



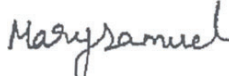
Analytical method for 3,6-dichlorosalicylic acid (DCSA), a degradate of dicamba, in water

- Reports:** ECM 1: EPA MRID No. 51052501. Allen, L. 2017. Dicamba. Dicamba - Validation of Draft Residue Method GRM022.09A for the Determination of Dicamba Metabolite NOA414746 (DCSA) in Water. Method Validation. Report No.: CEMR-7878. Study No.: CEMS-7878. Task No.: TK0312150. Report prepared by CEM Analytical Services Limited (CEMAS), Wokingham, United Kingdom, sponsored by Syngenta Ltd., Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 65 pages. Final report issued February 1, 2017.
- ECM 2: EPA MRID No. 51052502. Allen, L., and S. Brooks. 2017. Dicamba. Dicamba - Residue Method GRM022.09A for the Determination of Metabolite NOA414746 (DCSA) in Water. Analytical Method. Report No.: GRM022.09A. Task No.: TK0312150. Report prepared by CEM Analytical Services Limited (CEMAS), Wokingham, United Kingdom, sponsored by Syngenta Ltd., Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 52 pages. Final report issued January 31, 2017.
- ILV: EPA MRID No. 51154401. Wiesner, F., and N. Breyer. 2017. Dicamba. Dicamba - Independent Laboratory Validation of Analytical Method GRM022.09A for the Determination of the Metabolite NOA414746 (DCSA) in Water. Final Report Amendment 1. Report and Study No.: S17-00148. Task No.: TK0313784. Report prepared by Eurofins Agroscience Services Chem GmbH, Hamburg, Germany, sponsored by Syngenta Ltd., Berkshire, United Kingdom, and submitted by Syngenta Crop Protection, LLC, Greensboro, North Carolina; 42 pages. Final report issued March 28, 2017; report amendment dated May 4, 2017.
- Document No.:** MRIDs 51052501 & 51052502 & 51154401
- Guideline:** 850.6100
- Statements:** ECM 1: The study was conducted in accordance with United Kingdom Good Laboratory Practices (GLP) standards (1999, amended 2004), which are based on the OECD GLP (revised 1997), as well as UK Department of Health GLP (p. 3; Appendix 4, p. 65 of MRID 51052501). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 4, p. 65). A statement of the authenticity of the study report was included with the QA statement.
- ECM 2: The study was not claimed to be in accordance with OECD GLP (revised 1997), and there was no GLP study director for the study (p. 3 of MRID 51052502). Signed and dated No Data Confidentiality and GLP statements were provided (pp. 2-3). Quality Assurance and Authenticity statements were not included. A signed and dated summary of revisions to previous version was provided (p. 4).
- ILV: The study was conducted in accordance with German and OECD GLP standards which are accepted by regulation authorities throughout the

European Community, the US (FDA and EPA) and Japanese (MHW, MAFF, and METI; p. 3; Appendix 3, p. 42 of MRID 51154401). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4; Appendix 3, p. 42). A statement of the authenticity of the study report was included with the GLP statement.

Classification: This analytical method is classified as **supplemental** for surface water, ground water, and drinking water, but **unacceptable** for seawater. Since the reported method LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than LOQ. The ILV only validated the method in drinking water and surface water. The number of trials was not reported. Calibration curves and correlation coefficients were not provided for all matrices in the ECM. The LOD was not reported in the ILV.

PC Code: 029801 (for dicamba)

Reviewer:	Chuck Peck Senior Fate Scientist	Signature:		2021.11.17 13:53:26 -05'00'
	Lisa Muto, M.S., Environmental Scientist	Signature:		
CDM/CSS- Dynamac JV Reviewers:	Mary Samuel, M.S., Environmental Scientist	Signature:		
		Date:	06/25/2020	
		Date:	06/25/2020	

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

Executive Summary

This analytical method, Syngenta Analytical Method GRM022.09A, is designed for the quantitative determination of the dicamba metabolite 3,6-dichlorosalicylic acid (NOA414746; DCSA) at 0.05 µg/L in water using LC/MS/MS. The LOQ is less than the lowest toxicological levels of concern for DCSA in water (31 µg/L). Although two ECM MRIDs were submitted, ECM 2 (MRID 51052502; January 31, 2017, 52 pages) was a draft for ECM 1 (MRID 51052501; February 1, 2017, 65 pages), and essential ECM 1 and ECM 2 data were the same.

Based on the performance data submitted by the ILV and ECMs, the LLMV was equivalent to the reported method LOQ for DCSA (NOA414746) in water (surface water, ground water, and drinking water); however, natural seawater (salinity not reported) was included as a water matrix in the ECM validation, but a seawater/saltwater matrix was not included in the ILV. Since seawater/saltwater is typically considered to be a difficult water matrix, the method was not considered to be reproducible in seawater/saltwater since only one set of performance data was provided.

The number of ILV trials was not reported; however, the reviewer believed that the ILV validated Syngenta Analytical Method GRM022.09A with the first trial for DCSA in drinking and surface water at both fortification levels without method modifications, except for the use of slightly different analytical equipment and modified analytical parameters.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for DCSA, except the specificity of the method for seawater in the ECM. ECM representative chromatograms of the quantification ion transition showed multiple significant contaminants (peak heights *ca.* 10-50% of the LOQ peak) surrounding the analyte peak which interfered with peak identification, integration, and attenuation.

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						
DCSA ¹	51052501 ² & 51052502 ³	51154401 ⁴	Supplemental	Surface/ Ground/ Drinking Water	01/02/2017 (ECM 1)	Syngenta Crop Protection, LLC	LC/MS/MS	0.05 µg/L
		None submitted	Unacceptable	Seawater ⁵	31/01/2017 (ECM 2)			

¹ 3,6-Dichlorosalicylic acid; NOA414746.

² MRID 51052501 was designated as ECM 1. In the ECM 1, surface (river) water (Sample Reference CCON/116/010; pH 8.0, hardness 275 mg CaCO₃/L, 3.36 mg/L dissolved organic carbon) obtained from River Meon, United Kingdom, groundwater (Sample Reference CCON/116/005; pH 7.8, hardness 300 mg CaCO₃/L, 3.21 mg/L dissolved organic carbon) obtained from Fawley Lodge, Henley-on-Thames, United Kingdom, and seawater (Sample Reference CCON/116/011; pH 7.9, hardness 6606 mg CaCO₃/L, 8.80 mg/L dissolved organic carbon) obtained from Hayling Island, United Kingdom, were used in the study (p. 14; Table 1, p. 24 of MRID 51052501). The salinity of the seawater was not reported. Water characterization was carried-out under a separate GLP study.

³ MRID 51052502 was designated as ECM 2. In the ECM 2, the water matrices were those of ECM 1 since ECM 2 was a draft for ECM 1 (Table 1, p. 24 of MRID 51052502).

⁴ In the ILV, drinking water (pH 8.1, hardness 119.3 mg CaCO₃/L, 1.1 mg/L total organic carbon) obtained from a local tap at Groundwater-works, Süderelbmarsch, Hamburg, Germany, and surface (river) water (pH 7.8, hardness 185.1 mg CaCO₃/L, 5.9 mg/L dissolved organic carbon) obtained from River Alster, Hamburg, Germany, were used in the study (p. 15; Tables 1-2, p. 27 of MRID 51154401). Water characterization laboratory was not reported.

⁵ Seawater/saltwater is typically considered to be a difficult water matrix.

I. Principle of the Method

Samples (5 mL) of water were fortified with DCSA (NOA414746) fortification solutions in acetonitrile (10, 1.0, 0.1, or 0.01 $\mu\text{L}/\text{mL}$), as necessary, then acidified with 50 μL of concentrated HCl (pp. 15-16; Appendices 1-2, pp. 50-55 of MRID 51052501; pp. 10-11, 13-14; Appendix 4, p. 52 of MRID 51052502). The samples were applied to a reversed-phase Phenomenex Strata-X solid phase extraction (SPE) cartridge (100 mg, 3 mL) which was pre-conditioned with 3 mL each of acetonitrile then ultra-pure water. The cartridge was not allowed to become dry. After the samples were applied under gravity or low vacuum (*ca.* 200 mbar), the sample flask was rinsed with 2 mL of ultra-pure water. After the rinsate was added to the cartridge and drawn through via vacuum, the cartridge was dried under high vacuum (≤ 500 mbar) for 10 minutes. The method noted that longer drying times maybe necessary depending on the achievable vacuum. The analyte was eluted with 2 x 3 mL of 1% acetic acid in acetonitrile under gravity or low vacuum (*ca.* 200 mbar) in glass vials or 15-mL polypropylene centrifuge tubes. The sample was evaporated to dryness under a stream of clean, dry air or nitrogen at 40°C for *ca.* 1.5 hours (for samples ion glass vials) using a heating block. After the sample was reconstituted in 1 mL of acetonitrile:ultra-pure water (20:80, v:v), the sample was submitted to LC/MS/MS analysis.

Samples were analyzed for DCSA (NOA414746) using an Agilent 1200 series LC coupled to an AB Sciex 5500 triple quadrupole mass spectrometer operating in the negative electrospray (ESI, TurboIonSpray) ionization mode (Appendix 2, pp. 56-57 of MRID 51052501; pp. 15-16 of MRID 51052502). The following LC/MS/MS conditions were used: Ace Ultracore Super C18 column (50 m x 2.1 mm, 2.5 μm particle size; column temperature 30°C); gradient mobile phase of A) 0.1% (v:v) formic acid in ultra-pure water and B) 0.1% (v:v) formic acid in acetonitrile (percent A:B; 0 min. 95:5, 4.0 min. 30:70, 4.1-6.0 min. 95:5); MS temperature 400°C; MRM scan; and injection volume of 30 μL . Expected retention time was 3.1 minutes for DCSA (NOA414746). Two ion transitions were monitored (primary and confirmatory, respectively): m/z 205 \rightarrow 125 and m/z 205 \rightarrow 161.

The ILV performed the ECM method as written, except for the use of slightly different analytical equipment and modified analytical parameters (pp. 11, 17-19; Appendix 1, p. 40 of MRID 51154401). Samples were analyzed for DCSA (NOA414746) using a Agilent 1200 series LC coupled to an API 5500 mass spectrometer. All LC parameters were similar; MS parameters differed slightly. Expected retention time was *ca.* 3.8 minutes for DCSA (NOA414746). The two monitored ion transitions were the same as those in the ECM. No other ILV modifications were reported.

The Limit of Quantification (LOQ) for DCSA (NOA414746) was reported as 0.05 $\mu\text{g}/\text{L}$ in the ECMs (surface water, ground water, and seawater) and ILV (drinking water and surface water) (pp. 11-12, 21; Tables 9-11, pp. 27-28 of MRID 51052501; p. 20 of MRID 51052502; pp. 11, 24 of MRID 51154401). The Limit of Detection (LOD) in water for DCSA (NOA414746) was estimated as 0.002072-0.002518 $\mu\text{g}/\text{L}$, 0.000808-0.001781 $\mu\text{g}/\text{L}$, and 0.002373-0.011321 $\mu\text{g}/\text{L}$ for surface water, ground water, and seawater, respectively, in the ECM 1. In the ECM 2, LODs for the primary transitions were reported as 0.0025 $\mu\text{g}/\text{L}$, 0.0008 $\mu\text{g}/\text{L}$, and 0.0113 $\mu\text{g}/\text{L}$ for surface water, ground water, and seawater, respectively. The LOD was estimated as 0.015 $\mu\text{g}/\text{L}$ (30% of the LOQ) in the ILV for drinking and surface water. Since the LOQ was not based on

scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

II. Recovery Findings

ECM 1 & 2 (MRIDs 51052501 & 51052502): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD \leq 20%) for analysis of DCSA (NOA414746) in three water matrices at the LOQ (0.05 $\mu\text{g/L}$) and 10 \times LOQ (0.50 $\mu\text{g/L}$; Tables 3-4, p. 25 of MRID 51052501; Tables 2-3, p. 25 of MRID 51052502). Recovery results of the quantitative and confirmatory ion transitions were comparable. The surface (river) water (Sample Reference CCON/116/010; pH 8.0, hardness 275 mg CaCO_3/L , 3.36 mg/L dissolved organic carbon) obtained from River Meon, United Kingdom, groundwater (Sample Reference CCON/116/005; pH 7.8, hardness 300 mg CaCO_3/L , 3.21 mg/L dissolved organic carbon) obtained from Fawley Lodge, Henley-on-Thames, United Kingdom, and seawater (Sample Reference CCON/116/011; pH 7.9, hardness 6606 mg CaCO_3/L , 8.80 mg/L dissolved organic carbon) obtained from Hayling Island, United Kingdom, were used in the study (p. 14; Table 1, p. 24 of MRID 51052501; Table 1, p. 24 of MRID 51052502). The salinity of the seawater was not reported. Water characterization was carried-out under a separate GLP study.

ILV (MRID 51154401): Mean recoveries and RSDs met requirements for analysis of DCSA (NOA414746) in drinking (tap) water at the LOQ (0.05 $\mu\text{g/L}$) and 10 \times LOQ (0.50 $\mu\text{g/L}$) using matrix-matched calibration standards (Tables 3-4, p. 28). Recovery results of the quantitative and confirmatory ion transitions were comparable, but the recoveries in the surface water were notably higher than those of the drinking water. The drinking water (pH 8.1, hardness 119.3 mg CaCO_3/L , 1.1 mg/L total organic carbon) obtained from a local tap at Groundwater-works, Süderelbmarsch, Hamburg, Germany, and surface (river) water (pH 7.8, hardness 185.1 mg CaCO_3/L , 5.9 mg/L dissolved organic carbon) obtained from River Alster, Hamburg, Germany, were used in the study (p. 15; Tables 1-2, p. 27). Water characterization laboratory was not reported. Although the number of trials was not reported, the reviewer believed that the method was validated by the ILV with the first trial for DCSA (NOA414746) in drinking and surface water at both fortification levels without method modifications, except for the use of slightly different analytical equipment and modified analytical parameters (pp. 11-12, 17-19, 25). No updated ECM was required.

Table 2. Initial Validation Method Recoveries for 3,6-Dichlorosalicylic Acid (DCSA; NOA414746) in Water^{1,2,3}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ⁴	Relative Standard Deviation (%)
Surface (River) Water						
Quantitation ion transition						
DCSA	0.05 (LOQ)	5	76-98	93	9	10.1
	0.50	5	92-93	92	1	0.6
Confirmation ion						
DCSA	0.05 (LOQ)	5	78-100	93	9	9.4
	0.50	5	94-95	95	1	0.6
Groundwater						
Quantitation ion transition						
DCSA	0.05 (LOQ)	5	80-90	84	4	4.5
	0.50	5	69-77	73	3	4.0
Confirmation ion transition						
DCSA	0.05 (LOQ)	5	80-87	83	3	3.2
	0.50	5	70-75	73	2	3.0
Seawater						
Quantitation ion transition						
DCSA	0.05 (LOQ)	5	82-92	88	4	4.7
	0.50	5	86-94	91	3	3.3
Confirmation ion transition						
DCSA	0.05 (LOQ)	5	86-98	93	4	4.7
	0.50	5	87-93	91	3	2.7

Data (uncorrected results, pp. 18-19) were obtained from Tables 3-4, p. 25 of MRID 51052501; Tables 2-3, p. 25 of MRID 51052502; DER Attachment 2. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is considered the lowest level of method validation (LLMV) rather than an LOQ.

1 3,6-Dichlorosalicylic acid; NOA414746.

2 The surface (river) water (Sample Reference CCON/116/010; pH 8.0, hardness 275 mg CaCO₃/L, 3.36 mg/L dissolved organic carbon) obtained from River Meon, United Kingdom, groundwater (Sample Reference CCON/116/005; pH 7.8, hardness 300 mg CaCO₃/L, 3.21 mg/L dissolved organic carbon) obtained from Fawley Lodge, Henley-on-Thames, United Kingdom, and seawater (Sample Reference CCON/116/011; pH 7.9, hardness 6606 mg CaCO₃/L, 8.80 mg/L dissolved organic carbon) obtained from Hayling Island, United Kingdom, were used in the study (p. 14; Table 1, p. 24 of MRID 51052501; Table 1, p. 24 of MRID 51052502). Water characterization was carried-out under a separate GLP study.

3 Two ion transitions were monitored (primary and confirmatory, respectively): *m/z* 205→125 and *m/z* 205→161.

4 Standard deviations were reviewer-calculated since the values were not reported in the study report. Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for 3,6-Dichlorosalicylic Acid (DCSA; NOA414746) in Water^{1,2,3}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ⁴	Relative Standard Deviation (%)
Drinking (Tap) Water						
Quantitation ion transition						
DCSA	0.05 (LOQ)	5	64-80	74	6	8.1
	0.50	5	69-86	82	7	9.0
Confirmation ion transition						
DCSA	0.05 (LOQ)	5	70-84	78	7	8.6
	0.50	5	67-87	81	8	10
Surface (River) Water						
Quantitation ion transition						
DCSA	0.05 (LOQ)	5	74-97	84	10	12
	0.50	5	92-108	101	7	6.7
Confirmation ion transition						
DCSA	0.05 (LOQ)	5	73-88	79	7	9.1
	0.50	5	92-106	100	6	5.5

Data (uncorrected results, Appendix 1, p. 44) were obtained from Tables 3-4, p. 28 of MRID 51154401; DER Attachment 2. Since the LOQ was not based on scientifically acceptable procedures defined in 40 CFR Part 136, the reported LOQ is the lowest level of method validation (LLMV) rather than an LOQ.

1 3,6-Dichlorosalicylic acid; NOA414746.

2 The drinking water (pH 8.1, hardness 119.3 mg CaCO₃/L, 1.1 mg/L total organic carbon) obtained from a local tap at Groundwater-works, Süderelbmarsch, Hamburg, Germany, and surface (river) water (pH 7.8, hardness 185.1 mg CaCO₃/L, 5.9 mg/L dissolved organic carbon) obtained from River Alster, Hamburg, Germany, were used in the study (p. 15; Tables 1-2, p. 27 of MRID 51154401). Water characterization laboratory was not reported.

3 Two ion transitions were monitored (primary and confirmatory, respectively): m/z 205→125 and m/z 205→161. The monitored ions of the ILV were the same as those of the ECM.

4 Standard deviations were reviewer-calculated since the values were not reported in the study report. Rules of significant figures were followed.

III. Method Characteristics

The LOQ for DCSA (NOA414746) was reported as 0.05 µg/L in the ECM 1 and ECM 2 (surface water, groundwater, and seawater) and ILV (drinking water and surface water) (pp. 11-12, 21; Tables 9-11, pp. 27-28 of MRID 51052501; p. 20 of MRID 51052502; pp. 11, 24 of MRID 51154401). In the ECM 2, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of 20% has been obtained. No calculations for the LOQ were reported in the ILV or ECMs. The LOQ was reported in the ILV without justification. In the ECM 1, the LOD in water was estimated for each test matrix using the following equation:

Estimated LOD = (baseline noise × 3) ÷ height of standard × standard concentration ÷ sample concentration.

Estimated LODs were calculated as 0.002072-0.002518 µg/L, 0.000808-0.001781 µg/L, and 0.002373-0.011321 µg/L for surface water, ground water, and seawater, respectively. In the ECM 2, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time and estimated as three times the background noise. In the ECM 2, LODs for the primary transitions were reported as 0.0025 µg/L, 0.0008 µg/L, and 0.0113 µg/L for surface water, ground water, and seawater, respectively. The LOD was estimated as 0.015 µg/L (30% of the LOQ) in the ILV.

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

Table 4. Method Characteristics

		DCSA ¹
Limit of Quantitation (LOQ)*	ECM 1	0.05 µg/L
	ECM 2	
	ILV	
Limit of Detection (LOD)	ECM 1	0.002072-0.002518 µg/L (SW)
	ECM 2 ²	0.000808-0.001781 µg/L (GW) 0.002373-0.011321 µg/L (SEW)
	ILV	0.015 µg/L (30% of the LOQ)
Linearity (calibration curve r and concentration range) ³	ECM 1	r = 0.9999 (Q, SW) r = 1.0000 (C, SW)
	ECM 2	r = 0.9998 (Q, GW) r = 0.9986 (C, GW) r = 0.9999 (Q/C, SEW)
	ILV	r = 0.9999 (Q) r = 0.9996 (C)
	Range	0.075-5 µg/L
Repeatable	ECM 1 ⁴	Yes for LOQ and 10×LOQ in characterized surface (river) water, groundwater, and seawater (salinity not reported).
	ECM 2 ⁵	
	ILV ^{6,7}	Yes for LOQ and 10×LOQ in characterized drinking (tap) water and surface (river) water.

Reproducible		Yes for 0.05 µg/L (LLMV)* and 0.50 µg/L in surface/drinking/ground water matrices. No for 0.05 µg/L (LLMV)* and 0.50 µg/L in seawater/saltwater matrices since only one set of performance data was submitted.
Specific	ECM 1	Yes for SW and GW, no matrix interferences were observed; however, baseline noise interfered with analyte peak integration and attenuation. Minor contamination/baseline noise was observed near the analyte peak. Peak tailing was observed.
	ECM 2 ⁵	No for SEW (based on Q), no matrix interferences were quantified; however, multiple significant contaminants (peak heights <i>ca.</i> 10-50% of the LOQ peak) surrounded the analyte peak interfering with peak identification, integration, and attenuation. ⁸
	ILV	Yes, no matrix interferences were observed; however, baseline noise interfered with analyte peak integration and attenuation. ⁹ Peak tailing was observed in the drinking water matrix.

Data were obtained from pp. 11-12, 21; Tables 3-4, p. 25 (recovery data); Tables 6-8, pp. 26-27 (linearity data); Tables 9-11, pp. 27-28 (LOQ/LOD); Figures 1-18, pp. 31-48 (chromatograms & calibration curves) of MRID 51052501; p. 20 (LOQ/LOD); Tables 2-3, p. 25 (recovery data); Tables 6-7, p. 28 (linearity data); Figures 2-17, pp. 31-46 (chromatograms & calibration curves) of MRID 51052502 pp. 11, 24 (LOQ/LOD); Tables 3-4, p. 28 (recovery data); Figures 1-2, pp. 31-32 (linearity data & calibration curves); Figures 4-12, pp. 34-38 (chromatograms) of MRID 51154401; DER Attachment 2. Q = quantitative ion transition; C = confirmatory ion transition; SW = surface water; GW = groundwater; SEW = seawater; DW = drinking water.

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

1 3,6-Dichlorosalicylic acid; NOA414746.

2 Only the quantitation ion transition values were reported in ECM 2.

3 Reported r values were reviewer-calculated from r² values reported in the study reports (Tables 6-8, pp. 26-27 of MRID 51052501; Tables 6-7, p. 28 of MRID 51052502; Figures 1-2, pp. 31-32 of MRID 51154401; DER Attachment 2). Values were reported to four significant figures.

4 MRID 51052501 was designated as ECM 1. In the ECM 1, surface (river) water (Sample Reference CCON/116/010; pH 8.0, hardness 275 mg CaCO₃/L, 3.36 mg/L dissolved organic carbon) obtained from River Meon, United Kingdom, groundwater (Sample Reference CCON/116/005; pH 7.8, hardness 300 mg CaCO₃/L, 3.21 mg/L dissolved organic carbon) obtained from Fawley Lodge, Henley-on-Thames, United Kingdom, and seawater (Sample Reference CCON/116/011; pH 7.9, hardness 6606 mg CaCO₃/L, 8.80 mg/L dissolved organic carbon) obtained from Hayling Island, United Kingdom, were used in the study (p. 14; Table 1, p. 24 of MRID 51052501). The salinity of the seawater was not reported. Water characterization was carried-out under a separate GLP study.

5 MRID 51052502 was designated as ECM 2. In the ECM 2, the water matrices, performance data, and representative chromatograms and calibration curves were those of ECM 1 since ECM 2 was a draft for ECM 1 (Table 1, p. 24 of MRID 51052502).

6 In the ILV, drinking water (pH 8.1, hardness 119.3 mg CaCO₃/L, 1.1 mg/L total organic carbon) obtained from a local tap at Groundwater-works, Süderelbmarsch, Hamburg, Germany, and surface (river) water (pH 7.8, hardness 185.1 mg CaCO₃/L, 5.9 mg/L dissolved organic carbon) obtained from River Alster, Hamburg, Germany, were used in the study (p. 15; Tables 1-2, p. 27 of MRID 51154401). Water characterization laboratory was not reported.

7 Although the number of trials was not reported, the reviewer believed that the method was validated by the ILV with the first trial for DCSA (NOA414746) in drinking and surface water at both fortification levels without method modifications, except for the use of slightly different analytical equipment and modified analytical parameters (pp. 11-12, 17-19, 25 of MRID 51154401). No updated ECM was required.

8 Based on Figure 13, p. 43 of MRID 51052501 and Figure 10, p. 39 of MRID 51052502.

9 The reviewer noted that the analyte peak integration range in drinking water (*ca.* RT 3.75-4.0 min.) was significantly broader than that of the surface water (*ca.* RT 3.6-3.75 min.; Figures 8-12, pp. 36-38 of MRID 51154401). The extension of the analyte peak integration range in drinking water appeared to allow for improved recoveries (76% for the Q LOQ in Figure 8, p. 36).

IV. Method Deficiencies and Reviewer's Comments

1. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 11-12, 21; Tables 9-11, pp. 27-28 of MRID 51052501; p. 20 of MRID 51052502; pp. 11, 24 of MRID 51154401). In the ECM 2, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated and a mean recovery of 70-110% with a relative standard deviation of 20% has been obtained. No calculations for the LOQ were reported in the ILV or ECMs. The LOQ was reported in the ILV without justification. In the ECM 1, the LOD in water was estimated for each test matrix using the following equation: $\text{Estimated LOD} = (\text{baseline noise} \times 3) \div \text{height of standard}] \times \text{standard concentration} \div \text{sample concentration}$. In the ECM 2, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time and estimated as three times the background noise. The LOD was estimated as 30% of the LOQ in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

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

2. Although two ECM MRIDs were included in this DER, ECM 2 (MRID 51052502; January 31, 2017, 52 pages) was a draft for ECM 1 (MRID 51052501; February 1, 2017, 65 pages). In the finalization of ECM 2 to ECM 1, the following changes were noted: 1) elimination of Stephen Brooks as a study author; 2) GLP compliance and Quality Assurance statements were added/updated; 3) the study references were modified; 4) tables regarding stability and fortification were added; and 5) GLP Certificate was added. ECM 1 and ECM 2 data were reported together when possible for DER clarity. Both method dates for ECMs 1 & 2 were included in Table 1 of the DER.
3. Natural seawater was included as a water matrix in the ECM validation, but a seawater/saltwater matrix was not included in the ILV. Seawater/saltwater is typically considered to be a difficult water matrix; therefore, the method was not considered to be reproducible in seawater/saltwater since only one set of performance data was provided.

The salinity of the ECM seawater obtained from Hayling Island, United Kingdom, was not reported; however, other general water characterization data was reported (p. 14; Table 1, p. 24 of MRID 51052501).

4. The specificity of the method in seawater/saltwater was not supported by ECM representative chromatograms of the quantification ion transition since multiple significant contaminants (peak heights *ca.* 10-50% of the LOQ peak) surrounded the analyte peak interfering with peak identification, integration, and attenuation (Figure 13, p. 43 of MRID 51052501; Figure 10, p. 39 of MRID 51052502).

5. The number of trials was not reported, the reviewer believed that the method was validated by the ILV with the first trial for DCSA (NOA414746) in drinking and surface water at both fortification levels without method modifications, except for the use of slightly different analytical equipment and modified analytical parameters (pp. 11-12, 17-19, 25 of MRID 51154401). No updated ECM was required.
6. The specificity of the method in drinking water was not supported by ILV representative chromatograms. The reviewer noted that the analyte peak integration range in drinking water (*ca.* RT 3.75-4.0 min.) was significantly broader than that of the surface water (*ca.* RT 3.6-3.75 min.; Figures 8-12, pp. 36-38 of MRID 51154401). The extension of the analyte peak integration range in drinking water appeared to allow for improved recoveries (76% for the Q LOQ in Figure 8, p. 36).
7. The ILV reported that no communications occurred between the ILV laboratory and method developer during the study (pp. 11, 25 of MRID 51154401). The Syngenta Study Sponsor was reported as Laurence Berthod for the ILV and Paul Edwards for the ECM 1 (p. 6 of MRID 51052501; p. 6 of MRID 51154401). No Syngenta Study Monitor was reported for ECM 2.
8. In the ECMs, no significant matrix effects (<20%) were observed for river water and groundwater samples, but significant matrix effects were observed for seawater samples (p. 21; Table 5, p. 26 of MRID 51052501; p. 26 of MRID 51052502). Matrix-matched calibration standards were used for all analyses. In the ILV, no significant matrix effects (<20%) were observed for the drinking and surface water matrices, so solvent-based calibration standards were used for quantitation (p. 24; Table 5, p. 29 of MRID 51154401).
9. In the ECMs, DCSA in final sample extracts fortified at the LOQ and stored in acetonitrile:ultra-pure water (20:80, v:v) were stable at 2-8°C for up to 6-8 days (p. 21; Tables 12-14, pp. 28-29 of MRID 51052501; p. 27 of MRID 51052502). The stock standard solutions and working solutions in acetonitrile were stable at 2-8°C for up to 6-8 days (Tables 15-16, p. 29 of MRID 51052501)
10. The time requirement for the method in the ILV was reported as 1 day (8-hour work period) for the analysis of a batch of 24 samples (p. 23 of MRID 51154401).

V. References

- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319, and Revision 2; 1994 and 2016.

Attachment 1: Chemical Names and Structures**3,6-Dichlorosalicylic acid (NOA414746; DCSA)****IUPAC Name:** 3,6-Dichloro-2-hydroxybenzoic acid**CAS Name:** 3,6-Dichloro-salicylic acid**CAS Number:** 3401-80-7**SMILES String:** ClC1=CC=C(Cl)C(O)=C1C(O)=O