Pesticide Waste Treatment Monitoring of s-Triazines Using Immunoassay†

Mark T. Muldoon‡,§,|| and Judd O. Nelson*,§

Soil Microbial Systems Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, and Department of Entomology, University of Maryland, College Park, Maryland 20742

Pesticide waste disposal monitoring currently utilizes conventional methods of analysis such as gas–liquid chromatography and high-performance liquid chromatography. Enzyme-linked immunosorbant assays (ELISAs) have been developed for s-triazine herbicides and many of their environmental degradation products. This paper reports the use of an s-triazine herbicide class-specific ELISA in conjunction with an ELISA for the intermediate product chlorodiamino-s-triazine (CAAT) for measuring s-triazine herbicide ozonation followed by microbiological treatment. For the treatment of atrazine, the ELISAs were very accurate and precise for measuring atrazine and CAAT. The geometric mean regression equation of the amount found by ELISA on the amount found by HPLC for both analytes was $Y = 1.12X - 7.13$, standard error = 0.038 μM, $R = 0.95$, df = 43. The information obtained by the two ELISAs could be used for treatment process control. This ELISA system may save time and expense in s-triazine herbicide treatment monitoring applications.

Keywords: *Atrazine; biodegradation; ELISA*

INTRODUCTION

Normal agricultural pesticide spraying operations may generate large volumes of excess aqueous pesticide-containing materials. This consists of excess pesticide product, leftover tank mixtures, and equipment rinsates. Pesticide concentrations can range from 1.0 to 10,000 ppm (Seiber, 1987). Appropriate on-site management of these materials depends on the particular situation involved. The options include reuse, recycling as subsequent makeup water, or, if necessary, disposal (Dwinell, 1992). Improper disposal of unusable materials has been identified as an important point source of environmental contamination (Aharonson et al., 1987).

On-site disposal methods have been developed for these high-volume, relatively low concentration wastes. The methods include physical (evaporation, adsorption, filtration), chemical (hydrolysis, oxidation, incineration), biological (composting, landfarming, enzymatic, bioreactors), and combinations of various methods (Ferguson, 1991; Seiber, 1992). With each technology, analytes have traditionally been monitored using conventional analytical methods such as gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC) to ensure the effectiveness of the treatment and to determine end-points for multiple-step processes. These analytical methods are usually multiresidue techniques; however, they are often expensive and time-consuming, require highly trained personnel for operation, and may not be suitable for on-site analyses. Depending on the treatment process and application, monitoring for the loss of parent pesticides may not be sufficient for end-point determinations since transformation products may possess pesticidal activity or assume some other undesirable characteristic (Seiber, 1987). Therefore, it may be necessary to monitor for specific characteristics or transformation products.

Enzyme-linked immunosorbent assays (ELISAs) have been developed for many pesticide analytes (Sherry, 1992). They are simple to perform and can be made field adaptable (Bushway et al., 1988). ELISA kits are commercially available for many different environmental contaminants. These features make immunoassay technology particularly attractive for use in pesticide management applications.

A combined ozonation and microbial metabolism process currently under development utilizes ozone to oxidize recalcitrant pesticide substrates to more biolabile intermediates which are readily mineralized by indigenous soil and sludge microorganisms (Kearney et al., 1988; Somich et al., 1988, 1990; Leeson et al., 1993). The s-triazines were shown to be among the most recalcitrant of the substrates studied and were considered to be useful indicator analytes for treatment effectiveness. Ozonation of the chloro-s-triazine herbicides (Figure 1) proceeded by direct N-dealkylation or the addition of oxygen to either or both of the *N*-alkyl side chains followed by the loss of the acetamido moieties, resulting in the accumulation of chlorodiamino-s-triazine (CAAT, Table 1). The ozonation process was shown to be most efficient at pH 9–10. The disposal process was completed with the mineralization of CAAT by soil or sludge microorganisms (Kearney et al., 1988; Leeson et al., 1993). It was necessary to monitor for the loss of the parent pesticide and accumulation of the terminal ozonation product CAAT and subsequent biodegradation of this intermediate.

We have evaluated the use of multiple immunoassays for the discrimination and quantitation of individual components in mixtures of s-triazine herbicides in

* Author to whom correspondence should be addressed.

† Contribution 8721, Scientific Article A-6514, of the Maryland Agricultural Experiment Station.

‡ U.S. Department of Agriculture.

§ University of Maryland.

|| Present address: Food Animal Protection Research Laboratory, USDA–ARS, Route 5, Box 810, College Station, TX 77845.

0021-8561/94/1442-1686$04.50/0

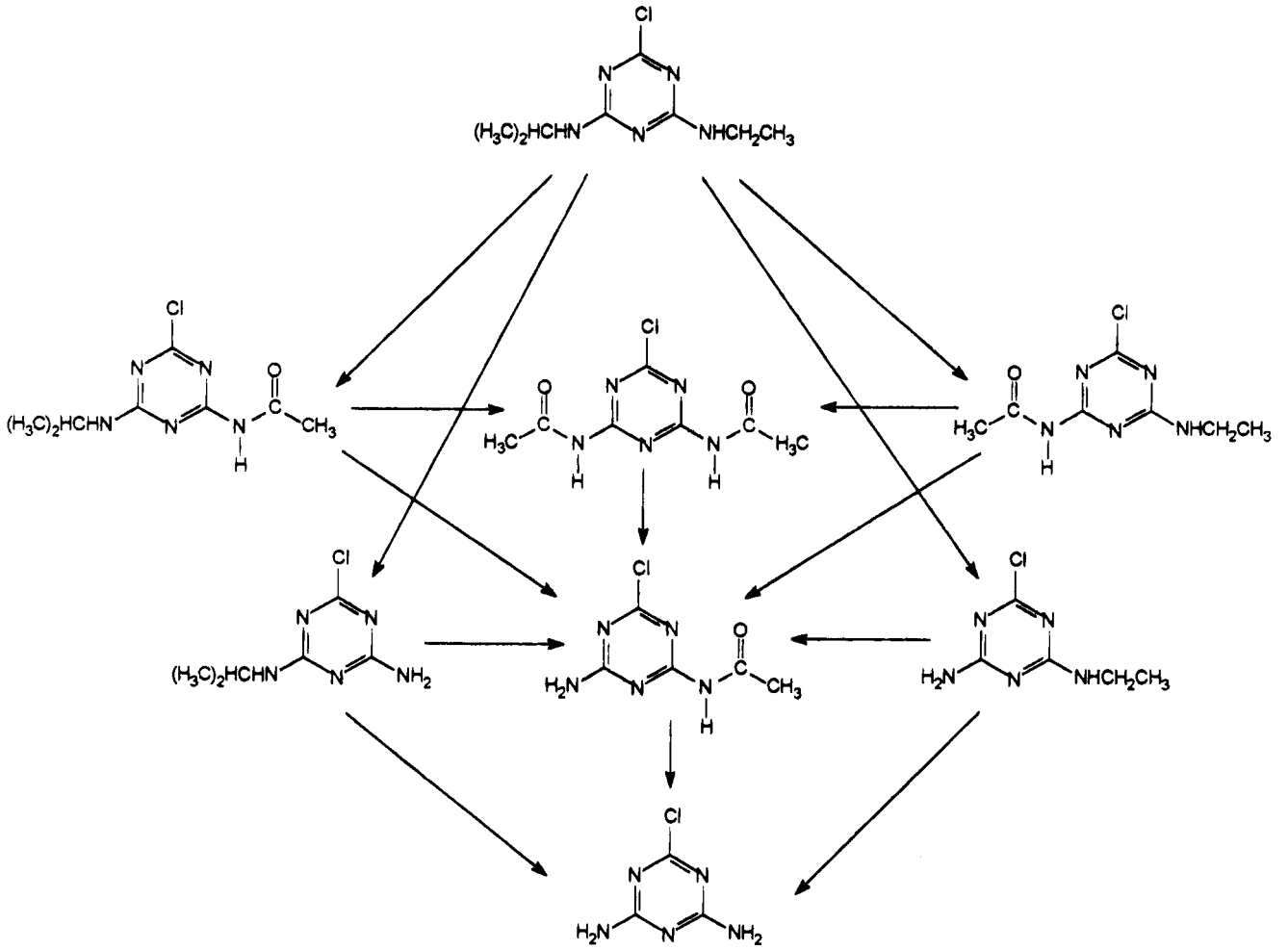
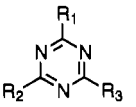


Figure 1. Pathway of atrazine ozonation. Atrazine (CIET, Table 1) undergoes either direct deamination or addition of oxygen to either or both alkyl side chains followed by loss of this acetamido moiety, resulting in the formation of chlorodiamino-*s*-triazine (CAAT). Reproduced with permission from Hapeman-Somich et al. (1992). Copyright 1992 American Chemical Society.

Table 1. Structure and Nomenclature of *s*-Triazines



common name	Cook system[a]	R_1	R_2	R_3
atrazine	CIET	Cl	$NH[CH(CH_3)_2]$	$NH(CH_2CH_3)$
	CDDT	Cl	$NH[C(O)CH_3]$	$NH[C(O)CH_3]$
	CDIT	Cl	$NH[C(O)CH_3]$	$NH[CH(CH_3)_2]$
	CDET	Cl	$NH[C(O)CH_3]$	$NH(CH_2CH_3)$
	CDAT	Cl	$NH[C(O)CH_3]$	NH_2
deethylatrazine	CIAT	Cl	$NH[CH(CH_3)_2]$	NH_2
deisopropylatrazine	CEAT	Cl	$NH(CH_2CH_3)$	NH_2
chlorodiamino-*s*-triazine	CAAT	Cl	NH_2	NH_2
	COAT	Cl	OH	NH_2
	CEPrT	Cl	$NH(CH_2CH_3)$	$NH(CH_2)_2COOH$
	CAHeT	Cl	NH_2	$NH(CH_2)_5COOH$

[a] T, *s*-triazine ring; C, chlorine; A, amino; O, hydroxy; I, isopropylamino; E, ethylamino; D, acetamido; Pr, aminopropanoic acid; He, aminohexanoic acid. Adapted from Cook (1987).

pesticide waste and rinsate generated from actual use (Muldoon et al., 1993). An immunoassay was developed for CAAT, the key intermediate in the ozonation and microbial metabolism disposal process, to monitor the complete disposal process for chloro-*s*-triazine herbicides (Muldoon et al., 1994). The assay was validated by HPLC and shown to be highly accurate and precise for CAAT quantitation in treated pesticide waste and rinsate. The purpose of the current study is to evaluate the use of this immunochemical approach for process monitoring in the treatment of *s*-triazine herbicides.

MATERIALS AND METHODS

Chemicals. Analytical standard grade atrazine (CIET, Table 1) and the monodealkylated metabolites CIAT and CEAT were gifts from Ciba-Geigy Corp. (Agricultural Division, Greensboro, NC). Chlorodi-

amino-s-triazine (CAAT) was from Aldrich Chemical Co. (Milwaukee, WI). The acetamido derivatives CDAT, CDET, CDIT, and CDDT were synthesized as previously described (Hapeman-Somich et al., 1992). 2-Chloro-4-hydroxy-6-amino-s-triazine (COAT) was prepared from CAAT using resting cells of *Pseudomonas* sp. strain A (Cook and Hutter, 1981) grown on melamine as a nitrogen source according to the method of Grossenbacher et al. (1984). Aatrex Nine-O wettable powder (85.5% atrazine, 4.5% related ingredients) was from Ciba-Geigy.

Immunochemicals. Mouse monoclonal s-triazine-herbicide specific antibody AM7B2.1 (primary antibody) was donated by Dr. A. E. Karu, Department of Plant Pathology, University of California, Berkeley, CA. The ascites fluid preparation was centrifuged, and the supernatant was used unpurified. The development of mouse polyclonal chlorodiamino-s-triazine-specific antibody PAb 1 (primary antibody) was previously described (Muldoon et al., 1994). The ascites fluid was centrifuged, and the supernatant was used unpurified. Alkaline phosphatase (AP), goat anti-mouse IgG (H+L) (trapping antibody), bovine serum albumin (BSA), and p-nitrophenyl phosphate (enzyme substrate) were purchased from Sigma Chemical Co. (St. Louis, MO). The s-triazine hapten CEPrT was synthesized according to the method of Goodrow et al. (1990). CAHeT was synthesized as previously reported (Muldoon et al., 1994). Hapten–alkaline phosphatase enzyme conjugates CEPrT–AP and CAHeT–AP (Table 1) were made as previously described (Muldoon et al., 1993, 1994).

Buffers. Phosphate-buffered saline (pH 7.5) containing Tween 20 and sodium azide (PBSTA) was used for dilution of immunoreagents and samples prior to immunoassay and for microtiter plate washing. Sodium carbonate buffer (pH 9.6) was used in coating microtiter plates with antibodies. Enzyme substrate buffer was diethanolamine, pH 9.8. The compositions of the various buffers used have been described in detail elsewhere (Lucas et al., 1991).

Equipment. The microtiter plates were Nunc Immunoplate II Maxisorp (Nunc, No. 442964). Optical density (OD) measurements and calculations were made with a ThermoMax microplate reader with associated SOFTmax software (Molecular Devices Corp., Menlo Park, CA) on an IBM PC.

HPLC measurements were made using a Waters 712 WISP automatic sample injector, two Waters Model 510 HPLC pumps, a Waters Model 490 UV detector (210, 220, and 230 nm monitored), and a NEC APC-IV controller with Maxima 820 software. The column was a Waters Nova-Pak 4-μm C_{18} in a 8 mm × 10 cm radial compression module. The solvent system was 0–75% acetonitrile/phosphoric acid, pH 2, 15-min gradient (Waters curve 10) at a flow rate of 2.0 mL/min. The final condition was maintained for 5 min. Analyte concentrations were calculated on the basis of standard curves for each of the individual compounds using authentic analytical standards.

ELISA Formats. Development of each of the haptenated enzyme competitive inhibition ELISA formats was previously reported using either antibody AM7B2.1 (CIET ELISA) (Muldoon et al., 1993) or PAb 1 (CAAT ELISA) (Muldoon et al., 1994). Briefly, microtiter plates were coated with trapping antibody diluted in coating buffer, incubated for 18 h at 4 °C, and then washed. One hundred microliters of a predetermined amount of primary antibody diluted in 0.5 mg/mL BSA in PBSTA was applied to the plate, incubated for 60 min, and frozen with the liquid remaining in the wells. When needed, the plate was thawed and washed. The protocols for ELISA sample analyses for parent herbicide and CAAT were slightly different. For the CIET ELISA, 40 μL of sample (±s-triazine) was mixed with 200 μL of CEPrT–AP in a separate uncoated well. Aliquots (50 μL) were applied in replicate to the antibody-coated plate and incubated for 30 min and then the plate was washed. Enzyme substrate was added, and the plate OD measurements were made at 30 min. For the CAAT ELISA, 100 μL of sample (±s-triazine) was mixed with 100 μL of CAHeT–AP in a separate uncoated well. Aliquots (50 μL) were applied in replicate to the antibody-coated plate and incubated for 30 min, and then the plate was washed. Enzyme substrate was added, and the plate OD measurements were made at 60 min. For treatment monitoring, each plate consisted of a standard curve of 10 concentrations of either CIET (200 to 7.63×10^{-4} μM, CIET ELISA) or CAAT (1000 to 3.80×10^{-3} μM, CAAT ELISA), a zero-dose control, a blank (no haptenated enzyme added), and 4 dilutions of 5 samples.

Determination of Antibody Cross-Reactivity for Atrazine Ozonation Products. Both ELISAs were characterized for cross-reactivity toward CIET and the various ozonation products depicted in the degradation scheme shown in Figure 1. Eleven concentrations of each compound plus a zero-dose control were assayed in duplicate. Cross-reactivites were expressed as IC_{50} values (concentration of analyte that produces a 50% decrease in the maximum normalized response) generated from the four-parameter logistic curve fitting function in SOFTmax (parameter C) and were interpreted relative to CIET for the CIET ELISA or to CAAT for the CAAT ELISA according to the formula

$$\% \text{ reactivity} = (IC_{50} \text{ CIET or CAAT}/IC_{50} \text{ analog}) \times 100 \quad (1)$$

Bench-Scale Ozonation. Two hundred and fifty milliliters of a 100 mg/L solution of Aatrex Nine-O was made basic (pH 10.5) with the addition of 1 N NaOH. Ozonation was carried out using a photoreactor fitted with a sintered glass frit at the bottom for the introduction of ozone gas (Somich et al., 1988). Ozone was generated using a PCI Model GL-1B ozone generator (PCI Ozone Corp., West Caldwell, NJ) with oxygen feed gas at a rate of 1.0 L/min. Ozone output was approximately 1.0% (w/w) as determined using a PCI Model HC ozone monitor. The pH of the reaction solution was monitored using pH indicator paper and maintained at a pH value of 9.5–10.5 with the addition of 1 N NaOH. Samples were taken periodically and were purged with nitrogen to remove residual ozone prior to analysis. Samples were analyzed either undiluted or diluted 1:2 with acetonitrile (for CIET, CIAT, and CEAT HPLC analysis of 0–30-min samples). Ozonation was monitored by HPLC and was carried out until CIET was converted to CAAT (150 min). Samples were diluted in PBSTA and analyzed by ELISA.

Pilot-Scale Ozonation. The pilot-scale unit was a newly designed system and is described in detail elsewhere (C. J. Hapeman, D. R. Shelton, J. S. Karns, M. T. Muldoon, unpublished results). Two hundred and eight liters (55 gal) of 100 mg/L of Aatrex Nine-O was ozonated without pH adjustment. Ozone was generated using the equipment described above with oxygen feed gas introduced at a rate of 1.0 L/min. Ozone output was

Table 2. Cross-Reactivities of CIET and CAAT ELISAs for Atrazine Ozonation Products[g]

common name	Cook system[a]	cross-reactivity[b] CIET ELISA	cross-reactivity[b] CAAT ELISA[c]
atrazine	CIET	100	0.2
	CDDT	0.1	1.1
	CDIT	9.0	1.1
	CDET	10.6	1.1
	CDAT	0.1	2.3
deethylatrazine	CIAT	0.5	2.2
deisopropylatrazine	CEAT	0.5	92.8
chlorodiamino-*s*-triazine	CAAT	ni[d]	100
	COAT[e]	nd[f]	0.3

[a] Adapted from Cook (1987); structures are given in Table 1. [b] Cross-reactivities were calculated relative to CIET (atrazine) for the CIET ELISA and CAAT (chlorodiamino-*s*-triazine) for the CAAT ELISA. [c] From Muldoon et al. (1994), except COAT data. [d] ni, no inhibition at 1 mM. [e] COAT was approximately 90% pure by HPLC. [f] nd, not done. [g] IC_{50}s were determined by assaying a zero-dose control (PBSTA) and 11 concentrations of each analog and deriving the value from the four-parameter logistic curve fitting function. Each concentration was assayed in duplicate wells of an antibody-coated plate.

approximately 3.0% (w/w). Samples were taken periodically and were purged with nitrogen to remove residual ozone prior to analysis. Samples were analyzed either undiluted or diluted 1:2 with acetonitrile (for CIET HPLC analysis of the 0-h sample). Ozonation was monitored by HPLC and was carried out until CIET was converted to CDAT or CAAT (18.5 h). The solution was fortified to 2 mM KOH, resulting in a pH value of 10.7 to hydrolyze CDAT to CAAT (3 h). The solution was brought to pH 6.6 by fortification to 5 mM KH_2PO_4. Samples were diluted in PBSTA and analyzed by ELISA.

Biodegradation of Ozonated Aatrex Using DRS-1. One hundred and fifty milliliters of ozonated Aatrex was fortified to 10 mM phosphate buffer, pH 7.0, 0.1% (w/v) Tru-Sweet high fructose corn syrup (American Fructose–Decatur, Decatur, AL), 0.5 mM $MgCO_3$, 50 μM $CaCO_3$, 50 μM $MnSO_4$, and 5 μM $FeCl_3$. The solution was inoculated with 20 mL of a culture of *Klebsiella terragena* strain DRS-1-S (Leeson et al., 1993) which had been grown on the above media for 24 h (Klett 660 = 30 units). Biodegradation was monitored by HPLC and was carried out until CAAT was no longer detected (<0.05 ppm, 100-μL injection). Samples were removed from the flask and centrifuged at 15000*g* for 2 min to remove cellular material. An aliquot was injected immediately on HPLC and the remainder frozen (−20 °C). Samples were thawed and diluted in PBSTA prior to ELISA analysis.

RESULTS AND DISCUSSION

ELISA Cross-Reactivities with the Various Ozonation Products. The monoclonal antibody AM7B2.1 was originally developed as a *s*-triazine herbicide class-specific antibody (Karu et al., 1991). This antibody has been used for soil, water (Lucas et al., 1991; Goh et al., 1992), and pesticide waste analysis (Muldoon et al., 1993). For use in ozonation monitoring, it was important to characterize the antibody cross-reactitivies toward the acetamido ozonation intermediates CDDT, CDIT, CDET, and CDAT. These compounds have not been previously studied using monoclonal antibody AM7B2.1. Table 2 shows the cross-reactivity of the various compounds identified in chloro-*s*-triazine herbicide ozonation with the two assays used in this study.

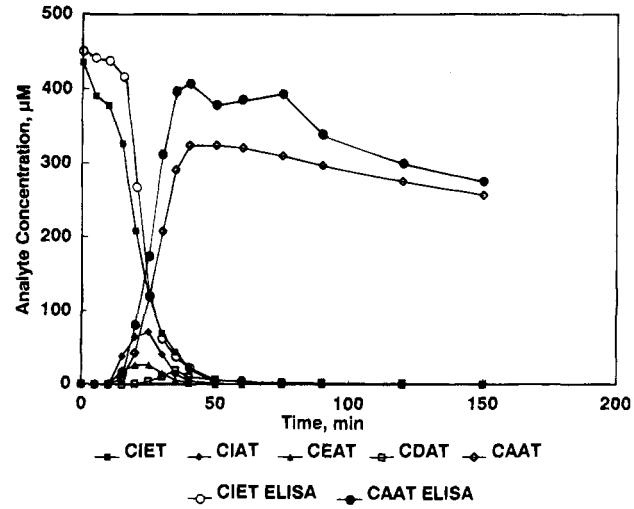


Figure 2. Reaction profile of bench-scale ozonation of 250 mL of 100 ppm Aatrex carried out under alkaline conditions as measured by HPLC and ELISAs.

For the CIET ELISA, addition of a single oxygen atom to either alkyl side chain (CDIT, CDET) greatly reduced antibody recognition. Antibody recognition was lost when two oxygen atoms were added to the alkyl side chains. Removal of alkyl moieties resulted in complete loss of recognition by antibody AM7B2.1. The CAAT ELISA was previosly characterized with most of these structures (Muldoon et al., 1994). In summary, addition of an ethyl group (CEAT) did not have an effect on recognition by antibody PAb 1. However, additional of an isopropyl or acetyl group resulted in a large decrease in antibody recognition. Additional substitution led to loss of antibody recognition. COAT is a secondary ozonation product and an important hydrolysis product of CAAT (Kearney et al., 1988) and has been identified as an intermediate in CAAT degradation in the environment (Grossenbacher et al., 1984). Although attempts to purify it from other minor contaminants (ammeline and ammelide) were unsuccessful, use of technical material allowed for an estimate of cross-reactivity with this system. COAT was not recognized by PAb 1. This was not surprising since ammeline and ammelide were not recognized either (Muldoon et al., 1994).

These data indicated that the CIET ELISA using antibody AM7B2.1 should give an accurate estimate of CIET since the antibody showed little or no recognition of the various breakdown products. The CAAT ELISA was shown to recognize CEAT to the same extent as CAAT; therefore, this assay should provide a summed estimate of CAAT and CEAT concentrations. Since CEAT does not accumulate in the ozonation of CIET and is not a relevant degradation product in propazine (CIIT) ozonation (Hapeman, 1993), the CAAT ELISA can be used for monitoring the accumulation of CAAT in these processes. However, CEAT is a very important intermediate degradation product in simazine (CEET) ozonation and may complicate the interpretation of CAAT ELISA results in this situation.

Bench-Scale Ozonation Monitoring. Ozonation of formulated atrazine (Aatrex Nine-O) was monitored by HPLC followed by ELISA using each of the ELISA systems to demonstrate the applicability of these ELISAs to CIET ozonation monitoring. Figure 2 shows the product profile as determined by HPLC in addition to the CIET and CAAT ELISA measurements during the ozonation process. With both ELISAs there was an

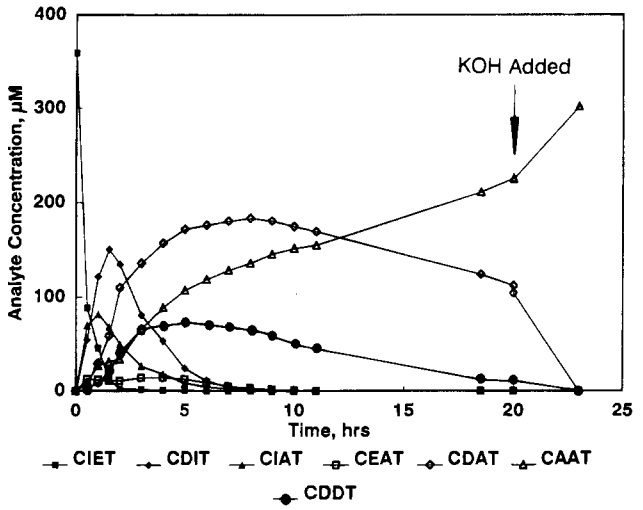


Figure 3. Reaction profile of pilot-scale ozonation of 208 L (55 gal) of 100 ppm Aatrex as measured by HPLC. At 20 h, the solution was made basic with the addition of KOH to convert the acetamido components CDAT and CDDT to CAAT.

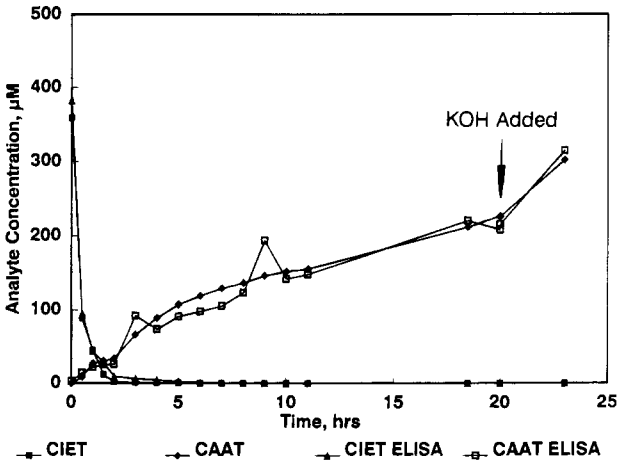


Figure 4. Reaction profile of pilot-scale ozonation of 208 L (55 gal) of 100 ppm Aatrex as measured by HPLC and ELISAs. At 20 h, the solution was made basic with the addition of KOH to convert the acetamido components CDAT and CDDT to CAAT.

overestimation of analyte content, particularly at the higher concentrations. This could be due to the presence of some unidentified cross-reactants in the samples or more probably is a result of errors in sample dilution since the samples with high analyte concentrations required considerable dilution to be quantitated by ELISA (up to 1000-fold for the CIET ELISA and 80-fold for the CAAT ELISA). Sample dilution should have the benefit of diluting out potential nonspecific sample interferences such as surfactant-type materials present in agricultural formulations. Foaming action was observed during ozonation but rapidly diminished during the course of the reaction and was not apparent after 30 min. This was an indication that surfactant-type materials were degraded during the treatment process. Nevertheless, the concentrations obtained by the ELISAs were highly correlated with HPLC data for both assays. The geometric mean regression equation for the amount found by the CIET ELISA on the amount found by HPLC was $Y = 1.15X - 1.17$, standard error = 0.032 μM, $R = 0.99$, df = 13; the CAAT ELISA gave $Y = 1.23X + 0.00$, standard error = 0.046 μM, $R = 0.98$, df = 13. Y is the concentration obtained by ELISA (micromolar analyte equivalents) and X is the concentration obtained by HPLC (micromolar analyte equivalents). Use of only CIET or CAAT concentrations found by HPLC for regression (versus a summed response) did not change the correlations found (CIET ELISA $R = 0.99$, CAAT ELISA $R = 0.97$), indicating that the ELISAs can be used to measure these two analytes exclusively in the treatment process.

Pilot-Scale Ozonation. Pilot-scale ozonation of Aatrex was performed without any pH adjustment to measure the formation and disappearance of the intermediate products. The HPLC analysis was in support of an ongoing pesticide waste disposal project at USDA. Figure 3 shows product formation and disappearance during the reaction. Decomposition of CDDT and CDAT to CAAT is slow under neutral to acidic conditions (the pH decreased from 6.5 to 3.8 during ozonation), and this is evidenced by the large amount of CDDT and CDAT present long after the disappearance of CIET. At 20 h, the acetamido compounds were converted to CAAT by raising the pH to 10.7 for 3 h. Figure 4 shows the loss of CIET and production of CAAT as measured by HPLC along with the CIET and CAAT ELISA measurements. The geometric mean regression equation for the amount found by the CIET ELISA on the amount found by HPLC was $Y = 1.06X - 1.70$, standard error = 0.013 μM, $R = 0.99$, df = 16; the CAAT ELISA was $Y = 1.08X - 22.07$ standard error = 0.060 μM, $R = 0.95$, df = 16. Correlations were not different when only CIET or CAAT HPLC concentrations were used in regression. From these data it can be seen that the ELISAs are very selective toward CIET and CAAT. However, the insensitivities of the ELISAs to CDDT and CDAT may not be advantageous in this circumstance since the operator could not obtain a mass balance of substrate and products. From Figure 3 it can be seen that ozonation could have been stopped at 11 h and alkaline conversion of the acetamido products to CAAT initiated. Experiments were carried out in which 1-mL samples were made alkaline to convert acetamido products to CAAT prior to sample analysis by ELISA to measure "CAAT-convertible products" during ozonation. These were unsuccessful due to the slow rate of conversion measured by HPLC. In addition, higher alkalinities resulted in CAAT decomposition. Ozonation at alkaline pH (9–10) not only enhanced rates of CIET decomposition (Kearney et al., 1988) but also simplified analysis by ELISA.

Biodegradation of Ozonated Aatrex. *Klebsiella* sp. DRS-1 was isolated from sewage sludge for its ability to utilize the *s*-triazine ring nitrogen of CAAT as a sole source of nitrogen in the presence of ammonia nitrogen (Leeson et al., 1993). This is an important feature since pesticide waste materials may contain considerable amounts of nitrogen fertilizer which may be more readily utilized by microorganisms. Figure 5 shows the loss of CAAT as measured by HPLC and the CAAT ELISA. The geometric mean regression equation for the amount found by the CAAT ELISA on the amount found by HPLC was $Y = 0.903X + 2.66$, standard error = 0.036 μM, $R = 0.98$, df = 13. The CAAT ELISA estimates of CAAT concentration were slightly lower than the HPLC estimates. This was probably due to the presence of residual enzymatic activity in the sample supernatants which were sampled immediately by HPLC, frozen, thawed, and assayed by ELISA. The HPLC aliquots were reinjected after standing at room temperature for 12 h and were found to have decomposed considerably. Only the data from the first HPLC sample injections could be used. These data indicate

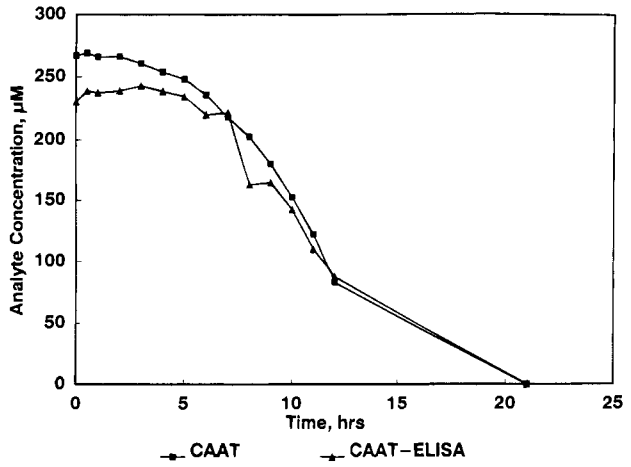


Figure 5. CAAT degradation by the sludge microorganism DRS-1 as measured by HPLC and CAAT ELISA.

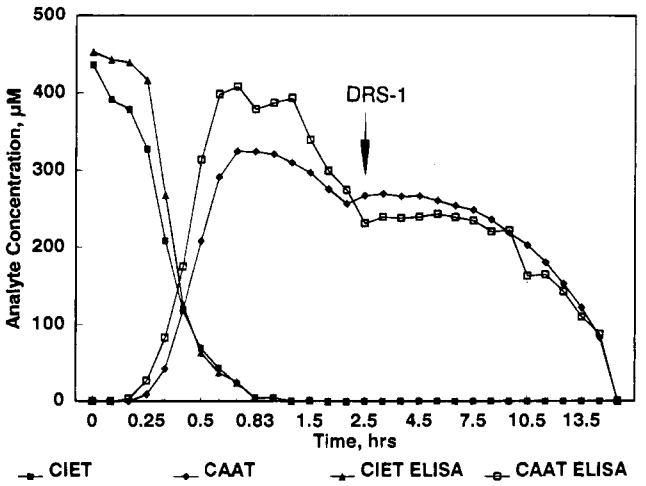


Figure 6. Comparison of HPLC and ELISA results for atrazine ozonation and CAAT biodegradation monitoring.

that the CAAT ELISA is a reliable method for monitoring the biodegradation of ozonated Aatrex with minimal sample preparation.

Complete Treatment Process Monitoring by ELISA. Figure 6 shows a comparison of the HPLC and ELISA methods used for monitoring the complete treatment process. The geometric mean regression equation for the amount found by the two ELISAs on the amount found by HPLC was $Y = 1.12X - 7.13$, standard error $= 0.038\ \mu M$, $R = 0.95$, df $= 43$. The ELISAs can be used to obtain much of the same monitoring information as the HPLC data. The binary treatment process of ozonation followed by microbial degradation of the products first requires monitoring for the complete disappearance of the parent chloro-*s*-triazine herbicide substrate and conversion to the intermediate CAAT. When this is accomplished, the ozonation process is considered complete and the material is then subjected to biological treatment. The criteria for determining this end-point can be reliably measured using the two ELISAs. The slow loss of accumulated CAAT as measured by ELISA (and HPLC) during ozonation could also be used as supportive evidence that the more readily oxidizable substrates (CIET, CIAT, CEAT) have been substantially depleted. The second phase requires monitoring for the biodegradation of the ozonated material. The CAAT ELISA could be used to monitor biodegradation and was found to be a very reliable indicator for the degradation of CAAT. When CAAT was no longer detected (ELISA limit of detection = 0.5 μM), COAT was present at an approximate concentration of 10 μM (HPLC), indicating that this intermediate does not accumulate during the biodegradation process.

Conclusions. Immunoassay technology offers many advantages over conventional analytical methods for on-site treatment monitoring. We have used the *s*-triazine class-specific antibody AM7B2.1 in an ELISA for measuring the disappearance of parent herbicide in conjunction with an ELISA for measuring the accumulation of the key intermediate CAAT during the ozonation of atrazine. Biodegradation of the ozonation product was then measured using the CAAT ELISA. The ELISAs were very accurate and precise for the quantitation of these two analytes and should provide a valuable tool for process monitoring of chloro-*s*-triazine herbicide treatment and disposal. Coupled with a conventional multiresidue method such as GLC or HPLC for initial and final waste characterization, the use of this method should save time and expense in treatment monitoring.

ACKNOWLEDGMENT

We gratefully acknowledge Dr. A. E. Karu, Hybridoma Facility, University of California, Berkeley, CA, for generously providing antibody AM7B2.1 used in this study.

LITERATURE CITED

Aharonson, N.; Cohen, S. Z.; Drescher, N.; Gish, T. J.; Gorbach, S.; Kearney, P. C.; Otto, S.; Roberts, T. R.; Vonk, J. W. Potential contamination of ground water by pesticides. *Pure Appl. Chem.* **1987**, *59*, 1419–1446.

Bushway, R. J.; Perkins, B.; Savage, S. A.; Lekousi, S. J.; Ferguson, B. S. Determination of atrazine residues in water and soil by enzyme immunoassay. *Bull. Environ. Contam. Toxicol.* **1988**, *40*, 647–654.

Cook, A. M. Biodegradation of s-triazine xenobiotics. *FEMS Microbiol. Rev.* **1987**, *46*, 93–116.

Cook, A. M.; Hutter, R. *s*-Triazines as nitrogen sources for bacteria. *J. Agric. Food Chem.* **1981**, *29*, 1135–1143.

Dwinell, S. E. Federal laws affecting the design and management of pesticide mixing and loading facilities. In *National Symposium on Pesticide and Fertilizer Containment: Design and Management*; Midwest Plan Service: Ames, IA, 1992; pp 5–12.

Ferguson, T. D. *Proceedings of International Workshop on Research in Pesticide Treatment / Disposal / Waste Minimization*;. EPA/600/9–91/047; U.S. Government Printing Office: Washington, DC, 1991; 193 pp.

Goh, K. S.; Spurlock, F.; Lucas, A. D.; Kollman, W.; Schoenig, S.; Braun, A. L.; Stoddard, P.; Biggar, J. W.; Karu, A. E.; Hammock, B. D. Enzyme-linked immunosorbant assay (ELISA) of simazine for Delhi and Yolo soils in California. *Bull. Environ. Contam. Toxicol.* **1992**, *49*, 348–353.

Goodrow, M. H.; Harrison, R. O.; Hammock, B. D. Hapten synthesis, antibody development, and competitive inhibition enzyme immunoassay for s-triazine herbicides. *J. Agric. Food Chem.* **1990**, *38*, 990–996.

Grossenbacher, H.; Horn, C.; Cook, A. M.; Hutter, R. 2-Chloro-4-amino-1,3,5-triazine-6(5H)-one: a new intermediate in the biodegradation of chlorinated *s*-triazines. *Appl. Environ. Microbiol.* **1984**, *48*, 451–453.

Hapeman, C. J. Oxidation of *s*-triazine pesticides. In *Emerging Technologies in Hazardous Waste Management IV*; Tedder, D. W., Pohland, F. G., Eds.; ACS Symposium Series 554; American Chemical Society: Washington, DC, 1994.

Hapeman-Somich, C. J.; Gui-Ming, Z.; Lusby, W. R.; Muldoon, M. T.; Waters, R. Aqueous ozonation of atrazine. Product identification and description of the degradative pathway. *J. Agric. Food Chem.* **1992**, *40*, 2294–2298.

Karu, A. E.; Harrison, R. O.; Schmidt, D. J.; Clarkson, C. E.; Grassman, J.; Goodrow, M.H.; Lucas, A.; Hammock, B. D.; White, R. J.; Van Emon, J. M. Monoclonal immunoassay of triazine herbicides: development and implementation. In *Immunoassays for Trace Chemical Analysis: Monitoring Toxic Chemicals in Humans, Foods, and Environment*; Vanderlaan, M., Stanker, L. H., Watkins, B. E., Roberts, D. W., Eds.; ACS Symposium Series 451; American Chemical Society: Washington, DC, 1991; pp 59–77.

Kearney, P. C.; Muldoon, M. T.; Somich, C. J.; Ruth, J. M.; Voaden, D. J. Biodegradation of ozonated atrazine as a wastewater disposal system. *J. Agric. Food Chem.* **1988**, *36*, 1301–1306.

Leeson, A.; Hapeman, C. J.; Shelton, D. R. Biomineralization of atrazine ozonation products. Application to the development of a pesticide waste disposal system. *J. Agric. Food Chem.* **1993**, *41*, 983–987.

Lucas, A. D.; Schneider, P.; Harrison, R. O.; Seiber, J. N.; Hammock, B. D.; Biggar, J.W.; Rolston, D. E. Determination of atrazine and simazine in water and soil using polyclonal and monoclonal antibodies in enzyme-linked immunosorbent assays. *Food Agric. Immunol.* **1991**, *3*, 155–167.

Muldoon, M. T.; Fries, G. F.; Nelson, J. O. Evaluation of ELISA for the multianalyte analysis of *s*-triazines in pesticide waste and rinsate. *J. Agric. Food Chem.* **1993**, *41*, 322–328.

Muldoon, M. T.; Fries, G. F.; Nelson, J. O. Hapten synthesis and immunoassay development for the analysis of chlorodiamino-*s*-triazine in treated pesticide waste and rinsate. *J. Agric. Food Chem.* **1994**, *42*, 747–755.

Seiber, J. N. Pesticide waste disposal: review and 1985 national workshop summary. In *Proceedings: National Workshop on Pesticide Waste Disposal*; EPA/600/9–87/001; U.S. Government Printing Office: Washington, DC, 1987; pp 11–19.

Seiber, J. N. Current technologies for pesticide waste disposal. In *Pesticide Waste Management. Technology and Regulation*; Bourke, J. B., Felsot, A. S., Gilding, T. J., Jenson, J. K., Seiber, J. N., Eds.; ACS Symposium Series 510; American Chemical Society: Washington, DC, 1992; pp 138–147.

Sherry, J. P. Environmental chemistry: the immunoassay option. *Crit. Rev. Anal. Chem.* **1992**, *23*, 217–300.

Somich, C. J.; Kearney, P. C.; Muldoon, M. T.; Elsasser, S. Enhanced soil degradation of alachlor by treatment with ultraviolet light and ozone. *J. Agric. Food Chem.* **1988**, *36*, 1322–1326.

Somich, C. J.; Muldoon, M. T.; Kearney, P. C. On-site treatment of pesticide waste and rinsate using ozone and biologically active soil. *Environ. Sci. Technol.* **1990**, *24*, 745–749.

Received for review October 26, 1993. Revised manuscript received February 17, 1994. Accepted June 16, 1994.[⊗] Mention of specific products or suppliers is for identification and does not imply endorsement by the U.S. Department of Agriculture to the exclusion of other suitable products or suppliers.

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1994.