

Test Material: Thiamethoxam

MRID: 49667101

Title: Environmental Chemistry Method: Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection

MRID: 49383301

Title: Independent Laboratory Validation: Syngenta Method No. T003103-03: "Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, NOA-459602 in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection" (p. 9)

EPA PC Code: 060109

OCSPP Guideline: 850.6100

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Analytical method for thiamethoxam (CGA-293343) and its transformation products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in water

- Reports:** ECM: EPA MRID No.:49667101. Mayer, T. 2004. Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406 and NOA-459602 in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection. Report prepared, sponsored, and submitted by Syngenta Crop Protection, Inc., Greensboro, North Carolina; 105 pages. Syngenta Study No.: T003103-03 (p. 9). Final report issued March 17, 2004.
ILV: EPA MRID No. 49383301. Nelson, S., and C. Blenkinsop. 2005.

Independent Laboratory Validation: Syngenta Method No. T003103-03: "Analytical Method for the Determination of CGA-293343 and its Degradates CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, NOA-459602 in Water by Direct Injection High Performance Liquid Chromatography with Mass Spectrometric Detection" (p. 9). Report prepared by Enviro-Test Laboratories (ETL), Alberta, Canada, sponsored and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 100 pages. ETL Study No.: 04ILV04SYN and Report No.: 04SYN144.REP (p. 9). Syngenta Study No. and Task No.: T017172-04 (pp. 1, 9). Final report issued June 6, 2005.
- Document No.:** MRIDs 49667101 & 49383301
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49667101 ECM). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). A statement certifying the authenticity of the study report was not provided; a signature (Report Approval) page was included (p. 4).
ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49383301). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 5). A statement certifying the authenticity of the study report was not provided; a signature (Report Approval) page was included (p. 4).
- Classification:** This analytical method is classified as **Acceptable**. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The ILV did not report LODs. ECM validation water matrices were not characterized. The LOQ was less than the lowest toxicological level of concern in water for thiamethoxam and clothianidin based on the Agency's Aquatic Life Benchmarks.
- PC Code:** 060109
- Reviewer:** Christopher M. Koper, M.S., Chemist **Date:** July 29, 2015

Executive Summary

This analytical method, Syngenta Method T003103-03, is designed for the quantitative determination of thiamethoxam (CGA-293343) and its transformation products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.05 ppb ($\mu\text{g}/\text{kg}$, $\mu\text{g}/\text{L}$). **The LOQ of 0.05 $\mu\text{g}/\text{L}$ is less than the lowest toxicological level of concern in water for thiamethoxam (acute invertebrates = 17.5 $\mu\text{g}/\text{L}$) and clothianidin (chronic invertebrates = 1.1 $\mu\text{g}/\text{L}$) based on the Agency's Aquatic Life Benchmarks http://www.epa.gov/oppefed1/ecorisk_ders/aquatic_life_benchmark.htm.** The independent laboratory validated the method for analysis of thiamethoxam, CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 at the LOQ and 10x LOQ in ground and surface water matrices after one trial. No major modifications were made by the independent laboratory.

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						
Thiamethoxam (CGA-293343)	49667101	49383301		Ground and Surface Water	17/03/2004	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 ppb ($\mu\text{g}/\text{kg}$, $\mu\text{g}/\text{L}$)
CGA-322704								
CGA-355190								
CGA-353042								
NOA-404617								
NOA-407475								
SYN-501406								
NOA-459602								

I. Principle of the Method

Water (10.0 g) was fortified with a mixed standard solution of thiamethoxam (CGA-293343), CGA-322704, CGA-355190, CGA-353042, NOA-407475, NOA-404617, SYN-501406, and NOA-459602 in 5% methanol in water + 0.1% acetic acid for procedural recoveries (pp. 19-20; Tables 3-8, pp. 32-55 of MRID 49667101). Ground water was obtained from the Mark Grunenwald Residence, Colfax, North Carolina (p. 9). Surface water matrices were obtained from City Lake of High Point, Guilford County, North Carolina, and Buffalo Creek, Greensboro, North Carolina. Turbid samples should be centrifuged to obtain a clear supernatant, rather than filtered, as the analytes may be absorbed onto the filter (p. 19). Water samples (volume variable) are acidified with acetic acid to 0.1% acetic, then analyzed directly using LC/MS/MS (p. 15; Figure 2, p. 67).

LC/MS/MS system I for thiamethoxam (CGA-293343), CGA-322704, CGA-355190, NOA-404617, NOA-459602, and SYN-501406: Samples are analyzed using a Perkin-Elmer Series 200 HPLC system and a PE Sciex API 4000 triple quadrupole MS with Turbo Ion-Spray interface (pp. 13, 15-16; Table 1, pp. 24-28 of MRID 49667101 (ECM)). The following LC conditions were used: Phenomenex Develosil RP Aqueous column (3 mm x 150 mm, 3 μm , column temperature 40°C) preceded by a Phenomenex C-18 Guard column (dimensions not reported) plus an Upchurch (A-318) pre-column filter (0.5 μm), using a mobile phase of (A) 0.1% acetic acid in water and (B)

0.1% acetic acid in methanol [percent A:B (v:v) at 0-1 min. 95:5, 3-5 min. 0:100, 8-9.5 min 95:5]; final gradient step interval 8-9.5 min. (obtained from Figure 6, pp. 74-79). Injection volume was 50 μL (50-100 μL dependent upon instrument sensitivity). The following MS/MS conditions were used: positive ion mode for thiamethoxam (CGA-293343), CGA-322704, CGA-355190, and NOA-404617, negative ion mode for NOA-459602 and SYN-501406, and multiple reaction monitoring (MRM). Analytes are identified using one ion transition. Ion transitions monitored were as follows: m/z 292.09 \rightarrow 211.15 for thiamethoxam (CGA-293343), m/z 250.12 \rightarrow 131.85 for CGA-322704, m/z 248.12 \rightarrow 174.95 for CGA-355190, m/z 237.08 \rightarrow 174.95 for NOA-404617, m/z 335.85 \rightarrow 204.74 for NOA-459602, and m/z 293.88 \rightarrow 190.85 for SYN-501406. Expected retention times were *ca.* 3.87, 6.04, 6.83, 7.14, 7.27, and 7.57 minutes for NOA-459602, SYN-501406, thiamethoxam (CGA-293343), CGA-322704, CGA-355190, and NOA-404617, respectively. A confirmatory method was not used (p. 17).

LC/MS/MS system II for CGA-353042 and NOA-407475: The same LC/MS/MS instrumentation was utilized (Table 2, pp. 29-31 of MRID 49667101 (ECM)). The following LC conditions were used: MAC-MOD Analytical Zorbax 300-SCX column (2.1 mm x 150 mm, 5 μm , column temperature 40°C) preceded by a Zorbax SCX Guard column (dimensions not reported) plus an Upchurch (A-318) pre-column filter (0.5 μm), using an isocratic mobile phase of 50% acetonitrile/25mM ammonium acetate. Injection volume was 20 μL (20-100 μL dependent upon instrument sensitivity). The following MS/MS conditions were used: positive ion mode and MRM. Ion transitions monitored were as follows: m/z 116.09 \rightarrow 85.95 for CGA-353042 and m/z 247.10 \rightarrow 132.05 for NOA-407475. Expected retention times were *ca.* 3.46 and 3.30 minutes for CGA-353042 and NOA-407475, respectively.

ILV: Test compounds were supplied by Syngenta (p. 11 of MRID 49383301). Ground water was obtained from a rural well near Rimbey, Alberta, Canada, and surface water from Gull Lake, Alberta, Canada (p. 12). The following modifications to the ECM were made: a PE Sciex API 4000 MS/MS system was used in place of the PE Sciex API triple quadrupole MS, individual stock standard solutions were made up in 100-mL volumetric flasks, an Aquasil C18 column (3 x 150 mm, 3 μm) was used instead of the Devosil C18 column, the gradient mobile phase was adjusted to (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in methanol [percent A:B (v:v) at 0.10-1.0 min. 95:5, 7.0-10.0 min. 0:100, 15.0 min. 98:2, 16.0 min. 95:5], and NOA-404617 was analyzed with thiamethoxam (CAG-293343), CGA-322704, and CGA-355190 rather than separately, plus additional minor modifications to optimize LC/MS/MS conditions (p. 13; Tables 1-11, pp. 18-25). Injections volumes were 20-100 μL . Using the Aquasil C18 column, approximate retention times were 3.09-3.13, 4.04-4.31, 6.36-6.85, 6.38-6.97, 6.64-7.22, and 7.07-7.66 minutes for NOA-459602, SYN-501406, NOA-404617, thiamethoxam (CGA-293343), CGA-322704, and CGA-355190, respectively. Using the Zorbax 300-SCX column, approximate retention times were 3.91-4.29 and 4.13-4.46 minutes for CGA-353042 and NOA-407475, respectively.

LOQ and LOD: In the ECM and ILV, the LOQ (or "limit of determination" in the ECM) for all analytes was 0.05 ppb (pp. 13, 21 of ECM MRID 49667101; pp. 10, 13 of MRID 49383301). In the ECM, the LOD for all analytes was defined as 0.025 $\text{pg}/\mu\text{L}$ (ng/mL), equivalent to 1.25 pg injected for NOA-459602, SYN-501406, thiamethoxam (CGA-29343), CGA-322704, CGA-355190, and NOA-404617, and 0.5 pg injected for CGA-353042 and NOA-407475 (p. 13; Tables 3-8, pp. 32-55 of ECM MRID 49667101). In the ILV, LODs were not specified.

II. Recovery Findings

ECM (MRID 49667101): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of thiamethoxam (CGA-293343) and its transformation products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in surface (lake, creek) and ground (well) water matrices at fortification levels of 0.05 ppb (pg/mg, μ g/kg, LOQ) and 0.5 ppb (10x LOQ; pp. 20-21; Tables 3-8, pp. 32-55; DER Attachment 2). Analytes were identified and quantified using one ion transition; a confirmatory method was not used. The water matrices were not characterized.

ILV (MRID 49383301): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of thiamethoxam (CGA-293343) and its transformation products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in ground (well) and surface (lake) water matrices at fortification levels of 0.05 ppb (ng/mL, μ g/L, LOQ) and 0.5 ppb (10x LOQ; Table 14, p. 29; Table 16, p. 33; Figures 1-16, pp. 36-51). The method was validated for all analytes at both fortification levels in both water matrices after one trial (pp. 15-17). The water matrices were characterized by the independent laboratory (pp. 12-13; Appendices 1-2, pp. 52-55).

Table 2. Initial Validation Method Recoveries for Thiamethoxam and Its Transformation Products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in Surface and Ground Water

Analyte	Fortification Level (ppb) ¹	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface (City Lake) Water						
Thiamethoxam (CGA-293343)	0.05 (LOQ)	5	92-102	96	3.94	4.10
	0.5	5	93-97	95	1.64	1.73
CGA-322704	0.05 (LOQ)	5	77-87	83	3.78	4.53
	0.5	5	77-82	80	1.79	2.24
CGA-355190	0.05 (LOQ)	5	88-95	92	2.68	2.91
	0.5	5	92-95	93	1.64	1.76
CGA-353042	0.05 (LOQ)	5	92-101	97	4.51	4.66
	0.5	5	92-97	95	2.00	2.11
NOA-404617	0.05 (LOQ)	5	72-86	80	5.67	7.08
	0.5	5	77-82	79	2.00	2.53
NOA-407475	0.05 (LOQ)	5	90-97	94	3.21	3.43
	0.5	5	98-103	100	2.00	2.00
SYN-501406	0.05 (LOQ)	5	94-105	98	4.21	4.30
	0.5	5	100-103	102	1.30	1.28
NOA-459602	0.05 (LOQ)	5	90-99	95	3.78	3.96
	0.5	5	99-102	101	1.14	1.13
Surface (Buffalo Creek) Water						
Thiamethoxam (CGA-293343)	0.05 (LOQ)	5	103-109	105	2.30	2.18
	0.5	5	96-103	99	2.55	2.58
CGA-322704	0.05 (LOQ)	5	92-102	98	4.04	4.14
	0.5	5	86-91	88	1.92	2.18
CGA-355190	0.05 (LOQ)	5	103-111	108	3.13	2.89
	0.5	5	100-106	103	2.30	2.24
CGA-353042	0.05 (LOQ)	5	91-98	96	2.95	3.08
	0.5	5	96-98	97	0.89	0.92
NOA-404617	0.05 (LOQ)	5	97-109	102	4.67	4.60
	0.5	5	85-90	87	2.17	2.50
NOA-407475	0.05 (LOQ)	5	88-106	96	6.75	7.03
	0.5	5	102-107	105	1.87	1.78
SYN-501406	0.05 (LOQ)	5	100-114	107	5.36	5.02
	0.5	5	101-105	102	1.67	1.63
NOA-459602	0.05 (LOQ)	5	101-108	106	2.92	2.75
	0.5	5	103-107	104	1.79	1.72
Ground (Well) Water						
Thiamethoxam (CGA-293343)	0.05 (LOQ)	5	100-108	104	4.10	3.96
	0.5	5	97-102	100	1.79	1.79
CGA-322704	0.05 (LOQ)	5	101-108	104	2.70	2.59
	0.5	5	97-102	100	1.82	1.82
CGA-355190	0.05 (LOQ)	5	96-106	102	3.96	3.88
	0.5	5	98-103	101	2.17	2.14
CGA-353042	0.05 (LOQ)	5	82-92	86	4.04	4.67
	0.5	5	88-91	90	1.41	1.57
NOA-404617	0.05 (LOQ)	5	94-107	100	5.89	5.88
	0.5	5	96-103	100	2.59	2.58

Analyte	Fortification Level (ppb)¹	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
NOA-407475	0.05 (LOQ)	5	83-96	89	5.15	5.78
	0.5	5	94-101	98	2.77	2.83
SYN-501406	0.05 (LOQ)	5	89-99	94	4.00	4.26
	0.5	5	96-100	99	1.73	1.75
NOA-459602	0.05 (LOQ)	5	93-95	94	1.10	1.17
	0.5	5	99-101	100	0.84	0.83

Means, standard deviations, and relative standard deviations for each analyte per fortification level and matrix were determined by the reviewer using uncorrected recovery results, because the study author only provided summary results (Tables 9-10, pp. 56-63 of ECM MRID 49667101); DER Attachment 2).

1 Ppb analyte = pg/mg or µg/kg (pp. 20-21).

Table 3. Independent Validation Method Recoveries for Thiamethoxam and Its Transformation Products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in Surface and Ground Water

Analyte	Fortification Level (ppb) ¹	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground (Well) Water						
Thiamethoxam (CGA-293343)	0.05 (LOQ)	5	86-98	92	4.7	5.1
	0.5	5	93-102	96	3.8	4.0
CGA-322704	0.05 (LOQ)	5	103-108	105	2.3	2.2
	0.5	5	92-93	92	0.45	0.49
CGA-355190	0.05 (LOQ)	5	98-108	102	4.9	4.8
	0.5	5	92-99	96	3.4	3.5
CGA-353042	0.05 (LOQ)	5	92-101	98	3.7	3.8
	0.5	5	95-103	100	3.0	3.0
NOA-404617	0.05 (LOQ)	5	78-104	89	9.6	11
	0.5	5	88-93	91	1.9	2.1
NOA-407475	0.05 (LOQ)	5	73-85	79	4.9	6.2
	0.5	5	95-101	98	2.2	2.2
SYN-501406	0.05 (LOQ)	5	71-114	91	16	18
	0.5	5	91-99	94	3.1	3.3
NOA-459602	0.05 (LOQ)	5	99-107	102	3.3	3.2
	0.5	5	99-101	100	0.89	0.89
Surface (Lake) Water						
Thiamethoxam (CGA-293343)	0.05 (LOQ)	5	88-101	92	5.1	5.5
	0.5	5	105-109	107	2.0	1.9
CGA-322704	0.05 (LOQ)	5	70-96	89	11	12
	0.5	5	81-95	90	5.7	6.3
CGA-355190	0.05 (LOQ)	5	81-101	90	10	11
	0.5	5	100-105	103	1.9	1.8
CGA-353042	0.05 (LOQ)	5	87-120	107	13	12
	0.5	5	85-115	100	14	14
NOA-404617	0.05 (LOQ)	5	83-96	91	6.2	6.8
	0.5	5	82-91	86	3.3	3.8
NOA-407475	0.05 (LOQ)	5	86-117	103	14	14
	0.5	5	107-119	113	4.4	3.9
SYN-501406	0.05 (LOQ)	5	86-107	100	9.0	9.0
	0.5	5	109-117	114	3.0	2.6
NOA-459602	0.05 (LOQ)	5	89-117	100	11	11
	0.5	5	106-116	113	4.0	3.5

Data (uncorrected recovery results) were obtained from Table 14, p. 29; Table 16, p. 33; Figures 1-16, pp. 36-51 of MRID 49383301.

¹ Ppb analyte = ng/mL or µg/L (Table 13, pp. 26-28; Table 15, pp. 30-32).

III. Method Characteristics

In the ECM and ILV, the LOQ (or "limit of determination" in the ECM) for thiamethoxam (CGA-293343) and its transformation products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in water was 0.05 ppb (pp. 13, 21 of ECM MRID 49667101); pp. 10, 13 of MRID 49383301). The ECM defined the LOQ as the lowest fortification specified by the method which yields adequate recovery according to USEPA guidelines (mean 70-120%; RSD \leq 20%). The ECM defined the LOD as the lowest standard amount injected during the chromatographic run, which for this ECM was 0.025 pg/ μ L (ng/mL), equivalent to 1.25 pg injected for NOA-459602, SYN-501406, thiamethoxam (CGA-29343), CGA-322704, CGA-355190, and NOA-404617, and 0.5 pg injected for CGA-353042 and NOA-407475 (p. 13; Tables 3-8, pp. 32-55 of ECM MRID 49667101). In the ILV, LODs were not specified.

Table 4. Method Characteristics for Thiamethoxam (CGA-293343) and Its Transformation Products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in Surface and Ground Water

		Thiamethoxam	CGA-322704	CGA-355190	CGA-353042	NOA-404617	NOA-407475	SYN-501406	NOA-459602	
Limit of Quantitation (LOQ)		0.05 ppb (µg/kg, µg/L)								
Limit of Detection (LOD)		0.025 pg/µL (ng/mL), equivalent to 1.25 pg injected for NOA-459602, SYN-501406, thiamethoxam (CGA-29343), CGA-322704, CGA-355190, and NOA-404617, and 0.5 pg injected for CGA-353042 and NOA-407475.								
Linearity (calibration curve r^2 and concentration range)	ECM:	$r^2 = 0.9994-1.0000$	$r^2 = 0.9990-0.9998$	$r^2 = 0.9996-0.9998$	$r^2 = 0.9992-0.9996$	$r^2 = 0.9996-0.9998$	$r^2 = 0.9992-1.0000$	$r^2 = 0.9998-1.0000$	$r^2 = 0.9998-1.0000$	
		Range: 0.025-1.0 ng/mL (pg/µL)								
	ILV: ²	Well:	$r^2 = 0.9964$	$r^2 = 0.9986$	$r^2 = 0.9990$	$r^2 = 0.9974$	$r^2 = 0.9980$	$r^2 = 0.9986$	$r^2 = 0.9827$	$r^2 = 0.9956$
		Surface:	Range: 0.05-1.50 ng/mL							
			$r^2 = 0.9994$	$r^2 = 0.9996$	$r^2 = 0.9994$	$r^2 = 0.9884$	$r^2 = 0.9992$	$r^2 = 0.9988$	$r^2 = 0.9992$	$r^2 = 0.9994$
		Range: 0.025-10.0 ng/mL								
Repeatable	ECM:	Yes at LOQ and 10x LOQ.								
	ILV:	Yes at LOQ and 10x LOQ.								
Reproducible		Yes.								
Specific ³	ECM:	Yes; interferences at the analyte retention times were <50% of the LOD (based on lowest calibration standard and peak heights); however, NOA-404617 chromatograms show an interference at <i>ca.</i> .7.73-7.74 minutes that overlaps tailing edge of NOA-404617 peak (retention time <i>ca.</i> 7.67 minutes).								
	ILV:	Yes; interferences at the analyte retention times were <50% of the LOD (based on 0.025 ng/mL calibration standard and peak areas).								

Data were obtained from pp. 13, 21; Tables 3-8, pp. 32-55; Figure 6, p. 74; Figure 7, p. 80; Figures 8-10, pp. 85-87; Figure 11, p. 90; Figure 12, p. 93; Figure 13, p. 96; Figure 14, p. 99; Figure 15, p. 102 of ECM MRID 49667101.; pp. 10, 13; Figures 1-16, pp. 36-51; Appendix 3, pp. 71-72, 93-94 of MRID 49383301; and DER Attachment 2.

Linearity is satisfactory when $r^2 \geq 0.995$.

1 ECM and ILV coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (Tables 3-8, pp. 32-55 of ECM MRID 49667101).; Figures 1-16, pp. 36-51 of MRID 49383301; DER Attachment 2). ECM r values determined with linear slope through zero. ILV r values determined with 1/x weighting.

2 Calibration curves are separated by sample set (analyte/matrix), but are not matrix matched standards (pp. 19-20 of ECM MRID 49667101); p. 13 of MRID 49383301). The ILV r^2 values are presented separately in this table because different calibration standard ranges were used for the well water and surface water sample sets.

3 A confirmatory method was not used; however, a confirmatory method is typically not required where LC/MS methods are used as the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. This analytical method, Syngenta Method T003103-03, is reported as an alternate method to methods 442-00 and RAM 396/01 previously validated for the determination of thiamethoxam (CGA-293343) and its transformation products CGA-322704, CGA-355190, CGA-353042, NOA-404617, NOA-407475, SYN-501406, and NOA-459602 in water (p. 13 of ECM MRID 49667101).
2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. The ECM defined the LOQ (or "limit of determination") as the lowest fortification specified by the method which yields adequate recovery according to USEPA guidelines (mean 70-120%; RSD \leq 20%; p. 13 of ECM MRID 49667101). The ECM defined the LOD as the lowest standard amount injected during the chromatographic run, which for this ECM was 0.025 pg/ μ L (ng/mL), equivalent to 1.25 pg injected for NOA-459602, SYN-501406, thiamethoxam (CGA-293343), CGA-322704, CGA-355190, and NOA-404617, and 0.5 pg injected for CGA-353042 and NOA-407475 (p. 13; Tables 3-8, pp. 32-55).

In the ILV, LODs were not specified. The independent laboratory reported that the API 4000 instrument detection limits were explored, but did not specify the LODs (p. 15 of MRID 49383301). In reporting individual recovery results, method blank and matrix blank results were reported as "<0.017 ng/mL", even though calibration standard ranges were 0.05-1.50 ng/mL for well water sample sets and 0.025-10.0 ng/mL for surface water sample sets (Table 13, p. 26; Table 15, p. 30; Figures 1-16, pp. 36-51).

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.

3. The ground (well) and surface (lake and creek) water matrices used in the ECM validation were not characterized.
4. Communication between the independent laboratory and Syngenta was not adequately documented. The ILV study authors only reported that "minimal communication was required" (Table 17, p. 34 of MRID 49383301).
5. For the ILV, chromatograms for reagent blanks were not provided. For the calibration standards, only chromatograms of 0.05 and 0.5 ng/mL standards were provided; calibration standard ranges were 0.05-1.50 ng/mL for well water sample sets and 0.025-10.0 ng/mL for surface water sample sets (Figures 1-16, pp. 36-51; Appendix 3, pp. 69-70, 77-78, 91-92, 99-100 of MRID 49383301). Linearity of the standard curves was not always \geq 0.995 (see **Table 4** above).
6. For the ECM, individual calibration standard data with regression curve analyses were provided, but plots of the standard curves were not provided (Tables 3-8, pp. 32-55 of ECM MRID 49667101). Chromatograms of the analytes at the lowest spiking level show attenuation to where one can measure the peak accurately, except for NOA-404617 which

sometimes had an interference on the tailing edge of the analyte peak (Figure 10, p. 88; Figure 12, p. 94; Figure 14, p. 100 of ECM MRID 49667101).

7. The following typographical error was noted in the ECM: Table 7 (p. 48) subtitle "NOA-459617" should read NOA-459602.
8. A confirmatory method was not employed; however, typically, a confirmatory method is not required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.
9. For the ECM, means, standard deviations, and relative standard deviations for each fortification level and matrix were determined by the reviewer, because the study author only provided summary results (Tables 9-10, pp. 56-63; DER Attachment 2).
10. The equipment substitutions and modifications to optimize LC and MS conditions implemented by the independent laboratory (see section **I. Principle of the Method, ILV:** above for details) are not considered substantial changes to the ECM.
11. It was reported for the ILV that 8 person-hours are required to complete one set of twelve samples, including instrumental data analysis (p. 16 of MRID 49383301).

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**Thiamethoxam [Cruiser 5FS, CGA293343, CGA-293343, Cruiser, A9765N, CGA 293343, Actara, Platinum, Centric]**

IUPAC Name: (E Z)-3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine.

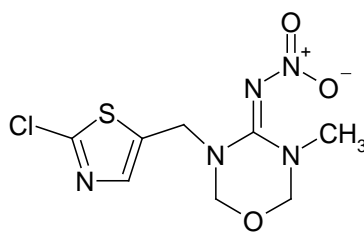
3-(2-Chloro-thiazol-5-ylmethyl)-5-methyl-[1,3,5]oxadiazinan-4-ylidene-N-nitroamine.

CAS Name: 3-[(2-Chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazin-4-imine.

4H-1,3,5-Oxadiazin-4-imine-3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-.

CAS Number: 153719-23-4.

SMILES String: CN2COCN(Cc1cnc(Cl)s1)C2=NN(=O)=O

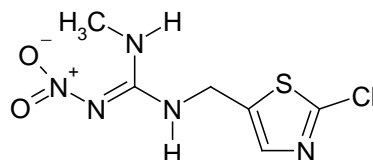
**CGA-322704 [CGA 322704]**

IUPAC Name: Not reported.

CAS Name: Guanidine, N-[(2-chloro-5-thiazolyl)methyl]-N'-methyl-N''-nitro-.

CAS Number: 131748-59-9.

SMILES String: CNC(=NN(=O)=O)NCc1cnc(Cl)s1

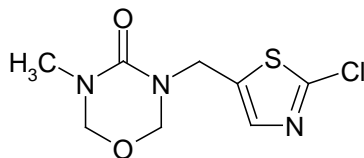
**CGA-355190 [CGA 355190, CGA355190]**

IUPAC Name: Not reported.

CAS Name: 4H-1,3,5-Oxadiazine-4-one, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-.

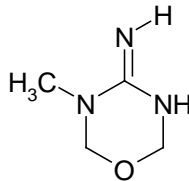
CAS Number: Not reported.

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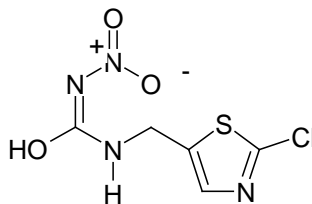


CGA-353042 [CGA 353042]

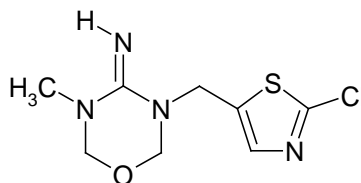
IUPAC Name: Not reported.
CAS Name: 2H-1,3,5-Oxadiazine-4-amine, 3,6-dihydro-3-methyl-.
CAS Number: Not reported.
SMILES String: Not found.

**NOA-404617 [NOA 404617, NOA404617]**

IUPAC Name: Not reported.
CAS Name: Urea, N-[(2-chloro-5-thiazolyl)methyl]-N'-nitro-.
CAS Number: Not reported.
SMILES String: Not found.

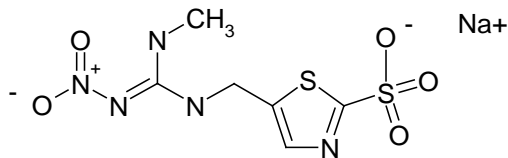
**CGA-407475 [NOA-407475, NOA 407475, NOA407475]**

IUPAC Name: Not reported.
CAS Name: 4H-1,3,5-Oxadiazine-4-imine, 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-.
CAS Number: Not reported.
SMILES String: Not found.

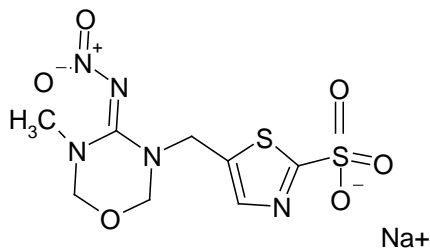


SYN-501406 [SYN 501406]

IUPAC Name: Sodium; 5-(N'-Methyl-N''-nitro-guanidinomethyl)-thiazole-2-sulfonate
CAS Name: Sodium; 5-(N'-Methyl-N''-nitro-guanidinomethyl)-thiazole-2-sulfonate.
CAS Number: Not reported.
SMILES String: Not found.

**NOA-459602 [NOA 459602]**

IUPAC Name: Sodium; 5-{5-methyl-4-[nitroimino]-[1,3,5]oxadiazinan-3-ylmethyl}-thiazole-2-sulfonate.
CAS Name: Sodium; 5-{5-methyl-4-[nitroimino]-[1,3,5]oxadiazinan-3-ylmethyl}-thiazole-2-sulfonate.
CAS Number: Not reported.
SMILES String: Not found.

**Attachment II: Calculations**

calcs