Evaluation of an ELISA Method for Acetochlor Analysis in the Le Sueur River Watershed

MINNESOTA DEPARTMENT OF AGRICULTURE
PESTICIDE AND FERTILIZER MANAGEMENT

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Cover Photo: Beauford Ditch (MDA)

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Abstract

In the spring of 2009, the Minnesota Department of Agriculture (MDA) conducted an evaluation of the use of enzyme-linked immunosorbent assays (ELISA) for the analysis of acetochlor in surface water samples collected from within the Le Sueur River Watershed in south central Minnesota. Both composite and grab samples were collected from a network of existing stream gage locations and submitted to the University of Wisconsin-Stevens Point Trace Organics Laboratory for ELISA analysis. Of the 90 samples submitted for ELISA analysis 39 were also submitted for base neutral pesticide analysis including acetochlor, using gas chromatography/mass spectrometry (GC/MS) at the MDA Laboratory. ELISA results indicated that 11 percent of the samples exceeded an acetochlor concentration of 1 µg/L. No samples collected had concentrations greater than the current Minnesota aquatic life standard for acetochlor of 3.6 µg/L. An acetochlor cross-reactivity analysis was completed using samples that had both ELISA and GC/MS analysis. The results of the acetochlor analysis for the two methods indicated a strong relationship existed ($R^2=0.80$) between the ELISA and GC/MS methods. The relationship improved ($R^2=0.92$) when the acetochlor ELISA results were compared to the cumulative GC/MS results of acetochlor and metolachlor. Approximately 10 percent of the ELISA samples were submitted for additional liquid chromatography/mass spectrometry (LC/MS) analysis for eight chloroacetanilide degradates. There did not appear to be cross-reactivity between the acetochlor ELISA method and the chloroacetanilide degradates although the data collected in this study was limited. Metolachlor appeared to be the primary analyte causing cross-reactivity concerns in the ELISA method. Results of this study strongly suggest that the acetochlor ELISA analysis is a reasonable screening tool for evaluating acetochlor concentration in surface water especially when combined with GC/MS analysis of an appropriate number of split samples above a pre-determined critical concentration to verify the presence of acetochlor and metolachlor. However because of the potential for cross-reactivity with metolachlor, which is a common contaminant in Minnesota waters, and a slight bias towards high (more protective) values in sampling results, the acetochlor ELISA is not suitable as a method for the precise quantification of the concentration of acetochlor.

Introduction

The objectives of this study were to evaluate the use of an enzyme-linked immunosorbent assays (ELISA) method and to enhance our understanding of the spatial and temporal distribution of acetochlor detections in the Le Sueur River Watershed during the 2009 monitoring season (April through June). There is a need for an effective surrogate to analyze for pesticides such as acetochlor and atrazine due to the high cost of conventional laboratory analysis. Through comparison of split sample results obtained with ELISA and conventional gas chromatography mass spectrometry (GC/MS) or liquid chromatography tandem mass spectrometry (LC-MS/MS) methods, it was theorized a meaningful evaluation as to the reliability of the ELISA method would be possible.

Background

The MDA has been monitoring pesticides in the Le Sueur River located in south-central Minnesota since 1999. In 2008, the Minnesota Pollution Control Agency (MPCA) listed portions of the Le Sueur River as impaired due to the presence of acetochlor, a common corn herbicide, at levels above the currently applicable aquatic life standard (ALS) established by the Minnesota Pollution Control Agency (MPCA). The impairments were based on acetochlor concentrations following storm events in 2001 and 2005 near the mouth of the Le Sueur River and in a small tributary known as the Beauford Ditch.

The Le Sueur River Watershed (LRW), (HUC= 7020011) is located in the south central portion of Minnesota within the greater Minnesota River Basin, the state's largest tributary to the Mississippi River (Figure 1). The LRW covers a total area of about 1,110 square miles in the counties of Blue Earth (33%), Waseca (31.8%), Faribault (22%), Freeborn (9.7%), Steele (3.2%), and Le Sueur (0.3%) (Figure 1) (Folle et al., 2009).

In the LRW, the soils are predominantly loamy in texture residing on till plains with scattered potholes and lacustrine areas. Precipitation in the watershed ranges from 29 to 33 inches annually. Land use is dominated by row crops (83%), with the rest being in residential/commercial development (6.5%), grass/pasture/hay (4%), wetlands (3.5%), and open water (2%). Of the areas in row crop production, 93% is in a two-year corn/soybean rotation (USDA, 2009).

Acetochlor is a herbicide used for the control of most annual grasses and certain broadleaf weeds in corn and other crops. It is typically applied pre-emergence and belongs to the class of chloroacetanilide herbicides that includes alachlor, dimethanamid and metolachlor. The rate of application can vary but typical applications in the LRW currently consist of reduced rate pre-emergence for early season weed control. The United States Environmental Protection Agency (USEPA) has stated that acetochlor is "moderately persistent and moderately to very mobile in soil, depending on the characteristics of the soil where it is applied." As a result, there is a relatively high potential for acetochlor residues to reach ground and surface water" (USEPA).

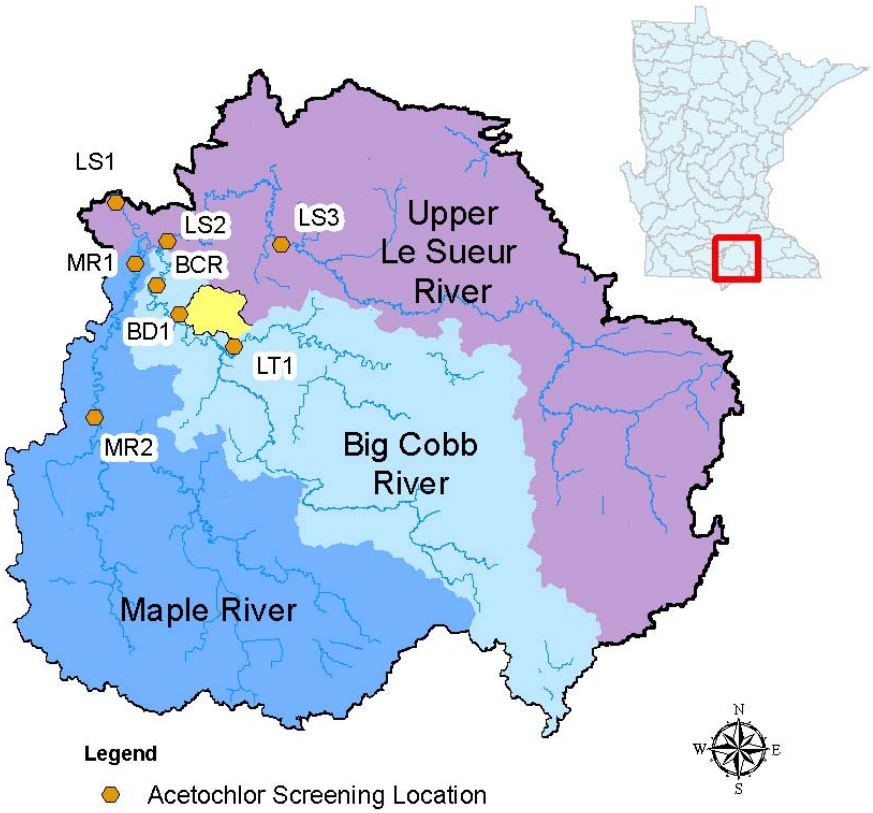

Figure 1. Le Sueur River Watershed with the three major sub-watersheds and the Beauford Ditch Watershed identified along with the sampling locations.

A primary concern in the use of ELISA for evaluating acetochlor concentrations is the potential for cross-reactivity with other chloroacetanilide herbicides and/or their degradates. Based on a review of the acetochlor ELISA information provided by the manufacturer, Abraxis, metolachlor represented the greatest potential for cross-reactivity (see Appendix A). MDA pesticide data obtained for the Le Sueur River and Beauford Ditch through GC/MS analysis over the last several years indicated a range of metolachlor concentrations from non-detect to 3.7 µg/L, with concentrations in excess of 1 µg/L representing less than five percent of the samples collected. In the instances where metolachlor concentrations exceeded 1 µg/L, there was typically a corresponding acetochlor concentration in excess of 1 µg/L. The exceptions to this pattern were samples collected in February, March and early April that exhibit elevated metolachlor concentrations without a corresponding elevated acetochlor concentration. The presence of metolachlor in these samples was generally attributed to fall application of the product. Although registered for fall application in Minnesota, acetochlor is typically not applied in the fall. Due to these issues, the experimental design for this project considered the timing and anticipated concentrations of the two chemicals. In addition, analytical laboratory GC/MS confirmation of all ELISA sample concentrations that exceeded 1 µg/L was performed.

The eight locations sampled as part of this study in the LRW are presented in Table 1 and Figure 1. The watersheds included as part of this study range in size from approximately 5,100 acres to over 700,000 acres. The Upper Le Sueur, Big Cobb Outlet and Maple River Outlet represent the three major tributaries to the Le Sueur River. The channels of all of the major tributaries become more deeply incised and the landscape changes to include many deep ravines and gullies as they approach the mainstem of the Le Sueur River. The Le Sueur River near St. Clair, Little Cobb River, Beauford Ditch and Upper Maple River represent areas typical of the upper portions of the LRW. These watersheds consist of flatter landscapes where the channels are not as deeply incised and networks of agricultural drainage ditches and subsurface tile facilitate water movement and enhance agricultural productivity.

Table 1. The Le Sueur River Watershed sampling locations for ELISA analysis.

Site Name (Road Crossing)	Site Code	Project	Drainage Acres
Le Sueur River Outlet (Hwy 66)	LS1	MDA	710,041
Upper Le Sueur River (CR 8)	LS2	MSU	285,189
Le Sueur River near St. Clair (CR 28)	LS3	MSU	225,078
Big Cobb River Outlet (CR 16)	BCR	MSU	195,145
Little Cobb River(CR 16)	LT1	MDA/MSU	82,868
Beauford Ditch (Hwy 22)	BD1	MDA/MSU	5,111
Maple River Outlet (CR 35)	MR1	MR CWP	216,879
Upper Maple River (CR 18)	MR2	MR CWP	197,362

MDA - Minnesota Department of Agriculture
MSU - Minnesota State University Mankato Water Resources Center
MR CWP – Maple River Clean Water Partnerships

Previous Studies

Several published studies have verified the utility of ELISA or immunoassay type of analysis for screening water samples and many have compared ELISA analysis with more conventional techniques, such as GC/MS, with good results. A study published in the Journal of Environmental Science and Technology (Casino et al., 2001) looked at detection limits for metolachlor, alachlor, and acetochlor ELISA analysis under best conditions (e.g., laboratory prepared and analyzed samples), all of which were less than 1 µg/L. The ELISA analysis was found to be highly specific, showing little or no cross-reactivity to other similar compounds. Recoveries obtained (mean values ranging between 90 and 98 percent) confirmed the potential usefulness of ELISA-type analysis in water quality studies.

A study conducted in North Carolina (Holman et al., 2000) evaluated pesticide presence in surface water supply intakes using ELISA, with GC/MS confirmation for detections over 1 µg/L. Results indicated that the ELISA tests for the 11 pesticides evaluated (which included atrazine, metolachlor, acetochlor and 2,4-D) provided good evidence as to the presence or absence of pesticides. Percent recoveries in spiked samples ranged

from 87 to 103 percent for the individual compounds. Acetochlor was not detected in the intake supplies using ELISA, so it was only evaluated in the laboratory portion of the study where recoveries ranged from 96 to 98 percent.

Methods

Sampling Methods

Samples collected as part of this study consisted of grab and equal-time increment composites. Grab samples were collected at the majority of the sites either by using extension poles with the sample bottle attached to the end, weighted samplers lowered from a bridge deck or wading out and simply lowering the sample bottles into a representative portion of the stream. Composite sample collection occurred at the Le Sueur River Outlet site (LS1) and at Beauford Ditch (BD1). The composite samples were collected by ISCO autosamplers that were activated based on an increase in stage, and pulsed every hour or every two hours to deliver a 48 or 96 hour equal-time increment composite sample. Composite samples were collected in glass jars under refrigerated conditions. Grab samples were also collected at these two locations. Samples were not simultaneously collected from all locations. Samples were collected during the same storm events and an effort was made to collect samples from each major storm event.

Sampled water was placed in 950 ml glass amber bottles, from which the 50 ml ELISA aliquot was removed for pesticide analysis. Grab and composite samples were stored in coolers with ice or in a refrigerator until analysis was completed. Because the ELISA analysis was used to screen samples for concentrations greater than 1 μg/L for eventual analysis by GC/MS, it was necessary to hold and refrigerate the remaining 900 ml portion of the sample pending analysis results.

Analytical Methods

Acetochlor ELISA analysis was conducted at the University of Wisconsin-Stevens Point Trace Organics Laboratory. The ELISA kit, manufactured by Abraxis, was used in this study, which has a reported detection range of 0.10 to 2.5 μg/L (Appendix A). All ELISA samples were pre-filtered at the laboratory with a 0.45 micron filter prior to analysis of the water. Abraxis reports cross-reactivity with other chloroacetanilides and/or their degradates in the Acetochlor ELISA kit fact sheet, which is a primary concern in using ELISA for evaluation of acetochlor. Based on a review of the acetochlor ELISA information provided by Abraxis, metolachlor represented the greatest potential for cross-reactivity when compared to historical Le Sueur River pesticide monitoring data.

To evaluate cross-reactivity and general ELISA performance, approximately one half of the samples collected for pesticide analysis were submitted to the MDA laboratory for GC/MS analysis for the analytes listed in Appendix B. A smaller subset of samples (eight) were analyzed using LC/MS-MS for eight chloroacetanilide degradates including the degradates of acetochlor and metolachlor. The LC/MS-MS analyte list is presented in Appendix C. This total included all ELISA samples having acetochlor concentrations in excess of 1 μg/L. Because there was a delay pending the ELISA results, some of the GC/MS samples submitted exceeded the normal three week holding time for pesticide

analysis. Holding times generally ranged from three to five weeks prior to analysis by GC/MS.

Results

Acetochlor ELISA Duplicate Analysis

Approximately 90 acetochlor ELISA samples were collected in this study. Ten of the 90 samples represented duplicates that were submitted for ELISA analysis, which included a mixture of grab and composite samples from multiple sites. A comparison of the samples collected for duplicate ELISA analysis is presented in Figure 2. The mean relative percent difference (RPD) for the 10 sample pairs was 19 percent.

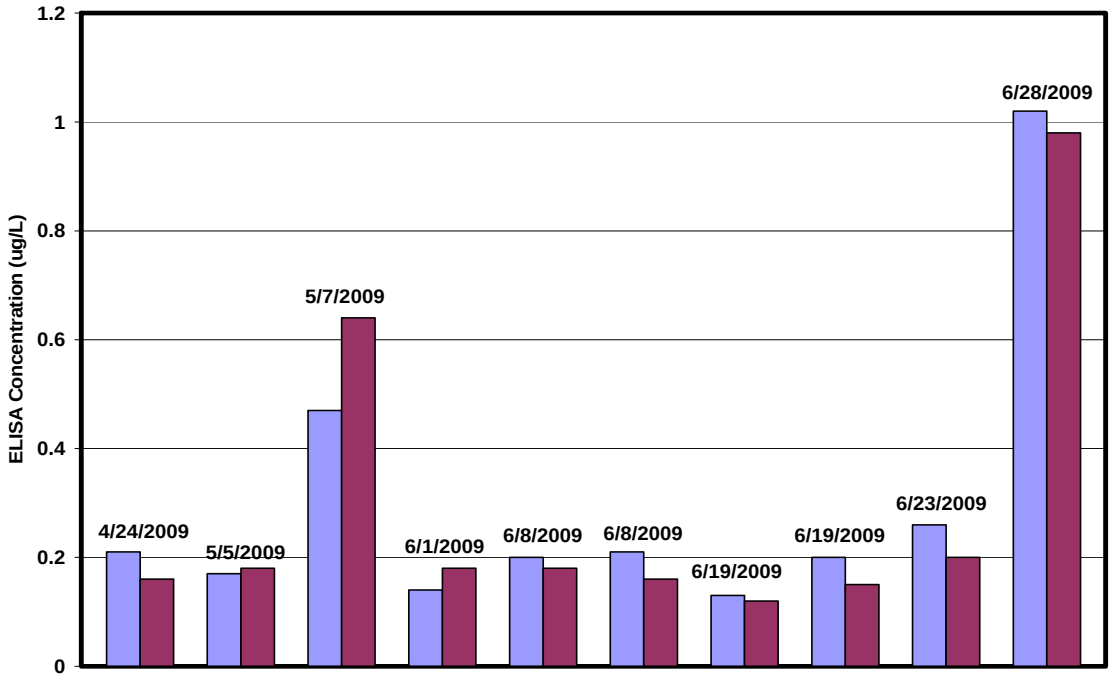

Figure 2. A comparison of ELISA duplicate analysis from the Le Sueur River Watershed sites.

ELISA Detections

Without the ten duplicate samples presented in Figure 2, there were 80 non-QA/QC samples submitted for acetochlor ELISA analysis. Of these, only one sample was reported to have a concentration below the 0.10 µg/L method detection level. The median concentration of acetochlor in the ELISA samples was 0.22 µg/L; the maximum concentration detected in the ELISA analysis was 2.28 µg/L. These values are below the 3.6 µg/L aquatic life standard established by the Minnesota Pollution Control Agency. Detection statistics for the ELISA analysis are presented in Table 2. Samples were equally distributed between baseflow and stormflow conditions with slightly higher median and maximum concentrations occurring during stormflow periods.

Table 2. Summary statistics from the acetochlor ELISA analysis in the Le Sueur River watershed.

	Sample #	Frequency of Detection	Minimum (µg/L)	Median (µg/L)	Maximum (µg/L)
Baseflow	41	100%	0.12	0.21	1.47
Stormflow	39	97%	< 0.10	0.31	2.28
All Samples	80	99%	< 0.10	0.22	2.28

Acetochlor ELISA results for the individual sampling locations are presented in Table 3. Median concentrations ranged from 0.18µg/L to 0.29 µg/L. The highest median concentration measured came from station LS1, which is near the mouth of the Le Sueur River. Coincidently, LS1 also had the most samples collected from it out of the eight sites sampled. There does not appear to be a clear relationship between the size of the individual watersheds and the concentrations measured.

Of the eight sites that were sampled, all sites had relatively similar median concentrations as presented in Table 3. This likely relates to the fact that a large percentage of the LRW has similar land use, agronomic systems, and hydrology. Without specific information on acetochlor usage in the watersheds, it is difficult to ascertain if the variability observed is related to product use and/or management, climate factors or other characteristics of the watersheds. In general there does not appear to be a clear relationship between the size of the individual watersheds and the concentrations measured. The data shows that samples collected at LS1 (Le Sueur outlet) are fairly consistent with acetochlor concentrations in contributing subwatersheds within the basin.

Table 3. Summary statistics from the acetochlor ELISA analysis in the Le Sueur River watershed for each of the eight sampling locations.

	LS1	LS2	LS3	BCR	LT1	BD1	MR1	MR2
Sample #	14	8	10	9	9	10	11	9
Watershed Acres	710,041	285,189	225,078	195,145	82,868	5,111	216,879	197,362
Median (µg/L)	0.29	0.19	0.18	0.23	0.23	0.24	0.18	0.21
Max (µg/L)	1.13	0.74	1.56	1.38	1.07	0.89	1.89	2.28
Min (µg/L)	0.13	0.12	0.17	0.16	0.13	0.13	0.15	<0.10

GC/MS Verification

Of the 80 non-QA/QC samples collected for ELISA analysis, 39 samples were submitted to the MDA laboratory for Base Neutral (BN) analysis using GC/MS (Appendix B). In addition, eight samples were submitted for chloroacetanilide degradate analysis by the MDA laboratory. Ten of the samples had a reported acetochlor ELISA concentration greater than 1 µg/L. A comparison of ELISA analysis results with the GC/MS results is presented in Figure 4. The graph suggests a strong relationship between the two methods ($R^2 = 0.80$).

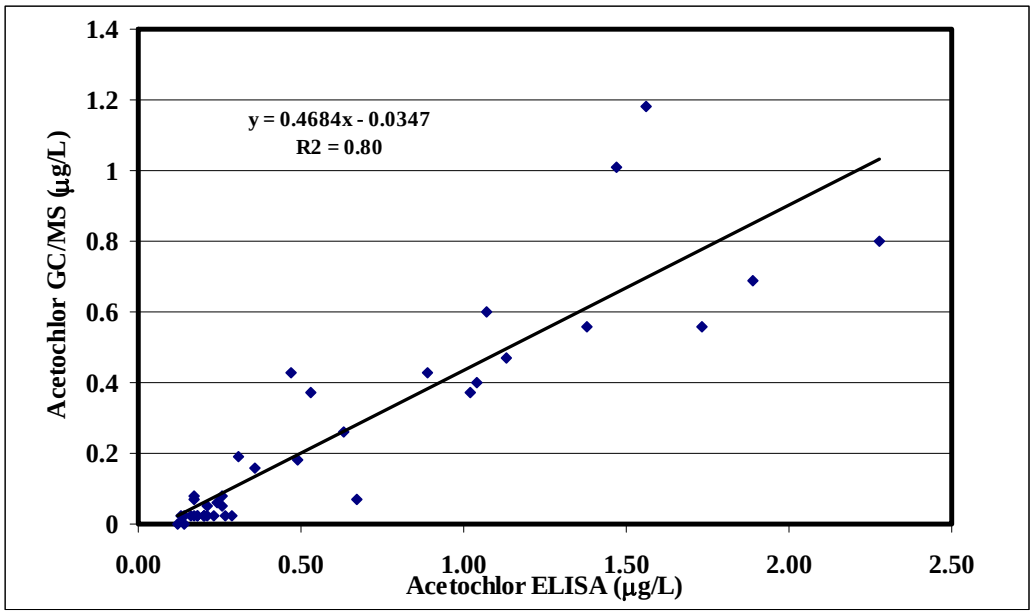

Figure 3. A comparison of acetochlor ELISA analysis and GC/MS analysis.

As was discussed in the "Background" section, the primary cross-reactivity concern with the samples from the LRW appeared to be related to the presence of metolachlor. Figure 5 presents the acetochlor ELISA concentration compared to the cumulative pesticide concentration for acetochlor and metolachlor from the GS/MS analysis. Combining the two compounds gives a stronger correlation than acetochlor alone (R^2=*0.92*) suggesting cross-reactivity with metolachlor may be occurring.

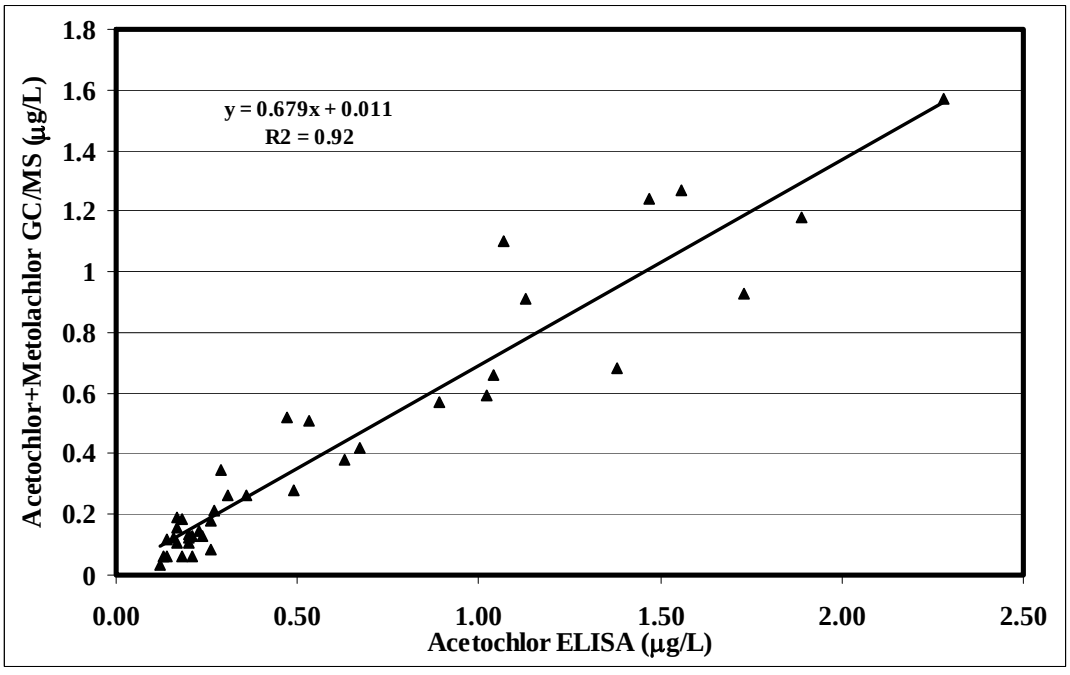

Figure 4. Relationship between the acetochlor ELISA analysis and the combined acetochlor and metolachlor GC/MS concentrations.

It is possible that the presence of metolachlor in samples with a higher acetochlor concentration may have a greater impact on the ELISA analysis as noted by the greater variability at higher concentrations. The greater variability may also be due to the presence of other chemicals. It is also apparent that the ELISA analysis generally tends to over-predict the amount of acetochlor present in the sample.

The results of the chloroacetanilide degradate analysis are presented in Table 4. There appears to be no clear relationship between the ELISA method and the presence of degradates. However, the number of samples collected and the acetochlor ELISA concentration range were both too small to adequately evaluate the effects of the individual degradates. Based on the literature provided by the manufacturer (appendix A), the concentration of chloroacetanilide degradates measured in this study should not have resulted in significant cross-reactivity in the acetochlor ELISA analysis.

Table 4. Results from the acetochlor ELISA analysis compared with GC/MS results for acetochlor and metolachlor and related LC/MS results for chloroacetanilide degradates measured at LS1 and BD1.

Date	ELISA Acetochlor (µg/L)	GC/MS Acetochlor (µg/L)	GC/MS Metolachlor (µg/L)	LC/MS Acetochlor ESA (µg/L)	LC/MS Acetochlor OXA ((µg/L)	LC/MS Alachlor ESA (µg/L)	LC/MS Metolachlor ESA (µg/L)	LC/MS Metolachlor OSA (µg/L)
5/29/09	0.14	ND	0.12	0.69	0.32	0.23	0.86	0.14
6/8/09	0.20	P	0.11	0.72	0.24	0.19	0.88	0.14
6/29/09	0.12	ND	P	0.75	0.21	0.24	0.96	0.18
5/5/09	0.17	0.08	0.11	0.39	0.2	0.11	0.71	0.13
6/1/09	0.14	P	P	0.44	0.2	0.18	0.79	0.18
6/8/09	0.21	0.05	0.08	0.57	0.37	0.21	0.79	0.26
6/15/09	0.24	0.06	0.07	0.57	0.4	0.19	1.32	0.24
6/23/09	0.26	0.08	0.1	1.84	2.29	0.1	0.74	0.28

- "ND" not-detected at method reporting limit.
- "P" indicates the compound was present but below quantifiable levels.

Conclusions

The acetochlor ELISA method produced comparable results to the GC/MS methods ($R^2 = 0.80$). In general, the acetochlor ELISA method tended to slightly over-predict the acetochlor concentration when compared to the acetochlor concentration measured by GC/MS. There appeared to be a cross-reactivity issue with metolachlor, as the total concentration (acetochlor and metolachlor summed) from the GC/MS method showed a stronger correlation with the acetochlor ELISA concentration ($R^2 = 0.92$). Analysis of chloroacetanilide degradates showed limited to no cross-reactivity to the ELISA acetochlor method but the number of samples was limited.

Comparing acetochlor concentrations from the eight sites in the Le Sueur watershed yielded relatively similar median concentrations for the period evaluated. It is possible that the similarity of concentrations is a function of watershed characteristics, hydrology and climate as well as similar acetochlor usage and management although no information is available on acetochlor use in the watershed.

This study indicates that the acetochlor ELISA method is a reasonable screening tool for evaluating acetochlor concentrations in surface water and, when combined with GC/MS verification for split samples above a pre-determined critical concentration, is a reasonable surrogate for high-cost, conventional laboratory analysis. However because of the potential for cross-reactivity with metolachlor, which is a common contaminant in Minnesota waters, and a slight bias towards high (more protective) values in sampling results, the acetochlor ELISA is not suitable as a method for the precise quantification of the concentration of acetochlor.

References

Acetochlor ELISA Kit, 100T, PN 500021. Manufactured by Abraxis LLC, Warminster, PA. WEB: http://www.abraxiskits.com

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Appendix A. Abraxis Acetochlor ELISA Fact Sheet

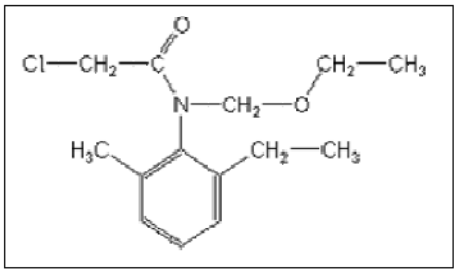
ELISA Kit for Agricultural Pollutants

Acetochlor ELISA Kit

(Magnetic Particle Format)

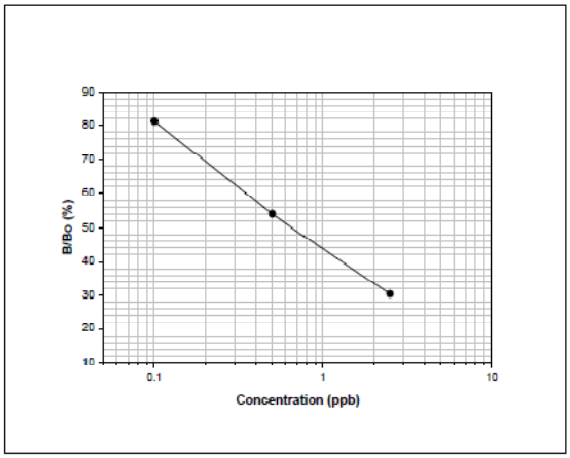
- ◇ The antibody binds Acetochlor and related Chloroacetanilides and does not cross-react with other non-related agricultural compounds.
- ◇ The assay range is between 0.10 ppb and 2.5 ppb. This supersensitive assay allows the determination of Acetochlor and related Chloroacetanilides in a range of environmental samples (water, soil, sediment, fish plasma, etc.).
- ◇ Direct sample. No time-consuming sample extraction or the use of hazardous organic solvents.
- ◇ Total time for measurement is less than 45 minutes.
- ◇ The kit (100 Tests), a magnetic particle format with ready to use reagents, enables faster assay kinetics, super sensitivity, and the simultaneous measurement of multiple samples at a reasonable cost.

Chemical Structure



Acetochlor is used for the control of most annual grasses and certain broadleaf weeds and yellow nutsedge. Crops include cabbage, citrus, coffee, corn, cotton, green peas, maize, onions, orchards, peanuts, potatoes, rape, soybeans, sugarbeets, sunflower, and vineyards. Acetochlor is applied pre-emergence and belongs to the class of Chloroacetanilide herbicides that includes alachlor and metolachlor. Acetochlor has been found under certain conditions to contaminate ground water, but it is mostly found in surface water. This ELISA test kit detects Acetochlor and related Chloroacetanilides in environment samples at the ppt levels.

Acetochlor Standard Curve



Samples containing Acetochlor within the dynamic range (0.1-2.5 ppb) can be directly tested in the assay after filtration.

ABRAXIS

Basic Test procedure

- Add 200 uL of sample, 250 uL enzyme conjugate, and 500 uL of antibody coupled magnetic particles. Vortex.
- Incubate for 20 minutes.
- Separate using the magnetic separator, decant and wash.
- Add 500 uL of color solution.
- Incubate 20 minutes.
- Stop the reaction and read color at 450 nm. Quantitate results.

Cross-reactivity Pattern

Cross-reactivity of the Abraxis Acetochlor ELISA expressed as the least detectable dose (LDD) which is estimated at 90% B/Bo and at the concentration required to displace 50% (50% B/Bo).

Compound	LDD (ppb)	50% B/Bo (ppb)
Acetochlor	0.042	0.60
Alachlor	0.045	0.70
Metolachlor	0.110	1.60
Butachlor	0.92	20
Alachlor Sulfonic Acid	9.2	224
Acetochlor Sulfonic Acid	15	78
Alachlor Oxalinic Acid	16.8	496
Metalaxyl	68	1,600
Acetochlor Oxalinic Acid	130	680
Propachlor	8,000	>10,000

The following compounds demonstrated no reactivity in the Acetochlor Assay when tested at concentrations up to 1,000 ppb: aldicarb, aldicarb sulfoxide, aldicarb sulfone, atrazine, ametryn benomyl, butachlor, butylate, captan, carbaryl, carbendazim, carbofuran, 2,4-D, 1,3-dichloropropene, dinoseb, MCPA, metribuzin, PCP, picloram, propazine, simazine, terbufos, thiabendazole, thiophanate-methyl.

Kit Format

Acetochlor ELISA Kit (Magnetic Particle format, 100T) PN 500021

Manufactured by
Abraxis LLC
54 Steamwhistle Drive
Warminster, PA 18974
Phone: (215) 357-3911
FAX: (215) 357-5232
Email: info@abraxiskits.com
WEB: www.abraxiskits.com

Appendix B: Base Neutral Pesticide List.

Common Name	Type	MRL (µg/L)
Acetochlor	Herbicide	0.05
Alachlor	Herbicide	0.05
Atrazine	Herbicide	0.05
Boscalid	Fungicide	0.30
Chlorpyrifos	Insecticide	0.10
Cyanazine	Herbicide	0.20
De-ethyl Atrazine	Metabolite	0.05
De-isopropyl atrazine	Metabolite	0.20
Diazinon	Insecticide	0.12
Dimethenamid	Herbicide	0.05
Dimethoate	Insecticide	0.22
EPTC	Herbicide	0.23
Fonofos	Insecticide	0.10
Malathion	Insecticide	0.09
Metolachlor	Herbicide	0.07
Metribuzin	Herbicide	0.10
Metribuzin DA	Metabolite	1.00
Metribuzin DADK	Metabolite	1.00
Metribuzin DK	Metabolite	1.00
Methyl Parathion	Insecticide	0.12
Myclobutanil	Fungicide	0.20
Pendimethalin	Herbicide	0.08
Phorate	Insecticide	0.12
Propiconazole	Fungicide	0.20
Tebucanazole	Fungicide	0.20
Tebuprimiphos	Fungicide	0.10
Terbufos	Insecticide	0.19
Tetraconazole	Fungicide	0.15
Trifluralin	Herbicide	0.17

Appendix C: Chloroacetanilide Degradates Analyte List.

Compound	MRL [µg/L]
Acetochlor ESA	0.07
Acetochlor OXA	0.07
Alachlor ESA	0.07
Alachlor OXA	0.07
Dimethenamid ESA	0.07
Dimethenamid OXA	0.07
Metolachlor ESA	0.07
Metolachlor OXA	0.07