Analytical method for Atrazine, Simazine, Propazine, G30033, G28279, G28273 and Metolachlor in water

Reports:	ECM: MRID 48346602. Huang, S.-B. 2010. Atrazine - Analytical Method for the Determination of Atrazine, Simazine, Propazine, G30033, G28279, G28273 and Metolachlor in Water Using Direct-Aqueous-Injection Liquid Chromatography-Electrospray lonization Tandem Mass Spectrometry (LC-ESI/MS/MS) with Stable Isotope Analogues as Quantification Internal Standard - Method. Syngenta Report No.: GRM014.02A and Task No.: T001943-09. Report prepared, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 94 pages. Final report issued February 16, 2010. ILV: MRID 48331501. Swaim, L. 2010. Atrazine - Independent Laboratory Validation of Analytical Method GRM014.02 A for the Determination of Atrazine, Simazine, Propazine, G30033, G28279, G28273, and Metolachlor in Water Using Direct-Aqueous-Injection Liquid Chromatography-Electrospray lonization Tandem Mass Spectrometry (LC-ESI/MS/MS) with Stable Isotope Analogues as Quantification Internal Standard – Final Report. Syngenta Study No. 52-99. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, and sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 161 pages. Final report issued November 1, 2010.
Document No.:	MRIDs 48346602 & 48331501
Guideline:	850.6100
Statements:	ECM: The study was not a validation and was not required to be conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 48346602). Signed and dated No Data Confidentiality, and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. ILV: The study was conducted in accordance with the USEPA GLP standards (p. 3 of MRID 48331501). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as **Supplemental**. ECM performance data was not reported for the 10×LOQ analysis of metolachlor. The ECM study report did not provide chromatograms for all fortifications/matrices tested and individual recovery data. The LOD was not reported in the ILV.
PC Code:	108801 (metolachlor/S-metolachlor)/080803 (atrazine)/ 080807 (simazine)/080808 (propazine)
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Dynamac JV Reviewers:	Environmental Scientist	Date:	5/10/18
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		Date:	5/10/18

Executive Summary

The analytical method, Syngenta Method No. GRM014.02A, is designed for the quantitative determination of atrazine, simazine, propazine, G30033, and G28279 in water at the LOQ of 0.05 µg/L using LC/MS/MS, of G28273 in water at the LOQ of 0.50 µg/L using LC/MS/MS, and of metolachlor in water at the LOQ of 0.10 µg/L using LC/MS/MS. The method was not designed to resolve the stereoisomers of metolachlor. The ECM test material was S-metolachlor; the ILV test material was metolachlor**. For metolachlor, simazine and propazine, the LOQs in water of 0.10, 0.05 and 0.05 ug/L are less than the lowest toxicological levels of concern for aquatic organisms (non-vascular plant aquatic life benchmarks = 8.00, 2.24, 24.8 ug/L)[1]. For atrazine, the LOQ in water of 0.05 ug/L is currently less than the lowest toxicological level of concern for aquatic organisms (non-vascular plant endpoint = 1 ug/L)[2].** The ECM validated the method using four characterized water matrices; the ILV validated the method using three characterized water matrices. The ILV validated the method for all analytes in the three water matrices after the first trial, with insignificant analytical instrument and parameter modifications, except for the treated (finished) water samples at the 10×LOQ fortification which needed to be re-analyzed due to a laboratory fortification error. Analytes were identified and quantified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate data. Quantification was based on the ratio of the analyte response to the response of a radiolabeled internal standard. All ILV data was satisfactory regarding accuracy, precision, linearity, and specificity. All ECM data was satisfactory regarding accuracy, precision, and linearity, except that no samples were prepared for the 10×LOQ analysis of S-metolachlor/metolachlor; therefore, reproducibility for the 10×LOQ analysis of S-metolachlor/metolachlor was not supported by the data. Additionally, the ECM study report did not provide chromatograms for all fortifications/matrices tested and any individual recovery data. The LOD was not reported in the ILV. Supplementary data for Method GRM014.02A was presented in the ECM to demonstrate that atrazine, simazine, propazine, G30033, G28279, G28273, and metolachlor can be determined in water samples from selected ECO and AMP programs containing certain types of preservatives.

[1] **https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk**

[2] **Error! Main Document Only.**USEPA, 2016. Refined Ecological Risk Assessment for Atrazine, Office of Chemical Safety and Pollution Prevention, Washington, DC. April 12, 2016; D418317.

Table 1. Analytical Method Summary.

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Atrazine	48346602[1]	48331501[2]		Water	16/02/2010	Syngenta Crop Protection, Inc.	LC/MS/MS	0.05 µg/L
Simazine								
Propazine								
G30033								
G28279								
G28273								0.50 µg/L
Metolachlor								0.10 µg/L

1 In the ECM, the deionized, ground (well), surface, and treated (finished) water matrices used in the study were transferred from a previous study (T001681-06). Deionized water (pH 6.4, hardness 3.0 mg equivalent $CaCO_3$/L, 4.0 ppm total dissolved solids) was collected from a Picopure purification system in Syngenta laboratory L-2021. Ground (well; pH 7.1, hardness 43 mg equivalent $CaCO_3$/L, 108 ppm total dissolved solids) and treated (finished; pH 6.2, hardness 15 mg equivalent $CaCO_3$/L, 14 ppm total dissolved solids) waters were collected from two separate residential water supplies. Surface water (pH 7.5, hardness 81 mg equivalent $CaCO_3$/L, 162 ppm total dissolved solids) was collected from a local municipality reservoir. The water characterization was provided by Agvise Laboratories (Northwood, North Dakota).

2 In the ILV, the ground (well), surface, and treated (finished) water matrices used in the study were provided and characterized by Agvise Laboratories (Northwood, North Dakota). Ground water (well; pH 7.5, hardness 677 mg equivalent $CaCO_3$/L, 814 ppm total dissolved solids) was collected from a well owned by Bob Deutsh at Agvise Laboratories. Treated water (finished; pH 7.9, hardness 117 mg equivalent $CaCO_3$/L, 114 ppm total dissolved solids) was tap water collected from Agvise Laboratories. Surface water (pH 8.2, hardness 643 mg equivalent $CaCO_3$/L, 1156 ppm total dissolved solids) was collected from Goose River in Northwood, North Dakota.

I. Principle of the Method

Samples (9.0 mL) was fortified, as necessary, with 1.0 mL of a 0.0005 µg/mL standard solution (pp. 17-18 of MRID 48346602). The sample was further diluted, if necessary. An aliquot (900 µL) was transferred to an autosampler vial and 100 µL of internal standard (IS) solution (50 pg/L concentration) was added prior to analysis by LC/MS/MS.

Samples were analyzed using a Surveyor Plus LC pump coupled to a Thermo Electron TSQ Quantum Ultra MS (pp. 19-22 of MRID 48346602). The following LC conditions were used: Zorbax SB-Aq column (4.6 mm x 50 mm, 3.5 µm, column temperature 20°C), ColumnSaver filter, mobile phase of (A) 0.1% formic acid in HPLC grade water and (B) 0.1% formic acid in HPLC grade methanol [percent A:B (v:v) at 0.0-0.5 min. 95:5, 1.0-1.5 min. 70:30, 2.5-5.5 min. 10:90, 5.6-7.5 min. 95:5], and injection volume of 50 µL. The following MS/MS conditions were used: positive mode (temperature 350°C) and multiple reaction monitoring (MRM). Analytes were identified using one ion pair transition as follows: *m/z* 146.00→104.00 for G28273, *m/z* 174.05→132.00 for G28279, *m/z* 188.05→145.95 for G30033, *m/z* 202.10→132.00 for simazine, *m/z* 216.10→174.10 for atrazine, *m/z* 230.10→146.10 for propazine, and *m/z* 284.10→252.10 for S-metolachlor. Expected retention times were not reported. The study author reported that the method was not designed to resolve the stereoisomers of metolachlor. Quantification was based on the ratio of the analyte response to the IS response.

The independent laboratory performed the ECM as written, except for the use of metolachlor as a test material instead of S-metolachlor and for insignificant modifications of analytical instrumentation and parameters: the injection volume was reduced from 50 µL to 35 µL (pp. 17-19 of MRID 48331501). An Applied Biosystems/Sciex API 5000 Waters Acquity UPLC coupled to a Sciex API 5000 LC/MS/MS was used. The following LC conditions were used: Zorbax SB-Aq column (4.6 mm x 50 mm, 3.5 µm, column temperature 25°C) and injection volume of 50 µL; the mobile phase and gradient was the same as the ECM. The following MS/MS conditions were used: positive mode (temperature 350°C) and multiple reaction monitoring (MRM). Analytes were identified using one ion pair transition as follows: *m/z* 146.6→104.0 for G28273, *m/z* 174.0→104.0 for G28279, *m/z* 188.0→146.0 for G30033, *m/z* 202.0→132.0 for simazine, *m/z* 216.0→174.0 for atrazine, *m/z* 230.0→146.0 for propazine, and *m/z* 284.0→176.0 for Metolachlor. Expected retention times were not reported. The ILV modifications did not warrant an updated ECM.

In the ECM and ILV, Limit of Quantification (LOQ) in water was 0.05 µg/L for atrazine, simazine, propazine, G30033 and G28279, 0.50 µg/L for G28273, and 0.10 µg/L for metolachlor (pp. 11, 26-27 of MRID 48346602; pp. 9 of MRID 48331501). In the ECM, the Limits of Detection (LODs) in water were 0.90 pg (0.02 pg/µL) for atrazine, simazine, propazine, G30033 and G28279, 9.0 pg (0.20 pg/µL) for G28273, and 2.25 pg (0.05 pg/µL) for metolachlor. The LODs were not reported in the ILV.

II. Recovery Findings

ECM (MRID 48346602): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of atrazine, simazine, propazine, G30033, and G28279 in four water matrices at fortification levels of 0.05 µg/L (LOQ), 0.10 µg/L (2×LOQ), 0.50 µg/L (10×LOQ), and 5.0 µg/L (100×LOQ; Tables 1-7, pp. 30-36). Mean recoveries and RSDs were within guidelines for analysis of G28273 in four water matrices at fortification levels of 0.50 µg/L (LOQ) and 5.0 µg/L (10×LOQ). Mean recoveries and RSDs were within guidelines for analysis of S-metolachlor in four water matrices at fortification levels of 0.10 µg/L (LOQ), 0.50 µg/L (5×LOQ), and 5.0 µg/L (50×LOQ); no samples were fortified at 10×LOQ. RSDs for the 5.0 µg/L fortification of the analytes in surface water were reviewer-calculated using mean and standard deviation since the RSDs of the study report were erroneously omitted from the data tables. Individual data was not reported for any analysis. Analytes were identified and quantified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate data. According to the calculation method, recovery results were corrected when residues were quantified in the controls. The deionized, ground (well), surface, and treated (finished) water matrices used in the study were transferred from a previous study (T001681-06; pp. 24-25). Deionized water (pH 6.4, hardness 3.0 mg equivalent $CaCO_3$/L, 4.0 ppm total dissolved solids) was collected from a Picopure purification system in Syngenta laboratory L-2021. Ground (well; pH 7.1, hardness 43 mg equivalent $CaCO_3$/L, 108 ppm total dissolved solids) and treated (finished; pH 6.2, hardness 15 mg equivalent $CaCO_3$/L, 14 ppm total dissolved solids) waters were collected from two separate residential water supplies. Surface water (pH 7.5, hardness 81 mg equivalent $CaCO_3$/L, 162 ppm total dissolved solids) was collected from a local municipality reservoir. The water characterization was provided by Agvise Laboratories (Northwood, North Dakota).

ILV (MRID 48331501): Mean recoveries and RSDs were within guidelines for analysis of atrazine, simazine, propazine, G30033, and G28279 in three water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/L (10×LOQ; p. 10). Mean recoveries and RSDs were within guidelines for analysis of G28273 in three water matrices at fortification levels of 0.50 µg/L (LOQ) and 5.0 µg/L (10×LOQ). Mean recoveries and RSDs were within guidelines for analysis of metolachlor in three water matrices at fortification levels of 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ). Analytes were identified and quantified using one ion transition; a confirmatory method is not usually required when LC/MS or GC/MS is used as the primary method to generate data. According to the calculation method, recovery results were corrected when residues were quantified in the controls. The ground (well), surface, and treated (finished) water matrices used in the study were provided and characterized by Agvise Laboratories (Northwood, North Dakota; pp. 15-16). Ground water (well; pH 7.5, hardness 677 mg equivalent $CaCO_3$/L, 814 ppm total dissolved solids) was collected from a well owned by Bob Deutsh at Agvise Laboratories. Treated water (finished; pH 7.9, hardness 117 mg equivalent $CaCO_3$/L, 114 ppm total dissolved solids) was tap water collected from Agvise Laboratories. Surface water (pH 8.2, hardness 643 mg equivalent $CaCO_3$/L, 1156 ppm total dissolved solids) was collected from Goose River in Northwood, North Dakota. The method was validated for all analytes in the three water matrices at both fortification levels after the first trial, with insignificant analytical instrument and parameter modifications, except for the treated (finished) water samples at the 10×LOQ fortification which needed to be re-analyzed due to a laboratory fortification error (pp. 17-19, 21).

Table 2. Initial Validation Method Recoveries for Atrazine, Simazine, Propazine, G30033, G28279, G28273 and Metolachlor in Water.[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Deionized Water					
Atrazine	0.05 (LOQ)	5	Not Reported	106	14	13
	0.10	5		100	2.7	2.7
	0.50	5		103	2.3	2.2
	5.0	5		99.7	1.9	1.9
Simazine	0.05 (LOQ)	5		103	4.9	4.8
	0.10	5		101	1.3	1.3
	0.50	5		99.7	2.1	2.1
	5.0	5		98.0	1.2	1.2
Propazine	0.05 (LOQ)	5		97.5	5.6	5.7
	0.10	5		93.4	4.6	4.9
	0.50	5		99.6	1.6	1.6
	5.0	5		97.6	2.0	2.0
G30033	0.05 (LOQ)	5		98.5	5.2	5.3
	0.10	5		98.5	2.7	2.8
	0.50	5		101	1.6	1.6
	5.0	5		101	2.4	2.4
G28279	0.05 (LOQ)	5		93.6	6.5	7.0
	0.10	5		96.3	6.2	6.4
	0.50	5		98.7	3.5	3.6
	5.0	5		98.0	1.8	1.8

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
G28273	0.50 (LOQ)	5		91.0	3.2	3.5
	5.0	5		101	1.2	1.2
S-Metolachlor	0.10 (LOQ)	5		80.0	5.4	6.8
	0.50	5		110	5.9	5.3
	5.0	5		98.8	3.5	3.5
	Ground (Well) Water					
Atrazine	0.05 (LOQ)	5	Not Reported	91.9	6.5	7.1
	0.10	5		105	7.6	7.2
	0.50	5		101	3.3	3.3
	5.0	5		101	1.8	1.8
Simazine	0.05 (LOQ)	5		105	7.8	7.4
	0.10	5		101	5.0	4.9
	0.50	5		102	2.8	2.8
	5.0	5		103	3.1	3.0
Propazine	0.05 (LOQ)	5		100	1.9	1.9
	0.10	5		101	2.7	2.7
	0.50	5		98.6	1.0	1.0
	5.0	5		99.5	3.4	3.4
G30033	0.05 (LOQ)	5		88.9	4.9	5.5
	0.10	5		98.9	4.7	4.7
	0.50	5		102	0.8	0.8
	5.0	5		101	3.0	3.0
G28279	0.05 (LOQ)	5	Not Reported	99.8	7.0	7.0
	0.10	5		98.7	5.4	5.5
	0.50	5		98.2	2.5	2.6
	5.0	5		99.6	3.2	3.2
G28273	0.50 (LOQ)	5		98.3	6.2	6.3
	5.0	5		101	2.1	2.1
S-Metolachlor	0.10 (LOQ)	5		100	9.4	9.4
	0.50	5		103	7.1	6.9
	5.0	5		94.9	2.6	2.7
	Finished Water					
Atrazine	0.05 (LOQ)	5	Not Reported	104	10	9.8
	0.10	5		101	2.9	2.9
	0.50	5		102	1.9	1.8
	5.0	5		102	1.5	1.5
Simazine	0.05 (LOQ)	5		95.4	9.3	9.8
	0.10	5		94.6	7.1	7.5
	0.50	5		103	2.9	2.8
	5.0	5		102	2.3	2.3
Propazine	0.05 (LOQ)	5		92.8	9.0	9.7
	0.10	5		93.2	5.9	6.3
	0.50	5		100	1.7	1.7
	5.0	5		100	2.0	2.0
G30033	0.05 (LOQ)	5		102	8.2	8.1
	0.10	5		101	3.2	3.1

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.50	5		104	0.5	0.5
	5.0	5		102	1.2	1.2
G28279	0.05 (LOQ)	5		103	13	12
	0.10	5		102	3.9	3.8
	0.50	5		106	1.5	1.4
	5.0	5		104	2.7	2.6
G28273	0.50 (LOQ)	5		101	4.5	4.5
	5.0	5		105	2.3	2.2
S-Metolachlor	0.10 (LOQ)	5		98.9	7.6	7.7
	0.50	5		97.1	9.5	9.8
	5.0	5		99.5	1.8	1.8
	Surface Water					
Atrazine	0.05 (LOQ)	5	Not Reported	93.4	7.9	8.5
	0.10	5		104	6.4	6.1
	0.50	5		102	2.4	2.3
	5.0	5		102	1.3	**1.3**
Simazine	0.05 (LOQ)	5		96.7	9.5	9.8
	0.10	5		100	6.0	6.0
	0.50	5		100	3.6	3.6
	5.0	5		101	2.6	**2.6**
Propazine	0.05 (LOQ)	5		93.6	13	13
	0.10	5		101	2.4	2.4
	0.50	5	Not Reported	99.4	1.8	1.8
	5.0	5		98.9	0.7	**0.7**
G30033	0.05 (LOQ)	5		93.7	7.7	8.3
	0.10	5		100	8.0	8.0
	0.50	5		99.0	2.2	2.2
	5.0	5		99.4	1.0	**1.0**
G28279	0.05 (LOQ)	5		98.7	10	10
	0.10	5		103	5.3	5.1
	0.50	5		102	2.5	2.5
	5.0	5		101	3.0	**3.0**
G28273	0.50 (LOQ)	5		100	5.1	5.1
	5.0	5		102	3.1	**3.0**
S-Metolachlor	0.10 (LOQ)	5		90.7	13.3	14.7
	0.50	5		99.3	7.7	7.7
	5.0	5		99.1	5.2	**5.2**

Data (recovery results were corrected when residues were quantified in the controls; pp. 22-23) were obtained from Tables 1-7, pp. 30-36 of MRID 48346602. Bolded RSDs were reviewer-calculated using mean and standard deviation reported and the following equation: standard deviation ÷ mean × 100 = RSD; the RSDs of the study report were erroneously omitted from the data tables. Individual data was not reported.

1 The deionized, ground (well), surface, and treated (finished) water matrices used in the study were transferred from a previous study (T001681-06; pp. 24-25). Deionized water (pH 6.4, hardness 3.0 mg equivalent $CaCO_3$/L, 4.0 ppm total dissolved solids) was collected from a Picopure purification system in Syngenta laboratory L-2021. Ground (well; pH 7.1, hardness 43 mg equivalent $CaCO_3$/L, 108 ppm total dissolved solids) and treated (finished; pH 6.2, hardness 15 mg equivalent $CaCO_3$/L, 14 ppm total dissolved solids) waters were collected from two separate residential water supplies. Surface water (pH 7.5, hardness 81 mg equivalent $CaCO_3$/L, 162 ppm total dissolved solids) was collected from a local municipality reservoir. The water characterization was provided by Agvise

Laboratories (Northwood, North Dakota).

2 Analytes were identified using one ion pair transition as follows: *m/z* 146.00→104.00 for G28273, *m/z* 174.05→132.00 for G28279, *m/z* 188.05→145.95 for G30033, *m/z* 202.10→132.00 for simazine, *m/z* 216.10→174.10 for atrazine, *m/z* 230.10→146.10 for propazine, and *m/z* 284.10→252.10 for S-metolachlor. The study author not that the method was not designed to resolve the stereoisomers of metolachlor.

Table 3. Independent Validation Method Recoveries for Atrazine, Simazine, Propazine, G30033, G28279, G28273 and Metolachlor in Water.[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Groundwater					
Atrazine	0.05 (LOQ)	5	88.0-101	95.0	4.6	4.8
	0.50	5	103-105	105	0.88	0.84
Simazine	0.05 (LOQ)	5	91.4-102	96.8	4.0	4.1
	0.50	5	100-106	104	2.6	2.5
Propazine	0.05 (LOQ)	5	90.8-96.0	92.7	2.1	2.3
	0.50	5	102-104	103	1.0	1.0
G30033	0.05 (LOQ)	5	83.4-110	94.6	9.9	11
	0.50	5	98.2-103	101	1.8	1.7
G28279	0.05 (LOQ)	5	91.6-116	107	11	10
	0.50	5	93.8-106	102	5.0	4.9
G28273	0.50 (LOQ)	5	90.4-106	99.8	6.2	6.2
	5.0	5	96.4-101	99.9	2.0	2.0
Metolachlor	0.10 (LOQ)	5	73.6-92.5	83.8	8.2	9.8
	1.0	5	90.7-96.6	93.5	2.2	2.3
	Treated Water					
Atrazine	0.05 (LOQ)	5	88.4-98.2	93.8	3.8	4.1
	0.50	5	98.2-104	100	2.2	2.2
Simazine	0.05 (LOQ)	5	89.6-99.4	95.5	3.8	4.0
	0.50	5	97.3-106	101	3.2	3.2
Propazine	0.05 (LOQ)	5	90.0-99.8	93.8	3.8	4.0
	0.50	5	101-103	102	1.0	1.0
G30033	0.05 (LOQ)	5	74.8-92.6	85.0	7.4	8.7
	0.50	5	96.8-103	100	2.2	2.2
G28279	0.05 (LOQ)	5	79.2-119	98.6	15	15
	0.50	5	90.6-105	99.5	5.6	5.6
G28273	0.50 (LOQ)	5	94.2-101	97.0	2.8	2.9
	5.0	5	92.8-107	102	5.6	5.5
Metolachlor	0.10 (LOQ)	5	76.2-93.5	86.7	6.8	7.9
	1.0	5	90.4-98.9	95.0	3.9	4.1
	Surface Water					
Atrazine	0.05 (LOQ)	5	94.4-105	100	3.9	3.9
	0.50	5	99.4-106	102	2.7	2.6
Simazine	0.05 (LOQ)	5	92.8-105	98.6	4.7	4.7
	0.50	5	102-110	106	2.9	2.8
Propazine	0.05 (LOQ)	5	97.6-103	100	2.2	2.2
	0.50	5	101-107	103	2.2	2.1
G30033	0.05 (LOQ)	5	90.2-111	103	7.8	7.6

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.50	5	100-104	102	1.6	1.6
G28279	0.05 (LOQ)	5	102-114	110	5.3	4.9
	0.50	5	95.2-105	101	3.8	3.8
G28273	0.50 (LOQ)	5	92.2-100	95.8	4.1	4.3
	5.0	5	87.4-105	98.2	6.6	6.7
Metolachlor	0.10 (LOQ)	5	85.9-109	96.1	8.8	9.1
	1.0	5	93.2-103	97.2	4.0	4.1

Data (recovery results were corrected when residues were quantified in the controls; pp. 19-20) were obtained from p. 10 of MRID 48331501.

1 The ground (well), surface, and treated (finished) water matrices used in the study were provided and characterized by Agvise Laboratories (Northwood, North Dakota; pp. 15-16). Ground water (well; pH 7.5, hardness 677 mg equivalent $CaCO_3$/L, 814 ppm total dissolved solids) was collected from a well owned by Bob Deutsh at Agvise Laboratories. Treated water (finished; pH 7.9, hardness 117 mg equivalent $CaCO_3$/L, 114 ppm total dissolved solids) was tap water collected from Agvise Laboratories. Surface water (pH 8.2, hardness 643 mg equivalent $CaCO_3$/L, 1156 ppm total dissolved solids) was collected from Goose River in Northwood, North Dakota.

2 Analytes were identified using one ion pair transition as follows: *m/z* 146.6→104.0 for G28273, *m/z* 174.0→104.0 for G28279, *m/z* 188.0→146.0 for G30033, *m/z* 202.0→132.0 for simazine, *m/z* 216.0→174.0 for atrazine, *m/z* 230.0→146.0 for propazine, and *m/z* 284.0→176.0 for metolachlor.

III. Method Characteristics

In the ECM and ILV, LOQ in water was 0.05 µg/L for atrazine, simazine, propazine, G30033 and G28279, 0.50 µg/L for G28273, and 0.10 µg/L for metolachlor (pp. 11, 26-27 of MRID 48346602; pp. 9 of MRID 48331501). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample set at which the methodology has been validated, i.e. demonstrating a mean recovery of 70-120% with an RSD ≤20%. In the ECM, the LODs in water were 0.90 pg (0.02 pg/µL) for atrazine, simazine, propazine, G30033 and G28279, 9.0 pg (0.20 pg/µL) for G28273, and 2.25 pg (0.05 pg/µL) for metolachlor. The LODs were defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. While an estimate of the LOD can be taken as four times the background noise, the method LOD in this study is defined as the smallest standard amount injected during the chromatographic run. The LOD typically corresponds to an amount of the analyte equivalent to *ca*. 50% of the theoretical amount for a recovery sample at the method LOQ. The method LOD also may vary from instrument-to-instrument, depending on the injection volume and concentrations needed to obtain adequate analyte response on a specific model of mass instruments. The LODs were not reported in the ILV. No LOQ calculations were reported in ECM or ILV; no LOD calculations were reported in ECM.

Table 4. Method Characteristics for Atrazine, Simazine, Propazine, G30033, G28279, G28273 and Metolachlor in Water.

Analyte		**Atrazine**	**Simazine**	**Propazine**	**G30033**	**G28279**	**G28273**	**Metolachlor (S-metolachlor)**
Limit of Quantitation (LOQ)		0.05 µg/L					0.50 µg/L	0.10 µg/L
Limit of Detection (LOD)	ECM	0.02 pg/µL (0.90 pg)					0.20 pg/µL (9.0 pg)	0.05 pg/µL (2.25 pg)
	ILV	**Not reported**						
Linearity (calibration curve r^2 and concentration range)	ECM[1]	r^2 = 0.9997	r^2 = 0.9999	r^2 = 0.9999	r^2 = 0.9998	r^2 = 0.9997	r^2 = 0.9982	r^2 = 0.9984
		0.90-450 pg (0.02-10 pg/µL)					9.0-450 pg (0.2-10 pg/µL)	2.25-450 pg (0.05-10 pg/µL)
	ILV[2,3]	r^2 = 0.9994-1.0000	r^2 = 0.9998-1.0000	r^2 = 0.9994-1.0000	r^2 = 0.9996-0.9997	r^2 = 0.9994-0.9998	r^2 = 0.9992-1.0000	r^2 = 0.9997-1.0000
		0.02-10 pg/µL (groundwater and treated water) 0.02-10 pg/µL [treated water (2nd trial) and surface water]					0.20-10 pg/µL (groundwater and treated water) 0.20-10 pg/µL [treated water (2nd trial) and surface water]	0.05-10 pg/µL (groundwater and treated water) 0.05-10 pg/µL [treated water (2nd trial) and surface water]
Repeatable	ECM[4]	Yes at LOQ, 2×LOQ, 10×LOQ, and 100×LOQ					Yes at LOQ and 10×LOQ	Yes at LOQ, 5×LOQ and 50×LOQ. **No** 10×LOQ samples prepared
		(four characterized water matrices)						
	ILV[5,6]	Yes at LOQ and 10×LOQ (three characterized water matrices)						
Reproducible		Yes at LOQ and 10×LOQ						Yes at LOQ; **No** at 10×LOQ

Analyte		**Atrazine**	**Simazine**	**Propazine**	**G30033**	**G28279**	**G28273**	**Metolachlor (S-metolachlor)**
Specific	ECM	Representative chromatograms were only provided for the surface water matrix. Representative chromatograms were not provided for all fortifications, including 10×LOQ for some analytes.						
		Yes, matrix interferences were <LOD (based on peak area ratio).	Yes, matrix interferences were *ca*. 6% of the LOQ (based on residues quantified).	Yes, matrix interferences were <LOD (based on peak area ratio).				
	ILV[7]	The LOQ was fairly small compared to the height of the baseline noise in the majority of the chromatograms.						
		Yes, matrix interferences were *ca*. 10-21% of the LOQ (based on peak area).	Yes, no matrix interferences were observed in the ground or finished water matrices, and matrix interferences were *ca*. 19% of the LOQ (based on peak area) in surface water.	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed in the ground or finished water matrices, and matrix interferences were *ca*. 18% of the LOQ (based on peak area) in surface water.	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; however, a minor peak whose retention time was near that of the analyte was noted.	Yes, no matrix interferences were observed in the ground water matrix, and, matrix interferences were *ca*. 16-19% of the LOQ (based on peak area) in surface and finished water matrices.

Data were obtained from pp. 11, 26-27; Tables 1-7, pp. 30-36 (recovery results); Figures 1-7, pp. 42-62 (chromatograms); Figures 8-14, pp. 63-69 (calibration curves) of MRID 48346602; pp. 9-10 and Appendix 1, pp. 130-157 (recovery results); p. 21; Figure 1, pp. 31-44 (calibration curves); Figures 2-4, pp. 45-128 (chromatograms) of MRID 48331501; DER Attachment 2.

1 Plots were Area Ratio (Analyte/IS) versus amount (pg).

2 Quadratic equations were generated by the ILV study author. Plots were Area Ratio (Analyte/IS) versus Concentration Ratio (Analyte/IS).

3 ILV coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (1/x weighting; matrices combined; Tables 5-7, pp. 36-53 of MRID 48346602; DER Attachment 2).

4 In the ECM, the deionized, ground (well), surface, and treated (finished) water matrices used in the study were transferred from a previous study (T001681-06; pp. 24-25 of MRID 48346602). Deionized water (pH 6.4, hardness 3.0 mg equivalent $CaCO_3$/L, 4.0 ppm total dissolved solids) was collected from a Picopure purification system in Syngenta laboratory L-2021. Ground (well; pH 7.1, hardness 43 mg equivalent $CaCO_3$/L, 108 ppm total dissolved solids) and treated (finished; pH 6.2, hardness 15 mg equivalent $CaCO_3$/L, 14 ppm total dissolved solids) waters were collected from two separate residential water supplies. Surface water (pH 7.5, hardness 81 mg equivalent $CaCO_3$/L, 162 ppm total dissolved solids) was collected from a local municipality reservoir. The water characterization was provided by Agvise Laboratories (Northwood, North Dakota).

5 In the ILV, the ground (well), surface, and treated (finished) water matrices used in the study were provided and characterized by Agvise Laboratories (Northwood, North Dakota; pp. 15-16 of MRID 48331501). Ground water (well; pH 7.5, hardness 677 mg equivalent $CaCO_3$/L, 814 ppm total dissolved

solids) was collected from a well owned by Bob Deutsh at Agvise Laboratories. Treated water (finished; pH 7.9, hardness 117 mg equivalent $CaCO_3$/L, 114 ppm total dissolved solids) was tap water collected from Agvise Laboratories. Surface water (pH 8.2, hardness 643 mg equivalent $CaCO_3$/L, 1156 ppm total dissolved solids) was collected from Goose River in Northwood, North Dakota.

6 The ILV validated the method for all analytes in the three water matrices at both fortification levels after the first trial, with insignificant analytical instrument and parameter modifications, except for the treated (finished) water samples at the 10×LOQ fortification which needed to be re-analyzed due to a laboratory fortification error (pp. 17-19, 21 of MRID 48331501).

7 Matrix interference percentages based on the peak areas were generally in agreement with the percentages of area ratios and residues quantified reported in Appendix 1, pp. 130-157 of MRID 48331501.

IV. Method Deficiencies and Reviewer's Comments

1. In the ECM, no samples were prepared at the 10×LOQ fortification for the analysis of S-metolachlor in four water matrices (Tables 1-7, pp. 30-36 of MRID 48346602). The reproducibility for the 10×LOQ analysis of S-metolachlor/metolachlor was not supported by the data.

2. In the ECM, individual recovery values and recovery ranges were not reported (Tables 1-7, pp. 30-36 of MRID 48346602). Raw data was not reported; raw data should be reported to help assess the validity of the results. The reviewer noted that the ECM reported that it was not a validation and was not required to be conducted in accordance with the USEPA FIFRA (GLP) standards (40 CFR Part 160; p. 3).

3. The ECM representative chromatograms did not provide full support for the specificity of the method because chromatograms were only provided for the surface water matrix (one of the four matrices) and chromatograms were not provided for all fortifications tested, including 10×LOQ for some analytes (Figures 1-7, pp. 42-62 of MRID 48346602). Representative chromatograms for all fortifications/matrices should be provided for review. The reviewer noted that the ECM reported that it was not a validation and was not required to be conducted in accordance with the USEPA FIFRA (GLP) standards (40 CFR Part 160; p. 3).

4. The reviewer noted that the LOQ was fairly small compared to the height of the baseline noise in the majority of the ILV chromatograms; however, baseline noise was less than one-fourth of the LOQ peak height (Figures 2-4, pp. 45-128 of MRID 48331501).

5. The reviewer noted that the amounts of analyte, i.e. size of the analyte peak, observed in the control samples of the ECM analyses for atrazine and metolachlor were very large (Figure 1, pp. 42-44; Figure 7, pp. 60-62 of MRID 48346602). Based on peak area percentages, the matrix interferences were *ca*. 47% and *ca*. 81% for atrazine and metolachlor, respectively; however, quantitation was based on the analyte/IS area peak ratio not only peak areas. Based on the analyte/IS peak ratio, matrix interferences were <LOD (LOD = *ca*. 40-50% of the LOQ).

6. In the ECM, the study author reported that the method was not designed to resolve the stereoisomers of metolachlor (pp. 19-22 of MRID 48346602). The ECM test material was S-metolachlor; the ILV test material was metolachlor (Appendix 2, pp. 73-75 of MRID 48346602; pp. 11-15; Appendix 3, Amendment 2, p. 161 of MRID 48331501). The use of S-metolachlor was written in the DER for consistency with the ECM study report.

7. Sample recoveries of the ECM and ILV were corrected for residues quantified in the controls.

8. The ILV study author provided a communication log between Lisa Swaim (ILV study author), Louis Mayer (role not reported), Summao Chen (Sponsor Representative), and Del Koch (role not reported; p. 3; Appendix 2, pp. 158-160 of MRID 48331501). These

communications included protocol approval, trial outcome communication, and trial success. Communications were almost completely one-sided from the ILV to the Sponsor. The role/titles of Louis Mayer and Del Koch should have been reported.

9. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11, 26-27 of MRID 48346602; pp. 9 of MRID 48331501). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample set at which the methodology has been validated, i.e. demonstrating a mean recovery of 70-120% with an RSD ≤20%. In the ECM, the LODs were defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time. While an estimate of the LOD can be taken as four times the background noise, the method LOD in this study is defined as the smallest standard amount injected during the chromatographic run. The LOD typically corresponds to an amount of the analyte equivalent to *ca*. 50% of the theoretical amount for a recovery sample at the method LOQ. The method LOD also may vary from instrument-to-instrument, depending on the injection volume and concentrations needed to obtain adequate analyte response on a specific model of mass instruments. The LODs were not reported in the ILV. No LOQ calculations were reported in ECM or ILV; no LOD calculations were reported in ECM. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

10. Supplementary data for Method GRM014.02A was presented in Appendix 3 of the ECM to demonstrate that atrazine, simazine, propazine, G30033, G28279, G28273 and metolachlor can be determined in water samples from selected ECO and AMP programs containing certain types of preservatives (Appendix 3, pp. 76-94 of MRID 48346602). For ECO-81580 water samples, overall recoveries were 105 ±9.3, RSD 8.9% for atrazine, 97.7 ±8.6, RSD 8.8% for G30033, 83.7 ±8.0, RSD 9.5% for G28279, 106 ±17, RSD 16% for G28273, 91.2 ±18, RSD 19% for simazine, 102 ±5.8, RSD 5.7% for propazine, and 102 ±17, RSD 17% for metolachlor (Table A7, p. 91). For AMP-80909 (raw) water samples, overall recoveries were 100 ±4.2, RSD 4.2% for atrazine, 87.0 ±7.9, RSD 9.1% for G30033, 106 ±5.3, RSD 5.0% for G28279, 93.6 ±4.5, RSD 4.8% for G28273, 102 ±12, RSD 11% for simazine, 99.5 ±4.7, RSD 4.7% for propazine, and 90.5 ±8.0, RSD 8.8% for metolachlor (Table A8, p. 92). For AMP-80910 (finished) water samples, overall recoveries were 93.5 ±8.8, RSD 9.4% for atrazine, 99.9 ±5.5, RSD 5.5% for G30033, 95.2 ±14, RSD 14% for G28279, 99.4 ±20, RSD 20% for G28273, 101 ±4.4, RSD 4.3% for simazine, 103 ±7.3, RSD 7.1% for propazine, and 97.5 ±6.6, RSD 6.8% for metolachlor (Table A9, p. 93). Fortifications were prepared at 0.05 µg/L, 0.10 µg/L and 0.50 µg/L for atrazine, simazine, propazine, G30033 and G28279, 0.50 µg/L for G28273, and 0.10 µg/L and 0.50 µg/L for metolachlor. Two to three samples were prepared at each fortification level; correlation coefficients (R) were provided for each analyte/matrix. No matrix characterization or representative chromatograms were provided.

11. It was reported for the ILV that each validation trial of 23 injections required *ca*. 2 hours for preparation, *ca*. 4 hours for HPLC/MS/MS analysis, and *ca*. 3 hours for data processing and verification (p. 24 of MRID 48331501).

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

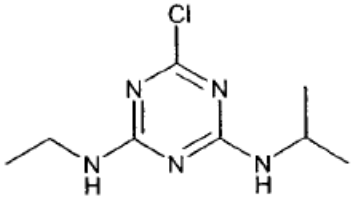
VI. Calculations


ECM ILV calcs

DER Attachment 1. Chemical Names and Structures.

Atrazine (G30027)

IUPAC Name:	6-Chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine
CAS Name:	6-Chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine. 6-Chloro-N-ethyl-N'-isopropyl-1,3,5-triazine-2,4-diamine. 1,3,5-Triazine-2,4-diamine, 6-chloro-N-ethyl-N'-(1-methylethyl)-.
CAS Number:	1912-24-9
SMILES String:	Not found



Simazine (G27692)

IUPAC Name:	6-Chloro-N^2,N^4-diethyl-1,3,5-triazine-2,4-diamine
CAS Name:	6-Chloro-N,N'-diethyl-1,3,5-triazine-2,4-diamine. 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-diethyl-.
CAS Number:	122-34-9
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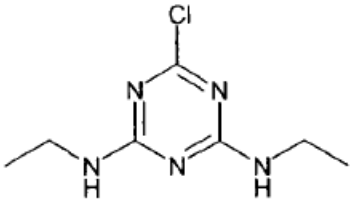
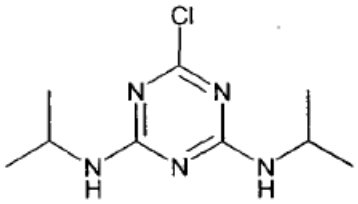


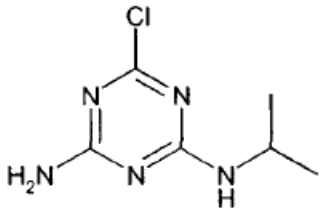
Propazine (G30028)

IUPAC Name:	6-Chloro-N^2,N^4-diisopropyl-1,3,5-triazine-2,4-diamine
CAS Name:	6-Chloro-N,N'-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine. 6-Chloro-N,N'-diisopropyl-1,3,5-triazine-2,4-diamine. 1,3,5-Triazine-2,4-diamine, 6-chloro-N,N'-bis(1-methylethyl)-.
Chemical Name:	2-Chloro-4,6-bis(isopropylamino)-s-triazine.
CAS Number:	139-40-2
SMILES String:	Not found



G30033

IUPAC Name: Not reported
CAS Name: 6-Chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine
CAS Number: 6190-65-4
SMILES String: Not found



G28279

IUPAC Name: Not reported
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CAS Number: 1007-28-9
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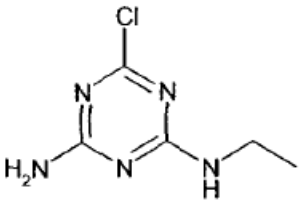

G28273

IUPAC Name: Not reported
CAS Name: 6-Chloro-1,3,5-triazine-2,4-diamine
CAS Number: 3397-62-4
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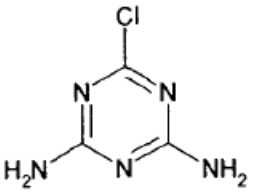

S-metolachlor (CGA-77102)

IUPAC Name:	Not reported
CAS Name:	(S)-2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide
CAS Number:	87392-12-9
SMILES String:	Not found