

Test Material: Prometryn

MRID: 46839502

Title: ATRAZINE: METHOD AMENDMENT - 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

MRID: 46839501

Title: INDEPENDENT LABORATORY VALIDATION: 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 - FINAL REPORT

EPA PC Code: 080805

OCSPP Guideline: 850.6100

**For CDM Smith**

**Primary Reviewer:** Lynne Binari

**Signature:**



**Date:** 9/5/14

**Secondary Reviewer:** Lisa Muto

**Signature:**



**Date:** 9/5/14

**QC/QA Manager:** Joan Gaidos

**Signature:**



**Date:** 9/5/14

**Analytical method for prometryn and its products GS-11354, GS-11355, and GS-26831 in water**

- Reports:** ECM: EPA MRID No.: 46839502. Perez, R., J. Allen, S. Huang, T. Mayer, S. Perez, and R. Yokley. 2005. ATRAZINE: METHOD AMENDMENT - 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. ADPEN Study No.: ADPEN-2K5-0106. Syngenta Study No.: T010097-04. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and Syngenta Crop Protection, Inc., Greensboro, North Carolina, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 119 pages. Amended final report issued November 11, 2005.
- ILV: EPA MRID No. 46839501. Reed, R. 2006. INDEPENDENT LABORATORY VALIDATION: 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 - FINAL REPORT. Morse Labs Project No.: ML05-1270-SYN. Syngenta Study No.: T011223-05. Report prepared by Morse Laboratories, Inc., Sacramento, California, sponsored and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 319 pages. Final report issued April 10, 2006.
- Document No.:** MRIDs 46839502 & 46839501
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in compliance with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 46839502). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5-6). An Authenticity Certification statement was not provided.
- ILV: The study was conducted in compliance with USEPA GLP standards (pp. 3, 5 of MRID 46839501). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5, 7). A statement of the authenticity of the study report was included as part of the Quality Assurance Statement (p. 7).
- Classification:** This analytical method is classified as Supplemental. The ECM did not provide performance data at 10x LOQ. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. LODs differed in the ECM and ILV. A finalized ECM report with implemented ILV major modifications was not provided. The method did not include the degradates of prometryn: GS-11526, GS-16141, M2, and GS-17794. The registrant is requested to submit a state-of-art method that includes prometryn degradates.

**PC Code:** 080805

**Reviewer:** Mohammed A. Ruhman,

**Signature:**



Senior Scientist, EFED, EPA

**Date:** 03/20/2017

Page citations refer to the page numbers located in the bottommost right corner of MRID 46839501 (ILV, since this report contains the amended ECM), unless noted otherwise.

## Executive Summary

This analytical method, Syngenta Number T010097-04, is designed for the quantitative determination of prometryn and its products GS-11354, GS-11355, and GS-26831 in ground water and surface water using HPLC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.10 µg/L. The LOQ is less than the lowest toxicological level of concern in water. The independent laboratory validated the method after one trial. LODs were set at 0.01 µg/L in the ECM and 0.05 µg/L in the ILV. Based on the ILV, Syngenta decided that this method is not suitable to determine prometryn and its products in water matrices potentially containing chlorine, such as tap water; however, a finalized ECM report specifying this modification was not provided.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide	MRID		EPA Review	Matrix <sup>1</sup>	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Prometryn	46839502	46839501		Ground water & Surface water	11/11/2005	Syngenta	HPLC/MS/MS	0.10 µg/L
GS-11354								
GS-11355								
GS-26831								

<sup>1</sup> This method is not suitable to analyze for prometryn and its products in water potentially containing chlorine, such as tap water, due to instability (oxidation) of the analytes during fortification (p. 28; Appendix 1, p. 109).

## I. Principle of the Method

Samples (200 µL) of water were diluted with 800 µL of methanol:HPLC water (5:95, v:v) in an auto-sampler vial, then analyzed directly using an Applied Biosystems MDS Sciex API 4000 LC/MS/MS (Appendix 2, pp. 128, 142).

Samples were analyzed for prometryn and its products GS-11354, GS-11355, and GS-26831 by HPLC (Agilent Zorbax SB-CN, 4.6 mm x 75 mm, 3.5 µm column, 45°C) using a mobile phase of (A) HPLC water and (B) HPLC methanol [percent A:B at 0.0-0.5 min. 95:5, 0.5-2.5 min. linear to 35:65, 2.5-10.5 min. 35:65, 10.5-18.6 min. immediate step gradient to initial 95:5 conditions for 8.0 min.] with MS/MS-ESI (electrospray ionization, positive ion mode) detection and multiple reaction monitoring (MRM; Appendix 2, pp. 142-143). Injection volumes were 50 µL for ground water and 100 µL for surface and finished waters (Appendix 2, pp. 132-133). Prometryn and its products were identified and quantified using one ion transition. Ion transitions monitored were as follows:  $m/z$  242.0→158.2 for prometryn,  $m/z$  200.3→158.0 for GS-11354,  $m/z$  186.1→96.0 for GS-11355,  $m/z$  158.2→110.0 for GS-26831. A confirmatory method was not reported.

The ILV was performed by diluting a 10 mL water sample to 50 mL with methanol:water (5:95, v:v; pp. 22-23), adjusting the HPLC mobile phase composition [percent A:B at 0.0-0.5 min. 95:5, 1.5-5.0 min. 50:50, 7.5-10.5 min. 35:65, 10.6-18.6 min. 95:5], injection volume of 50 µL for all

water matrices, and using 1/x weighting for calculation of calibration curves (pp. 23-25). The modifications are not considered substantial changes to the ECM.

The LOQ for prometryn and its products was the same in the ECM and ILV at 0.1 µg/L (ppb; p. 16; Appendix 2, p. 133). The LOD was set at 0.01 µg/L (pg/µL, equivalent to 0.5 pg for 50 µL injection and 1.0 pg for 100 µL injection) in the ECM and 0.05 µg/L in the ILV (p. 16; Appendix 2, pp. 132-133; Appendix 4, p. 298).

## II. Recovery Findings

This analytical method is designed for the quantitative determination of other analytes (atrazine, simazine and their products G-30033, G-28279, and G-28273; ametryn; and metolachlor) in addition to prometryn and its products GS-11354, GS-11355, and GS-26831 in water (Appendix 2, p. 125). This review only concerns the performance data relating to prometryn and its products.

ECM (MRID 46839502 & 46839501): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of prometryn and its products GS-11354, GS-11355, and GS-26831 in ground water, surface (lake) water, and finished (tap) water at fortification levels of 0.10 µg/L (LOQ), 0.20 µg/L (2x LOQ), and 3.0 µg/L (30x LOQ; Appendix 2, pp. 136-141). Performance data at 10x LOQ were not reported. Analytes were identified and quantified using a single ion transition; a confirmatory method was not used. The ground water and surface water matrices were characterized (Appendix 2, p. 176). Representative water quality test results were provided for the finished water matrix.

ILV (MRID 46839501): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of prometryn and its products in ground (well) water and surface (river) water at fortification levels of 0.10 µg/L (LOQ) and 1.0 µg/L (10x LOQ; pp. 13-14). Analytes were identified and quantified using a single ion transition. The method was validated for all analytes at both fortification levels in both matrices after one trial, with minor method and instrument parameter modifications (pp. 22-23, 27-28). The water matrices were characterized (Appendix 5, pp. 311-312).

The first ILV trial for analysis of prometryn and its products in finished (tap) water failed (pp. 27-28; Appendix 4, p. 300; Appendix 6, p. 315). Syngenta informed the independent laboratory that water samples potentially containing residual chlorine, such as tap water, required degassing prior to fortification, even though this procedure was not described in the ECM (Appendix 4, p. 301). Two additional trials using degassed tap water were conducted, and, while recovery results for prometryn and its products were acceptable, results for other analytes (metolachlor in second trial; atrazine, simazine, G-30003, and G-28279 in third trial) were unacceptable (Appendix 6, pp. 316-319). Syngenta proposed that the degassing procedures altered the sample, thus affecting the recoveries of some of the other analytes (Appendix 4, p. 309). Syngenta ultimately decided that this method "was not intended to, nor designed to determine" thiomethyl compounds ametryn and prometryn and their products GS-11354, GS-11355, and GS-26831 in water potentially containing chlorine, such as tap water (pp. 27-28; Appendix 1, p. 109; Appendix 4, p. 309). Morse Labs issued a protocol amendment to exclude analysis of ametryn, prometryn and their products GS-11354, GS-11355, and GS-26831 in finished (tap) water (Appendix 1, p. 109).

**Table 2. Initial Validation Method Recoveries for Prometryn and Its Products in Water**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Ground Water</b>						
Prometryn	0.10 (LOQ)	5	84.4-88.5	86.1	1.81	2.1
	0.2	5	86.5-90.0	88.2	1.30	1.5
	3.0	5	86.3-91.3	88.8	2.09	2.4
GS-11354	0.10 (LOQ)	5	100-107	105	3.05	2.9
	0.2	5	105-110	107	2.46	2.3
	3.0	5	101-105	103	1.71	1.7
GS-11355	0.10 (LOQ)	5	98.7-105	101	2.58	2.6
	0.2	5	101-107	104	2.02	1.9
	3.0	5	98.3-101	99.7	1.05	1.1
GS-26831	0.10 (LOQ)	5	106-113	109	3.11	2.9
	0.2	5	112-117	114	2.48	2.2
	3.0	5	103-108	106	1.71	1.6
<b>Surface (Lake) Water</b>						
Prometryn	0.10 (LOQ)	5	81.0-99.0	89.3	8.43	9.4
	0.2	5	89.8-102	96.9	4.32	4.5
	3.0	5	88.2-90.3	89.2	0.87	1.0
GS-11354	0.10 (LOQ)	5	81.5-102	93.5	9.94	10.6
	0.2	5	91.3-107	102	6.31	6.2
	3.0	5	95.0-100	97.6	2.35	2.4
GS-11355	0.10 (LOQ)	5	92.5-101	98.1	3.19	3.3
	0.2	5	95.3-105	101	3.63	3.6
	3.0	5	96.2-99.3	97.9	1.41	1.4
GS-26831	0.10 (LOQ)	5	98.5-104	101	2.18	2.2
	0.2	5	100-103	102	1.33	1.3
	3.0	5	96.8-103	99.7	2.30	2.3
<b>Finished (Tap) Water</b>						
Prometryn	0.10 (LOQ)	5	93.0-99.5	96.4	2.48	2.6
	0.2	5	91.8-92.8	92.1	0.49	0.5
	3.0	5	91.7-92.3	91.9	0.25	0.3
GS-11354	0.10 (LOQ)	5	95.5-103	99.6	2.75	2.8
	0.2	5	94.5-97.5	96.0	1.31	1.4
	3.0	5	96.2-99.5	97.7	1.28	1.3
GS-11355	0.10 (LOQ)	5	95.5-105	100	3.58	3.6
	0.2	5	90.0-97.5	95.3	3.07	3.2
	3.0	5	97.2-102	99.9	1.66	1.7
GS-26831	0.10 (LOQ)	5	93.5-103	98.9	3.58	3.6
	0.2	5	88.3-102.5	96.2	5.13	5.3
	3.0	5	99.3-102	100	0.93	0.9

Data (uncorrected recovery results) were obtained from Appendix 2, pp. 136-141.

1 Ground water from Muen County, Georgia (although, currently there is no Muen County), and surface water from High Point City Lake, North Carolina, were characterized by Agvise Laboratories, Inc., Northwood, North Dakota (Appendix 2, pp. 131, 176). Tap water from Greensboro, North Carolina, was not characterized, but representative water characterization analyses results from the 2003 Drinking Water Quality Test Results, City of Greensboro, Water Resources Department were reported.

**Table 3. Independent Validation Method Recoveries for Prometryn and Its Products in Water<sup>1</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Ground (Well) Water</b>						
Prometryn	0.10 (LOQ)	5	96-102	98	2.2	2.2
	1.0	5	100-104	102	1.5	1.5
GS-11354	0.10 (LOQ)	5	102-110	106	3.5	3.3
	1.0	5	101-104	103	1.3	1.3
GS-11355	0.10 (LOQ)	5	96-114	109	7.5	6.9
	1.0	5	104-110	107	2.2	2.1
GS-26831	0.10 (LOQ)	5	95-115	107	8.1	7.6
	1.0	5	104-112	107	3.3	3.1
<b>Surface (River) Water</b>						
Prometryn	0.10 (LOQ)	5	92-108	100	6.3	6.4
	1.0	5	109-114	111	1.9	1.7
GS-11354	0.10 (LOQ)	5	86-101	94	5.4	5.8
	1.0	5	95-106	103	4.4	4.3
GS-11355	0.10 (LOQ)	5	84-92	89	3.4	3.9
	1.0	5	95-108	103	4.8	4.7
GS-26831	0.10 (LOQ)	5	74-106	89	14	15
	1.0	5	90-111	101	8.0	7.9

Data (uncorrected recovery results) were obtained from Table 1, p. 35; Table 2, p. 37.

<sup>1</sup> Ground water from a residential well in Elk Grove, California, and surface water from the American River, Sacramento, California, were characterized by Agvise Laboratories, Inc., Northwood, North Dakota (p. 19; Appendix 5, pp. 310-313).

### III. Method Characteristics

In the ECM and ILV, the LOQ for prometryn and its products GS-11354, GS-11355, and GS-26831 in water was 0.10 µg/L (ppb; p. 16; Appendix 2, p. 133). The LOQ was defined as the lowest analyte concentration in a sample at which the method has been validated. The LOD was 0.01 µg/L (pg/µL) in the ECM and 0.05 µg/L (50% of LOQ) in the ILV. In the ECM, the LOD was set at the lowest concentration of standard injected and used to construct the calibration curve (Appendix 2, pp. 125, 133, 191). However, the Syngenta study monitor defined the LOD as 50% of the LOQ for the purposes of the ILV (Appendix 4, p. 301).

**Table 4. Method Characteristics for Prometryn and Its Products in Ground Water and Surface Water<sup>1</sup>**

	Prometryn	GS-11354	GS-11355	GS-26831
Limit of Quantitation (LOQ)	0.10 µg/L			
Limit of Detection (LOD)	0.05 µg/L <sup>2</sup>			
Linearity (calibration curve r <sup>2</sup> and concentration range) <sup>3</sup>	r <sup>2</sup> = 0.9999	r <sup>2</sup> = 1.0000	r <sup>2</sup> = 0.9999	r <sup>2</sup> = 0.9993
	(0.01-0.5 ng/mL)			
Repeatable	Yes			
Reproducible	Yes			
Specific <sup>4</sup>	Yes			

Data were obtained from pp. 16, 30; Table 1, p. 35; Table 2, p. 37; Figure 2, pp. 63-64; Figure 3, pp. 68-69; Figure 4, pp. 80-81.

- 1 This method is not suitable to analyze for prometryn and its products in water potentially containing chlorine, such as tap water, due to instability (oxidation) of the analytes during fortification (p. 28; Appendix 1, p. 109).
- 2 LOD was set at 0.01 µg/L in the ECM, based on the lowest calibration standard (Appendix 2, pp. 125, 133). For the ILV, the Syngenta study monitor set the LOD at 50% of the LOQ (Appendix 4, p. 301).
- 3 Linearity of the ILV calibration curves were verified by the reviewer (DER Attachment 2). ECM reported r values were 0.9980-1.0000 (Appendix 2, pp. 183-185, 190); linearity of those curves could not be verified because individual peak area count data were not reported. However, reviewer-calculated curves were generated from provided chromatograms of calibration standards and yielded r<sup>2</sup> values of 0.9991-1 (DER Attachment 2).
- 4 A confirmation method was not used; however, OCSPP 850.6100 guidelines specify that a confirmatory procedure is not typically necessary where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

#### IV. Method Deficiencies and Reviewer's Comments

1. This analytical method is designed for the quantitative determination of atrazine and simazine and their products G-30033, G-28279, and G-28273; ametryn and prometryn and their products GS-11354, GS-11355, and GS-26831; and metolachlor in water (Appendix 2, p. 125). This review is only concerned with the performance data relating to prometryn and its products GS-11354, GS-11355, and GS-26831.

An initial validation of the ECM was performed by ADPEN using a MDS Sciex API 3000 LC/MS/MS; however, RSDs were >20% for GS-26831 at 0.4 µg/L (ppb, LOQ) in surface water, and GS-11354 and GS-11355 at 0.2 µg/L (LOQ) in ground water (Appendix 2, pp. 131-132, 211-217). A second validation of the ECM was performed by Syngenta using a MDS Sciex API 4000 LC/MS/MS which yielded acceptable recovery results at a lower LOQ for all analytes (Appendix 2, pp. 132-133, 136-143). And, while the API 3000 was not excluded for analyses, the API 4000 was determined the instrument of choice. Detailed data results and chromatograms for the API 3000 were not provided with the ECM (Appendix 2, p. 132).

2. The ECM did not provide performance data at 10x LOQ for any analyte.



3. The determination of the LOQ and LOD were not based on scientifically acceptable procedures. The LOQ (0.1 µg/L, ppb) was defined as the lowest analyte concentration in a sample at which the method has been validated (Appendix 2, p. 133). The ECM set the LOD at the lowest concentration of standard injected and used to construct the calibration curve (0.01 µg/L; Appendix 2, pp. 125, 133, 191). However, the Syngenta study monitor defined the LOD as 50% of the LOQ for the purposes of the ILV (0.05 µg/L), with no justification (Appendix 4, p. 301).

Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

4. A finalized ECM report with implemented ILV major modifications should be provided. Based on the ILV recovery results (see **II. Recovery Findings** above), Syngenta ultimately decided that this method "was not intended to, nor designed to determine" thiomethyl compounds ametryn and prometryn and their products GS-11354, GS-11355, and GS-26831 in water potentially containing chlorine (pp. 27-28; Appendix 1, p. 109; Appendix 4, p. 309; Appendix 6, pp. 317, 319).

Additionally, the ratio of the methanol:water dilution solvent is listed as 10:90 (v:v) in the method flow diagram, rather than the 5:95 (v:v) ratio specified in the method text (Appendix 2, pp. 125-126, 128, 179). The ILV used the latter ratio (5:95, v:v) for sample preparation and did not refer to the method diagram (pp. 16, 23). The method flow diagram should be corrected.

A finalized ECM specifying that the method is suitable for analysis of prometryn and its products GS-11354, GS-11355, and GS-26831 in ground water and surface water, but not in water potentially containing chlorine, such as tap water, plus correction of the method flow diagram should be provided.

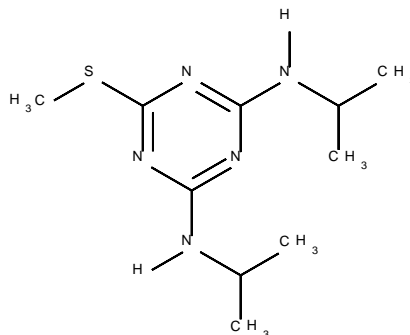
5. The ECM residue calculations specify correcting recovery results for any residues detected in the matrix control samples (Appendix 2, pp. 130-131). However, no residues were detected in either the matrix blank controls for the ECM, or in the reagent blank and matrix control samples for the ILV.
6. For the ECM, the individual peak area count data used to generate the provided four-point standard curves were not reported (Appendix 2, pp. 183-185, 190). The reviewer generated six-point curves using peak area count data from provided chromatograms of calibration standards (Appendix 2, pp. 191-16; DER Attachment 2). For both the ECM and ILV, chromatograms of reagent blank samples were not included.
7. A confirmatory method was not used. However, OCSPP 850.6100 guidelines specify that a confirmatory procedure is not typically necessary where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.
8. It was reported for the ILV that one analyst could complete a single set of thirteen samples in 2 hours with LC/MS/MS analysis performed overnight (p. 31).

## V. References

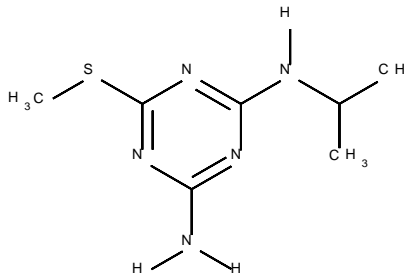
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**Attachment 1: Chemical Names and Structures****Prometryn (G-34161)**

**IUPAC Name:** N<sup>2</sup>,N<sup>4</sup>-diisopropyl-6-methylthio-1,3,5-triazine-2,4-diamine  
**CAS Name:** N,N'-bis(1-methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine  
**CAS Number:** 7287-19-6  
**SMILES String:** CSc1nc(NC(C)C)nc(NC(C)C)n1

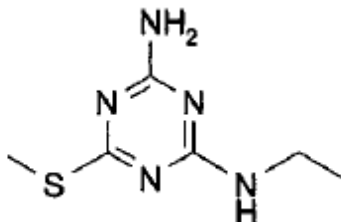
**GS-11354**

**IUPAC Name:** N-Isopropyl-6-methylsulfanyl-[1,3,5]triazine-2,4-diamine  
**CAS Name:** N-(1-Methylethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine  
**CAS Number:** 4147-57-3  
**SMILES String:** CC(C)Nc1nc(nc(n1)SC)N



**GS-11355**

**IUPAC Name:** Not reported  
**CAS Name:** N-Ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine  
**CAS Number:** 4147-58-4  
**SMILES String:** Not reported

**GS-26831**

**IUPAC Name:** 6-Methylsulfanyl-1,3,5-triazine-2,4-diamine  
**CAS Name:** 6-(Methylthio)-1,3,5-triazine-2,4-diamine  
**CAS Number:** 5397-01-3  
**SMILES String:** CSc1nc(nc(n1)N)N

