# MULTIRESIDUE ANALYTICAL METHOD FOR THE DETERMINATION OF ACETOCHLOR, ALACHLOR, ATRAZINE, DIMETHENAMID AND METOLACHLOR IN AQUEOUS ENVIRONMENTAL SPECIMENS

## 1 INTRODUCTION

### 1.1 Scope

This multiresidue analytical method has been developed for the determination of acetochlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-ethoxymethylacetamide; alachlor, 2-chloro-N-(2,6-diethylphenyl)-N-methoxymethylacetamide; atrazine, 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine; dimethenamid, 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide and metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxypropan-2-yl)-acetamide in ground water, raw surface water and finished surface water. The structures are shown in Figure 1. This methodology was developed in support of the ground and surface water programs implemented by the Acetochlor Registration Partnership (ARP).

### 1.2 Principle

This analytical method describes the analysis of the multiresidue analytes in ground water and surface water. The method involves addition of deuterated internal standard to the specimen, concentration of analytes by collection on a solid phase extraction column, elution of analytes, concentration to volume and analysis by gas chromatography/mass spectrometry.

The accuracy of the analytical method is estimated based on the recovery of known concentrations of each herbicide fortified into control water samples which are then carried through the analytical procedure.

Because regulatory action levels are set at 0.10 ppb, the method was evaluated at 0.05 ppb to ensure acceptable precision and accuracy at the action level. Based on actual fortification data for 1995, the common limit of quantitation (LOQ) for all the analytes is estimated to be 0.05 ppb[1].

## 2 MATERIALS AND REAGENTS

The following materials, equipment, and reagents are required to perform the analysis. Appropriate substitution for items is left to the discretion of the analyst unless otherwise noted and should be recorded in the raw data. Glassware and other equipment must be thoroughly cleaned to minimize contamination. Control water samples are analyzed routinely to confirm a minimum contribution of interferences to actual specimens from the reagents, solvents and glassware.

## 2.1 Equipment and Example Part Numbers

- Mettler electronic analytical balance, model AE 163
- 12 port vacuum manifold for SPE columns with transparent side walls and pressure gauge, Baxter No. 9400DK
- Reservoir, 75 mL, Varian Sample Preparation Products No. 1213-1012
- Reservoir, 75 mL with frit, Varian Sample Preparation Products No. 1213-1018
- SPE Adapter, 8 mL, Varian Sample Preparation Products No. 1213-1015
- Graduated cylinder, 250 mL, Kimax No. 20024D-250 (tolerance ± 1.4 mL)
- Octadecyl ($C_{18}$) SPE Disposable Extraction Column, 2.8 mL capacity, 500 mg sorbent weight, Varian Sample Preparation Products No. 1210-2028
- Silica (Si) SPE Disposable Extraction Column, 500 mg, Burdick & Jackson No. 9054
- Sea sand, washed, Fisher No. S25-3
- Volumetric flask, 100 mL, Kimax No. 28014100
- Graduated centrifuge tube, 5 mL, Fisher No. 05-538-35A
- Volumetric glass pipette, various sizes
- Pasteur pipette, $5^3/_4$ and 9 inch length, Fisher Nos. 13-6678-6A and B
- DuPont/Sorvall RC-5B refrigerated centrifuge, No. 50253
- Nalgene centrifuge bottle, 250 mL, Fisher No. 05-564-1
- 2 mL glass screw cap autosampler vial with teflon lined septum, National Scientific No. C4000-82W
- Fisons Instruments TRIO-2000 gas chromatograph/mass spectrometer/MassLynx data system (GC/MS/DS) or equivalent
- J & W DB-1 fused silica open tubular column (FSOT), 30 meters x 0.25 mm ID x 0.25 μm film, J & W No. 122-1032
- Guard column, Base Deactivated, 5 meter x 0.25mm ID, Restek No. 10000
- Press-Tight connector, Restek No. 20400

## 2.2 Reagents

- Acetochlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-ethoxymethylacetamide, analytical grade, ≥ 95% purity

- Alachlor, 2-chloro-N-(2,6-diethylphenyl)-N-methoxymethylacetamide, analytical grade, ≥ 95% purity

- Atrazine, 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine, analytical grade, ≥ 95% purity

- Dimethenamid, 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide, analytical grade, ≥ 95% purity

- Metolachlor, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxypropan-2-yl)-acetamide, analytical grade, ≥ 95% purity

- Deuterated acetochlor, 2-chloro-N-(2-(2'-trideutero)-ethyl-6-methylphenyl)-N-ethoxymethylacetamide, analytical grade, ≥ 92% purity

- Deuterated alachlor, 2-chloro-N-(2,6-pentadeuterodiethyl-3,4,5-deuterophenyl)-N-methoxymethylacetamide, analytical grade, ≥ 95% purity

- Deuterated atrazine, 6-chloro-N-pentadeuteroethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine, analytical grade, ≥ 95% purity

- Deuterated dimethenamid, 2-chloro-N-(2-methyl-4-trideuteromethyl-3-thienyl)-N-(2-methoxy-1-methylethyl)-acetamide, analytical grade, ≥ 95% purity

- Deuterated metolachlor, 2-chloro-N-(2-(2'-trideutero)-ethyl-6-methylphenyl)-N-(2-methoxypropan-2-yl)-acetamide, analytical grade, ≥ 84% purity

- Ethyl acetate (EtOAc), Optima Grade, Fisher No. E196

- 2,2,4-Trimethylpentane (isooctane, IO), Optima Grade, Fisher No. O301

- Methanol ($CH_3OH$), Optima Grade, Fisher No. A454

- Water, Optima Grade, Fisher No. W7-4

- Ethanol ($C_2H_5OH$), absolute-200 proof, Midwest Grain Products No. 6810-00-242-3645

- Sodium Sulfate, Anhydrous, Certified ACS, Fisher No. S421-500

- Deionized water (DI water) from a Milli-Q water purification system (Millipore Co.). This system consists of an activated carbon cartridge for the removal of organics in series with two mixed-bed ion-exchange cartridges for the removal of ionic species.

## 2.3 Reagent Preparation

Prepare an adequate quantity of the following solutions and reagents.

10% (v/v) EtOAc/IO

Using a graduated cylinder, combine 1 part of ethyl acetate and 9 parts of isooctane and mix.

50% (v/v) MeOH/water

Using a graduated cylinder, combine 1 part of methanol and 1 part of Optima water and mix.

Pre-Washed Sodium Sulfate

Combine 1500 g of sodium sulfate and 1.0 L of 10% EtOAc/IO and agitate for 15 minutes. Filter and wash with approximately 250 mL of fresh solvent under vacuum.

## 2.4 Analytical Standards

Analytical standards are prepared for two purposes: for fortifying control matrices to determine analytical accuracy and for calibrating the response of the analyte in the gas chromatographic detector. The purity of all standards must be verified before preparation of the stock solutions. All standards should be refrigerated (2-10° C) in clean amber glass bottles with foil/teflon lined screw caps. The absolute volume of the standard solutions may be varied at the discretion of the analyst, as long as the correct proportions of the solute and solvent are maintained and the changes are documented. Calibrate the analytical balance prior to weighing any analytical standard material for this method.

### 2.4.1 Standard Stock Solutions

1000 µg/mL Individual Herbicide Solutions

Weigh 0.1000 ± 0.0010 g (weight adjusted for purity) of each analytical grade herbicide into individual 100 mL volumetric flasks, dilute to volume with absolute ethanol and mix to insure complete dissolution. This solutions contains 1000 ± 10 µg/mL of herbicide. Repeat for all herbicides.

100.0 µg/mL Mixed Herbicide Solution (for extended standards)

Pipet 10.0 mL of each of the five herbicides, from their individual 1000 µg/mL solutions, into a single 100 mL volumetric flask. Dilute to volume with absolute ethanol and mix. This solution contains 100.0 µg/mL each of the five herbicides. This solution is only required for the preparation of extended analytical standards.

10.0 µg/mL Mixed Herbicide Solution

Pipet 1.0 mL of each of the five herbicides, from their individual 1000 µg/mL solutions, into a single 100 mL volumetric flask. Dilute to volume with absolute ethanol and mix. This solution contains 10.0 µg/mL each of the five herbicides.

1.00 µg/mL Mixed Herbicide Solution

Pipet 10.0 mL of the 10.0 µg/mL mixed herbicide solution into a 100 mL volumetric flask, dilute to volume with absolute ethanol and mix. This solution contains 1.00 µg/mL each of the five herbicides.

500 µg/mL Individual Deuterated Herbicide Solutions

Weigh 0.0500 ± 0.0010 g (weight adjusted for purity) of each analytical grade deuterated herbicide into individual 100 mL volumetric flasks, dilute to volume with absolute ethanol and mix to insure complete dissolution. This solutions contains 500 ± 10 µg/mL of deuterated herbicide. Repeat for all herbicides.

10.0 µg/mL Mixed Deuterated Herbicide Solution

Pipet 2.0 mL of each of the five deuterated herbicides, from their individual 500 µg/mL solutions, into a single 100 mL volumetric flask. Dilute to volume with absolute ethanol and mix. This solution contains 10.0 µg/mL each of the five deuterated herbicides.

### 2.4.2 Fortification Solutions

In order to estimate the analytical accuracy of the method with a given set of water specimens, it is necessary to fortify a certain number of control water samples with a known amount of each herbicide. Control water samples are fortified at different analyte levels across the range of anticipated concentrations. For example, 0.010 µg of analyte is necessary for a 0.05 ppb fortification of a 200 mL sample. This would be accomplished by adding 1.0 mL of a 0.010 µg/mL solution to the sample. The deuterated standards are not incorporated in the fortification solutions. Since deuterated standards are added to all control and fortified samples, recoveries are internally corrected. The solutions used to fortify control water samples may be prepared in the following manner.

1.00 µg/mL Mixed Herbicide Fortification Solution

Pipet 10.0 mL of the 10.0 µg/mL mixed herbicide solution into a 100 mL volumetric flask, dilute to volume with absolute ethanol and mix. This solution contains 1.00 µg/mL each of the five herbicides.

0.200 µg/mL Mixed Herbicide Fortification Solution

Pipet 2.00 mL of the 10.0 µg/mL mixed herbicide solution into a 100 mL volumetric flask, dilute to volume with absolute ethanol and mix. This solution contains 0.200 µg/mL each of the five herbicides.

0.020 µg/mL Mixed Herbicide Fortification Solution

Pipet 10.0 mL of the 0.200 µg/mL mixed herbicide fortification solution into a 100 mL volumetric flask, dilute to volume with absolute ethanol and mix. This solution contains 0.020 µg/mL each of the five herbicides.

0.010 µg/mL Mixed Herbicide Fortification Solution

Pipet 5.00 mL of the 0.200 µg/mL mixed herbicide fortification solution into a 100 mL volumetric flask, dilute to volume with absolute ethanol and mix. This solution contains 0.010 µg/mL each of the five herbicides.

The above solutions are adequate to fortify 200 mL control water samples in the range of 0.05 to 20.0 ppb of each analyte. Samples fortified at levels above 5.00 ppb are analyzed only with extended calibration standards. Typical fortification levels may be achieved in the following manner.

0.05 ppb fortification

Pipet 1.00 mL of the 0.010 µg/mL fortification solution into a 200 mL sample.

0.10 ppb fortification

Pipet 1.00 mL of the 0.020 µg/mL fortification solution into a 200 mL sample.

0.20 ppb fortification

Pipet 2.00 mL of the 0.020 µg/mL fortification solution into a 200 mL sample.

0.50 ppb fortification

Pipet 0.50 mL of the 0.200 µg/mL fortification solution into a 200 mL sample.

1.00 ppb fortification

Pipet 1.00 mL of the 0.200 µg/mL fortification solution into a 200 mL sample.

2.00 ppb fortification

Pipet 2.00 mL of the 0.200 µg/mL fortification solution into a 200 mL sample.

5.00 ppb fortification

Pipet 1.00 mL of the 1.00 µg/mL fortification solution into a 200 mL sample.

10.0 ppb fortification

Pipet 2.00 mL of the 1.00 µg/mL fortification solution into a 200 mL sample.

20.0 ppb fortification

Pipet 4.00 mL of the 1.00 µg/mL fortification solution into a 200 mL sample.

### 2.4.3 Deuterated Internal Standard Solution

0.10 µg/mL Mixed Deuterated Herbicide Internal Standard Solution

Pipet 10.0 mL of the 10.0 µg/mL mixed deuterated herbicide solution into a 1000 mL volumetric flask, dilute to volume with absolute ethanol and mix. This solution contains 0.10 µg/mL each of the five deuterated herbicides. Addition of 1.00 mL of this solution to each control and fortified water sample and each water specimen will result in 0.10 µg/mL each of the five deuterated herbicides in the final analytical sample when the volume is 1.00 mL.

### 2.4.4 Detector Calibration Standards

The detector calibration standards are made at convenient concentrations of each analyte. All standards should be refrigerated (2-10° C) in clean amber glass bottles with foil/teflon lined screw caps. The absolute volume of the standard solutions may be varied at the discretion of the analyst, as long as the correct proportions of the solute and solvent are maintained and the changes are documented.

Experience has shown a linear range from 5 to 1000 µg/L with our instrumentation. This is equivalent to 0.05 to 5.0 ppb in a 200 mL water sample. The extended standards are only prepared and used when the analytes exceed approximately 6.00 ppb (120% of 5 ppb).

The following is an example of GC/MS calibration standard levels. Concentrations other than the ones shown below also may be prepared and used if necessary. The GC/MS calibration standards may be prepared in the following manner.

| Vol. of 1.00 µg/mL Herbicide Solution (mL) | Vol. of 10.0 µg/mL Deuterated Solution (mL) | Final Volume (mL) | Final Analyte Concentration (µg/L) |
|---|---|---|---|
| 0.50 | 1.00 | 100 | 5.0 |
| 2.50 | 1.00 | 100 | 25.0 |

| Vol. of 10.0 µg/mL Herbicide Solution (mL) | Vol. of 10.0 µg/mL Deuterated Solution (mL) | Final Volume (mL) | Final Analyte Concentration (µg/L) |
|---|---|---|---|
| 1.00 | 1.00 | 100 | 100.0 |
| 2.00 | 1.00 | 100 | 200.0 |
| 4.00 | 1.00 | 100 | 400.0 |
| 7.00 | 1.00 | 100 | 700.0 |

| Vol. of 100.0 µg/mL Herbicide Solution (mL) | Vol. of 10.0 µg/mL Deuterated Solution (mL) | Final Volume (mL) | Final Analyte Concentration (µg/L) |
|---|---|---|---|
| 1.00 | 1.00 | 100 | 1000.0 |

Extended Standards:

| Vol. of 100.0 µg/mL Herbicide Solution (mL) | Vol. of 10.0 µg/mL Deuterated Solution (mL) | Final Volume (mL) | Final Analyte Concentration (µg/L) |
|---|---|---|---|
| 2.00 | 1.00 | 100 | 2000 |
| 3.00 | 1.00 | 100 | 3000 |
| 5.00 | 1.00 | 100 | 5000 |
| 10.00 | 1.00 | 100 | 10000 |
| 20.00 | 1.00 | 100 | 20000 |

Dilute each of the detector calibration standards to a final volume of 100 mL with 10% EtOAc/IO. The final concentration of each deuterated component is 100 µg/L in all analytical standards.

## 3 ANALYTICAL PROCEDURE

The following is a general method for ground and surface water specimens. Unique interferences in particular specimens may require modification of this method. If modifications are necessary, they should be fully documented in the raw data. The normal analytical specimen size is 200 mL. The volume may be varied depending on the concentration of analytes in the specimen. The workable volume is expected to range between 5 to 1000 mL. In cases where the analytes are very concentrated and small volumes are used (i.e. 5 mL), the specimens should be diluted to approximately 50 mL with Optima Grade bottled water prior to solid phase extraction. This method has been validated with a 200 mL sample volume.

### 3.1 Aqueous Specimen Preparation

Specimens are generally prepared and analyzed in sets of 24, which includes at least one control and one fortified control water sample.

The aqueous specimens are removed from chilled storage, mixed and gross particulates are allowed to settle before removing the analytical aliquot. Depending on the appearance of the specimens, begin with one of the following two paragraphs.

**If the specimens have a large amount of particulate matter,** transfer approximately 220 mL of specimen to a 250 mL centrifuge bottle and centrifuge for 10 minutes at 11,000 rpm. After centrifugation, transfer 200 ± 4 mL of specimen to a 250 mL graduated cylinder. Skip the next paragraph and continue.

**If the specimens are clear or just cloudy,** transfer 200 ± 4 mL of specimen to a 250 mL graduated cylinder. Continue to the next paragraph.

Fortification to control water samples must be made at this point by adding the correct volume of the appropriate fortification solution. Optima Grade bottled water is used for the control and fortified matrices for all water types. Add 1.00 mL of the 0.10 µg/mL mixed deuterated herbicide internal standard solution to each control and fortified water sample and each water specimen. A 200 mL volume is sufficient to quantitate to levels of 0.05 ppb.

### 3.2 Solid Phase Extraction

Place the $C_{18}$ solid phase extraction (SPE) column on the vacuum manifold and prepare for extraction by washing sequentially with 3 x approximately 3 mL volumes of methanol and Optima water, respectively. Following the final water wash, allow a small volume of liquid to remain on top of the resin bed (never allow the column to become dry prior to sample addition). **If the initial water specimens were clear, skip the next paragraph and continue.**

**If the initial water specimens had a large amount of particulate matter or were cloudy,** remove the $C_{18}$ column and place a 75 mL fritted reservoir on the vacuum manifold. Add approximately 10 g of sea sand to the reservoir. Wash the sea sand with approximately 20 mL volumes of 10% EtOAc/IO, methanol and Optima water, respectively. Following the final water wash, allow a small volume of liquid to remain on top of the sea sand bed. Place the washed $C_{18}$ column onto the manifold and piggy-back the reservoir containing the washed sea sand to it. Continue to the next paragraph but omit the first sentence (Add a 75 mL reservoir (with or without a frit) to the top of the $C_{18}$ column.) of the paragraph.

Add a 75 mL reservoir (with or without a frit) to the top of the $C_{18}$ column. Transfer the sample quantitatively to the reservoir. With a small vacuum of 5-10 in. Hg applied to the chamber, slowly open the valve to the $C_{18}$ column until a flow of 4-8 mL/minute is achieved. Discard the sample eluent from the $C_{18}$ column. When the sample has eluted through the $C_{18}$ column, wash the graduated cylinder with approximately 10 mL of Optima water and add to the reservoir. After the entire sample and wash volume have eluted through the $C_{18}$ column, remove the 75 mL reservoir and wash the $C_{18}$ column with 2 x 2.0 mL of 50% MeOH/water. Place a silica column on the $C_{18}$ column and dry the column under vacuum for approximately 20 minutes to remove all the water. The silica serves to prevent possible atmospheric contamination of the $C_{18}$ column. Remove and retain the silica column. Piggy-back the $C_{18}$ column to an 8 mL reservoir containing approximately 2 mL of pre-washed sodium sulfate. Return the $C_{18}$ column to its original position on the vacuum manifold. Place a 5 mL centrifuge tube in the rack below the $C_{18}$ column. Add 2 x 2.5 mL of 10% EtOAc/IO to the $C_{18}$ column. Slowly apply vacuum to the manifold and elute at a rate of approximately 2-4 mL/minute.

### 3.3 Analytical Sample Preparation

Inspect the centrifuge tubes in the manifold to determine if there is a lower water layer in the EtOAc/IO eluent for any sample. If a water layer is present, quantitatively transfer the organic phase into a clean centrifuge tube using a small amount of additional solvent as necessary. Return the centrifuge tube containing the organic extract to its proper location in the manifold rack. Once again piggy-back the silica column on top of the $C_{18}$ column. With the sample remaining in the centrifuge tube, continue to apply vacuum to the manifold to remove excess solvent. When the solvent volume is < 1 mL, discontinue vacuum and allow the sample to return to room temperature. Adjust the sample volume in the centrifuge tube to 1.00 mL with 10% EtOAc/IO. Transfer the entire sample into an autosampler vial for GC/MS analysis.

# 4 INSTRUMENTATION

## 4.1 Detector Calibration

A calibration curve is generated for every set of samples with a minimum of 5 standards. The standards are placed among the analytical samples for each set. The first and last sample in each analytical sample set must be a standard. Typically, the standards are randomly selected by concentration and placed in the set with several samples between the standard levels. The calibration curve is generated by plotting the ratio of the peak areas of each analyte and its deuterated analog against the concentration of each calibration standard. Least squares estimates of the data points are used to define the calibration curve. Linear, exponential or quadratic calibration curves may be used, but the analyte levels for all the samples from the same protocol must be analyzed with the same curve fit. In the event analyte responses exceed the calibration range by more than approximately 20%, the samples are reanalyzed with the extended standards. Only those analytes out of range will normally be quantitated with the extended calibration curve. This reanalysis necessitates a reduction in instrument sensitivity which is accomplished by reducing the electron multiplier voltage.

## 4.2 Gas Chromatography/Mass Spectrometry

A gas chromatograph/mass spectrometer (GC/MS) is used for separation and quantitation of the herbicides. Using selected ion monitoring (SIM) in the electron ionization (EI) mode, the GC/MS gives superior specificity and similar sensitivity compared to conventional GC/ECD. The improved specificity eliminates interferences typically found in GC/ECD analyses. Data acquisition is with a data system which provides complete instrument control of the mass spectrometer.

The instrument is tuned and mass calibrated in the electron ionization (EI) mode. Typically, four ions are monitored for each analyte, 2 ions for each herbicide and 2 ions for the deuterated analog. If there are interferences with the quantitation ion, the confirmation ion may be used for quantitation purposes. The typical quantitation and confirmation ions for the analytes are shown below. Alternate ions may be used if they provide better data.

| Analyte | Quantitation Ion (daltons) | Confirmation Ion (daltons) |
|---|---|---|
| acetochlor | 162 | 146 |
| $d_3$-acetochlor | 165 | 149 |
| alachlor | 188 | 160 |
| $d_{13}$-alachlor | 200 | 171 |
| atrazine | 200 | 215 |
| $d_5$-atrazine | 205 | 220 |
| dimethenamid | 154 | 230 |
| $d_3$-dimethenamid | 157 | 233 |
| metolachlor | 162 | 240 |
| $d_3$-metolachlor | 165 | 243 |

Operate the gas chromatograph at the following conditions. The conditions may require modification to achieve satisfactory sensitivity and separation.

| | |
|---|---|
| Column: | J & W DB-1 fused silica open tubular column (FSOT), 30 meters x 0.25 mm ID x 0.25 µm film |
| Carrier Gas: | Helium |
| Flow Rate: | 0.7-1.0 mL/minute |
| Injection Volume: | 1-5 µL |
| Injection Mode: | Splitless |
| Injector Temp: | 240° C |
| Ion Source Temp: | 200° C |
| Interface Temp: | 270° C |
| Column Temp: | 100° - 185° C at 20°/minute<br>185° - 195° C at 1°/minute<br>195° - 300° C at 30°/minute<br>300° C for 8 minutes |
| Total Run Time: | about 30 minutes, injection to injection |

### 4.3 Column/Injector Maintenance

The use of a guard column, in addition to scheduled injector maintenance, has been found to provide significantly improved GC performance. Loss of performance manifests itself in poor peak shape and reduced sensitivity. This is particularly noticeable with atrazine but also negatively effects the other analytes. A maintenance schedule should be employed that provides: removal of a 1-2 foot section of the guard column and replacement of the silylated glass injection port liner and associated seals and o-rings. In the HP 5890 GC, this would include replacement of the gold plated metal seal at the base of the injector. The frequency of this scheduled maintenance is at the discretion of the analyst and is based on the quality and number of samples analyzed and the historical performance of the method. The maintenance schedule should be based on the number of samples injected rather than a fixed time interval. With the method as written, at least 300 environmental specimens have been analyzed on our system without a significant negative effect on performance.

### 4.4 Detection Criteria

Analyte detection will be determined by the criteria in ARP SOP 14B, current version.

## 5 INTERFERENCES

### 5.1 Specimen Matrix

At least one control water sample must be run concurrently with the water specimens to determine the presence of matrix interferences and/or background levels of the herbicides. Optima Grade bottled water is used for the control and fortified matrices for all water types because it is difficult to obtain ground and surface water specimens that are completely free of the herbicides.

Our experience with ground and surface waters has demonstrated an abundance of

low level interferences present in these matrices. The 50% MeOH/water wash in the SPE phase of the sample workup is intended to minimize these interferences while maintaining quantitative recovery of the analytes.

### 5.2 Other Pesticides

Interferences from other pesticides are unknown because none have been examined. However, none are expected due to the high level of specificity of the EI/GC/MS/SIM analysis.

Random instances of background contamination have been noted. The source of contamination may be atmospheric. Hence, the method employs a silica column to "scrub" all air pulled through the $C_{18}$ column during the drying and evaporation steps. No instances of contamination have been found since incorporating a silica scrubber into the method.

### 5.3 Solvents

A solvent blank may be injected with the samples as part of an analytical set to confirm the cleanliness of a solvent used.

### 5.4 Labware

Interferences related to improperly cleaned labware have not been noted. A solvent blank sample may be run through the method from extraction to quantitation to determine if contamination from glassware occurs.

## 6 CONFIRMATORY TECHNIQUES

The samples are analyzed by mass spectrometry. A confirmatory technique is not needed because the analysis identifies the analyte by its retention time and specific fragment ions.

## 7 ANALYSIS TIME

A set of 24 specimens may be taken from extraction to chromatography in about 1 day. Analysis of samples on the GC/MS is about 30 minutes per injection.

## 8 POTENTIAL PROBLEMS

### 8.1 Safety Concerns

The method requires general lab safety awareness.

### 8.2 Chromatographic Interferences

Chromatographic interferences may be a problem in some matrices. Use of the mass spectrometer with selected ion monitoring analysis greatly reduces the interferences for these samples.

## 9 CALCULATIONS

Linear, exponential or quadratic calibration curves may be used to quantitate the amount of analyte in each sample. Quantitation of each analyte is made independently.

### 9.1 Quantitation Using A Linear Calibration Curve

The concentration of the analyte in the injected sample is determined based on the height or area of the analyte peak and interpolation of the internal or external standard linear calibration curve according to the following equation:

$$(\mu g/L \text{ Analyte})\ m + b = PKR_{analyte}$$

Where,

$PKR_{analyte}$ is the detector response (as peak height, area or ratio of the natural isotope over the deuterated analog; i.e., 162/165), of the analyte.

m is the slope of the linear least squares fit of the calibration curve.

b is the Y-intercept of the linear least squares fit of the calibration curve.

The resulting µg/L value represents the concentration of the analyte in the injected sample. Now that the concentration of analyte in the injected sample, the final volume and the volume of water extracted are known, the concentration of analyte present in the water specimen can be calculated. The ppb of herbicide in the water specimen is calculated by multiplying the analyte concentration (µg/L) by the final volume (mL) then dividing by the water specimen volume (mL).

$$\text{ppb (herbicide)} = \frac{\mu g/L \text{ herbicide found} \times \text{final volume in mL}}{\text{water specimen volume in mL}}$$

### 9.2 Quantitation Using An Exponential Calibration Curve

The concentration of the analyte in the injected sample is determined based on the height or area of the analyte peak and interpolation of the internal or external standard exponential calibration curve according to the following equation:

$$\ln (PKR_{analyte}) = A + B \ln (\mu g/L \text{ Analyte})$$

Thus,

$$\ln (\mu g/L \text{ Analyte}) = [\ln (PKR_{analyte}) - A]/B$$

$$\ln (\mu g/L \text{ Analyte}) = (-A/B) + (1/B) \ln (PKR_{analyte})$$

Where,

$PKR_{analyte}$ is the detector response (as peak height, area or ratio of the natural isotope over the deuterated analog; i.e., 162/165), of the analyte.

A is the Y-intercept of the curve of the natural log of the concentration and detector response.

B is the slope of the curve of the natural log of the concentration and detector response.

Once the µg/L concentration of the analyte is determined in the injected sample, the remainder of the calculations are the same as the linear calibration curve.

### 9.3 Quantitation Using A Quadratic Calibration Curve

The concentration of the analyte in the injected sample is determined based on the height or area of the analyte peak and interpolation of the internal or external standard quadratic calibration curve according to the following equation:

$$A\ (\mu g/L\ Analyte)^2 + B\ (\mu g/L\ Analyte) + C = PKR_{analyte}$$

Where,

$PKR_{analyte}$ is the detector response (as peak height, area or ratio of the natural isotope over the deuterated analog; i.e., 162/165), of the analyte.

A, B and C are curve constants

Once the µg/L concentration of the analyte is determined in the injected sample, the remainder of the calculations are the same as the linear calibration curve.

### 9.4 Analytical Accuracy

The mixture of all deuterium labeled internal standards is added to each control and fortified water sample and each water specimen prior to extraction. This does not prevent the loss of the unlabeled herbicides from the sample in subsequent processing steps, but a proportional loss of the deuterated internal standard precludes the need to correct for recovery. It is inappropriate to refer to recovery in this type of analysis although it is important to monitor the accuracy of this method.

The estimated analytical accuracy of the method can be obtained from the mean of the accuracies of each individual fortification using the following equation:

$$\text{Estimated Accuracy} = \frac{\sum Cf / Af\ (100\%)}{Ni}$$

Where,

$C_f$ is the concentration of herbicide found in the fortified control sample
$A_f$ is the concentration fortified into the control sample
$N_i$ is the number of fortified control samples

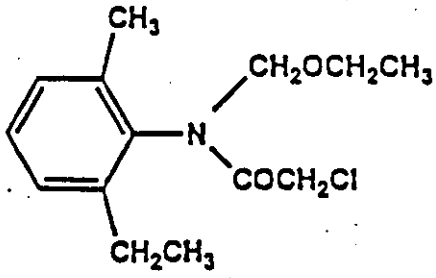
There should be near equal numbers of fortifications at each level so the estimated analytical accuracy will not be disproportionately weighted.

If a control water sample to be fortified is found to contain a significant concentration of any of the five herbicides, then this concentration is subtracted from the amount found in the fortified control sample in order to calculate the accuracy for the sample. This is done for those samples that have been found to contain low concentrations with respect to the fortification level. As a general rule, the amount fortified should be at least twice that concentration found in the identical sample that is not fortified. In this case, the variable $C_f$ in the above equation should be replaced by the expression $(C_f - C_b)$, where $C_b$ is the concentration found in the identical sample that was not fortified.

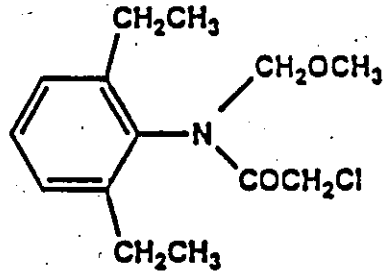
## 11 REFERENCES

1. J. D. Fuhrman, "GC/MS Analytical Methodology to Support the Acetochlor Registration Partnership Surface Water and Ground Water Monitoring Studies", tentative completion date April, 1996, Monsanto Progress Report Number MSL-14562.

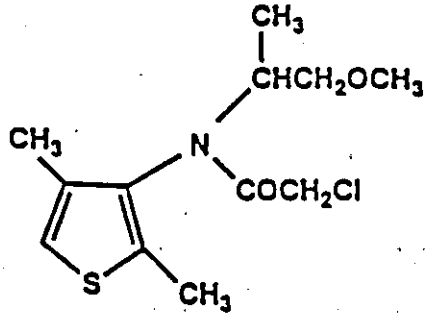
**Figure 1: Herbicide Structures**



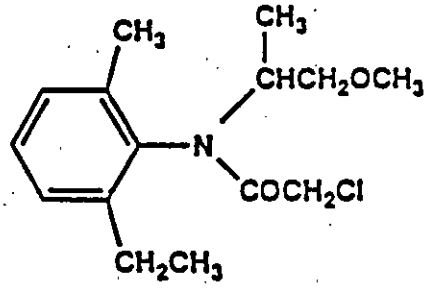

Acetochlor



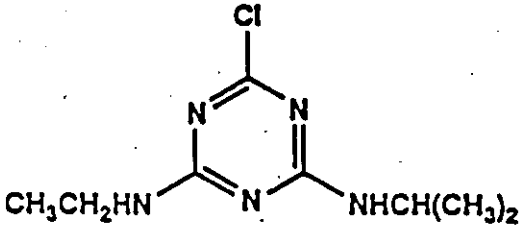

Alachlor




Dimethenamid

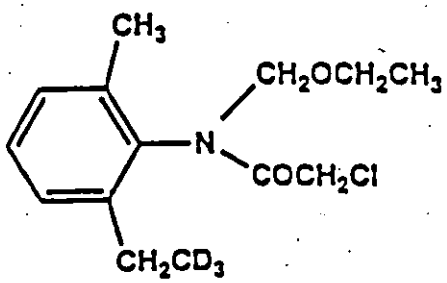



Metolachlor

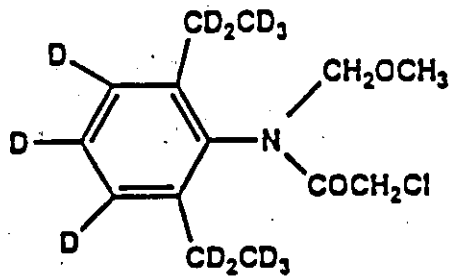



Atrazine

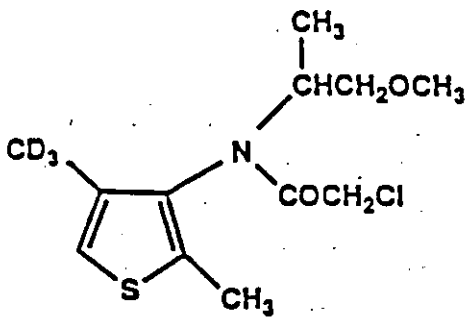
**Figure 2: Deuterated Herbicide Structures**



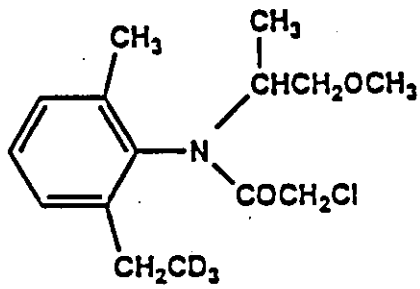

$d_3$-Acetochlor



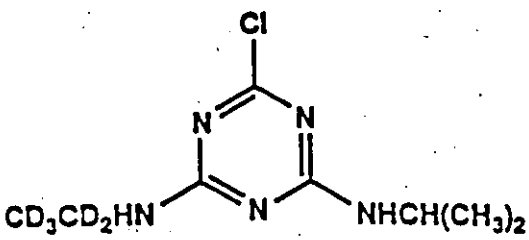

$d_{13}$-Alachlor




$d_3$-Dimethenamid




$d_3$-Metolachlor




$d_5$-Atrazine