
Immunoassay Methods of Acetochlor Detection

Review of Existing Immunoassay Kits for Screening of Acetochlor and Other Acetanilides in Water

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I. SUMMARY

As part of the conditional registration of acetochlor in March 1994, the Acetochlor Registration Partnership (ARP) agreed to provide immunoassay methods for cost effective state monitoring of ground and surface water samples

for the presence of acetanilide herbicides. This report is submitted to the Environmental Protection Agency in fulfillment of that agreement.

At the time of registration of acetochlor, only specific acetanilide assays, directed toward the detection of a single analyte had been reported. Whilst engaged in an assessment of the potential utility of these specific immunoassays, the ARP became aware of a class-specific assay in pre-commercial development by Millipore Corporation. This acetanilide immunoassay will be available in a microtiter plate format for use in experienced laboratories with appropriate equipment and an automated format available as an analytical service from the Environmental Health Laboratories (EHL) in South Bend, Indiana.

A summary of the performance and characteristics of the existing analyte specific assays has been compiled and included within this report. Also experimental data are presented for an ARP sponsored independent evaluation of the Millipore assay at the Water Quality Laboratory (WQL) of Heidelberg College in Tiffin, Ohio and the results of the analysis of blind fortified surface and ground water samples at the both the WQL and the EHL.

Five commercially available analyte specific acetanilide immunoassays were evaluated for cross-reactivity with acetochlor. Two of the assays were designed for metolachlor and three were directed toward alachlor. Three of the assays were reported to have some cross-reactivity towards acetochlor but the sensitivities as measured by I50 (concentration of analyte resulting in a 50% inhibition of control response) were from 6.55 to 70 ppb acetochlor. Based on the typical characteristics of immunoassay dose-response curves, these levels of sensitivity were considered inadequate to provide sufficient precision of measurement at lower concentrations of interest.

The pre-commercial acetanilide assay of Millipore was reported to have I50's for metolachlor, acetochlor and alachlor of 0.26 ppb, 1.7 ppb and 4.9 ppb, respectively, suggesting that it offers the best prospects for an acetanilide screen including acetochlor. The independent WQL evaluation of this kit confirmed the sensitivity of the assay for the reported acetanilides as well as demonstrating that dimethenamid is also detected with an I50 of 0.5 ppb. Atrazine and the common soil metabolites of alachlor and acetochlor were not detected by the assay at concentrations up to 500 ppb. Calibration of the assay using acetochlor calibrators provided by Millipore was reproducible and an acceptable level of accuracy for acetochlor in reagent water was demonstrated at 0.1 and 1.0 ppb. The within-assay and between-assay precision varied with the level of measurement but were typical of this type of immunoassay (% CV 8.3 to 35.2%). The automated assay available from the EHL gave similar results except with slightly better precision as would be anticipated for instrumented operation.

The analysis of blind surface and ground water samples fortified with acetochlor at 0.1 or 1.0 ppb provided a direct comparison of the manual operation of the

acetanilide assay at WQL with the automated version available at the EHL using samples with a typical matrix composition. In addition, since these samples were analyzed before and after fortification by GC/MS for corn herbicides, it was possible to establish potential interferences due to the presence of other acetanilides. The results of the blind analyses by the WQL and the EHL were in general agreement but differed significantly from the GC/MS in their estimation of acetochlor level for the unfortified and the 0.1 ppb fortifications. These discrepancies were shown to be due to the presence of an average of 0.091 ppb metolachlor in the surface water samples. Improved agreement among all assays was obtained for the 1.0 ppb fortifications.

The results described in this report indicate that the acetanilide immunoassay in pre-commercial development by Millipore when used as a plate kit or in an automated format has sufficient accuracy and precision to serve as a screen for acetochlor in water at 1.0 ppb. Use of the assay as a screen at 0.1 ppb was considered to be unadvisable due to low precision at this level and the fact that the lowest calibrator provided in the kit was 0.1 ppb acetochlor. The assay was shown to have improved precision at 0.2 ppb and to be within the linear range of the standard curve at this level. These observations suggest that 0.2 ppb would be a reasonable low level threshold for application of the assay.

The acetanilide immunoassay of Millipore is not spectific and does not differentiate among alachlor, acetochlor, metolachlor and dimethenamid but rather detects their presence in varying degrees. Therefore, it is imperative that any positive detects obtained using this immunoassay as a plate kit or in an automated format be confirmed with another analytical method, such as GC/MS, to establish the presence and level of acetochlor in water.

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II. INTRODUCTION

As part of the conditional registration of acetochlor in March 1994, the EPA requested that the Acetochlor Registration Partnership provide immunoassay methods for cost effective state monitoring of ground and surface water samples for the presence of acetanilide herbicides.

The ARP has adopted a structured approach towards fulfilling this requirement. In the first instance, this has involved discussions with commercial immunoassay kit manufacturers, in order to gain a clear understanding of the potential issues associated with the development, validation, and use of appropriate methods. Subsequent discussions with possible end-users have served to define additional criteria for the acceptability of such methods in routine use.

On the basis of these initial findings, the ARP has reviewed the potential applicability of currently available immunoassays against the criteria listed below:

- Only commercially available immunoassays are suitable for the proposed application. This reflects the need to ensure a guaranteed, quality-assured supply to the end-user, with an appropriate level of after-sales support.
- The chosen assay must be sufficiently rugged to operate reliably in the hands of end-users who may not have extensive experience in the use of this technology.
- The assay must offer the prospect of a rapid, high-throughput screen for samples prior to confirmatory analysis of positive detects.
- The assay should perform with a suitably high degree of reliability at the requisite levels. The incidence of "false negatives" (samples incorrectly identified as not containing acetanilides above a specified threshold) must be suitably low. The permissible incidence of "false positives" may be relatively higher, but should not be so high that the potential benefits are negated by unwarranted repeat analysis.
- The assay should be able to detect those acetanilides currently used as corn herbicides, namely alachlor, acetochlor, dimethenamid and metolachlor.
- The possible inadvertent detection of interfering compounds ("cross-reactants") must be defined. The incidence of such detects must not prevent the application of the assay for detection of the specified analytes.

At the time of registration of acetochlor, only specific acetanilide assays, directed towards the detection of a single analyte, had been reported: this encompassed both commercially available assays and those developed by agrochemical companies1,2. Consequently, the ARP concentrated attention on commercially available assays (see Section IV) designed for specific acetanilides; the reported levels of cross-reactivity for related compounds in these assays were used to provide an initial assessment of their potential utility for class-specific determination of acetanilides. Additionally, whilst engaged in this process, the ARP became aware of a potential class-specific assay in pre-commercial development. The basic performance characteristics of all these assays were subsequently compared as a means of prioritizing them for further evaluation.

The factors taken into account in comparing the assays are discussed in Section III. The assays are described and compared in Section IV.

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III. FACTORS USED IN COMPARISON OF IMMUNOASSAYS

A. Dose-Response Curves

Immunoassay dose-response curves are usually defined in terms of the inhibition of binding of a labelled ligand to an antibody as a function of concentration of the analyte in a test sample. The concentration of the analyte is related to the absorbance (color) produced by the bound, labelled ligand. As the concentration of the test analyte increases, the color produced by the labelled ligand decreases. The resulting absorbance versus log [concentration] curves are sigmoidal and express the inverse relationship between concentration of the test analyte and intensity of color.

Two pieces of information are often quoted to define these curves: I50 and I10 or I20. The I50 concentration defines the limiting analyte concentration producing 50% inhibition and is the mid-point of the dose-response curve. The I10 or I20 concentration defines the limiting analyte concentration producing a 10% or 20% inhibition of color formation, respectively. A sigmoidal dose-response curve is necessarily steepest at the I50 and shallowest at its upper and lower (I10) extremes. Consequently, the relative precision is higher at the I50 and lower at concentrations represented by the lower inhibition values3.

B. Analytes of Interest

The analytes of interest are acetochlor, alachlor, metolachlor and dimethenamid. A suitable class-specific assay must be capable of identifying any sample in which any of these analytes occur at or above their respective threshold levels. It is, however, unlikely that any assay will be capable of measuring all four analytes with the same degree of sensitivity. Under these circumstances, it is important to evaluate the candidate assays in terms of their performance for all four potential analytes.

C. Cross-Reactants

An important criterion of immunoassay suitability is the selectivity or specificity of the assay for the analytes of interest. Interference caused by materials of related structure can lead to a high level of "false positives" and severely limit the usefulness of the assay for measuring acetochlor, alachlor, metolachlor and dimethenamid. Previous studies with an alachlor immunoassay have shown the potential of metabolites such as alachlor ethane sulfonic acid to cause false positive results4,5.

The state ground and surface water programs outlined in the acetochlor registration agreement do not include degradates of acetochlor since these materials are not of toxicological concern. Therefore, an assessment of the cross- reactivity of degradates or metabolites of the analytes is a key factor in determining the suitability of an assay.

D. Threshold Screening Levels

The use of immunoassays to screen for the presence of analytes in environmental samples has been successfully tested for a number of analytes including alachlor6 and atrazine7. The need for standardized validation of screening procedures has been recognized and a number of groups including the Association of Official Analytical Chemists (AOAC), Analytical Environmental Immunochemical Consortium (AEIC) and International Union of Pure and Applied Chemistry (IUPAC) are reported to be developing guidelines for the evaluation of immunoassay kits8. The need to verify immunoassay results at a specified threshold point by an alternative technique such as GC/MS is generally recognized as a requirement of a suitable validation program.

The acetochlor registration agreement has specified the detection of acetochlor at 0.10 ppb in ground water in the state monitoring program as a "trigger" for further regulatory action. In other monitoring programs outside of the Prospective Ground Water (PGW) program or the State Monitoring Programs, detection of acetochlor at 0.20 ppb requires follow up action. Regulatory action for the State Surface Water Monitoring Program is based on exceeding an annual time-weighted mean concentration of 2.0 ppb for acetochlor or a single peak concentration of 8.0 ppb for this analyte.

In determining the suitability of an immunoassay for use as a screen for threshold levels of acetochlor and other corn herbicides two action levels are worthy of consideration:

1. 0.1 - 0.2 ppb and
2. 1.0 - 2.0 ppb.

Sufficient sensitivity at the lower level would provide an assay capable of satisfying both thresholds since the more concentrated samples could be easily diluted.

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VI. COMPARISON OF COMMERCIALLY AVAILABLE IMMUNOASSAYS FOR ACETANILIDES

Acetanilide immunoassays either currently commercially available, or in pre-commercial development, are listed below:

Metolachlor (Ohmicron)
Metolachlor 1.0 (Idetek/Quantix)
Alachlor 1.0 (Idetek/Quantix)

Alachlor (Millipore)
Alachlor (Ohmicron)
Acetanilide (Millipore)

The first five of these are already commercially available, and are promoted as specific assays for the title compound. The class-specific acetanilide kit is currently in evaluation/development by Millipore. The properties of these assays are described below.

. **Metolachlor (Ohmicron)**

The Metolachlor Ohmicron RaPID Assay Kit9 is intended for the detection of metolachlor in water and soil. The key assay characteristics are outlined in Table 1. The assay shows high sensitivity towards metolachlor (I10 = 0.05 ppb; I50 = 0.85 ppb), and some cross-reactivity towards acetochlor (I10 = 0.06 ppb; I50 = 6.55 ppb). The level of cross-reactivity towards alachlor is, however, comparatively low (I10 = 1.30 ppb; I50 = 84.0 ppb), making the assay of limited use for detection of the latter.

Notably, metalaxyl, N-(2,6-dimethyl-phenyl)-N-(methloxyacetyl)alanine methyl ester, also shows strong cross-reactivity, comparable to that of acetochlor, and might therefore constitute a significant source of false positive detects for chloroacetanilides were this kit to be used.

A. Metolachlor 1.0 (Idetek/Quantix)

This assay10 shows high specificity towards metolachlor (I50 = 0.75 ppb). The levels of cross-reactivity to both alachlor and acetochlor are very low (I50 = 100 ppb and 70 ppb, respectively). I10/20 values are not quoted in the technical data sheet, but the I50 values indicate that this assay would be of no use for the detection of either alachlor or acetochlor.

B. Alachlor 1.0 (Idetek/Quantix)

This assay11 is specific for alachlor (I50 = 0.48 ppb). The levels of cross-reactivity towards acetochlor (I50 = 23 ppb) and metolachlor (I50 = 80 ppb) indicate that the assay would not be of use for the detection of the compounds. Limited cross-reactivity was demonstrated for alachlor metabolites as indicated: alachlor sodium oxanilate (I50 = 50 ppb), alachlor sulfonic acid (I50 = 10 ppb) and alachlor sulfinyl acetic acid (I50 = 7 ppb). I10/20 were not reported.

C. Alachlor (Millipore)

The EnviroGard Alachlor Plate Kit12 has a least detectable dose for alachlor of 0.046 ppb and an I50 of 0.6 ppb. The least detectable dose of metolachlor is 0.6 ppb, with an I50 of 40 ppb, again indicating that the assay would be of little utility for metolachlor detection. No data describing the cross-reactivity of acetochlor in this assay were reported.

D. Alachlor (Ohmicron)

The characteristics of the alachlor Ohmicron RaPID Assay Kit13 are summarized in Table 2. The assay does not have sufficient cross-reactivity towards metolachlor (I10/20) to form the basis of a useful screen for acetanilides. In addition, there is significant inadvertent cross-reactivity to the ethane sulfonic acid metabolite of alachlor.

E. Acetanilide (Millipore)

The EnviroGard Acetanilide Plate Kit14 is the only one of the assays described which is primarily intended as a class-specific screen for acetanilides including acetochlor. The assay characteristics are summarized in Table 3. The assay shows the highest sensitivity towards metolachlor (I10 = 0.02 ppb; I50 = 0.25 ppb), but also has strong cross-reactivity towards acetochlor (I10 = 0.02 ppb; I10 = 1.7 ppb) and alachlor (I10 = 0.55 ppb; I50 = 4.9 ppb).

F. Summary

Table 4 shows a direct comparison of the six assays with respect to their capabilities to detect metolachlor, acetochlor and alachlor. Published data for dimethenamid were not available for any kit. The metolachlor (Idetek and Ohmicron) and alachlor (Idetek, Millipore and Ohmicron) assays are all clearly deficient in their capabilities to detect at least one of the three analytes. This is most readily demonstrated where I10/20 values, corresponding to the least detectable doses, are in excess of the concentrations of interest. In those cases where I10/20 values are not quoted, large I50 values indicate that the sigmoidal dose-response curves must be very shallow, and therefore imprecise, if indeed even measurable, at the low concentrations of interest.

The Millipore Acetanilide assay offers the best prospects for a acetanilide screen including acetochlor. The least detectable doses for alachlor, acetochlor and metolachlor are relatively low, and the ranges of the respective dose-response curves appear sufficiently narrow to enable reasonably precise measurements to be made.

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VII. EVALUATION OF ENVIROGARD™ ACETANILIDE PLATE KIT

. Background

The pre-commercial development of an acetanilide immunoassay by Millipore Corporation was confirmed on December 15, 1994 with Brian A. Skoczenski, Vice President of Research and Development of Immunosystems Inc., a division of Millipore. Based on this information it was clear that the EnviroGard™ Acetanilide Plate Kit offered the best prospects for an acetanilide screen and the ARP was able to obtain, by special order, twelve kits for independent evaluation.

The ARP selected the Water Quality Laboratory at Heidelberg College in Tiffin, Ohio to conduct an evaluation of the acetanilide kit because of their extensive experience in water analysis using a variety of methods and their prior work with Millipore immunoassays4. A protocol was written which specified the design of the study and was finalized by the signature of the study director on January 4, 1995. The study was conducted with clear guidelines for record keeping and documentation but not under the specific dictates of Good Laboratory Practices (GLP) as written in 40 CFR Part 160.

The objective of the evaluation was to determine how the kit performed for the analysis of acetochlor and other corn herbicides when used by experienced investigators. Included within this evaluation was an analysis of blind coded surface and ground water samples that had been fortified with acetochlor at 0.10 and 1.0 ppb levels.

A. Principles of the Assay

The Millipore assay is a competitive ELISA method that uses a microtiter plate format (12 strips of 8 antibody-coated wells each, in a strip holder) supplied by the manufacturer with wells precoated using the acetanilide antibody. Water samples of calibration standards are added to individual wells of the microtiter plates, followed by an acetochlor-enzyme conjugate for binding to the limiting concentration of antibody during a fixed incubation period. Following a wash step, which serves to remove any unbound acetochlor-enzyme conjugate, an enzyme substrate is added. This results in the formation of a blue color in the presence of the bound acetochlor-enzyme conjugate. The intensity of the color is inversely related to the concentration of acetanilide in the sample. The actual concentration is measured by reference to a standard curve of log[concentration] versus absorbance which is generated concurrently during the analysis using standards of known concentration. Since

acetochlor calibrators are used, the concentration is expressed as acetochlor equivalents. However, since the assay has broad specificity for a number of acetanilides including alachlor, acetochlor and metolachlor, a positive response in the assay does not mean that acetochlor is present in the sample; the assay will only detect unspecified cross-reacting acetanilides.

The method performance has been evaluated in terms of the following assay characteristics:

- Reproducibility of the standard curve
- Cross-reactivity towards related analytes
- Precision and accuracy of measurement of replicate analyses of fortified samples in reagent water

The results of these evaluations are reported and discussed below.

B. Reproducibility of the Standard Curve

The acetanilide kit relies on a three point calibration curve using the following acetochlor calibrators in reagent water: 0.0 ppb (negative control or blank), 0.1 ppb, 0.5 ppb and 5.0 ppb. The standard curve is expressed as %Bo [(average absorbance of calibrator)/(average absorbance of negative control) x 100%] versus log [acetochlor]. The reproducibility of the standard curve was evaluated by analyzing in triplicate a series of acetochlor standard solutions on ten separate days. The data indicates acceptable reproducibility (%CV of 5.7 to 14.5%).

C. Cross-Reactivity

The ARP provided a certified sample of acetochlor as well as the following reference materials for testing:

0. Alachlor
1. Metolachlor
2. Dimethenamid
3. Atrazine
4. Alachlor Sulfonic Acid
5. Alachlor Oxanilic Acid
6. Alachlor Sulfinyl Acetic Acid
7. Acetochlor Oxanilic Acid
8. Aceotchlor Sulfonic Acid
9. Acetochlor Sulfinyl Acetic Acid

Compounds 5-10 represent the major soil metabolites of alachlor and acetochlor. A stock solution of each standard was prepared in methanol

and dilutions in water gave a series of solutions between 500 ppb and 0.1 ppb. Each of the aqueous solutions were tested using the acetanilide kit, sigmoidal plots of %Bo versus log [concentration] were prepared and the values of I10 (10% inhibition) and I50 (50% inhibition) were determined from the graphs. The results are shown in Table 5.

The sensitivity of the assay for acetochlor, alachlor and metolachlor was confirmed as demonstrated by the I50 values (Table 5). The acetanilide data sheet from Millipore for this assay lists a lower I50 for metolachlor than acetochlor contrary to the results of the Heidelberg College determination. The reason for this discrepency is not known but one possibility could be the use of linear plots of %Bo versus log [concentration] by Millipore. Since Heidelberg College used sigmoidal rather than linear fit of the data different estimates would be obtained. Dimethenamid was shown to be a sensitive analyte in the assay with a lower I50 than acetochlor. Atrazine and the soil metabolites of alachlor and acetochlor were unreactive even at 500 ppb demonstrating that these materials have a very low probability of giving false positives at typical environmental concentrations.

D. Accuracy and Precision

The acetanilide assay was used to measure samples, generally in triplicate, on ten different days. At total of twenty nine individual measurements were made at each fortification.

At 0.1 ppb, individual concentration measurements ranged from 0.09 - 0.29 ppb. Mean concentrations from daily measurements ranged from 0.10 - 0.26 ppb and the overall mean was 0.16 ppb.

At 1.0 ppb, individual concentrations ranged from 0.72 - 1.90 ppb. Mean daily concentrations ranged from 0.83 - 1.48 ppb, while the overall mean was 1.06 ppb.

The within-assay precision of measurement of samples fortified at 0.1 ppb ranged between 10.0 - 23.2% (CV). The between-assay precision was 32.5% (CV).

The within-assay precision of measurement of samples fortified at 1.0 ppb ranged between 8.3 - 35.2% (CV). The between -assay precision was 17.6%.

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X. ENVIROMENTAL HEALTH LABORATORIES (EHL) ACETANILIDE ASSAY

. Background

A draft Standard Operating Procedure for an automated acetanilide immunoassay was received by the Partnership on December 7, 1994 from Jerry Thoma, President of Environmental Health Laboratories (EHL) located in South Bend, Indiana. The EHL was founded in 1986 with the goal of providing drinking water analysis to both the public and private water suppliers as well as complex analytical testing for the commercial sector and state and federal contracting agencies. The laboratory is certified in 33 states and EPA Regions to perform laboratory analyses for regulated parameters monitored by Public Water Supplies for compliance with the Safe Drinking Water Act.

The EHL, in an exclusive agreement with Millipore, adapted the EnviroGard Acetanilide Plate Kit for an automated assay format. The goal of the program was to provide an automated acetanilide immunoassay service to public and private water suppliers. The first circular advertising the service was distributed January 12, 1995 and more information is available by contacting the Client Services Department of the EHL at (800) 332-4345.

A. Principle of the Assay

EHL assay relies on reagents supplied by Millipore Corporation and has been fully automated using a Bio-Tek Els1000 integrated microplate system. In all other respects the assay resembles the typical competitive ELISA method that is the basis for the EnviroGard Acetanilide Plate Kit. The Standard Operation Procedure for the EHL method 1294A contains information on the performance on the assay which will be summarized below.

B. Reproducibility of Standard Curve

The EHL method relies on a four-point standard curve using the following acetochlor calibrators in reagent water: 0.0 ppb (negative control or blank), 0.1 ppb, 0.25 ppb, 1.0 ppb and 5.0 ppb. The standard curve is determined in the same way as the acetanilide plate kit by expressing %Bo versus log [acetochlor].

Ten observations of the standard curve, each in duplicate, were made on separate occasions between October 27, 1994 and December 1, 1994. The reproducibility of the automated EHL method is acceptable and somewhat better than that observed for the manual plate kit assay (see Table 5.).

C. Cross-Reactivity

The cross-reactivity results obtained with the EHL method are shown in Table 6 and are in general agreement with the results reported in the acetanilide plate kit data insert. The EHL analysis indicates that metolachlor is a more sensitive analyte than acetochlor with I50's of 0.25 and 0.71 ppb, respectively.

D. Accuracy and Precision

The EHL assay was used to measure samples in triplicate on each of seventeen days, giving a total of fifty one individual determinations at each concentration.

At 0.1 ppb individual concentration measurements ranged from 0.081 - 0.126 ppb. Mean concentration measurements from daily determinations ranged from 0.088 - 0.119 ppb while the overall mean was 0.103 ppb.

At 1.0 ppb, individual concentrations ranged from 0.845 - 1.309 ppb. Mean concentrations from daily measurements ranged from 0.934 - 1.228 ppb, and the overall mean was 1.100 ppb.

The within-assay precision of measurement of samples fortified at 0.1 ppb ranged between 0.9 - 13.1% (CV). The mean between-assay precision was 7.9% (CV, n=17).

The within-assay precision of measurement for samples fortified at 1.0 ppb ranged from 1.7 - 17.0% (CV). The mean between-assay precision was 7.0% (CV, n=17).

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XIII. PERFORMANCE TESTING OF ACETANILIDE IMMUNOASSAYS

. Purpose

This performance test was conducted as part of the evaluation of the EnviroGard™ Acetanilide Plate Kit and the automated acetanilide immunoassay test.

A. Experimental Design

Water samples were collected from 14 finished surface water sites in the greater metropolitan area surrounding St. Louis, Missouri and from one raw surface water site on the Missouri River near Defiance, Missouri. In addition ground water from two Missouri wells, one well in Illinois and two wells in Wisconsin were also collected. These samples were analyzed by GC/MS for the presence of corn herbicides and then fortified with

acetochlor at either 0.1 or 1.0 ppb. The samples were reanalyzed by GC/MS to confirm the fortification level and identical coded sets were sent to the Water Quality Laboratory (WQL) at Heidelberg College in Tiffin, Ohio and to the Environmental Health Laboratories (EHL) in South Bend, Indiana for analysis using an acetanilide immunoassay.

B. Results and Discussion

No acetochlor or dimethenamid was detected in the surface and ground water samples analyzed by GC/MS. Low levels of atrazine and metolachlor which averaged 0.225 ppb and 0.091 ppb, respectively, were found primarily in the surface water. Alachlor was detected at trace levels that were <0.05 ppb in all cases.

Analysis of the unfortified water samples by immunoassay using the EnviroGard™ Acetanilide Plate Kit (WQL, Heidelberg College) and an automated acetanilide assay (EHL) gave results which were in general agreement but differed significantly from the acetochlor analysis by GC/MS. No acetochlor was present in these samples and yet the WQL analysis indicated an average of 0.19 ppb acetanilide with five samples <0.10 ppb and the EHL results gave an average level of 0.32 ppb acetanilide with 9 samples <0.10 ppb. The discrepancy was due primarily to the presence of metolachlor which has been shown to be a strong cross-reactant in this immunoassay.

Following fortification at 0.10 ppb with acetochlor and reanalysis by GC/MS, the water samples were shown to contain 0.091 ppb acetochlor in good agreement with the theoretical concentration. Immunoassay at WQL and EHL gave acetanilide results that averaged 0.26 and 0.37 ppb, respectively, once again reflecting the presence of other chloroacetanilides in addition to acetochlor. Fortification at 1.0 ppb with acetochlor gave immunoassay values that were more precise and accurate and in better agreement with the GC/MS. WQL and EHL measured an average of 1.20 ppb acetanilide compared with 1.09 ppb acetochlor obtained by GC/MS. This agreement between ELISA and GC/MS at 1.0 ppb acetochlor was due to the fact that interfering acetanilides were minor compared with acetochlor and the values were within the range of the immunoassay where good precision for this analyte was obtained (I50).

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XVI. CONCLUSIONS

The sensitive and specific ELISA immunoassays developed for alachlor and metolachlor have been shown in some cases to cross-react with acetochlor. However, the I50's (concentration of acetochlor required to achieve 50%

inhibition) in these assays were between 6.55 and 70 ppb (see Table 4) making them unsuitable for measuring acetochlor in water at 0.1 to 2 ppb.

A new kit,the EnviroGard Acetanilide Plate Kit, in pre-commercial development by Millipore Corporation has been shown to have greater sensitivity for acetochlor (I50 = 0.7 ppb) and broad cross-reactivity for other acetanilides including metolachlor and alachlor with I50's of 0.26 ppb and 4.9 ppb, respectively. These characteristics of the new Millipore kit give it the potential for use as a screen for the acetanilide corn herbicides including alachlor, metolachlor, acetochlor and dimethenamid in water. The Millipore acetanilide kit has also been adapted for use in an automated format by the Enviromental Health Laboratories in South Bend, Indiana and offered as a commercial service to public and private water suppliers by this analytical laboratory.

With the support of the Acetochlor Registration Partnership, an evaluation of the EnviroGard Acetanilide Plate Kit was conducted by the Water Quality Laboratory of Heidelberg College in Tiffin, Ohio. A similar evaluation of the automated format for the Millipore acetanilide kit was provided by Enviromental Health Laboratories and presented in their Standard Operating Procedure for EHL Method 1294A.

For this report four criteria were used to assess the characteristics and performance of the two assays:

Reproducibility of calibration curve
Cross-reactivity toward related analytes
Accuracy and precision of replicate analyses of acetochlor fortified reagent water
Accuracy of blind fortified surface and ground water samples

Since both assays are based on the same antibody and reagents from the same company (Millipore Corporation), similar results would be expected. Differences are likely to reflect the precision afforded by instrumented transfer and reproducible plate mixing, washing and incubation provided by the automated assay.

Both assays gave reproducible standard curves. The acetanilide plate kit utilizes a three point calibration curve with acetochlor calibrators at 0.1 ppb, 0.5 ppb and 5.0 ppb. The EHL method relies on a four point calibration that includes calibrators of 0.1 ppb, 0.25 ppb, 1.0 ppb and 5.0 ppb. Somewhat better precision was observed for the automated EHL assay.

Cross-reactivity results for both methods were consistent with those reported in the acetanilide kit technical data sheet from Millipore although differences in the absolute values for the I50's were observed. Dimethenamid was found to be a sensitive analyte for the assay with comparable levels of cross-reactivity to that

observed for metolachlor. The assay was shown to be non responsive to atrazine and the major soil metabolites of alachlor and metolachlor at levels up to 500 ppb.

Investigation of the accuracy and precision using acetochlor fortified in reagent water indicated improved accuracy and between-assay precision evident at 1.0 ppb.

A final aspect of the immunoassay evaluation was an analysis of representative blind surface and ground water samples fortified with acetochlor at 0.1 and 1.0 ppb. The surface and ground water samples were analyzed by GC/MS prior to fortification to measure the presence of indigenous acetanilides and after fortification to verify the level of acetochlor. The results of the two ELISA immunoassay systems were found to be relatively inaccurate for measuring acetochlor at 0.1 ppb but satisfactory at 1.0 ppb acetochlor. The inaccuracy at low levels of acetochlor appeared to be due to the presence metolachlor in the surface water samples.

In summary, the EnviroGard Acetanilide Plate Kit and the EHL automated acetanilide assays have sufficient accuracy and precision to serve as a screen for acetochlor in water at 1.0 ppb. Use of the assay as a screen at 0.1 ppb was considered to be unadvisable due to low precision at this level and the fact that the lowest calibrator provided in the kit was 0.1 ppb acetochlor. At 0.2 ppb, values were found to range between 0.13 ppb and 0.34 ppb and improved precision was obtained. In addition, 0.2 ppb was shown to be within the linear range of the standard curve. These observations suggest that 0.2 ppb would be a reasonable low level threshold for application of the assay.

The acetanilide immunoassay of Millipore is not specific and does not differentiate among alachlor, acetochlor, metolachlor and dimethenamid but rather detects their presence to varying degrees. Therefore, it is imperative that any positive detects obtained using this immunoassay as a plate kit or in an automated format be confirmed with another analytical method, such as GC/MS, to establish the presence and level of acetochlor in water.

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XVII. TABLES/FIGURES

Table 1.
Cross-Reactivity of Metolachlor Ohmicron RaPID Assay Kit for Acetanilides

Compound	Least Detectable Dose (I10 in ppb)	Dose-Response Mid-Point (I50 in ppb)
Metolachlor	0.05	0.85

Table 1.
Cross-Reactivity of Metolachlor Ohmicron RaPID Assay Kit for Acetanilides

Compound	Least Detectable Dose (I10 in ppb)	Dose-Response Mid-Point (I50 in ppb)
Acetochlor	0.06	6.55
Metalaxyl	0.06	5.60
Butachlor	0.26	52.0
Propachlor	1.00	2500
Alachlor	1.30	84.0

Table 2.
Cross-Reactivity of Alachlor Ohmicron RaPID Assay Kit for Acetanilides

Compound	Least Detectable Dose (I10 in ppb)	Dose-Response Mid-Point (I50 in ppb)
Alachlor	0.05	ca. 1.0
Acetochlor	n/aa	n/a
Butachlor	6.0	ca. 100
Metolachlor	5.6	ca. 80
Propachlor	6000	n/a
Alachlor sulfonic acid	0.03	n/a

XIX. a n/a = not available

Table 3.
Cross-Reactivity of EnviroGard Acetanilide Plate Kit for Acetanilides

Compound	Least Detectable Dose (I10 in ppb)	Dose-Response Mid-Point (I50 in ppb)
Metolachlor	0.02	0.25
Acetochlor	0.02	1.7
Metalaxyl	0.02	0.24
Butachlor	0.13	4.9
Propachlor	0.18	9.2
Alachlor	0.55	4.9

Table 4.
Comparison of Cross-Reactivity of Immunoassays for Corn Herbicides

		Metolachlor		**Acetochlor**		**Alachlor**	
Assay (Company)	**I10 (ppb)**	**I50 (ppb)**	**I10 (ppb)**	**I50 (ppb)**	**I10 (ppb)**	**I50 (ppb)**	
Metolachlor (Ohmicron)	0.05	0.85	0.06	6.55	1.30	84.0	
Metolachlor	n/aa	1.3	n/a	70	n/a	100	

Table 4.
Comparison of Cross-Reactivity of Immunoassays for Corn Herbicides

	Metolachlor		Acetochlor		Alachlor	
	I10 (ppb)	**I50 (ppb)**	**I10 (ppb)**	**I50 (ppb)**	**I10 (ppb)**	**I50 (ppb)**
(Idetek)						
Alachlor (Idetek)	n/a	80	n/a	23	n/a	0.6
Alachlor (Millipore)	0.6	40	n/a	n/a	0.15	5.0
Alachlor (Ohmicron)	5.6	ca.80	n/a	n/a	0.05	1.0
Acetanilide (Millipore)	0.02	0.26	0.02	1.7	0.55	4.9

XXI. a n/a = not available

Table 5.
Cross-Reactivity of EnviroGard Acetanilide Plate Kit (WQL)

Compound	**Least Detectable Dose (I10 in ppb)**	**Dose-Response Mid-Point (I50 in ppb)**
Acetochlor	ca. 0.1	1.0
Alachlor	2.0	5.0
Metolachlor	0.2	1.5
Dimethenamid	0.1	0.5
Atrazine	n/da	> 500
Alachlor Sulfonic Acid	n/d	> 500
Alachlor Oxanilic Acid	n/d	> 500
Alachlor Sulfinyl Acetic Acid	n/d	> 500
Acetochlor Oxanilic Acid	n/d	> 500
Acetochlor Sulfonic Acid	n/d	> 500
Acetochlor Sulfinyl Acetic Acid	n/d	> 500

XXII. a n/d = not determined

Table 6.
Cross-Reactivity of Automated Acetanilide Assay (EHL)

Compound	**Least Detectable Dose (I10 in ppb)**	**Dose-Response Mid-Point (I50 in ppb)**

Table 6.
Cross-Reactivity of Automated Acetanilide Assay (EHL)

Compound	Least Detectable Dose (I10 in ppb)	Dose-Response Mid-Point (I50 in ppb)
Metolachlor	0.04	0.25
Acetochlor	0.08	0.71
Metalaxyl	0.02	0.13
Butachlor	0.81	8.9
Alachlor	0.52	3.9

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XXV. REFERENCES

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