3.0 MATERIALS and METHODS

3.1 Reference Substances

The analytical (reference) standards used in this study were:

Compound:	Atrazine (G-30027)
CAS RN:	1912-24-9
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-ethyl-N'-(1-methylethyl)-
Analytical standard no.:	410313
Purity:	96.2 ± 0.5%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	September, 2006

Compound:	G-30033
CAS RN:	6190-65-4
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-(1-methylethyl)-
Analytical standard no.:	S92-1618
Purity:	94%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	August, 2008

Compound:	G-28279
CAS RN:	1007-28-9
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-*N*-ethyl-
Analytical standard no.:	S87-1225
Purity:	96%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	March, 2007

Compound:	G-28273
CAS RN:	3397-62-4
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro
Analytical standard no.:	S87-1195
Purity:	97%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	August, 2008

Compound:	Simazine (G-27692)
CAS RN:	122-34-9
Chemical Name:	1,3,5-Triazine-2,4-diamine, 6-chloro-*N,N'*-diethyl-
Analytical standard no.:	443460
Purity:	96.5 ± 0.5%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	April, 2008

Compound:	Ametryn (G-34162)
CAS RN:	834-12-8
Chemical Name:	1,3,5-Triazine-2,4-diamine, *N*-ethyl-*N'*-(1-methylethyl)-6-methylthio)-
Analytical standard no.:	410430
Purity:	98.3 ± 0.5%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	November, 2009

Compound:	Prometryn (G-34161)
CAS RN:	7287-19-6
Chemical Name:	1,3,5-Triazine-2,4-diamine, *N,N'*-bis(1-methylethyl)-6-(methylthio)-
Analytical standard no.:	410566
Purity:	99.7 ± 0.5%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	January, 2010

Compound:	GS-11354
CAS RN:	4147-57-3
Chemical Name:	2-amino-4-isopropylamino-6-methylthio-s-triazine
Analytical standard no.:	S85-0804
Purity:	97%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	April, 2007

Compound:	GS-11355
CAS RN:	4147-58-4
Chemical Name:	1,3,5-Triazine-2,4-diamine,*N*-ethyl-6-(methylthio)-
Analytical standard no.:	DAH-XXXI-29-1
Purity:	98%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	May 31, 2006

Compound:	GS-26831
CAS RN:	5397-01-3
Chemical Name:	2,4-bis-amino-6-methylthio-s-triazine
Analytical standard no.:	S85-0802
Purity:	99%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	April, 2007

Compound:	Metolachlor
CAS RN:	51218-45-2
Chemical Name:	Acetamide, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-
Analytical standard no.:	412778
Purity:	98.0 ± 0.5%
Source:	Syngenta Crop Protection, Inc.
Date received:	October 21, 2005
Expiration date:	May, 2009

Characterization data for the reference substances are maintained by the Technology Support/Chemistry Group of Syngenta Crop Protection, Greensboro, NC.

The test and reference substances used in this study were procured and stored as directed on "Analytical Standard Certificate" or by the Study Monitor. All solutions made from the reference substances were stored according to the method.

3.2 Test Systems

Three types of water were evaluated in this study: ground, surface, and finished. All waters were obtained by Morse Labs personnel in one-gallon plastic containers from local sources. Ground (well) water was obtained December 3, 2005 from a residence in Elk Grove, CA. Surface (river) water was collected December 3, 2005 from the American River near Watt Avenue in Sacramento, CA. Finished (tap) water was collected December 3, 2005 from a residence in Sacramento, CA.

Upon receipt of the samples at the laboratory (within 2 hours of collection) they were immediately placed in refrigerated storage (typically 1-8°C), where they remained pending analysis for suitability. Once determined by analysis to be suitable for the study, an aliquot of each bulk sample was removed for subsequent characterization analysis.

The waters were characterized under GLP for pH, calcium, magnesium, sodium, hardness, conductivity, sodium absorption ratio (SAR), total dissolved solids, and turbidity by Agvise Laboratories, Inc. of Northwood, ND. Five hundred (500)-mL aliquots of each control water sample (3) were sent to Agvise Labs for analysis. The results are summarized below:

	Ground Water	**Surface Water**	**Finished Water**
Location	Elk Grove (Well)	Sacramento (American River)	Sacramento (Tap)
State	CA	CA	CA
Sample ID	1270B	1270C	1270A
pH	7.5	7.5	8.0
Calcium (ppm)	18	5.8	12
Magnesium (ppm)	12	2.0	6.5
Sodium (ppm)	14	2.4	20
Hardness (mg equiv. $CaCO_3$/L)	95	23	58
Conductivity (mmhos/cm)	0.23	0.05	0.20
Sodium Absorption Ratio (SAR)	0.62	0.22	1.14
Total Dissolved Solids (ppm)	150	10	118
Turbidity (NTU)	0.20	1.82	0.27

The Agvise analysis report is found in Appendix 5.

3.3 Equipment and Reagents

The equipment and reagents used for the method validation were as outlined in the method (Section 2.0 Materials and Methods and Appendices 1 and 2). Identical or equivalent apparatus and materials were used, as permitted by the method.

3.4 Standard Solution Preparation

3.4.1 Stock Standard Solutions

The following concentrations of stock standard solutions were prepared. These solutions were prepared to contain each targeted analyte individually.

All targeted analytes except G-30033, G-28279, and G-28273:

Ten (10.0) mg (corrected for purity) of each applicable analytical standard were accurately weighed and quantitatively transferred to individual 100-mL volumetric flasks and brought to volume with methanol. The resulting concentration was 100 µg/mL.

G-30033, G-28279, and G-28273:

Five (5.0) mg (corrected for purity) of each applicable analytical standard were accurately weighed and quantitatively transferred to individual 100-mL volumetric flasks and brought to volume with methanol. The resulting concentration was 50 µg/mL.

3.4.2 Laboratory (Procedural) Fortification Standard Solutions

The following concentrations of fortification standard solutions were prepared. These solutions were prepared as mixtures containing all targeted analytes. The first concentration listed is for all analytes except G-28273, which is represented by the second concentration listed:

2 µg/mL/
10 µg/mL: 2.0 mL each of the 100 µg/mL stock standard solutions prepared for atrazine, simazine, ametryn, prometryn, GS-11354, GS-11355, GS-26831, and metolachlor and 4.0 mL each of the 50 µg/mL stock standard solutions prepared for G-30033 and G-28279 and 20.0 mL of the 50 µg/mL standard solution prepared for G-28273 were transferred to a 100-mL volumetric flask. The solution was brought to a final volume of 100 mL with methanol:water (5:95, v/v). The solution was mixed well.

0.2 µg/mL/
1 µg/mL: 10.0 mL of the 2 µg/mL/10 µg/mL standard solution mixture prepared above were transferred to a 100-mL volumetric flask. The solution was brought to a final volume of 100 mL with methanol:water (5:95, v/v). The solution was mixed well.

0.02 µg/mL/
0.1 µg/mL: 10.0 mL of the 0.2 µg/mL/1 µg/mL standard solution mixture prepared above were transferred to a 100-mL volumetric flask. The solution was brought to a final volume of 100 mL with methanol:water (5:95, v/v). The solution was mixed well.

3.4.3 Instrumentation (Calibration) Standard Solutions

The following concentrations of calibration standard solutions were prepared. These solutions were prepared as mixtures containing all targeted analytes. The first concentration listed is for all analytes except G-28273, which is represented by the second concentration listed:

0.0005 µg/mL/
0.0025 µg/mL: 2.5 mL of a 0.02 µg/mL/0.1 µg/mL mixed fortification standard solution were transferred to a 100-mL volumetric flask. The contents were brought to volume with methanol:water (5:95, v/v). The solution was mixed well.

0.0001 µg/mL/
0.0005 µg/mL: 500 µL of a 0.02 µg/mL/0.1 µg/mL mixed fortification standard solution were transferred to a 100-mL volumetric flask. The contents were brought to volume with methanol:water (5:95, v/v). The solution was mixed well.

0.00005 µg/mL/
0.00025 µg/mL: 250 µL of a 0.02 µg/mL/0.1 µg/mL mixed fortification standard solution were transferred to a 100-mL volumetric flask. The contents were brought to volume with methanol:water (5:95, v/v). The solution was mixed well.

0.00002 µg/mL/
0.00010 µg/mL: 100 µL of a 0.02 µg/mL/0.1 µg/mL mixed fortification standard solution were transferred to a 100-mL volumetric flask. The contents were brought to volume with methanol:water (5:95, v/v). The solution was mixed well.

0.00001 µg/mL/
0.00005 µg/mL: 50 µL of a 0.02 µg/mL/0.1 µg/mL mixed fortification standard solution were transferred to a 100-mL volumetric flask. The contents were brought to volume with methanol:water (5:95, v/v). The solution was mixed well.

3.5 Analytical Method

The method found in Syngenta Amended Report T010097-04 entitled "Analytical Method T010097-04 for the Determination of Atrazine, Simazine, G-30033, G-28279, G-28273, Ametryn, Prometryn, GS-11354, GS-11355, GS-26831, and Metolachlor in Water Using Direct Injection LC-ESI/MS/MS Including Validation Data" was used for the analyses in this study. See Appendix 2 for the complete text of the method. The following is a summary of the method.

> Samples were diluted 1 to 5 (10 mL to a final volume of 50 mL in a 50 mL-volumetric flask) with methanol:water (5:95, v/v). The diluted samples were, without any cleanup, submitted directly to HPLC analysis. Determination and quantitation of the targeted analytes was conducted using high performance liquid chromatography (HPLC) employing electrospray ionization mass spectrometric (ESI/MS/MS) detection. The limit of quantitation (LOQ) was 0.1 ppb and the limit of detection (LOD) was 0.05 ppb for all analytes except G-28273, whose quantitation and detection limits were 0.5 ppb and 0.25 ppb, respectively.

3.6 Sample Preparation and Fortification

Each validation set per water type consisted of one reagent blank, two control samples, five control samples fortified at the LOQ (0.1 ppb for all analytes except G-28273 which was 0.5 ppb) for each analyte and five control samples fortified at 10 × LOQ for each analyte.

Twelve 10.0-mL portions of a specific water type control (untreated) sample were used as samples for each validation set. Samples were designated as controls or fortified controls. Fortified controls were each fortified with all eleven analytes at either the LOQ (50 µL of a mixed standard solution containing 0.02 µg/mL of all analytes except G-28273 which was at 0.1 µg/mL) or 10 × LOQ (50 µL of a mixed standard solution containing 0.2 µg/mL of all analytes except G-28273 which was at 1.0 µg/mL). Once fortified, the fortified control samples were swirled, then diluted to 50 mL as described in the method summary.

3.7 Modifications, Interpretations, and Critical Steps

The analytical method was run exactly as written except as follows:

1) Alternate quantitation ions were used for some analytes:

Ametryn:

Primary quantitation transition specified:	*m/z* 228.3 to186.1
Alternate quantitation transition used:	*m/z* 228.3 to138.0

Simazine:

Primary quantitation transition specified:	*m/z* 202.1 to132.1
Alternate quantitation transition used:	*m/z* 202.1 to124.0

G-28279:

Primary quantitation transition specified:	*m/z* 174.2 to 96.2
Alternate quantitation transition used:	*m/z* 174.2 to 104.0

2) The gradient elution profile was changed as described in Section 3.8 of this report.

3) The initial 1 to 5 sample dilution was carried out by diluting 10 mL of sample to a final volume of 50 mL with methanol:water (5:95, v/v) rather than mixing 200µL of sample with 800 µL of methanol:water (5:95, v/v).

4) Calculations were carried out using 1/x weighting and slightly different equations.

No steps in the method were determined to have to be followed so specifically (or critically) that they required special care and/or specific instructions in order to avoid posing the risk of method failure.

3.8 Instrumentation

The HPLC conditions employed on the first attempt for each water type evaluated [ground (well) water, surface (river) water, and finished (tap) water], which produced acceptable data for all applicable analytes as reported herein, were as follows:

HPLC/MS/MS system:	Applied BioSystems/MDS Sciex API 4000 LC/MS/MS system with a Shimadzu SIL-HTA autosampler, an integrated Shimadzu chromatograph consisting of (2) LC-10AD*vp* Liquid Chromatograph units and a DGU-14A Degasser. The system is controlled and data processed by Applied BioSystems/MDS Sciex Analyst Software.
HPLC analytical column:	Zorbax SB-CN, 75 mm × 4.6 mm i.d., 3.5 µm particle size (Agilent P/N 866953-905)
Mobile phase:	Fisher Water, Burdick and Jackson Methanol

Gradient:

Time (min)	% Water	% Methanol
0-0.5	95	5
1.5-5.0	50	50
7.5-10.5	35	65
10.6-18.6	95	5

Divert valve:	Programmed to divert LC flow from column to waste (bypassing detector) from 0 to 3.0 minutes and again from 12.0 to 18.6 minutes. LC flow is directed to detector during the 3.0 to 12.0 minute window. Diversion time settings can be adjusted as necessary depending on the retention times of the analytes.
Flow rate:	0.4 mL/min
Interface:	TIS/ES (turbo ion spray/electrospray)
Ionization mode:	positive (+)
Acquisition mode:	MRM
Source temperature:	700 °C
Curtain gas:	Nitrogen @ 10
Collision gas:	Nitrogen @ setting of "8"
Transitions monitored:	Atrazine: *m/z* 216.1 to 174.1 Simazine: *m/z* 202.1 to 124.0 G-30033: *m/z* 188.1 to 146.1 G-28279: *m/z* 174.2 to 104.0 G-28273: *m/z* 146.0 to 103.9 Ametryn: *m/z* 228.3 to 138.0 Prometryn: *m/z* 242.1 to 158.2 GS-11354: *m/z* 200.1 to 158.2 GS-11355: *m/z* 186.1 to 96.1 GS-26831: *m/z* 158.0 to 110.0 Metolachlor: *m/z* 284.2 to 176.3
Injection volume:	50 µL
Column temperature:	45 °C

Retention times:	Atrazine:	~7.7 min.
	Simazine:	~6.8 min.
	G-30033:	~5.7 min.
	G-28279:	~5.3 min.
	G-28273:	~4.6 min.
	Ametryn:	~9.1 min.
	Prometryn:	~10 min.
	GS-11354:	~6.5 min.
	GS-11355:	~5.8 min.
	GS-26831:	~4.9 min.
	Metolachlor:	~9.9 min.

3.9 Calculations

Calculations for instrumental analysis were conducted using a validated software application to create a standard curve based on linear regression. The regression functions were used to calculate a best fit line (from a set of standard concentrations in µg/mL versus peak response) and to determine concentrations of the analyte found during sample analysis from the calculated best fit line. 1/x weighting was employed.

The equation used for the least squares fit is:

$$y = mx + b$$

where:

y	=	peak response (area)
x	=	µg/mL found for peak of interest
m	=	slope
b	=	y-intercept

The calculations for ppb found and percent recovery (for fortified samples) are:

1. The amount of analyte (in ppb) found in the sample is calculated according to the following equation:

$$ppb = \mu g/mL\ found \times \frac{final\ vol.(mL)}{sample\ wt.(g)} \times \frac{1000\ ng}{1\ \mu g} \times HPLC\ dil.\ factor$$

where:

µg/mL found	=	µg/mL of analyte determined from standard curve

final vol. (mL)	=	final volume of sample following dilution (typically 50.0 mL), an aliquot of which is submitted to HPLC analysis
sample wt. (g)	=	gram weight equivalence of sample taken through procedure (typically 10.0 g)
HPLC dil. factor	=	the magnitude of dilution required to bracket the response of the sample within the standard curve responses. When the sample requires no dilution, the HPLC dilution factor = 1

2. The percent recovery for fortified control samples is calculated as follows:

$$\%\ Recovery = \frac{ppb\ found\ in\ fortified\ control}{ppb\ added} x\ 100$$

Example Calculations

All targeted analyte residues were calculated in an identical manner for all water types. Only examples of atrazine residue calculations in ground (well) water will be provided and thus serve to illustrate the calculations for all other analytes in all water types.

1. ML ticket #83680, Atrazine, Well water, Set #3, 1270B, **Control** 6 [Figure 3 (1)]:

$$0\ peak\ response \rightarrow 0.000\ \mu g/mL$$

$$ppb = 0.000 \mu g/mL \times \frac{50.0\ mL}{10.0 g} \times \frac{1000\ ng}{1\ \mu g} \times 1$$

$$ppb = 0.000$$

$$reported = < 0.1\ ppb$$

2. ML ticket #83680, Atrazine, Well water, Set #3, 1270B, **Fortified Control** 11 @ 0.1 ppb [Figure 3 (2)]:

$$22900\ peak\ response \rightarrow 0.0000199\ \mu g/mL$$

$$ppb = 0.0000199\,\mu g/mL \times \frac{50.0\,mL}{10.0\,g} \times \frac{1000\,ng}{1\,\mu g} \times 1$$

$$ppb = 0.0995$$

$$reported = 0.0995\ ppb$$

$$\%\,Recovery = \frac{0.0995\ ppb}{0.10\ ppb} \times 100$$

$$= 100\%$$

APPENDIX 1. LC Operating Paramenters

Table 2. DI-ESI-LC/MS/MS API 4000 Operating Parameters

HPLC: Perkin Elmer Series 200
Column: Zorbax SB-CN, 4.6 x 75 mm, 3.5 µm particle size (Agilent P/N 866953-905)
Flow rate: 0.40 mL/min
Column Temperature: 45°C
Injection volume: 100 µL or 50 µL
Mobile Phase: A = HPLC grade water
B = HPLC grade methanol
Time = Method Run time after injection

Time	%A	%B	Description
0.0 - 0.5 min.	95	5	isocratic condition for 0.5 min.
0.5 - 2.5 min.	35	65	linear gradient for 2 min.
2.5 – 10.5 min.	35	65	isocratic conditions for 8.0 min.
10.5 – 18.5	95	5	step gradient immediately to initial conditions for 8.0 min.

MS/MS Conditions & Parameters:
Instrumentation: Applied Biosystems, MDS Sciex API 4000 triple quadrupole
Software: Analyst 1.4
Ionization: Electrospray (Positive mode)
Source Temperature: 700°C

Equivalent equipment, conditions or other columns may be used as long as equivalent recovery results are obtained. Any changes must be documented in the raw data.