

Test Material: Naled

MRID: 48802301

Title: Naled: Validation of Analytical Methodology for the Determination of Residues in Water

MRID: 48802302

Title: NALED: Independent Laboratory Validation of the Methodology for the Determination of Residues of Naled and Dichlorvos in Water (Surface and Ground Water)

EPA PC Code: 034401

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto

Signature: 

Date: 1/14/16

Secondary Reviewer: Kathleen Ferguson

Signature: 


Date: 1/14/16

QC/QA Manager: Joan Gaidos

Signature: 

Date: 1/14/16

Analytical method for naled and dichlorvos in water

Reports:	ECM: EPA MRID No. 48802301. Brewin, S. 2012. Naled: Validation of Analytical Methodology for the Determination of Residues in Water. Huntingdon Life Sciences Project Identity: BDG0130. Report prepared by Huntingdon Life Sciences Ltd., Eye Research Centre, Suffolk, England; sponsored and submitted by AMVAC Chemical Corporation, Newport Beach, California; 93 pages. Final report issued April 5, 2012. ILV: EPA MRID No. 48802302. Pawula, M. 2012. NALED: Independent Laboratory Validation of the Methodology for the Determination of Residues of Naled and Dichlorvos in Water (Surface and Ground Water). Huntingdon Life Sciences Project Identity: BDG0074. Report prepared by Huntingdon Life Sciences Ltd., Huntingdon Research Centre, Cambridgeshire, United Kingdom; sponsored and submitted by AMVAC Chemical Corporation, Commerce, California; 75 pages. Final report issued April 16, 2012.
Document No.:	MRIDs 48802301 & 48802302
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with the UK and OECD Good Laboratory Practices (GLP), UK Department of Health and EC Commission Directive 2004/10/EC, which are accepted by USA and Japanese Regulation Authorities, with the exception that the water characterization was performed in separate, non-GLP studies (p. 3; Appendix 4, p. 93 of MRID 48802301). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix 4, p. 93). A statement of authenticity was included with the GLP statement (p. 3). ILV: The study was conducted in accordance with SANCO/825/00 and SANCO/3029/99, as well as UK, UK Department of Health and OECD GLP and EC Commission Directive 2004/10/EC (p. 3; Appendix 2, p. 75 of MRID 48802302). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4; Appendix 2, p. 75). A statement of authenticity was included with the GLP statement (p. 3).
Classification:	This analytical method is classified as supplemental. 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 for dichlorvos by the representative chromatograms due to significant residues in the controls. The ILV ground water matrix was not characterized. The number of trials was not reported in the ILV. Reagent blanks were not included in the ECM or ILV.
PC Code:	034401
Reviewer:	Ronald D. Parker, Ph.D. Senior Environmental Engineer, U.A. EPA Date: 01/11/17 

Executive Summary

This analytical method, Huntingdon Life Sciences Project BDG0130, is designed for the quantitative determination of naled and dichlorvos in ground and surface water at the LOQ of 0.1 µg/L using LC/MS/MS. **The lowest aquatic toxicity endpoint is NOAEC = 0.098 ug/L and LOAEC = 0.180 ug/L in the chronic daphnid study (MRID 42908801).** The LOQ is approximately equal to the lowest toxicological level of concern in water. The reviewer assumed that the method was validated by the ILV with the first trial using characterized surface water and uncharacterized ground water with insignificant modifications to the analytical method. The specificity of the method was not validated for dichlorvos by the representative ILV chromatograms due to significant residues (*ca.* 17-28% of LOQ) were observed in both ions of the surface water control matrix and the quantification ion of the ground water control matrix. Additionally, it could not be determined if the ILV was provided with the most difficult ground water matrix with which to assess the method for both analytes. Reagent blanks were not included in the ECM or ILV.

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						
Naled	48802301	48802302		Water ^{1,2}	05/04/2012	AMVAC Chemical Corporation	LC/MS/MS	0.1 µg/L

1 In the ECM, surface water (pH 8.07, total organic carbon 16.198 mgC/L), obtained from Diss Mere, and ground water (pH 7.62, total organic carbon 1.8 mgC/L), obtained from Anglian Water Denton Lodge Borehole 1, were used (p. 14). The water sources were not specified further.

2 In the ILV, surface water (pH 7.52, total organic carbon 6.4 mg/L), obtained from Calwich Abbey, and ground water (uncharacterized), obtained from Anglian Water Denton Lodge Borehole 1, were used (p. 14). Both water samples were obtained from the Department of Bioanalysis at Huntingdon Life Sciences.

I. Principle of the Method

Samples (10 mL) of water in 15-mL polypropylene tubes were fortified, as necessary, then mixed with 1 mL of methanol (p. 15; Appendix 3, pp. 90-91 of MRID 48802301). The sample was purified using an Oasis HLB solid phase extraction (SPE) cartridge (60-mg, 3-mL). The SPE column was pre-conditioned with methanol and water (3 mL each). The sample was applied to the column. The column was washed with 3 mL of methanol:water (10:90, v:v), and the eluate was discarded. After drying the column for about 30 seconds, the analytes were eluted with a 4.5-mL aliquot of methanol into 15-mL polypropylene tube. The volume of the sample was adjusted to 5.0 mL using methanol. An aliquot of the sample was transferred to a LC/MS/MS vial for analysis.

Samples were analyzed for naled and dichlorvos using an AB Sciex API 4000 LC/MS/MS (Appendix 3, p. 92 of MRID 48802301). The instrumental conditions consisted of an Acquity UPLC® BEH C₁₈ column (2.1 x 50 mm, 1.7- μ m; column temperature 45°C), a mobile phase of (A) methanol:water (90:10, v:v) + 0.01M ammonium formate + 0.1% formic acid and (B) methanol:formic acid (100:0.1, v:v) [percent A:B (v:v) at 0.0-0.2 min. 80:20, 2.0-2.5 min. 5:95, 3-4 min. 80:20], MS/MS detection in Ionspray positive ion mode with Multiple Reaction Monitoring (MRM), and injection volume 10 μ L. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): m/z 381 \rightarrow 127 and m/z 383 \rightarrow 127 for naled, and m/z 221 \rightarrow 127 and m/z 223 \rightarrow 127 for dichlorvos. Retention times were observed at *ca.* 1.5 and 1.3 min. for naled and dichlorvos, respectively.

The ILV was performed exactly as above using the same analytical instruments (pp. 15-17 of MRID 48802302). The study author noted that minor changes to the analytical parameters were necessary for optimization; however, the only apparent change was the specified sample temperature of “+4°C” (pp. 15, 17). The two monitored parent-daughter ion transitions were the same as the ECM. Retention times were observed at *ca.* 1.9 and 1.6 min. for naled and dichlorvos, respectively.

In the ECM and ILV, the LOQ and LOD were reported as 0.1 μ g/L and 0.025 μ g/L (0.05 ng/mL), respectively (pp. 11, 18-19; Appendix 3, p. 92 of MRID 48802301; pp. 11, 17, 21 of MRID 48802302).

II. Recovery Findings

ECM (MRID 48802301): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of naled and dichlorvos in surface and ground water matrices at the LOQ (0.1 $\mu\text{g/L}$) and $10\times$ LOQ (1 $\mu\text{g/L}$; uncorrected recovery results; p. 17; Tables 5-12, pp. 25-32; DER Attachment 2). Quantitation and confirmation ion recoveries were comparable. Standard deviations were reviewer-calculated from the data in the study report since the study author only reported means and RSDs. The water matrices were fully characterized (p. 14). The surface water (pH 8.07, total organic carbon 16.198 mgC/L) was obtained from Diss Mere. The ground water (pH 7.62, total organic carbon 1.8 mgC/L) was obtained from Anglian Water Denton Lodge Borehole 1. The water sources were not specified further.

ILV (MRID 48802302): Mean recoveries and RSDs were within guidelines for analysis of naled and dichlorvos in surface and ground water matrices at the LOQ (0.1 $\mu\text{g/L}$) and $10\times$ LOQ (1 $\mu\text{g/L}$; uncorrected recovery results; p. 18; Tables 5-12, pp. 25-32). Quantitation and confirmation ion recoveries were comparable. The reviewer assumed that the method was validated with the first trial with insignificant modifications (pp. 11, 15, 17, 22). The surface water (pH 7.52, total organic carbon 6.4 mg/L) was fully characterized and obtained from Calwich Abbey (p. 14). The ground water (uncharacterized) was obtained from Anglian Water Denton Lodge Borehole 1. Both water samples were obtained from the Department of Bioanalysis at Huntingdon Life Sciences.

Table 2. Initial Validation Method Recoveries for Naled and Dichlorvos in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantification ion						
Naled	0.1 (LOQ)	5	91-98	95	3	3.4
	1	5	90-93	92	1	1.2
Dichlorvos	0.1 (LOQ)	5	89-94	92	3	2.7
	1	5	92-98	95	2	2.3
Confirmation ion						
Naled	0.1 (LOQ)	5	83-97	91	6	6.3
	1	5	92-98	95	3	3.2
Dichlorvos	0.1 (LOQ)	5	92-98	94	3	2.7
	1	5	90-99	94	4	4.1
Ground Water						
Quantification ion						
Naled	0.1 (LOQ)	5	94-98	95	2	1.7
	1	5	88-97	92	3	3.5
Dichlorvos	0.1 (LOQ)	5	89-98	93	4	4.1
	1	5	92-96	94	2	2.2
Confirmation ion						
Naled	0.1 (LOQ)	5	89-100	94	4	4.8
	1	5	93-98	95	2	2.0
Dichlorvos	0.1 (LOQ)	5	84-103	95	9	9.2
	1	5	91-98	94	3	3.9

Data (uncorrected recovery results, p. 17) were obtained from Tables 5-12, pp. 25-32 of MRID 48802301 and DER Attachment 2. Standard deviations were reviewer-calculated from the data in the study report since the study author only reported means and RSDs (see DER Attachment 2).

1 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively):

m/z 381 \rightarrow 127 and m/z 383 \rightarrow 127 for naled, and m/z 221 \rightarrow 127 and m/z 223 \rightarrow 127 for dichlorvos.

2 The water matrices were fully characterized (p. 14). The surface water (pH 8.07, total organic carbon 16.198 mgC/L) was obtained from Diss Mere. The ground water (pH 7.62, total organic carbon 1.8 mgC/L) was obtained from Anglian Water Denton Lodge Borehole 1. The water sources were not specified further.

Table 3. Independent Validation Method Recoveries for Naled and Dichlorvos in Water^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Quantification ion						
Naled	0.1 (LOQ)	5	82.5-106.8	94.6	8.71	9.20
	1	5	100.8-106.7	103.1	2.23	2.16
Dichlorvos	0.1 (LOQ)	5	87.7-121.3	103.4	13.87	13.42
	1	5	95.4-105.6	100.7	3.70	3.68
Confirmation ion						
Naled	0.1 (LOQ)	5	96.7-109.5	100.7	5.11	5.07
	1	5	98.7-105.6	101.5	2.65	2.61
Dichlorvos	0.1 (LOQ)	5	92.9-126.4	104.0	13.72	13.18
	1	5	96.1-105.8	100.4	3.98	3.96
Ground Water						
Quantification ion						
Naled	0.1 (LOQ)	5	86.0-100.4	95.6	5.94	6.22
	1	5	101.2-107.2	103.9	2.77	2.67
Dichlorvos ³	0.1 (LOQ)	5	78.6-121.7	99.3	17.61	17.75
	1	5	100.1-104.5	101.9	1.73	1.70
Confirmation ion						
Naled	0.1 (LOQ)	5	100.9-111.5	105.6	3.82	3.62
	1	5	103.3-108.1	105.0	1.94	1.85
Dichlorvos ³	0.1 (LOQ)	5	78.4-121.9	96.5	17.62	18.26
	1	5	98.1-107.2	103.5	3.44	3.33

Data (uncorrected recovery results, p. 18) were obtained from Tables 5-12, pp. 25-32 of MRID 48802302.

- 1 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): m/z 381 \rightarrow 127 and m/z 383 \rightarrow 127 for naled, and m/z 221 \rightarrow 127 and m/z 223 \rightarrow 127 for dichlorvos.
- 2 The surface water (pH 7.52, total organic carbon 6.4 mg/L) was fully characterized and obtained from Calwich Abbey (p. 14). The ground water (uncharacterized) was obtained from Anglian Water Denton Lodge Borehole 1. Both water samples were obtained from the Department of Bioanalysis at Huntingdon Life Sciences.
- 3 The recovery for one of the two control samples was 0.0443 µg/L (44.3%) and 0.0488 µg/L (48.8%) for the quantification and confirmation ions, respectively. The study author considered these values to be anomalous and the controls to be free of residues based on the other control sample recovery of "ND".

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD were reported as 0.1 µg/L and 0.025 µg/L (0.05 ng/mL), respectively (pp. 11, 18-19; Appendix 3, p. 92 of MRID 48802301; pp. 11, 17, 21 of MRID 48802302). In the ECM and ILV, the LOQ was defined as the lowest fortification level with acceptable recoveries. In the ECM, the LOD was defined the concentration of the lowest calibration standard chromatographed which gave rise to a measureable chromatographic response. In the ILV, the LOD was defined as the concentration of the lowest calibration standard chromatographed which gave rise to a peak height to baseline noise ratio ≥ 3 .

Table 4. Method Characteristics in Water

		Naled	Dichlorvos
Limit of Quantitation (LOQ)		0.1 µg/L	
Limit of Detection (LOD)	ECM:	0.025 µg/L	
	ILV:	(calculated using the lowest calibration standard, 0.05 ng/mL)	
Linearity (calibration curve r^2 and concentration range)	ECM ¹ :	$r^2 = 0.9988-0.9990$ (Q) $r^2 = 0.9996-0.9998$ (C)	$r^2 = 0.9994-0.9998$ (Q) $r^2 = 0.9986-0.9998$ (C)
	ILV ² :	$r^2 = 0.9986$ (Q) $r^2 = 0.9990$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9978$ (C)
		0.05-2.5 ng/mL	
Repeatable	ECM ³ :	Yes at LOQ and 10×LOQ (n = 5). (characterized surface and ground water matrices)	
	ILV ⁴ :	Yes at LOQ and 10×LOQ (n = 5). (characterized surface and uncharacterized ground water matrix)	
Reproducible		Yes at LOQ and 10×LOQ.	
Specific	ECM:	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). Some baseline noise was included in the peak integration of a few analyte signals. ⁵	
	ILV:	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).	Significant residues (ca. 17-28% of LOQ) were observed in both ions of the surface water control matrix and the quantification ion of the ground water control matrix. ⁶ Minor residues (ca. 7% of LOQ) were observed in the confirmation ion of the ground water control matrix.
		Peak integration was not uniform throughout the chromatograms; varied amounts of baseline noise was included in or excluded from the peak integration of most of the analyte signals. ⁷	

Data were obtained from pp. 11, 18-19; Tables 1-12, pp. 23-32; Figures 1-44, pp. 34-77; Appendix 2, pp. 80-87; Appendix 3, p. 92 of MRID 48802301; pp. 11, 17, 21; Tables 1-12, pp. 23-32; Figures 1-40, pp. 33-72 of MRID 48802302; 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 naled and dichlorvos from reported r values of 0.9993-0.9998 (Q & C; calculated from data in Tables 1-4, pp. 23-24; Appendix 2, pp. 80-87 of MRID 48802301; see DER Attachment 2).

2 ILV standard curves were weighted 1/x. ILV r^2 values are reviewer-generated for naled and dichlorvos from reported r values of 0.9989-0.9995 (Q & C; calculated from data in Tables 1-4, pp. 23-24 of MRID 48802302; see DER Attachment 2).

- 3 In the ECM, surface water (pH 8.07, total organic carbon 16.198 mgC/L), obtained from Diss Mere, and ground water (pH 7.62, total organic carbon 1.8 mgC/L), obtained from Anglian Water Denton Lodge Borehole 1, were used (p. 14). The water sources were not specified further.
- 4 In the ILV, surface water (pH 7.52, total organic carbon 6.4 mg/L), obtained from Calwich Abbey, and ground water (uncharacterized), obtained from Anglian Water Denton Lodge Borehole 1, were used (p. 14). Both water samples were obtained from the Department of Bioanalysis at Huntingdon Life Sciences.
- 5 Based on Figures 25-26, pp. 58-59; Figures 31-32, pp. 64-65; Figure 41, p. 74; Figure 43, p. 76 of MRID 48802301.
- 6 Based on Figures 23-24, pp. 55-56; Figures 26-27, pp. 58-59; Figures 35-36, pp. 67-68 of MRID 48802302. All of these control residue values were reported as “ND” in the tables of the study report (Tables 5-12, pp. 25-32; see Reviewer Comment #2).
- 7 Based on Figures 17-40, pp. 49-72 of MRID 48802302.

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 ECM and ILV, the LOQ was defined as the lowest fortification level with acceptable recoveries (pp. 11, 18-19; Appendix 3, p. 92 of MRID 48802301; pp. 11, 17, 21 of MRID 48802302). In the ECM, the LOD was defined the concentration of the lowest calibration standard chromatographed which gave rise to a measureable chromatographic response. In the ILV, the LOD was defined as the concentration of the lowest calibration standard chromatographed which gave rise to a peak height to baseline noise ratio ≥ 3 . 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 for dichlorvos was not validated by the representative chromatograms. Significant residues (*ca.* 17-28% of LOQ) were observed in both ions of the surface water control matrix and the quantification ion of the ground water control matrix (Figures 23-24, pp. 55-56; Figures 26-27, pp. 58-59; Figures 35-36, pp. 67-68 of MRID 48802302). All of these control residue values were reported as “ND” in the tables of the study report (Tables 5-12, pp. 25-32) and <30% of the LOQ in the figures (Figures 23-24, pp. 55-56; Figures 26-27, pp. 58-59; Figures 35-36, pp. 67-68). However, the LOD was 25% of the LOQ, so these significant residues were *ca.* 70-100% of the LOD. Additionally, the recovery for one of the two control samples of dichlorvos in ground water was 0.0443 $\mu\text{g/L}$ (44.3% of the LOQ) and 0.0488 $\mu\text{g/L}$ (48.8% of the LOQ) for the quantification and confirmation ions, respectively (Tables 11-12, pp. 31-32). The study author considered these values to be anomalous and the controls to be free of residues based on the other control sample recovery of “ND”. Chromatograms of these “anomalous” controls were not included in the study report.

Representative chromatograms of the reagent blank were not included in ECM or ILV; a reagent blank was not included (p. 15; Tables 5-12, pp. 25-32 of MRID 48802301; p. 16; Tables 5-12, pp. 25-32 of MRID 48802302).

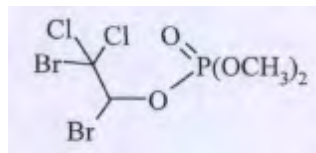
3. The ILV ground water matrix was not characterized (p. 14 of MRID 48802302). The source was reported. It could not be determined if the ILV was provided with the most difficult ground water matrix with which to assess the method.

The locations of water sources for the ECM water matrices were not fully specified (p. 14 of MRID 48802301).

4. The number of trials was not reported in the ILV. The reviewer assumed that the method was validated with the first trial (pp. 11, 22 of MRID 48802302)
5. The reviewer noted that the third column of the ILV Recovery Tables 5-12 were always entitled “Naled detected ($\mu\text{g/L}$)”, even when the recovery table presented data for dichlorvos (Tables 5-12, pp. 25-32 of MRID 48802302).
6. The communication with the sponsor was not reported in the ILV.
7. Although the laboratory which performed the ILV, Huntingdon Life Sciences Ltd., was the same company as the originating laboratory, the location of the independent laboratory was Cambridgeshire, United Kingdom, while the location of the originating laboratory was Suffolk, England. So, the laboratory which performed the ILV was independent of the performing laboratory, as well as the study sponsor, AMVAC Chemical Corporation.
8. Matrix effects were studied and found to be insignificant in the ECM (p. 20; Tables 22-25, pp. 88-89 of MRID 48802301).
9. In the ECM, the final sample extracts were found to be stable when stored at *ca.* -20°C for up to 7 days (p. 19; Table 13, p. 33 of MRID 48802301).
10. The minor ILV modifications of the ECM had no effect on the outcome of the study. The majority of the ILV was performed exactly as in the ECM using the same analytical instruments (pp. 15-17 of MRID 48802302). The study author noted that minor changes to the analytical parameters were necessary for optimization; however, the only apparent change was the specified sample temperature of “ $+4^{\circ}\text{C}$ ” (pp. 15, 17). The two monitored parent-daughter ion transitions were the same as the ECM. Retention times were observed at *ca.* 1.9 and 1.6 min. for naled and dichlorvos, respectively. These minor modifications had no impact on the outcome of the study.
11. The time requirement for completion of the method was reported as *ca.* 1 calendar day for one batch of 12 samples in the ILV (p. 21 of MRID 48802302). LC/MS/MS was run overnight in one run.

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**Naled (Dibrom Pestanal)****IUPAC Name:** 1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate**CAS Name:****CAS Number:** 300-76-5**SMILES String:****Dichlorvos (DDVP; Pestanal)****IUPAC Name:** 2,2-Dichlorovinyl dimethyl phosphate**CAS Name:****CAS Number:** 62-73-7**SMILES String:**