

Addendum #1 to Data Evaluation Record

MRIDs: 49809001, 49809002

PC Code: 036201

OPPTS Guidelines: 850.6100

Date: June 14, 2021

DER Study Titles: ECM: EPA MRID No.: 49809001. Lucash, K. 2015. Method Validation for 3-(trifluoromethyl)-4-nitrophenol (TFM) in Ecotoxicology Media. ABC Study No.: 81341. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, and sponsored and submitted by Great Lakes Fishery Commission, Ann Arbor, Michigan; 59 pages. Final report issued March 5, 2015.

ILV: EPA MRID No. 49809002. Wu, X. 2015. Independent Laboratory Validation (ILV) of the Analytical Method for Determination of TFM in Water by HPLC-UV. Smithers Viscient Study No.: 14099.6100. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored and submitted by Great Lakes Fishery Commission, Ann Arbor, Michigan; 56 pages. Final report issued October 16, 2015.

Action: Amend study classification for TFM 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,*

Analytical method for TFM [3-(trifluoromethyl)-4-nitrophenol] in freshwater and ecotoxicology media

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Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards, with the exception of the latest water characterizations in April 2014 (p. 3 of MRID 49809001). 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 49809002). 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 confirmatory HPLC/UV method. The test material of the ECM did not match that of the ILV for one of the matrices. Performance data to validate the method at 10×LOQ were not reported in the ECM. ILV chromatograms were indecipherable. The LOQ of the ILV for freshwater differed slightly from that of the ECM; the LODs for both matrices differed between the ECM and ILV.

PC Code: 036201

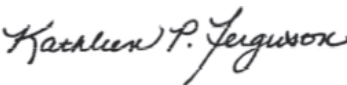
Final EPA Reviewer: Cheryl Sutton, Ph.D.
Environmental Scientist

Signature:
Date: July 31, 2017

Lisa Muto,
Environmental Scientist

Signature: 
Date: 1/10/17

CDM/CSS-Dynamac JV Reviewers: Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 
Date: 1/10/17

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. 81341, is designed for the quantitative determination of TFM [3-(trifluoromethyl)-4-nitrophenol] in freshwater at the LOQ of 0.0608 mg/L and in 20X Freshwater Algal Assay Procedure (20XAAP; 20XFWAM) medium at the LOQ of 0.02 mg/L using HPLC/UV. In the ILV, the LOQ for freshwater was reported as 0.06 mg/L. In the ECM, performance data to validate the method at 10×LOQ was not reported for either matrix. 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. The freshwater in the ECM and ILV was prepared by adjusting the total hardness of well water to 130 to 160 mg/L as CaCO₃; the preparations varied between the two laboratories. In the ECM, the freshwater was fortified with TFM HP Sea Lamprey Larvicide (TFM-HP), and the 20XAAP was fortified with TFM. No confirmation method was employed to support the HPLC/UV analyte identification and quantification. The ILV validated the method after one trial with insignificant modifications to the analytical method, except that only TFM was used as a test material and 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 both matrices 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						
TFM ^{1,2}	49809001 ³	49809002 ⁴		Freshwater	05/03/2015	Great Lakes Fishery Commission	HPLC/UV	0.0608 mg/L ⁵
				20XFWAM				0.02 mg/L

1 4-Nitro-3-(trifluoromethyl)phenol; 3-(Trifluoromethyl)-4-nitrophenol.

2 Freshwater was fortified with TFM-HP in the ECM and with TFM in the ILV. 20XFWAM was fortified with TFM in the ECM and ILV.

3 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 CaCO₃ (see below for prep; p. 10 of MRID 49809001). Chemical characteristics of ABC well water were reported in Appendix B, pp. 43-46; further characterization was not reported. 20X Freshwater Algal Assay Procedure medium (20XAAP; 20XFWAM) was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (see below for prep; pp. 10-11). No matrix characterization of the 20XAAP was reported.

4 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. 11 of MRID 49809002). The well water was not characterized. The preparation 20X Freshwater Algal Assay Procedure medium was not reported. No matrix characterization of 20XAAP was reported.

5 In the ILV, the LOQ for freshwater was reported as 0.06 mg/L (pp. 9, 20 of MRID 49809002).

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 CaCO₃ (p. 10 of MRID 48306934). Prepared freshwater samples (8 mL) were fortified with TFM HP Sea Lamprey Larvicide and diluted with methanol (unspecified final volume; pp. 12-14). 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. 10 of MRID 48306934). 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- μ m filter. The 20XAAP medium was adjusted to pH 7.5 \pm 0.1 with 0.1N HCl and filtered through Millipore 0.22- μ m filters. Prepared 20XAAP samples were fortified with TFM and diluted with methanol (unspecified final volume; pp. 12-14). 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 and 20XAAP samples were analyzed for 3-(trifluoromethyl)-4-nitrophenol (TFM) using Agilent 1100 HPLC system (Waters Symmetry C18 column, 4.6 mm x 75 mm, 3.5 μ m column; column temperature 25°C) using an isocratic mobile phase of 58 mM acetate buffer in water:methanol (30:70, v:v) coupled with a UV Detector (295 nm; p. 12 of MRID 48306934). Injection volume was 50 μ L. Approximate retention time was *ca.* 1.9 minutes (Figures 2-6, pp. 27-31; Figures 8-12, pp. 33-37).

In the ILV, the ECM was performed as written with the final volume after methanol dilution specified as 10.0 mL, except that only TFM was used as a test material (TFM HP Sea Lamprey Larvicide was not a test material; pp. 11-15 of MRID 49809002). 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. 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 μ m; confirmatory column: Agilent Zorbax SB-C18 column, 4.6 x 75 mm, 3.5 μ m) and UV detection (295 nm). Approximate retention times were *ca.* 2.4 and 2.1 minutes on the primary and confirmatory column. No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for freshwater were 0.0608 mg/L and 0.06 mg/L in the ECM and ILV, respectively (Tables 2-3, pp. 19-20 of MRID 49809001; pp. 9, 20 of MRID 49809002). The LOQ for 20XAAP was the same in the ECM and ILV at 0.02 mg/L. The Limit of Detection (LOD) in the ECM was reported as 0.0182 mg/L for freshwater and 0.006 mg/L for 20XAAP. The LOD in the ILV was reported as 0.005 mg/L for all matrices.

II. Recovery Findings

ECM (MRID 49809001): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of TFM [3-(trifluoromethyl)-4-nitrophenol] in freshwater at fortification levels of 0.0608 mg/L (LOQ) and 125 mg/L (2056x LOQ; Table 2, p. 19). Mean recoveries and RSDs were within guideline requirements for analysis of TFM in 20X Freshwater Algal Assay Procedure medium (20XAAP; 20XFWAM) at fortification levels of 0.02 mg/L (LOQ) and 125 mg/L (6250x LOQ; Table 3, p. 20). No samples were prepared at 10 \times LOQ for either water matrix. One sample of each matrix was fortified at the LOD; recoveries were 112% in freshwater (LOD 0.0182 mg/L) and 107% in 20XAAP (LOD 0.006 mg/L). Both water matrices were analyzed for percent recovery of TFM; however, the freshwater was fortified with TFM-HP (TFM HP Sea Lamprey Larvicide), and the 20XAAP was fortified with TFM. TFM 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 CaCO₃ (see above for prep; p. 10). No further characterization of the freshwater was reported. Chemical characteristics of ABC well water were reported, but further characterization was not reported. 20XAAP was prepared by addition of the appropriate reagent grade salts to autoclaved ABC reagent water (see above for prep; pp. 10-11). No matrix characterization of 20XAAP was reported.

ILV (MRID 49809002): Mean recoveries and RSDs were within guideline requirements for analysis of TFM in freshwater at fortification levels of 0.06 mg/L (LOQ) and 0.6 mg/L (10x LOQ; Tables 1-4, pp. 23-26). Mean recoveries and RSDs were within guideline requirements for analysis of TFM in 20XAAP at fortification levels of 0.02 mg/L (LOQ) and 0.2 mg/L (10x LOQ). TFM 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. 11). The well water was not characterized. 20XAAP preparation was not reported; no matrix characterization was reported. The method was validated for both matrices after one trial with insignificant modifications to the analytical method, except that only TFM was used as a test material (TFM HP Sea Lamprey Larvicide was not a test material) and a confirmatory HPLC/UV method was employed (pp. 11-16, 19-20).

Table 2. Initial Validation Method Recoveries for TFM in Water

Analyte	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Freshwater fortified with TFM-HP^{1,2}						
TFM	0.0182 (LOD)	1	112	*	*	*
	0.0608 (LOQ)	5	95-100	98	2.3	2.4
	125	5	99-101	100	0.6	0.6
20XAAP fortified with TFM³						
TFM	0.006 (LOD)	1	107	*	*	*
	0.02 (LOQ)	5	100-105	103	2.1	2.0
	125	5	101-103	102	1.1	1.1

Data (uncorrected recovery results, pp. 12-13) were obtained from Tables 2-3, pp. 19-20 of MRID 49809001.

* Not calculated, n = 1.

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 CaCO₃ (see above for prep; p. 10). Chemical characteristics of ABC well water were reported in Appendix B, pp. 43-46; further characterization was not reported.

2 TFM-HP = TFM HP Sea Lamprey Larvicide (pp. 12-14).

3 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; pp. 10-11). No matrix characterization was reported.

Table 3. Independent Validation Method Recoveries for TFM in Water

Analyte	Fortification Level (mg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Freshwater¹						
Primary Method						
TFM	0.06 (LOQ)	5	93.3-103	97.4	3.49	3.59
	0.6	5	89.4-101	95.8	4.14	4.32
Confirmatory Method						
TFM	0.02 (LOQ)	5	79.0-99.8	89.4	9.09	10.2
	0.2	5	92.5-114	100	8.10	8.09
20XAAP²						
Primary Method						
TFM	0.06 (LOQ)	5	99.1-103	101	1.45	1.44
	0.6	5	95.4-98.2	96.7	1.15	1.19
Confirmatory Method						
TFM	0.02 (LOQ)	5	90.8-101	96.3	4.73	4.91
	0.2	5	94.5-105	99.5	4.68	4.70

Data (uncorrected recovery results, pp. 17-18) were obtained from Tables 1-4, pp. 23-26 of MRID 49809002.

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. 11). The well water was not characterized.

2 20X Freshwater Algal Assay Procedure medium; 20XFWAM (p. 11). The preparation was not reported. No matrix characterization was reported.

III. Method Characteristics

The LOQ for freshwater were 0.0608 mg/L and 0.06 mg/L in the ECM and ILV, respectively (Tables 2-3, pp. 19-20 of MRID 49809001; pp. 9, 18, 20 of MRID 49809002). The LOQ for 20XAAP was the same in the ECM and ILV at 0.02 mg/L. No justification was provided for the LOQ in the ECM or ILV. The LOD in the ECM was reported as 0.0182 mg/L for freshwater and 0.006 mg/L for 20XAAP. 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; pp. 13, 15). The MDL was calculated as the standard deviation of the seven replicates multiplied by 3.14 and equaled 0.000584 mg/L. The PQL was calculated as five times the MDL and equaled 0.00292 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		TFM	
		Freshwater ¹	20XAAP
Limit of Quantitation (LOQ)	ECM	0.0608 mg/L	0.02 mg/L
	ILV	0.06 mg/L	
Limit of Detection (LOD)	ECM	0.0182 mg/L	0.006 mg/L
	ILV	0.005 mg/L	
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.999816$	$r^2 = 0.999956$
	ILV	$r^2 = 0.99933$ (1)	$r^2 = 0.99751$ (1)
		$r^2 = 0.99975$ (2)	$r^2 = 0.99975$ (2)
Concentration Range	(0.005-0.2 mg/L)		
Repeatable	ECM ²	No samples were prepared at 10×LOQ	
		Yes at LOQ and 2056×LOQ	Yes at LOQ and 6250×LOQ
	ILV ^{3,4}	Yes at LOQ and 10×LOQ	
Reproducible	Yes at LOQ and 10×LOQ		
Specific	ECM	HPLC/UV used; No confirmation method was employed. 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 pp. 14-15; Tables 2-3, pp. 19-20 (recovery data); Figures 1-12, pp. 26-37 (calibration curves and chromatograms) of MRID 49809001; pp. 9, 18, 20; Tables 1-4, pp. 23-26 (recovery data); Figures 1-22, pp. 27-48 (calibration curves and chromatograms) of MRID 49809002. 1 = primary method; 2 = confirmatory method.

1 Freshwater was fortified with TFM-HP in the ECM and with TFM in the ILV.

2 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 CaCO₃ (see above for prep; p. 10 of MRID 49809001). Chemical characteristics of ABC well water were reported in Appendix B, pp. 43-46; 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; pp. 10-11). No matrix characterization of the 20XAAP was reported

3 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. 11 of MRID 49809002). The well water was not characterized. The preparation 20X Freshwater Algal Assay Procedure medium was not reported. No matrix characterization of 20XAAP was reported.

4 The ILV validated the method after one trial with insignificant modifications to the analytical method, except that only TFM was used as a test material (TFM HP Sea Lamprey Larvicide was not a test material) and a confirmatory HPLC/UV method was employed (pp. 11-16, 19-20 of MRID 49809002).

IV. Method Deficiencies and Reviewer's Comments

1. An updated ECM/ILV method validation set should be submitted for TFM in freshwater and 20XAAP which contains the same LOQs and LODs, test materials and analytical methods. In the ECM MRID 49809001, the freshwater was fortified with TFM HP Sea Lamprey Larvicide (TFM-HP), and the 20XAAP was fortified with TFM. No confirmation method was employed to support the HPLC/UV analyte identification and quantification. In the ILV MRID 49809002, only TFM was used as a test material in both freshwater and 20XAAP and a confirmatory HPLC/UV method was employed. An updated ECM should be submitted incorporating the confirmatory HPLC/UV method. The test material for each matrix should be the same between the ECM and ILV. The LOQ of the ILV for freshwater (0.06 mg/L) differed slightly from that of the ECM (0.0608 mg/L); the LODs for both matrices differed between the ECM and ILV (Tables 2-3, pp. 19-20 of MRID 49809001; pp. 9, 18, 20 of MRID 49809002). The LOQ and LOD of the method for each matrix should be the same between the ECM and ILV.
2. In the ECM, performance data at 10×LOQ was not reported to validate the method (Tables 2-3, pp. 19-20 of MRID 49809001). 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.
3. In the ILV, chromatograms were too faint to be interpreted to confirm specificity of the primary and confirmatory HPLC/UV methods (Figures 1-22, pp. 27-48 of MRID 49809002). An updated ILV should be submitted with acceptable chromatographic support. Representative chromatograms from all fortification levels should be provided for review of method specificity.
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 2-3, pp. 19-20 of MRID 49809001; pp. 9, 18, 20 of MRID 49809002). 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 CaCO₃; the preparations varied between the two laboratories (pp. 10-11 of MRID 49809001; p. 11 of MRID 49809002). 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. 17 of MRID 49809002). The full list of communications was maintained with the study raw data.
7. In the ECM, no significant matrix effects (>20%) were observed for 20XAAP and freshwater (p. 15; Table 4, p. 21 of MRID 49809001).
8. In the ECM, recovery of TFM in 20XAAP and TFM-HP in freshwater was found to be acceptable (>90%) after 7 days of refrigerated storage (pp. 14, 16; Tables 5-7, pp. 22-25 of MRID 49809001). Samples were prepared at the method LOQ and high spike fortifications (125 mg/L). Samples from the stock solutions were also tested and found to be stable after 7 days of refrigerated storage.
9. 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. 17 of MRID 49809002).

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**TFM****IUPAC Name:** 4-Nitro-3-(trifluoromethyl)phenol**CAS Name:** 3-(Trifluoromethyl)-4-nitrophenol**CAS Number:** Not reported**SMILES String:** Not found