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Evaluation of Enzyme Linked Immunosorbent Assay (ELISA) for Diaminoatrazine Analysis of Water Samples in Comparison to Gas Chromatography

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Abstract

In Wisconsin the widespread use of the herbicide atrazine has led to the presence of atrazine and it's metabolites in some groundwaters. One of the metabolites, diaminoatrazine, is of particular interest because it is reported to pose a greater health threat (to those drinking the water) than the other metabolites. Diaminoatrazine can be measured by conventional gas chromatography techniques, but the test is time consuming, expensive, and less robust than desirable. However, a new test for diaminoatrazine, using enzyme linked immunosorbent assay (ELISA) technology, has been commercially developed (although it is not yet on the market). The purpose of the present study was to evaluate this new ELISA by comparing the results of split samples analyzed by both the new ELISA and the conventional technique. Approximately 70 groundwater samples from Wisconsin wells, many of which were known by the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) to be contaminated by atrazine and atrazine metabolites, were collected and split. DATCP's laboratory analyzed one set of the split samples using conventional extraction and chromatographic analysis techniques. The other set of split samples were analyzed using the new diaminoatrazine ELISA. This same set of samples was also analyzed by an atrazine ELISA, a test that has been in use for about ten years. The results indicate that while the new diaminoatrazine ELISA produces results that correlate with conventional measurements, the ELISA produced results that were higher (usually about double) than results obtained from the conventional technique. The higher results may relate to the fact that atrazine (parent compound) can cross react (i.e., the new ELISA is not as specific to diaminoatrazine as desired). The new diaminoatrazine atrazine ELISA appears to be very sensitive, as it detected the presence of diaminoatrazine (or possibly cross reactants) when the conventional technique was not able to detect it. Some samples in which neither atrazine nor any of its metabolites could by detected by conventional gas chromatography had detects using the diaminoatrazine ELISA. Interestingly, total atrazine measured by the conventional GC technique (sum of the parent compound plus all metabolites measured) gave similar results to the sum of the diaminoatrazine ELISA and the older atrazine ELISA for the samples studied. Despite the fact that the new diaminoatrazine ELISA does not produce the same results as conventional analyses, it still could be a useful (and relatively inexpensive) test for determining waters that could contain the diaminoatrazine metabolite (and thus pose an elevated drinking water health risk).

Introduction and Background

Long term use of the herbicide atrazine in Wisconsin has resulted in the contamination of Wisconsin's groundwater in many areas of the state. While atrazine has been restricted or banned in certain areas of the state that have been shown to be susceptible to groundwater contamination, it is still being used in other areas as an effective means to control weeds in corn crops. While the detection of atrazine led to the original environmental concerns, it has also been recognized that degradation products or metabolites of atrazine, which can be just as toxic as the parent compound, can exist for a long time before they are further degraded or reduced in concentration by dilution.

In Wisconsin atrazine and atrazine metabolites are regulated in combination. The maximum contamination limit (MCL) for the sum of atrazine and atrazine metabolites is 3.0 parts per billion (micrograms per liter). While the standard method for detection of the compounds is conventional gas chromatography, a much lower cost atrazine enzyme linked immunosorbent assay (ELISA) has been used at the Wisconsin State Laboratory of Hygiene (WSLH) for many years to screen water samples for the presence of atrazine. The atrazine ELISA used at the WSLH primarily detects the parent compound, but has some cross reactivity to some atrazine metabolites, mainly desethyl-atrazine. Of the known atrazine metabolites that can exist in groundwater, diaminoatrazine is of the most interest because of its suspected toxicity. However, diaminoatrazine is a relatively difficult, and thus expensive, analyte to measure using conventional chromatographic techniques. Consequently a new diaminoatrazine ELISA test has recently been introduced.

The work described in this report provides an evaluation of the diaminoatrazine ELISA to determine its value for use as a routine groundwater-monitoring tool. In this study, 74 water samples, mostly groundwater, were analyzed for atrazine and atrazine metabolites using conventional gas chromatographic methods at the Wisconsin Department of Agriculture Trade and Consumer Protection's (WDATCP) pesticide laboratory. Aliquots of those 74 samples were also analyzed using both the atrazine and diaminoatrazine ELISA at the WSLH. An evaluation of the ELISA results, relative to the conventional chromatographic measurements, was made.

Materials and Methods

Samples

The majority of the water samples were collected from groundwater wells that WDATCP routinely monitors due to the wells' history of atrazine and atrazine metabolite contamination. Additionally groundwater samples from random wells that tested negative for atrazine by ELISA were also analyzed for diaminoatrazine, as were a few surface water samples.

ELISA Assays

Both ELISA tests were performed according to the manufacturer's instructions. The Atrazine ELISA technology has been previously described by others.[1] The test kit used in this study for the diaminoatrazine ELISA is not currently commercially available. Beacon Analytical Systems, Inc. of Portland, ME, in conjunction with Syngenta Crop Protection, Inc., has developed a prototype ELISA for diaminoatrazine. They allowed the WSLH to use their ELISA kit for this study. The diaminoatrazine test is a competitive ELISA test method using polyclonal antibodies that bind both diaminoatrazine and diaminoatrazine-enzyme conjugate molecules attached to the inside surface of test tubes. Samples of water are added to these tubes along with diaminoatrazine molecules with a specific enzyme attached (conjugated) and allowed to incubate. During this incubation period, diaminoatrazine molecules from the sample compete with diaminoatrazine-enzyme conjugate molecules for a limited number of antibody binding sites on the wall of the test tube. If there is a lot of diaminoatrazine in the sample, most of the binding sites capture the diaminoatrazine compound. If there is little diaminoatrazine in the sample, most of the binding sites are then occupied with the diaminoatrazine-enzyme conjugated compound. After the incubation period the unbound sample and conjugate are washed from the antibody coated tubes. This step is analogous to sample extraction and clean up normally used for chromatography. A substrate solution is then added to the tubes, which reacts with the enzyme portion of the diaminoatrazine-enzyme conjugate molecule to form a colored product. If there's a low concentration of diaminoatrazine in the unknown sample, the binding sites will be filled with the enzyme conjugate molecules and a dark color will develop. Conversely, if the sample being tested is rich in diaminoatrazine, most of the sites would be filled with the compound leaving few sites with the enzyme present, thus resulting in a minimum level of color production. The intensity of the color is inversely proportional to the concentration of the diaminoatrazine in the sample. Actual concentrations of the compound can be estimated by comparison to a standard curve.

GC assays

All gas chromatography was performed by the Wisconsin DATCP pesticide laboratory using standard techniques.[2] Triazine pesticides were extracted from water samples with methylene chloride followed by ethyl acetate. The extracts were mixed, the solvent was evaporated to dryness and the sample reconstituted with methyl tert-butyl ether (MTBE). The MTBE solution was then analyzed by gas chromatography using a nitrogen phosphorous detector. Confirmation of detections using a different column or detector was done routinely. Quantification was done by using a calibration curve that bracketed the concentration of the sample or by peak-to-peak comparison of the unknown to a standard (whose peak height is ten percent of the unknown peak height). More details on the method may be found in Method 633 of the DATCP Laboratory Services Manual.[2]

Results and Discussion

ELISA Cross reactivity

The first step in carrying out this study was to interpret the diaminoatrazine ELISA cross reactivity data provided by the manufacturers, to determine if compounds that may be in Wisconsin's groundwater would also react in the assay.(Tables 1 and 2) Cross reactivity is the ability of an ELISA to detect related compounds to varying degrees. It is a difficult concept to quantify especially when more than one cross-reactant is present in a sample. It should not be concluded from the cross-reactivity table that the cross reactants are cumulative (for example, using the atrazine cross reactivity table (table 1), if there were 0.1ppb atrazine and 0.1ppb propazine present in a sample, the atrazine immunoassay would not necessarily provide a result of 0.2ppb). From a public health standpoint cross-reactivity can be a positive or a negative feature of the assay depending on the intended use of the results.

Cross-reactivity to metabolites would be a positive attribute if screening samples for a group or a certain class of compounds were the objective. Using the atrazine ELISA as an example, if there is an ELISA result of <0.062 ppb, it can be concluded (from the cross reactivity, Table 1) that atrazine, propazine, ametryn, prometryn, prometon, desethyl atrazine, are all less than 0.062 ppb, as 0.062 ppb is the highest minimum detection level (MDL) concentration of that group. That finding would rule out the occurrence (at levels above 0.062 ppb) of several chemicals with one simple test. On the other hand, a positive atrazine ELISA detection in a sample could be due to any one or a combination of the cross-reactants and the results of the assay must be interpreted within the constraints of that knowledge.

Both the diaminoatrazine ELISA, and the commercially available atrazine ELISA, have some cross reactivity to other atrazine metabolites as well as to other triazine herbicides and their metabolites. The manufacturers have tested cross reactivity to some compounds for both test kits (Tables 1. and 2). Some of the samples used in this study had known concentrations of deisopropylatrazine. Tables 1 and 2 do not indicate that deisopropylatrazine is a significant cross reactant and therefore will not skew the results. This lack of reactivity to deisopropylatrazine could hamper the effectiveness of either assay in determining the safety of the rare drinking water sample that may have a high concentration of deisopropylatrazine, but little or no significant concentrations of other atrazine or atrazine metabolites.

Recovery

Recovery of analyte would also be a factor affecting comparability of the methods. Beacon Analytical Systems did not provide diaminoatrazine recovery data for the new ELISA test. Subsequently, recovery data was acquired during this study by implementing a 2.0 ppb diaminoatrazine spiked blank that was analyzed in five different analytical runs. The average spike recovery over the five runs was 100% (STD. DEV. = 0.094074). This recovery is very favorable when compared to the recoveries for the conventional DATCP GC method, which varied between 50-100%. [(2)]

Comparison of ELISA vs GC results

In this study results were compared both qualitatively and quantitatively. Quantitative comparison means that respective results for both methods were mathematically and graphically compared by calculating and analyzing slope, intercept, and correlation coefficients. For the qualitative comparisons the respective ELISA and GC results were stratified by concentration range and compared as to the respective public health interpretations that could be made based on the results. For example, the paired results could be stratified and compared as to whether or not they are above or below the MCL.

Table 3 is a side-by-side compilation of all the results generated in this study. The sample number is in column 1, the diaminoatrazine ELISA results in column 2, and the GC diaminoatrazine results are in column 5. As a first step in understanding the data, the ELISA diaminoatrazine results were graphed against the GC diaminoatrazine results and the slope, intercept, and correlation coefficients were calculated. For this analysis the "below detection" results are assumed to be zero. The results are presented in figure 1. This data analysis suggests that the concentration results for the two methods match quite well. The correlation coefficient is 0.9134 and the slope is 0.5037. A correlation of greater than .90 is indicative that a values from column 2 (ELISA diaminoatrazine results) will accurately track with the values in column 5 (GC diaminoatrazine results). The slope, however tells you the GC value will track at approximately 50% of the ELISA value, or the ELISA results are biased towards yielding results that are double the GC results. The higher ELISA concentrations are probably due to low detection limits of the ELISA method, better recovery at lower concentrations, and some cross-reactivity to other triazine compounds.

In order to better understand the public health implications of the bias identified in figure 1, the data was stratified into ranges of values based on the levels that are currently used in advising well owners about actions they might wish to take based on levels of atrazine found in their wells. Currently, owners are advised that the water is potable if no atrazine is detected. If detectable levels are found that are below the MCL (3.0 ppb), owners are told to continue periodic testing to see if levels increase and to consider not using the water for drinking. If the level is above the 3 ppb MCL, they are advised to not consume the water. A comparison of the two methods with the data stratified into groups is presented in table 4. In addition to the strata based on health advisories an additional strata was include based on the limit of detection of the GC method. The data indicates that using the diaminoatrazine ELISA test as a surrogate for the GC test would result in 11 of the 74 wells testing above the MCL. Similarly, the ELISA test would have found only 13 wells were below detection, while the GC test would have produced 53 wells where no diaminoatrazine was detected.

While it is interesting to understand how the new diaminoatrazine ELISA test system performed when compared to the diaminoatrazine GC test, it is more important from a drinking water safety standpoint to make comparisons with total atrazine. "Total atrazine" is the sum of the atrazine, desethylatrazine, diaminoatrazine, and deisopropylatrazine concentrations. Column 4 of table 3 contains the sum concentrations of atrazine and diaminoatrazine determined using ELISA. Column 9 contains the sum of atrazine plus all

metabolite concentrations (total atrazine) determined with GC. Statistical and graphical comparisons of the combined values are presented in figures 2a and 2b. Figure 2a, an analysis of all the data points, shows a correlation coefficient of 0.8223 indicating that the GC results track fairly well with the ELISA results. The slope value of 1.1332 suggests that there is a small bias towards higher values using the ELISA tests. Figure 2b is the same set of data with one outlier result removed. The outlier sample had an unusually high GC concentration of deisopropylatrazine, a metabolite the ELISA test has low sensitivity to. Figure 2a shows a slope of 0.9893 and a correlation coefficient of 0.9118 which is a very favorable ELISA vs. GC comparison. As described above, it is helpful to stratify the data and further compare the two methods. The stratified data is presented in table 5. The agreement suggested by the statistical analysis is apparent in this table. The GC method produced 12 wells above the MCL while the ELISA method produced 11. The GC method had 47 samples with concentrations of 1.0 ppb or less. In the 1.0 ppb or less range, the ELISA method had 43 samples in agreement with GC and 10 results that were higher than this range. This is most likely due to the lower limit of detection inherent in the ELISA technology. The other strata in the table show good agreement between the methods.

Additional statistical analyses were performed comparing other possible combinations of ELISA vs. GC results. The atrazine ELISA result alone was not a good predictor of total atrazine plus metabolites (figures 3a and 3b). The correlation coefficient of figure 3a was only 0.4937 and the slope was 4.2420. When the GC outlier result (fig. 3b) is removed, the slope and correlation coefficient improve only slightly. ELISA diaminoatrazine (figures. 4a & 4b) is a somewhat better predictor (when compared to the ELISA atrazine test) of total atrazine by GC results. The correlation coefficient was 0.7848 with a slope of 1.2880. If the GC outlier is removed, the slope and correlation coefficient improves to 1.1195 and 0.8681 respectively.

Other Considerations

As figures. 2a and 2b indicate, "total atrazine" as measured by conventional GC techniques (the sum of all the metabolites plus atrazine) was usually close to the sum of the ELISA results for diaminoatrazine and the ELISA results for the atrazine test. [Note that it is not chemically correct to add the concentrations of compounds of different molecular weights, but because (1) the state regulations are viewed in this way, that is, they consider atrazine and atrazine to be additive when assessing the standard and (2) the difference in concentration if all concentrations were expressed as atrazine would be generally minimal]. These data suggest that the occurrence of atrazine plus atrazine metabolites in a water sample can be accurately screened using the new diaminoatrazine ELISA evaluated in this study and the established (Strategic Diagnostics) atrazine ELISA assay. If both ELISA assays were performed on a sample, the cost would be on the order of $50. This cost is perhaps an order of magnitude less expensive than the gas chromatographic analysis. Table 6 summarizes the ELISA vs. GC method agreement above and below the MCL. The ELISA combination of atrazine and diaminoatrazine correctly predicted whether the sample was above or below the MCL for 73 of the 74 samples. Using the ELISA diaminoatrazine method alone in a stratified fashion would yield accurate results in 72 out of 74 cases with respect to being above or below the total

atrazine MCL. Stratified data suggests that the ELISA diaminoatrazine method would be a good choice for screening private water samples.

Table 7 shows the results of the two different ELISAs performed on randomly chosen private well water samples (i.e., there was no known history that these wells might contain high levels of atrazine or atrazine metabolites). The table shows that some diaminoatrazine would be missed if only the atrazine ELISA were used to test the samples. Six samples (out of eleven total) indicated measurable concentrations of diaminoatrazine were present while none of the samples measured by the atrazine ELISA produced detects. No GC results were run for this set of samples. One sample had a diaminoatrazine concentration of 0.55 ppb. So this further suggests that the diaminoatrazine might be a useful screen. From a regulatory perspective, perhaps a concentration for the diaminoatrazine ELISA could be established whereby samples with concentrations less than that value would have very little chance of having a total atrazine concentration greater than the MCL. For example, there are no sample cases in table 3 with ELISA diaminoatrazine concentrations less than 1.0 ppb that have GC total atrazine concentrations greater than the MCL.

Conclusions

Overall the new diaminoatrazine ELISA is straightforward to perform, the test following a similar procedure as other ELISAs. The test is inexpensive to perform compared to conventional gas chromatographic procedures. Interpretation of the results is complicated by fact that other triazine compounds, including the parent compound atrazine, can cross react. Nevertheless, given the public health concern for diaminoatrazine, the test, which was found to respond to very low concentrations of diaminoatrazine, the ELISA test might be used as valuable screening tool or as a supplemental analysis. More specific conclusions are given below.

1. While the new diaminoatrazine ELISA correlates with conventional gas chromatographic measurements of diaminoatrazine, the ELISA produced results that were generally higher (about double) than those obtained from gas chromatography.
2. Some samples in which neither atrazine nor any of its metabolites could be detected by conventional gas chromatography, had detects using the diaminoatrazine ELISA.
3. Total atrazine measured by the conventional GC technique (sum of the parent compound concentration and the concentration of all detected metabolites) gave similar results to the sum of the diaminoatrazine ELISA concentration and the older atrazine ELISA for the samples studied.

It is hoped that the ELISA diagnostic test industry will soon develop kits that will simultaneously detect multiple compounds and avoid the problem of cross-reactivity. Assays using Immuno-flourescent conjugates have been developed for other classes of analytes using differing wave lengths of light on various analyte-conjugates that can detect and quantify several components in one pass through the immunoassay system.

Immuno-flourescent technology could possibly give results that are analogous to gas chromatography, that is, speciation and low level quantification. Further, as more ELISAs become available for testing atrazine metabolites (an immunoassay that is specific for desethylatrazine, an atrazine metabolite, is expected to be made available later this year), there will be more tools for data interpretation.

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Table 1

Cross Reactivity Chart for the atrazine ELISA
(Strategic Diagnostics, Inc. data)

Analyte	MDL[1]	LOQ[2]	IC50[3]
	ppb	ppb	ppb
Atrazine	0.046	0.1	0.72
Propazine	0.033	0.1	0.74
Ametry	0.053	0.05	0.39
Prometryn	0.054	0.09	0.64
Prometon	0.056	0.31	2.22
Desethylatrazine	0.062	0.45	3.21
Terbutryn	0.09	0.76	5.5
Terbutylazine	0.31	2.15	15.5
Simazine	0.34	0.68	4.9
Deisopropylatrazine	0.8	30.1	217
Cyanazine	1	>1000	>10000
6-Hydroxy Atrazine	1.1	20.6	148

1. The lowest concentration that can be detected with the atrazine ELISA
2. The lowest concentration that can be quantified with the atrazine ELISA
3. The concentration required to inhibit one-half of the color produced by the negative control.

Table 2

Diaminoatrazine ELISA cross-reactivity chart
(Beacon Analytical Systems, Inc. Data

Analyte	MDL[1]	LOQ[2]	IC50[3]
	ppb	ppb	ppb
diaminoatrazine	0.05	0.05	
deisopropylatrazine			4.7
desethylatrazine			25.5
Atrazine		>10,000	>10,000
OH-Atrazine		>10,000	>10,000
Propazine		>10,000	>10,000
Cyanazine		>10,000	>10,000
Simazine		>10,000	>10,000

1. The lowest concentration that can be detected with the atrazine ELISA
2. The lowest concentration that can be quantified with the atrazine ELISA
3. The concentration required to inhibit one-half of the color produced by the negative control.

Table 3

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9
	ELISA	ELISA	ELISA	GC	GC	GC	GC	GC
Sample	Diamino	Atrazine	Atra.+Dia.	Diamino	Desethyl	De-iso	Atrazine	Total Atra[1]
Number	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
02-3887	4.23	1.06	5.29	1.500	1.540	0.539	0.504	4.083
02-3888	4.26	1.04	5.3	1.940	1.350	0.643	0.585	4.518
02-3889	>5.0	0.86	>5.86	3.000	2.420	0.623	0.459	6.502
02-3890	0.59	0.05	0.64	ND	ND	ND	ND	0.000
02-3891	2.39	<0.05	<2.44	1.010	ND	ND	ND	1.010
02-3892	1.33	0.05	1.38	0.563	ND	ND	ND	0.563
02-3893	0.22	<0.05	<0.27	ND	ND	ND	ND	0.000
02-3894	0.44	0.36	0.8	ND	0.964	ND	ND	0.964
02-3895	0.97	0.95	1.92	ND	1.240	ND	0.310	1.550
02-3896	0.73	0.37	1.1	ND	1.070	ND	0.158	1.858
02-3897	0.13	0.11	0.24	ND	0.346	ND	ND	0.346
02-4330	0.60	0.43	1.03	ND	0.436	ND	0.243	0.679
02-4331	0.87	0.12	0.99	ND	ND	0.315	ND	0.315
02-4332	0.43	0.14	0.57	ND	ND	ND	ND	0.000
02-4333	0.20	0.14	0.34	ND	ND	ND	ND	0.000
02-4334	0.19	0.21	0.4	ND	ND	ND	0.299	0.299
02-4335	0.66	0.32	0.98	ND	0.693	ND	0.299	0.299
02-4336	0.30	0.19	0.49	ND	0.497	ND	0.158	0.655
02-4337	0.22	0.28	0.5	ND	ND	ND	0.299	0.299
02-4338	0.12	0.05	0.17	ND	ND	ND	ND	0.000
02-4339	0.35	0.17	0.52	ND	0.404	ND	0.168	0.572
02-4340	1.55	1.22	2.77	0.693	1.460	0.746	1.070	3.969
02-4341	0.36	0.21	0.57	ND	0.342	ND	0.158	0.500
02-4342	0.49	0.29	0.78	ND	0.463	ND	0.317	0.780
02-4688	0.64	0.41	1.05	ND	1.770	ND	0.347	2.117
02-4689	0.27	0.14	0.41	ND	0.850	ND	ND	0.850
02-4690	0.16	0.20	0.36	ND	0.731	ND	0.201	0.932
02-4691	0.15	0.06	0.21	ND	ND	ND	ND	0.000
02-4692	0.53	0.35	0.88	ND	1.670	ND	0.298	1.968
02-4693	0.19	0.23	0.42	ND	0.731	ND	0.239	0.770
02-4694	0.32	0.18	0.5	ND	1.050	ND	ND	0.105
02-4695	<0.05	<0.05	<0.05	ND	ND	ND	ND	0.000
02-4696	3.93	0.26	4.19	1.990	1.420	1.040	0.218	4.668
02-4697	2.08	0.14	2.22	1.540	0.439	0.519	ND	2.498
02-4698	1.25	<0.05	<1.30	0.652	ND	ND	ND	0.652
02-4699	<0.05	<0.05	<0.05	ND	ND	ND	ND	0.000
02-4700	0.05	<0.05	<0.1	ND	ND	ND	ND	0.000
02-4701	0.06	<0.05	<0.11	ND	ND	ND	ND	0.000

1. Total Atrazine is the sum of Atrazine, Diaminoatrazine, Desethylatrazine, and Deisopropylatrazine.

Table 3 Continued

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9
	ELISA	ELISA	ELISA	GC	GC	GC	GC	GC
Sample	Diamino	Atrazine	Atra.+Dia.	Diamino	Desethyl	De-iso	Atrazine	Total[1]
Number	ppb	ppb	ppb	ppb	ppb	ppb	ppb	ppb
02-4702	<0.05	<0.05	<0.05	ND	ND	ND	ND	0.000
02-4703	<0.05	0.17	<0.22	ND	ND	ND	0.353	0.353
02-4704	<0.05	0.06	<0.11	ND	ND	ND	ND	0.000
02-4705	<0.05	0.08	<0.13	ND	ND	ND	0.268	0.268
02-4706	<0.05	<0.05	<0.10	ND	ND	ND	ND	0.000
02-9015	0.08	0.11	0.19	ND	ND	ND	ND	0.000
02-9016	0.61	0.41	1.02	ND	ND	1.300	0.152	1.452
02-9017	<0.05	<0.05	<0.10	ND	ND	ND	ND	0.000
02-9065	<0.05	<0.05	<0.10	ND	ND	ND	ND	0.000
02-9066	<0.05	<0.05	<0.10	ND	ND	ND	ND	0.000
02-9067	0.09	0.48	0.57	ND	ND	ND	0.295	0.295
02-9068	1.32	0.07	1.39	ND	ND	2.020	ND	2.020
02-9069	<0.05	<0.05	<0.10	ND	ND	ND	ND	0.000
02-9110	<0.05	<0.05	<0.10	ND	ND	ND	ND	0.000
02-9111 neat	4.82	1.08	5.9	2.520	1.250	8.150	0.685	12.605
02-9111 dil. 1:1	5.30		5.3	2.520	1.250	8.150	0.685	12.605
02-9685	<0.05	<0.05	,0.10	ND	ND	ND	ND	0.000
02-9686	0.09	<0.05	<0.14	ND	ND	ND	ND	0.000
02-10290 Chippewa River	0.06	0.14	0.2	ND	ND	ND	ND	0.000
02-10291 St. Croix R.	0.06	0.08	0.14	ND	ND	ND	ND	0.000
02-10292 Mississippi R.	0.08	0.14	0.22	ND	ND	ND	ND	0.000
02-10293 Black R	0.08	0.53	0.61	ND	ND	ND	0.401	0.401
02-12479	4.20	0.59	4.79	1.75	0.538	1.72	0.297	4.305
02-12480	>5.00	1.00	>6.0	2.82	0.852	2.16	0.562	6.394
02-12481	>5.00	0.70	>5.7	2.25	0.496	3.94	0.279	6.965
02-9111 dup.	4.86	1.21	6.07	2.52	1.25	8.15	0.685	12.605
02-10945	0.88	0.80	1.68	0.657	1.07	ND	0.428	2.155
02-10946	3.96	0.51	4.47	1.96	1.29	0.474	ND	3.724
02-10947	0.42	0.58	1	nd	nd	nd	0.439	0.439
02-10948	1.16	1.09	2.25	0.705	0.454	0.415	0.771	2.345
02-10949	1.60	0.57	2.17	0.747	0.636	0.403	0.312	2.098
02-10998	1.29	1.02	2.31	0.647	0.386	0.503	0.718	2.254
02-11618	1.40	0.09	1.49	0.548	nd	0.765	nd	1.313
02-11382	0.20	1.16	1.36	nd	0.655	nd	0.796	1.451
02-11383	2.14	0.50	2.64	nd	0.552	0.608	0.216	1.376
02-11384	1.17	0.18	1.35	nd	nd	0.835	nd	0.835
02-12478	4.06	0.90	4.96	1.94	1.14	1.21	0.377	4.667
LOQ	0.05	0.05		0.500	0.300	0.300	0.150	

1. Total Atrazine is the sum of Atrazine, Diaminoatrazine, Desethylatrazine, and Deisopropylatrazine.

Table 4

Range stratified comparison of diaminoatrazine, ELISA vs. GC. N=74			
GC Conc. Range (number of samples in GC range)	Number of ELISA results in GC range	Number of ELISA results Under GC range	Number of ELISA results over GC range
0 -1.0 ppb (61)	51		10
1.1 - 2.0 ppb (8)			8
2.1 - 3.0 ppb (5)			5
>3.0 ppb (0)			11

Table 5

Range stratified comparison of the sum of ELISA diaminoatrazine & atrazine compared with GC total atrazine [1]. N=74

GC Conc. Range (number of samples in GC conc. range	Number of ELISA results in same GC range	Number of ELISA results below GC range	Number of ELISA results above GC range
0 -1.0 ppb (47)	43		4
1.1 - 2.0 ppb (8)	5	1	2
2.1 - 3.0 ppb (7)	4	3	
>3.0 ppb (12)	11	1	

1. Total atrazine = atrazine+diaminoatrazine+desethylatrazine+deisopropylatrazine

Table 6

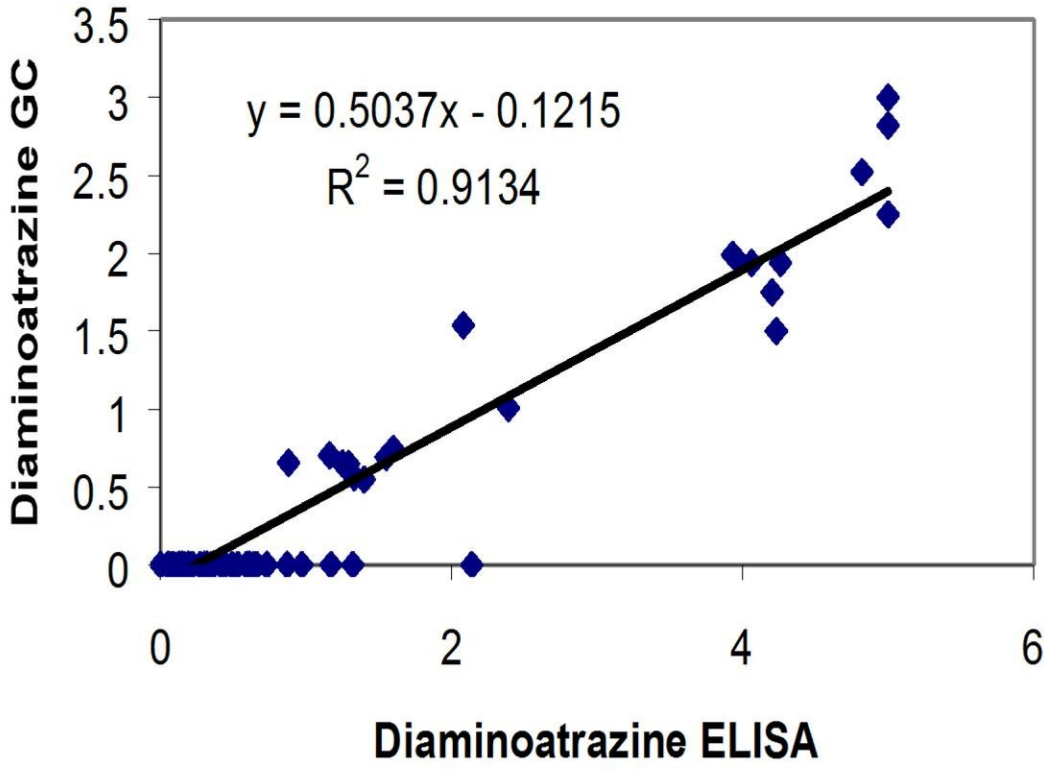
Comparison of data stratified above and below the 3.0 ppb MCL N=74					
ELISA analytes	GC analytes	GC and ELISA results both <3.0 ppb (agreement)	GC <3.0 and ELISA >3.0 “over estimate”	GC >3.0 and ELISA <3.0 "under estimate"	GC and ELISA results both >3.0 ppb "agreement"
Diaminoatrazine	Diaminoatrazine	66	8	0	0
Diaminoatrazine + Atrazine	Diaminoatrazine + Atrazine	63	7	0	4
Diaminoatrazine + Atrazine	Total Atrazine [1]	62	0	1	11
Atrazine	Total Atrazine [1]	62	0	12	0
Diaminoatrazine	Total Atrazine [1]	61	1	1	11

Table 7

Results of atrazine ELISA and diamino-atrazine ELISA of well waters with no known atrazine contamination

Sample number	Atrazine ELISA (ppb)	Diamino-atrazine ELISA (ppb)
1	<0.05	<0.05
2	<0.05	0.19
3	<0.05	<0.05
4	<0.05	<0.05
5	<0.05	0.08
6	<0.05	0.07
7	<0.05	0.15
8	<0.05	<0.05
9	<0.05	<0.05
10	<0.05	0.05
11	<0.05	0.55

Figure 1. Diaminoatrazine ELISA vs. GC



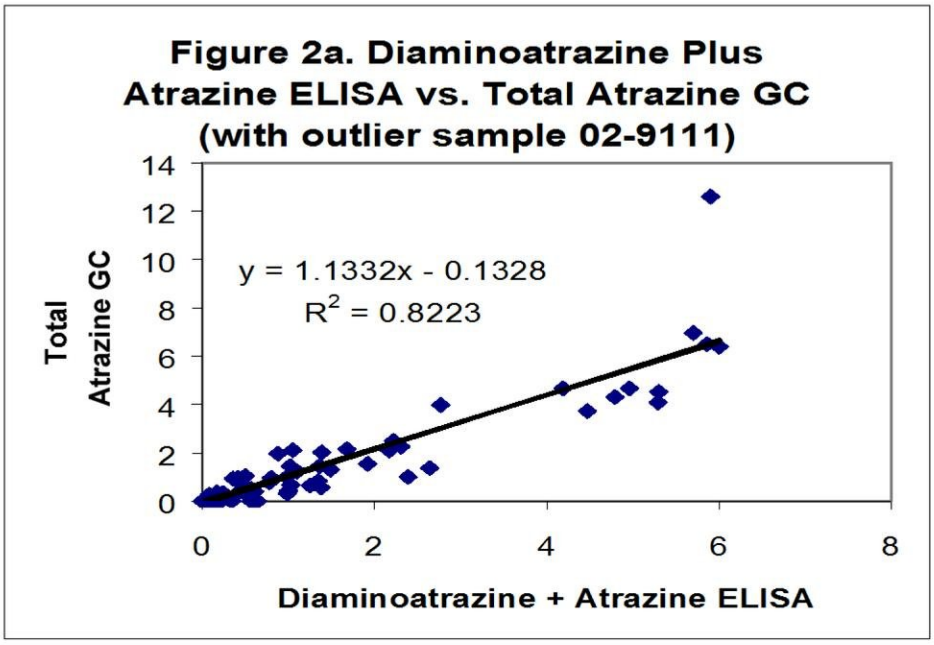


Figure 2a. Diaminoatrazine Plus Atrazine ELISA vs. Total Atrazine GC (with outlier sample 02-9111)
Total Atrazine GC
y = 1.1332x - 0.1328
R2 = 0.8223
0
2
4
6
8
10
12
14
0
2
4
6
8
Diaminoatrazine + Atrazine ELISA

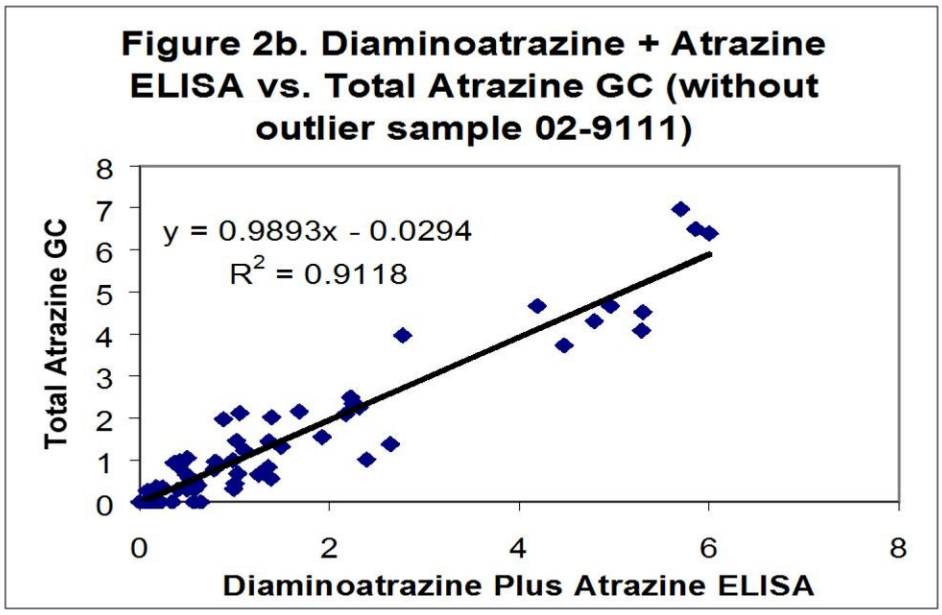


Figure 2b. Diaminoatrazine + Atrazine ELISA vs. Total Atrazine GC (without outlier sample 02-9111)
Total Atrazine GC
y = 0.9893x - 0.0294
R2 = 0.9118
0
1
2
3
4
5
6
7
8
0
2
4
6
8
Diaminoatrazine Plus Atrazine ELISA

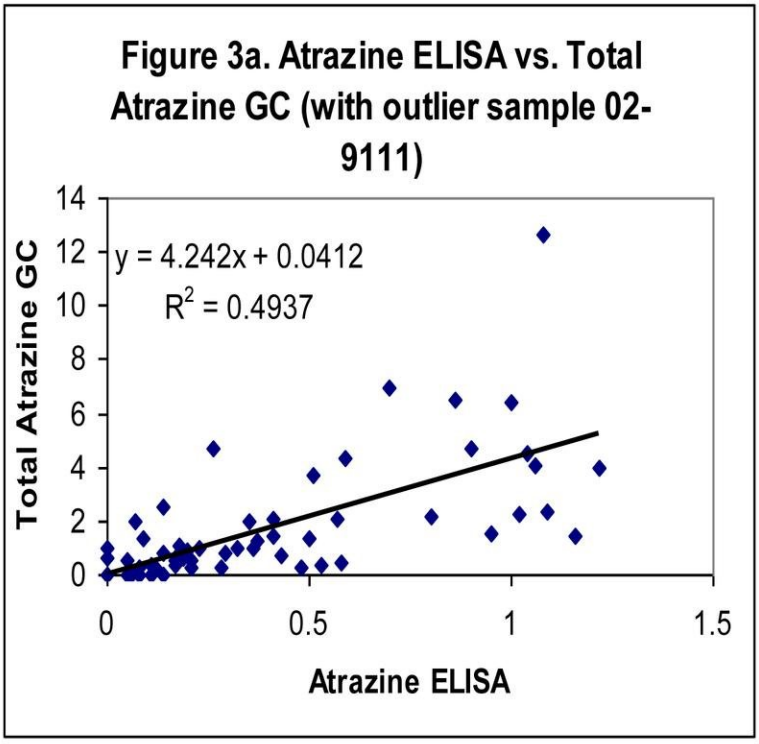


Figure 3a. Atrazine ELISA vs. Total Atrazine GC (with outlier sample 02-9111)
y = 4.242x + 0.0412
R2 = 0.4937
Total Atrazine GC
Atrazine ELISA
0
2
4
6
8
10
12
14
0
0.5
1
1.5

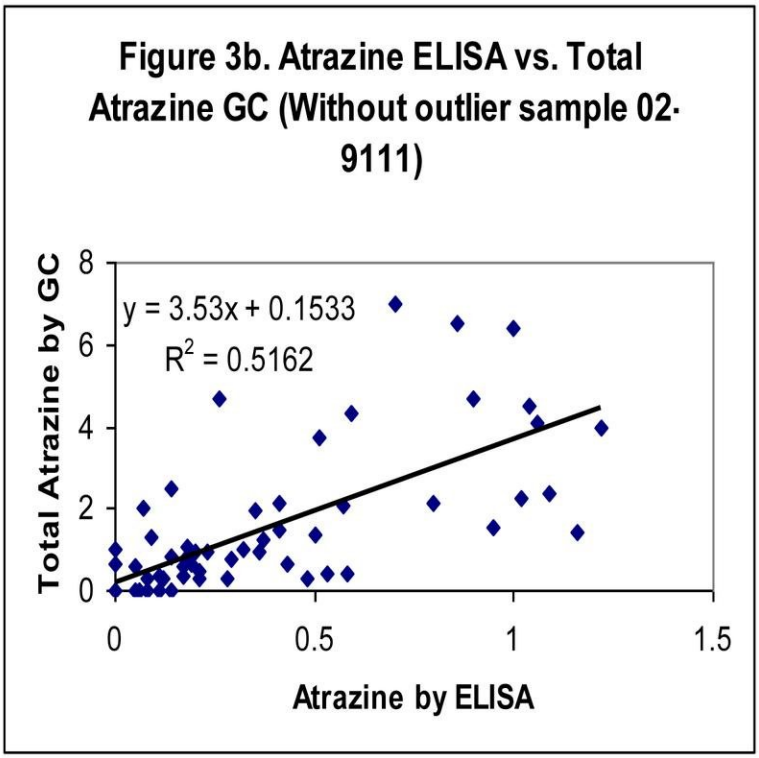


Figure 3b. Atrazine ELISA vs. Total Atrazine GC (Without outlier sample 02-9111)
y = 3.53x + 0.1533
R2 = 0.5162
Total Atrazine by GC
Atrazine by ELISA
0
2
4
6
8
0
0.5
1
1.5

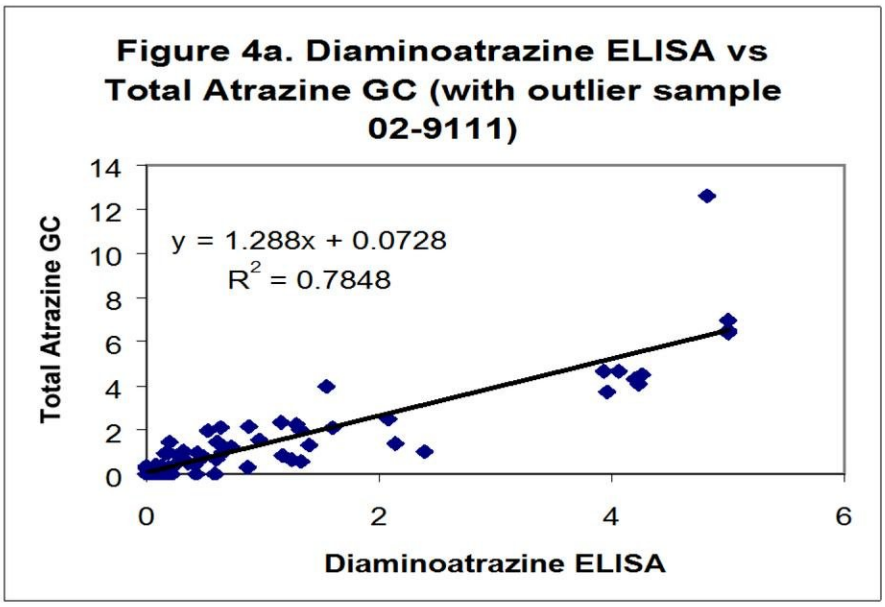


Figure 4a. Diaminoatrazine ELISA vs Total Atrazine GC (with outlier sample 02-9111)
y = 1.288x + 0.0728
R2 = 0.7848
Total Atrazine GC
Diaminoatrazine ELISA
0
2
4
6
8
10
12
14

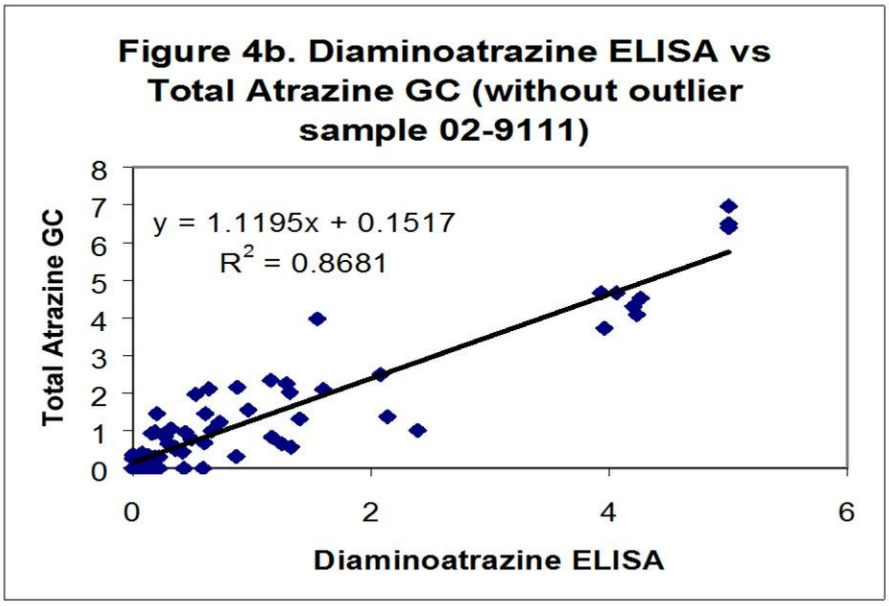


Figure 4b. Diaminoatrazine ELISA vs Total Atrazine GC (without outlier sample 02-9111)
y = 1.1195x + 0.1517
R2 = 0.8681
Total Atrazine GC
Diaminoatrazine ELISA
0
1
2
3
4
5
6
7
8