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Enzyme Immunoassay for the Determination of Atrazine Residues in Soil

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Atrazine, an s-triazine herbicide, is used in California to control weeds in rights-of-way, corn, landscape areas, sorghum, industrial areas, conifers, etc. (California Department of Food and Agriculture (CDFA) 1989). Effective Jan. 1, 1989, atrazine was placed on the "Groundwater Protection List" that lists pesticides having the potential to pollute groundwater (State of California 1989). Also, agricultural, outdoor institutional, and outdoor industrial use of atrazine are prohibited in atrazine "Pesticide Management Zones" which are areas sensitive to groundwater leaching by atrazine in California.

Because of the concerns of groundwater contamination and its potential adverse health impacts from drinking contaminated water ("Lifetime Health Advisory level" for atrazine in drinking water was set at 3 ppb by U.S. EPA (1989); also the State of California has adopted a "Maximum Contaminant Level" of 3 ppb), the presence and transport of atrazine in water and soil are being monitored and researched by the CDFA, and these activities generate hundreds of samples requiring residue analysis. Earlier studies showed that enzyme immunoassay (EIA) could be used as a rapid analytical method for the screening of atrazine residues (Bushway et al. 1988; Goh et al. 1990).

This study was conducted to optimize and evaluate a commercially available EIA kit to quantify atrazine residues in soil by i) determining the variability of the calibration line through time; ii) comparing the extraction efficiency of water, methanol, methanol+water, and acetonitrile; and iii) quantifying atrazine concentration in field soil samples using the EIA and comparing the results to gas chromatographic (GC) analysis.

MATERIALS AND METHODS

Description of the EIA kits (Res-I-Mune™, ImmunoSystems Inc.,

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Maine, USA) is given in Bushway et al. (1988) and Goh et al. (1990). Standards preparation and procedures for the assay are as described in Goh et al. (1990) except that instead of distilled water, extract of untreated soil was used for the control and for making the standards because our earlier study indicated that our field soil contained background substances that interfered with the EIA. A control sample and three standards were run in each set of ten assay tubes (Goh et al. 1990).

In the first experiment to determine calibration line variability, three sets of standards were generated by fortifying a water extract of untreated soil with technical atrazine (98.2% US EPA) at a) 0.5, 2.0, 6.0; b) 0.5, 2.0, 10; c) 0.5, 5, 10 ng/mL. Each set was tested on two random dates. Calibration lines were calculated for four replicates on each date. The experiment formed a completely nested design with replicates nested in days within standard sets. Data were analyzed using a nested analysis of variance (ANOVA) with standard sets as a fixed factor and days as a random factor.

In the second experiment, the extraction efficiency of water, methanol, methanol+water, and acetonitrile were compared. Aliquots (25 g) of untreated Delhi Loamy Sand (Soil Conservation Service, 1971) with average content of 89.3% sand, 5.2% silt, 5.4% clay and 0.1% organic carbon obtained from a farm at the California State University of Fresno (CSUF) were fortified at 20, 100, 500, and 1,000 ppb. The soil was extracted with two aliquots of solvents of 25-mL each. The solvents were distilled water, 99.9% methanol, and 99.9% acetonitrile. The methanol+water extraction consisted of prewetting the soil with 10 mL of methanol (99.9%) and followed with 15 mL of water; the second aliquot was 25 mL of water. The soil and solvent were shaken for 10 min on a platform shaker; the extract was decanted; a second aliquot of solvent was added and extraction repeated. The two extracts were composited and filtered through a syringe type 25 mm, 0.2-um nylon cartridge filter (Gelman Sciences, MI, USA) and diluted as appropriate (Table 1) for differential optical density reading at 450 nm with a portable Artel ™ photometer (ME, USA). For the 20-ppb fortified soil, only water or methanol+water was used for the extraction because higher methanol (>10%) and acetonitrile (>4%) levels in the final dilutions were shown to interfere with the EIA system (Goh et al. 1990). Extraction for each solvent at each fortified level was replicated three times.

In the third experiment, soil samples were obtained from a field plot (Field 15, CSUF) where atrazine was applied at 3.97 kg/ha (3.5 lb ai/acre) and immediately flood irrigated. Soil samples were collected at various depths and days after flood irrigation. The best solvent,

methanol+water, as determined in experiment two, was used for all the extractions. To determine the possible range of atrazine concentrations, two soil samples representing the extreme conditions (day and depth) were first analyzed using three dilutions of the extracts (0, 5, 50) to bring the concentrations to within the immunoassay's linear range (0.5-10.0 ppb) shown in Table 1. Over 95% of the samples

Table 1. Scheme for the extraction of soil (25 g) of known atrazine concentration range using 50 mL of solvents and appropriate dilution to final concentration within the immunoassay's linear range (0.5-10 ng/mL).

Soil Conc. (ppb)	Extract Conc.[1] (ng/mL)	Dilution factor	Final Conc. (ng/mL)
2,000	1,000	100x	10.0
1,000	500	50x	10.0
50	25	"	0.5
100[2]	50	5x	10.0
5	2.5	"	0.5
5[3]	2.5	0x	2.5
1.0	0.5	"	0.5

[1]Extracting 25 g soil twice with 25 mL solvent each, giving maximum concentration of atrazine assuming 100% recovery

[2]Use water or methanol (10 mL)+water only

[3]Use water only as extraction solvent

were within 10-90 ppb and hence, only a 5x dilution was needed (Table 1). Forty-eight soil samples were split and analyzed using both EIA and GC. The GC analysis was done by Agriculture and Priority Pollutants Laboratories, Inc. (CA), on a Hewlett-Packard 5890 instrument equipped with a nitrogen-phosphorous detector. The column was 30 m x 0.25 mm i.d. SPB-5 fused-silica capillary column, temperature programed from 67 to 290°C at 20°C/min. The soil samples (100 g each) for GC analysis were extracted using three sequential volumes of 40, 40, and 30 mL of ethyl acetate (>90% recovery) and concentrating the pooled extract to 10 mL. The results from immunoassay were compared to those obtained from GC analysis using linear regression. Immunoassay calibration lines were calculated by linear regression of optical density on the logarithm of standard concentration. All statistical analyses were performed using the SAS system (SAS Institute, Inc., 1987).

RESULTS AND DISCUSSION

Calibration lines produced on different days have different intercepts, but similar slopes and goodness of fits (Table 2). However, slopes were different between standard sets, but intercepts were similar. This means that at concentrations near 1 ppb (corresponding to the intercept of the calibration line) the standard sets give similar sample concentration readings, but as concentration increased the magnitude of the discrepancies increased. Examination of the mean slopes revealed that the slope for the set (a) was higher than for set (b) or (c) (slopes of 0.10, 0.08 and 0.07, respectively). This could indicate that 10 ng/mL is above the linear range of the EIA as shown in Fig. 1 generated independently with a series of atrazine concentrations. If the reduced slope is due to nonlinearity, it should be accompanied by poorer fit of the calibration line, but ANOVA on fit (mean squared errors) of the calibration lines showed that the effect of standard sets was non-significant. However, with the calibration lines based on only three points, such an effect on fit might not be apparent. Table 2 shows that most of the variability in fit is among replicates. That is, the precision of the calibration line varies from replicate to replicate, but there are no systematic effects on precision due to standard sets or days. This indicates that a new calibration curve does need to be generated for each replicate of the immunoassay. The variability may be due to either operator error or a lack of uniformity in the EIA kit itself or both.

In experiment two, the extraction efficiency for the four solvents depended on the concentration of atrazine (Table 3). For all four solvents, there was greater variability in recovery at the low (20 and 100 ppb) levels than at the high (500 and 1,000 ppb) levels of the atrazine concentrations tested. Results for the methanol or methanol+water extraction were more consistent than for water or acetonitrile extraction. Methanol alone cannot be used for extracting atrazine concentrations <50 ppb because high levels of methanol (20%) in the final dilution interferes with the EIA. Therefore, methanol+water was selected as the solvent for subsequent field sample extractions.

A previous study (Goh et al. 1990) showed that atrazine in fortified soil could be extracted efficiently and detected with the kit at concentrations of 0.5-5 ppb using a water extraction and at concentrations of 5-20 ppb using methanol extraction and this study showed that the kit could be used at 20-1,000 ppb using methanol+water extraction. Hence, the theoretical detectable range of atrazine in soil using the EIA kit is 0.5-2,000 ppb. The upper limit being the maximum concentration of atrazine attainable in soil when atrazine is applied at the label rate of 4.48 kg AI/ha.

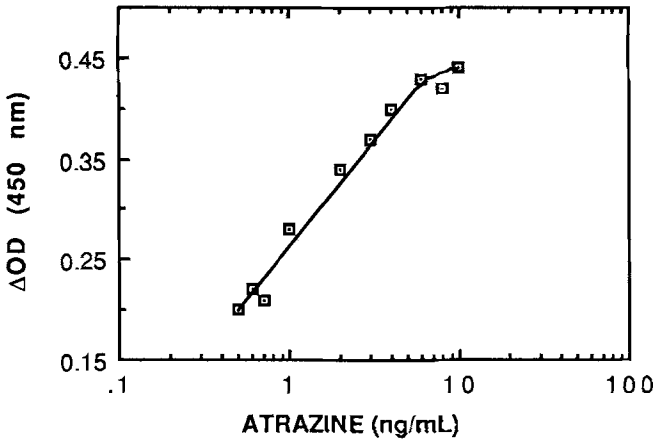

Figure 1. Standard curve for atrazine concentration (n=4 per concentration) of the enzyme immunoassay kit.

Table 2. Analysis of variance tables for the intercept, slope and fit (mean squared error) of calibration lines (SAS PROC NESTED).

Dependent Variable	Source	df	Type I Mean Square	F	% of Total Variance Accounted
Intercept	Std sets	2	0.00989	1.29	8.4
	Days (std sets)	3	0.00765	5.59**	51.2
	Reps (Days)	16	0.00137		40.4
Slope	Std sets	2	0.00157	10.27*	67.5
	Days (std sets)	3	0.00015	2.18	8.0
	Reps (Days)	16	0.00007		24.5
Goodness of fit	Std sets	2	7.4×10^{-7}	5.28	13.6
	Days (std sets)	3	1.4×10^{-7}	0.264	0.0
	Reps (Days)	16	5.3×10^{-7}		86.4

* Significant at the p=0.05 level
** Significant at the p=0.01 level

Although the extraction solvent and procedure, hence extraction efficiency, were dissimilar for the EIA kit and GC, the atrazine concentrations in the split samples (48 pairs) as determined by immunoassay and GC were highly correlated (Fig. 2). However, the slope of the regression line was significantly greater than 1 ($t=7.79$, $df=46$, $p<0.001$) while the intercept was significantly less than 0 ($t=-3.170$, $df=46$, $p<0.01$), indicating that significant bias was present. At concentrations below ca. 52 ppb, the EIA kit gives lower measured

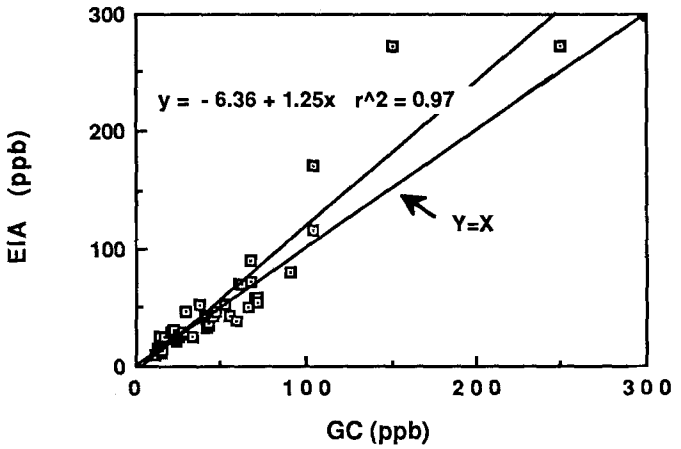

Figure 2. Linear regressions of the concentration of atrazine in soil (n=48) as determined by enzyme immunoassay (EIA) versus gas chromatography (GC).

Table 3. Extraction efficiency (25 g soil) of various solvents (25 mL) on soil fortified with various levels of atrazine (n=3).

Fortified level (ppb)	Solvent	% Recovery±SE
20	water	93.3±14.8
	methanol+water	102.9±12.3
100	water	76.8±18.6
	methanol	108.6±13.9
	methanol+water	106.3±14.7
	acetonitrile	76.3±11.7
500	water	99.3±4.5
	methanol	98.2±6.0
	methanol+water	95.0±6.1
	acetonitrile	95.0±6.1
1,000	water	90.7±5.3
	methanol	94.2±8.7
	methanol+water	90.4±1.0
	acetonitrile	71.9±5.5

concentrations than GC; while above 52 ppb, EIA gives higher concentrations than GC. The significant bias was mostly due to the 100x dilution necessary to bring the 3 soil samples with atrazine concentrations of >100 ppb to the detectable linear range of the kit, as indicated by the regression line (slope=0.90) when the 3 samples were

excluded: $y=4.67 + 0.90x$ ($r^2=0.84$).

The EIA kit is a useful analytical tool giving results comparable to the GC method in determining concentration of atrazine residues in the soil type tested, especially for the range of field concentration of 5-100 ppb. Because the kit is a non-specific method, i.e., cross reactive for some of the metabolites of atrazine such as hydroxyatrazine and desethylatrazine and all the s-triazine herbicides, confirmation by GC or HPLC/mass spectroscopy is necessary for definitive determination of a particular atrazine molecule. The kit is suitable for use when a single known triazine is applied to the soil under experimental condition or for fields with known pesticide application history.

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