceeded by addition of oxygen to the alkylamino group, dealkylation, deamination, and dehalogenation. Product distribution was substantially shifted toward the more polar *s*-triazines as the pH was increased. Atrazine metabolism in soil increased as the pH values of the preozonated solutions were adjusted to more basic conditions.

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] is one of the most widely used herbicides in the United States. Annual production was estimated at 35 913 metric tons in 1982 (Gianessi, 1986). Atrazine has been detected in groundwater in the parts per billion (ppb) range (Cohen et al., 1986).

Safe disposal of pesticide wastewater of this herbicide and related compounds is a major problem for the farmer, commercial applicator, or small-scale formulator. Improper pesticide wastewater disposal may be a contributing factor to groundwater contamination. Two national workshops on pesticide disposal (Bridges, 1985, 1987) have reviewed the physical, chemical, and biological options available and stressed the need for a low-cost, versatile, and simple system for the low-level pesticide user. The use of ultraviolet (UV) irradiation in the presence of O_2 or O_3 has been investigated for several pesticides as a pretreatment step prior to soil disposal (Kearney et al., 1983–1985). The use of ozone alone as an oxidant for pretreatment for several herbicides has been compared to combined UV-O_3 for 11 major pesticides and gave comparable rates of oxidation (Kearney et al., 1987b). Ozone may be a viable alternative pretreatment compared to UV-O_3, since ozone generators are more readily available than large-scale lamp units. Ozone has been used to oxidize several pesticides, and this literature has recently been summarized by Rice (1986). Glaze (1987) has reviewed the chemistry of ozone as an oxidant in drinking water treatment, where ozone reacts with natural organic and synthetic substances to produce low molecular weight oxygenated byproducts that are generally more biodegradable than their precursors.

Biodegradation of the *s*-triazines, including atrazine as a wastewater disposal option, has been studied extensively by Cook (1987). Several microorganisms were obtained from nitrogen-limited enrichment cultures that were able to degrade synthetic *s*-triazine industrial byproducts as sources of nitrogen. The consortium of microorganisms developed by Cook and co-workers was unsuccessful, however, as a wastewater disposal strategy because of the low specific activity of some enzymes, inhibition by salts in the wastewater, and expensive carbon sources.

The objective of the present study was to investigate (1) the rates of ozonation of analytical and formulated atrazine solutions, (2) the products resulting from ozonation of atrazine, and (3) the rates of metabolism of those ozonated products by indigenous and selected soil microorganisms. Our major focus was directed at formulated solutions, since these are the materials that will be encountered in any real world disposal situation. The ultimate objective of this research is a better understanding of the reactions occurring in a two-chamber unit designed for on-site disposal of pesticide wastewater. The unit combines ozonation and soil metabolism in a simple system described by Kearney et al. (1987a).

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METHODS AND MATERIALS

Rate Studies. Formulated atrazine (Aatrex 4L containing 480 g/L atrazine) was obtained from CIBA-GEIGY Corp., Greensboro, NC 27419. Analytical standards (>98% pure) of atrazine were obtained from the Pesticide Reference Standards Laboratory, EPA, OPP, Analytical Chemistry Section, BARC-East, Beltsville, MD 20705. [U-*ring*-^{14}C]Atrazine (sp act. 8.7 mCi/mM) was purchased from Pathfinder Laboratories, Inc., St. Louis, MO 63141. Labeled solutions were purified to greater than 99% by thin-layer chromatography.

Laboratory small-volume studies (100–1000 mL) were conducted with enriched ozone fed into a glass cylinder from a Model GTC-1B ozone generator (Griffin Technics Corp., 178 Route 46, Lodi, NJ 07644). For those experiments, ozonation was carried out in a graduated cylinder with ozone introduced through a stainless steel airstone at the bottom of the cylinder. All reactions were carried out in a well-ventilated hood. Ozone was generated at a rate of 2.1 μmol/min as measured by iodometric titration (Flamm, 1977).

One liter solutions containing 33 mg/L analytical and 100 mg/L active ingredient formulated atrazine spiked with [U-*ring*-^{14}C]atrazine were ozonated and samples removed periodically for analysis. To determine the effect of pH on the rate of degradation and product distribution, 100-mL atrazine solutions were adjusted to pH 8.0 with phosphate buffer and pH 10.0 with a sodium carbonate–bicarbonate buffer. The solutions were ozonated for 120 min.

Degradation of the herbicide was measured by high-pressure liquid chromatography using a Waters C-18 Nova-Pak radially compressed cartridge column. The pumps were Waters M6000A connected to a Waters Model 660 solvent programmer. The detector system consisted of a Waters 990 photodiode array detector interfaced with a NEC APC III computer. The solvent system used for monitoring the loss of the parent compounds was 60% acetonitrile in 0.75 mM phosphoric acid, pH 2 (solvent A), at a flow rate of 2.0 mL/min. Atrazine products were separated with a 15-min concave gradient (Waters curve 10) starting at 0% acetonitrile and ending at 75% aceto-

nitrile in solvent A at a flow rate of 2.0 mL/min. Labeled products were separated by the HPLC system coupled to a LKB2211 Superac fraction collector. Radioactivity was monitored on a Beckman LS 6800 liquid scintillation counter.

Product Studies. Products were derivatized with trimethylsilyl reagent (Me_3Si) and analyzed by use of a gas chromatograph–mass spectrometer. The mass spectrometer is a Finnigan Model 4021 with an Incos data system, operated in the electron impact mode, with an electron energy of 70 eV. The column was Supelco SPB-5 (5% diphenyl/94% dimethyl/1% vinylpolysiloxane) 30 m × 0.25 mm (i.d.) fused silica column (0.25-μm film). The gas chromatograph oven temperature was held for 1 min at 90 °C and programmed from 90 to 230 °C at 5 °C/min for the trimethylsilyl derivatives. For underivatized products the temperatures were 60 °C for 1 min and 15 °C/min to 120 °C and then 3 °C/min to 230 °C.

To conserve space, the nomenclature system for *s*-triazine products proposed by Cook (1987) is used in the text. In this system A = amino, C = chloro, E = ethylamino, I = isopropylamino, O = hydroxy, and T = triazine ring structure. For example, atrazine would be CIET.

Ammeline (OAAT), ammelide (OOAT), cyanuric acid (OOOT), and 2-chloro-4,6-diamino-*s*-triazine (CAAT) were purchased commercially. 2-Chloro-4-amino-6-(isopropylamino)-*s*-triazine (CIAT) and 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (CEAT) were obtained gratis from CIBA-GEIGY. The following compounds were synthesized:

2-Chloro-4-amino-6-hydroxy-s-triazine (COAT) was synthesized by alkaline hydrolysis of 2,4-dichloro-6-amino-*s*-triazine (CCAT) (Kim et al., 1978). CCAT (10.5 mg, 0.061 mmol) was suspended in 0.37 mL of distillled water, 0.63 mL of 0.2 N NaOH (0.061 mmol) was added, and the mixture was continuously vortexed for 30 min. HPLC analysis revealed the presence of OOAT, OOOT, and COAT in the mixture. For GC–MS analysis, 0.5 mL of the mixture was removed and evaporated to dryness in vacuo. The residue was treated with 0.1 mL of pyridine, and 0.1 mL of *N,O*-bis(trimethylsilyl)trifluoroacetamide was added. The silylated mixture gave mass spectra of OOAT, OOOT, and COAT TMS derivates, as recorded previously for standards and/or ozonation products.

2-Chloro-4-acetamido-6-(isopropylamino)-s-triazine was synthesized by adding 0.094 g (0.5 mmol) of CIAT to 20 mL (215 mmol) of acetic anhydride. The solution was magnetically stirred under reflux for 1.5 h. The acetic anhydride was evaporated, and a white precipitate was obtained. The precipitate was recrystallized twice from acetone. A 500 mg/L solution was prepared in acetonitrile and analyzed by HPLC and GC–MS. A single peak was obtained on HPLC. The structure of the 2-chloro-4-acetoamido-6-(isopropylamino)-*s*-triazine was confirmed by the mass spectrum showing strong peaks at m/z 229 (molecular ion), 214 (M – CH_3), and 172 (base peak, M – 57). The loss of 57 may be accounted for as successive losses of CH_3 and CH_2CO.

2-Chloro-4-amino-6-acetamido-s-triazine was synthesized by adding 0.5 g (3.45 mmol) of CAAT to 25 mL (270 mmol) of acetic anhydride. The slurry was magnetically stirred under reflux for 40 min. The solution was filtered to remove undissolved starting material. A white precipitate formed from solution upon cooling. The solution was filtered and the filtrate washed with acetic anhydride, with acetic acid, and several times with methanol and ethanol. The filtrate was positive for chlorine by the Beilstein test. A 100 mg/L solution was prepared in acetonitrile and analyzed by HPLC and GC–MS. Two peaks were seen on HPLC, presumably representing the mono- and diacetylated compounds. The mass spectrum of the monoacetylated product had strong peaks at m/z 187 (molecular ion), 159 (M – 28), 145 (presumably from loss of CH_2CO in a rearrangement with retention of a hydrogen), 110 (145 – Cl), and 68 (110 – H_2NCN). The last three peaks make up the spectrum of the unsubstituted CAAT.

Metabolism Studies. Soil metabolism studies were conducted in a Sassafras silt loam (14% organic matter, pH 4.2, sand, silt and clay contents of 56, 20 and 24% respectively, and moisture content of 57% at $^1/_3$ bar) obtained from Salisbury, MD. Soil metabolism studies were conducted in biometer flasks (Bartha and Pramer, 1965) containing 50 g of an unsterile Sassafras soil and sand mixture. The soil was amended with 5 mL of labeled herbicide solution that was preozonated under either of the various pH conditions. Degradation was measured by trapping metabolic $^{14}CO_2$ in 10 mL of 0.1 N NaOH and counting via liquid scintillation.

Bacterial strains that utilize various *s*-triazines, as described by Cook (1987), including *Pseudomonas* strain A and *Pseudomonas* strain D, were generously supplied by Dr. Homer LeBaron, CIBA-GEIGY. Nutrient composition and culture conditions for these organisms was the same as described by Cook and Hutter (1984). For microbial metabolic studies, 1 L of atrazine solution (100 mg/L) was ozonated for 8 h at pH 10, adjusted to pH 2.0 to remove the chlorine, and neutralized to pH 7.0, and 25 mL was added to a biometer flask. These flasks were inoculated with 0.5 mL of cells (containing approximately 1.4×10^{11} cells) taken from a 5-mL culture grown overnight from a single-plate colony. These cells were in the early stationary phase as determined by OD measurements.

Soil column studies were conducted in perfusion units described by Audus (1946). Each unit was filled with 30 g of an unsterile 50% Sassafras soil and sand mixture. Sand was added to improve the flow rate through the columns. Duplicate units were set up containing soil and soil inoculated with *Pseudomonas* strain A. The column was first equilibrated with 10 mM phosphate buffer, pH 7, for 24 h. Next the buffer was replaced with 250 mL of 10 mM phosphate buffer, pH 7, containing 10 mM lactate, 1.5 mM ammonium sulfate, and trace metals as designated by Cook and Hutter (1981). The unit was then inoculated with 5 mL (containing approximately 1.4×10^{11} cells) taken from a 50-mL culture in the early stationary phase. Inoculated columns were equilibrated over 4 days, and then each unit received 150 mL of the same solution of the ozonated atrazine used in the previous microbial studies containing 10 mM phosphate buffer, 25 mM succinate, and Cook's trace metals. Before the ozonated atrazine solution was added, a loop of the equilibrated solution was streaked on agar plates containing 20 mM glycerol and 0.5 mM cyanuric acid. After 24 h at 27 °C the plates were observed for bacterial growth. No attempt was made to enumerate the number of colonies in the inoculated columns. Exhaust air from the perfusion unit was passed through a gas dispersion unit containing 10 mL of 1.0 N KOH. Metabolism was measured by removing 0.1 mL of the trapping solution and measuring evolved $^{14}CO_2$. Samples were taken periodically over the 15-day period.

RESULTS AND DISCUSSION

Rate Studies. The loss of analytical (33 mg/L) and formulated (100 mg/L) atrazine in unbuffered aqueous solution (1000 mL) by ozonation is shown in Figure 1. The degradation rate followed first-order kinetics ($t_{1/2}$ = 16 min) for the formulated solution. This rate is slower than

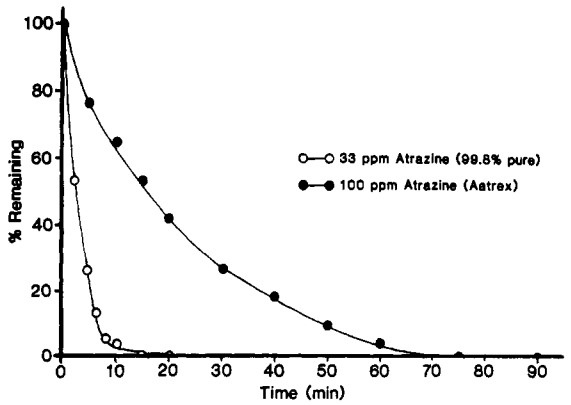


Figure 1. Ozonation of purified and formulated atrazine in unbuffered aqueous solutions. Loss of atrazine is based on the disappearence of active ingredient using HPLC.

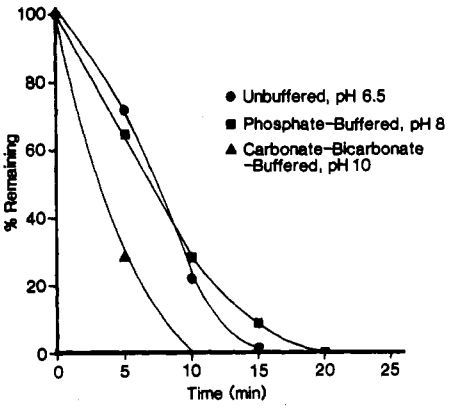


Figure 2. Ozonation of 100 mg/L formulated atrazine in unbuffered pH 6.5, pH 8, and pH 10.0 aqueous solutions. Loss of atrazine is based on the disappearence of active ingredient using HPLC.

that of formulated alachlor ($t_{1/2}$ = 1.9 min) at the same concentration under identical conditions of ozonation (Somich et al., 1988). The low reactivity of atrazine toward ozonation has been reported elsewhere (Legube et al., 1987). In their study designed to measure the reactivity of four nitrogen heterocyclic compounds toward ozone in slightly acidic aqueous solutions, the susceptibility to oxidation decreased in the order amitrole > 2-benzotriazoles > atrazine.

Only the formulated solutions of atrazine were compared in subsequent studies because of the ease of measuring comparable concentration and our focus on the material most likely to be found in a disposal situation. As pointed out by Glaze (1987), the chemistry of ozone in water is very complex, and the overall rate of ozonation depends on the rate of mass transfer of ozone from the gas phase to the aqueous phase, the rate of decomposition of ozone in water, and the rate of chemical reactions in solution that drives ozone dissolution into water.

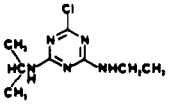
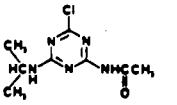
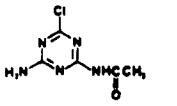
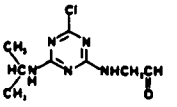
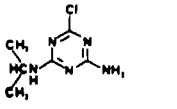
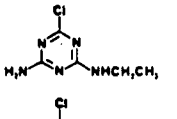
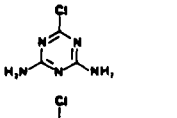
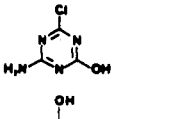
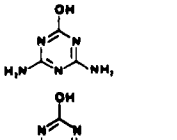
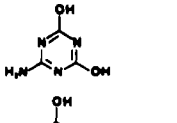
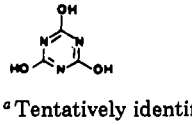
Also in contrast to alachlor (Somich et al., 1988), there was no measurable loss of ring-labeled carbon from the heterocyclic *s*-triazine ring after 60 min and only 6.4% loss after 24 h. There was a change in pH during the course of this study, starting at pH 6.5 and decreasing to pH 3.7 after 120 min. An increase in pH has been found to decrease the stability of ozone in water due to the catalytic effect of hydroxy ions on the O_3 decomposition process. This, in turn, enhances hydroxy radical formation and subsequently the overall rate of oxidation (Hoigne and Bader, 1976).

To determine whether maintaining the solutions at higher pH would accelerate the rate of atrazine degradation, the 100 mg/L formulated solutions (100 mL) were adjusted to pH 8 and 10 and the rate studies repeated in the presence of the [U-^{14}C]atrazine (Figure 2). An increase to pH 10 did accelerate the destruction of atrazine. There was no major change in pH (8.0–7.6 and 9.9–9.9 after 120 min) in these buffered solutions and no loss of ^{14}C from fragmentation of the *s*-triazine ring. Attempts to conduct ozone studies at pH 11 and 12 led to major foaming problems with the formulated solutions.

Product Studies. A list of products identified, or tentatively identified, from derivatized and underivatized ozonated atrazine products are shown in Table I. The reaction appears to proceed by oxidation of the alkylamino groups at the 4- and 6-positions of the *s*-triazine ring to yield two products of molecular weight 229. One product was identified as the amide derivative of atrazine or 2-chloro-4-acetamido-6-(isopropylamino)-*s*-triazine by comparison of their retention time and mass spectral pattern with that of the synthesized product. The second product has not been identified to date but would appear to be the aldehyde of the ethyl or isopropyl groups. These products are under investigation.

There were also two compounds at molecular weight 187

Table I. Products Identified, or Tentatively Identified, from Derivatized and Underivatized Ozonated Atrazine Reactions

structure	name	abbrev
	atrazine	CIET
	atrazine amide	
	deisopropylatrazine amide	
	atrazine aldehyde[a]	
	deethylatrazine	CIAT
	deisopropylatrazine	CEAT
	chlorodiamino-*s*-triazine	CAAT
	chlorohydroxyamino-*s*-triazine	COAT
	ammeline	OAAT
	ammelide	OOAT
	cyanuric acid	OOOT

[a] Tentatively identified.

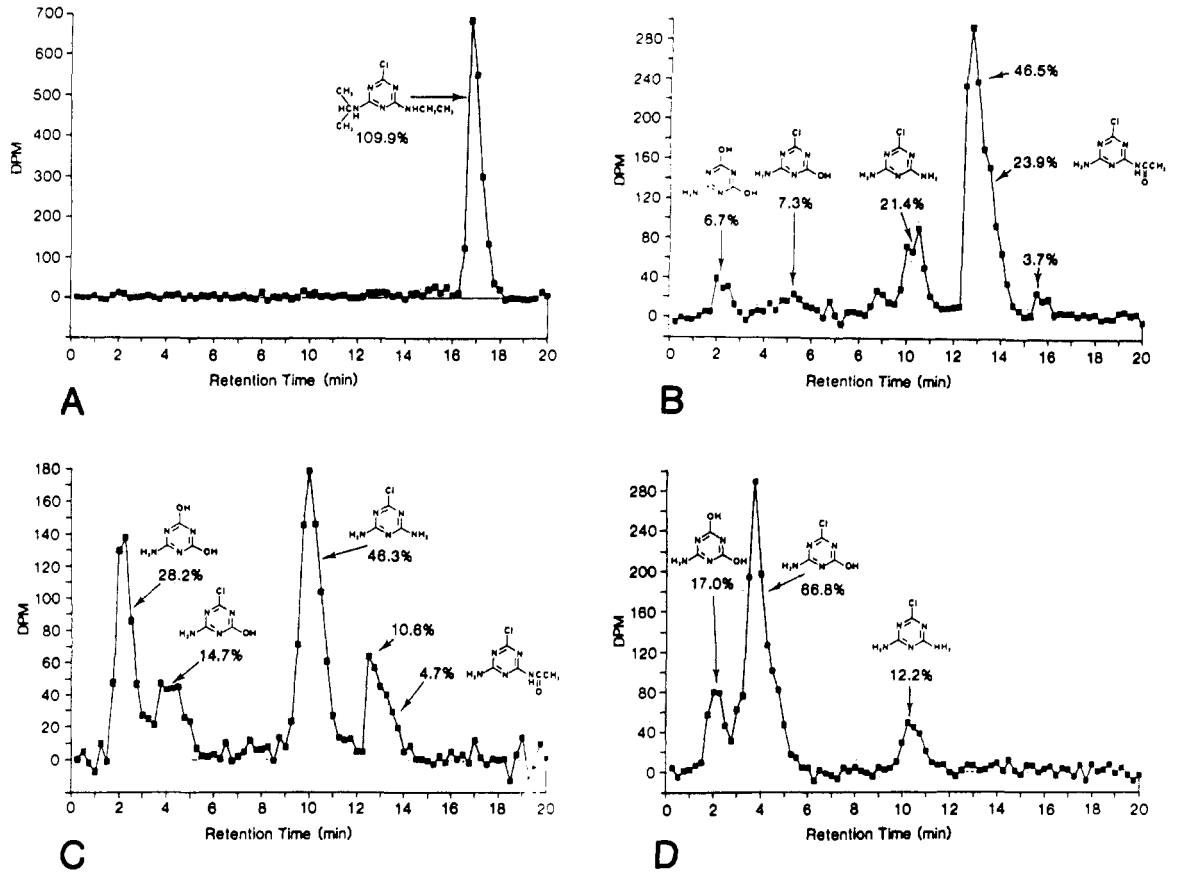


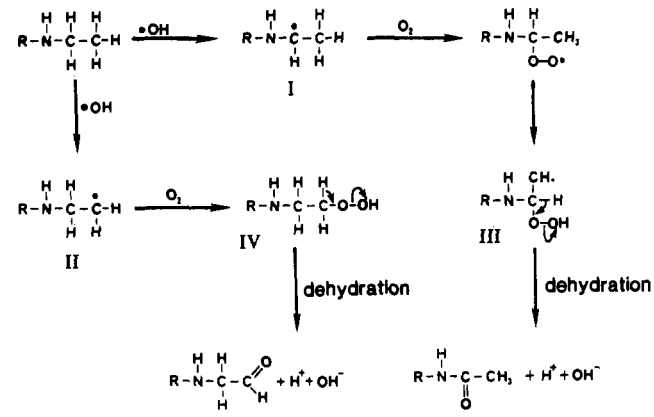
Figure 3. Distribution of ^{14}C-labeled products from 120-min ozonation of formulated atrazine (100 mg/L) plus [U-*ring*-^{14}C]atrazine (1 μCi) (A), at pH 6.5 (B), at pH 8.0 (C), and at pH 10.0 (D). Products were separated by high-pressure liquid chromatography coupled with a fraction collector.

that retained the chloro substituent and may represent the two compounds at molecular weight 229, minus the isopropyl group. One of these products was identified as the 2-chloro-4-acetamido-6-amino-*s*-triazine or the deisopropylatrazine amide, when compared to the synthesized product. By analogy, the other molecular weight 187 product could be the deisopropyl amino acetaldehyde of atrazine. Loss of the alkyl groups to yield deethylatrazine (CIAT) and deisopropylatrazine (CEAT) also appear to be early products. Both products were identified by GC–MS. A comparison of the ion chromatograph suggests that the ratio of CIAT to CEAT is about 10 to 1. The 2-chloro-4-hydroxy-6-amino-*s*-triazine (COAT) was produced in large amounts at pH 10, and at lesser amounts at lower pH levels.

Isolated COAT gave the same retention time and fragmentation pattern as the synthetic product. OAAT, OOAT, and OOOT were all identified by retention times, UV, and GC–MS when compared to commercial standards. Hydroxyatrazine (OEIT) was not detected in any ozonated solutions of atrazine.

The distribution of ^{14}C-labeled products from atrazine ozonation for 120 min in a 100 mg/L formulated solution at pH 8, 10, and unbuffered (pH 6.5) is shown in Figure 3. Solutions of formulated atrazine maintained at pHs 8 and 10 were stable and showed no degradation prior to the ozonation step. The recovery of ^{14}C in all the cumulative peaks for each pH study was essentially quantitative, and therefore, there were no major undetected atrazine derived peaks in these studies. Our inability to detect isopropylammelide (OOIT) and isopropylammeline (OIAT) as major products as reported by Legube et al. (1987) cannot be accounted for by the loss of a major compound during chromatography.

Scheme I



While there is no difference in the rate of atrazine degradation between unbuffered and pH 8 solutions, there is a major difference in product distribution. In unbuffered solutions, the major product (46%) is still unknown. The peak at 13.5 min is the deisopropylatrazine amide (24%) with smaller peaks for CAAT (21%), COAT (7%), and OOAT (6%). At pH 8.0 there is a major shift toward the more oxidized products, with the major peak being CAAT (46%) and peaks at COAT (15%) and OOAT (28%). At pH 10.0 there is complete loss of the parent and the early peaks. A major peak was COAT (67%) with CAAT (12%) and OOAT (17%). Increasing the pH thus resulted in an acceleration of the oxidation process. COAT appeared to be unstable and slowly converted to OOAT. COAT was rapidly converted to OOAT at pH 2.0.

On the basis of products identified, a mechanism can be proposed for the early reactions responsible for the

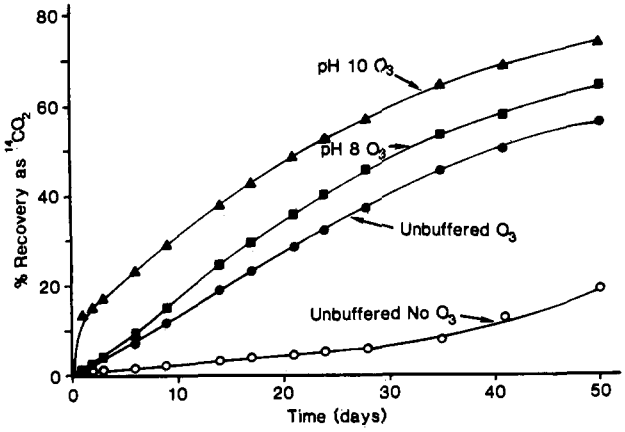


Figure 4. Metabolism of formulated atrazine (100 mg/L) plus [U-*ring*-^{14}C]atrazine (1 μCi) in Sassafras silt loam after preozonation at pH 6.5, pH 8.0, and pH 10.0 for 120 min.

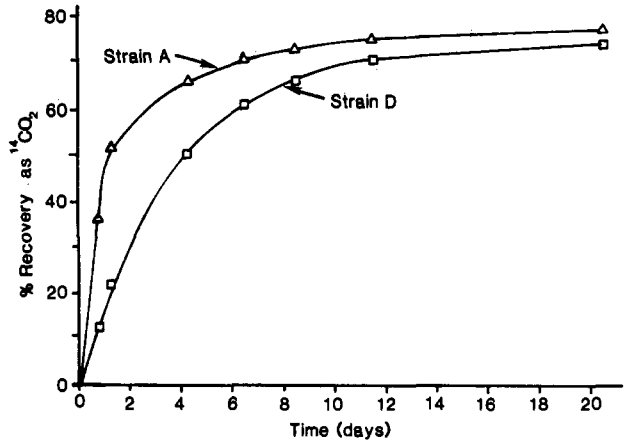


Figure 5. Metabolism of formulated atrazine (100 mg/L) plus [U-*ring*-^{14}C]atrazine (1 μCi) in separate solutions incubated with *Pseudomonas sp.* strain A and strain D after preozonation of atrazine at pH 10.0.

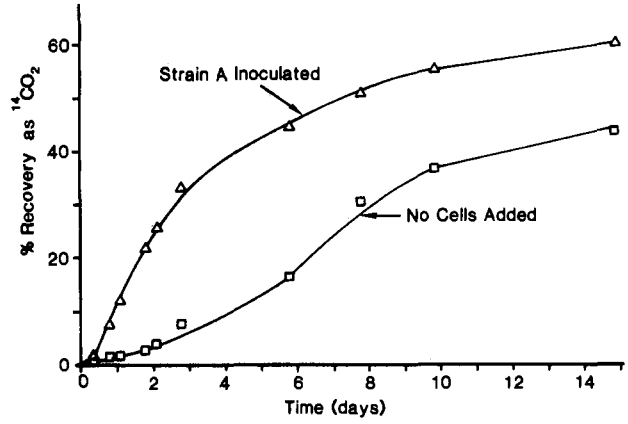


Figure 6. Metabolism of formulated atrazine (100 mg/L) plus [U-*ring*-^{14}C]atrazine (1 μCi) in soil columns containing Sassafras silt loam and Sassafras silt loam inoculated with *Pseudomonas sp.* strain A after the atrazine was preozonated at pH 10.0.

chemical oxidation of the ethyl group of atrazine. By this proposed mechanism (Scheme I), the hydroxyl free radical first reacts with the ethyl group to produce water and an ethyl free radical I. The oxidative attack takes place preferentially at the methylene carbon as opposed to the terminal methyl carbon, which would be the free radical II. I reacts with molecular oxygen to form the ethyl hydroperoxide III, which subsequently undergoes dehydration to form the amide, or, in the case of atrazine, the 2-chloro-4-acetoamido-6-(isopropylamino)-*s*-triazine. If the unknown *m/z* 229 peak is the aminoacetaldehyde derivative of atrazine, then the reaction involving free radical II forming the hydroperoxide and the aldehyde make this a plausible mechanism.

Hydroxyl radicals generated by chemical systems have been shown to dealkylate *s*-triazine herbicides (Plimmer et al., 1971). Fenton's reagent (ferrous sulfate–hydrogen peroxide), a hydroxyl radical generating system, reacts with atrazine to yield CIAT, CEAT, and CAAT. Simazine and propazine were also dealkylated in the same system. We isolated a product from the oxidation of simazine (CEET) that also had *m/z* 187, which was assumed to be the 2-chloro-4-(methylamino)-6-(ethylamino)-*s*-triazine. This product would arise from oxidation of the terminal carbon atom of the *N*-ethyl group to a carboxylic acid, followed by decarboxylation. In retrospect, the *m/z* 187 product from simazine could have been the deethyl derivative of the simazine amide similar to the deisopropyl atrazine amide described in the current study.

Metabolism Studies. Soil metabolism of ozonated atrazine at pH 6.5 (unbuffered), 8, and 10 is shown in Figure 4. Degradation of atrazine was substantially slower than alachlor pretreated under essentially the same conditions (Somich et al., 1988). When the formulated atrazine solution was adjusted to pH 8, ozonated, and then added to soil, metabolism was more rapid than in the unbuffered solution. After 50 days, about 65% of the ring-labeled carbon had been metabolized to $^{14}CO_2$. When formulated atrazine was oxidized at pH 10, neutralized, and added to soil, the rate of metabolism was again increased and amounted to about 75% of the ring appearing as $^{14}CO_2$. Less than 20% of the unozonated atrazine was degraded at 50 days.

Microbial metabolism of ozonated atrazine in a soil-free solution by *Pseudomonas sp.* strains A and D was very rapid (Figure 5). Essentially 70% of the ring carbons in ozonated products of atrazine was released as metabolic $^{14}CO_2$ after 6 days in strain A. Strain D was somewhat slower than strain A, but both yielded about 75 and 78%, respectively, of the *s*-triazine carbons as $^{14}CO_2$ after 20 days. Cook (1987) reported that *Pseudomonas sp.* strain A possesses several aminohydrolases (EC 3.5.4) capable of quantitative ring deaminations and ureases responsible for ring cleavage. Strain A can carry out the following reactions, OAAT → OOAT → OOOT → biuret → urea → CO_2 + NH_3, and it also converts CAAT to COAT and presumably COOT. The rapid metabolism demonstrated by the bacterial strains suggested that soil metabolism could be accelerated by amending the Sassafras sand mixture with an inoculum of *Pseudomonas sp.* strain A.

Metabolism rates of ozonated atrazine in soil columns and soil columns fortified with *Pseudomonas* strain A are shown in Figure 6. There was an initial rapid burst of metabolism in the amended soil columns during the first 3 days, followed by a slower rate of metabolism for the next 7 days and a leveling off at about 60% on day 15. Indigenous soil microorganisms degraded ozonated atrazine more slowly and achieved about 40% mineralization after 15 days. The soil inoculated with strain A increased the amount of $^{14}CO_2$ evolved by about 20% over the native soil population. Studies are under way to determine whether introduced bacteria can be maintained in these columns.

The degree and rate at which these various ozonated solutions were metabolized by indigenous or introduced soil microorganisms are directly related to the biodegradability of products produced by pretreatment with ozone. In an unpublished study on the soil degradation of nine ring- and side-chain-labeled *s*-triazines (Loos and Kearney, 1979), starting with the most labile and progressing to the

most persistent, the biodegradability was OOOT* = OOAT* = OAAT* > OOE*T > OOE*T > OAE*T > OAET* ≫ OEE*T > OEET* (the dot indicates the labeled moiety, i.e., E* = ^{14}C ethylamino and T* = ^{14}C ring). Alkyl substitutions on the amino groups bonded to the *s*-triazine ring retarded degradation; OOOT, OOAT, and OAAT were almost completely degraded within 30 days, whereas OOET and OEAT were degraded about 2–5% in 90 days and OEET only 0.6% or less in 90 days. The persistent hydroxyatrazine OIET was not detected in ozonated atrazine solutions, which is an advantage in the overall degradation scheme that depends on microbial metabolism of preoxidized products. Any combination of time, pH, and ozone that produces the more biodegradable OAAT, OOAT, or OOOT enhances the overall degradation process.

In summary, ozonation of atrazine resulted in a number of products in which the alkyl groups were altered and eventually removed, the halogen was slowly removed, and the *s*-triazine remained essentially intact. The rate of chemical interconversions of these products was accelerated at higher pH values. The rate of subsequent soil metabolism of these products is enhanced by prior oxidative dealkylation, deamination, and dehalogenation reactions. These reactions can be scaled up to provide a fairly efficient and inexpensive disposal option for on-site wastewater treatment. Such a simple system is currently under development in our group.

Registry No. CIET, 1912-24-9; CIAT, 6190-65-4; CEAT, 1007-28-9; CAAT, 3397-62-4; COAT, 38862-29-2; OAAT, 645-92-1; OOAT, 645-93-2; OOOT, 108-80-5; atrazine amide, 83364-15-2; deisopropylatrazine amide, 115339-34-9; atrazine aldehyde, 115339-35-0; deethylatrazine, 6190-65-4.

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