


**Analytical method for starlicide in water**

- Reports:** ECM: EPA MRID No. 50468101. Griffin, D. L., and B. Abbo. 2016. Validation of the Environmental Analytical Chemistry Method for DRC-1339 in Surface Water. Laboratory Project ID: QA-2499. Report prepared, sponsored, and submitted by National Wildlife Research Center, USDA/APHIS/WS, Fort Collins, Colorado; 92 pages. Final report issued November 1, 2016.
- ILV: EPA MRID No. 50468102. Keenan, D. 2017. Independent Laboratory Validation of the Method: Determination of 3-Chloro-*p*-Toluidine Hydrochloride (CPTH) in Environmental Surface Water by GC-MS/MS. EAG Project No.: 2870W. Report prepared by EAG Laboratories, Life Sciences Division, Hercules (Formerly PTRL West), Hercules, California, sponsored and submitted by USDA APHIS WS National Wildlife Research Center, Fort Collins, Colorado, and USDA APHIS, Riverdale, Maryland; 74 pages. Final report issued November 3, 2017.
- Document No.:** MRIDs 50468101 & 50468102
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160), with the exceptions of the test material characterization and the synthesis and purity assay of the deuterated internal standard (p. 3 of MRID 50468101). 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 QA statement.
- ILV: The study was conducted in accordance with USEPA GLP (FIFRA) standards (40 CFR Part 160), except that the certification of the test materials was not specified whether analyses were conducted under GLP (p. 3 of MRID 50468102). 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 **acceptable**. The LOQ of the ECM was not equivalent to the LOQ of the ILV, although sample fortifications in the ECM and ILV were the same. No ECM or ILV samples were prepared at the LOQ reported in the ECM. The reproducibility of the method at 10×LOQ could not be determined since no samples were prepared at 10×LOQ of the respective LOQ values in the ECM or ILV. The number of trials required to validate the method was not reported by the ILV. Representative ECM chromatograms were not submitted for all fortification levels.
- PC Code:** 009901

**EFED Final  
Reviewer:**Stephen P. Wente, Ph.D.,  
Senior Scientist**Signature:**   
**Date:** 11/14/2018**CDM/CSS-  
Dynamac JV  
Reviewers:**Lisa Muto, M.S.,  
Environmental Scientist**Signature:** **Date:** 09/05/2018Joan Gaidos, Ph.D.,  
Environmental Scientist**Signature:** **Date:** 09/14/2018

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

**Executive Summary**

The analytical method, National Wildlife Research Center (NWRC) Method No. 175A, is designed for the quantitative determination of starlicide (DRC-1339; CPTH) in water using GC/MS. The LOQ was reported as 1 ng/mL (1 µg/L) in the ILV, calculated as 0.41-0.44 ng/mL (0.41-0.44 µg/L) in the ECM, and calculated as 1.1 ng/mL in the ILV. A method LOQ which was a general concentration threshold identified with the method regardless of matrix source was not reported in the ECM. The LOQs are less than the lowest toxicological level of concern in water for starlicide (70 µg a.i./L). The ECM and ILV were performed using two characterized surface water matrices from the same sources; sample fortifications in the ECM and ILV were the same (1 ng/mL, 20 ng/mL, and 300 ng/mL). Three ion transitions were monitored, but only one was quantified; a confirmatory method is not usually required when LC/MS and/or GC/MS is the primary method used to generate study data. Starlicide was quantified based on the analyte response ratio compared with deuterated starlicide using a quadratic equation. The number of trials was not reported in the ILV, but the reviewer assumed that the method for starlicide was validated in the first trial with insignificant modifications. The minor recommendation reported in the ILV could be added to the ECM as an amendment and does not require a full updated ECM. No ECM or ILV samples were prepared at the LOQ reported in the ECM. The reproducibility of the method at 10×LOQ could not be determined since no samples were prepared at 10×LOQ of the respective LOQ values in the ECM or ILV. For the prepared sample fortifications, all ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory, except that representative ECM chromatograms were not submitted for all fortification levels.

**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						
Starlicide (DRC-1339; CPTH)	50468101	50468102		Water <sup>1,2</sup>	01/11/2016 (Final Report) 22/02/2016 (Method) <sup>3</sup>	National Wildlife Research Center USDA/ APHIS/ WS <sup>4</sup>	GC/MS	1.0 ng/mL (ILV) 0.41-0.44 ng/mL (ECM) <sup>5</sup>

1 In the ECM, surface (lake) water (pH 8.2, conductivity 1.47 mmhos/cm, hardness 683 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (pH 8.4, conductivity 1.45 mmhos/cm, hardness 691 mg/L as CaCO<sub>3</sub>, alkalinity 302 mg/L as CaCO<sub>3</sub>) were used in the study (p. 9; Appendix V, pp. 40-41 of MRID 50468101). The surface (lake) water was sourced from Golden Lake in North Dakota. The surface (river) water was sourced from Goose River in North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

2 In the ILV, surface (lake) water (PTRL 2870W-003; pH 8.7, conductivity 1.48 mmhos/cm, hardness 674 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (PTRL 2870W-004; pH 8.3, conductivity 1.26 mmhos/cm, hardness 521 mg/L as CaCO<sub>3</sub>, alkalinity 323 mg/L as CaCO<sub>3</sub>) were used in the study (p. 14; Appendix C, pp. 57-58 of MRID 50468102). The surface (lake) water was sourced from Golden Lake in Steele County, North Dakota. The surface (river) water was sourced from Goose River in Grand Forks County, North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The two surface water matrices of the ILV were sourced from the same bodies of water as those of the ECM.

3 NWRC Method No. 175A (Appendix II, p. 17 of MRID 50468101).

4 National Wildlife Research Center, Wildlife Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture.

5 The fortification level 1 ng/mL was not reported as the LOQ in the ECM. The Method LOQ was estimated as 0.41-0.44 ng/mL in the ECM (pp. 10, 26 of MRID 50468101). A method LOQ which was a general concentration threshold identified with the method regardless of matrix source was not reported in the ECM.

## I. Principle of the Method

### Starlicide

Water samples (25.0 mL) were fortified with 25, 50, or 75 µL of starlicide intermediate standard solution in 50 mL plastic centrifuge tubes (pp. 7, 9; Appendix II, pp. 20-21, 24-25; Appendix VI, pp. 54-55, 58-59 of MRID 50468101). The deuterated starlicide internal standard (50 µL; CPTH-*d*<sub>6</sub>) and one drop of water soluble food coloring (to aid in differentiating the aqueous and organic layers) were added to the sample. The sample was extracted by adding 2.5 mL of 2.0M NaOH, *ca.* 5 g of NaCl and 10 mL of hexane. After vortexing for 10 seconds, the sample was centrifuged for 1 minute at *ca.* 4000 rpm. The upper hexane layer was removed, and the extraction was repeated twice more with 5 mL hexane (2 x 5mL). The combined extracts were applied to an IST-Si (1 g/6 mL) solid phase extraction (SPE) cartridge (pre-conditioned with 2 mL of n-butyl acetate and 5 mL of hexane). The method noted that the solvents and sample should be eluted via gravity, and the column should not be allowed to dry. After the sample was passed through the column, the column was dried via vacuum for *ca.* 1 minute. The starlicide was eluted with 2 mL of n-butyl acetate into a clean 10-mL glass screw-cap tube. Vacuum was applied to the column to fully elute the analyte. An aliquot was transferred to an autosampler vial for analysis by GC/MS.

Water samples were analyzed for starlicide (CPTH) using an Agilent 7890A gas chromatograph equipped with a purged ultimate union (PUU) [Column 1 (Agilent HP-5MS UI column, 0.25 mm x 15 m, 0.25  $\mu$ m thickness); Column 2 (Fused Silica column, 0.15 mm x 0.60 m, thickness not reported)] using a column temperature program (70°C for 2.00 min., 70°C to 175°C at 20°C/min., 175°C to 300°C at 100°C/min., 300°C for 2.00 min.) and helium carrier gas coupled with an Agilent G7000B QQQ mass selective detector (source temperature 230°C) using electron impact (EI) source in Multiple Reaction Monitoring (MRM) mode (Appendix II, pp. 21-22; Appendix VI, pp. 55-56 of MRID 50468101). Injection volume was 1  $\mu$ L (splitless; injection temperature 250°C). Three ion transitions were monitored for CPTH as follows (quantitation, confirmation 1, and confirmation 2, respectively):  $m/z$  140.9 $\rightarrow$ 106.2,  $m/z$  139.9 $\rightarrow$ 105.2 and  $m/z$  139.9 $\rightarrow$ 77.2. Three ion transitions were also monitored for CPTH- $d_6$  as follows (quantitation, confirmation 1, and confirmation 2, respectively):  $m/z$  148.9 $\rightarrow$ 112.2,  $m/z$  146.9 $\rightarrow$ 112.2 and  $m/z$  112.2 $\rightarrow$ 81.2. Retention time was *ca.* 5.8 minutes for CPTH and CPTH- $d_6$ .

### ILV

In the ILV, the ECM was performed as written, except that the SPE cartridge was vacuum dried prior to addition of extract and the use of a different analytical equipment (pp. 13, 17-21, 26; Figure 1, p. 32 of MRID 50468102). The GC/MS system was an Agilent 7890 Series gas chromatograph equipped with an Agilent HP-5MS column (0.25 mm x 15 m, 0.25  $\mu$ m thickness) and Restek guard column (1 m x 0.15 mm) and coupled to an Agilent 7000B triple-quad mass selective detector. The GC injection port liner was a double gooseneck liner instead of the splitless, single taper liner with wool; other parameters were the same as the ECM. The same ion transitions were monitored as in the ECM. Retention time was *ca.* 5.9 minutes for CPTH and CPTH- $d_6$ . No other modifications to the ECM were reported.

The Limit of Quantification (LOQ) in water for starlicide (CPTH) was estimated as 0.41-0.44 ng/mL in the ECM and 1.1 ng/mL in the ILV (p. 10; Appendix II, p. 26 of MRID 50468101; pp. 10, 23; Tables 2-3, pp. 30-31; Figure 10, p. 41 of MRID 50468102). The Limit of Detection (LOD) in water for CPTH was estimated as 0.12-0.13 ng/mL in the ECM and 0.3 ng/mL in the ILV. Estimations of LOQ and LOD were calculated by comparing the peak height of the control samples and the peak height of the 1 ng/mL recovery samples. The ILV also reported that 1 ng/mL was the LOQ for the study.

## II. Recovery Findings

ECM (MRID 50468101): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of starlicide in two surface water matrices at the fortification levels of 1.00 ng/mL (*ca.* 2.5 $\times$ LOQ), 20.0 ng/mL (*ca.* 50 $\times$ LOQ), and 300 ng/mL (*ca.* 750 $\times$ LOQ) using GC/MS (p. 11). The fortification level 1 ng/mL was not reported as the LOQ in the ECM. The Method LOQ was estimated as 0.41-0.44 ng/mL in the ECM (p. 10; Appendix II, p. 26). No samples were prepared at LOQ or 10 $\times$ LOQ. Three ion transitions were monitored, but only one was quantified; a confirmatory method is not usually required when LC/MS and/or GC/MS is the primary method used to generate study data. The surface (lake) water (pH 8.2, conductivity 1.47 mmhos/cm, hardness 683 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (pH 8.4, conductivity 1.45 mmhos/cm, hardness 691 mg/L as CaCO<sub>3</sub>, alkalinity 302 mg/L as CaCO<sub>3</sub>) were used in the study (p. 9; Appendix V, pp. 40-41). The surface (lake) water was sourced from Golden Lake in North Dakota. The surface (river) water was sourced from Goose River in North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50468102): Mean recoveries and RSDs were within guideline requirements for analysis of starlicide in two surface water matrices at the fortification levels of 1.00 ng/mL (LOQ), 20.0 ng/mL (20 $\times$ LOQ), and 300 ng/mL (300 $\times$ LOQ) using GC/MS (Table 1, p. 29). The fortification level 1 ng/mL was reported as the LOQ in the ILV. No samples were prepared at 10 $\times$ LOQ. Three ion transitions were monitored, but only one was quantified; a confirmatory method is not usually required when LC/MS and/or GC/MS is the primary method used to generate study data. The surface (lake) water (PTRL 2870W-003; pH 8.7, conductivity 1.48 mmhos/cm, hardness 674 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (PTRL 2870W-004; pH 8.3, conductivity 1.26 mmhos/cm, hardness 521 mg/L as CaCO<sub>3</sub>, alkalinity 323 mg/L as CaCO<sub>3</sub>) were used in the study (p. 14; Appendix C, pp. 57-58). The surface (lake) water was sourced from Golden Lake in Steele County, North Dakota. The surface (river) water was sourced from Goose River in Grand Forks County, North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The two surface water matrices of the ILV were sourced from the same bodies of water as those of the ECM. The number of trials was not reported in the ILV, but the reviewer assumed that the method for starlicide was validated in the first trial with insignificant modifications, including the vacuum-drying of the SPE cartridge prior to addition of extract and the use of a different analytical equipment (pp. 10-11, 26-27). The ILV reported one minor method recommendation: that the approximate retention time was changed to *ca.* 4.8 in the typical GC conditions since this appeared to be a typographical error based on the ECM chromatograms.

**Table 2. Initial Validation Method Recoveries for Starlicide (CPTH) in Water<sup>1,2</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Surface (Lake) Water</b>						
Quantitation Ion Transition						
Starlicide (CPTH)	1.00 <sup>3</sup>	7	88.0-93.3	91.0	2.0	2.2
	20.0	7	90.6-96.5	93.3	2.1	2.3
	300	7	93.7-99.0	96.6	1.6	1.7
<b>Surface (River) Water</b>						
Quantitation Ion Transition						
Starlicide (CPTH)	1.00 <sup>3</sup>	7	88.6-93.5	90.4	1.7	1.9
	20.0	7	91.1-96.0	92.9	1.8	1.9
	300	7	96.4-101	98.2	1.6	1.6

Data (uncorrected recovery results, Appendix II, pp. 23-24) were obtained from p. 11 of MRID 50468101.

1 For starlicide, three ion transitions were monitored as follows (quantitation, confirmation 1, and confirmation 2, respectively):  $m/z$  140.9→106.2,  $m/z$  139.9→105.2 and  $m/z$  139.9→77.2; however, recovery results were only quantified for the quantitation ion transition (Appendix II, p. 22).

2 The surface (lake) water (pH 8.2, conductivity 1.47 mmhos/cm, hardness 683 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (pH 8.4, conductivity 1.45 mmhos/cm, hardness 691 mg/L as CaCO<sub>3</sub>, alkalinity 302 mg/L as CaCO<sub>3</sub>) were used in the study (p. 9; Appendix V, pp. 40-41). The surface (lake) water was sourced from Golden Lake in North Dakota. The surface (river) water was sourced from Goose River in North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

3 The fortification level 1 ng/mL was not reported as the LOQ in the ECM. The Method LOQ was estimated as 0.41-0.44 ng/mL in the ECM (pp. 10, 26).

**Table 3. Independent Validation Method Recoveries for Starlicide (CPTH) in Water<sup>1,2</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Surface (Lake) Water</b>						
Quantitation Ion Transition						
Starlicide (CPTH)	1 (LOQ)	5	100-107	102	3	3
	20	5	91-99	95	3	4
	300	5	96-98	96	1	1
<b>Surface (River) Water</b>						
Quantitation Ion Transition						
Starlicide (CPTH)	1 (LOQ)	5	97-103	99	3	3
	20	5	95-99	97	1	2
	300	5	98-99	99	1	1

Data (recovery results were corrected when residues were quantified in the controls, pp. 21-23) were obtained from Table 1, p. 29 of MRID 50468102.

1 For starlicide, three ion transitions were monitored as follows (quantitation, confirmation 1, and confirmation 2, respectively):  $m/z$  140.9→106.2,  $m/z$  139.9→105.2 and  $m/z$  139.9→77.2; however, recovery results were only quantified for the quantitation ion transition (p. 21).

2 The surface (lake) water (PTRL 2870W-003; pH 8.7, conductivity 1.48 mmhos/cm, hardness 674 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (PTRL 2870W-004; pH 8.3, conductivity 1.26 mmhos/cm, hardness 521 mg/L as CaCO<sub>3</sub>, alkalinity 323 mg/L as CaCO<sub>3</sub>) were used in the study (p. 14; Appendix C, pp. 57-58). The surface (lake) water was sourced from Golden Lake in Steele County, North Dakota. The surface (river) water was sourced from Goose River in Grand Forks County, North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The two surface water matrices of the ILV were sourced from the same bodies of water as those of the ECM.

### III. Method Characteristics

The LOQ in water for starlicide (CPTH) was estimated as 0.41-0.44 ng/mL in the ECM and 1.1 ng/mL in the ILV (p. 10; Appendix II, p. 26 of MRID 50468101; pp. 10, 23-24, 26; Tables 2-3, pp. 30-31; Figure 10, p. 41 of MRID 50468102). The LOD in water for CPTH was estimated as 0.12-0.13 ng/mL in the ECM and 0.3 ng/mL in the ILV. Estimations of LOQ and LOD were calculated by comparing the peak height of the control samples and the peak height of the 1 ng/mL recovery samples using the following equation:

$$\text{Estimated MLOD} = (\text{Avg. Height}_{\text{ctrl}}(\text{counts})) \times 3 \times (\text{Avg. Conc. (ng/mL)} / (\text{Avg. Height}_{\text{Fort}}(\text{counts})))$$

And

$$\text{Estimated MLOQ} = (\text{Avg. Height}_{\text{ctrl}}(\text{counts})) \times 10 \times (\text{Avg. Conc. (ng/mL)} / (\text{Avg. Height}_{\text{Fort}}(\text{counts})))$$

Where, MLOD is the Method LOD, MLOQ is the Method LOQ, Avg. Height<sub>ctrl</sub> is the mean peak height of the control, Avg. Conc. is the mean fortification at the 1 ng/mL fortification level and Avg. Height<sub>Fort</sub> is the mean peak height of the fortified controls at the 1 ng/mL fortification level.

The ILV also reported that 1 ng/mL was the LOQ for the study. The calculated values support the LOQ established for the study by the ILV.

**Table 4. Method Characteristics**

Analyte		Starlicide	
Limit of Quantitation (LOQ)	ECM	Method <sup>1</sup>	Not reported
		Calculated <sup>2</sup>	0.44 ng/mL (lake) 0.41 ng/mL (river)
	ILV	Method <sup>1</sup>	1 ng/mL
		Calculated <sup>2</sup>	1.1 ng/mL
Limit of Detection (LOD)	ECM	Method <sup>1</sup>	Not reported
		Calculated <sup>2</sup>	0.13 ng/mL (lake) 0.12 ng/mL (river)
	ILV	Method <sup>1</sup>	Not reported
		Calculated <sup>2</sup>	0.3 ng/mL
Linearity (calibration curve $r^2$ and concentration range) <sup>3,4</sup>	ECM		$r^2 = 0.99992$ 5.05-5200 ng/mL
	ILV		$r^2 = 0.99989098$ (lake) $r^2 = 0.99990015$ (river) 5-5000 ng/mL
Repeatable	ECM <sup>3,5</sup>		Yes at 1.00 ng/mL ( <i>ca.</i> 2.5×LOQ), 20.0 ng/mL ( <i>ca.</i> 50×LOQ), and 300 ng/mL ( <i>ca.</i> 750×LOQ) fortifications in two characterized surface water matrices. <b>No samples prepared at LOQ or 10×LOQ.</b>
	ILV <sup>3,6,7</sup>		Yes at 1 ng/mL (LOQ), 20 ng/mL (20×LOQ), and 300 ng/mL (300×LOQ) fortifications in two characterized surface water matrices. <b>No samples prepared at 10×LOQ.</b>
Reproducible			Yes at 1 ng/mL, 20 ng/mL, and 300 ng/mL fortifications. <b>No samples prepared at 10×LOQ.</b> <b>ILV LOQ ≠ ECM LOQ.</b>
Specific	ECM <sup>3</sup>		Yes, matrix interferences were <5% of the LOQ (based on peak height). Some minor peak tailing at 1 ng/mL fortification was observed. <b>No</b> representative chromatograms of 20 ng/mL and 300 ng/mL were provided.
	ILV <sup>3</sup>		Yes, matrix interferences were <2% of the LOQ (based on peak area).

Data were obtained from p. 10; p. 11 (recovery data); Appendix II, p. 25 (calibration data); Appendix II, p. 26 (LOQ/LOD); Appendix II, Figures 1-5, pp. 29-31 (chromatograms) of MRID 50468101; pp. 10, 23; Table 1, p. 29 (recovery data); Tables 2-3, pp. 30-31; Figures 6-7, pp. 37-38 (calibration curves); Figure 10, p. 41 (LOD/LOQ); Figures 8-12, pp. 39-43 (chromatograms) of MRID 50468102.

1 Referring to a general concentration threshold identified with the method regardless of matrix source.

2 Estimated using the comparison of the analyte response in the controls and the 1 ng/mL fortification. Termed MLOQ/MLOD (Method LOQ/Method LOD) in the ECM and ILV.

3 Data referring to quantitation ion transition only. Three ion transitions were monitored, but only one was quantified; a confirmatory method is not usually required when LC/MS and/or GC/MS is the primary method used to generate study data.

4 Quadratic equation used to evaluation calibration curve. Calibration curve depicted Relative Response versus Relative Concentration, as a comparison of CPTH and CPTH-*d6*.

5 In the ECM, surface (lake) water (pH 8.2, conductivity 1.47 mmhos/cm, hardness 683 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (pH 8.4, conductivity 1.45 mmhos/cm, hardness 691 mg/L as CaCO<sub>3</sub>, alkalinity 302 mg/L as CaCO<sub>3</sub>) were used in the study (p. 9; Appendix V, pp. 40-46 of MRID 50468101). The surface (lake) water was sourced from Golden Lake in North Dakota. The surface (river) water was sourced from Goose River in North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota.

6 In the ILV, surface (lake) water (PTRL 2870W-003; pH 8.7, conductivity 1.48 mmhos/cm, hardness 674 mg/L as CaCO<sub>3</sub>, alkalinity 199 mg/L as CaCO<sub>3</sub>) and surface (river) water (PTRL 2870W-004; pH 8.3, conductivity 1.26 mmhos/cm, hardness 521 mg/L as CaCO<sub>3</sub>, alkalinity 323 mg/L as CaCO<sub>3</sub>) were used in the study (p. 14;



Appendix C, pp. 57-58 of MRID 50468102). The surface (lake) water was sourced from Golden Lake in Steele County, North Dakota. The surface (river) water was sourced from Goose River in Grand Forks County, North Dakota. Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The two surface water matrices of the ILV were sourced from the same bodies of water as those of the ECM.

- 7 The number of trials was not reported in the ILV, but the reviewer assumed that the method for starlicide was validated in the first trial with insignificant modifications, including the vacuum-drying of the SPE cartridge prior to addition of extract and the use of a different analytical equipment (pp. 10-11, 26-27 of MRID 50468102). The ILV reported one minor method recommendation: that the approximate retention time was changed to *ca.* 4.8 in the typical GC conditions since this appeared to be a typographical error based on the ECM chromatograms.

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

1. The LOQ of the ECM was not equivalent to the LOQ of the ILV, although sample fortifications in the ECM and ILV were the same (1 ng/mL, 20 ng/mL, and 300 ng/mL). A method LOQ which was a general concentration threshold identified with the method regardless of matrix source was not reported in the ECM. The ILV identified this method LOQ as 1 ng/mL (pp. 10, 24, 26; Figure 10, p. 41 of MRID 50468102). Estimated LOQ and LOD values based on the comparison of the analyte response in the controls and the 1 ng/mL fortification were calculated. This value was termed MLOQ/MLOD (Method LOQ/Method LOD) in the ECM and ILV (p. 10; Appendix II, p. 26 of MRID 50468101; pp. 23-24; Tables 2-3, pp. 30-31 of MRID 50468102). The MLOQ of the ECM (0.41-0.44 ng/mL) differed from the MLOQ (1.1 ng/mL) and general method LOQ (1 ng/mL) of the ILV.
2. No ECM or ILV samples were prepared at the MLOQ of the ECM (p. 11 of MRID 50468101; Table 1, p. 29 of MRID 50468102).
3. The reproducibility of the method at 10×LOQ could not be determined since no samples were prepared at 10×LOQ of the respective LOQ or MLOQ values in the ECM or ILV (p. 11 of MRID 50468101; Table 1, p. 29 of MRID 50468102).
4. The number of trials was not reported in the ILV, but the reviewer assumed that the method for starlicide was validated in the first trial with insignificant modifications, including the vacuum-drying of the SPE cartridge prior to addition of extract and the use of a different analytical equipment (pp. 10-11, 26-27 of MRID 50468102). The ILV reported one minor method recommendation: that the approximate retention time was changed to *ca.* 4.8 in the typical GC conditions since this appeared to be a typographical error based on the ECM chromatograms. The reviewer agreed with the ILV about the typographical error (Appendix II, Figures 1-5, pp. 29-31 of MRID 50468101). This minor ILV recommendation could be added to the ECM as an amendment and does not require a full updated ECM.
5. ECM representative chromatograms of 20 ng/mL and 300 ng/mL were not provided (Appendix II, Figures 1-5, pp. 29-31 of MRID 50468101). Representative chromatograms from all fortification levels and matrices should be submitted to allow the reviewer to assess the specificity of the method.
6. The ECM water matrices were chosen as two surface water matrices in response to an email from EPA to APHIS on November 3, 2015 specifying that both environmental water matrices should be surface water and that water matrices from North Dakota purchased from Agvise Laboratories would suffice (Appendix I, pp. 14-15 of MRID 50468101).

The ILV two surface water matrices of the ILV were sourced from the same bodies of water as those of the ECM, although the water characteristics differed slightly (p. 14; Appendix C, pp. 57-58 of MRID 50468102).

7. The ILV study author reported that no communications between the ILV and ECM occurred (p. 26 of MRID 50468102).
8. The determinations of LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 13, 23-24 of MRID 50468101; pp. 12-13, 36 of MRID 50468102). In the ECM, the LOQ was defined as the lowest level fortified and analyzed during each validation set. No justifications or calculations were provided to support the LOQ in the ILV. In the ECM, the theoretical LOD was defined as the product of the lowest calibration standard analyzed and the dilution factor of the blank and LOQ sample. In the ECM, LOD for determination of starlicide and DFB alcohol in water were calculated using the standard deviation from the respective LOQ recovery results. The LOD was calculated as the standard deviation multiplied by the t-statistic (3.747). In the ILV, the LOD was defined as 40% of the LOQ for starlicide and 25% of the LOQ for DFB alcohol. The calculated values support the LOQ and LOD established for the study. Detection limits should not be based on arbitrary values.
9. The time required to perform the method for one sample set (one solvent blank, one reagent blank, two controls, and five fortified water samples at each of the three fortification levels) was reported as *ca.* 10 hours in the ILV, including *ca.* 4 hours for preparation of standard solutions, *ca.* 4 hours for sample preparation, and *ca.* 2 hours for GC/MS analysis and data processing (not including automated sample analysis; pp. 23-24 of MRID 50468102).

## 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****Starlicide (DRC-1339; CPTH; CPT HCl; CI-47676)****IUPAC Name:** 3-Chloro-*p*-toluidine hydrochloride**CAS Name:** 3-Chloro-4-methylaniline hydrochloride**CAS Number:** 7745-89-3**SMILES String:** Not found