Food & Agricultural Immunology (1991) *3*, 155–167

Determination of Atrazine and Simazine in Water and Soil Using Polyclonal and Monoclonal Antibodies in Enzyme-linked Immunosorbent Assays

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(*Received for publication 24 June 1991*)

An enzyme-linked immunosorbent assay (ELISA) was developed as a rapid, reproducible and cost-effective method for routine analysis of atrazine and simazine in environmental samples. Atrazine recoveries from C_{18} solid phase extractions (SPE) of water spiked from 0·1 ppb to 100 ppb showed good correlations with gas chromatography (GC), [^{14}C] atrazine radioassay and ELISA methods. The C_{18} cartridges demonstrated very good recovery for extracting and concentrating the herbicide by all three analytical methods. Comparison between ELISA and GC for analysis of 75 well water samples showed no false negatives and a low (5%) occurrence of false positives. Soil extracts from a controlled simazine spill were also analyzed by GC and ELISA, with excellent correlation between the two methods. Characterization of the s-*triazine assay for tolerance to organic solvents and salts demonstrated the method to be resistant to such modifiers. Additionally, comparison of two monoclonal and two polyclonal antibodies raised against triazine structures were made to assess the performance of the two types of bioreagents. The ELISA method offered sensitivity, accuracy and precision which were competitive with the GC methods. The throughput and cost of the ELISA method offers advantages over traditional extraction and analysis of the* s-*triazines on a routine basis.*

Keywords: *analysis of atrazine, ELISA, enzyme immunoassay,* s-*triazine, soil, water*

INTRODUCTION

The triazine herbicides represent a widely used pesticide class. In a recent survey of state agencies testing groundwater for pesticide contamination, both atrazine and

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simazine were detected in over half the states testing for these compounds. Some wells were found to contain atrazine and simazine (structures shown in Figure 1) in levels above the EPA health advisory limit (Parsons & Witt, 1989). The growing concern of pesticide residues in drinking water, food and air calls for the development and use of efficient and cost-effective means of detection applicable to a variety of matrices. These herbicides have been analyzed extensively by chromatographic methods (Ott *et al.*, 1971; Vickery *et al.*, 1980; Gorder & Dahm, 1981; Lee & Chau, 1983; Lopez-Avila *et al.*, 1985; Huang, 1989), several enzyme-linked immunosorbent assays (ELISAs) (Huber, 1985; Bushway *et al.*, 1988; Hock, 1989; Schlaeppi *et al.*, 1989; Wittmann & Hock, 1989; Dunbar *et al.*, 1990), and with commercially available ELISA kits. However, published chromatrographic methods require expensive instruments and often laborious extraction techniques employing large volumes of organic solvents. Although chromatographic methods are advantageous for multiresidue analysis, the methods are sequential. On the other hand, numerous immunoassays can be run in parallel. Here, we report an ELISA for the *s*-triazines which demonstrates sensitivity, ruggedness and stability as well as resistance to matrix effects. Use of solid phase extraction (SPE) to facilitate the analysis of triazines has been reported (Wachob, 1984; Bardalaye & Wheeler, 1986; Sherma, 1986; Junk & Richard, 1988; Brooks, *et al.*, 1989), reducing time and cost of traditional chromatographic methods. Coupling an immunoassay with an efficient means of extraction has led to sensitive methods for the routine analysis of the herbicide molinate (Li *et al.*, 1989). By interfacing an ELISA with an SPE method, we have developed an efficient and reliable means of detection for atrazine and simazine in soil and water.

A series of antibody and antigen combinations were compared based upon available haptens (Goodrow *et al.*, 1990), and polyclonal (Harrison *et al.*, 1991) and monoclonal (Karu *et al.*, 1990) antibodies. Of the combinations examined in this study, a monoclonal antibody, AM7B2·1, performed best and was used for all subsequent studies to determine *s*-triazine concentrations in soil and water. Results from extracting and concentrating atrazine from water using solid phase extraction cartridges were verified using gas chromatography (GC), [^{14}C] radioassay using ring-labeled atrazine, and ELISA. Soil samples were extracted and analyzed by GC prior to solvent exchange and ELISA quantification. Detailed studies of the precision and error of the assay and evaluation of the intrinsic background noise levels were also performed. We have designed and characterized an ELISA suitable for routine analysis of atrazine and simazine.

MATERIALS AND METHOD

Reagents

All solvents were of residue grade from JT Baker (Phillipsburg, NJ) or Fisher (Pittsburgh, PA); salts were purchased from Fisher and of certified ACS grade. SPE cartridges (C_{18}, 3 ml volume, 500 mg packing) were obtained from Analytichem International, Inc. (Harbor City, CA). A Sybron/Barnstead Nanopure II water purification system set at 16·7 MΩ cm provided water for all spiking experiments and aqueous solutions. For studies involving the examination of [^{14}C] ring-labeled triazine, an LKB model 1217 Rackbeta liquid scintillation counter was employed. The [^{14}C] atrazine ($7{\cdot}25 \times 10^5$ Bq nmol^{-1}) and the analytical standards atrazine (98·8%) and simazine (99·7%) were a gift from the Ciba-Geigy Corporation (Greensbourgh, NC). Microtiter plates were obtained from both Nunc (#4-42404) and Dynatech Laboratories, Inc (001–012–9200). The *p*-nitrophenyl phosphate enzyme substrate tablets were obtained from Sigma Chemical Co. (St Louis, MO), the goat anti-mouse antibody and the goat anti-rabbit antibody from Boehringer Mannheim (Indianapolis, IN). Absorbance measurements were made with a UVmax kinetic microplate reader

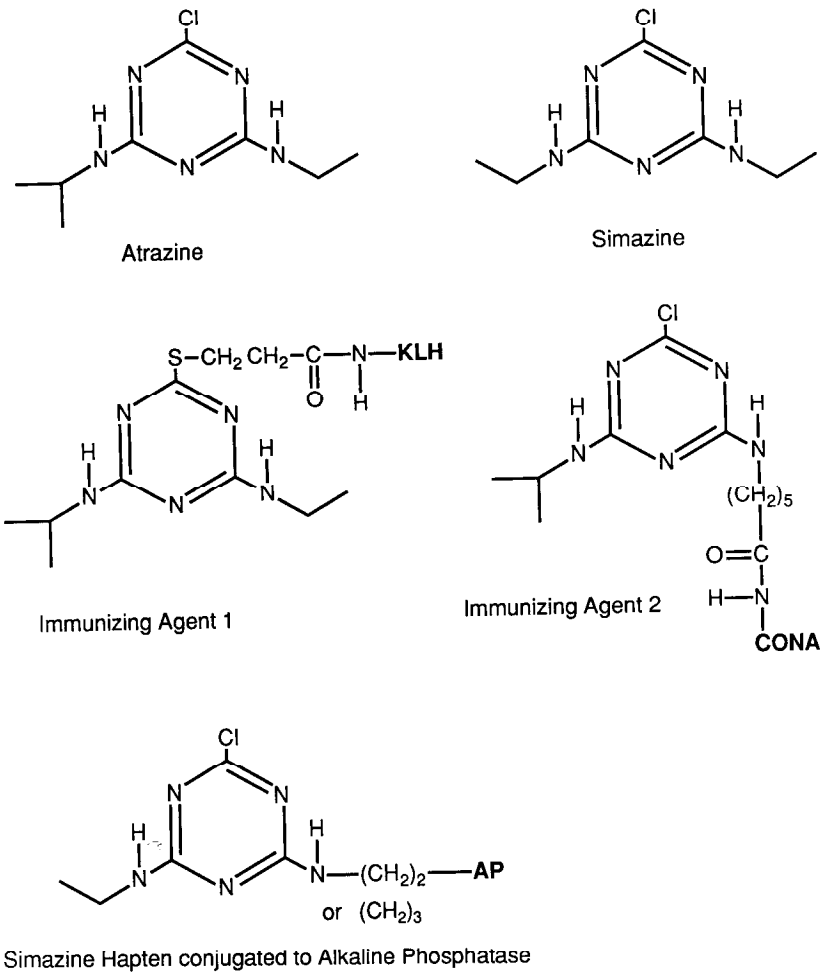

FIG. 1. Structures of atrazine and simazine, the immunizing agents for the two monoclonal antibodies and polyclonal #194 (Immunizing Agent 1, (3-{[4-[ethylamino]-6-[(1-methylethyl)amino]-1,3,5-triazin-2-yl]thio}propanoic acid conjugated to KLH)), and for the polyclonal antibody #357 (Immunizing Agent 2, (6-{[4-chloro-6-[(1-methylethyl)amino]-1,3,5-triazin-2-yl]amino}-hexanoic acid conjugated to CONA)). Also pictured are the simazine haptens conjugated to the alkaline phosphatase (AP) enzyme used in a competitive inhibition assay.

from Molecular Devices in a dual wavelength mode, at 405 nm minus 650 nm. The software package Softmax (v. 2·01, Molecular Devices) was used for fitting the 12-point sigmoidal standard curve based on the four parameter logistic method of Rodbard (1981), and for interpolation of unknown sample values.

Extraction of Water

Solid Phase Extraction. C_{18} SPE cartridges were used for extraction and concentration of atrazine from well water samples. After pre-washing the column with two column volumes of hexane, followed by two column volumes (approximately 5 ml) each of ethyl acetate, methanol and purified water in that order, 100 ml water samples were

pulled by vacuum through the cartridges at an average rate of 8–12 ml min^{-1}. After rinsing with 2–5 ml purified water, the cartridges were dried by pulling air through them with a vacuum for 5–10 min. Elution of the sample with 2 ml of ethyl acetate under gentle positive pressure yielded an eluate suitable for GC analysis. The efficiency of the SPE method was verified by extracting water containing various levels of radiolabeled atrazine and counting the eluate. For samples to be evaluated with the ELISA method, the extracts were placed under a gentle stream of nitrogen at room temperature and evaporated to near dryness before reconsitution with 1 ml of a phosphate-buffered saline solution (10 g l^{-1} NaCl, 0·25 g l^{-1} KH_2PO_4, 0·25 g l^{-1} KCl, 1·44 g l^{-1} Na_2HPO_4) with 0·05% Tween 20, v/v, and 0·02%, w/v, NaN_3 (PBSTA).

Liquid–liquid extraction. Liquid-liquid extractions of well water samples were done by the California Department of Food and Agriculture, Chemistry Laboratory Services, Sacramento, CA. Samples were collected from wells in the Sacramento Valley, with a sequential series of four 1 l bottles taken from each well head and numbered consecutively. One of the samples was extracted three times with methylene chloride, the combined extract dried over Na_2SO_4, evaporated to near dryness and reconstituted in methanol.

Soil Extraction

Panoche soil samples of a clay–loam type were taken from a controlled simazine spill experiment conducted at the University of California, Davis, CA in a study designed to examine the movement of pesticides in soil. All samples were frozen until analysis, then thawed, air dried and ground to a fine powder. Ethyl acetate (10 ml) was added to 10 g of soil and the suspension shaken for 15 min on a rotary shaker. The soil mixture was next sonicated for 5 min and dried on a column of anhydrous Na_2SO_4. The collected extract was then directly analyzed by GC.

Within one day after the soil was extracted and analyzed by GC, an aliquot of the extract was dried under a gentle stream of nitrogen and then reconstituted in a volume of PBSTA ranging from 1 to 50 ml depending on analyte concentration. For samples containing extremely high concentrations of simazine, indicated by a white precipitate, more PBSTA and a small amount of methanol (less than 5% in sample volume) was used to solubilize the triazine, with further dilutions as necessary. The corresponding ELISA standard curves were run with the identical quantity of methanol used in the final sample dilution.

Based on these initial studies, a protocol for routine analysis was developed which involved drying the original ethyl acetate extract under a stream of nitrogen prior to addition of 50 μl of methanol to an unknown soil sample. Subsequently, the PBSTA buffer was added, usually 1 ml, and serial dilutions were made to place the response of the soil sample on scale. The aqueous samples were analyzed by the ELISA method within two days.

Gas Chromatographic Analysis

Spiked water. For laboratory recovery studies, a Hewlett-Packard model 5890 gas chromatograph equipped with a nitrogen–phosphorus detector and a fused silica J&W DB5 column, 30 m × 0·53 mm (i.d.) with a film thickness of 1·5 μm was used. Column temperature was programmed from 180°C to 220°C at 10°C min^{-1} with a final hold of 5 min. The detector temperature was maintained at 240°C and that of the injector at 240°C. Gas flows were air, 90·0 ml min^{-1}, hydrogen, 3·5 ml min^{-1}, helium carrier gas, 12·0 ml min^{-1} and helium make-up gas, 18·0 ml min^{-1}.

Well water. The final methanolic extract was analyzed using a Varian model 3700 TSD with a 10 m×0·53 mm HP-17 column (Hewlett-Packard). The temperature was held at 170°C for 5 min, then increased to 220°C at a rate of 10°C min^{-1}. The hydrogen pressure was maintained at 23 psig and the helium make-up gas was set to obtain a column flow rate of 10 ml min^{-1}. The injector temperature was 210°C and that of the detector 250°C.

Soil. A Varian model 3700 with a nitrogen-phosphorus detector was used for analysis of ethyl acetate extracts of soil. The column temperature was 220°C, that of the injector 240°C and that of the detector 270°C. Gas flows were maintained at 50 ml min^{-1} for hydrogen, 40 ml min^{-1} air and 30 ml min^{-1} for nitrogen through SPB-35 column 30 m long with an inner diameter of 0·75 mm.

ELISA of Soil and Water

Single-coated antibody format. Briefly, a microtitre plate (Nunc #4-42404) was coated with one antibody, either a 1:2000 (v:v) dilution of goat anti-mouse antibody for mouse monoclonal antibodies or 1:1000 dilution of goat anti-rabbit for polyclonal antibodies in a coating buffer consisting of 1·59 g l^{-1} Na_2CO_3, 2·93 g l^{-1} $NaHCO_3$ and 0·02 g l^{-1} NaN_3. In a separate plate (Dynatech #001–012–9200), a competitive inhibition between a mouse anti-triazine monoclonal antibody or a rabbit polyclonal serum (100 μl), a standard or sample extract (40 μl), and a simazine hapten conjugated to an alkaline phosphatase enzyme (100 μl) was allowed to react (structures shown in Figure 1). For monoclonal antibodies, the N-$(CH_2)_2$-alkaline phosphatase form was used, while N-$(CH_2)_3$-alkaline phosphatase form was used for polyclonals. After 1 h, a 50 μl portion of the contents of the competitive inhibition well was added to a well on a plate coated with the goat anti-mouse (monoclonals) or goat anti-rabbit antibody (polyclonals). The mixture was allowed to react for another hour, washed, and a 1 mg ml^{-1} *p*-nitrophenyl phosphate solution was added. After a 1 h period, the plates were read at 405 nm minus 650 nm. The development of a yellow color was invesely related to the amount of triazine present.

Double coated antibody format. Two antibodies are coated sequentially. First, a 1:2000 (v:v) dilution of the goat anti-mouse antibody was prepared, in an identical manner as the single coated antibody format. The plate was coated again with a 1:3200 (v:v) dilution of the monoclonal AM7B2.1. The competitive inhibition consisted of equal volumes of the sample and the simazine hapten conjugated to alkaline phosphatase (Figure 1) diluted to 1:3200 and incubated at room temperature for 30 min. This particular format yielded a 10-fold increase in assay sensitivity over that using only the goat anti-mouse antibody in coating. However, the single-coated antibody format proved more resistant to matrix and modifiers in the sample, gave adequate sensitivity for the current samples and was used for all ELISA analysis.

RESULTS AND DISCUSSION

Antibody Comparison

Two monoclonal and two polyclonal antibodies were evaluated for sensitivity, reproducibility, and general performance in a series of soil extracts of known simazine concentration. The two monoclonals (AM7B2·1 and AM5D1·1) and one polyclonal (#194) were raised against the same hapten (shown as immunizing agent 1 in Figure 1), 3-{{4-[ethylamino]-6-[(1-methylethyl)amino]-1,3,5-triazin-2-yl}thio}propanoic acid conjugated to the protein KLH (keyhole limpet hemocyanin). The second polyclonal,

#357, was raised against 6-{[4-chloro-6-[(1-methylethyl)amino]-1,3,5-triazin-2-yl]amino}hexanoic acid conjugated to CONA (Conalbumin). The structure of this compound is shown in Figure 1 and referred to as immunizing agent 2. Details of polyclonal antibody development and assay optimization can be found in Harrison *et al.* (1991); monoclonal antibody information in Karu *et al.* (1990).

TABLE 1. Comparison of the parameters describing the standard curve for two monoclonal and two polyclonal antibodies for atrazine (top), and simazine (bottom). For comparisons, only the single coated antibody format was use and all parameters were either identical or optimized for the antibody used

Standard curve parameter	Monoclonal		Polyclonal	
	AM7B2·1	Am5D1·1	357	194
Atrazine standard curve				
A Maximum absorbance	1·06	0·475	0·407	0·364
B Slope	1·23	1·34	0·93	1·13
C IC_{50}	13·2	25·5	19·9	32·6
D Minimum absorbance	−0·005	0·002	0·007	0·002
Simazine standard curve				
A Maximum absorbance	0·505	0·512	0·372	0·446
B Slope	1·46	1·37	1·47	1·37
C IC_{50}	19·7	42·0	167	165
D Minimum absorbance	0·002	−0·006	0·069	0·111

Table 1 compares the four parameters defining the standard curve generated individually by each of the four antibodies for both atrazine and simazine using the single-coated antibody format. The parameter *A* depicts the maximum absorbance, or signal, of the assay. Background level is indicated by *D*, i.e. the minimum signal. The *B* and *C* values are more critical as they define the sensitivity and range of the assay, much as a standard linear GC curve. *B* is a number which describes the slope of the linear portion of the curve. The parameter *C* is the midpoint of the curve. It is used as an indicator of relative assay sensitivity and is also referred to as the IC_{50}. We generally consider absorbance above 0·4, a slope (*B* value) of 1 or greater, and the lowest IC_{50} and background (*D* value) possible ideal. The equation describing the four-parameter standard curve is

$$y = (A\text{-}D)/[\{1 + (x/C)B\} + D]$$

where y is the absorbance and x is the concentration. Details concerning this equation can be found in Rodbard (1981).

The lowest IC_{50} value, in $\mu g\ l^{-1}$, is generated by using the monoclonal AM7B2·1 assay for both atrazine and simazine. Both the monoclonal assays had equivalent values for the remaining parameters. The polyclonal antibody assays both showed greater recognition of atrazine, illustrated by the lower IC_{50} number, than simazine. In addition, the monoclonal assays showed no significant change in response with addition of up to 10% methanol in the soil extract samples, while both polyclonal assays demonstrated a significant drop in analyte recognition. Both of the monoclonal assays showed excellent correlation with GC soil results for soil samples. Monoclonal AM7B2·1 had superior sensitivity (IC_{50}) and a consistently lower background. Both of the polyclonal assays demonstrated a higher noise level (larger *D*), slow color development (lower *A*) and less sensitivity (higher IC_{50}). The three antibodies raised against the same hapten had roughly parallel slopes when regression data with actual results from simazine soil samples were calculated. These slopes indicate similar recognition of the analyte in the matrix. However, the intercept of the assay using polyclonal #194

was much larger, indicating a matrix effect, while the monoclonal assays were almost superimposable. For the superior performance in recognition of atrazine and in resisting matrix effects, the monoclonal AM7B2·1 was used for all soil and water work.

Format Comparison

In a series of experiments involving addition of various modifiers to the sample, the single- and double-coated antibody formats were compared. Both ELISA procedures proved rugged when evaluated with water and soil samples, as seen in Figure 2. Addition of methanol, acentonitrile, or acetone to 10%, tetrahydrofuran to 2·5%, or trifluoroacetic acid to 0·05% of the total assay volume did not adversely affect assay performance. A consideration in the practical working assay is the volume of sample in relation to the total assay volume. Although both single- and double-coated antibody formats demonstrated the same modifier tolerance in relation to the total assay volume, the single-coated antibody form can withstand a larger percentage of modifier in the sample itself. As noted in the Materials and Methods section, the single-coated antibody method used only 40 μl of sample per 240 μl total assay volume (16·7%), where the double-coated format requires an equal volume of haptenated enzyme to sample (50%). The double-coated format is somewhat more sensitive using the antibody, but the single-coated antibody format yielded a lower minimum absorbance value (background), and was used for other aspects of this study.

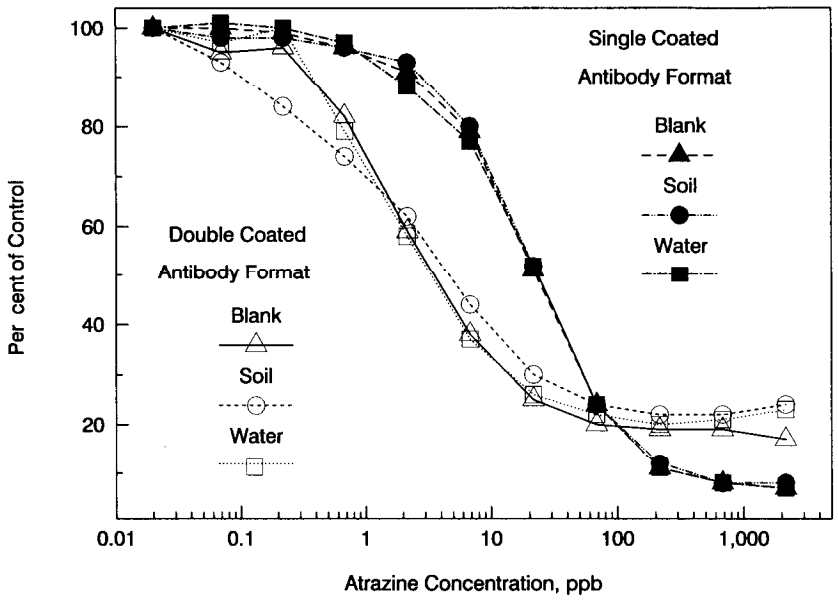

FIG. 2. A comparison of the effect of soil extract (circles) and well water (squares) on the control (triangle) single- (closed) and double- (open) coated antibody formats. Data are the average of four replicates with confidence intervals similar to those shown in Figure 6. Methanol, acetonitrile and acetone to 10%, tetrahydrofuran to 2·5% or trifluoroacetic acid to 0·05% of the total assay volume did not adversely affect assay performance and yielded curves similar to those shown here.

Addition of salts increased the sensitivity of both formats. Figure 3 shows the change in IC_{50} value with increasing amounts of salt in the sample for the double-coated antibody format. The single-coated format demonstrated the same trend (data

not shown). Although this is not a significant increase in sensitivity, the trend to lower IC_{50} numbers is evident. There is no apparent advantage in using one salt over another, however the time needed to obtain an adequate signal for the assay significantly increased in every case as the salt concentration and sensitivity increased. In analysis of samples with potentially large amounts of salt, this bias must be addressed by analyte extraction, desalting techniques, or similar measures.

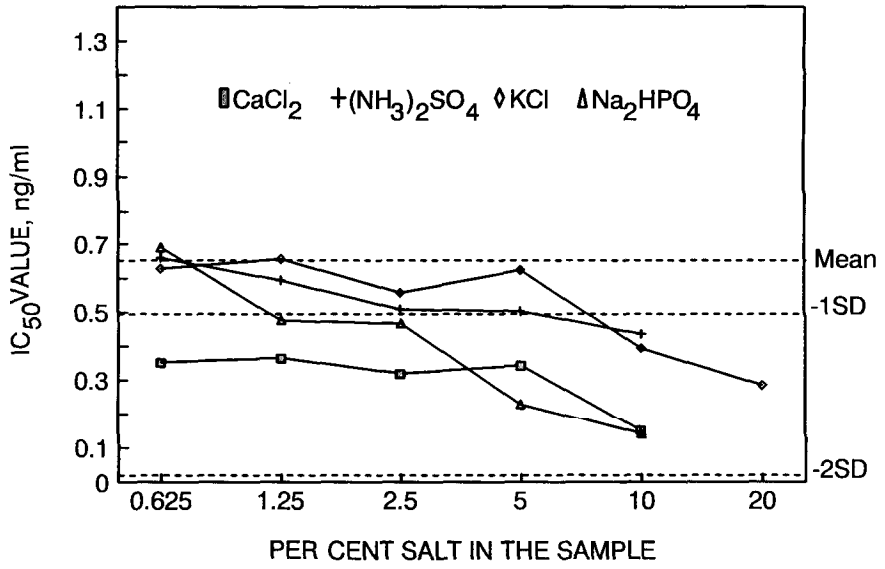

FIG. 3. The effect of various salts at specified sample concentrations on the IC_{50} value of the double-coated antibody format. Single-coated format demonstrated the same trend, increased sensitivity with increased amount of salt.

Recoveries

Recoveries of atrazine from the C_{18} SPE columns were examined by GC (NP detector) and by radioassay using ring-labeled [^{14}C] atrazine (Figure 4). For spiked samples quantified by GC, sensitivity to the standard was excellent, but background contaminants from the extraction of pure, spiked water dampened the detector response at concentrations below 10 μg l^{-1}. Injection of the standard directly after blanks or spikes of less than 10 μg l^{-1} reduced peak area of the standard by 40%–60%. As the limit of quantification for the GC was 10 μg l^{-1}, [^{14}C] ring-labeled atrazine was used to assess recoveries down to 10 ng l^{-1}. The efficiency of the C_{18} cartridge for extraction of atrazine from water was essentially 100% (Figure 4).

Recoveries of atrazine from water using the SPE scheme with quantification by the ELISA were determined over the concentration range 10 ng l^{-1} to 10 mg l^{-1}. Good recoveries were obtained from 0·1 to 1000 ng l^{-1}, but levels below 0·1 ng l^{-1} for 75 ml extracts were not quantitatively distinguishable from background noise. At the 10 mg l^{-1} level the recovery dropped significantly (Figure 4). This may be due to the solubility limit of atrazine in this buffer system. Although somewhat lower than the reported 33 ppm solubility of atrazine in pure water (Herbicide Handbook, 1983), the effect of the buffer salts could decrease this value. This salting-out phenomenon is supported by a visible precipitation above the 1 mg l^{-1} level and by decreasing reproducibility and recovery.

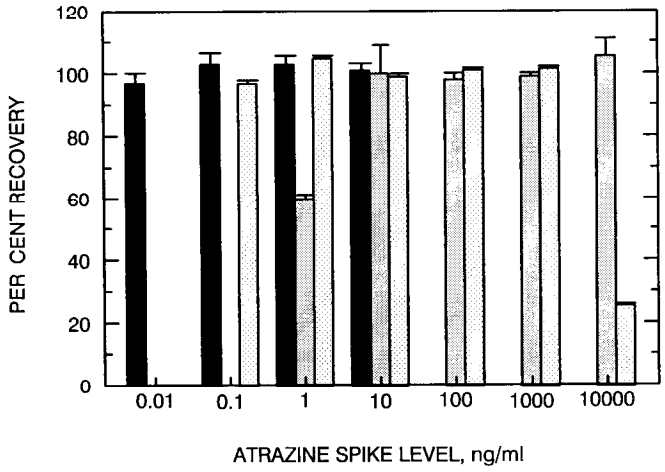

FIG. 4. Recovery of atrazine from spiked water using solid phase extraction cartridge for GC (dark shading), [^{14}C] atrazine (solid bar), and ELISA (light shading). Bars represent means of three extractions of 75 ml samples, each measured in duplicate with the error bar indicating one standard deviation.

Background Characterization of the ELISA

Examination of a series of control blanks of differing volumes carried through the standard procedure to analysis demonstrate minimal intrinsic noise. For a 100 ml water sample, the average background level was 50·1 ng $l^{-1} \pm 0{\cdot}9\%$, indicating a limit of quantification at 100 ng l^{-1} for this volume. Use of larger volumes of sample lowered the limit of detection, but from the practical standpoint of routine analysis of samples, a 100 ml volume was sufficient in balancing time and sensitivity needs. The source of background noise in reasonably constant when samples are evaluated prior to calculating in the concentrating factor (Table 2). After adjusting for the initial volume of water used in the SPE extraction, the noise level for samples greater than 1 l are in the 2 ng l^{-1} range. Examining the entire analytical procedure by using a blank control at each step, the major source of the background signal was attributed to glassware, solvents and solution, and not the water analyzed. The 0 l blank involved prewashing and then eluting with ethyl acetate, the control blank consisted of 2 ml of ethyl acetate. Both of these sample sets were subsequently evaporated, reconstituted and analyzed. The nitrogen blank was begun at the evaporation stage (a vial placed under the gas for the same length of time as the extracted samples), and the PBSTA blank was composed of the buffer in the sample vials. Table 2 details the background detected from these control blanks.

Results of Well Water Analysis

Seventy-five well water samples previously analyzed by California Department of Food and Agriculture using GC were analysed subsequently by ELISA in a blind fashion. The agreement of these two methods is illustrated by finding only one false negative and no false positives by the ELISA when compared with GC for the entire set of water samples. As the sampling technique used was sequential rather than split samples, direct comparison of the GC and ELISA results is not possible. The recovery data shown in Figure 4 indicate the ELISA and GC techniques to correlate very well when water subsamples were taken from the same spiked water sample and analyzed within one day.

TABLE 2. Background levels for different volumes of water (run in triplicate), and for control blanks begun at different points in the extraction and analytical procedure. Details can be found in the text

	Atrazine equivalents detected			
Sample	Non-adjusted ($\mu g\ l^{-1}$)	Adjusted for original volume ($ng\ l^{-1}$)	Non-adjusted values SD	Non-adjusted values CV (%)
0 l	2·73		0·65	23·9
0·1 l	5·01	50·1	0·90	18·0
0·5 l	5·15	10·3	0·81	15·7
1·0 l	1·86	1·86	0·68	36·7
2·0 l	4·07	2·04	0·17	4·10
Control	1·87		0·56	29·9
Nitrogen	1·58		0·15	9·50
PBSTA	0·69		0·62	90·0

Soil Results

The agreement between the two different methods each performed in a different laboratory for the soil extracts is very good, as demonstrated by the correlation shown in Figure 5. Prior to the controlled simazine spill, 12 blank soil samples were taken from the site for evaluation of background levels and matrix effects. Analysis indicated a minimal matrix effect, with the baseline signal increased above the buffer blank an average of 7·3% with a 3·3% CV.

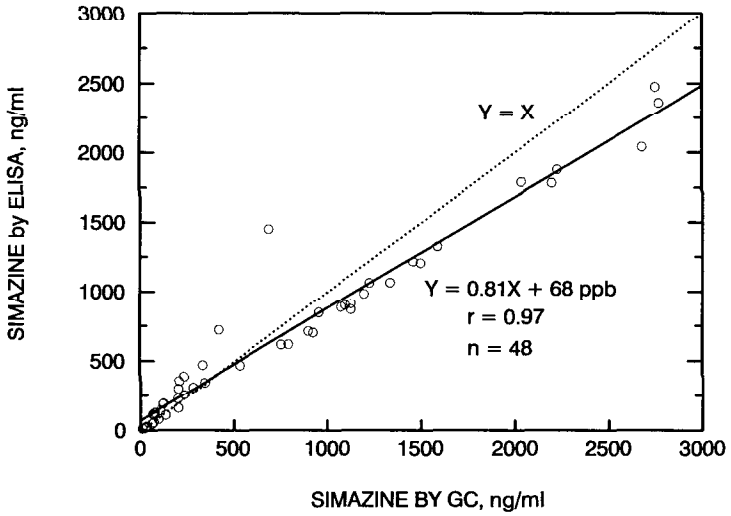

FIG. 5. A comparison of the results of 48 soil samples analyzed by GC and the monoclonal *s*-triazine ELISA using the single coated antibody format by a linear regression.

CONCLUSIONS

The ELISA method described in this paper is suitable for routine use in analyzing field samples. Use of an ELISA procedure to screen or analyze for environmental contamination on a routine basis by the widely used triazine herbicides has advantages in

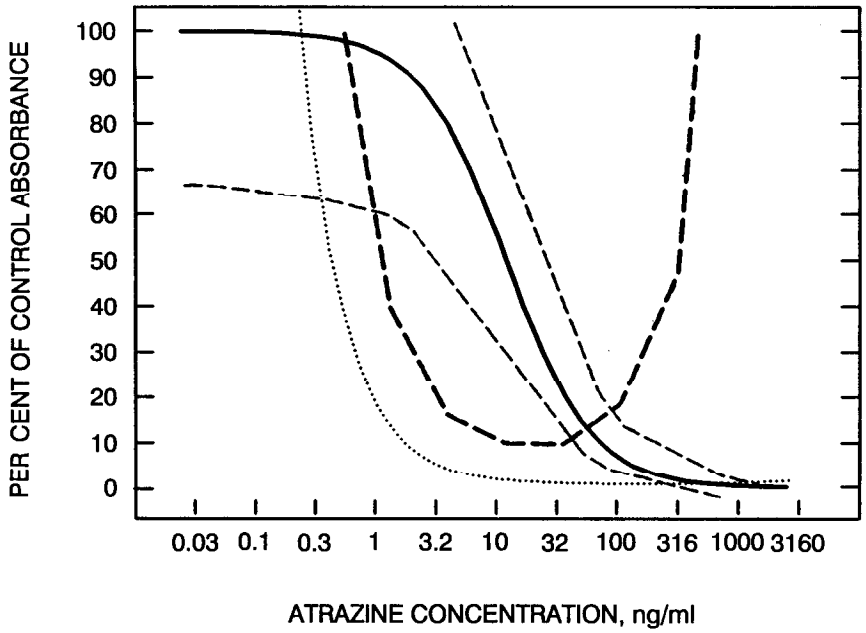

FIG. 6. Stability of an ELISA for atrazine run over a period of six months. These curves represent a summation of over 3500 datum points. The standard curve was generated using 12 concentrations and is represented by the heavy solid line. The two light dashed lines represent the standard deviation of the average curve generated by a four parameter logit fit standard curve of the data using commercial software. The relative error of these data expressed as a per cent (coefficient of variation/average analyte concentration) is represented by the dotted line. The classic precision profile is indicated by the heavy dashed line.

speed, sensitivity and cost. It took one day for one trained analyst to complete the 75 well water samples, from extraction with the SPE C_{18} cartridge to generation of final triazine concentration values. The extra time and effort spent in using SPE cartridges with an enzyme immunoassay reduces the detection limit. The ethyl acetate extract from the SPE cartridges can be directly analyzed by GC for confirmation and the extraction of the water by a cartridge eliminates potential interference such as pH extremes, high ionic strengths, etc. The use of SPE cartridges also increases confidence in the data by eliminating potentially immunoreactive metabolites.

The triazines are less volatile than most of the commonly analyzed pesticides. In applying ELISAs to other pesticides, especially those with a high vapor pressure of those difficult to resolubilize, use of a glycol trapping solution in the bottom of the test tube prior to nitrogen evaporation (Li *et al.*, 1989) would be prudent. Alternate treatments of the SPE cartridges, such as centrifugation to elute compounds rather than pressure, minimizes the loss of analyte.

Figure 6 demonstrates the accuracy and precision of the ELISA standard curve over a six-month period and 73 individual assays, of 12 concentrations each of which was run in quadruplicate for a total of over 3500 datum points. The IC_{50} of this method was $12{\cdot}3 \pm 3{\cdot}3$ $\mu g\ l^{-1}$ for the entire time period. The slope of the standard curve, reflected in its B value, also remained quite steady at $1{\cdot}22 \pm 0{\cdot}1$. A and D, indicating absorbance values for the maximum and minimum standard curve values, were $0{\cdot}699 \pm 0{\cdot}23$ and $0{\cdot}001 \pm 0{\cdot}008$ respectively. As these values depended on the enzymatic kinetics of the alkaline phosphatase, higher errors due to time and temperature differences were expected. Within the range of quantification (the linear portion of the curve) the relative error is lowest—less than 5%. In addition, this particular assay has

been sent to other laboratories, which have obtained remarkably similar four-parameter values. Comparison between analysts for identical samples have yielded almost identical results. Also, this antibody hapten system has been reformatted, yielding a large increase in sensitivity.

Soil, a traditionally difficult matrix by classical methods, proved the ELISA to be rugged and resistant to matrix effects. Although the levels of simazine detected were largely above the usual residue analysis levels, it is the close agreement of the GC and ELISA results which is of major concern in this paper. The ELISA method shows good agreement with conventional GC techniques for evaluation of simazine and atrazine in soil and water.

Having more than one antibody available for use in an ELISA system is an advantage beyond screening and choosing the best bioreagent for the analytical task at hand. Noting the differences in IC_{50} values between AM7B2·1 and both of the polyclonal antibodies, the large difference in recognition between atrazine and simazine could be used for further evidence of analyte presence as well as relative proportions. Subsequent experiments utilizing this large difference in specificity in analysis of individual *s*-triazine in environmental samples need to be conducted as one of several possible approaches to confirmation.

ACKNOWLEDGEMENTS

We gratefully acknowledge the gift of the monoclonal antibodies from Alex Karu, University of California, Berkeley, CA and the expertise of Frank Spurlock and Scott Teeter of the Land, Air and Water Resources Department at UC Davis for the soil extracts. The water samples were provided by Adolf Braun and Joan Fleck of the CDFA, Environmental Monitoring and Pest Management Branch, and GC analysis was done by Karen Hefner, CDFA, Chemistry Laboratory Services.

This work was supported in part by NIEHS Superfund Grant ES04699–03, California Department of Food and Agriculture, the US Environmental Protection Agency Cooperative agreement CR-814709-01-0, and the UC Systemwide Toxics Program. BDH was a Burroughs Wellcome Toxicology Scholar and ADL is a TE Archer fellow in the Dept. of Environmental Toxicology and fellow of the Ecotoxicology Program at UCD.

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