April, 2002

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Determination of Atrazine, Bromacil, Diuron, Hexazinone, Norflurazon, Prometon, Simazine, Desethyl Atrazine (DEA), Desisopropyl Atrazine (ACET) and Diamino Chlorotriazine (DACT) in Well Water By Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry

Scope:

This method is applicable to the analysis of Atrazine, Bromacil, Diuron, Hexazinone, Norflurazon, Prometon, Simazine, Desethyl Atrazine (DEA), Desisopropyl Atrazine (ACET) and Diamino Chlorotriazine (DACT) in well water using LC/MS/MS. The MDLs for the analytes were as follows: 0.057 µg/L for DACT, 0.032 µg/L for ACET, 0.035 µg/L for DEA, 0.022 µg/L for Bromacil, 0.035 µg/L for Simazine, 0.082 µg/L for Hexazinone, 0.031 µg/L for Atrazine, 0.022 µg/L for Diuron, 0.021 µg/L for Nofflurazon, and 0.022 µg/L for Prometon. The reporting limit for all chemicals was 0.05 µg/L by APCI/LC/MS/MS, except DACT (0.1 µg/L) and Hexazinone (0.1 µg/L).

Equipment and Reagents:

Equipment:

Glassware and Miscellaneous Equipment

Balance

Erlenmeyer flask, 500 mL

Filter, Nylon Acrodisc, 0.2 micron, Gelman Sciences

Filter Flask, 1 L

Graduated Cylinder, various sizes

Nitrogen evaporator (Meyers Organomation Assoc.)

Solid phase extraction cartridges: Waters Oasis MCX 6cc, 60 micron particle size (Waters, Division of Millipore Corporation)

Syringes, microliter, various sizes

Test tube, 15 mL graduated

Turbovap LV (Zymark, Hopkinton, MA)

Volumetric flask, various sizes

Volumetric pipette, various sizes

Vortex mixer

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Reagents and Standards:

Solvents/Reagents

All solvents were HPLC grade unless noted:

Acetonitrile

Ammonium formate

Ammonium hydroxide

Formic Acid

Hydrochloric Acid

Methanol

Distilled Water

Standard Reference Substances

The reference standards were supplied or purchase as shown below:

Standard	Source	Lot Number	Expiration Date	Purity
Diamino Chlorotriazine (DACT)	Syngenta	S87-1195	November, 2003	97%
Desisopropyl Atrazine	ChemService Crescent Chemical	252-49B 7 1030	October, 2003 January, 2003	99% 99.5%
Desethyl Atrazine (DEA)	ChemService Crescent Chemical	249-21c 703 11	July, 2003 January, 2003	99% 95.5%
Bromacil	ChemService	244-84A	April, 2004	99%
Atrazine	ChemService Crescent Chemical	254- 140B 70326	November, 2003 January, 2003	98% 98.4%
Norflurazon	ChemService	237-34A	January, 2006	98.6%
Simazine	ChemService	249-29A	August, 2003	99%
Hexazinone	ChemService Crescent Chemical	245-14B 70109	May, 2005 Januarv. 2003	99% 99.2%
Diuron	ChemService Crescent Chemical	249-137A 90129	September, 2004 January, 2005	99% 97.5%
Prometon	ChemService	245-150B	July, 2006	99%
Propazine	ChemService	254-l 17B	November, 2004	98%

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The reference standards were concluded to be stable throughout the conduct of the study based on the comparison of chromatograms of the first and last analysis.

Analytical Procedures

Preparation of Sample:

All samples were received cool at PTRL West, Inc. and remained refrigerated until used for analysis.

Preparation of Standards:

Stock solutions of each reference standard were prepared in acetonitrile or methanol, as described under the "Method of Calculations" section. A 1 mg/mL stock solution was prepared in acetonitrile for the following analytes: ACET, DEA, atrazine, bromacil, diuron, hexazinone, prometon, norflurazon, and propazine. Due to the solubility properties of DACT and simazine, the stock solutions for these two analytes were prepared at 0.1 mg/mL in methanol. A 40 µg/mL propazine solution was prepared by dilution with methanol:water (75:25, v:v), which was further diluted to 1 ng/µL for the sample surrogate spike. Working solutions were made by diluting the stock standards to prepare fortification standards and calibration standard solutions, as described below. Microliter syringes, volumetric pipettes and volumetric flasks were used throughout.

Fortification Procedure:

Fortification of untreated well water was conducted to determine the percent recovery within each sample set for atrazine, bromacil, diuron, hexazinone, norflurazon, prometon, simazine, desethyl atrazine (DEA), desisopropyl atrazine (ACET) and diamino chlorotriazine @ACT). Fortification of control water was conducted in duplicate within sample sets. A mixed 5 µg/mL fortification stock was prepared by aliquoting 250 µL for each 1 mg/mL stock and 2.5 mL for the 0.1 mg/mL stocks into a 50 mL volumetric flask and diluting to the mark with methanol. A mixed 0.5 µg/mL fortification stock was prepared by aliquoting 50 µL for each 1 mg/mL stock and 0.5 mL for the 0.1 mg/mL stocks into a 100 mL volumetric flask and diluting to the mark with methanol.

The following fortifications were conducted:

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Method Validation:

Fortification Level (µg/L)	Triazines
10.0	1.0 mL of 5.0 ug/mL
5.0	0.5 mL of 5.0 ug/mL
2.0	400 µL of 5.0 ug/mL
1.0	200 µL of 5.0 ug/mL
0.2	200 µL of 0.5 ug/mL
0.1	100 µL of 0.5 ug/mL

Sample Set Analysis:

Fortification Level (µg/L)	Triazines
0.25	250 µL of 0.5 ug/mL

Preparation of Mixed Linearity Standards:

All mixed triazine dilutions made with methanol:water (75:25, v:v).

Concentration (µg/mL)	Preparation:
1.0	5 mL of 5.0 µg/mL mixed triazine stock plus 115 µL of 40 µg/mL propazine
0.7	3.5 mL of 5.0 µg/mL mixed triazine stock plus 115 µL of 40 µg/mL propazine
0.4	2 mL of 5.0 µg/mL mixed triazine stock plus 115 µL of 40 µg/mL propazine
0.2	10 mL of 0.5 µg/mL mixed triazine stock plus 115 µL of 40 µg/mL propazine
0.1	5 mL of 0.5 µg/mL mixed triazine stock plus 115 µL of 40 µg/mL propazine
0.04	2 mL of 0.5 µg/mL mixed triazine stock plus 115 µL of 40 µg/mL propazine

All dilutions were prepared in volumetric flasks using Hamilton syringes and volumetric pipettes.

A set of calibration curves were generated with each sample set to determine linearity and to quantitate each triazine, see “Methods of Calculation” for example.

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Extraction Method for Triazine in Well Water:

1. Allow sample to adjust to room temperature. Measure sample weight of ~500g or measure sample volume of 500 mL.
2. Fortify samples as needed. Add to each water sample 0.1µg of the internal standard propazine as a surrogate (100µL of 1ng/µL spiking solution in MeOH).
3. Adjust pH to ~3 with 6N HCl.
4. Connect two MCX 6cc cartridges in tandem onto a one-liter vacuum filter flask. Condition cartridges at 10mL/minute with 15mL of methanol followed by 15mL of purified water by applying vacuum. ***Do not let the cartridges run dry.***
5. Add sample to conditioned cartridge and allow to pass through cartridges at 10-15mL/minute.
6. Dry cartridges under vacuum for two minutes.
7. Turn off vacuum and reverse the order of the cartridge positions.
8. Elute compounds off cartridge into the previously calibrated 15mL conical shape test tube with 5mL of 5% ammonium hydroxide in methanol at a flow rate of 5mL/minute.
9. Concentrate the eluant to ~0.2mL in a 40°C waterbath using Turbovap LV.
10. Add 500µL of methanol:water (25:75, v:v). Vortex for 30sec. Measure the final volume using a 500µL syringe.
11. If patricles are observed in the final extract, filter extract through a microfilterfuge tube.
12. Transfer the final extract into an autosampler vial with insert.

Analysis Method

LCQ™ LC/MS System Components:

LC Pump: Spectra System P4000, Thermo Separations

MS Detector: Finnigan LCQ APCI Ionization Mass Spectrometer

Autosampler: Spectra SYSTEM AS3000 autosampler, Thermo Separations

A Finnigan MAT (San Jose, CA) LCQ Atmospheric Pressure Ionization Mass Spectrometer equipped with a Atmospheric Pressure Chemical Ionization (APCI) probe was used to obtain mass spectra in the positive ion mode.

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LC Column: Phenomenex Sepherex 5 C18: 150mm x 3.2mm, 5µm with 2 pm stainless steel frit pre-filter

Injection volume: 40 µL

Mobile Phase Program:

Solvents: C = 95:5 10mM ammonium formate:methanol, + 0.1% formic acid

D = 90: 10 methanol:0. 1 M ammonium formate, + 0.1% formic acid

Step	Time (min)	Flow Rate	%C	%D
1	0	0.75 mL/min	85	15
2	3.0	0.75 mL/min	85	15
3	4.0	0.75 mL/min	50	50
4	20.0	0.75 mL/min	50	50
5	21.5	0.75 mL/min	25	75
6	25.0	0.75 mL/min	5	95
7	30.0	0.75 mL/min	5	95
8	30.5	0.75 mL/min	85	15
9	35.0	0.75 mL/min	85	15

The APCI source settings for LCNS Method were conducted as follows:

Vaporizer Temp (°C):	550
Sheath Gas Flow Rate (arb):	90 (50 for DACT)
Auxiliary Gas Flow Rate (arb):	15 (0 for DACT)
Discharge Current (µA):	5
Discharge Voltage (kV):	4.5-5.5
Capillary Temp (°C):	210
Capillary Voltage (V):	3.0

Separation of the analyte was achieved by high performance liquid chromatography. The analytes were identified by the coincidence of their retention times with the reference standards, and quantitated by integration of the peak area for the relevant ion(s).

MS Detector Settings:

MS Run time: 29.0 minutes

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Divert valve: 0.00 minutes to waste, 1.50 minutes to source, 28.5 minutes to waste.

Method: 1000Wymsms7b

Segment 1 Information:

Duration time (min.): 4.00
Number of scan events: 1
Tune Method: 1000W-001b
Scan event details: Pos [147.0] ⇒ [100.0-160.0]
MS/MS: Amp.: 55.0% Q: 0.400 Time: 30.000 IsoW: 5.0

Segment 2 Information:

Duration time (min.): 4.23
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [175.0] ⇒ [125.9-190.0]
MS/MS: Amp.: 43.0% Q: 0.300 Time: 30.000 IsoW: 5.0

Segment 3 Information:

Duration time (min.): 4.25
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [262.0] ⇒ [185.0-275.0]
MS/MS: Amp.: 30.0% Q: 0.300 Time: 30.000 IsoW: 5.0

Segment 4 Information:

Duration time (min.): 4.75
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [217.0] ⇒ [160.0-230.0]
MS/MS: Amp.: 40.0% Q: 0.300 Time: 30.000 IsoW: 5.0

Segment 5 Information:

Duration time (min.): 3.89
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [235.0] ⇒ [60.0-315.0]
MS/MS: Amp.: 35.0% Q: 0.250 Time: 30.000 IsoW: 5.0

Segment 6 Information:

Duration time (min.): 3.16
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [231.0] ⇒ [170.0-250.0]
MS/MS: Amp.: 40.0% Q: 0.300 Time: 30.000 IsoW: 4.0

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Segment 7 Information:

Duration time (min.): 4.72
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [226.0] ⇒ [170.0-235.0]
MS/MS: Amp.: 40.0% Q: 0.300 Time: 30.000 IsoW: 4.0

Method: 1000Wymsms6e

Segment 1 Information:

Duration time (min.): 5.00
Number of scan events: 1
Tune Method: 1000W-001b
Scan event details: Pos [147.01 ⇒ [100.0-160.0]
MS/MS: Amp.: 55.0% Q: 0.400 Time: 30.000 IsoW: 5.0

Segment 2 Information:

Duration time (min.): 4.07
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [189.0] ⇒ [130.0-200.0]
MS/MS: Amp.: 38.0% Q: 0.300 Time: 30.000 IsoW: 4.0

Segment 3 Information:

Duration time (min.): 3.26
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [203.0] ⇒ [65.0-220.0]
MS/MS: Amp.: 40.0% Q: 0.300 Time: 30.000 IsoW: 4.0

Segment 4 Information:

Duration time (min.): 5.54
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [253.0] ⇒ [160.0-270.0]
MS/MS: Amp.: 30.0% Q: 0.300 Time: 30.000 IsoW: 4.0

Segment 5 Information:

Duration time (min.): 6.09
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [305.0] ⇒ [lOO.O-315.01
MS/MS: Amp.: 40.0% Q: 0.300 Time: 30.000 IsoW: 5.0

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Segment 6 Information:

Duration time (min.): 5.04
Number of scan events: 1
Tune Method: 1000W-001
Scan event details: Pos [226.0] ⇒ [170.0-235.0]
MS/MS: Amp.: 40.0% Q: 0.300 Time: 30.000 IsoW: 4.0

A typical injection sequence for triazine water samples as analyzed by LC/MS/MS was: 0.04 µg/mL mixed standard, 0.04 µg/mL mixed standard, solvent blank, solvent blank, control water sample, control water sample, 0.1 µg/mL mixed standard, 0.1 µg/mL mixed standard, fortified sample 1, fortified sample 1, fortified sample 2, fortified sample 2, 0.2 µg/mL mixed standard, 0.2 µg/mL mixed standard, treated water sample 1, treated water sample 1, treated water sample 2, treated water sample 2, 0.4 µg/mL mixed standard, 0.4 µg/mL mixed standard, treated water sample 3, etc.

Statistical Methods

The residue data included the following statistical calculations: means, averages, standard deviations, relative standard deviations and linear regression analysis.

Method Detection Limit

The limit of quantitation was determined according to SOP Number QAQCOO1.OO, wherein the 0.2 µg/mL mixed standard was injected seven times. The MDLs for the analytes were as follows: 0.057 µg/L for DACT, 0.032 µg/L for ACET, 0.035 µg/L for DEA, 0.022 µg/L for Bromacil, 0.035 µg/L for Simazine, 0.082 µg/L for Hexazinone, 0.031 µg/L for Atrazine, 0.022 µg/L for Diuron, 0.021 µg/L for Nofflurazon, and 0.022 µg/L for Prometon.

Unequivocal Identification

The analysis for each triazine pesticide was conducted by LC/MS/MS. This method of analysis involves the detection of a mass spectral ion specific for a triazine pesticide followed by fragmentation to form a daughter ion(s) uniquely related to the structure of the pesticide. Therefore, the retention time, analyte molecular ion mass and one or two specific daughter ion masses were used as a means of identifying and quantitating the analyte unequivocally. The LC/MS/MS ions for each analyte are presented in the following table.

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Triazine	Parent Mass m/z	Parent Ion	Daugther Ions m/z	Retention Time (minutes)
DACT	145	$(M+H)^+$	110	2.2
ACET	173	$(M+H)^+$	132, 138, 146, 148	6.3
DEA	187	$(M+H)^+$	145, 148	7.5
Bromacil	260	$(M+H)^+$	205, 207	9.6
Simazine	201	$(M+H)^+$	124, 132, 174	11.2
Hexazinone	252	$(M+H)^+$	171	14.0
Atrazine	215	$(M+H)^+$	174, 176	15.7
Diuron	232	(M+H)+	72	19.4
Norflurazon	303	$(M+H)^+$	284	21.5
Propazine	229	(M+H)+	188,190	23.0
Prometon	226	(M+H)+	184	25.5

Methods of Calculation

Preparation of Stock Standards:

$$\text{Volume of solvent (mL)} = \frac{(W) \times (P)}{(FC)}$$

where W = Milligrams of neat standard
P = Chemical purity of neat standard
FC = Final Concentration (mg/mL)

Residue in Water:

Linear regression formula for each analyte,

calibration curve $y = mx + b$

where y = peak area
x = ng/mL analyte injected
m = Slope
b = Calibration intercept

The residue in water was calculated as follows:

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$$\text{Analyte } (\mu g/L) = \frac{\mu g/mL \text{ (from standard curve) x final volume (mL)}}{\text{Sample weight (g)}} \times \frac{1000\,g}{L}$$

Residues in fortified water were corrected for background by subtracting residue in control water.

Method Performance:

Quality Control:

1. Sample Storage: All field samples were refrigerated at 4 °C until extracted.
2. Sample extraction: All extracts were kept refrigerated at 4 °C until analyzed.
3. For each set of samples, at least one matrix blank and two matrix spikes were included.

Recovery data:

The analytical method was validated by conducting 7 sets of samples using the provided background well water. Each set contained 5 different levels of spike and a matrix blank. Each set was processed through the entire analytical method on a different day. Each sample was injected twice on a Phenomenex Cl8 column. The results are presented in Appendix A.

Method Detection Limit (MDL):

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably, To determine the MDL, 7 replicate background samples were spiked at 0.20 µg/mL. The standard deviation from the spiked samples was used to calculate the MDL using the following equation:

$$MDL = tS$$

Where t is the Student t value for the 99% confidence level with n-l degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t = 3.143. See Appendix B for the recovery data from the determination of the Method Detection Limits.

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The Reporting Limit (RL) refers to the level at which quantitative results may be obtained. By convention, the RL is chosen in a range l-5 times the MDL. The Reporting Limit for this method was 0.05 µg/L for all analytes, except DACT and Hexazinone (0.1 µg/L).

Discussion:

The method provided to us was slightly modified by PTRL West. Sample sets were conducted through the elution with methanol into a test tube (Step 5). The samples were not concentrated until immediately prior to analysis, at which time they were brought up to -0.5 mL in methanol:water (75:25, v:v). For improved accuracy, the final volume was measured by drawing the sample into a 1 mL Hamilton syringe.

Propazine was used as a surrogate, where each sample was spiked with 0.1 µg of propazine and processed through the entire method. A standard curve consisting of 6 levels was used for each analysis set. Constant monitoring of the chromatography was necessary to adjust the mass spectral events to coincide with the changing retention times. Due to non-baseline separation of analytes, each sample was injected twice, using two different MS/MS programs which alternated the analysis of analytes according to their retention time (i.e. the first method determined a + c + e, etc. and the second method determined b + d + f, etc).

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Reference

"Determination of Atrazine, Bromacil, Cyanazine, Diuron, Hexazinone, Metribuzin, Norflurazon, Prometon, Prometryn, Simazine, Deethyl Atrazine (DEA), Deisopropyl Atrazine (ACET), and Diamino Chlorotriazine @ACT) in Well Water and River Water by Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry," Duc Tran and Pamela Fitch, California Center for Analytical Chemistry, July 21, 1999 (Revised February 5, 2001).

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Appendix A

Method Validation Results

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Method Validation Results for P1000W

METHOD VALIDATION RESULTS TABLE

California Department of Pesticide Regulation Study

Analysis Location: PTRL WEST, INC.
Analysis Method: LC/MS
Column: C18 Phenomenex

Percent Recovery for 0.1µg/L

Conc. ng/mL	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
MV1 (0.2µg/L)	57.6%	76.4%	73.1%	70.1%	74.4%	58.8%	63.8%	67.4%	70.8%	60.9%
MV2	59.5%	46.2%	56.3%	63.3%	52.8%	42.2%	57.0%	52.5%	58.5%	62.6%
MV3	73.9%	77.9%	74.5%	87.1%	74.9%	83.1%	72.5%	60.0%	71.5%	63.8%
MV4	97.0%	99.9%	104.0%	109.9%	103.9%	102.6%	101.6%	98.7%	101.9%	103.2%
MV5	113.0%	101.0%	105.0%	109.0%	103.0%	117.0%	102.0%	100.0%	100.0%	101.0%
MV6	86.0%	85.0%	85.0%	89.0%	83.0%	53.0%	81.0%	93.0%	89.0%	83.0%
MV7	109.0%	89.0%	80.0%	74.0%	88.0%	66.0%	79.0%	85.0%	80.0%	77.0%
Average =	95.8%	90.6%	89.7%	93.8%	90.6%	84.3%	87.2%	87.3%	88.5%	85.6%
Std Dev =	16.2%	9.9%	14.0%	15.4%	12.7%	26.1%	13.7%	16.4%	13.0%	16.6%
RSD=	16.9%	10.9%	15.6%	16.4%	14.0%	31.0%	15.7%	18.8%	14.7%	19.4%

Percent Recovery for 0.5µg/L

Conc. ng/mL	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
MV1 (1.0µg/L)	69.3%	81.1%	81.8%	80.6%	69.3%	83.0%	67.2%	72.0%	75.9%	69.3%
MV2	64.6%	87.2%	91.0%	83.2%	90.5%	83.3%	87.4%	76.7%	80.5%	77.3%
MV3	73.4%	88.7%	86.1%	89.4%	80.9%	87.2%	75.0%	78.4%	78.2%	75.3%
MV4	69.3%	82.6%	83.4%	83.8%	79.2%	69.9%	81.1%	76.4%	80.7%	74.5%
MV5	70.8%	78.0%	75.2%	79.8%	76.8%	85.0%	71.4%	72.4%	81.0%	71.8%
MV6	83.6%	99.2%	106.6%	93.6%	103.6%	90.0%	98.8%	95.2%	95.4%	94.0%
MV7	73.2%	89.8%	90.0%	92.2%	80.6%	112.8%	78.4%	83.4%	89.2%	77.8%
Average =	74.1%	87.7%	88.3%	87.8%	84.2%	89.0%	80.9%	81.2%	84.9%	78.7%
Std Dev =	5.6%	8.0%	11.6%	5.8%	11.0%	15.4%	10.6%	8.8%	7.2%	8.8%
RSD=	7.6%	9.1%	13.1%	6.6%	13.1%	17.3%	13.1%	10.8%	8.5%	11.2%

Percent Recovery for 1.0µg/L

Conc. ng/mL	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
MV1 (2.0µg/L)	67.6%	95.9%	92.1%	92.2%	81.1%	93.9%	78.0%	88.2%	88.1%	78.9%
MV2	72.6%	90.5%	88.8%	95.6%	84.5%	100.5%	79.6%	82.8%	83.4%	82.1%
MV3	95.8%	105.1%	107.1%	103.6%	98.3%	102.4%	94.8%	96.5%	97.8%	92.1%
MV4	79.8%	100.8%	99.8%	101.3%	91.9%	87.8%	94.0%	90.3%	98.3%	91.3%
MV5	86.5%	91.9%	91.3%	92.5%	85.2%	77.6%	81.9%	88.5%	94.0%	84.6%
MV6	75.6%	99.8%	104.3%	101.1%	94.5%	100.7%	93.1%	91.9%	98.1%	92.7%
MV7	79.4%	100.1%	98.9%	103.3%	88.3%	102.3%	85.9%	87.6%	92.9%	86.9%
Average =	83.4%	99.5%	100.3%	100.4%	91.6%	94.2%	89.9%	91.0%	96.2%	89.5%
Std Dev =	8.0%	4.8%	6.0%	4.5%	5.1%	11.1%	5.7%	3.5%	2.6%	3.6%
RSD=	9.6%	4.8%	6.0%	4.5%	5.6%	11.8%	6.3%	3.8%	2.7%	4.0%

Method Validation Results for P1000W

METHOD VALIDATION RESULTS TABLE

California Department of Pesticide Regulation Study

Analysis Location: PTRL WEST, INC.
Analysis Method: LC/MS
Column: C18 Phenomenex

Percent Recovery for 5.0µg/L

Conc. ng/mL	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
MV1	75.7%	98.1%	93.0%	97.3%	85.4%	80.9%	83.5%	90.8%	94.7%	86.3%
MV2	62.6%	82.3%	78.3%	85.0%	73.1%	84.5%	71.6%	76.4%	79.5%	78.7%
MV3	78.6%	98.3%	96.2%	91.0%	84.7%	88.2%	86.1%	83.5%	88.8%	87.3%
MV4	82.0%	99.1%	95.9%	92.1%	89.3%	71.3%	89.6%	93.2%	91.0%	91.9%
MV5	80.5%	97.3%	95.3%	91.0%	90.4%	76.9%	83.7%	83.2%	92.9%	85.6%
MV6	84.6%	101.1%	103.4%	100.4%	95.9%	86.9%	92.1%	90.7%	95.2%	93.1%
MV7	82.6%	98.4%	96.9%	97.2%	88.6%	93.1%	85.6%	82.5%	87.9%	85.2%
Average =	81.7%	98.8%	97.5%	94.3%	89.8%	83.3%	87.4%	86.6%	91.2%	88.6%
Std Dev =	2.3%	1.4%	3.3%	4.2%	4.0%	8.9%	3.4%	5.0%	3.0%	3.7%
RSD=	2.8%	1.4%	3.4%	4.5%	4.5%	10.7%	3.9%	5.8%	3.3%	4.2%

Percent Recovery for 10.0µg/L

Conc. ng/mL	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
MV1	58.2%	89.6%	85.9%	88.7%	79.9%	71.6%	77.8%	81.3%	85.7%	80.0%
MV2	51.9%	76.2%	76.1%	79.5%	68.3%	68.3%	67.5%	72.5%	75.4%	76.8%
MV3	80.2%	96.5%	92.0%	94.1%	88.0%	98.3%	80.3%	79.6%	84.7%	83.0%
MV4	89.0%	101.1%	94.6%	95.7%	93.5%	91.4%	89.7%	88.3%	92.5%	93.4%
MV5	91.0%	100.8%	96.3%	93.1%	89.2%	94.9%	87.2%	90.3%	93.5%	93.0%
MV6	71.6%	92.4%	92.8%	88.0%	88.9%	71.0%	84.2%	87.5%	84.7%	88.0%
MV7	81.9%	92.5%	95.3%	90.5%	84.9%	84.8%	81.9%	83.6%	83.2%	87.4%
Average =	82.7%	96.7%	94.2%	92.3%	88.9%	88.1%	84.7%	85.9%	87.7%	89.0%
Std Dev =	7.7%	4.3%	1.8%	3.0%	3.1%	10.8%	3.8%	4.3%	4.9%	4.3%
RSD=	9.3%	4.4%	1.9%	3.3%	3.5%	12.3%	4.5%	5.0%	5.6%	4.8%

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Appendix B

Method Detection Limit Results

Analytical Data Set for P1000W

METHOD DETECTION LIMITS

California Department of Pesticide Regulation Study

Description: Method Detection Limits - C18 Phenomenex 40µl inj. Vol.
Analysis Location: PTRL WEST, INC.
Analysis Method: LC/MS
Analysis Date: 6/21/01
Date of Extraction: 6/12/01

		Peak Area									
Sample Name	Sample Volume (mL)	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
0.2µg/mL MDL	500	2,131,650	47,919,128,099	230,272,339,863	122,947,295,903	34,255,724,178	163,299,289,769	341,506,438,262	20,350,029,226	NP	396,549,209,828
0.2µg/mL MDL	500	2,216,413	47,574,373,799	225,235,269,754	129,855,129,478	33,418,065,197	186,087,612,826	329,877,012,535	18,437,041,208	NP	385,345,171,189
0.2µg/mL MDL	500	1,923,144	45,321,325,336	216,844,993,697	124,622,415,559	32,163,282,404	173,929,389,124	323,362,726,481	18,925,301,154	631,961,539,689	365,820,367,248
0.2µg/mL MDL	500	2,329,677	46,721,206,311	216,497,273,296	130,118,254,967	32,222,054,943	177,292,614,828	322,129,324,006	18,863,385,993	616,245,729,898	379,289,168,092
0.2µg/mL MDL	500	2,501,297	50,580,865,678	237,659,771,515	136,090,908,298	37,540,285,473	232,125,751,648	363,038,658,271	20,253,628,042	667,850,268,655	399,846,452,762
0.2µg/mL MDL	500	2,548,511	51,221,109,437	249,837,882,756	121,249,880,511	36,865,051,436	247,007,551,172	370,835,880,748	20,838,010,805	NP	417,913,031,867
0.2µg/mL MDL	500	2,075,873	46,812,669,550	229,789,130,984	122,332,934,023	35,713,314,828	206,426,505,571	355,638,417,413	20,537,461,281	NP	398,254,220,892
	Std Dev =	227,603	2,138,583,371	11,749,330,498	5,431,211,366	2,164,980,642	31,600,483,368	19,696,094,817	966,723,100	26,451,263,248	16,752,860,100
	Average =	2,246,652	48,021,525,459	229,448,094,552	126,745,259,820	34,596,825,494	198,024,102,134	343,769,779,674	19,743,551,101	638,685,846,081	391,859,660,268
	%Dev=	10.1%	4.5%	5.1%	4.3%	6.3%	16.0%	5.7%	4.9%	4.1%	4.3%

		Calculated Conc. (µg/L)									
Sample Name	Sample Volume (mL)	DACT	ACET	DEA	Bromacil	Simazine	Hexazinone	Atrazine	Diuron	Norflurazon	Prometon
0.2µg/mL MDL	500	0.147	0.162	0.156	0.142	0.155	0.124	0.150	0.141	NP	0.138
0.2µg/mL MDL	500	0.153	0.160	0.152	0.151	0.150	0.142	0.144	0.128	NP	0.133
0.2µg/mL MDL	500	0.130	0.149	0.144	0.144	0.144	0.132	0.141	0.131	0.123	0.125
0.2µg/mL MDL	500	0.162	0.156	0.143	0.151	0.144	0.135	0.140	0.131	0.120	0.131
0.2µg/mL MDL	500	0.176	0.174	0.163	0.159	0.171	0.180	0.160	0.141	0.132	0.139
0.2µg/mL MDL	500	0.179	0.177	0.175	0.139	0.168	0.192	0.164	0.145	NP	0.147
0.2µg/mL MDL	500	0.142	0.156	0.156	0.141	0.162	0.159	0.157	0.143	NP	0.139
	Average =	0.156	0.162	0.156	0.147	0.156	0.152	0.151	0.137	0.125	0.136
	Std Dev =	0.018	0.010	0.011	0.007	0.011	0.026	0.010	0.007	0.007	0.007
	t Value =	3.143	3.143	3.143	3.143	3.143	3.143	3.143	3.143	3.143	3.143
	MDL =	0.057	0.032	0.035	0.022	0.035	0.082	0.031	0.022	0.021	0.022

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DCH 6-22-01

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File: Mdl 2 MDL

PTRL West, Inc. Cost Estimate
MOR Study: Herbicide on Tomatoes
for Syngenta Crop Protection, Inc.

Analysis will be conducted 244 samples of Tomato

Processing/Inventory		$6,000
Protocol		NC
Method Set-up (Standards prep/LC tryout)		1,200
Method Validation: set includes 1 Control, 1 Reagent Blank, 3 forts at 3 different levels		2,000
Sample Analysis ($180/sample)		
Analysis will be conducted with 10 samples per set (Controls from each site, 2 Fortified Controls and 6 Treated samples) for a total of 244 samples		43,920
LC/MS Confirmation		500
QA		2,400
Project Management/Report		6,000
	Total	$62,020