Environmental Chemistry Method (ECM) for Determination of Residues of Thiamethoxam in Samples from Dust Deposition Trials

Reports:	ECM: Thiamethoxam – Residue Analysis Method (GRM009.08A) for the Determination of Residues of Thiamethoxam and Clothianidin (CGA 322704) in Samples from Dust Deposition Trials ---Analytical Method
PC Code:	044309, 060109
Document No.:	[MRIDs 49158921]
Guideline:	EPA Guideline is not applicable to Dust Deposition Study. This Analytical Method for Dust Deposition is reviewed under EPA Guidelines 850.6100 (or 860.1340)
GLP Statements:	No claim of compliance with Environmental Protection Agency's Good Laboratory Practice Standards (40 CFR Part 160, October 16, 1989) or OECD Principles of Good Laboratory Practice (Revised 1997) is made for GRM030.05A There is no GLP study director for this volume
Classification:	The ECM method is classified as **Invalid** for analyzing thiamethoxam and CGA 322704 field dust samples. This ECM must be validated by an independent laboratory validation (ILV) as part of the pesticide analytical method petition. Especially, the limit of detection (LOD) and limit of quantification (LOQ) must be confirmed by an ILV method. This method is upgradable upon provision of the correction of the following deficiencies (Page 5).

EPA Primary Reviewer:	He Zhong, Ph.D. Biologist	Signature: Date: 4/23/2015
EPA Secondary Reviewer:	Kristina Garber Senior Science Advisor	Signature: Date: 4/23/2015

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Executive Summary

The analytical procedure is for the determination of residues of thiamethoxam in samples from the thiamethoxam dust deposition trials (glycerol/water dust traps, quartz sand petri-dish traps, air filters and gauze netting samples) using an external standard. The limit of quantification (LOQ) has been set at 1 ng per dust trap for the dust trap solutions (equivalent to 0.008 ng/mL for the glycerol/water (30/70, v/v)) 1 ng per quartz sand dust trap, 1 ng per gauze netting sample and 2.5 µg per air filter.

Table 1. Analytical Method Summary Using LC-MS/MS

Analyte(s)	MRID		Matrix	Date **(m/d/y)**	Analysis	Samplers (unit)	LOD / LOQ[1] (ng/trap)
	ECM	ILV					
Thiamethoxam	49158921	N/A	Dust	3/20/2013	LC-MS/MS	Dust trap (ng/trap)	0.24 / 1
						Quartz sand trap (ng/trap)	NA / 1
						Gauze netting (ng/trap)	NA / 1
						Air filter (µg/air filter)	0.053 / 2.5
CGA322704	49158921	N/A	Dust	3/20/2013	LC-MS/MS	Dust trap	0.06 / 1
						Quartz sand	NA / 1
						Gauze netting	NA / 1
						Air filter	0.125 / 2.5

[1]LOD = limit of detection and LOQ = limit of quantitation

I. Principle of the Method

Aqueous Dust Trap Solutions

A glycerol/chromasolv water dust trap solution (37.5/87.5, v/v) is shaken on a flatbed shaker for 30 minutes to dissolve any thiamethoxam residues adsorbed to particulate matter. An aliquot of the sample is diluted with ultra pure water and subjected to a Waters Oasis™ HLB solid phase extraction (SPE) procedure prior final determination by high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS).

Quartz Sand Dust Traps

Sand/glycerol/water samples consisted of 50 g of quartz sand that were moistened with 17 mL of glycerol/water (1/1, v/v) each. Water was added to the specimen material of sand/glycerol/water and the sample is shaken on a flatbed shaker at room temperature. After filtration the final extraction volume was made up to 200 mL using water. An aliquot of the sample is subjected to a Waters Oasis™ HLB solid phase extraction (SPE) procedure prior final determination by LC-MS/MS.

Gauze netting

50 x 50 cm gauze squares were moistened with approximately 15 mL of glycerol/water (1/1, v/v). A conditioned gauze square was extracted with methanol/water (1/1, v/v) by shaking on a flatbed shaker at room temperature. After filtration the final extraction volume was made up to 200 mL using water. The methanol amount of an aliquot was evaporated in a stream of nitrogen and the initial volume of the aliquot is restored by adding water. An aliquot of the sample is

subjected to a Waters Oasis™ HLB solid phase extraction (SPE) procedure prior final determination by LC-MS/MS.

Air Filters
An air filter sheet is shaken with methanol/ ultra pure water solution (50:50, v/v) to desorb thiamethoxam residues from the filter. An aliquot of the methanol/ultra pure water solutions is diluted with ultra pure water prior final determination by LC-MS/MS.

II. Recovery Findings

The mean recoveries and the relative standard deviations (RSD) of thiamethoxam and CGA322704 were within guideline requirements (mean 70-120%; RSD ≤20%) for ECM (**Table 2**).

Table 2. Environmental Chemistry Method (ECM) Recoveries for Thiamethoxam and CGA322704 in Trap and Filter Specimen

Analyte	Matrix	Fortification Level (µg/specimen)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	% RSD
Thiamethoxam Quantitation m/z 292.2/211.2	Glycero/Water Solution	0.001	5	81-91	85	6
		1	5	87-90	88	1
	Filter	2.5	5	98-121	108	8
		2.5	5	64-88	80	12
		25	5	79-107	95	11
Thiamethoxam Confirmation m/z 292.2/181.1	Glycero/Water Solution	0.001	5	97-101	99	2
		1	5	84-90	88	3
	Filter	2.5	5	98-113	105	6
		2.5	5	75-87	81	6
		25	5	81-111	97	11
CGA322704 Quantitation m/z 250.2/169.2	Glycero/Water Solution	0.001	5	84-109	91	11
		1	5	78-104	95	12
	Filter	2.5	5	55-88	77	17
		25	5	85-109	98	10
CGA322704 Confirmation m/z 250.2/132.1	Glycero/Water Solution	0.001	5	94-104	98	5
		1	5	90-104	95	6
	Filter	2.5	5	95-120	108	11
		2.5	5	67-94	79	16
		25	5	87-120	99	10

III. Method Characteristics

Protonated molecular ions generated in the ion source (m/z = 292.2 for thiamethoxam and m/z = 250.2 for CGA322704) were selected and subjected to further fragmentation by collisional activation. The most abundant ion or primary ion (m/z = 211.2 for thiamethoxam and m/z = 169.2 for CGA322704) in the resulting daughter spectra are then monitored and used for

quantitative analysis. Other abundant ions or confirmatory ion (m/z = 181.1 for thiamethoxam and m/z = 132.1 for CGA322704) were selected for confirmatory analysis.

Linearity is established in the calibration (y=a+bx) using external standards. The correlation coefficient of the calibration curves (R^2) exceeded 0.998 for the least squares equation. The **limit of quantification** (LOQ) is reported as 1 ng/dust trap and 2.5 µg/filter for thiamethoxam. The method in general satisfies the **repeatability** criteria with mean recoveries are in the range of 70-120% and RSDs are ≤20%. **Reproducibility** is to be verified by an Independent Laboratory Validation. This method using LCMS/MS demonstrated excellent **specificity** by selecting the following primary and confirmatory ions.

The ECM method characteristics are listed in **Tables 4** and **5**.

Table 4. Environmental Chemistry Method (ECM) of Thiamethoxam and CGA322704 Characteristics

	Thiamethoxam (m/z = 211.2)	CGA322704 (m/z – 181.1)
Limit of Quantitation (LOQ) -- Dust trap	1 ng/trap	N/A
Limit of Quantitation (LOQ) -- Filter	2.5 µg/filter	N/A
Limit of Detection (LOD) -- Dust trap	0.24 ng/trap	0.06 ng/trap
Limit of Detection (LOD) -- Filter	0.053 µg/filter	0.125 µg/filter
Linearity ([1]calibration curve r^2 and concentration range)	r^2 = 0.999 10 – 500 (pg) (injection volume 100µL)	r^2 = 0.998 10 – 500 (pg) (injection volume 100µL)
Repeatable	Yes	Yes
Reproducible	Yes	Yes
Specific	Yes	Yes

[1]calibration curve is based on linear regression (y=a+bx)

Table 5. Method Specificity—LC-MS/MS Parent and Daughter ions

Analyte	Primary ion	Confirmatory ion
Thiamethoxam	211.2	181.1
CGA 322704	169.2	132.2

IV. Method Deficiencies and Uncertainties

1. This ECM must be validated by an independent laboratory validation (ILV) as part of the pesticide analytical method petition. Especially, the limit of detection (LOD) and limit of quantification (LOQ) must be confirmed by an ILV method.
2. An example is needed to use the formula (page 25, e) in calculating LOQ = 0.008 ng/ml. It is unknown for the calculation from 125 ml or applying the density at 134 g. The final elution volumes after the solid phase extraction were not explained for different traps.
3. A table is needed for each fortification level and each type of traps.
4. The methods of fortification to each type of four traps are needed.
5. A summary table is needed for all LOD and LOQ values. It is unclear if the LOQ data for both thiamethoxam and CGA322704.
6. The dimension (or the surface area) of the traps are unknown. It is unclear that how these traps are used for the dust collection in the field.
7. It is unclear that 50 g of quartz sand is a portion or total amount of the sand used for Quartz Sand Trap and what is the trap surface areas.
8. It is unclear the screen size for the 50 x 50 cm gauze squares netting.

V. References

Crook, S. and Chen, L., 2012. Thiamethoxam – Residue Analysis Method (GRM009.08A) for the Determination of Residues of Thiamethoxam in Samples form Dust Deposition Trials --- Analytical Method. MRID 49158921