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Comparison of an Enzyme Immunoassay with Gas Chromatography for Atrazine Determination in Water and Soil

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Point and nonpoint source contamination of surface and ground waters has been an important environmental subject in recent years. Pesticide residues from urban and agricultural use may leach through the soil profile or be transported via water or soil sediment into rivers, lakes, or streams. Detection of pesticides in surface waters has been correlated with agricultural runoff following spring applications (Davis et al. 1993; Spalding et al. 1994). These pesticides may slowly reach the groundwater by direct downward leaching (Wehtje et al. 1984) or more rapidly by preferential flow through soil macropores (Edwards et al. 1992). Accurate detection of pesticide residues is essential not only to ensure a safe, potable water supply but is useful for crop management decisions involving planting of sensitive crops.

Investigating the fate of pesticides in soil and water is a major focus for many researchers, pesticide manufacturers, and regulatory agencies. Current pesticide detection methods include gas chromatography (GC) or high performance liquid chromatography (HPLC). These methods are usually very time consuming and expensive. Traditional methods require extensive personnel training to operate and maintain equipment and ensure proper residue analysis.

Many research and regulatory monitoring projects require analysis of numerous soil and water samples. These samples often contain pesticide quantities below a predetermined threshold concentration. A detection method that could screen out subthreshold and pesticide-free samples would reduce the number of samples requiring analysis by conventional methods, thereby reducing analytical costs (Aga and Thurman 1993; Hall et al. 1993). In a survey of analytical laboratories in Nebraska, the average cost for analysis of atrazine by GC was approximately $100 per sample, whereas the immunoassay (IA) technique was $25 per sample.

The objective of this study was to compare an IA system to a conventional method, gas chromatography, for detecting atrazine in fortified water and soil

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samples, as well as field samples, using no concentration or clean up step prior to IA analysis.

MATERIALS AND METHODS

A commercial IA kit (Ohmicron Corporation, Newtown, PA) was used to scan samples for atrazine in fortified and field samples.

Distilled water was fortified in triplicate with 20, 40, 60, 80, or 100 ng mL^{-1} atrazine. A 200 µL aliquot of each concentration was used for IA analysis. Duplicate 10 mL aliquots of each concentration were pipetted into 250 mL round-bottom flasks, evaporated to dryness, redissolved in 3 mL toluene, and an aliquot pipetted into a 0.8 mL vial for GC analysis. A 200 µL aliquot of each sample was added to a disposable test tube followed by 250 µL of enzyme conjugate and 500 µL of antibody-coated magnetic particles. This mixture was allowed to incubate for 15 minutes at room temperature allowing any atrazine present in the sample to compete with the enzyme conjugate for sites on the antibodies. Following the incubation period, the tubes were placed in a magnetic rack for two minutes and decanted. Two wash steps with 1 mL washing solution (deionized water) removed unbound or potentially interfering substances. A 500-µL aliquot of color reagent was added to each sample that reacted with the enzyme conjugate to generate a blue product. Following a 20 minute incubation period to allow color development, the reaction was stopped with 500 µL of sulfuric acid. The absorbance for each sample was determined using the RPA-1 photometric analyzer at 450 nm. Atrazine concentration in each water sample was estimated from a calibration curve prepared during the sample run.

Soil samples of a Hastings silty clay loam (fine, montmorillonitic, mesic Udic argiustoll) were collected from a research site at the South Central Research Station at Clay Center, Nebraska (Table 1). A Giddings hydraulic probe was used to extract 5 cm diameter cores to a depth of 360 cm that were placed in a box with dry ice and transported to a cold room (-20 C). Cores were collected from plots with a history of atrazine application and from plots with no atrazine history. Three cores from each plot were combined, sieved, and thoroughly mixed. The cores were divided into 30 cm increments and sieved through a 2-mm screen. The outer surface of each core was removed to avoid contamination of lower depths as the plastic sleeve was pushed through the soil profile.

Soil from the Ap, Bt, or C horizon was fortified with atrazine at 30, 50, 100, 150, or 200 ng g^{-1} in 250 mL polyethylene screw-cap bottles. Stock solutions were prepared in methanol with 98.7% technical grade atrazine. A 2-mL aliquot of the appropriate dilution was added to 40 g moist soil from each horizon to achieve the desired atrazine concentration. The methanol was allowed to evaporate while the soil samples remained at room temperature for 24 hours. The method to extract atrazine from soil was modified from that of Baer et al. (1992). Atrazine was

Table 1. Particle size, organic matter content, and pH of a Hastings silty clay loam.

Soil depth	Sand[a]	Silt	Clay	O.M.[b]	pH[c]
cm	%				
0-30	18	48	34	2.6	6.3
30-60	24	42	34	1.0	6.8
90-90	18	54	28	0.3	7.7
90-120	20	60	20	0.2	7.7
180-210	20	62	18	0.3	7.9
270-300	18	62	20	0.1	7.9

[a]Soil texture determined by hydrometer method
[b]Percent organic matter
[c]1:1 soil: water mixture

extracted with 120 mL of a 75% methanol-distilled water solution. Samples were shaken for one hour on a wrist-action shaker and filtered through Whatman No. 1 filter paper. After removal of a 0.1 mL aliquot from the filtered solution for IA analysis, the methanol was preferentially removed by rotary evaporation under vacuum on a 35 C water bath. Water was removed by the same process at 45 C leaving only a dry residue in the flask. The dried residue was redissolved in 3 mL of toluene using an ultrasonic bath. An aliquot of the toluene solution was pipetted into a 0.8 mL vial for analysis by gas chromatography. Immunoassay analysis for soil samples was conducted by diluting the 0.1 mL aliquot removed from the filtered solution with 4.9 mL of distilled water and following the IA procedure described earlier.

In field samples, combining the top 30 cm of soil diluted the atrazine concentration to below the IA detection limit (15 ng g^{-1}). Therefore, 30 soil samples were taken from 0 to 15 cm with a 2.5-cm diameter hand probe where atrazine was applied 12 consecutive years at 3.4 kg ha^{-1}. Atrazine was extracted for IA and GC analysis as described in the soil fortification experiments.

Quantitative analysis by gas chromatography used a 30-m wide bore (0.53 mm) capillary column with a bonded SPB-35 stationary phase for separation and thermionic nitrogen-phosphorus detection. Typical operating temperatures were 165 C (column oven), 290 C (injector port), and 290 C (detector port). Carrier gas was helium at 4 mL min^{-1}. Detector gases were air (160 mL min^{-1}) and hydrogen (2 mL min^{-1}). Detection limit for the GC is 1 ng g^{-1}.

The statistical model used in the analysis of variance (ANOVA) to test for

differences between methods was a split plot. The whole plot in the water fortification experiment was a completely randomized design. The soil fortification experiment was evaluated as a split plot with factorial whole plot treatments. In all experiments, the slope of the regression line was tested for significant differences from the straight line (Y= X), and the Y intercept was tested for significant differences from zero. Data were examined for normality using PROC UNIVARIATE and for homogeneity of variance using PROC DISCRIM. Recovery means were compared using a paired t-test. All statistical analyses were performed using the SAS system (SAS Institute Inc.).

RESULTS AND DISCUSSION

Data in the water fortification study were normally distributed and did not violate the assumption of homogeneity of variance, thus data were not transformed. The overall recoveries ranged from 103% to 125% for the IA and 100% to 115% for the GC (Table 2).

Table 2. Atrazine recovery from fortified distilled water by immunoassay and gas chromatography (GC).

Concentration of added atrazine	Atrazine recovery[a]	
	Immunoassay	GC
-- ng mL^{-1} ---	———— Percent ————	
20	103 ± 7	115 ± 16
40	125 ± 6	102 ± 8
60	116 ± 4	99 ± 6
80	110 ± 4	108 ± 1
100	105 ± 2	106 ± 7

[a]Percent recovery ± standard deviation

The mean recovery for the IA (112%) was not significantly different from the GC mean (106%) (p=0.14). Results from other papers indicate that recovery greater than 100% is not atypical (Ferguson et al. 1993; Itak et al. 1993). Good reproducibility was obtained with both detection methods. There was excellent correlation (r=0.98) between the two methods. The slope (0.96) and intercept (6.1) were not significantly different from 1 and 0, respectively (p=0.47, p=0.13). The ideal detection method would generate a slope of 1.0 and an intercept of 0. Deviations from the ideal slope and intercept in typical environmental samples may result from binding of similar structured compounds, interferences from naturally occurring substances, or particulate in the sample matrix. The analysis of variance indicated a significant difference between methods (p=0.003), which is likely due to the consistently higher recoveries by the IA.

Data from fortified soil samples were normally distributed and did not violate the assumption of homogeneous variance. Mean recoveries over all horizons ranged from 82% to 135% for the IA and 71% to 117% for the GC (Table 3).

Table 3. Atrazine recovery from fortified soil samples by immunoassay and gas chromatography.

Atrazine concentration	Horizon	Immunoassay recovery[a]	GC recovery[a]
--- ng g^{-1}---		%	
30	A	103 ± 15	72 ± 5
50		135 ± 17	78 ± 4
100		99 ± 7	88 ± 26
150		101 ± 17	94 ± 20
200		95 ± 14	88 ± 1
30	B	103 ± 55	72 ± 2
50		102 ± 12	76 ± 7
100		85 ± 40	83 ± 2
150		96 ± 18	112 ± 3
200		92 ± 5	90 ± 2
30	C	82 ± 25	71 ± 4
50		105 ± 10	79 ± 1
100		101 ± 43	86 ± 1
150		118 ± 2	117 ± 1
200		99 ± 25	89 ± 2

[a]Percent recovery ± standard deviation

There were no differences in recovery among horizons for either method. However, the IA recovered more atrazine than GC ($\bar{x}$=101% to $\bar{x}$=86%) over all horizons (p=0.001). This was primarily due to the difference in recovery from the A horizon ($\bar{x}$=107% for IA to $\bar{x}$ =84% for GC, p=0.007). Subsamples of reclaimed methanol were analyzed for atrazine that may have been removed with methanol during the rotary evaporation step; however, no atrazine was detected. Aga and Thurman (1993) observed no atrazine losses during evaporation to dryness at temperatures below 50 °C. Li et al. (1989) also observed higher recoveries and lower precision with IA compared to GC in soil samples. Regression analysis of GC vs. IA indicated good correlation (r=0.92) between methods. The slope (0.94) was not different from 1 (p=0.39), but the intercept (15.7) was significantly different from 0 (p=0.035). The analysis of variance indicated little difference between methods (p=0.11)

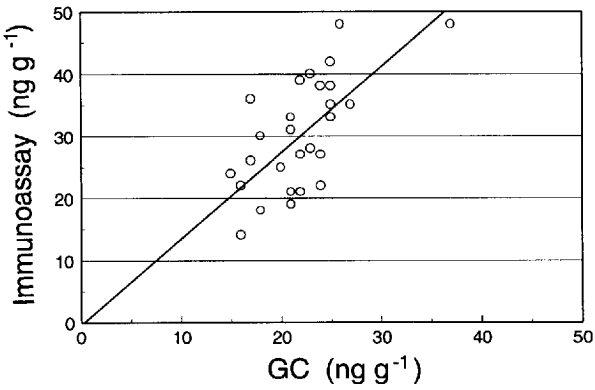

Figure 1. Comparison of atrazine recovery by immunoassay and GC from Soil treated annually for 12 years.

For field samples where soil had been treated with atrazine for 12 consecutive years, the mean concentration as determined by the IA (30 ng g^{-1}) was higher than by GC (22 ng g^{-1}). The correlation between methods (r=0.68) was much lower (Figure 1) compared to recovery from fortified samples. Presence of cross-reacting atrazine metabolizes may partially explain the higher IA recoveries. The cross-reactivity of atrazine related compounds may be represented as the dose of triazine analogue required to displace 50% of the enzyme conjugate (50% B/B_0) (Ohmicron 1994). At 50% B/B_0, deethylatrazine (DEA) is approximately 22% as reactive as atrazine (Ohmicron 1994). An estimate of atrazine concentration determined by the IA that is due to DEA cross-reactivity may be determined by multiplying the DEA concentration by 22%. Subtracting the concentration attributable to DEA gives an estimate of atrazine in the sample. Comparing the DEA-adjusted atrazine concentration for the IA to the GC concentrations did not improve the correlation coefficient (r=0.61). However, the average difference between IA and GC results ($\bar{x}$=8.5) was reduced by adjusting the atrazine concentration for DEA cross-reactivity ($\bar{x}$=6.4). Therefore, adjusting for the presence of the major atrazine metabolize (DEA) provided a partial explanation for the difference between IA and GC results. Although the GC method is the accepted technique, it can also be a source of error contributing to differences between the two methods. Deisopropylatrazine (DIA) was not detected in any samples by GC and hydroxyatrazine (HYA) could not be detected by this analytical procedure. DIA and HYA are major degradates of atrazine but are much less reactive to this IA compared to DEA, and probably had little effect on the results.

In summary, these studies compared an enzyme immunoassay procedure to gas chromatography for atrazine extraction and detection in water and soil samples. The IA compared favorably to GC results in atrazine-fortified water and soil samples. Atrazine recovery was generally more variable in soil than water samples. Correlation between the two methods was lower in field samples with

previous atrazine history. A more extensive extraction procedure, than used in this study, that removes potentially interfering substances may help improve correlation between methods.

Recent studies have indicated that solid-phase extraction (SPE) may increase immunoassay efficiency in complex matrices that contain interfering substances, such as naturally occurring particulate and structurally similar compounds. Aga and Thurman (1993) related the SPE-Immunoassay combination to a chromatographic separation where the SPE cartridge is the column and the immunoassay is the detector. Although SPE is an additional step, it is a simple and rapid sample-preparation technique (Aga and Thurman 1993). Solid-phase extraction may improve immunoassay reliability by removing interfering species and increasing the sensitivity by concentrating and purifying the analyte of interest (Aga and Thurman 1993). SPE has been used successfully in several studies involving water samples (Hall et al. 1993; Li et al. 1989) and even soil samples (Del Vane et al. 1994). Improving the sample preparation will lead to more accurate recoveries and reliability, Using an SPE-Immunoassay combination, Aga and Thurman (1993) obtained a high correlation with GC-MS ($r=0.95$) in groundwater samples with no false-positive or false-negative results. However, analysis without SPE on the same samples produced 2890 false-positive results and "no correlation" with GC-MS.

There are many variables that need consideration when deciding how immunoassay can be most useful. For example, the experimental objectives, compound specificity, detection level, accuracy, and cost required for a given project will influence the practicality of using immunoassay. Based on these results and those of others, the future of immunoassay appears promising for pesticide detection in soil and water. The U.S. Environmental Protection Agency (USEPA) Office of Solid Waste recently approved some assays to be used as a screening tool for research purposes (Lesnik 1994).

Immunoassay may be most useful for regulatory monitoring, identifying and isolating contaminated soils, remediation assessments, and testing for herbicide carryover in crop rotations. Immunoassay are most limited in situations where several analytes of interest may be present including breakdown products. They are typically optimized for a specific compound or class of compounds within which the specificity may differ greatly. The presence of cross-reacting compounds and complex matrix effects in natural environmental samples may require the need for an additional clean-up step such as solid-phase extraction to reduce variability and produce a more reliable quantitative estimate.

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