

## Analytical method for cycloate and its metabolites cycloate sulfoxide and N-ethylcyclohexylamine (ECHA) in soil

**Reports:** ECM: EPA MRID No. 50730602. DeVellis, S.R. 2018. Validation of an Environmental Chemistry Method for the Determination of Cycloate, Cycloate Sulfoxide, and N-ethylcyclohexylamine in Soil by LC-MS/MS. Smithers Viscient Study No.: 14113.6131. Report prepared by Smithers Viscient 790, Wareham, Massachusetts, and sponsored and submitted by Helm Agro US, Inc., Tampa, Florida; 82 pages. Final report issued October 15, 2018.

ILV: EPA MRID No. 50730604. Cashmore, A. 2018. Independent Laboratory Validation of Analytical Method 14113.6131 for the Determination of Cycloate in Soil Data Requirements. Laboratory Project ID: 3202259. Report prepared by Smithers Viscient (ESG) Ltd., North Yorkshire, United Kingdom, and sponsored and submitted by Helm Agro US, Inc., Tampa, Florida; 96 pages. Final report issued November 5, 2018.

**Document No.:** MRID 50730602 & 50730604

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, 40 CFR, Part 160, as well as OECD GLP, except for characterization of the test substances cycloate sulfoxide, and N-ethylcyclohexylamine (p. 3 of MRID 50730602). An authenticity statement was included with the QA statement.

ILV: The study was conducted in accordance with UK Health Department GLP Regulations, and OECD GLP standards, which are also accepted by the USEPA and Japanese authorities, except for the characterization of the soil and cycloate sulfoxide and ECHA test materials (p. 3; Appendix 2, p. 73 of MRID 50730604).

**Classification:** This analytical method is classified as acceptable. The cycloate dataset doesn't have an endpoint that can be used as a level of concern in soil.

**PC Code:** 041301

**EFED Final  
Reviewer:**

James Lin  
Environmental Engineer

**Signature:**   
Date: 03/23/2020

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*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## Executive Summary

The analytical method, Smithers Viscient Study No. 14113.6131, is designed for the quantitative determination of cycloate and its metabolites cycloate sulfoxide and N-ethylcyclohexylamine (ECHA) in soil at the LOQ of 10.0 µg/kg using LC/MS. The cycloate dataset doesn't have an endpoint that can be used as a level of concern in soil. The ECM and ILV were performed using one characterized soil matrix per analyte. In the ECM, the DU loam soil was used for cycloate validation; the DU clay loam soil was used for cycloate sulfoxide and ECHA validation. The same the DU loam soil was used in the ILV validation for all analytes. It could not be determined that the ILV was provided with the most difficult soil matrix with which to validate the method and if the ILV soil matrix was representative of terrestrial field dissipation soil matrices. The ILV validated the ECM method in the first trial for cycloate at 10×LOQ, cycloate sulfoxide and ECHA and in the second trial for cycloate at the LOQ with insignificant modifications to the analytical instrumentation and parameters. The first attempt for cycloate failed at the LOQ due to low recoveries for two of the samples after improper toluene evaporation. The ILV confirmed the ECM critical step of the evaporation of the toluene extracts. All ILV and ECM data regarding repeatability, accuracy, and precision were satisfactory for cycloate, cycloate sulfoxide, and ECHA. All ILV and ECM data regarding linearity and specificity were acceptable for cycloate sulfoxide. All ILV linearity was acceptable for cycloate and ECHA, but ECM linearity was not. All ILV and ECM specificity was acceptable for cycloate and ECHA, except that the specificity of the method was not supported by ILV representative chromatograms of ECHA where matrix interferences were *ca.* 26% of the LOQ (based on peak area) which was >50% of the LOD.

**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						
Cycloate	50730602	50730604		Soil <sup>1,2</sup>	15/10/2018	Helm Agro US, Inc.	LC/MS/MS	10.0 µg/kg
Cycloate sulfoxide								
N-ethylcyclohexylamine (ECHA)								

<sup>1</sup> In the ECM, the DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) and DU clay loam soil (Smithers Viscient Batch No. DU-L-PF 14DEC16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 1:1 soil:water ratio; WHC at 1/3 bar 31.5%)

were used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; pp. 14-15 of MRID 50730602). The DU loam soil was used for cycloate validation; the DU clay loam soil was used for cycloate sulfoxide and ECHA validation.

2 In the ILV, the DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 18/0000000/17; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) was used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; p. 16 of MRID 50730604). The soil was the same as one of the ECM test soils.

## I. Principle of the Method

### Sample Fortification

Soil samples (5 g dry weight) were fortified, if necessary, with 0.500 mL of 0.100 or 1.00 mg/L individual fortification solutions in 50-mL Nalgene centrifuge tubes (pp. 21-22 of MRID 50730602).

### For Cycloate

The sample was mixed with 10.0 mL of purified water, then 15.0 mL of toluene via vortex mixing (pp. 22-23 of MRID 50730602). After shaking on a shaker table at 300 rpm for one hour and centrifugation (3000 rpm for 10 minutes), *ca.* 10.0 mL of the top toluene layer was removed and transferred to a clean 50-mL volumetric flask. The extraction was repeated with 10.0 mL of toluene. The volume of the combined toluene extracts was adjusted to 50 mL using toluene, then an aliquot (1.00 mL) was reduced to 25-100  $\mu$ L under a gentle stream of nitrogen. The volume of the residue was adjusted to 1.00 mL using acetonitrile, then the volume was adjusted to 10.0 mL using acetonitrile:purified reagent water (50:50, v:v). The samples were further diluted into the calibration standard range with acetonitrile:purified reagent water (50:50, v:v) as needed. Note: the study report stated that the evaporation of the toluene extracts is an apparent critical step in the method. Evaporating to dryness could result in low sample recovery. Leaving greater than 100  $\mu$ L toluene in the final extract could also reduce the sample recovery by causing the organic phase to partition out of the final extract.

Samples were analyzed for cycloate using a Shimadzu LC-20AD HPLC system coupled to an AB MDS API 4000 mass spectrometer with ESI Turbo V source (pp. 13, 26-27 of MRID 50730602). The LC/MS conditions consisted of a Waters Atlantis T3 column (100 x 4.6 mm, 3  $\mu$ m particle size; column temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.01-0.50 min. 98.0:2.00, 0.60 min. 50.0:50.0, 6.00-7.00 min. 0.00:100, 7.10-8.50 min. 98.0:2.00] and electrospray ionization (ESI) interface MS detection in positive ion mode with MRM (source temperature 550°C). Injection volume was 50.0  $\mu$ L. The primary and confirmatory ion transitions were  $m/z$  215.99 $\rightarrow$ 83.05 and  $m/z$  215.00 $\rightarrow$ 154.18, respectively. Expected retention time was *ca.* 6.5 minutes for cycloate.

### For Cycloate Sulfoxide

The sample was mixed with 10.0 mL of saturated solution of NaCl in methanol:purified water (50:50, v:v), then 15.0 mL of toluene via vortex mixing (pp. 23-24 of MRID 50730602). After

shaking on a shaker table at 250 rpm for 30 minutes and centrifugation (3000 rpm for 5 minutes), 10.0 mL of the top toluene layer was removed and transferred to a disposable vial with PTFE-lined caps. The extraction was repeated three times with 10.0 mL each of toluene. The volume of the combined toluene extracts was adjusted to 40 mL using toluene. The volume of an aliquot (0.080 mL for LOQ samples, 0.025 mL for 10×LOQ samples) was adjusted to 10.0 mL using acetonitrile:purified reagent water (50:50, v:v).

Samples were analyzed for cycloate sulfoxide using a Shimadzu SIL-20ACXR HPLC system coupled to an MDS Sciex API 6500+ QTRAP mass spectrometer with ESI Turbo V source (pp. 13, 27-28 of MRID 50730602). The LC/MS conditions consisted of a Waters Atlantis T3 column (100 x 4.6 mm, 3 μm particle size; column temperature 35°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.01-1.00 min. 95.0:5.00, 4.00-5.00 min. 0.00:100, 5.10-6.00 min. 95.0:5.00] and electrospray ionization (ESI) interface MS detection in positive ion mode with MRM (source temperature 650°C). Injection volume was 25.0 μL. The primary and confirmatory ion transitions were  $m/z$  254.16→226.12 and  $m/z$  254.16→177.16, respectively. Expected retention time was *ca.* 4.0 minutes for cycloate sulfoxide.

#### For ECHA

The sample was mixed with 5.00 mL of methanol and 5.00 mL of 3M NaOH solution, then 10.0 mL of toluene via vortex mixing (pp. 24-25 of MRID 50730602). After shaking on a shaker table at 250 rpm for 10 minutes and centrifugation (3000 rpm for 5 minutes), 5.00 mL of the top toluene layer was removed and transferred to a disposable vial with PTFE-lined caps. The extraction was repeated three times with 5.00 mL each of toluene. The volume of the combined toluene extracts was 20 mL. The volume of an aliquot (0.060 mL for LOQ samples, 0.025 mL for 10×LOQ samples) was adjusted to 10.0 mL using acetonitrile:purified reagent water (50:50, v:v) by first adding 5.00 mL of acetonitrile than bringing to volume using purified reagent water.

Samples were analyzed for ECHA using a Shimadzu SIL-20ACXR HPLC system coupled to an MDS Sciex API 5000 mass spectrometer with ESI Turbo V source (pp. 13, 28-29 of MRID 50730602). The LC/MS conditions consisted of a Waters Atlantis T3 column (100 x 4.6 mm, 3 μm particle size; column temperature 40°C) with a mobile phase gradient of A) 0.1% formic acid in water and B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.01-0.50 min. 98.0:2.00, 3.00-4.00 min. 0.00:100, 4.10-5.00 min. 98.0:2.00] and electrospray ionization (ESI) interface MS detection in positive ion mode with MRM (source temperature 650°C). Injection volume was 50.0 μL. The primary and confirmatory ion transitions were  $m/z$  128.23→82.74 and  $m/z$  128.23→55.01, respectively. Expected retention time was *ca.* 1.3 minutes for ECHA.

#### ILV

In the ILV, the ECM was performed as written, except for the use of a different LC/MS system (pp. 20-26; Appendix 6, pp. 93-95 of MRID 50730604). A Shimadzu Nexera series HPLC system with AB Sciex API 5000 MS/MS detector was used for all analyses. All LC/MS conditions were the same for each analysis. The primary and confirmatory ion transitions for cycloate were  $m/z$  215.9→83.0 and  $m/z$  215.9→154.1, respectively; expected retention time was

*ca.* 6.2 minutes. The primary and confirmatory ion transitions for cycloate sulfoxide were  $m/z$  253.9→226.1 and  $m/z$  253.9→177.1, respectively; expected retention time was *ca.* 3.8 minutes. The primary and confirmatory ion transitions for ECHA were  $m/z$  128.2→83.0 and  $m/z$  128.2→55.1, respectively; expected retention time was *ca.* 1.2 minutes.

#### LOD/LOQ

The Limit of Quantification (LOQ) for cycloate, cycloate sulfoxide, and ECHA in soil was 10.0 µg/kg in the ECM and ILV (pp. 10, 30-32 of MRID 50730602; pp. 28-30 of MRID 50730604). The Limit of Detection (LOD) for cycloate, cycloate sulfoxide, and ECHA in soil was calculated to be 1-5 µg/kg in the ECM and 0.765-4.53 µg/kg in the ILV.

## II. Recovery Findings

ECM (MRID 50730602): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of cycloate, cycloate sulfoxide, and ECHA in one soil matrix at fortification levels of 10.0  $\mu\text{g}/\text{kg}$  (LOQ) and 100  $\mu\text{g}/\text{kg}$  (10 $\times$ LOQ; Tables 1-6, pp. 39-44). Analytes were identified using two ion transitions; primary and confirmatory recovery results were comparable. The DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) and DU clay loam soil (Smithers Viscient Batch No. DU-L-PF 14DEC16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 1:1 soil:water ratio; WHC at 1/3 bar 31.5%) were used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; pp. 14-15). The DU loam soil was used for cycloate validation; the DU clay loam soil was used for cycloate sulfoxide and ECHA validation.

ILV (MRID 50730604): Mean recoveries and RSDs were within guideline requirements for analysis of cycloate, cycloate sulfoxide, and ECHA in one soil matrix at fortification levels of 10.0  $\mu\text{g}/\text{kg}$  (LOQ) and 100  $\mu\text{g}/\text{kg}$  (10 $\times$ LOQ; p. 29; Tables 1-6, pp. 35-40). Analytes were identified using two ion transitions; primary and confirmatory recovery results were comparable. The DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 18/0000000/17; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) was used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; p. 16). The soil was the same as one of the ECM test soils. The ILV validated the ECM method in the first trial for cycloate at 10 $\times$ LOQ, cycloate sulfoxide and ECHA and in the second trial for cycloate at the LOQ with insignificant modifications to the analytical instrumentation and parameters (pp. 20-26, 30-31). The first attempt for cycloate failed at the LOQ due to low recoveries for two of the samples. It was suspected that too much toluene remained in the final extract, and was causing the organic layer to partition out. In the second validation attempt, the toluene extracts were re-aliquoted and carefully dried to between 25 and 100  $\mu\text{L}$  and ultrasonicated in acetonitrile. It was confirmed that the final extracts were not partitioning, and any remaining toluene was fully dissolved. The results were acceptable. The ILV confirmed the ECM critical step of the evaporation of the toluene extracts. Evaporating to dryness could result in low sample recovery. Leaving greater than 100  $\mu\text{L}$  toluene in the final extract could also reduce the sample recovery by causing the organic phase to partition out of the final extract.

**Table 2. Initial Validation Method Recoveries for Cycloate and its Metabolites Cycloate Sulfoxide and N-ethylcyclohexylamine (ECHA) in Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>DU Loam Soil</b>						
Quantitation ion transition						
Cycloate	10.0 (LOQ)	7	68.6-86.1	80.0	6.13	7.66
	100	5	94.4-111	102	6.90	6.75
Confirmation ion transition						
Cycloate	10.0 (LOQ)	7	79.4-90.6	83.5	4.38	5.25
	100	5	91.2-102	96.8	4.66	4.81
<b>DU Clay Loam Soil</b>						
Quantitation ion transition						
Cycloate Sulfoxide	10.0 (LOQ)	7	84.4-91.5	87.6	2.66	3.04
	100	5	81.2-87.5	83.8	2.60	3.11
ECHA	10.0 (LOQ)	7	76.8-108	88.2	11.8	13.4
	100	5	74.4-91.9	80.2	6.77	8.43
Confirmation ion transition						
Cycloate Sulfoxide	10.0 (LOQ)	7	88.3-94.7	90.8	2.30	2.54
	100	5	80.9-86.7	84.1	2.46	2.92
ECHA	10.0 (LOQ)	7	77.3-114	89.9	14.8	16.5
	100	5	79.4-90.1	84.3	4.44	5.27

Data (uncorrected recovery results, pp. 30-31) were obtained from Tables 1-6, pp. 39-44 of MRID 50730602.

1 The DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) and DU clay loam soil (Smithers Viscient Batch No. DU-L-PF 14DEC16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 1:1 soil:water ratio; WHC at 1/3 bar 31.5%) were used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; pp. 14-15).

2 The primary and confirmatory ion transitions for cycloate were  $m/z$  215.99→83.05 and  $m/z$  215.00→154.18, respectively. The primary and confirmatory ion transitions for cycloate sulfoxide were  $m/z$  254.16→226.12 and  $m/z$  254.16→177.16, respectively. The primary and confirmatory ion transitions for ECHA were  $m/z$  128.23→82.74 and  $m/z$  128.23→55.01, respectively.

**Table 3. Independent Validation Method Recoveries for Cycloate and its Metabolites Cycloate Sulfoxide and N-ethylcyclohexylamine (ECHA) in Soil<sup>1,2</sup>**

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>DU Loam Soil</b>						
Quantitation ion transition						
Cycloate	10.0 (LOQ)	7	73-81	77	3.3	4.3
	100	5	74-83	79	3.4	4.2
Cycloate Sulfoxide	10.0 (LOQ)	7	69-83	76	4.3	5.7
	100	5	81-88	84	3.0	3.6
ECHA	10.0 (LOQ)	7	90-107	99	6.5	6.5
	100	5	91-94	92	1.3	1.4
Confirmation ion transition						
Cycloate	10.0 (LOQ)	7	73-83	77	3.5	4.5
	100	5	75-84	79	3.2	4.0
Cycloate	10.0 (LOQ)	7	68-76	71	2.6	3.7
	100	5	83-89	85	2.4	2.8
Cycloate	10.0 (LOQ)	7	82-105	90	9.3	10.3
	100	5	85-92	90	2.7	3.0

Data (uncorrected recovery results, p. 27) were obtained from p. 29; Tables 1-6, pp. 35-40 of MRID 50730604.

- 1 The DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 18/0000000/17; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) was used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; p. 16). The soil was the same as one of the ECM test soils.
- 2 The primary and confirmatory ion transitions for cycloate were  $m/z$  215.9→83.0 and  $m/z$  215.9→154.1, respectively. The primary and confirmatory ion transitions for cycloate sulfoxide were  $m/z$  253.9→226.1 and  $m/z$  253.9→177.1, respectively. The primary and confirmatory ion transitions for ECHA were  $m/z$  128.2→83.0 and  $m/z$  128.2→55.1, respectively. These were similar to those of the ECM.

### III. Method Characteristics

The LOQ for cycloate, cycloate sulfoxide, and ECHA in soil was 10.0 µg/kg in the ECM and ILV (pp. 10, 30-32 of MRID 50730602; pp. 28-30 of MRID 50730604). The LOQ in the ECM was defined as the lowest fortification level; in the ILV, the LOQ was based on the lowest level validated. The LOD for cycloate, cycloate sulfoxide, and ECHA in soil was calculated to be 1-2 µg/kg, 1 µg/kg, and 4-5 µg/kg, respectively, in the ECM. The LOD for cycloate, cycloate sulfoxide, and ECHA in soil was calculated to be 1.01-1.03 µg/kg, 0.765-1.39 µg/kg, and 3.01-4.53 µg/kg, respectively, in the ILV. In the ECM and ILV, the LOD was calculated using the standard deviation of the average recovery in units of concentration of the seven samples fortified at the LOQ, multiplied by one-tailed t-statistic at the 99% confidence level for n-1 replicates. The LOD was calculated for each matrix using the following equation, according to the U.S. EPA, 2016, 1994, Definition and Procedure for the Determination of the Method Detection Limit, Revision 1.11 and Revision 2.:

$$\text{LOD} = t_{0.99} \times \text{SD} + \text{average residue in the untreated controls}$$

Where,  $t_{0.99}$  is the one-tailed t statistic for  $n = 7$  (3.143) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV

Additionally, the Method Detection Limit (MDL) was defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions. The MDL was calculated as 5.00 µg/kg for cycloate and cycloate sulfoxide and 6.67 µg/kg for ECHA in the ECM and ILV.

**Table 4. Method Characteristics**

Analyte		Cycloate	Cycloate Sulfoxide	ECHA
Limit of Quantitation (LOQ)	ECM	10.0 µg/kg		
	ILV			
Limit of Detection (LOD)	ECM	2 µg/kg (Q) 1 µg/kg (C)	1 µg/kg (Q & C)	4 µg/kg (Q) 5 µg/kg (C)
	ILV	1.03 µg/kg (Q) 1.01 µg/kg (C)	1.39 µg/kg (Q) 0.765 µg/kg (C)	3.01 µg/kg (Q) 4.53 µg/kg (C)
Linearity (calibration curve $r^2$ and concentration range)	ECM	$r^2 = 0.990$ (Q) $r^2 = 0.991$ (C)	$r^2 = 0.998$ (Q) $r^2 = 0.999$ (C)	$r^2 = 0.993$ (Q) $r^2 = 0.996$ (C)
	ILV	$r^2 = 0.9976$ (Q) $r^2 = 0.9963$ (Q) $r^2 = 0.9964$ (C, LOQ & 10×LOQ)	$r^2 = 0.9991$ (Q) $r^2 = 0.9997$ (C)	$r^2 = 0.9980$ (Q) $r^2 = 0.9982$ (C)
	Range	0.05-0.5 µg/L	0.005-0.05 µg/L	0.01-0.1 µg/L
Repeatable	ECM <sup>1</sup>	Yes at LOQ and 10×LOQ (characterized soil matrix)	Yes at LOQ and 10×LOQ (characterized soil matrix)	
	ILV <sup>2,3</sup>	Yes at LOQ and 10×LOQ (characterized soil matrix)		
Reproducible		Yes at LOQ and 10×LOQ		
Specific	ECM	Yes, matrix interferences were <10% of the LOQ (based on peak area).	Yes, matrix interferences were <10% of the LOQ (based on peak area). Nearby contaminants noted in baseline noise.	Yes, matrix interferences were <10% of the LOQ (based on peak area), but LOQ peak was small compared to baseline noise.
	ILV	Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed, but LOQ peak was small compared to baseline noise.	<b>No</b> , matrix interferences were <i>ca.</i> 26% of the LOQ (based on peak area). <sup>4</sup> The LOQ peak was small compared to baseline noise.

Data were obtained from pp. 11, 18-21 (LOQ/LOD); Tables 1-6, pp. 39-44 (recovery data); p. 33; Figures 16-21, pp. 66-71 (calibration data & curves); Figures 1-15, pp. 51-65 (chromatograms) of MRID 50730602; pp. 18-20 (LOQ/LOD); p. 29; Tables 1-6, pp. 35-40 (recovery data); p. 29; Figures 1-2, pp. 45-46; Figures 15-16, pp. 53-54; Figures 29-30, pp. 61-62 (calibration data & curves); Figures 3-14, pp. 47-52; Figures 17-28, pp. 55-60; Figures 31-42, pp. 63-68 (chromatograms) of MRID 50730604. Q = Quantitation ion transition; C = Confirmation ion transition.

1 In the ECM, the DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) and DU clay loam soil (Smithers Viscient Batch No. DU-L-PF 14DEC16 Soil-B; 40% sand, 28% silt, 32% clay; pH 5.4 in 1:1 soil:water ratio; WHC at 1/3 bar 31.5%) were used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; pp. 14-15 of MRID 50730602). The DU loam soil was used for cycloate validation; the DU clay loam soil was used for cycloate sulfoxide and ECHA validation.

2 In the ILV, the DU loam soil (Smithers Viscient Batch No. DU-L-PF 10JAN18 Soil-B; 18/000000/17; 31% sand, 44% silt, 25% clay; pH 6.7 in 1:1 soil:water ratio; WHC at 1/3 bar 45.1%) was used in the study and collected from Grand Forks, North Dakota (USDA soil texture classification; p. 16 of MRID 50730604). The soil was the same as one of the ECM test soils.

3 The ILV validated the ECM method in the first trial for cycloate at 10×LOQ, cycloate sulfoxide and ECHA and in the second trial for cycloate at the LOQ with insignificant modifications to the analytical instrumentation and parameters (pp. 20-26, 30-31 of MRID 50730604). The first attempt for cycloate failed at the LOQ due to low recoveries for two of the samples after improper toluene evaporation. The ILV confirmed the ECM critical step of the evaporation of the toluene extracts.

4 Based on Figures 37-40, pp. 66-67 of MRID 50730604.

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

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

1. It could not be determined that the ILV was provided with the most difficult soil matrix with which to validate the method since only one characterized soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies. A cycloate terrestrial field dissipation study was not submitted along with the method validation MRID 50730602 to determine if the ILV soil matrix was representative of terrestrial field dissipation soil matrices. The reviewer also noted that the ILV test soil was the same as one of the ECM test soils.
2. The specificity of the method was not supported by ILV representative chromatograms of ECHA. Matrix interferences were *ca.* 26% of the LOQ (based on peak area) which was >50% of the LOD (Figures 37-40, pp. 66-67 of MRID 50730604). Additionally, the LOQ peak was small compared to baseline noise.
3. The ECM linearity was not satisfactory for cycloate [ $r^2 = 0.990$  (Q) 0.991 (C)] and ECHA [ $r^2 = 0.993$  (Q); p. 33; Figures 16-21, pp. 66-71 of MRID 50730602]. Linearity is satisfactory when  $r^2 \geq 0.995$ . The reviewer noted that linearity deviations in the confirmation ion analyses do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.
4. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10, 30-32 of MRID 50730602; pp. 28-30 of MRID 50730604). The LOQ in the ECM was defined as the lowest fortification level; in the ILV, the LOQ was based on the lowest level validated. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV. Detection limits should not be based on arbitrary values. In the ECM and ILV, the LOD was calculated using the standard deviation of the average recovery in units of concentration of the seven samples fortified at the LOQ, multiplied by one-tailed t-statistic at the 99% confidence level for n-1 replicates. The LOD was calculated for each matrix using the following equation, according to the U.S. EPA, 2016, 1994, Definition and Procedure for the Determination of the Method

Detection Limit, Revision 1.11 and Revision 2.

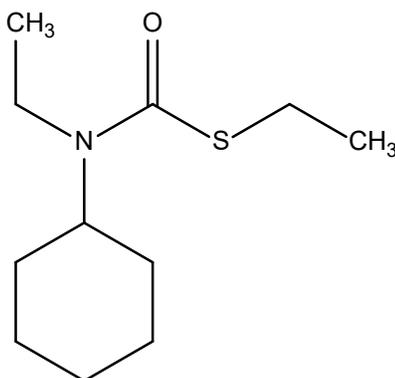
5. Communications between the Study Sponsor from Helm Agro US, Inc. and ILV reportedly involved exchange of study protocol and successful validation results (Appendix 4, p. 75 of MRID 50730604). The Study Sponsor provided technical guidance in order to overcome the failed cycloate LOQ validation; however, these suggestions did not solve the issues. The exact source of the technical guidance from the Study Sponsor was not reported; the Study Sponsor was not the ECM laboratory, Smithers Viscent 790. Details of communications were not reported.
6. The matrix effects were assessed in the ECM or ILV; solvent-based standards were used in the ECM and ILV since no matrix effects were observed (p. 31; Tables 7-12, pp. 45-50 of MRID 50730602; p. 30; Tables 7-9, pp. 41-43 of MRID 50730604).
7. It was reported for the ILV that one sample set of 15 samples required *ca.* 8 hours for sample processing and 6 hours for automated LC/MS/MS analysis (p. 17 of MRID 50730604).

## V. References

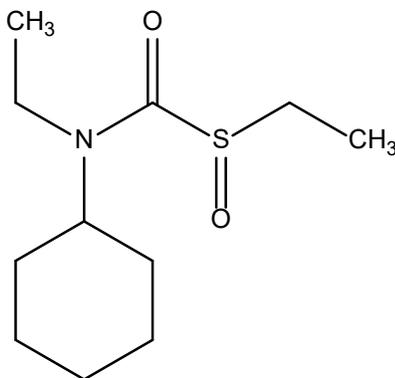
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition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

**Attachment 1: Chemical Names and Structures****Cycloate**

**IUPAC Name:** S-ethyl cyclohexyl(ethyl)thiocarbamate  
**CAS Name:** S-ethyl N-cyclohexyl-N-ethylcarbamothioate  
**CAS Number:** 1134-23-2  
**SMILES String:** CCSC(=O)N(CC)C1CCCCC1

**Cycloate sulfoxide**

**IUPAC Name:** N-cyclohexyl-N-ethyl-1-(ethylsulfinyl)methanamide  
**CAS Name:** Not reported  
**CAS Number:** Not reported  
**SMILES String:** O=C(S(CC)=O)N(CC)C1CCCCC1



**N-ethylcyclohexylamine (ECHA)****IUPAC Name:** N-ethylcyclohexylamine**CAS Name:** Not reported**CAS Number:** 5459-93-8**SMILES String:** [H]N(CC)C1CCCCC1