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ELISA of Simazine in Soil: Applications for a Field Leaching Study

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ELISAs have been used successfully in the determination of atrazine residues in soil (Bushway et al. 1988, Goh et al. 1990, Goh et al. 1991, Lucas et al. 1991). However, the ELISAs are non-specific; they are cross-reactive with most compounds in the s-triazine herbicide family (atrazine, simazine, propazine, cyanazine, prometon, prometryne, ametryne, terbutryne and their metabolites). Hence we have suggested that the current ELISAs are most useful for quantification of a known s-triazine in samples from fields where a specific s-triazine has been applied (Goh et al. 1991).

The California Department of Pesticide Regulation is mandated to protect groundwater from pesticide contamination (State of California, 1985). One way of controlling pesticide leaching to groundwater is by regulating the amount and timing of irrigation after pesticide application on the soil surface. Pignatello (1989) has reported that longer interaction time between pesticide residues and soil components leads to the formation of tightly bound residues which could be less mobile in soil water. To test this hypothesis, a field study was designed to determine whether a delay in irrigation after application of simazine to soil influences pesticide leaching. This generated soil samples containing the triazine herbicide, simazine, suitable for quantification using ELISA.

This study was conducted to evaluate the usefulness of ELISA in comparison with gas chromatography (GC) for the analysis of simazine residues in soil samples.

MATERIALS AND METHODS

Simazine was broadcast onto the soil surface at a rate of 4.4 kg/ha and immediately incorporated into the soil with a 1.25-cm sprinkler irrigation event. A basin-flood irrigation event containing 7.62 cm of water was applied either at 1, 7, or 14 days after pesticide application

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and incorporation into the soil. Each of the delay treatments was replicated three times. Soil from each replicate was sampled on two dates. The first sampling date, labelled 86B, occurred 24 hr after the first irrigation. Basin-floodings were continued 1 day/week for 6 weeks. A second sampling, labelled 86C, occurred 24 h after the sixth and final irrigation. Soil samples for the first sampling date (86B) were taken in 0.15-m increments down to the 1.5-m depth. Soil samples for the second sampling date (86C) were taken in 0.15-m increments down to the 3.0-m depth. Samples were collected with a hand auger. Soil from the auger was placed into a large plastic bag and tumbled in order to mix the contents. A sample of soil was taken from the bag and stored in a glass jar at -4°C until splitting for GC and ELISA analyses.

The double antibody, haptenated enzyme, competitive inhibition, University of California, triazine ELISA was previously described (Lucas et al. 1991). Briefly, Nunc® microtiter plates (Intermountain Scientific; Bountiful, UT) were coated with 100 uL/well of affinity-purified, goat anti-mouse antibody (Boehringer-Mannheim; Indianapolis, IN) in a coating buffer (Na_2CO_3, $NaHCO_3$; pH 9.6), sealed with a plate sealer (Dynatech; Chantilly, VA) and incubated overnight at 4°C. The "ELISA" plates were emptied and washed 5X with PBSTA (0.2M phosphate buffer with 0.8% NaCl, 0.05% Tween 20, 0.02% NaN_3 pH 7.5), "blocked" by adding 100 uL/well of 0.5% bovine serum albumin (Sigma) in buffer, incubated for 1 h, and washed 5X with PBSTA; then 50 uL/well of mixture from the competitive inhibition plate was added. The competitive inhibition plate (Dynatech) contained a mixture of the mouse anti-triazine monoclonal antibody (AM7B2.1), a standard or soil extract, and an enzyme tracer (simazine hapten conjugated to alkaline phosphatase) incubated together for 1 hr. The ELISA plate was sealed, incubated for 1 hr, drained, and washed 5X with PBSTA; then 100 uL/well of substrate solution was added (5 mg p-nitrophenyl phosphate tablet in 5 mL of 10% diethanolamine buffer). The ELISA plates were formatted with a 5-point standard curve, blanks, positive (35 ppb simazine in soil) and negative controls (0), and samples in triplicate (Fig. 1). The plates were read after 30-60 min using a Vmax® microtiter-plate reader at 450-650nM (Molecular Devices; Palo Alto, CA) and data were analyzed using Softmax® 2.01 software (Molecular Devices).

General procedure for extracting simazine from soil for ELISA by prewetting soil with methanol was discussed previously (Goh et al. 1991). Briefly, 25 g of soil was extracted with two aliquots of 10+15 mL of methanol+water each. Soil was shaken on a platform shaker for 10 min for each aliquot. The composited extract was filtered through a 0.2-um nylon cartridge filter (Gelman Sciences) using the Baker® 10 (J.T. Baker) vacuum system.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	BLANK	0	1.88	7.5	30	120	480	1.88	7.5	30	120	480	Standards (ppb)
B	"	"	"	"	"	"	"	"	"	"	"	"	
C	- Control	+ Control	1	2	3	4	5	6	7	8	9	10	Controls + Samples
D	"	"	"	"	"	SAM	PLES	"	"	"	"	"	
E	"	"	"	"	"	"	"	"	"	"	"	"	
F	11	12	13	14	15	16	17	18	19	20	21	22	Samples
G	"	"	"	"	"	SAM	PLES	"	"	"	"	"	
H	"	"	"	"	"	"	"	"	"	"	"	"	

Figure 1. ELISA plate layout with wells designated for 5-point standard curve (4 replicates), blanks, positive and negative controls, and 22 samples or sample dilutions (in triplicate).

In study 86B, 90 soil samples were split for ELISA and GC analyses. The GC analysis was done by Agricultural and Priority Pollutants Laboratories, Inc. (Fresno, CA). Soil samples (100 g each) for GC analysis were extracted using three sequential volumes of 40, 40, and 30 mL of ethyl acetate and concentrating the pooled extract to 10 mL. Samples were analyzed using a Hewlett-Packard model 5890 GC equipped with a nitrogen-phosphorous detector. The column was a 30 m x 0.25 mm id SPB-5 fused-silica, capillary column, temperature programed from 67 to 290°C at 20°C/min. The method detection limit (MDL) was 2 ppb, and average recovery was determined to be 90%.

In study 86C, 90 soil samples were split for ELISA and GC analyses. The GC analysis was done by the California Department of Food and Agriculture's Chemistry Lab Services using a method developed for general extraction of simazine, atrazine, prometon, bromacil, and diuron (Tran 1991). Soil (25.0 g) was extracted using 50 mL of hexane:acetone (60:40) and 30 g of sodium sulfate by shaking for 2 h at 210 rpm on a G-10 Gyrotory shaker (New Brunswick Scientific; Edison, NJ). The extract was decanted through 20-g sodium sulfate on a #1 filter paper. Another 20 mL of hexane:acetone were added to the soil and extracted for 1-2 min. The second extract was decanted as before and combined with the first extract. The filter funnel containing the sodium sulfate was then washed with 10 mL of hexane:acetone, and the final extract was brought to 75 mL with hexane:acetone. An aliquot (15 mL) of the extract was concentrated to 1 mL using a nitrogen evaporator (Myers Organomation Assoc., Inc.; South Berlin, MA) at 35°C. The sample was adjusted to 2 mL with hexane, and 2 g of sodium sulfate were added. The 2-mL sample was cleaned-up through a silica gel Sep-Pak® (Waters) which had been preconditioned with 4 mL of hexane. The hexane was discarded and the Sep-Pak was centrifuged at 1100 rpm for 30 s to remove remaining hexane. The analyte was eluted from the Sep-Pak with 10 mL of methanol, and the eluant was concentrated to 3

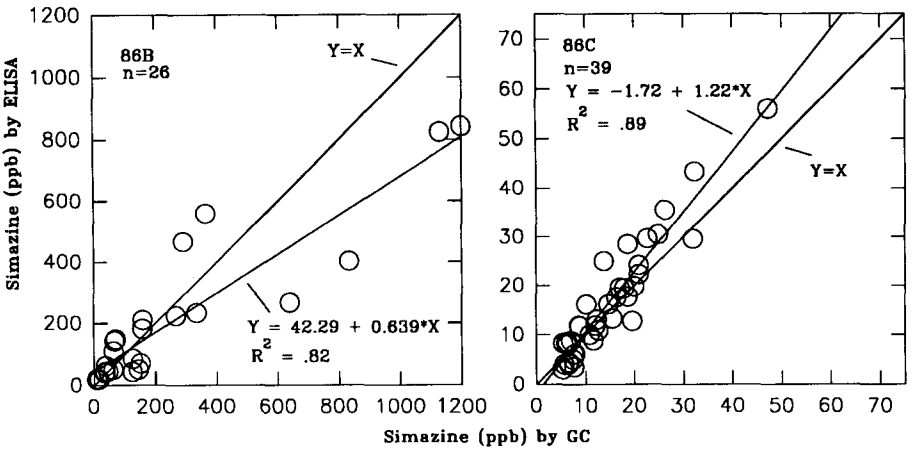

Figure 2. Linear regressions of simazine concentrations in soil measured by ELISA vs GC for sampling periods 86B (GC by APPL lab) and 86C (GC by CDFA lab), based on untransformed data. (Note the difference in scales for concentration).

mL using a nitrogen evaporator. The sample was analyzed using a Varian 6000 GC with a thermionic selective detector (Hewlett-Packard) equipped with a HP-carbowax 20M column, 30 m x 0.53 mm x 1.33-um film, and helium as a carrier gas at 20 mL/min. The inlet temperature was 210°C and detector was 250°C. The temperature was programmed at 15°C/min from 130 to 190°C, and at 25°C/min to 220°C with 1 min hold time. The MDL was 5 ppb, and average recovery was determined to be 90%.

ELISA and GC data were compared using linear regression. Non-detect data for both analyses were excluded from linear regression. Data were examined for normality using the Anderson-Darling test (Linnet 1988) and for violations of the assumptions of ordinary least squares regression. Data were transformed, when appropriate, using the Box-Cox family of power transformations (Draper and Smith 1981). Means and standard errors of measured concentrations for ELISA and GC within day and soil depth combinations were compared using two-sample t-tests (Steele and Torrie 1980). All statistical analyses were performed using the SAS system (SAS Institute, Inc., 1987).

RESULTS AND DISCUSSION

Simazine concentrations determined by ELISA and GC for sampling periods 86B and 86C were highly correlated (Fig. 2). Because the data in 86B were found to violate the assumption of homogeneity of

variance, log transformation was performed. The regression coefficient was significantly less than 1 (slope=0.78, t=-3.31, df=24, $p<0.01$) and the intercept was significantly greater than 0 (intercept=1.01, t=2.98, df=24, $p<0.01$). These results indicate that below 100-ppb values generated in the ELISA method tended to be higher than GC values but above 100-ppb ELISA values were lower than GC values. This significant bias was not corrected by removing samples that required dilution (>100 ppb) to be read by ELISA. This is in contrast to a previous study for a commercial ELISA kit for atrazine analysis where removal of samples >100 ppb corrected the bias by giving a slope which was not significantly different than 1 of the ELISA on GC regression line (Goh et al. 1990).

For 86C, the slope of the regression line was significantly greater than 1 (slope=1.22, t=3.14, df=37, $p<0.01$), but the intercept was not significantly different from 0 (intercept=-1.72, t=-1.43, df=37, $p>0.10$) (Fig. 2). The ELISA tended to give higher measured concentrations than GC at the low levels (below 60 ppb) in this experiment. This is in agreement with the results obtained for measurements below 100 ppb in 86B even though the GC analyses were done in different laboratories using distinct extraction methods (extraction efficiencies were similar for all 3 methods at an average of 90%) and different GC instrument and conditions. Results of 86B and 86C, however, are in contrast to our earlier study (Goh et al. 1991) on a commercial ELISA test-tube kit for atrazine analysis in which ELISA gave lower measured values than GC at lower concentrations (<52 ppb) and higher values at higher concentrations. These discrepancies and biases are not easily explained, and further investigation is needed. We should note, however, that the previous kit used polyclonal antibody and horseradish peroxidase tracer, while the ELISA in this study used monoclonal antibody and alkaline phosphatase tracer.

The general decrease in simazine concentration with increase in soil depth as well as through time (86B to 86C) reflect the combined effects of leaching and degradation under different irrigation schedules (Fig. 3). The standard errors (± 1) of the ELISA and GC concentration means derived from the 3 replicates overlapped for each sampling day by depth combination (Fig. 3). The mean concentrations of the two methods for each day by depth combination were not significantly different (t-test, $p<0.01$). The mean comparisons indicate that when replications were averaged results between ELISA and GC were similar within days and depth. This demonstrates that the triazine ELISA is a useful analytical tool for the analysis of simazine in soil samples from field experiments.

Furthermore, ELISA offers a cost savings of about 67% ($71 per sample) over GC. Commercial GC analysis cost was $108 per sample. Cost for ELISA per sample is $37: $21 material cost ($450 per plate for 22 samples), $10 analyst cost (20 person-hour at $20/h to run

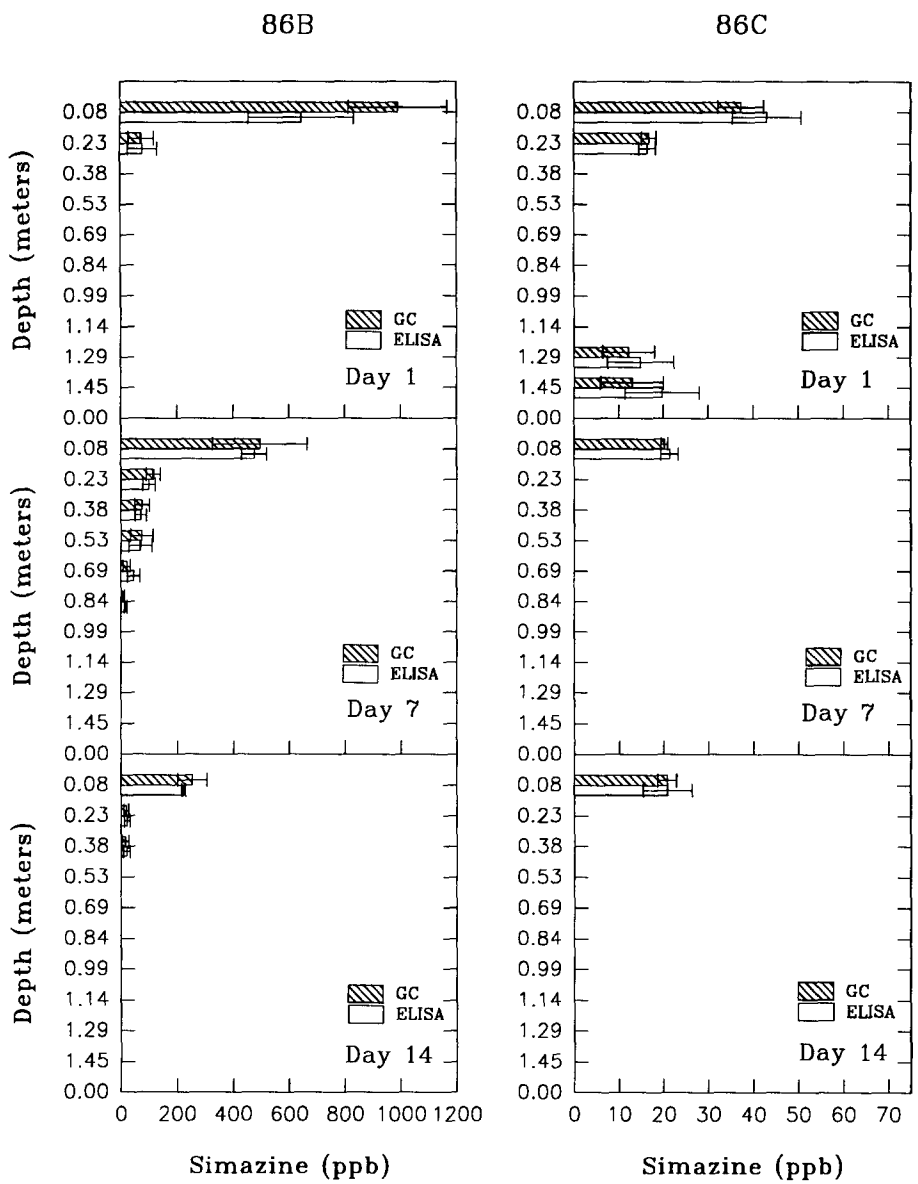

Figure 3. Concentrations (mean ± SE) of simazine measured by GC vs ELISA in field soil sampled at every 0.15 m down from field plots where irrigations were applied at 1, 7, or 14 days after simazine spray (86B) and from same plots after the sixth flood irrigation (86C).

40 samples), and $6 overhead cost (20%). By using ELISA for analysis of this simazine field study, we could save as much as $12,780 for a total of 180 samples.

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