

**Test Material:** Chlorpropham

**MRID:** 49642402

**Title:** Validation of an Analytical Method for the Determination of Chlorpropham in Drinking and Surface Water

**MRID:** 49642401

**Title:** Independent Lab Validation of the Analytical Method for the Determination of Chlorpropham Residues in Drinking and Surface Water

**EPA PC Code:** 018301

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lisa Muto

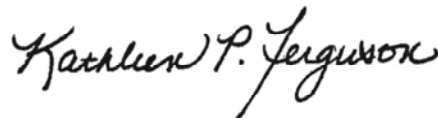
**Signature:**



**Date:** 1/6/16

**Secondary Reviewer:** Kathleen Ferguson

**Signature:**



**Date:** 1/6/16

**QC/QA Manager:** Joan Gaidos

**Signature:**



**Date:** 1/6/16

**Analytical method for chlorpropham in water**

- Reports:** ECM: EPA MRID No. 49642402. Stanislawski, T. 2014. Validation of an Analytical Method for the Determination of Chlorpropham in Drinking and Surface Water. PTRL Europe ID: P 3187 G. Report prepared by PTRL Europe, Ulm, Germany; sponsored by United Phosphorus Ltd, Warrington, United Kingdom; and Certis Europe, Brussel/Bruxelles, Belgium; and sponsored/submitted by Aceto Agricultural Chemicals Corporation, Port Washington, New York; 38 pages. Final report issued May 16, 2014. ILV: EPA MRID No. 49642401. Schlewitz, P. 2015. Independent Lab Validation of the Analytical Method for the Determination of Chlorpropham Residues in Drinking and Surface Water. Report No.: B4032; Final Report No.: R B4032. Report prepared by ANADIAG, Haguenau, France; sponsored by United Phosphorus Ltd, Warrington, United Kingdom; and Certis Europe, Brussel/Bruxelles, Belgium; and sponsored/submitted by Aceto Agricultural Chemicals Corporation, Port Washington, New York; 34 pages. Final report issued January 14, 2015.
- Document No.:** MRIDs 49642402 & 49642401
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with the German Good Laboratory Practices (GLP), which are based on the OECD Principles of GLP which are accepted by European, US FDA, US EPA and Japanese Regulation Authorities, as well as in accordance with EC Guidance documents (SANCO/825/00 and SANCO/3029/99; pp. 3, 5; Appendix 4, p. 38 of MRID 49642402). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5; Appendix 4, p. 38).  
ILV: The study was conducted in accordance with the OECD GLP, Article D523-8 (2007), EC Commission Directive 2004/10/EC (2004), EC Regulation 1107/2009, EU Regulation 284/2013, SANCO/825/00 and SANCO/3029/99, and EC Guidance documents (ENV/JM/MONO(2007)17; p. 3; Appendix VI, p. 34 of MRID 49642401). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix VI, p. 34). An authenticity statement was included with the QA statement.
- Classification:** This analytical method is classified as invalid. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. In the ILV, the specificity of the method was not validated by the representative chromatograms due to significant residues in the controls. In the ECM, chromatograms were not provided for 10×LOQ. The ILV drinking water matrix was not characterized. The number of trials was not reported in the ILV. Linearity in ILV was not satisfactory for the confirmation ion. Reagent blanks were not included in the ECM or ILV.

**PC Code:** 018301  
**Reviewer:** Karen Milians, Chemist

**Signature:**  
**Date:**

### Executive Summary

This analytical method, PTRL Europe ID P 3187 G, is designed for the quantitative determination of chlorpropham in drinking and surface water at the LOQ of 0.10 µg/L 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 water. The reviewer assumed that the method was validated by the ILV with the first trial using uncharacterized drinking water with minor modifications to the analytical method. The specificity of the method was not validated by the representative ILV chromatograms due to significant residues (*ca.* 0-30% of LOQ) in the quantification and confirmation ions of the drinking water control matrices. In the ILV, confirmation ion recoveries were only evaluated for the LOQ fortification level. Additionally, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. In the ECM, representative chromatograms were not provided for 10×LOQ.

**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						
Chlorpropham	49642402	49642401		Water <sup>1,2</sup>	16/05/2014	Aceto Agricultural Chemicals Corporation	LC/MS/MS	0.10 µg/L

1 In the ECM, drinking water (pH 7.23, total organic carbon 0.75 mg/L) was obtained from Ulm, Southern Germany. The surface water (pH 8.16, total organic carbon 1.9 mg/L) was obtained from River Danube in Ulm, Southern Germany (p. 12 of MRID 49642402).

2 In the ILV, drinking water was tap water obtained from the performing laboratory (ANADIAG; not characterized; p. 11 of MRID 49642401).

## I. Principle of the Method

Samples (1.0 mL) were dosed into an autosampler vial and fortified, as necessary, with 10  $\mu$ L of corresponding fortification solution (p. 14 of MRID 49642402). The sample was mixed with 10  $\mu$ L of 10% formic acid in water using a vortex machine. The sample was analyzed directly by HPLC.

Samples were analyzed for chlorpropham using an Agilent 1290 Infinity HPLC system coupled to an Applied Biosystems MDS Sciex API5500 triple quadrupole mass spectrometer with TurboIonSpray (ESI) source (pp. 14-15 of MRID 49642402). The HPLC/MS/MS conditions consisted of a Supelco Ascentis Express C<sub>18</sub> column (2.1 x 50 mm, 2.7- $\mu$ m, column temperature 35°C), a mobile phase gradient of (A) water:formic acid (99.9:0.1, v:v) and (B) methanol:formic acid (99.9:0.1, v:v) [percent A:B (v:v) at 0.00-2.00 min. 80:20, 2.1-5.00 min. 0:100, 5.10-8.00 min. 80:20], and MS/MS detection in TurboIon spray ESI positive ion mode with Multiple Reaction Monitoring (MRM). Two parent-daughter ion transitions were monitored (quantitative and confirmatory, respectively):  $m/z$  214  $\rightarrow$  172 and  $m/z$  214  $\rightarrow$  126. Injection volume was 40  $\mu$ L. Retention time was *ca.* 3.3 minutes for chlorpropham.

In the ILV, samples for SL-573 were prepared using the same method as the ECM, except that different analytical instruments and parameters were used (pp. 9, 12, 14-15 of MRID 49642401). Samples were analyzed using a XEVO-TQMS LC/MS/MS system. The injection volume was 90  $\mu$ L. Other instrumental conditions were equivalent of those in the ECM. The two monitored parent-daughter ion transitions were the same as the ECM. Retention time was *ca.* 3.2 minutes. These minor modifications had no impact on the outcome of the study.

In the ECM and ILV, the LOQ and LOD were reported as 0.10  $\mu$ g/L and 0.03  $\mu$ g/L, respectively (pp. 9-10 of MRID 49642402; p. 9, 16 of MRID 49642401).

## II. Recovery Findings

ECM (MRID 49642402): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of chlorpropham in drinking and surface water matrices at the LOQ (0.10  $\mu\text{g/L}$ ) and 10 $\times$ LOQ (1.0  $\mu\text{g/L}$ ; uncorrected recovery results; Tables 1-2, pp. 20-21; DER Attachment 2). Quantitation and confirmation ion recoveries were comparable. Percent recoveries, means, and standard deviations were reviewer-calculated based on the calibration equations (Figure 2, p. 26 and Figure 4, p. 28) because only peak area counts were reported in the study. The means were calculated from the mean peak area counts reported in the study report and verified by the reviewer. The water matrices were fully characterized (p. 12). The drinking water (pH 7.23, total organic carbon 0.75 mg/L) was obtained from Ulm, Southern Germany. The surface water (pH 8.16, total organic carbon 1.9 mg/L) was obtained from River Danube in Ulm, Southern Germany.

ILV (MRID 49642401): Mean recoveries and RSDs were within guidelines for analysis of chlorpropham in drinking water matrix at the LOQ (0.10  $\mu\text{g/L}$ ) and 10 $\times$ LOQ (1.0  $\mu\text{g/L}$ ; uncorrected recovery results; pp. 9, 17, 19; Appendix II, pp. 23-24). Quantitation and confirmation ion recoveries were fairly comparable at the LOQ; only quantitation ion results were provided for 10 $\times$ LOQ. The method was unaltered from that of the ECM, except that different analytical instruments and parameters were used (pp. 9, 12, 14-15). The reviewer assumed that the method was validated with the first trial (p. 19). The drinking water was tap water obtained from the performing laboratory (ANADIAG; not characterized; p. 11).

**Table 2. Initial Validation Method Recoveries for Chlorpropham in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking (Tap) Water</b>						
Quantification ion						
Chlorpropham	0.10 (LOQ)	5	77-83	80	3	2.9
	1.0	5	82-84	83	1	0.9
Confirmation ion						
Chlorpropham	0.10 (LOQ)	5	76-82	80	2	2.9
	1.0	5	80-82	81	1	0.9
<b>Surface (River) Water</b>						
Quantification ion						
Chlorpropham	0.10 (LOQ)	5	80-85	83	2	2.7
	1.0	5	79-81	80	1	1.1
Confirmation ion						
Chlorpropham	0.10 (LOQ)	5	82-85	83	1	1.6
	1.0	5	79-80	80	1	0.9

Data (uncorrected recovery results, Tables 1-2, pp. 20-21) were obtained from Tables 1-2, pp. 20-21 of MRID 49642402 and DER Attachment 2. Percent recoveries, means, and standard deviations were reviewer-calculated based on the calibration equations (Figure 2, p. 26 and Figure 4, p. 28) because only peak area counts were reported in the study. The means were calculated from the mean peak area counts reported in the study report and verified by the reviewer.

- Two parent-daughter ion transitions were monitored (quantitative and confirmatory, respectively):  $m/z$  214 → 172 and  $m/z$  214 → 126.
- The water matrices were fully characterized (p. 12). The drinking water (pH 7.23, total organic carbon 0.75 mg/L) was obtained from Ulm, Southern Germany. The surface water (pH 8.16, total organic carbon 1.9 mg/L) was obtained from River Danube in Ulm, Southern Germany.

**Table 3. Independent Validation Method Recoveries for Chlorpropham in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking (Tap) Water</b>						
Quantification ion						
Chlorpropham	0.10 (LOQ)	5	63.4-87.1	75.6	9.2	12.2
	1.0	5	71.9-104.8	89.5	14.0	15.6
Confirmation ion						
Chlorpropham	0.10 (LOQ)	5	81.2-103.0	85.7	11.9	13.8
	1.0	5	Not performed*			

Data (uncorrected recovery results, Appendix II, pp. 23-24) were obtained from pp. 9, 17, 19 of MRID 49642401.

\* Confirmation ion results were only provided for the LOQ fortification level.

- Two parent-daughter ion transitions were monitored (quantitative and confirmatory, respectively):  $m/z$  214 → 172 and  $m/z$  214 → 126.
- The drinking water was tap water obtained from the performing laboratory (ANADIAG; not characterized; p. 11).

### III. Method Characteristics

In the ECM and ILV, the LOQ was reported as 0.10 µg/L (pp. 9-10, 17 of MRID 49642402; p. 9, 16 of MRID 49642401). No justification or calculation to support the LOQ was provided in the ECM. In the ILV, the LOQ was defined as the lowest fortification level with acceptable recoveries (mean recoveries of 70-120% and RSDs of ≤20%). In the ECM and ILV, the LOD was reported as 0.03 µg/L. In the ECM, the LOD was defined as 30% of the LOQ. In the ILV, the LOD was defined as the lowest measureable standard concentration estimated at three times the background noise with the experimental analytical conditions.

**Table 4. Method Characteristics in Water**

		<b>Chlorpropham</b>
Limit of Quantitation (LOQ)		0.10 µg/L
Limit of Detection (LOD)	ECM:	0.03 µg/L (calculated using the lowest calibration standard, 0.2 ng/mL)
	ILV:	0.00333 µg/L (equivalent to 0.2 ng/mL)
Linearity (calibration curve $r^2$ and concentration range)	ECM <sup>1</sup> :	$r^2 = 0.9996-0.9998$ (Q & C) 0.03-10 ng/mL
	ILV <sup>2</sup> :	$r^2 = 0.9997$ (Q) $r^2 = \mathbf{0.9939}$ (C) 0.03-10 ng/mL
Repeatable	ECM <sup>3</sup> :	Yes at LOQ and 10×LOQ (n = 5). (characterized drinking and surface water matrices)
	ILV <sup>4</sup> :	Yes at LOQ and 10×LOQ (n = 5). (uncharacterized drinking water matrix)
Reproducible		Yes at LOQ and 10×LOQ. (confirmation ion not monitored at 10×LOQ in ILV) <sup>5</sup>
Specific	ECM:	Representative chromatograms were not provided for 10×LOQ. Minor residues (<5% of LOQ) at the analyte retention time, as well as insignificant baseline noise, were observed in both water control matrices (quantification and confirmation ions).
	ILV:	Significant residues (ca. 25-30% of LOQ) were observed in the quantification ion of the drinking water control matrices. <sup>6</sup> Significant residues (ca. 0-30% of LOQ) were observed in the confirmation ion of the drinking water control matrices. <sup>6</sup> However, the detailed validation data reported no residues in the controls, so there was no recovery data correction.

Data were obtained from pp. 9-10, 17; Tables 1-2, pp. 20-21; Figure 2, p. 26; Figure 4, p. 28; Figures 6-10, pp. 30-34 of MRID 49642402; p. 9, 16-17, 19; Appendix II, pp. 23-24; Appendices IV-V, pp. 29-33 of MRID 49642401; DER Attachment 2. Q = quantitation ion; C = confirmation ion.

1 ECM standard curves were weighted 1/x. ECM  $r^2$  values are reviewer-generated for chlorpropham from reported  $r$  values of 0.9998-0.9999 (Q & C; calculated from data in Figure 2, p. 26 and Figure 4, p. 28 of MRID 49642402; see DER Attachment 2).

2 ILV standard curves were weighted 1/x. ILV  $r^2$  values are reviewer-generated for chlorpropham from reported  $r$  values of 0.99986 (Q) and 0.99697 (C; calculated from data in Appendix V, pp. 32-33 of MRID 49642401; see DER Attachment 2).

3 In the ECM, drinking water (pH 7.23, total organic carbon 0.75 mg/L) was obtained from Ulm, Southern Germany. The surface water (pH 8.16, total organic carbon 1.9 mg/L) was obtained from River Danube in Ulm, Southern Germany (p. 12 of MRID 49642402).

4 In the ILV, drinking water was tap water obtained from the performing laboratory (ANADIAG; not characterized; p. 11 of MRID 49642401).

5 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.

6 Based on area count data presented in Appendix II, pp. 23-24 and Appendix IV, pp. 30-31 of MRID 49642401. Linearity is satisfactory when  $r^2 \geq 0.995$ .

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

1. The estimations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. In the ILV, the LOQ was defined as the lowest fortification level with acceptable recoveries (mean recoveries of 70-120% and RSDs of  $\leq 20\%$ ; pp. 9-10, 17 of MRID 49642402; p. 9, 16 of MRID 49642401). No justification or calculation to support the LOQ was provided in the ECM. In the ECM, the LOD was defined as 30% of the LOQ. In the ILV, the LOD was defined as the lowest measureable standard concentration estimated at three times the background noise with the experimental analytical conditions. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological levels of concern in water were not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.
2. In the ILV, the specificity of the method was not validated by the representative chromatograms. Significant residues (*ca.* 25-30% of LOQ) were observed in the quantification ion of the drinking water control matrices. Significant residues (*ca.* 0-30% of LOQ) were observed in the confirmation ion of the drinking water control matrices (Appendix II, pp. 23-24 and Appendix IV, pp. 30-31 of MRID 49642401). However, the detailed validation data reported no residues in the controls, so there was no recovery data correction (Appendix II, pp. 23-24).

In the ECM, chromatograms were not provided for  $10 \times \text{LOQ}$ . OCSPP guidelines recommend that representative chromatograms are provided for reagent blanks, matrix blanks, standard curves, and spiked samples at the LOQ and  $10 \times \text{LOQ}$  for all analytes in each matrix. Representative chromatograms of the reagent blank were not included in ECM or ILV; a reagent blank was not included (Tables 1-2, pp. 2-21 of MRID 49642402; Appendix II, pp. 23-24 of MRID 49642401).

3. The ILV drinking water matrix was not characterized (p. 11 of MRID 49642401). The source was reported. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.
4. The linearity in ILV was not satisfactory for the confirmation ion calibration curve ( $r^2 = 0.9939$ ; Appendix V, pp. 32-33 of MRID 49642401; DER Attachment 2).
5. The number of trials was not reported in the ILV. The reviewer assumed that the method was validated with the first trial (p. 19 of MRID 49642401).



6. The confirmation ion not monitored at 10×LOQ in ILV; however, 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 (Appendix II, pp. 23-24; pp. 9, 17, 19 of MRID 49642401).
7. The communication with the sponsor was not reported in the ILV.
8. Matrix effects were studied and found to be insignificant in the ECM and ILV (p. 14; Table 3, p. 22 of MRID 49642402; p. 21 of MRID 49642401). Matrix-matched standards were not used in the ECM; matrix-matched standards were not used in the ILV.
9. In the ECM, the calibration solutions, stock solutions and sample extracts were stored in a refrigerator (temperature not reported) for up to 6 days, 11 days and 5 days, respectively (p. 25; Tables 4-5, pp. 23-24 of MRID 49642402). In the ILV, the stock solutions and sample extracts were stored in a refrigerator (4°C) for up to 14 days and 18 days, respectively (p. 20 of MRID 49642402).
10. The minor ILV modifications of the ECM had no effect on the outcome of the study. A few different analytical instruments and parameters were used (pp. 9, 12, 14-15 of MRID 49642401). Samples were analyzed using a XEVO-TQMS LC/MS/MS system. The injection volume was 90 µL. Other instrumental conditions were equivalent of those in the ECM. These minor modifications had no impact on the outcome of the study.
11. The time requirement for completion of the method was not reported in the ILV. The time requirement for completion of the method was reported as *ca.* 1 calendar day in the ECM (p. 18 of MRID 49642402).

## 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  
**CAS Number:** 101-21-3  
**SMILES String:** CC(C)OC(=O)Nc1cccc(Cl)c1

