IMMUNOASSAY ANALYSIS FOR THE DETERMINATION OF PESTICIDES IN GROUNDWATER SAMPLES
THE TEXAS EXPERIENCE

Steve Musick, Alan Cherepon, Joseph Peters

Texas Natural Resource Conservation Commission
Office of Environmental Policy, Analysis and Assessment, Groundwater Assessment Section
MC-147, P.O. Box 13087
Austin, Texas 78711-3087

BIOGRAPHICAL SKETCH

Steve Musick is the Leader of the Groundwater Planning & Assessment Team of the Texas Natural Resource Conservation Commission (GPAT/TNRCC). His current responsibilities include program development and implementation for the state's groundwater protection program, the state's management plan for agricultural chemicals in groundwater. He has been with the agency since 1981. Mr. Musick has a BS in Geology from the University of Texas at Austin. Mr. Alan Cherepon has a BA in Geology from Rutgers University, New Jersey, and 20 years of hydrogeological and environmental experience. Mr. Cherepon is a Resource Conservation Specialist V for the GPAT/OEPAA of TNRCC, assisting Mr. Musick and Dr. Peters in related work. Dr. Joseph Peters is the Project Manager for the Pesticide Monitoring Program of the Texas State Management Plan for the Prevention of Pesticide Contamination of Groundwater, Groundwater Planning & Assessment Team, GPAT/OEPAA of the TNRCC. Dr. Peters has a PhD. in Agricultural Engineering from Texas A & M University.

ABSTRACT

The Texas Natural Resource Conservation Commission (TNRCC), with assistance from other state agencies and entities, prepared one of the first two state Generic Pesticide Management Plans (PMP) for the prevention of pesticide contamination of groundwater (June 1991). Texas has continued to develop and test its PMP to address the diversity of groundwater system characteristics in the state, and the budgetary dilemma of groundwater monitoring in a state the size of Texas.

The Texas PMP program has utilized immunoassay pesticide test kits for the following reasons; cost, speed, reliability and lower detection limits than most laboratory methods. Groundwater monitoring efforts related to the PMP program are in the testing and implementation stage in Texas. Magnetic-particle-based **e**nzyme-**l**inked **i**mmuno**s**orbant **a**ssay (ELISA) screening of samples enables the TNRCC to reduce the number of samples requiring laboratory analysis. It also provides fast results, which allows for more timely response. Due to lower costs, immunoassay also provides an efficient means of determining specific contaminated wells in a public water supply multiple well system. It also opens the door for cooperative monitoring with other agencies and groundwater management entities. To date, TNRCC has analyzed 72 samples from 62 wells at a lab cost of $15,220. Immunoassay analyses were conducted on 162 samples during the same time period and from the same projects, for a total cost estimate of $6000 (includes instrument, pipettes, reagents, etc.).

Program concerns focused on whether this method was reliable and acceptable to the State and USEPA for use in the PMP program. TNRCC staff needed to establish whether immunoassay was reliable and

comparable to laboratory methods, and to assess specifics for laboratory verification analysis. Sufficient samples have been analyzed and the results compared with those described in the existing literature, to gain confidence in the accuracy of the method and to verify that false negatives will not be a problem. The method chosen, involving sample splitting for both immunoassay and laboratory methods, is described, and a comparison of ELISA and laboratory results is presented. Results indicate satisfactory reliability of ELISA for screening purposes. Comparison of ELISA and laboratory results also shows a number of ELISA results as "false positive" detects, as reflected in the non detects (ND) results from analysis Method 525.2 for atrazine in drinking water. Probable explanations include metabolites and structurally-related triazines not analyzed for in Method 525.2 and 4670 (Reference 14). The triazine herbicides as atrazine in water immunoassay method was promulgated in 1999 by the Federal Government as USEPA Method 4670 (SW-846).

INTRODUCTION

Immunoassay analysis methods began as early as 1959, initially in the medical field, and were initially recommended for use in the environmental field in 1971 (Reference 1). Test kits have been commercially available since about 1990, but widespread environmental field application of immunoassay technology has occurred only since the mid-1990's. Presently, about 12 commercial manufacturers provide these kits. EPA only promulgated the immunoassay method for atrazine and triazine analysis in 1999, as EPA Method 4670.

Several papers during the mid-1990's have compared atrazine analysis results by immunoassay and laboratory GC/MS methods (References 2, 5, 8, 9, 10, 11 and 12), most notably in the three American Chemical Society Symposium Series publications. These papers agree that immunoassay and lab GC/MS results are comparable, but require verification analysis for a portion of the samples. Additionally, variations of up to 20% for immunoassay results are possible, depending on the analyst's training and skills. However, no single paper adequately addresses the more practical aspects of immunoassay for field screening activities, especially the "rules-of-thumb" for field personnel and project managers. The referenced papers primarily address the more tedious statistical side of comparing immunoassay and laboratory methods. This paper will address the more hands-on and practical aspects of immunoassay analysis for atrazine detection in groundwater, as related to a State Pesticide Management Plan (PMP) program.

Texas has aggressively sought to develop a PMP that is both effective and efficient. Groundwater resources are irreplaceable, and agriculture is a major part of the State's overall economy. Texas has more acres in farm lands than any state in the United States, and is second in agricultural production revenue (Reference 13). Atrazine is one of the most often used pesticides used for controlling weeds in corn, wheat, sorghum, and several other important crops. As analytical methods with increasingly lower detection levels are employed, an increasing number of detections are occurring in both surface water and groundwater in Texas.

The TNRCC is the State lead agency in the protection and regulation of the quality of groundwater resources in Texas. The TNRCC has the responsibility of chairing the Agricultural Chemicals Subcommittee and its parent Groundwater Protection Committee, the multi-agency bodies that direct and oversee protection of State groundwater resources from pesticide contamination. The Committee has responsibility advising the TNRCC for the development of the generic PMP and pesticide-specific PMPs. Two of the major components of the PMP are conducting groundwater monitoring in areas vulnerable to groundwater contamination by pesticides and the investigation of areas with detects of pesticides in the groundwater. The monitoring and investigative activities for a fully-implemented PMP will require considerable funding to cover the entire State.

One pilot site contamination response investigation in the Texas Panhandle expended nearly the entire annual sampling budget, leaving little funding for routine monitoring activities during the final quarter of the past fiscal year. The immunoassay method of pesticide analysis reduces the total number of costly lab analyses, which allows for increased sample coverage for a reliable and more efficient program. Immunoassay is generally useful for the detection of triazine herbicides, especially atrazine. Method descriptions are provided in several references, most notably Reference#5. The ELISA immunoassay method for atrazine is used by the TNRCC, and has proven to be useful for monitoring vulnerable areas and contamination response. The small sample volume required for immunoassay samples has helped facilitate (because of the small amount of additional ice chest space needed) recent cooperative monitoring efforts between TNRCC, other state agencies, and groundwater management districts. This cooperation may enable groundwater monitoring efforts to cover the entire state for a fraction of the cost of more traditional methods. Specifics of the Texas program and the results are presented below.

METHODOLOGY

The 1999 Draft Texas Generic Pesticide Management Plan (PMP) for Prevention of Pesticide Contamination of Groundwater specifies what groundwater protection programs are in place, detailing the potential scenarios requiring investigation. Included are two sampling scenarios; to indicate the source, source type, extent and magnitude of a pesticide impact, and to monitor areas vulnerable to pesticide contamination of groundwater, as well as high-use areas. A major aspect of the program is the Quality Assurance Project Plan (QAPP), which includes Standard Operating Procedures (SOP). The QAPP is the multi-part document that specifies groundwater sampling protocol and guidelines, acceptable analysis methods, and program standards.

Several commercially available immunoassay analysis products exist. The TNRCC uses the SDI/Ohmicron kits and instrument for magnetic-particle-based **e**nzyme-**l**inked **i**mmuno**s**orbant **a**ssay (ELISA) method of analysis of atrazine and metolachlor in water samples. The TNRCC immunoassay system includes the following:

- Ohmicron RPA-I RaPID Photometric analyzer (spectrophotometer)
- SDI RaPID Assay kits (reagents and test tubes) for atrazine and metolachlor
- Magnetic test tube rack and base (60 tube rack)
- Eppendorf repeating pipettes and disposable tips (1 is a repeating, the other a single dose)
- Vortex sample mixer and digital timer (quartz/battery)

IA method has resulted in occasional false positives, but no false negatives. In some cases, there may be little to no parent compound present, but high to moderate amounts of metabolites or structurally related compounds present, which would result in a false positive by immunoassay method. One recent high atrazine detection was the result of propazine, which is structurally similar to atrazine, and can typically result in half the propazine being detected by the atrazine immunoassay analysis as atrazine (References 4, 5, 12). The immunoassay concentration of atrazine indicated a value roughly 50% higher than the lab analysis concentration due to the presence of propazine. Until recently, a minimum of 10% of all samples collected were verified by lab analysis. Most samples indicating high (>0.3 ppb)concentrations of pesticides by the immunoassay method are verified by lab analysis. Samples used in this study included verification analysis of at least 10% of all samples analyzed by IA, which enabled determination of false positives or negatives.

DATA SOURCES

The data used in this paper has been accumulated from four pilot projects conducted from 1995 through 1999 under the same sampling team leader. The four projects include the monitoring of the Brazos River alluvium, the investigation of atrazine contamination in groundwater in and around the City of Friona, a preliminary investigation of four public water supplies having atrazine detects, and the monitoring of vulnerable areas in Texas. The data represents three different types of studies. 1) A research monitoring well field was included in the Brazos River alluvium monitoring. 2) Public water supply well fields were monitored in the City of Friona and the four public water supplies. And 3) Widely spaced wells, in mostly rural areas determined to be vulnerable, were monitored in the Brazos River alluvium and in other areas of the state. The various TNRCC data sources are summarized in Table 1 below, followed by a summary of some other state's and USGS experiences with the same. The comparison of immunoassay and lab GC/MS results are summarized in Table 2.

Table 1
Summary of TNRCC Data Sources

Project	Years	# of Wells	# IA	# Lab	# Both	IA DL (ppb)	Lab DL (ppb)	Lab EPA Method	# False Positives
Brazos River	'94-'95	43*	52*	30*	30*	0.05/5	0.5	8270B	6*
Brazos River	'95-'96	43*	52*	30*	30*	0.05/5	0.5	8141A	6*
Friona	'98-'99	60	38	38	21	0.05/5	0.1	525.2	11
PIs at 4 PWS	'99	39	56	12	12	0.05/5	0.1	525.2	4
Vuln. Areas	'98-'99	16	16	9	9	0.05/5	0.1	525.2	7
TOTAL	5 years	158	162	89	72	0.05/5	NA	NA	28

* Values for the Brazos River Study are totals for both years.

Summary of Several State and Federal Studies

Several other states (References 9, 10 and 11) and the USGS (References 2, 3, 4, 8, and 12) have also utilized the immunoassay method for screening samples for pesticides. The USGS, Illinois, Wisconsin, and several other state programs have conducted substantially more sampling and analysis by both immunoassay and lab methods. These studies are primarily from 1989 to 1994, and include surface water, soil, and groundwater media sampling. The USGS studies were primarily for surface water in the Midwest. The following results were consistent throughout most of these papers:

- IA is a reliable and effective screening tool for detection of pesticides, especially atrazine

- IA is faster, less costly, and more portable than laboratory methods for pesticide analysis
- The immunoassay method produces no false negatives but some false positives due to lower detection limits and cross-reaction of parent pesticide with metabolites and structurally-related pesticides

Generally speaking, few papers presented an actual side by side comparison of immunoassay to lab results. Several do not indicate lab method number or detection limits, or if they do, it is not overly clear or easy to find. Some reports indicate there is little correlation between low concentrations by immunoassay method and non-detects by laboratory GC/MS methods, while others note high correlation between the two. A more recent Wisconsin study (Reference 11) appears to support Texas' data, which indicate that triazine detects below 0.3 ppb by immunoassay analysis result in non-detects by GC/MS analysis. Another finding noted in a USGS paper (Reference 12) indicates best correlation of immunoassay to lab GC/MS results occur when atrazine concentrations are in the 0.4 ppb range. This is due to the 0.4 ppb being similar to the hepten used to make the antibodies in the test kits. A separate issue addresses the parent pesticide and metabolite concentrations as percentages of the total concentration, as a possible atrazine relative age indicator.

RESULTS

Table 2 presents comparison data of immunoassay and lab results from TNRCC sampling related to the PMP program. Most immunoassay values are near lab GC/MS values, with no false negatives present. The higher cost and Detection Limits (DL) for the USEPA Method 525.2 lab method, partially explains the relatively few in-house samples for which this paper's findings are based. Additionally, data from some of the referenced papers support these findings.

The Texas data included 162 total samples analyzed by immunoassay method, at a cost of about $6000. The number of these for which lab verification analysis by GC/MS methods totals 72 samples from 62 different wells (some were repeat samples on different days), at a cost of about $15,220. Lab and immunoassay results were in close agreement. Additionally, there were no false positive results when immunoassay concentrations were >0.3 ppb, and USEPA Method 525.2 was used for verification analysis. With the exception of well sample #5660 on 5/26/99 (Table 2), none of the immunoassay samples, after having atrazine concentrations below 0.3 ppb, resulted in atrazine detects by the lab analysis method. The immunoassay concentration was sufficiently low(0.13 ppb)and the lab analysis concentration was at the 0.1 ppb Detection Limit. The sample's results were potentially suspect, or at least of limited concern at these levels. The only other outliers in the data were for well samples 5659 on 3/23/99, 5693 on 12/6/99, 5751 on 12/7/99, 5709 on 12/7/99, and 5711 on 12/7/99, (Table 2). These immunoassay samples had atrazine concentrations less than the lab concentration. Since both methods detected the analyte, this would not be considered a false negative.

Results from other states further complicates the issue in some respects. The "false positives" are due at least in part from lab detection limits being higher than for the immunoassay method. The Wisconsin results present an alternative way of viewing the situation. Rather than discounting these differences between immunoassay and Lab results, they further define the results by testing for metabolites, then adding the three main metabolite totals to those of the parent compound to come up with similar concentrations of total atrazine compounds by both immunoassay and lab methods. By doing so, they consider low detects of atrazine by the immunoassay method as actual detects, and do not discount them because the lab method can detect and differentiate between parent atrazine, metabolites, and structurally-related pesticides. One USGS surface water study (Reference 3) indicates the parent atrazine to a specific metabolite (deethylatrazine) ratio can provide relative age of atrazine contamination. Personal communication with Postle (Reference 11) of Wisconsin indicates otherwise, revealing that they believe the age has less to do with how much of a metabolite is present. It is more important where the atrazine has been (in the soil, root zone, surface water, or groundwater), as to what processes would degrade the parent in any given time period.

The question arises, which approach should be adopted for the PMP program? By adopting the Wisconsin method, there is no real need for a cut-off concentration by immunoassay screening, and considerably more detects of atrazine at lower concentrations will need to be addressed. This would help trigger faster response to slowly developing problems, but would require more staff and sampling/response funds. By analyzing for parent atrazine alone, the more serious and immediate problems are addressed, and scarce state and federal funds can be stretched by establishing immunoassay method "cut-off" concentrations for limiting the number of samples sent for laboratory confirmation analysis.

The most promising result has been recent cooperative groundwater monitoring efforts between TNRCC, the Texas Water Development Board, and the High Plains Underground Water Conservation District #1 (Scheduled for 2000). The small volume of sample and relative ease of sample collection for immunoassay analysis has created a willingness to coordinate various monitoring efforts by these entities. As a result, TNRCC will analyze an estimated 800 groundwater samples for atrazine in the Panhandle Region at an estimated analytical cost of $4000. The budgetary constraints will only permit atrazine analysis, but TNRCC is continuing to negotiate for cooperation with other agencies to possibly analyze for metolachlor as well. As similar future cooperative efforts are anticipated, by piggybacking onto scheduled monitoring efforts, much of the state can be screened for atrazine for a fraction of the cost of lab analysis. The more expensive lab methods can be utilized in follow-up sampling for detects over 0.3 ppb.

CONCLUSIONS

The Texas data, and some of the other referenced data, indicate the immunoassay method of screening samples to decrease the number of expensive lab-bound samples is reliable, practical and cost-effective. This is further supported by the low discrepancies between immunoassay and lab concentrations, the lack of false negatives, and the lower detection limit for the immunoassay screening method. Additionally, the ease of collection and small volume required are also helping toward establishing an incredible cooperative monitoring effort with other agencies/entities in Texas, enabling greater groundwater monitoring coverage for a fraction of lab costs.

The advantages to using the immunoassay method for screening samples are as follow:

- The method is a reliable and effective screening tool, having a lower DL than lab GC/MS methods
- Decrease in lab fees by prioritizing specific samples for more expensive lab verification analysis
- Decrease in analysis turnaround time allows for additional sampling in areas where detects occur
- Can be used to narrow down the number of impacted wells in extensive well field systems
- Faster turnaround provides more time for planning additional investigative or remediation tasks, and enables field crews to direct field efforts
- Analytical funds can be stretched to expand sampling program, especially when State and local monitoring cooperation and coordination occur
- Quicker turnaround results means a quicker response
- Smaller sample volume required, for easier storage and shipping
- "Field" portable method
- Samples can be stored longer than lab samples (up to 8 weeks at 4 degrees Celsius)

- Sample preparation not as intensive as for lab methods
- Use of larger magnetic rack and base requires less staff analysis time and more samples to be run
- Use of the instrument calibration run memory for long-term projects or back-to-back sampling trips further reduces reagent cost and staff time

- Application of an 0.3 ppb cut-off concentration for atrazine can serve field personnel as a reasonable determinant of which samples to send in for lab verification analysis
- The method has been promulgated by the USEPA as Method 4670

Disadvantages of the immunoassay method include:
- It is not specific enough, in that concentrations of atrazine may include other triazines, metabolites/degradates, or has not yet been developed to include specific analyte detection
- Requires an initial outlay for instrument and equipment beyond some budgets
- Reagents require refrigeration, have a shelf life of one year, and reagents cannot be mixed
- Requires training and highly skilled field staff
- Requires consistent ambient temperatures of testing area, reagents and samples
- Lack of consistency in last two bullets can result in up to 20% result variance
- Greater responsibility on the part of the staff conducting the analysis
- False positives occur regularly with higher lab detection limits
- requires a certain percentage of samples be verified by lab GC/MS methods
- Reagent replacement kits must be shipped in ice packed and insulated container for next-day delivery, they are relatively expensive, and depending upon procurement system, requires a certain amount of time and coordination to secure for specific sampling needs and within Fiscal Year budgetary constraints.

RECOMMENDATIONS

The following recommendations are based on the review of the provided references and the authors' experiences with immunoassay for atrazine:
- Develop a thorough QA/QC program to address the many aspects of immunoassay to include; Training, Testing of instrument and equipment prior to each field use, Procedures, Documentation and Reagents (especially expiration dates and not mixing lots)
- Include laboratory confirmation analysis on samples having atrazine concentrations of 0.3 ppb or greater (this could be lowered in the future, should lab methods and detection limits improve)
- Utilize the "real-time" analysis results to help direct sample location determination in the field
- Coordinate immunoassay screening with other agency subdivisions, agencies, and local entities, to share in related efforts and expenses; One group may not be able to afford the equipment or use it enough to justify the purchase, but several can share in the expenses and benefits
- Compare commercially available instruments and kits, choosing the one that best suites your needs
- Keep abreast of developments in immunoassay technology, as less complicated methods are soon to be commercially available (a home-test kit, similar to pH paper), that may, or may not, pass more stringent QA/QC requirements
- Further research of immunoassay vs lab analysis for parent atrazine, metabolites, and structurally-related pesticides, to address the "false positives" issue

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TABLE 2 (continued)

13) USDA/TDA, 1997. Texas Agricultural Statistics Service, 1997.

14) USEPA Method 4670, SW-846, Chapter 4.4, or 540/R94-509, 1999.

TABLE 2

TNRCC FIFRA/GW Protection Program Water Sampling Results for Atrazine Comparison of Immunoassay\GC/MS Results (1994-1999)

Project	Sample #	Date Sampled	Lab\Atrazine Conc. (ppb)*	Lab Detection Limit	Immunoassay\Atrazine Conc. (ppb)
Brazos River	6 total	11/19/94	ND	0.5	ND-0.08
“”	5 total	3/11/95	ND	0.5	ND-0.12
“”	2 total	9/6/95	ND	0.5	ND
“”	4 total	6/13/96	ND	0.5	ND
“”	4 total	6/12/96	ND	0.5	ND-0.42
“”	3 total	7/9/96	ND	0.5	ND-0.55
“”	4 total	7/10/96	ND	0.5	ND
“”	2 total	7/11/96	ND	0.5	ND
Friona	5586	3/23/99	2.1	0.1	0.49
“”	5585	3/23/99	ND	0.1	0.08
“”	5598	3/23/99	ND	0.1	0.18
“”	5660	5/26/99	0.1	0.1	0.13
“”	5600	3/23/99	ND	0.1	0.23
“”	5599	3/23/99	2.0	0.1	3.87**
“”	5670	5/26/99	2.0	0.1	1.87
“”	5589	3/23/99	0.4	0.1	0.79
“”	5672	5/26/99	0.4	0.1	0.48
“”	5631	3/23/99	2.5	0.1	3.1

TABLE 2 (continued)

“”	5661	5/26/99	0.4	0.1	0.52
“”	5645	3/23/99	ND	0.1	0.3
“”	5663	5/26/99	ND	0.1	0.07
“”	5595	3/24/99	ND	0.1	0.18
Project	**Sample #**	**Date Sampled**	**Lab\Atrazine Conc. (ppb)***	**Lab Detection Limit**	**Immunoassay\Atrazine Conc. (ppb)**
Friona	5592	3/23/99	ND	0.1	0.21
“”	5639	3/23/99	ND	0.1	0.62
“”	5590	3/23/99	ND	0.1	0.14
“”	5640	3/23/99	ND	0.1	0.26
“”	5593	3/23/99	ND	0.1	0.36**
“”	SW-1	5/28/99	2.2	0.1	4.86
“”	SW-2	5/28/99	5.3	0.1	5.66
“”	5707	12/7/99	ND	0.1	0.13
“”	5711	12/7/99	0.951	0.1	0.89
“”	5697	12/7/99	0.871	0.1	0.89
“”	5706	12/7/99	0.382	0.1	0.42
“”	5703	12/7/99	ND	0.1	0.32
Hereford	5693	12/6/99	0.973	0.1	0.90
“”	5701	12/6/99	1.06	0.1	1.75
Dimmitt	5751	12/7/99	1.80	0.1	1.49
“”	5709	12/7/99	0.972	0.1	0.63
Tulia	5700	12/8/99	ND	0.1	0.35
“”	5720	12/8/99	2.08	0.1	3.41
Plainview	5710	12/8/00	ND	0.1	0.10
Hidalgo\Bailey	5 total				

TABLE 2 (continued)

Counties	Hidalgo	8/5-6/98	ND	0.1	0.07
“”	3 total Bailey	11/12/98 5/25/99	ND	0.1	0.06- 0.16

*ND is not detected above laboratory detection limits
1994-1995 samples analyzed by USEPA Method 8270B
1996 samples analyzed by USEPA Method 8141A
1998-1999 samples analyzed by USEPA Method 525.2 (Drinking Water Standards)
**Average of sample and duplicate