

**Analytical method for pethoxamid in water**

**Reports:** ECM: EPA MRID No. 49813522. Beck, I.C. 2008. Validation of Analytical Method(s) for the Confirmation of Residues of Pethoxamid in Soil and in Water. PTRL Study No.: P 1578 G. Report prepared by PTRL Europe GmbH, Ulm, Germany, and sponsored and submitted by Cheminova, Inc., Arlington, Virginia; 28 pages. Final report issued October 8, 2008.

ILV: EPA MRID No. 49813523. Tessier, V. 2013. Pethoxamid – Independent Laboratory Validation in Drinking Water. Eurofins Agroscience Services Chem SAS Study No.: S13-04101. Report prepared by Eurofins Agroscience Services Chem SAS, Vergèze, France, sponsored by Cheminova, A/S, Lemvig, Denmark, and submitted by Cheminova, Inc., Arlington, Virginia; 38 pages. Final report issued October 31, 2013.

**Document No.:** MRIDs 49813522 & 49813523

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with German Good Laboratory Practices (GLP) which are based on OECD GLP which are accepted by Regulatory Authorities throughout Europe, the USA (FDA and EPA, FIFRA (40 CFR Part 160)), and Japan (MHW, MAFF, and METI; pp. 3-6 of MRID 49813522). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-6).  
ILV: The study was conducted in accordance with OECD GLP standards (p. 3 of MRID 49813523). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement. The GLP Certificate of the Test Facility was also included (Appendix 3, p. 38).

**Classification:** This analytical method is classified as supplemental but upgradable. However, the registrant is encouraged to submit calculations for the limit of quantitation and limit of detection, any communications conducted with the registrant, blank measurements and other deficiencies found on page 9 of this document. Some of the deficiencies listed are: the reported method LOQ were not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest concentration tested in the method validation (LLMV) rather than an instrument response with high precision and accuracy, blank measurements. Only drinking water was included in the ILV validation, and the number of ILV trials required to validate the method was not reported. The ILV communications were not included in the study report.

**PC Code:** 090208

**EFED Final Reviewer:** Karen Milians, Ph.D., Chemist

Signature:  
Date:



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**CDM/CSS-Dynamac JV** Lisa Muto, M.S.,  
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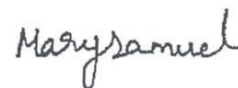
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08/13/2020

**Reviewers:**Mary Samuel, M.S.,  
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08/13/2020

EPA Reviewer: Katrina White, Ph.D., Senior Scientist

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

**Executive Summary**

This analytical method, PTRL Study No. P 1578 G, is designed for the quantitative determination of the pethoxamid in water at the LOQ of 0.1 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water (no observed adverse effect level (NOAEC) of 1.2 µg/L). Based on the performance data submitted by the ILV and ECM, the lowest level of method validation (LLMV) was equivalent to the reported method LOQ for pethoxamid in water. The ECM used two characterized water matrices, surface and drinking water, but the ILV only used one characterized drinking water matrix. The ILV validated the method in drinking water as written with insignificant modifications of analytical instrumentation and equipment. The number of ILV trials required to validate the method was not reported, but the reviewer assumed that the ILV validated the method in the first trial due to the lack of method deviations or issues. The ILV communications were not included in the study report, but the performing laboratories and personnel differed between the ECM and ILV. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for pethoxamid in the tested water matrices. The LOD was not reported in the ILV.

Analytical method PTRL Study No. P 1578 G also included a method for the analysis of pethoxamid in soil, but no accompanying ILV for the soil method was submitted for review and the soil method was not included in this review.

**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						
Pethoxamid	49813522 <sup>1</sup>	49813523 <sup>2</sup>	Karen Milians	Water	08/10/2008 <sup>3</sup>	Cheminova, Inc.	LC/MS/MS	0.1 µg/L

<sup>1</sup> In the ECM, the drinking (tap) water (pH 7.47, total hardness 2.2 mmol/L equivalent to 12 °dH) obtained from a tap at PTRL and surface (river) water (pH 7.8, total hardness 2.5 mmol/L equivalent to 14 °dH, total organic carbon 13 mg/L, dissolved organic carbon 4.0 mg/L) collected from the Danube River near Ulm, Germany were used in the study (pp. 11-12 of MRID 49813522). The surface water characterization performed by Institut Alpha in Ulm, Germany.

- 2 In the ILV, the drinking water (N°291; pH 6.9, total hardness 8.0°F, dissolved organic carbon <0.4 mg/L) was collected from Vergèze, France (p. 11 of MRID 49813523). The water characterization performed by Eurofins Institut Pasteur Lille in Montpellier, France (non-GLP facility, COFRAC certified).
- 3 ECM method was taken from Todd, M.A. August 17, 2000. TKC-94 - The Development and Validation of Methods of Analysis for the Determination of Residues in Soil and Water. Huntingdon Life Sciences Project ID TON 025 (pp. 7, 11 of MRID 49813522).

## I. Principle of the Method

Water samples (100 mL) were fortified with either 1.0 mL of the 10 ng/mL fortification solution or 0.10 mL of the 1000 ng/mL fortification solution, as necessary (pp. 11-14 of MRID 49813522). The samples were applied to a Varian Bond-Elut C<sub>18</sub> solid phase extraction (SPE) cartridge (500 mg, 6 mL) preconditioned with 3 mL of acetonitrile and 3 mL of water. After drawing the sample through dropwise, the cartridges were washed with 20 mL of deionized water then 3 mL of acetonitrile:water (1:1, v:v). The analyte was eluted with 2.5 mL of acetonitrile. An aliquot was transferred to an autosampler vial for analysis by LC/MS/MS.

Samples were analyzed using an Agilent 1100 HPLC series coupled to an Applied Biosystems MDS Sciex API 3000 Triple Quadrupole MS (pp. 14-15 of MRID 49813522). The following LC conditions were used: Thermo Aquasil C<sub>18</sub> (3.0 mm x 150 mm column; 3 µm particle size; column temperature 35°C), Thermo Aquasil Gold pre-column (3.0 mm x 10 mm column; 5 µm particle size), mobile phase of (A) 0.1% acetic acid and 10mM ammonium acetate in water:acetonitrile (80:20, v:v) and (B) 0.1% acetic acid and 10mM ammonium acetate in acetonitrile:water (80:20, v:v) [percent A:B (v:v) at 0.00 min. 30:70, 3.00-9.00 min. 0:100, 9.10-12.00 min. 30:70], and injection volume of 20 µL. The following MS/MS conditions were used: positive mode (source temperature 450°C), electrospray ionization (ESI) interface, and multiple reaction monitoring (MRM). Pethoxamid was identified using two ion pair transitions (quantitation and confirmation, respectively):  $m/z$  296.1→131.1 and  $m/z$  296.1→250.1. Expected retention time was *ca.* 8.5 minutes.

The independent laboratory performed the ECM (reported as method AGR/MOA/PTX-9 in the ILV) as written, except for insignificant modifications of analytical instrumentation and equipment (pp. 10, 12-16 of MRID 49813523). The same SPE cartridges were used. A Sciex API 4000 MS coupled with a Shimadzu LC20AD HPLC was used. All LC and MS parameters were similar to those of the ECM, except that the injection volume was 10 µL. Pethoxamid was identified with the same two ion pair transitions. Expected retention time was *ca.* 7.23 minutes.

The Limit of Quantification (LOQ) for pethoxamid was reported as 0.1 µg/L in water in the ECM and ILV (pp. 7, 17-19 of MRID 49813522; pp. 8, 18 of MRID 49813523). The Limit of Detection (LOD) for pethoxamid in water was reported as 0.02 µg/L in the ECM, but the LOD was not reported in the ILV. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than a value measured with accuracy and precision.

## II. Recovery Findings

ECM (MRID 49813522): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of pethoxamid in two water matrices at the LOQ (0.1  $\mu\text{g/L}$ ) and 10 $\times$ LOQ (1.0  $\mu\text{g/L}$ ; p. 7; Tables 2-3, pp. 21-22). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable. RSDs of the LOQ fortification in drinking water were 4xs to 6xs the RSDs of all other fortifications/matrices. Drinking (tap) water (pH 7.47, total hardness 2.2 mmol/L equivalent to 12 $^{\circ}$ dH) obtained from a tap at PTRL and surface (river) water (pH 7.8, total hardness 2.5 mmol/L equivalent to 14 $^{\circ}$ dH, total organic carbon 13 mg/L, dissolved organic carbon 4.0 mg/L) collected from the Danube River near Ulm, Germany were used in the study (pp. 11-12). The surface water characterization performed by Institut Alpha in Ulm, Germany.

ILV (MRID 49813523): Mean recoveries and RSDs met requirements for analysis of pethoxamid in one water matrix at the LOQ (0.1  $\mu\text{g/L}$ ) and 10 $\times$ LOQ (1.0  $\mu\text{g/L}$ ; p. 19). Two ion transitions were quantified; recovery results of the quantitative and confirmatory ion transitions were comparable. The drinking water (N $^{\circ}$ 291; pH 6.9, total hardness 8.0 $^{\circ}$ F, dissolved organic carbon  $<$ 0.4 mg/L) was collected from Vergèze, France (p. 11). The water characterization performed by Eurofins Institut Pasteur Lille in Montpellier, France (non-GLP facility, COFRAC certified). The method was validated by the ILV in one water matrix as written with insignificant modifications of analytical instrumentation and equipment (pp. 8-10, 12-16). The number of ILV trials required to validate the method was not reported, but the reviewer assumed that the ILV validated the method in the first trial due to the lack of method deviations or issues.

**Table 2. Initial Validation Method Recoveries for Pethoxamid 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>						
Quantitation ion transition						
Pethoxamid	0.10 (LOQ)	5	88-119	108	13	12
	1.0	5	89-95	92	2	2
Confirmation ion transition						
Pethoxamid	0.10 (LOQ)	5	91-115	107	12	12
	1.0	5	87-94	91	3	3
<b>Surface (River) Water</b>						
Quantitation ion transition						
Pethoxamid	0.10 (LOQ)	5	103-112	105	4	4
	1.0	5	103-109	105	2	2
Confirmation ion transition						
Pethoxamid	0.10 (LOQ)	5	105-114	108	4	4
	1.0	5	103-107	105	2	2

Data (uncorrected recovery results; pp. 16-17) were obtained from p. 7; Tables 2-3, pp. 21-22 of MRID 49813522. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Pethoxamid was identified using two ion pair transitions (quantitation and confirmation, respectively):  $m/z$  296.1→131.1 and  $m/z$  296.1→250.1.

2 The drinking (tap) water (pH 7.47, total hardness 2.2 mmol/L equivalent to 12°dH) obtained from a tap at PTRL and surface (river) water (pH 7.8, total hardness 2.5 mmol/L equivalent to 14°dH, total organic carbon 13 mg/L, dissolved organic carbon 4.0 mg/L) collected from the Danube River near Ulm, Germany were used in the study (pp. 11-12). The surface water characterization performed by Institut Alpha in Ulm, Germany.

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

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>3</sup>	Relative Standard Deviation (%)
<b>Drinking Water</b>						
Quantitation ion transition						
Pethoxamid	0.10 (LOQ)	5	76-94	89	8	8
	1.0	5	93-97	96	2	2
Confirmation ion transition						
Pethoxamid	0.10 (LOQ)	5	77-95	89	7	8
	1.0	5	91-97	95	2	3

Data (uncorrected recovery results; p. 17) were obtained from p. 19 of MRID 49813523 and DER Attachment 2. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 Pethoxamid was identified using two ion pair transitions (quantitation and confirmation, respectively):  $m/z$  296.1→131.1 and  $m/z$  296.1→250.1. These were the same as those in the ECM.

2 The drinking water (N°291; pH 6.9, total hardness 8.0°F, dissolved organic carbon <0.4 mg/L) was collected from Vergèze, France (p. 11). The water characterization performed by Eurofins Institut Pasteur Lille in Montpellier, France (non-GLP facility, COFRAC certified).

3 Standard deviations were not reported in the study but were reviewer-calculated based on recovery results (DER Attachment 2). Rules of significant figures were followed.

### III. Method Characteristics

The LOQ for pethoxamid was reported as 0.1 µg/L in water in the ECM and ILV (pp. 7-8, 17-19 of MRID 49813522; pp. 8, 18 of MRID 49813523). No justification of the LOQ was reported in the ECM. In the ILV, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated. No calculations to support the LOQ were reported in the ECM or ILV. The LOD for pethoxamid in water was reported as 0.02 µg/L in the ECM (20% of the LOQ), but the LOD was not reported in the ILV. No calculations or comparisons to baseline noise were reported for the LOQ or LOD in the ECM or ILV.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the LLMV rather than a calculated concentration with high precision and accuracy.



**Table 4. Method Characteristics**

		Pethoxamid
Limit of Quantitation (LOQ)*	ECM	0.1 µg/L
	ILV	
Limit of Detection (LOD)	ECM	0.02 µg/L (20% of the LOQ)
	ILV	<b>Not reported</b>
Linearity (calibration curve r and concentration range)	ECM <sup>1</sup>	r = 0.9997 (Q) r = 0.9998 (C)
		0.1-25 ng/mL
	ILV <sup>1,2</sup>	r = 0.9984 (Q) r = 0.9990 (C)
		0.05-25 ng/mL
Repeatable	ECM <sup>3</sup>	Yes for LOQ and 10×LOQ in two characterized water matrices (drinking and surface)
	ILV <sup>4,5</sup>	Yes for LOQ and 10×LOQ in one characterized drinking water matrix
Reproducible		Yes for 0.1 µg/L (LLMV)* and 1.0 µg/L
Specific	ECM	Yes, no matrix interferences were observed. Some minor baseline noise affected peak integration and attenuation at the LOQ in surface water.
	ILV	Yes, no matrix interferences were observed.

Data were obtained from pp. 7-8, 17-19 (LOQ/LOD); p. 7; Tables 2-3, pp. 21-22 (recovery data); ; p. 17; Figure 1, p. 23 (calibration curves); Figure 2, p. 24; Figures 4-5, pp. 26-27 (chromatograms) of MRID 49813522; pp. 8, 18 (LOQ/LOD); p. 19 (recovery data); p. 20; Figure 2, p. 26; Figure 8, p. 32 (calibration curves); Figures 3-13, pp. 27-37 (chromatograms) of MRID 49813523; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition.

\* Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ. The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV.

1 Solvent-based calibrations were used in the ECM and ILV (p. 13 of MRID 49813522; p. 12 of MRID 49813523).

2 Reported r values (1/x weighted) were reviewer-calculated using the r<sup>2</sup> values provided in the study report (Figure 2, p. 26; Figure 8, p. 32 of MRID 49813523; DER Attachment 2).

3 In the ECM, the drinking (tap) water (pH 7.47, total hardness 2.2 mmol/L equivalent to 12°dH) obtained from a tap at PTRL and surface (river) water (pH 7.8, total hardness 2.5 mmol/L equivalent to 14°dH, total organic carbon 13 mg/L, dissolved organic carbon 4.0 mg/L) collected from the Danube River near Ulm, Germany were used in the study (pp. 11-12 of MRID 49813522). The surface water characterization performed by Institut Alpha in Ulm, Germany.

4 In the ILV, the drinking water (N°291; pH 6.9, total hardness 8.0°F, dissolved organic carbon <0.4 mg/L) was collected from Vergèze, France (p. 11 of MRID 49813523). The water characterization performed by Eurofins Institut Pasteur Lille in Montpellier, France (non-GLP facility, COFRAC certified).

5 The ILV validated the method in one water matrix as written with insignificant modifications of analytical instrumentation and equipment (pp. 8-10, 12-16 of MRID 49813523). The number of ILV trials required to validate the method was not reported, but the reviewer assumed that the ILV validated the method in the first trial due to the lack of method deviations or issues.

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

1. The submitted ECM MRID 49813522 (Validation of Analytical Method(s) for the Confirmation of Residues of Pethoxamid in Soil and in Water) included methods for analysis of pethoxamid in water, as well as soil. Only the method for water was reported in this DER. No accompanying ILV for the soil method was submitted for review, so a DER for the soil method was not prepared.
2. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than LOQ (pp. 7-8, 17-19 of MRID 49813522; pp. 8, 18 of MRID 49813523). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for pethoxamid in water.
3. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. Only drinking water was included in the ILV validation. Both drinking water and surface water were included in the ECM validation. Ground water was not included in the ECM or ILV validations.
4. The number of ILV trials required to validate the method was not reported, but the reviewer assumed that the ILV validated the method in the first trial due to the lack of method deviations or issues (pp. 8-10, 12-16 of MRID 49813523).
5. ILV communications were not reported, summarized, or provided in the study report. The communications should be included in the study report to assess whether the ILV was performed independently from the ECM. The performing laboratories and personnel differed between the ECM and ILV (pp. 1, 6 of MRID 49813522; pp. 1, 5 of MRID 49813523).
6. No reagent blank was included in the ECM (p. 11 of MRID 49813522).
7. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 7-8, 17-19 of MRID 49813522; pp. 8, 18 of MRID 49813523). No justification of the LOQ was reported in the ECM. In the ILV, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been successfully validated. No calculations to support the LOQ were reported in the ECM or ILV. The LOD for pethoxamid in water was reported as 20% of the LOQ in the ECM, but the LOD was not reported in the ILV. No calculations or comparisons to baseline noise were reported for the LOQ or LOD in the ECM or ILV. Detection Limit should not be based on the arbitrarily selected lowest concentration in the spiked samples.

Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.



8. In the ILV, the final extracts in the drinking water matrix were determined to be stable for up to 8 days at 4°C based on recovery results of 92-99% in the quantitation ion transition analysis (pp. 9, 21 of MRID 49813523).
9. In the ILV, no significant matrix effects were observed (<20%; pp. 9, 21 of MRID 49813523). Solvent-based calibration standards were used in the ECM and ILV (p. 13 of MRID 49813522; p. 12 of MRID 49813523).
10. The time requirement for the method was not reported in the ECM or ILV.

## 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, and Revision 2; 1994 and 2016.

**Attachment 1: Chemical Names and Structures****Pethoxamid****IUPAC Name:** 2-Chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenylprop-1-enyl)acetamide**CAS Name:** 2-Chloro-*N*-(2-ethoxyethyl)-*N*-(2-methyl-1-phenyl-1-propenyl)acetamide**CAS Number:** 106700-29-2**SMILES String:** Not found