Immunoassay Analysis and Gas Chromatography Confirmation of Atrazine Residues in Water Samples from a Field Study Conducted in the State of Wisconsin

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In 1990, Ciba Crop Protection entered into a cooperative study with the State of Wisconsin Department of Agriculture, Trade and Consumer Protection and Department of Natural Resources. The objective of the study was to assess the level of atrazine in Wisconsin groundwater by immunoassay techniques and confirm detections of regulatory significance by gas chromatography. The study was conducted in two phases. In phase I, 2177 rural well water samples were screened at an immunoassay response level equivalent to 0.10 ppb or greater of atrazine. In phase II, all wells that produced an immunoassay response equivalent to 0.35 ppb or greater were resampled and assayed by immunoassay and gas chromatography with mass selective (GC/MS) and thermionic specific detection (nitrogen mode) (GC/TSD). Immunoassay results were shown to correlate highly with results obtained by GC/MS ($r = 0.95$) and GC/TSD ($r = 0.95$). Immunoassay analyses conducted in Ciba and Wisconsin laboratories also correlated well ($r = 0.93$).

Keywords: *Atrazine; immunoassay; gas chromatography; confirmation*

INTRODUCTION

Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] has received the most attention of any agrichemical by immunochemists due to its widespread use and long product history. Introduced to the marketplace in 1959, approximately 75 million pounds are applied each year throughout the U.S. cornbelt for control of a variety of annual broadleaf and grass weeds (U.S. EPA, 1994).

Investigators's efforts have been primarily directed to various aspects of method development including the synthesis of haptens (Huber, 1985; Dunbar, 1985; Schlaeppi et al., 1989; Wittman and Hock, 1989; Dunbar et al., 1990; Goodrow et al., 1990; Harrison et al., 1991), production of polyclonal or monoclonal antibodies (Schlaeppi et al., 1989; Dunbar et al., 1990; Goodrow et al., 1990; Giersch and Hock, 1990; Giersch, 1993), and optimization of assay systems (Huber, 1985; Wittman and Hock, 1989, 1990; Giersch and Hock, 1990; Giersch, 1993; Rubio et al., 1991; Lucas et al., 1991). Unfortunately, application of these assays has largely been limited to the analysis of laboratory-fortified samples. The results of such work may have little bearing on the use of immunochemical technology for the analysis of real-world samples. To date, only a few workers have analyzed samples with incurred atrazine residues. Fleeker and Cook (1991) and Thurman et al. (1990) analyzed groundwater from the midwestern United States. Both groups found their immunoassay results correlated well with those obtained by gas chromatography with, respectively, thermionic (GC/TSD) and mass selective detection (GC/MS). Goolsby et al. (1991) screened midwestern streams over a 10-state region. These workers concluded immunoassay was an excellent qualitative screening tool prior to chromatographic analysis, although the correlation between immunoassay and GC/MS results was nonlinear. Bushway et al. (1992) surveyed Maine groundwater by immunoassay and HPLC. This group also found their immunochemical data to compare favorably with chromatographic results.

All of these investigators used antibody-coated tubes to conduct their analyses. This format has limited utility when an analyst is confronted with hundreds or thousands of samples. Antibody-coated microtiter plates may offer a more practical approach. An opportunity to evaluate methodology based on microtiter plates came in 1990 when Ciba Crop Protection entered into an unprecedented agreement with the State of Wisconsin. The objective of that agreement was to conduct a statewide survey to assess the level of atrazine in groundwater by immunoassay methods. Detections equal to or greater than a predetermined level of significance would be confirmed by additional sampling and reanalysis by gas chromatography and immunoassay techniques.

MATERIALS AND METHODS

Apparatus. *Immunoassay.* Envirogard triazine plate kits were obtained from Immunosystems, Inc., Scarborough, ME. Eight-channel digital pipets were purchased from Labsystems, Marlboro, MA. Polystyrene microtiter plates were obtained from ICN Biomedicals, Costa Mesa, CA. Immunoassay plates

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were incubated on a Model 4625 Labline microtiter plate shaker, purchased from Labline Instruments, Melrose Park, IL. Ciba Crop Protection personnel measured absorbance readings on an ICN MCC 340/MK II microplate reader controlled by an IBM PC/XT computer. Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) personnel collected absorbance readings using an ICN Titertek Multiskan PLUS microplate reader. This instrument was controlled by a Zenith Model ZWL-183-93 laptop computer.

Gas Chromatography (GC/MS). Analyses were performed on a Hewlett-Packard 5890 gas chromatograph interfaced with a Hewlett-Packard 5970 mass selective detector. Sample components were separated on a J&W DB-5 capillary column, 0.25 mm in diameter by 30 m in length, coated with a 0.25 μm film. Flow rate of the helium carrier gas was maintained at 1.0 mL/min. The instrument was operated in the selected ion monitoring mode (SIM) with temperature programming. The initial oven temperature was 110 °C, raised to 170 °C at 70 °C/min. A second gradient increased the temperature to 205 °C at 8 °C/min. The final ramp raised the oven temperature to 260 °C at 5 °C/min. This temperature was maintained for 2 min.

GC/TSD. Atrazine residues in phase II samples were quantitated on a Varian 3700 gas chromatograph fitted with a glass column, 4 mm in diameter by 1.22 m in length, filled with 10% SP-2401 packing. Residues were detected by a thermionic specific detector in the nitrogen mode (TSD). These analyses were carried out under isothermal conditions at 190 °C.

Residues quantified by this system were confirmed by reanalysis of the sample extract on a similar Varian 3700 instrument also outfitted with a TSD. This chromatograph contained a 4 mm diameter by 1.83 m long glass column packed with 1% Reoplex. Column temperature was maintained at 200 °C. Gas flows for both Varian instruments were set at 30.8 mL/min nitrogen, 3.6 mL/min hydrogen, and 175 mL/min air.

Sampling. Wells sampled in this study were selected on a first-come, first-serve basis in response to newspaper and radio announcements across Wisconsin. Farmers, homeowners, and other interested parties contacted DATCP personnel by a toll-free telephone number created for this study. Participants received a mailer containing a 4-oz amber Boston round bottle with a polyseal cap, a protective styrofoam liner, and sampling instructions. After collecting a sample, study participants mailed the capped bottle back to DATCP along with a check for $16.00.

Upon receipt, DATCP personnel labeled each sample bottle with a Wisconsin unique well number (WUWN). Two-20 mL aliquots were decanted into separate scintillation vials each labeled with the original WUWN. One vial from each sample was shipped under refrigerated conditions to Ciba Crop Protection and maintained at 4 °C until brought to room temperature prior to analysis. The other replicate was refrigerated until analysis at DATCP facilities.

Both laboratories conducted the enzyme immunoassay (EIA) analysis of all samples in duplicate. If any replicate in either laboratory produced an immunoassay response equivalent to or greater than 0.35 ppb of atrazine, Wisconsin Department of Natural Resources personnel resampled the well and collected a 4-L sample which was immediately refrigerated. Two liters of each followup sample was shipped to Ciba Crop Protection for a GC/MS confirmatory analysis and a repeat immunochemical assay. The remaining 2 L was sent to the DATCP Bureau of Laboratory Services (BLS) for analysis by GC/TSD.

Wisconsin personnel selected 0.35 ppb as the value that would trigger a followup sampling and analysis. This level corresponds to the Wisconsin preventive action limit (PAL), the concentration of atrazine that would initiate regulatory measures to prevent the amount of compound from exceeding the Wisconsin enforcement standard (ES) for atrazine in water, 3.5 ppb.

Enzyme Immunoassay. The immunoassay methodology is described in Ciba Crop Protection analytical method AG-568. Briefly, the analyst recorded the location of standard and sample solutions in the antibody-coated microtiter plate on a plate layout sheet which mimicked the 8 × 12 well array of the plate. All samples and standards were analyzed in duplicate. According to the layout sheet, approximately 120 μL of a sample or standard solution was pipetted to the appropriate wells of an uncoated polystyrene microtiter plate (the reservoir plate). When the reservoir plate was completed, the analyst transferred an 80-μL aliquot from each well to the corresponding well of the antibody-coated assay plate using a multichannel pipet. Fifty microliters of the enzyme conjugate solution was also added to each well. The plate was then incubated with shaking (approximately 90 oscillations/min) at room temperature for 1 h. The shaker was covered with a cardboard box to protect the plate from drafts.

After the initial incubation, the contents of the wells were removed with an eight-channel pipet or washing manifold. The wells were washed three times with distilled, deionized water. The plate was then inverted and tapped on a dry paper towel to remove the final traces of liquid in the wells.

One hundred microliters of a freshly prepared substrate solution (two parts of "substrate solution" combined with one part of "chromogen solution") was added to each well. The plate was returned to the shaker and incubated as previously described for about 30−45 min at the discretion of the analyst. When the analyst judged the intensity of color development was sufficient, the plate was removed from the shaker and 50 μL of 2 M sulfuric acid added to each well to terminate substrate conversion. The absorbance of the acidified solution in each well was measured at 450 nm.

For purposes of monitoring the absorbance of each well, the antibody-coated plate was treated as a collection of 96 spectrophotometric cuvettes. Thus, the absorbance of each well within the microtiter plate was measured prior to transfer of sample or standard solutions and after termination of color production. Net absorbances for each well were calculated by subtracting the absorbances of the empty plate from those of the plate containing the colored reaction products. The net absorbances were used to calculate the standard curve and to determine the amount of atrazine equivalents (AE) in each sample.

GC/MS. A half liter of sample was combined in a 1-L flask with 35 mL of a 2% disodium hydrogen phosphate/water solution, two to five drops of 10% sodium hydroxide, and 160 g of sodium chloride dissolved. The solution was transferred to a 1-L separatory funnel and extracted by partitioning with ethyl acetate (2 × 75 mL) and methylene chloride (2 × 75 mL). The organic extracts were combined and reduced to dryness on a rotary evaporator. The residues were dissolved in 10 mL of methanol and transferred to a concentration tube. The methanol was evaporated to dryness under a stream of nitrogen and the residues were reconstituted in 0.5 mL of acetone. Two microliters was injected for each GC/MS analysis.

Two ions were monitored for detection of atrazine (MW = 215). One ion (m/z = 200) was used for quantification and a second (m/z = 215) for confirmation. The confirmation (qualifier) ion/quantification ion peak area ratio was determined on a daily basis from the responses of atrazine standards. The identity of a sample peak was considered to be confirmed if the ratio was within ±20% of the value established from the analytical standards.

The GC/MS technique was a multiresidue method analyzing for a variety of test substances. Most phase II samples were also analyzed for simazine (CEET) and the chloro-degradates of atrazine: 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (CIAT), 2-chloro-4-(ethylamino)-6-amino-*s*-triazine (CEAT), and 2-chloro-4,6-diamino-*s*-triazine (CAAT) (Cook, 1987).

GC/TSD. Samples were analyzed according to BLS method 178. One liter of sample was extracted with methylene chloride (3 × 100 mL) in a 2-L separatory funnel. The organic fractions were dried over anhydrous sodium sulfate and collected in a 500-mL round-bottom flask. The sodium sulfate was rinsed with additional methylene chloride (2 × 20 mL), which was added to the round-bottom flask. The contents of the flask were reduced to near dryness on a rotary evaporator.

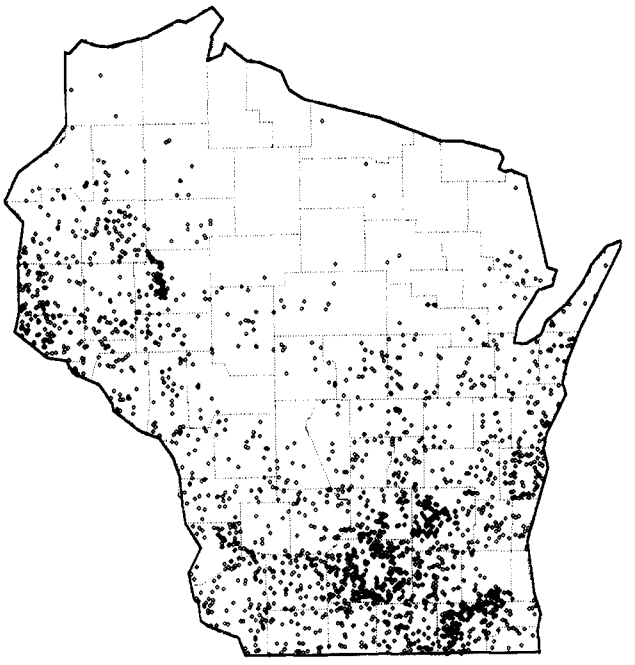

Figure 1. Locations of drinking water wells sampled in phase I of the Wisconsin well water study. Note the high concentration of wells from southern Wisconsin included in the study.

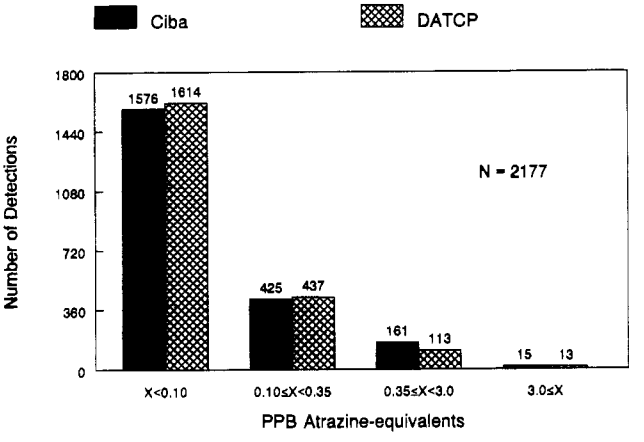

Figure 2. Immunoassay results from phase I generated at Ciba and DATCP laboratories.

After the final traces of methylene chloride were removed under a gentle stream of air, sample residues were brought up in 5 mL of hexane. Two milliliters of the hexane extract was injected for each analysis.

Statistical Evaluation. Since immunoassay analyses yielded two results per sample and a single result was obtained by GC/MS or GC/TSD, the results between methods were compared on the basis of the following rules. If both immunoassay values were less than 0.10 ppb of AE, a zero was assigned to represent those results. If only one replicate was found to contain equal to or greater than 0.10 ppb of AE, its value was used. If the results of both replicates were equal to or greater than 0.10 ppb of AE, the mean of those values was used. These rules were also applied to the comparison of immunoassay results obtained in Ciba and DATCP laboratories.

RESULTS

Nearly 2200 well water samples from across Wisconsin were screened in phase I (Figure 1). Analytical results are summarized in Figure 2. It is evident from these data that both laboratories obtained similar results. The percentage of detections in each of the ranges is nearly identical. Of the 2177 samples analyzed, approximately 27% gave a response equal to or greater than 0.10 ppb of AE in either Ciba Crop Protection or DATCP testing. Of those samples that gave a positive response, about 70% of the Ciba results and 78% of the DATCP results were less than the 0.35 ppb PAL.

Table 1. Results of Phase I Quality Control Samples Analyzed by Ciba Crop Protection Personnel

fortification level, ppb	N	mean ppb of AE found ± SD
0	5	all <0.10
0.30	9	0.38 ± 0.15
0.50	24	0.59 ± 0.12
0.60	10	0.93 ± 0.36
0.70	27	0.77 ± 0.11
1.0[a]	2	8.7 ± 0.52

[a] Samples were fortified at 10 ppb instead of 1.0 ppb.

Samples collected from 768 wells were found to contain at least 0.10 ppb of AE by either laboratory. Within this group, 396 wells were positive by both Ciba Crop Protection and DATCP. Approximately 48% of all samples containing detectable residue (372 samples) were positive at only one facility (205 samples at Ciba Crop Protection and 167 samples at DATCP). Nearly all of these samples (370 or 99%) produced screening values less than the PAL. The two samples that triggered follow-up analyses were both determined to be false positives at 0.35 ppb by gas chromatography. When immunoassay results between laboratories were compared by regression analysis, their relationship was calculated to be DATCP = 0.86 Ciba − 0.04 (r = 0.93, N = 768). Limiting the sample set to those samples determined to contain residues by both laboratories confirms this relationship (DATCP = 0.88 Ciba − 0.08, r = 0.94, N = 396). Slope values less than one are consistent with observations that sample results generated at the Ciba laboratory were often greater than those produced in Wisconsin. These differences suggest results obtained by AG-568 may be, in part, analyst-dependent.

Detections in 176 samples were found to meet or exceed 0.35 ppb of AE in Ciba laboratories compared to 126 at DATCP facilities (Figure 2). Of these samples, Ciba Crop Protection and DATCP personnel determined 15 and 13, respectively, to contain greater than 3.0 ppb of AE. This corresponds to approximately 0.7 or 0.6% of all samples containing atrazine in excess of 3.0 ppb, the federal maximum contaminant level for atrazine in drinking water.

As a quality control measure, DATCP chemists included among the phase I samples a series of vials containing Madison, WI, municipal tap water fortified with known amounts of atrazine. The identity of these samples was unknown to Ciba analysts. The results of these analyses indicate that no false negatives were observed at the PAL (Table 1). The immunoassay results did display a positive bias, however.

In phase II of the study, 201 follow-up samples were analyzed by immunoassay, GC/MS, and GC/TSD (Figures 3 and 4). Although immunoassay results correlated well with both types of chromatographic analyses (r = 0.95), the immunoassay data showed a strongly positive bias compared to chromatographic results, especially in the range of values equal to or greater than 0.35 ppb. The following relationships were determined by regression analysis: GC/MS = 0.44 EIA + 0.19 (r =

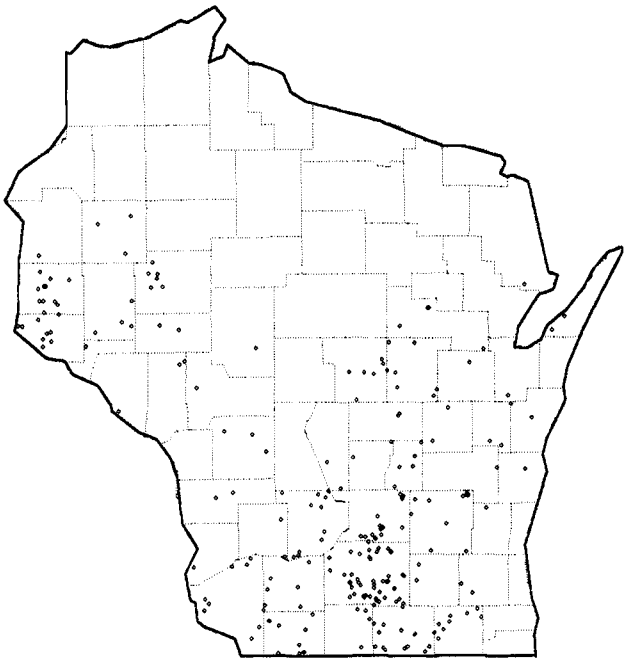

Figure 3. Locations of drinking water wells included in phase II of the well water study. Many of these wells are clustered in the southern and western areas of the state.

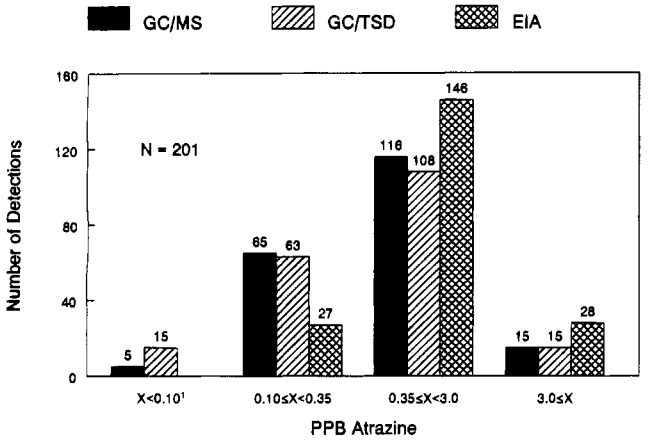

Figure 4. Phase II analytical results for all methods. The strongly positive bias of the immunoassay data is evident in samples determined to contain atrazine residue equal to or exceeding the PAL. [1]The detection limit for the GC/TSD method is 0.15 ppb.

0.96) and GC/TSD = 0.60 EIA − 0.08 (r = 0.95, N = 201 for both comparisons).

Gas chromatographic results between laboratories correlated well, but mathematical comparisons suffered from the effects of two extreme outlying points (greater than 10 ppb). With all 201 points included in the sample set, regression analysis found GC/TSD = 1.25 GC/MS − 0.23 (r = 0.92). Excluding the outliers yielded a relationship of GC/TSD = 0.91 GC/MS + 0.04 (r = 0.90, N = 199). The latter expression is more indicative of the higher values generally obtained by Ciba personnel. This bias is probably due to BLS analysts not correcting GC/TSD results for the mean percent recovered of the procedural recovery samples since it is likely that atrazine recoveries of less than 100% were obtained. Ciba Crop Protection results were corrected if procedural recoveries were less than 100%. Sample results were not corrected if the procedural recoveries were in excess of 100% since doing so would minimize the amount of residue found.

DISCUSSION

This study clearly demonstrated the efficacy of EIA as a cost-effective screening tool for estimating the concentration of atrazine in groundwater. Approximately 1600 samples were found by Ciba Crop Protection and DATCP personnel to contain less than 0.10 ppb of AE (Figure 2). An additional 430 samples yielded EIA results greater than 0.10 ppb of AE but less than 0.35 ppb of AE. In total, analysis of samples collected from 2030 or about 93% of all sites in the study yielded results less than 0.35 ppb of AE. If these samples were analyzed by GC/MS or GC/TSD, approximately $304 500.00 would have been expended, assuming $150.00 per analysis conducted by a laboratory operating under good laboratory practice (GLP) guidelines. Nearly 79% of these funds, or $240 000.00, would have been spent without even detecting atrazine. The actual cost of immunoassay screening of these samples totaled $32 480.00, about one-tenth of the estimated cost of chromatographic analyses. These funds were generated by the $16.00/sample charge DATCP assessed each study participant.

Screening large numbers of samples by EIA techniques also resulted in substantial savings of the "hidden costs" associated with pesticide residue analysis. Unlike GC analyses, immunoassays do not generate large volumes of waste organic solvent. The cost of purchase and subsequent disposal of these solvents is eliminated. Moreover, the time and expense required to train personnel to conduct GC analyses and maintain the instrument are far greater than that needed to train staff to conduct immunological assays. Maintenance of the microtiter plate reader is minimal, usually consisting of changing the light source on a semiannual basis.

Prior to initiating the study, Wisconsin personnel selected 0.35 ppb of AE as the immunoassay response level that would trigger a follow-up sampling and analysis. The PAL was ideally suited to AG-568 because 0.35 is near the middle of the standard curve, in the range where an analytical method should be most accurate. By predetermining a level of regulatory concern for initiating follow-up analyses, DATCP personnel were able to take advantage of the cost-effective aspects of an immunoassay screen without the expense of confirming all positive detections. The laboratory space, staff, and monetary resources of Ciba Crop Protection and the State of Wisconsin were thus appropriately directed to samples containing residues of regulatory concern.

The results of the present study compare favorably with those of a 1988 dairy well study in which DATCP personnel collected samples from 534 grade A dairy farm wells selected at random (LeMasters and Doyle, 1989). Analytical results from 7.5% of the samples in the current study equaled or exceeded the PAL compared to 7% in excess of the PAL in the dairy well study. In a similar fashion, a maximum of 0.7% of the samples from the well water study yielded results greater than the MCL and 0.6% of the dairy study samples produced data in excess of the ES.

The percentage of detects found in both studies differs markedly, however. The differences in the number of detects, 28% for the current study and 12% from the previous project, may be attributed to two factors. First, the detection limit of the immunoassay screen is lower (0.10 ppb) than that of the GC method (0.15 ppb) used in the dairy well study. It is reasonable to assume that as the limit of detection decreases, the number of

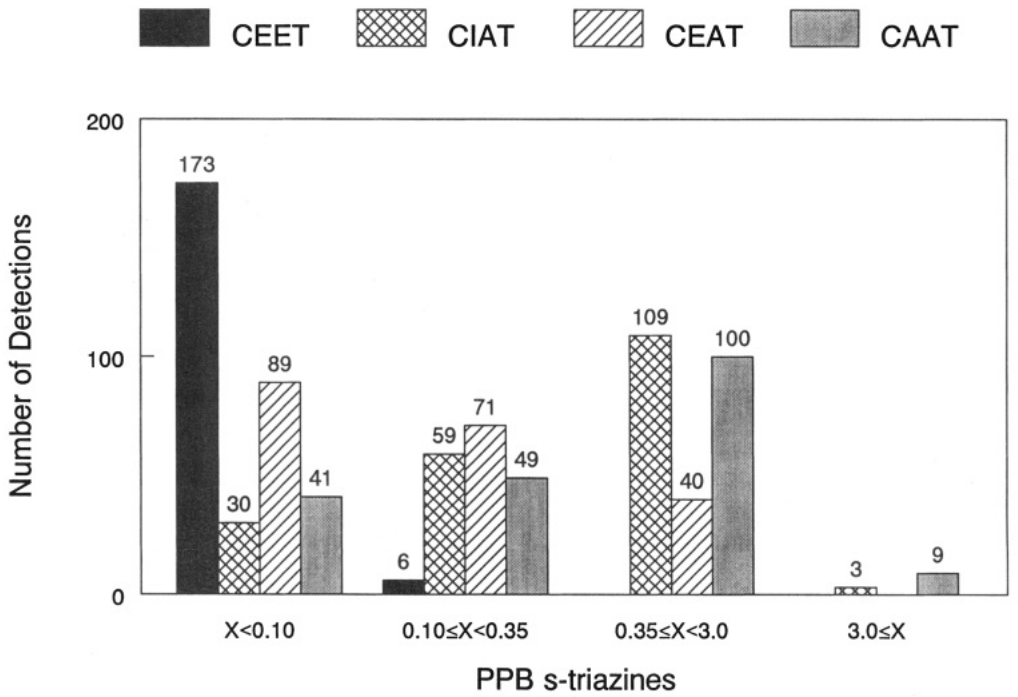

Figure 5. Results of phase II analyses for *s*-triazines other than atrazine. Not all samples from phase II were analyzed for all test substances.

detections will increase. In fact, at least one replicate of each of 243 samples, or 11.2% percent of all samples screened by EIA, had a value greater than or equal to 0.10 ppb but less than 0.15 ppb. Second, farms from which samples were collected in the previous survey were selected at random across the state while the wells in the present study were sampled on a first-come, first-serve basis. Many wells sampled in the present study are located in the south-central and southwest parts of Wisconsin, areas known for a high percentage of atrazine groundwater detections (Figure 1). As a result, a large number of samples (960 or 44% of all samples) originating from susceptible areas were included in the study.

To successfully apply immunoassay methodology to groundwater monitoring, some limitations of the technique must be recognized. Analysts should be aware that EIA methods have often demonstrated a positive bias compared to results obtained by chromatographic analysis of the same sample set. Fleeker and Cook (1991), for example, noted a tendency of an atrazine immunoassay to yield false positives but were unable to correlate these responses with sample pH or conductivity. Feng et al. (1990) observed that only 48% of samples determined to contain at least 1 ppb of alachlor by enzyme-linked immunosorbent assay could be verified by GC/MS. The tendency of immunoassays to produce positively biased data can be beneficial to a screening program, however, since the likelihood of generating false negative results is greatly reduced. The analyst should also recognize that while some samples whose screening values were inflated by the immunoassay measurement will unnecessarily undergo a confirmatory analysis, the percentage of such samples should be quite small and not jeopardize the overall cost-savings aspect of EIA.

Previous investigators have expressed concern that the cross-reactivity characteristics of immunoassays may have a bearing on assay results (Hock et al., 1992; Baker et al., 1993). Indeed, the cross-reactivity profile of antibodies can provide insight into potential inhibitors. In this study, the Immunosystems assay employed polyclonal antibodies that are cross-reactive to a variety of parent *s*-triazines but essentially nonreactive to triazine metabolites (Thurman et al., 1990). Among a number of *s*-triazines potentially reactive to the assay, phase II confirmatory analyses found simazine in only six samples (Figure 5). This is consistent with the limited application of simazine to apples, cherries, and Christmas trees (*Wisconsin 1985 Pesticide Use*, 1986). Detections of the chloro-degradate metabolites of atrazine, on the other hand, were found in 188 samples. The relationship of EIA results and the combined GC/MS data for parent atrazine, CIAT, and CEAT was determined by multiple regression analysis (CAAT does not react with the immunoassay and was excluded from the statistical evaluation). This analysis yielded a correlation coefficient of 0.95. The coefficient did not change when data on either or both of the degradates were removed from the analysis. As a result, the presence of atrazine metabolites in the samples did not have a bearing on immunoassay results. Consequently, it is unlikely the positive bias shown by the EIA results is due to the cross-reactive nature of the antibodies used.

Although it is tempting to assign the bias shown by immunoassay results to nonspecific matrix effects, insufficient data have been collected to support such a hypothesis. Chemical characterization of phase II samples was limited to determination of the nitrate concentration and pH. Nitrate measurements were conducted because Wisconsin groundwater is known to contain nitrate from fertilizer applications and human and animal waste. Groundwater containing nitrate in excess of 10 ppm should not be consumed by infants (*Fed. Regist.*, 1991). Sample pH was monitored because antibody function is pH-dependent (Kimball, 1983). Although 84 samples or about 42% of phase II samples contained nitrate in excess of 10 ppm, nitrate concentration did not correlate with immunoassay bias, with bias defined as the difference between EIA and GC/MS results. Regression analysis found their relationship to be $[NO_3] = 0.360$ EIA bias + 12.57, $r = 0.094$, $N = 201$.

The pH of phase II samples ranged from 6.1 to 8.5 with only 24 samples or 12% having a pH less than 7 (N = 198). It is not surprising, then, that pH was not significantly related to EIA bias (pH = 0.0004 EIA bias + 7.37, r = 0.021). Unfortunately, additional characterization of samples was beyond the scope of this work. The cause of signal depression thus remains unresolved. Until this question can be satisfactorily answered, users of the technology should be aware of this aspect of assay performance to aid in interpretation of analytical results.

Analysts must take care with regard to interpretation of EIA data. As noted above, immunoassay results in this study are defined as atrazine equivalents. The immunochemical technique applied here measures a colored aqueous solution at 450 nm, indirectly measuring the amount of atrazine in a sample. Since the analyte is not measured directly, the measurement cannot be used to identify the source of inhibition. Thus, immunoassay results cannot be regarded as more than "analyte equivalents" given that the responses of undefined samples are compared to the responses of defined standard solutions containing known amounts of analyte.

This study is the first large-scale groundwater survey ever to be conducted on a cooperative basis between a state agency and an agrochemical manufacturer. This effort spanned nearly two and a half years from its inception to completion. Over that time, some valuable lessons were learned.

First, state personnel are eager to learn about immunoassay and apply the technique. Wisconsin personnel conducted their analyses in the first phase of the project after only two to three weeks of training. They obtained results that correlated well with those generated by Ciba Crop Protection personnel who had much more experience with the technique.

Second, homeowners who participated in this study collected their own samples and forwarded them to DATCP. At the outset, it was recognized that phase I sampling would not be conducted in compliance with GLP guidelines. From a practical viewpoint, the consequences of nonprofessionals conducting sample collections were unknown. Several unusual occurrences can be observed in retrospect. One well, for instance, was shared by two homes and sampled by each of the homeowners and thus appears twice in the data base. The largest value in phase I (22 ppb of AE) resulted from a sample collected by a farmer using a hose just removed from a mixing tank in which a batch of atrazine for application was prepared. A follow-up sampling identified this situation and confirmed the level of atrazine in the well used for drinking water to be less than the detection limit for all methods used in phase II.

Finally, although immunoassay is a rapid screening method, the logistics associated with a project of this magnitude required that sample tracking, analysis processing, data collection, and report generation procedures be worked out in advance. There is simply no substitute for good planning and an efficient computer system tailored to the needs of the study.

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LITERATURE CITED

Baker, D. B.; Bushway, R. J.; Adams, S. A.; Macomber, C. Immunoassay screens for alachlor in rural wells: false positives and an alachlor soil metabolite. *Environ. Sci. Technol.* **1993**, *27*, 562–564.

Bushway, R. J.; Hurst, H. L.; Perkins, L. B.; Tian, L.; Guiberteau Cabanilllas, C.; Young, B. E. S.; Ferguson, B. F.; Jennings, H. S. Atrazine, alachlor, and carbofuran contamination of well water in central Maine. *Bull. Environ. Contam. Toxicol.* **1992**, *49*, 1–9.

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

Dunbar, B. D.; Niswender, G. D.; Hudson, J. M. U.S. Pat. 4,530,786, 1985.

Dunbar, B.; Riggle, B.; Niswender, G. Development of enzyme immunoassay for the detection of triazine herbicides. *J. Agric. Food Chem.* **1990**, *38*, 433–437.

Fed. Regist. **1991**, *56*, 3588.

Feng, P. C. C.; Wratten, S. J.; Horton, S. R.; Sharp, C. R.; Logusch, E. W. Development of an enzyme-linked immunosorbent assay for alachlor and its application to the analysis of environmental water samples. *J. Agric. Food Chem.* **1990**, *38*, 159–163.

Fleeker, J. R.; Cook, L. W. Reliability of commercial enzyme immunoassay in detection of atrazine in water. In *Immunoassays for trace analysis*; Vanderlaan, M., Stanker, L. H., Watkins, B. E., Roberts, D. W., Eds.; ACS Symposium Series; American Chemical Society: Washington, DC, 1991; pp 78–85.

Giersch, T. A new monoclonal antibody for the sensitive detection of atrazine with immunoassay in microtiter plate and dipstick format. *J. Agric. Food Chem.* **1993**, *41*, 1006–1011.

Giersch, T.; Hock, B. Production of monoclonal antibodies for the determination of s-triazines with enzyme immunoassays. *Food Agric. Immunol.* **1990**, *2*, 85–97.

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.

Goolsby, D. A.; Thurman, E. M.; Clark, M. L.; Pomes, M. L. Immunoassay as a screening tool for triazine herbicides in streams. In *Immunoassays for trace analysis*; Vanderlaan, M., Stanker, L. H., Watkins, B. E., Roberts, D. W., Eds.; ACS Symposium Series 451; American Chemical Society: Washington, DC, 1991; pp 86–99.

Harrison, R. O.; Goodrow, M. H.; Hammock, B. D. Competitive inhibition ELISA for the s-triazine herbicides: assay optimization and antibody characterization. *J. Agric. Food Chem.* **1991**, *39*, 122–128.

Hock, B.; Giersch, T.; Kramer, K. Immunoassays for environmental analysis. *Analusis* **1992**, *20*, 29–33.

Huber, S. J. Improved solid-phase enzyme immunoassay in the ppt range for atrazine in fresh water. *Chemosphere* **1985**, *14*, 1795–1803.

LeMasters, G.; Doyle, D. J. *Grade A Dairy Farm Well Water Quality Survey*; Wisconsin Department of Agriculture, Trade and Consumer Protection and Wisconsin Agricultural Statistics Service: Madison, WI, 1989.

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 immunoassays. *Food Agric. Immunol.* **1991**, 3, 155–167.

Rubio, F. M.; Itak, J. A.; Scutellaro, A. M.; Selisker, M. Y.; Herzog, D. A. Performance charactersitics of a novel magnetic particle based ELISA for the quantitative analysis of atrazine and related triazines in water samples. *Food Agric. Immunol.* **1991**, *3*, 113–125.

Schlaeppi, J. M.; Fory, W.; Ramsteiner, K. Hydroxyatrazine and atrazine determination in soil and water using specific monoclonal antibodies. *J. Agric. Food Chem.* **1989**, *37*, 1532–1538.

Thurman, E. M.; Meyer, M.; Pomes, M.; Perry, C. A.; Schwab, A. P. Enzyme-linked immunosorbent assay compared with

gas chromatography for the determination of triazine herbicides in water. *Anal. Chem.* **1990**, *62*, 2043–2048.

U.S. EPA. *Pesticides Industry Sales and Usage, 1992 and 1993 Market Estimates*; U.S. Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances; U.S. Government Printing Office: Washington, DC, 1994; EPA 733-K-94-001.

Wisconsin 1985 Pesticide Use; Wisconsin Agricultural Statistics Service: Madison, WI, 1986.

Wittmann, C.; Hock, B. Improved enzyme immunoassay for the analysis of s-triazines in water samples. *Food Agric. Immunol.* **1989**, *1*, 211–224.

Wittman, C.; Hock, B. Evaluation and performance characteristics of a novel ELISA for the quantitative analysis of atrazine in water, plants and soil. *Food Agric. Immunol.* **1990**, *2*, 65–74.

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