

## Addendum #1 to Data Evaluation Record

**MRIDs:** 49808201, 49808202

**PC Code:** 077401

**OPPTS Guidelines:** 850.6100

**Date:** June 14, 2021

**DER Study Titles:** ECM: EPA MRID No.: 49808201. Leak, T. 2015. Method Validation for Niclosamide in Ecotoxicology Media. ABC Study No.: 81340. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, and sponsored and submitted by Great Lakes Fishery Commission, Ann Arbor, Michigan; 63 pages. Final report issued February 25, 2015.

ILV: EPA MRID No. 49808202. Wu, X. 2015. Independent Laboratory Validation (ILV) of the Analytical Method for Determination of Niclosamide in Water and Sediment by HPLC-UV. Smithers Viscient Study No.: 14099.6101. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored and submitted by Great Lakes Fishery Commission, Ann Arbor, Michigan; 72 pages. Final report issued October 1, 2015.

**Action:** Amend study classification for niclosamide ECM/ILV.

**Changes Made:** Study classification is being upgraded from unacceptable to **supplemental**.

**Rationale for Upgrade:** The registrant submitted additional information including a copy of the final study report with acceptable (clear) representative chromatograms which were not fully visible in the initial submission. Additionally, a letter was submitted to the Agency from Ms. Kim Fredericks of the U.S. Geological Survey indicating that the analytical method for monitoring the compound was fully available and established. In the memo, she stated:

*“As noted in the TFM and niclosamide reviews (PC 036201 and PC 077401, respectively), the results of the ECM and ILV demonstrated that recoveries for both TFM and niclosamide were within guidelines and the ILV studies primary and confirmatory analyses were comparable; indicating that HPLC detection methods are accurate, robust, and precise in terms of recovery, detection, and retention times. No interferences that would interfere with sample quantification were reported for either TFM or niclosamide. The primary methods have demonstrated specificity and linearity, which is confirmed in the ECM and ILV studies for both TFM and niclosamide.”*

Additionally, Ms. Fredericks noted that *“The analysis of the lampricides TFM and niclosamide in water using high performance liquid chromatography (HPLC) was developed in the early 1980s to provide a quick and accurate determination of lampricide concentrations during stream treatments (Dawson 1982). In the subsequent 38 years, HPLC has become widely accepted for both TFM and niclosamide analysis as demonstrated by its use in numerous peer-reviewed publications (TFM: Bills and Johnson 1992, Hubert et al. 2005, Boogaard et al. 2009, McConville,*

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*et al. 2017; niclosamide: Hubert et al. 1999, Waller et al. 2001, Boogaard and Johnson 2006, Ceballos et al. 2015). HPLC is also routinely used as an analysis tool when the lampricides are applied during field operations (Barber and Steeves 2019).*

*Due to the combination of the long-term use and wide acceptance of HPLC for TFM and niclosamide analyses with the evidence provided by the submitted ECM and ILV for TFM and niclosamide, we are requesting a waiver from the need to provide updated ECM or ILV methods as requested in the June 28, 2018 correspondence to Dr. Lantz from Dr. Anderson. It is in our expert opinion that the established methods and the preponderance of evidence demonstrate that primary HPLC/UV methods are sufficient and there is no need to expend additional resources to submit updated studies with additional confirmatory HPLC/UV methods.”*

EFED has concluded that the submitted ECM & ILV studies are sufficient for meeting monitoring needs and, therefore, meet the intention of the required guideline studies under guideline OCSPP 850.6100 and may be upgraded to supplemental.

**Revised by:** Cheryl Sutton, Ph.D. CHERYL SUTTON Digitally signed by CHERYL SUTTON Date: 2021.06.15 09:14:26 -04'00' **Date:** June 14, 2021

**Secondary reviewed by:** Katrina White, Ph.D. White, Katrina Digitally signed by White, Katrina Date: 2021.06.15 10:03:43 -04'00' **Date:** June 14, 2021

## Literature Cited

**Analytical method for niclosamide in ecotoxicology media [freshwater, 20X FWAM (20XAAP), and artificial sediment]**

**Reports:** ECM: EPA MRID No.: 49808201. Leak, T. 2015. Method Validation for Niclosamide in Ecotoxicology Media. ABC Study No.: 81340. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, and sponsored and submitted by Great Lakes Fishery Commission, Ann Arbor, Michigan; 63 pages. Final report issued February 25, 2015.

ILV: EPA MRID No. 49808202. Wu, X. 2015. Independent Laboratory Validation (ILV) of the Analytical Method for Determination of Niclosamide in Water and Sediment by HPLC-UV. Smithers Viscient Study No.: 14099.6101. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored and submitted by Great Lakes Fishery Commission, Ann Arbor, Michigan; 72 pages. Final report issued October 1, 2015.

**Document No.:** MRIDs 49808201 & 49808202

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160) and TSCA (40 CFR Part 792) and OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 49808201). 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 (p. 4).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49808202). 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 (p. 4).


**Classification:** This analytical method is classified as not acceptable. An updated ECM should be submitted incorporating the ILV confirmatory HPLC/UV method. The LOQ of the ILV for freshwater and 20XAAP differed slightly from that of the ECM; the LODs for all three matrices differed between the ECM and ILV. ILV chromatograms were indecipherable. Performance data to validate the method at 10×LOQ were not reported in the ECM.

**PC Code:** 077401

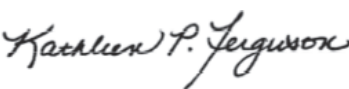
**EFED Final Reviewer:** Cheryl Sutton, Ph.D.  
Environmental Scientist

Signature:  
Date: July 31, 2017

**CDM/CSS- Dynamac JV Reviewers:** Lisa Muto,  
Environmental Scientist

Signature:   
Date: 2/21/17

**Reviewers:** Kathleen Ferguson, Ph.D.,  
Environmental Scientist

Signature:   
Date: 2/21/17

*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.*

## **Executive Summary**

This analytical method, ABC Study No. 81340, is designed for the quantitative determination of niclosamide in freshwater and 20X Freshwater Algal Assay Procedure (20XAAP; 20XFWAM) medium at the LOQ of 0.0209 mg/L and in artificial sediment at the LOQ of 0.200 mg/kg using HPLC/UV. In the ILV, the LOQ for freshwater and 20XAAP was reported as 0.02 mg/L. The LOQ is less than the lowest toxicological level of concern in freshwater. The LOQ is less than the lowest toxicological level of concern in 20XAAP. Sediment toxicity levels are not available for comparison with the LOQ in sediment. In the ECM, performance data to validate the method at 10×LOQ was not reported for any matrix. The freshwater in the ECM and ILV was prepared by adjusting the total hardness of well water to 130 to 160 mg/L as CaCO<sub>3</sub>; the preparations varied between the two laboratories. The artificial sediment was prepared in the ECM and ILV using different raw materials to result in a component composition (% dry weight) of 75, 20 and 5 for sand, clay, and peat, respectively. In the ECM, no confirmation method was employed to support the HPLC/UV analyte identification and quantification. The ILV validated the method after one trial for all three matrices with insignificant modifications to the analytical method, except that a confirmatory HPLC/UV method was employed. An updated ECM should be submitted incorporating the confirmatory HPLC/UV method. ILV chromatograms were too faint to be interpreted to confirm specificity of the primary and confirmatory HPLC/UV methods. An updated ILV should be submitted with acceptable chromatographic support. The LODs for all matrices also differed between the ECM and 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						
Niclosamide	49808201 <sup>1</sup>	49808202 <sup>2</sup>		Freshwater	25/02/2015	Great Lakes Fishery Commission	HPLC/UV	0.0209 mg/L <sup>3</sup>
				20XFWAM				
				Artificial Sediment				0.200 mg/kg

<sup>1</sup> In the ECM, the freshwater was prepared by blending naturally hard well water with RO well water to achieve a total hardness of 130 to 160 mg/L as CaCO<sub>3</sub> (see above for prep; pp. 11-12 of MRID 49808201). Chemical characteristics of ABC well water were reported in Appendix B, pp. 49-51; further characterization was not reported. 20X Freshwater Algal Assay Procedure medium (also abbreviated as 20XAAP) was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (see above for prep). No matrix characterization was reported. The formulated sediment (Lot No. ASD190614) was prepared by mixing 7500 g of sand, 2000 g of clay and 588.4 g of peat, resulting in a component composition (% dry weight) of 75, 20 and 5 for sand, clay, and peat, respectively. The moisture of the artificial sediment was adjusted to 35% by mixing 250.232 g of artificial sediment with 83.638 g of deionized water. No matrix characterization was reported.

<sup>2</sup> In the ILV, freshwater used in the study was laboratory well water reconstituted for hardness [according to the formula for hard water (USEPA 1975)] and filtered (Amberlite XAD-7 resin column) to remove any potential organic contaminants (p. 12 of MRID 49808202). The well water was not characterized. The preparation of 20XAAP was not reported; no matrix characterization was reported. The artificial sediment (lot # 111814) was prepared by combining sphagnum peat moss, kaolin clay and fine silica sand at approximate amounts of 5, 20 and 75% (dry weight), respectively (total % organic carbon 2%).

<sup>3</sup> In the ILV, the LOQ for freshwater and 20XFWAM was reported as 0.020 mg/L (pp. 10, 23 of MRID 49808202).

## I. Principle of the Method

Freshwater was prepared by blending naturally hard well water with well water that was demineralized by reverse osmosis (RO); well water and RO water were blended in order to achieve a total hardness of 130 to 160 mg/L as CaCO<sub>3</sub> (p. 11 of MRID 49808201). Prepared freshwater samples (8 mL) were fortified with niclosamide and diluted with methanol (unspecified final volume; pp. 13-15). The samples were further diluted with methanol:water (20:80, v:v), if necessary, to a concentration within the range of the standard curve and analyzed by HPLC/UV.

20X Algal Assay Procedure (20XAAP) medium was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (p. 11 of MRID 49808201). ABC reagent water was prepared by passing RO water through a series of deionization tanks, a laboratory water purification system consisting of carbon, de-mineralization, and organic adsorption cartridges, and then through a 0.2- $\mu$ m filter. The 20XAAP medium was adjusted to pH 7.5  $\pm$  0.1 with 0.1N HCl and filtered through Millipore 0.45- $\mu$ m filters. Prepared 20XAAP samples (8 mL) were fortified with niclosamide and diluted with methanol (unspecified final volume; pp. 13, 15). The samples were further diluted with methanol:water (20:80, v:v), if necessary, to a concentration within the range of the standard curve and analyzed by HPLC/UV.

The formulated sediment (Lot No. ASD190614) was prepared by mixing 7500 g of sand, 2000 g of clay and 588.4 g of peat, resulting in a component composition (% dry weight) of 75, 20 and 5 for sand, clay, and peat, respectively (pp. 11-12 of MRID 49808201). The moisture of the artificial sediment was adjusted to 35% by mixing 250.232 g of artificial sediment with 83.638 g of deionized water. Prepared artificial sediment samples (1 g, dry wt.) were fortified with niclosamide and extracted three times with methanol (2 mL x 3) via shaking for 30 minutes and centrifuging for 10 minutes at 3400 rpm (pp. 13, 15). The combined supernatants were reduced to 2 mL under nitrogen. The reduced extract was diluted with 8 mL of HPLC water. The samples were further diluted with methanol:water (20:80, v:v), if necessary, to a concentration within the range of the standard curve and analyzed by HPLC/UV.

Freshwater, 20XAAP and artificial sediment samples were analyzed for niclosamide using Agilent 1100 HPLC system (Waters Symmetry C18 column, 4.6 mm x 75 mm, 3.5  $\mu$ m column; column temperature 25°C) using an isocratic mobile phase of 58 mM acetate buffer in water:methanol (15:85, v:v) coupled with a UV Detector (335 nm; p. 13 of MRID 49808201). Injection volume was 50  $\mu$ L. Approximate retention time was *ca.* 3.1 minutes (Figures 3-6, pp. 29-32; Figures 8-12, pp. 34-38; Figures 14-18, pp. 40-44).

In the ILV, the ECM was performed as written, except that sediment samples were centrifuged at 3000 rpm instead of 3400 rpm during extractions; the injection volume was increased to 200  $\mu$ L for sediment samples; the final volume after methanol dilution of the freshwater and 20XAAP samples specified as 10.0 mL; and the final sediment extracts were centrifuged at 13000 rpm for 5 minutes prior to HPLC/UV analysis (pp. 12-13, 15-19 of MRID 49808202). The freshwater used in the study was laboratory well water reconstituted for hardness [according to the formula for hard water (USEPA 1975)] and filtered (Amberlite XAD-7 resin column) to remove any potential organic contaminants. 20X AAP was also used. The artificial sediment (lot # 111814) was prepared by combining sphagnum peat moss, kaolin clay and fine silica sand at approximate amounts of 5, 20 and 75% (dry weight), respectively (total % organic carbon 2%). A primary and confirmatory HPLC/UV method was employed using an Agilent Infinity Series 1260 LC System (primary column: Water Symmetry C18 column, 4.6 x 75 mm, 3.5  $\mu$ m; confirmatory column: Agilent Zorbax SB-C18 column, 4.6 x 75 mm, 3.5  $\mu$ m) and UV detection (335 nm). Approximate retention times for the aqueous samples were *ca.* 2.7 and 2.2 minutes on the primary and confirmatory column; approximate retention times for the sediment samples were *ca.* 2.9 and 2.3 minutes on the primary and confirmatory column. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for freshwater and 20XAAP was 0.0209 mg/L and 0.020 mg/L in the ECM and ILV, respectively (Tables 2-4, pp. 21-23 of MRID 49808201; pp. 10, 23 of MRID 49808202). The LOQ for sediment was the same in the ECM and ILV at 0.200 mg/kg. The Limit of Detection (LOD) in the ECM was reported as 0.00627 mg/L for freshwater and 20XAAP and 0.0602 mg/kg for sediment. The LOD in the ILV was reported as 0.005 mg/L for all matrices.



## II. Recovery Findings

ECM (MRID 49808201): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of niclosamide in freshwater at fortification levels of 0.0209 mg/L (LOQ), 62.8 mg/L (*ca.* 3005 $\times$ LOQ) and 127 mg/L (*ca.* 6077 $\times$ LOQ; Tables 2-4, pp. 21-23). Mean recoveries and RSDs were within guideline requirements for analysis of niclosamide in 20X Freshwater Algal Assay Procedure medium (20XAAP; 20XFWAM) at fortification levels of 0.0209 mg/L (LOQ), 63.4 mg/L (*ca.* 3033 $\times$ LOQ) and 127 mg/L (*ca.* 6077 $\times$ LOQ). Mean recoveries and RSDs were within guideline requirements for analysis of niclosamide in artificial sediment at fortification levels of 0.20 mg/kg (LOQ) and 203 mg/kg (1015 $\times$ LOQ). No samples were prepared at 10 $\times$ LOQ for any matrix. One sample of each matrix was fortified at the LOD; recoveries were 106% in freshwater (LOD 0.00627 mg/L), 127% in 20XAAP (LOD 0.00627 mg/L) and 92% in artificial sediment (LOD 0.0602 mg/kg). Niclosamide was identified using HPLC/UV; no confirmation method was employed. The freshwater was prepared by blending naturally hard well water with RO well water to achieve a total hardness of 130 to 160 mg/L as CaCO<sub>3</sub> (see above for prep; pp. 11-12). Chemical characteristics of ABC well water were reported in Appendix B, pp. 49-51; further characterization was not reported. 20X Freshwater Algal Assay Procedure medium (20XAAP; also abbreviated as 20XFWAM) was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (see above for prep). No matrix characterization was reported. The formulated sediment (Lot No. ASD190614) was prepared by mixing 7500 g of sand, 2000 g of clay and 588.4 g of peat, resulting in a component composition (% dry weight) of 75, 20 and 5 for sand, clay, and peat, respectively. The moisture of the artificial sediment was adjusted to 35% by mixing 250.232 g of artificial sediment with 83.638 g of deionized water. No matrix characterization was reported.

ILV (MRID 49808202): Mean recoveries and RSDs were within guideline requirements for analysis of niclosamide in freshwater and 20XAAP at fortification levels of 0.02 mg/L (LOQ) and 0.2 mg/L (10 $\times$ LOQ; Tables 1-6, pp. 26-31). Mean recoveries and RSDs were within guideline requirements for analysis of niclosamide in artificial sediment at fortification levels of 0.2 mg/kg (LOQ) and 2.0 mg/kg (10 $\times$ LOQ). Niclosamide was identified using a primary and confirmatory HPLC/UV method; performance data (recovery results) from primary and confirmatory analyses were comparable. The freshwater used in the study was laboratory well water reconstituted for hardness [according to the formula for hard water (USEPA 1975)] and filtered (Amberlite XAD-7 resin column) to remove any potential organic contaminants (p. 12). The well water was not characterized. 20XAAP preparation was not reported (p. 12). No matrix characterization was reported. The artificial sediment (lot # 111814) was prepared by combining sphagnum peat moss, kaolin clay and fine silica sand at approximate amounts of 5, 20 and 75% (dry weight), respectively (total % organic carbon 2%; p. 12). The method was validated for all three matrices after one trial with insignificant modifications to the analytical method, except that a confirmatory HPLC/UV method was employed (pp. 12-13, 15-19).

**Table 2. Initial Validation Method Recoveries for Niclosamide in Ecotoxicological Media**

Analyte	Fortification Level (mg/L or mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Freshwater<sup>1</sup></b>						
Niclosamide	0.00627 (LOD)	<b>1</b>	106	*	*	*
	0.0209 (LOQ)	5	100-105	102	1.7	2
	62.8	5	99-103	101	2.1	2
	127	5	86-97	88	4.7	5
<b>20XAAP<sup>2</sup></b>						
Niclosamide	0.00627 (LOD)	<b>1</b>	<b>127</b>	*	*	*
	0.0209 (LOQ)	5	103-113	106	4.0	4
	63.4	5	79-112	93	12.6	14
	127	5	98-101	99	1.2	1
<b>Artificial Sediment<sup>3</sup></b>						
Niclosamide	0.0602 (LOD)	<b>1</b>	92			
	0.200 (LOQ)	5	83-91	86	3.3	4
	203	5	87-90	88	1.2	1

Data (uncorrected recovery results, pp. 13-14) were obtained from Tables 2-4, pp. 21-23 of MRID 49808201.

\* Not calculated, n = 1.

- The freshwater was prepared by blending naturally hard well water with RO well water to achieve a total hardness of 130 to 160 mg/L as CaCO<sub>3</sub> (see above for prep; p. 11). Chemical characteristics of ABC well water were reported in Appendix B, pp. 49-51; further characterization was not reported.
- 20X Freshwater Algal Assay Procedure medium (also abbreviated as 20XFWAM) was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (see above for prep; p. 11). No matrix characterization was reported.
- The formulated sediment (Lot No. ASD190614) was prepared by mixing 7500 g of sand, 2000 g of clay and 588.4 g of peat, resulting in a component composition (% dry weight) of 75, 20 and 5 for sand, clay, and peat, respectively (pp. 11-12). The moisture of the artificial sediment was adjusted to 35% by mixing 250.232 g of artificial sediment with 83.638 g of deionized water. No matrix characterization was reported.



**Table 3. Independent Validation Method Recoveries for Niclosamide in Ecotoxicological Media**

Analyte	Fortification Level (mg/L or mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Freshwater<sup>1</sup></b>						
Primary Method						
Niclosamide	0.02 (LOQ)	5	98.8-118	106	7.05	6.68
	0.2	5	99.0-110	102	4.46	4.39
Confirmatory Method						
Niclosamide	0.02 (LOQ)	5	96.4-109	104	5.26	5.06
	0.2	5	94.8-103	99.1	3.85	3.89
<b>20XAAP<sup>2</sup></b>						
Primary Method						
Niclosamide	0.02 (LOQ)	5	99.6-110	106	4.42	4.18
	0.2	5	98.1-113	109	6.26	5.76
Confirmatory Method						
Niclosamide	0.02 (LOQ)	5	97.5-106.3	101	3.59	3.54
	0.2	5	95.9-111	101	5.72	5.64
<b>Artificial Sediment<sup>3</sup></b>						
Primary Method						
Niclosamide	0.20 (LOQ)	5	108-118	112	3.98	3.55
	2.00	5	87.0-96.7	91.4	4.12	4.51
Confirmatory Method						
Niclosamide	0.20 (LOQ)	5	80.3-83.4	82.0	1.13	1.38
	2.00	5	84.2-90.0	87.1	2.31	2.66

Data (uncorrected recovery results, pp. 20-21) were obtained from Tables 1-6, pp. 26-31 of MRID 49808202.

- 1 The freshwater used in the study was laboratory well water reconstituted for hardness [according to the formula for hard water (USEPA 1975)] and filtered (Amberlite XAD-7 resin column) to remove any potential organic contaminants (p. 12). The well water was not characterized.
- 2 20X Freshwater Algal Assay Procedure medium; 20XFWAM (p. 12). The preparation was not reported. No matrix characterization was reported.
- 3 The artificial sediment (lot # 111814) was prepared by combining sphagnum peat moss, kaolin clay and fine silica sand at approximate amounts of 5, 20 and 75% (dry weight), respectively (total % organic carbon 2%; p. 12).

### III. Method Characteristics

The LOQ for freshwater and 20XAAP was 0.0209 mg/L and 0.020 mg/L in the ECM and ILV, respectively (Tables 2-4, pp. 21-23 of MRID 49808201; pp. 10, 23 of MRID 49808202). The LOQ for sediment was the same in the ECM and ILV at 0.200 mg/kg. No justification was provided for the LOQ in the ECM or ILV. The LOD in the ECM was reported as 0.00627 mg/L for freshwater and 20XAAP and 0.0602 mg/kg for sediment. No justification for the LOD was reported in the ECM. However, in the ECM, the Method Detection Limit (MDL) and Practical Quantification Limit (PQL) were calculated using seven replicate samples of the lowest calibration standard (0.005 mg/L; p. 16; Table 1, p. 20). The MDL was calculated as the standard deviation of the seven replicates multiplied by 3.14 and equaled 0.000606 mg/L. The PQL was calculated as five times the MDL and equaled 0.00303 mg/L. The LOD in the ILV was reported as 0.005 mg/L for all matrices; the LOD was defined as the lowest calibration standard used in

the quantitation. No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method.

**Table 4. Method Characteristics**

Analyte		Niclosamide		
		Freshwater	20XAAP	Formulated Sediment
Limit of Quantitation (LOQ)	ECM	0.0209 mg/L		0.20 mg/kg
	ILV	0.02 mg/L		
Limit of Detection (LOD)	ECM	0.00627 mg/L		0.0602 mg/kg
	ILV	0.005 mg/L		
Linearity (calibration curve $r^2$ and concentration range)	ECM	$r^2 = 0.999944$	$r^2 = 0.999762$	$r^2 = 0.999898$
	ILV	$r^2 = 0.99972$ (1) $r^2 = 0.99980$ (2)	$r^2 = 0.99974$ (1) $r^2 = 0.99988$ (2)	$r^2 = 0.99995$ (1) $r^2 = 0.99998$ (2)
	Concentration Range	(0.005-0.2 mg/L)		
Repeatable	<b>No</b> samples were prepared at 10×LOQ			
	ECM <sup>1</sup>	Yes at LOQ, <i>ca.</i> 3005×LOQ and <i>ca.</i> 6077×LOQ	Yes at LOQ, <i>ca.</i> 3033×LOQ and <i>ca.</i> 6077×LOQ	Yes at LOQ and 1015×LOQ
	ILV <sup>2,3</sup>	Yes at LOQ and 10×LOQ		
Reproducible	Yes at LOQ and 10×LOQ			
Specific	ECM	HPLC/UV used; <b>No</b> confirmation method was employed.		
		No matrix interferences were observed. Chromatograms were not provided for all fortification levels.		No matrix interferences were observed.
	ILV	HPLC/UV used; primary and confirmatory methods employed. No matrix interferences were reported; however, provided chromatograms were too faint to interpret.		

Data were obtained from Tables 2-4, pp. 21-23 (recovery data); Figure 1, p. 27; Figure 7, p. 33; Figure 13, p. 39 (calibration curves); Figures 2-6, pp. 28-32; Figures 8-12, pp. 34-38; Figures 14-18, pp. 40-44 (chromatograms) of MRID 49808201; pp. 10, 23; Tables 1-6, pp. 26-31 (recovery data); Figures 1-3, pp. 32-34; Figures 17-19, pp. 48-50 (calibration curves); Figures 5-16, pp. 36-47; Figures 21-32, pp. 52-64 (chromatograms) of MRID 49808202. 1 = primary method; 2 = confirmatory method.

- In the ECM, the freshwater was prepared by blending naturally hard well water with RO well water to achieve a total hardness of 130 to 160 mg/L as CaCO<sub>3</sub> (see above for prep; pp. 11-12 of MRID 49808201). Chemical characteristics of ABC well water were reported in Appendix B, pp. 49-51; further characterization was not reported. 20X Freshwater Algal Assay Procedure medium (20XAAP; also abbreviated as 20XFWAM) was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (see above for prep). No matrix characterization was reported. The formulated sediment (Lot No. ASD190614) was prepared by mixing 7500 g of sand, 2000 g of clay and 588.4 g of peat, resulting in a component composition (% dry weight) of 75, 20 and 5 for sand, clay, and peat, respectively. The moisture of the artificial sediment was adjusted to 35% by mixing 250.232 g of artificial sediment with 83.638 g of deionized water. No matrix characterization was reported.
- In the ILV, freshwater used in the study was laboratory well water reconstituted for hardness [according to the formula for hard water (USEPA 1975)] and filtered (Amberlite XAD-7 resin column) to remove any potential organic contaminants (p. 12 of MRID 49808202). The well water was not characterized. The preparation of 20XAAP was not reported; no matrix characterization was reported. The artificial sediment (lot # 111814) was prepared by combining sphagnum peat moss, kaolin clay and fine silica sand at approximate amounts of 5, 20 and 75% (dry weight), respectively (total % organic carbon 2%).
- The ILV validated the method after one trial with insignificant modifications to the analytical method, except that only NICLOSAMIDE was used as a test material (NICLOSAMIDE HP Sea Lamprey Larvicide was not a test material) and a confirmatory HPLC/UV method was employed (pp. 11-16, 19-20 of MRID 49808202).

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

1. An updated ECM/ILV method validation set should be submitted for niclosamide in freshwater and 20XAAP which contains the same LOQs, LODs and analytical methods. Also an updated ECM/ILV method validation set should be submitted for niclosamide in artificial sediment which contains the same LODs and analytical methods. In the ECM MRID 49808201, no confirmation method was employed to support the HPLC/UV analyte identification and quantification. In the ILV MRID 49808202, a confirmatory HPLC/UV method was employed. An updated ECM should be submitted incorporating the confirmatory HPLC/UV method. The LOQ of the ILV for freshwater and 20XAAP (0.02 mg/L) differed slightly from that of the ECM (0.0209 mg/L); the LODs for all three matrices (freshwater, 20XAAP and artificial sediment) differed between the ECM and ILV (Tables 2-4, pp. 21-23 of MRID 49808201; pp. 10, 23 of MRID 49808202). The LOQ and LOD of the method for each matrix should be the same between the ECM and ILV.
2. In the ILV, chromatograms were too faint to be interpreted to confirm specificity of the primary and confirmatory HPLC/UV methods (Figures 5-16, pp. 36-47; Figures 21-32, pp. 52-64 of MRID 49808202). An updated ILV should be submitted with acceptable chromatographic support. Representative chromatograms from all fortification levels should be provided for review of method specificity. An updated ILV should be submitted with acceptable chromatographic support.
3. In the ECM, performance data at 10×LOQ was not reported to validate the method (Tables 2-4, pp. 21-23 of MRID 49808201). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.  
  
ECM representative chromatograms not provided for the mid-level fortifications for freshwater and 20XAAP, 62.8 mg/L and 63.4 mg/L, respectively (Figures 2-6, pp. 28-32; Figures 8-12, pp. 34-38 of MRID 49808201). Representative chromatograms should be provided for all fortification levels for review.
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 ILV (Tables 2-4, pp. 21-23 of MRID 49808201; pp. 10, 23 of MRID 49808202). No justification was provided for the LOQ in the ECM or ILV. No justification for the LOD was reported in the ECM. In the ILV, the LOD was defined as the lowest calibration standard used in the quantitation. No calculations or comparisons to background levels were reported to justify the LOQ and LOD for the method.
5. The freshwater in the ECM and ILV was prepared by adjusting the total hardness of well water to 130 to 160 mg/L as CaCO<sub>3</sub>; the preparations varied between the two laboratories (pp. 11-12 of MRID 49808201; p. 12 of MRID 49808202). Full water characterization was not provided for the freshwater in the ECM and ILV. Also, the 20XAAP preparation

- was not reported in the ILV. Water characterization of the 20XAAP was not provided in the ECM and ILV.
6. Communications between the ILV and study monitor were summarized as 1) clarification/approval of the protocol and method, 2) acquisition of analytical standard, and 3) approval of final ILV results (p. 19 of MRID 49808202). The full list of communications was maintained with the study raw data.
  7. In the ECM, recovery of niclosamide in freshwater, 20XAAP and sediment extracts was found to be acceptable (>90%) after 11, 9 and 7 days of refrigerated storage, respectively (pp. 16-18; Tables 5-7, pp. 24-26 of MRID 49808201). Samples were prepared at the method LOQ and “high” spike fortifications (62.8 mg/L, 63.4 mg/L and 203 mg/kg). Regardless of the storage stability results, the study author recommended that samples are analyzed “as soon as possible after sampling”. Samples from the stock solutions were also tested and found to be stable “during other phases of the study”; further details of storage stability of the stock solutions was not discussed (p. 18).
  8. It was reported for the ILV that two batches of thirty-two samples (nineteen samples per batch) required one working day (8 hours) with HPLC/UV performed overnight (p. 20 of MRID 49808202).

## 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****Niclosamide****IUPAC Name:** 2',5-Dichloro-4'-nitrosalicylanilide**CAS Name:** 5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide**CAS Number:** 50-65-7**SMILES String:** Clc1cc(N(=O)=O)ccc1NC(=O)c2cc(Cl)ccc2O