

Test Material: Chlorpropham

MRID: 49373403

Title: VALIDATION OF A METHOD FOR THE DETERMINATION OF CHLORPROPHAM IN SOIL FOR SUPPORT OF TERRESTRIAL FIELD DISSIPATION STUDIES

MRID: 49457204

Title: Independent Laboratory Validation of an Analytical Method for the Determination of Chlorpropham in Soil by LC/MS/MS

EPA PC Code: 018301

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: 

Date: 4/22/15

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QC/QA Manager: Joan Gaidos

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Date: 4/22/15

Analytical method for chlorpropham in soil

Reports: ECM: EPA MRID No. 49373403. MacGregor, J.A., E.S. Bodle. 2014. VALIDATION OF A METHOD FOR THE DETERMINATION OF CHLORPROPHAM IN SOIL FOR SUPPORT OF TERRESTRIAL FIELD DISSIPATION STUDIES. Wildlife International Project No.: 535C-148. Report prepared by Wildlife International, Evans Analytical Group, Easton , Maryland; sponsored and submitted by 1,4GROUP, Inc., Boise, Idaho; 46 pages. Final report issued March 25, 2014.
ILV: EPA MRID No. 49457204. Mannella, L. 2014. Independent Laboratory Validation of an Analytical Method for the Determination of Chlorpropham in Soil by LC/MS/MS. PTRL West Project No.: 2602W. Report prepared by PTRL West (a division of EAG Inc.), Hercules, California; sponsored and submitted by 1,4GROUP, Inc., Boise, Idaho; 49 pages. Final report issued August 8, 2014.

Document No.: MRIDs 49373403 (ECM) & 49457204 (ILV)

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160) and OECD Principles of GLP (p. 3 of MRID 49373403). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A signatures page was provided, but a Certification of Authenticity was not provided (p. 5).
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 49457204). Signed and dated No Data Confidentiality, GLP, Certification of Authenticity and Quality Assurance statements were provided (pp. 2-4). The authenticity statement was included in the QA statement. A signatures page was also provided (p. 5).

Classification: This analytical method is classified as invalid. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures as only two different concentrations were tested. The registrant failed to demonstrate how well the method performs.

PC Code: 018301

Reviewer: Karen Milians, Chemist

Signature:

Date:

Executive Summary

This analytical method, Wildlife International Project No. 535C-148, is designed for the quantitative determination of chlorpropham in soil at the stated LOQ of 0.05 mg/kg using LC/MS/MS. The LOQ reported is not reliable and therefore, it cannot be determined whether the method is less than or equal to/greater than the lowest toxicological level of concern in soil. The number of trials was not specified, but the reviewer assumed that the ILV successfully validated the method for chlorpropham after one trial. A sandy loam soil (5.6% organic matter, 9% clay) was used in the ECM and ILV; however, the registrant failed to analyze and demonstrate how well the method performs in that soil matrix.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Chlorpropham	49373403	49457204		Soil*	03/25/2014	1,4GROUP, LLC	LC/MS/MS	0.05 mg/kg

* The soil matrix was sandy loam (USDA textural class; 5.6% organic matter, pH 6.7, 9% clay 21% silt 70% sand) in the ECM and ILV.

I. Principle of the Method

Soil (10.0 g) in a 50 mL plastic graduated centrifuge tube was fortified then extracted with 25 mL of acetonitrile via hand shaking and vortexing, then sonication for *ca.* 1 minute using a Bransonic ultrasonic disruption sample processor at an amplitude setting of *ca.* 45% (pp. 12-13; Figure 1, p. 21 of MRID 49373403). After centrifugation (*ca.* 5 minutes at *ca.* 4500 rpm), the supernatant was removed. The soil pellet was extracted with 25 mL of acetonitrile using a gyratory shaker table apparatus (*ca.* 250 excursions per minute for *ca.* 15 minutes). After centrifugation (*ca.* 5 minutes at *ca.* 4500 rpm), the supernatant was removed. The soil pellet was extracted a final time with 25 mL of acetonitrile:water (75:25, v:v) with shaking as described above. After centrifugation, the supernatant was removed. The volume of the combined supernatants was adjusted to 75.0 mL using acetonitrile. An aliquot (*ca.* 3-5 mL) was filtered using an assembly of a 5-mL BD disposable plastic syringe connected to a 0.2 µm Whatman paradisk 25 TF syringe filter into a 20-mL glass scintillation vial. A 1.00-mL aliquot of the filtered extract was transferred to a 15-mL plastic graduated centrifuge tube and brought to 10.0 mL final volume using acetonitrile:water [45:55, v:v; final solvent composition of acetonitrile:water (50:50, v:v)]. Aliquots of the final filtered, diluted extracts were analyzed by LC/MS/MS.

Samples were analyzed for chlorpropham using an Agilent Technologies 1200 Infinity Series HPLC coupled with an Applied Biosystems MDS Sciex API 3000 Tandem Mass Spectrometer (MS/MS) using a Turbo-Ion Spray source operated in positive, multiple reaction monitoring

(MRM; p. 13; Table 1, p. 18 of MRID 49373403). The following LC conditions were used: THERMO EC Betasil C-18 column (50 mm x 2.1 mm, 5 µm, column temperature 40°C) with a THERMO EC Betasil C-18 guard column (20 mm x 2.1 mm) using a mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [percent A:B (v:v) at 0.00-1.00 min. 80:20, 4.00-6.00 min. 5:95, 6.10-10.0 min. 80:20]. Injection volume was 50.0 µL. Chlorpropham was identified using two ion transitions; one for quantitation (Q, "primary") and one for confirmation (C). Ion transitions monitored were as follows: m/z 214→172 (Q) and m/z 214→154 (C). Expected retention time was *ca.* 6.24 minutes.

ILV

The samples were processed using the same general procedure as that of the ECM, except that the agitation techniques/equipment (Branson sonicator for 5 minutes versus Bransonic ultrasonic disruption sample processor and Wrist-Action™ shaker versus gyratory shaker table apparatus), the centrifuge speed and time (samples centrifuged at 4000 rpm for 10 minutes), and the filter equipment (0.2 µm syringeless filter versus BD syringe/0.2 µm Whatman Puradisk 25 TF syringe filter) varied from that used in the ECM (pp. 14, 17-19, 23; Figure 1, p. 30 of MRID 49457204). Additionally, samples were analyzed for chlorpropham using an Agilent Technologies 1200 Series HPLC coupled with an Applied Biosystems MDS Sciex API 3200 Tandem Mass Spectrometer (MS/MS) using a Turbo-Ion Spray source operated in positive, multiple reaction monitoring (MRM). The following LC conditions used by the ILV varied from those of the ECM: ACE Excel 2 C18-AR column (50 mm x 2.1 mm, 2 µm or 50 mm x 2.0 mm, 2 µm; see Reviewer's Comment #6) with a Phenomenex C18 guard column (4 mm x 2 mm). All other LC/MS/MS conditions were the same as those of the ECM (the reviewer noted a typographical error in the LC mobile phase solvent; see Reviewer's Comment #6).

In the ECM and ILV, the LOQ and LOD were 0.0500 mg/kg (0.05 ppm) and 0.015 mg/kg (0.015 ppm), respectively (p. 14 of MRID 49373403; p. 22 of MRID 49457204).

II. Recovery Findings

ECM (MRID 49373403): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD ≤20%) for analysis of chlorpropham in soil at the LOQ and 10×LOQ (Tables 2-3, pp. 19-20). Analytes were identified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The soil matrix was sandy loam (USDA textural class; 5.6% organic matter, pH 6.7, 9% clay 21% silt 70% sand) which was fully characterized by Agvise Laboratories, Northwood, North Dakota (p. 11; Appendix 4, p. 45). The soil was chosen to best represent the soil type for the region of the potential terrestrial field dissipation study.

ILV (MRID 49457204): Mean recoveries and RSDs were within guidelines for analysis of chlorpropham in soil at the LOQ and 10×LOQ (p. 10; Table I, p. 28). Analytes were identified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The soil matrix was provided by the sponsor and was the same as that used in the ECM (pp. 15, 27). The number of trials was not specifically reported; the reviewer assumed that the method was validated with the first trial.

Table 2. Initial Validation Method Recoveries for Chlorpropham in Soil*

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) ¹
Quantitation Ion						
Chlorpropham <i>m/z</i> 214→172	0.05 (LOQ)	5	93.4-102	97.7	3.13	3.20
	0.5	5	99.6-103	101	1.38	1.37
Confirmation Ion						
Chlorpropham <i>m/z</i> 214→154	0.05 (LOQ)	5	89.6-99.6	93.2	3.79	4.07
	0.5	5	97.6-106	102	3.01	2.95

Data (uncorrected recovery results, p. 15) were obtained from Tables 2-3, pp. 19-20 of MRID 49373403.

* The soil was sandy loam (USDA textural class) which was fully characterized by Agvise Laboratories, Northwood, North Dakota (p. 11; Appendix 4, p. 45).

Table 3. Independent Validation Method Recoveries for Chlorpropham in Soil*

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) ¹
Quantitation Ion						
Chlorpropham <i>m/z</i> 214→172	0.05 (LOQ)	5	87-100	93	5	5
	0.5	5	83-102	89	7	8
Confirmation Ion						
Chlorpropham <i>m/z</i> 214→154	0.05 (LOQ)	5	88-99	91	5	5
	0.5	5	87-99	94	4	4

Data (uncorrected recovery results, pp. 21-22; Appendix C, pp. 48-49) were obtained from Table I, p. 28 of MRID 49457204.

* The soil was provided by the sponsor and was the same as that used in the ECM (pp. 15, 27).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD were 0.0500 mg/kg (0.05 ppm) and 0.015 mg/kg (0.015 ppm), respectively (p. 14 of MRID 49373403; p. 22 of MRID 49457204). The LOQ was defined as the lowest fortification level which was validated by the analytical method. No calculation was provided for the LOQ. No comparison was made to chromatogram background levels. The LOD was calculated as the product of the lowest calibration standard, 0.200 µg/L, and dilution factor of the matrix blank samples (75.0).

Table 4. Method Characteristics in Soil

		Chlorpropham
Limit of Quantitation (LOQ)		0.0500 mg/kg
Limit of Detection (LOD)		0.015 mg/kg
Linearity (calibration curve r^2 and concentration range) ¹	ECM:	$r^2 = 0.9995$ (quantitative ion) ²
	ILV:	$r^2 = 0.9996$ (quantitative and confirmative ions)
	Range:	0.200-10.0 ng/mL
Repeatable		Yes at LOQ and 10x LOQ (sandy loam soil)
Reproducible		Yes at LOQ and 10x LOQ (sandy loam soil) ³
Specific	ECM:	Yes; interferences at the analyte retention times were $\leq 30\%$ of the LOQ (<LOD). No interferences were observed.
	ILV:	Yes; interferences at the analyte retention times were $\leq 20\%$ (based on peak height) of the LOQ. A small peak in the reagent blank which eluted at the same retention time as chlorpropham was observed, but it represented <LOD. ⁴

Data were obtained from pp. 11, 14; Tables 2-3, pp. 19-20; Figure 2, p. 22; Figures 5-10, pp. 25-30 of MRID 49373403; pp. 15, 22, 25; Tables I-II, pp. 28-29; Figures 4-5, pp. 33-35; Figures 7-10, pp. 37-40 of MRID 49457204; DER Attachment 2.

- 1 The reviewer calculated ECM and ILV coefficient of determination (r^2) values from the provided r values (DER Attachment 2).
- 2 One calibration curve was also provided; the reviewer assumed that the calibration curve corresponded to the quantitative ion since the representative calibration LC/MS/MS spectra were for the quantitative ion (Figures 2-4, pp. 22-24).
- 3 The soil matrix of the ILV was the same soil sample which was used in the ECM (pp. 15, 27 of MRID 49457204).
- 4 The provided representative chromatograms of the ILV were not good quality. The chromatogram spectra could be clearly seen, including baseline; however, all reported data (numbers and letters) were of poor resolution and could not be read. The reviewer noted the position of the decimal place in the peak area values of chromatograms to compare the area of the interferences in the controls versus the LOQ analyte peak area.

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. No justification or calculation was provided for the LOQ. No comparison was made to chromatogram background levels. The LOD was reported in the ECM based on the lowest concentration standard. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.
2. The same sandy loam soil (5.6% organic matter, 9% clay) was used in the ECM and ILV; therefore, the registrant failed to select the most difficult analytical sample condition to analyze to demonstrate how well the method performs. The ECM study authors reported that the soil was chosen to best represent the soil type for the region of the potential terrestrial field dissipation study.
3. ILV modifications of the ECM included the agitation techniques/equipment, the centrifuge speed and time, the filter equipment and LC/MS/MS equipment (the LC

column and guard column; pp. 14, 17-19, 23; Figure 1, p. 30 of MRID 49457204). None of these modifications was considered significant and had any impact on the outcome of the study.

4. In the ILV, chromatograms were provided for one of the calibration standards (0.200 µg/L), solvent blank, reagent blank, matrix blank, LOQ and 10×LOQ for each ion measured (Figures 5-10, pp. 35-40 of MRID 49457204). Calibration curves and full LC/MS and MS/MS product ion spectra of chlorpropham were also included (Figures 2-4, pp. 31-34).

In the ECM, chromatograms were provided for two of the calibration standards (0.200 µg/L and 10.0 µg/L), reagent blank and matrix blank for the quantitative ion only (spectra for the confirmation ion were not included; Figures 3-10, pp. 23-30 of MRID 49373403). Representative chromatograms were provided for LOQ and 10×LOQ for each ion measured. One calibration curve was also provided; the reviewer assumed that the calibration curve corresponded to the quantitative ion since the representative calibration LC/MS/MS spectra were for the quantitative ion (Figures 2-4, pp. 22-24). The calibration curve for the confirmation ion should have been provided since percent recovery of the confirmation ion was presented in the study report. However, a confirmatory method is typically not required where GC/MS and LC/MS methods are used as the primary method.

5. In the calculations of the ILV, procedural recoveries were corrected for residues found in the controls; however, the raw data showed that no residues were quantified in the controls (pp. 21-22; Appendix C, pp. 48-49 of MRID 49457204).
6. The reviewer noted the following typographical error in the ILV: the mobile phase A of the LC conditions was reported as “0.1% formic acid in HPLC grade **Soil**” versus “0.1% formic acid in HPLC grade **Water**” (p. 18 of MRID 49457204).

The reviewer noted an additional typographical error in the ILV: LC column was reported as ACE Excel 2 C18-AR column (50 mm x **2.1** mm, 2 µm) on p. 18 and ACE Excel 2 C18-AR column (50 mm x **2.0** mm, 2 µm) on p. 23 (MRID 49457204). The equipment list on p. 14 did not list the LC column so the reviewer was unsure which LC column description was correct.

7. The ECM study authors noted that the calibration standard solutions must be refrigerated (Figure 1, p. 21 of MRID 49373403).
8. The communication with the sponsor or originating laboratory was not reported or discussed in the ILV.
9. The test material of the study was PIN-NIP[®], 98% chlorpropham (pp. 10-11 of MRID 49373403). The fortification rates of the fortification solutions were based on chlorpropham (corrected for purity).

10. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in *ca.* 5 hours (*ca.* 3.5 hours for sample preparation/extraction and *ca.* 1.5 hours for analysis; p. 22 of MRID 49457204). These time requirements do not include preparation of calibrants and fortification solutions.

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**Chlorpropham**

IUPAC Name: Isopropyl 3-chlorocarbanilate
CAS Name: 1-Methylethyl N-(3-chlorophenyl)carbamate
1-Methylethyl (3-chlorophenyl)carbamate
CAS Number: 101-21-3
SMILES String: CC(C)OC(=O)Nc1cccc(Cl)c1

