Analytical method for triallate and its metabolite TCPSA in water

Reports: ECM: EPA MRID No.: 51130401. Benet, F.L. 2016. Validation of Analytical Methodology for the Determination of Residues of Triallate and its Metabolite TCPSA in Water (Drinking, Ground and Surface Waters). Report prepared by Laboratorio de Anâlisis de Residuos de Plaguicidas, Avda. Vicent Sos Baynat s/n, Universität Jaume I, Castellón, Spain, sponsored by GOWAN Comércio Internacional e Serviços Limitada, Madeira, Portugal, monitored by SCC Scientific Consulting Company, Chemisch-Wissenschaftliche Beratung GmbH, Bad Kreuznach, Germany (p. 9), and submitted by Gowan Company, Yuma, Arizona; 195 pages (including pages 1A-3A and one unpaginated page). Study No.: 268-16. SCC Project No.: 272-071 (p. 4). Final report issued November 7, 2016.

ILV: EPA MRID No. 51130402. Gaag, S. 2018. Independent Laboratory Validation of an Analytical Method for the Determination of Residues of Tri-allate and its Metabolite TCPSA in Drinking Water. Report prepared by CIP Chemisches Institut Pforzheim GmbH, Pforzheim, Germany, sponsored by Gowan Crop Protection Limited, Berkshire, United Kingdom, monitored by SCC Scientific Consulting Company, Chemisch-Wissenschaftliche Beratung GmbH, Bad Kreuznach, Germany (p. 9), and submitted by Gowan Company, Yuma, Arizona; 56 pages (including pages 1A-3A and one unpaginated page). CIP Study Code: 17G10105-01-VMWA. SCC Project No.: 272-071 (p. 4). Final report issued July 13, 2018.

Document No.: MRIDs 51130401 & 51130402

Guideline: 850.6100

Statements: ECM: The study, with the exception of the water characterization, was conducted in accordance with OECD Good Laboratory Practice (GLP) standards [ENV/MC/CHEM (98)17 (as revised in 1997)], which are accepted by Regulatory Authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF, and METI) and are consistent with USEPA FIFRA (40 CFR Part 160; pp. 3A, 3-4 of MRID 51130401; the reviewer noted that it is possible that MHW was intended to be MHLW). The study GLP statement also cited compliance with European Directive 2004/10/EC and Spanish GLP. Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2A-3A, 3-5). Authenticity statement was included with the QA statement (p. 5).
ILV: The study was conducted in accordance with OECD Good Laboratory Practice (GLP) standards [ENV/MC/CHEM (98)17 (as revised in 1997)], which are accepted by Regulatory Authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHLW, MAFF, and METI), but the study was not specified as in accordance with USEPA FIFRA (40 CFR Part 160; pp. 3A, 3, 7 of MRID 51130402). The study GLP statement also cited compliance with German GLP. Signed and dated No Data Confidentiality, GLP, and Quality

	Assurance statements were provided (pp. 2A-3A, 3, 7-8). Authenticity statement was included with the GLP and QA statements (pp. 7-8). A confidentiality statement was included with the GLP statement (p. 7).
Classification:	This analytical method is classified as **Unacceptable**. The reproducibility of the ECM was difficult to evaluate since the ILV implemented multiple modifications of the analytical parameters, equipment, and instruments. No ILV communications were summarized or reported for review, so the independence of the ILV is unknown. The number of trials required to validate the ECM was not reported in the ILV. The ECM should be updated with the recommendation of the use of matrix-matched calibration standards for all analyses.
PC Code:	078802
EFED Final Reviewer:	A'ja V. Duncan, Ph.D. Chemist
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CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist
	Signature: Lisa Muto Date: 02/26/2021
	Mary Samuel, M.S., Environmental Scientist
	Signature: Marysamuel Date: 02/26/2021

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, Gowan Study No. 268-16, is designed for the quantitative determination of triallate and its metabolite TCPSA in water at the LOQ of 0.100 µg/L using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern (14 µg/L; USEPA, 2014) in water for the two analytes. Since the reported method 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. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for triallate and TCPSA in the tested water matrices (0.100 µg/L).

The ECM validated the method using characterized drinking, ground, and surface water matrices, while the ILV validated the method using only characterized drinking water. Although the number and type of matrices required for independent laboratory validations is not specified in OCSPP Guideline 850.6100, the ILV should be performed with matrices which are similar to or more rigorous matrices than those of the ECM to show equivalent reproducibility.

The ILV validated the ECM method (Gowan Study No. 268-16) for the quantitation and confirmation analyses of triallate and TCPSA in one water matrix with multiple modifications of the analytical parameters, equipment, and instruments. While no sample processing modifications were made by the ILV during validation, the LC columns of the ILV differed from those used in the ECM. The ILV LC columns were also longer than those of the ECM, and the mobile phase polarity switches were adjusted because of the longer LC column length. Since the ECM method (Gowan Study No. 268-16) was a dilution rather than direct injection for triallate and centrifugation/filtration then direct injection for TCPSA, the specifics of the method mainly involved the analytical portion. No ILV communication or discussion was provided; therefore, it could not be determined if the ECM analytical portion of the method was repeatable as written and if the ILV was performed independently from the ECM. Additionally, the number of trials required to validate the ECM was not reported in the ILV.

All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for both analytes in tested water matrices. The ECM should be updated with the recommendation of the use of matrix-matched calibration standards for all analyses because matrix-matched calibration standards were used in the ECM and ILV for matrices which did not exhibit significant (>20%) matrix effects.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Triallate	51130401	51130402		Water[1,2]	07/11/2016	Gowan Company	LC/MS/MS	0.100 µg/L
TCPSA								

1 In the ECM, two water matrices per water type were collected for the study: drinking water matrices were VAL268/1 (from a public water supply in Grao de Castellón, Spain) and VAL268/2 (from a public water supply in Castellón, Spain); surface water matrices were VAL268/13 (from Clot de la Mare de Deu in Burriana, Spain) and VAL268/4 (from Millars River in Castellón, Spain ; and ground water matrices were VAL268/5 (from a well in Castellón, Spain) and VAL268/6 (from a well in Burriana, Spain; p. 36 of MRID 51130401). Each water matrix sample was divided into different subsamples which were denoted by sequential alphabetical naming. For triallate validation, drinking water (Sample ID: VAL268/1/C; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/C; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/5/C; pH 8.5, <1.0 mg/L dissolved oxygen content, 700 mg/L hardness, 1356 µS/cm conductivity at 20°C) were used (pp. 36-37, 45, 50, 54, 75, 79, 83; Appendix 2, pp. 106-111). For TCPSA validation, drinking water (Sample ID: VAL268/1/A; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/A; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/6/A; 8.4, 1.2 mg/L dissolved oxygen content, 834 mg/L hardness, 1554 µS/cm conductivity at 20°C) were used. Water characterization was performed by Eurofins IPROMA Laboratorio y Asesoría, Castellón, Spain. The reviewer noted that water matrices VAL268/2 and VAL268/3 were not used in the validations and different ground water matrices were used for the triallate and TCPSA validations.

2 In the ILV, drinking water (pH 7.66, 0.7 mg/L total organic carbon, 2.42 mmol/L hardness, 423 µS/cm conductivity at 25°C) obtained from a local water supplier in Pforzheim, Germany, was used in the study (p. 16 of MRID 51130402).

I. Principle of the Method

For triallate, 100 µL of the fortification solution (9.90 µg/L or 99.0 µg/L) was transferred to 10-mL volumetric flask and the volume was adjusted to 10 mL with the water sample (pp. 37-39 of MRID 51130401). An aliquot (0.5 mL) of the fortified water sample was mixed with 0.5 mL of HPLC water and transferred to an autosampler vial for LC-MS/MS analysis.

Samples were analyzed for triallate using an LC-MS/MS Xevo TQD or LC-MS/MS Xevo TQS (pp. 28, 38-39 of MRID 51130401). The LC/MS conditions for triallate consisted of a Phenomenex Kinetex XB-C_{18} column (2.1 x 50 mm, 5-µm; column temperature room temperature), a mobile phase of (A) HPLC water with 0.01% formic acid and (B) HPLC methanol with 0.1% formic acid [percent A:B (v:v) at 0.00-1.00 min. 60:40, 4.00-8.00 min. 5:95, 8.10 min. 60:40] and MS/MS detection in positive ion mode (source temperature 150°C, desolvation temperature 650°C). Injection volume was 15 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 304→86 and *m/z* 304→128 for triallate. Retention time was 6.9 minutes for triallate.

For TCPSA, 50 µL of the fortification solution (10.6 µg/L or 106 µg/L) was transferred to 5-mL volumetric flask and the volume was adjusted to 5 mL with the water sample (pp. 40-42 of MRID 51130401). An aliquot (unspecified volume) of the fortified water sample was centrifuged (if necessary), filtered (0.2 µm PTFE), and transferred to an autosampler vial for LC-MS/MS analysis.

Samples were analyzed for TCPSA using an LC-MS/MS Xevo TQD or LC-MS/MS Xevo TQS (pp. 28, 40-42 of MRID 51130401). The LC/MS conditions for TCPSA consisted of a (primary analysis) Phenomenex Phenyl Hexyl column (2.1 x 50 mm, 5-µm; column temperature room temperature) or (confirmatory analysis) Phenomenex Kinetex C18 column (2.1 x 50 mm, 5-µm; column temperature room temperature), a mobile phase of (A) HPLC water with 0.01% formic acid and (B) HPLC acetonitrile [percent A:B (v:v) at 0.00 min. 90:10, 6.00-9.00 min. 10:90, 9.10-15.0 min. 90:10] and MS/MS detection in negative ion mode (source temperature 150°C, desolvation temperature 650°C). Injection volume was 25 µL. One ion transition was monitored for TCPSA primary and confirmatory analysis: *m/z* 223→80. Retention times were 3.3-3.4 and 1.9-2.0 minutes for TCPSA for primary and confirmatory analysis, respectively.

In the ILV, the ECM was performed as written, except for multiple modifications of the analytical parameters, equipment, and instruments (pp. 11-13; 16-22 of MRID 51130402). For both analytes, a Dionex Ultimate 3000 with AB Sciex API 5500 QTrap LC-MS/MS was used. The LC/MS conditions for triallate were as follows: Phenomenex Luna C18(2) column (2.0 x 150 mm, 5.0-µm; column temperature 30°C), a mobile phase of (A) water with 0.01% formic acid and (B) methanol with 0.1% formic acid [percent A:B (v:v) at 0.00-1.00 min. 60:40, 10.0-15.0 min. 5:95, 15.1-19.0 min. 60:40] and MS/MS detection in positive ion mode (interface temperature 550°C). Injection volume was 30 µL (increased from 15 µL). Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 304→86 and *m/z* 304→128 for triallate. These were the same as those of the ECM. Retention time was *ca*. 14.4 minutes for triallate. The LC/MS conditions for TCPSA primary analysis were as follows: Thermo Betasil Phenyl-Hexyl column (2.1 x 150 mm, 3.0-µm; column temperature 30°C), a

mobile phase of (A) water with 0.01% formic acid and (B) acetonitrile [percent A:B (v:v) at 0.00 min. 90:10, 8.00-11.0 min. 10:90, 11.1-16.0 min. 90:10] and MS/MS detection in negative ion mode (interface temperature 650°C). Injection volume was 25 µL. The LC/MS conditions for TCPSA confirmatory analysis were as follows: Phenomenex Luna C18(2) column (2.0 x 150 mm, 5.0-µm; column temperature 30°C), a mobile phase of (A) water with 0.01% formic acid and (B) acetonitrile [percent A:B (v:v) at 0.00 min. 90:10, 6.00-11.0 min. 10:90, 11.1-16.0 min. 90:10] and MS/MS detection in negative ion mode (interface temperature 650°C). Injection volume was 15 µL (decreased from 25 µL). One ion transition was monitored for TCPSA primary and confirmatory analysis: *m/z* 223→80. This was the same as that of the ECM. Retention time was *ca*. 6.6 minutes for TCPSA primary and confirmatory analysis. The LC columns were longer than those of the ECM, and the mobile phase polarity switches were adjusted because of the longer LC column length. The RTs were also affected by the LC column switches.

The Limit of Quantification (LOQ) was 0.100 µg/L for triallate and TCPSA in water in the ECM and ILV (pp. 10-11, 57-58, 86-87 of MRID 51130401; p. 11 of MRID 51130402). The Limit of Detection (LOD) in drinking, surface, and ground water was 0.02 µg/L for triallate and 0.005-0.006 µg/L for TCPSA in the ECM. The LOD was reported in the ILV as 30% of the LOQ (0.03 µg/L) for both analytes in drinking water. Since the reported method 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.

II. Recovery Findings

ECM (MRID 51130401): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of triallate and its metabolite TCPSA in three water matrices at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ; Tables 14-25, pp. 46-57; Tables 50-61, pp. 75-86). Performance data (recovery results) from primary and confirmatory analyses were comparable, except for the LOQ analyses of triallate in drinking and ground waters and the 10×LOQ analysis of TCPSA in ground water. Two water matrices per water type were collected for the study: drinking water matrices were VAL268/1 (from a public water supply in Grao de Castellón, Spain) and VAL268/2 (from a public water supply in Castellón, Spain); surface water matrices were VAL268/13 (from Clot de la Mare de Deu in Burriana, Spain) and VAL268/4 (from Millars River in Castellón, Spain); and ground water matrices were VAL268/5 (from a well in Castellón, Spain) and VAL268/6 (from a well in Burriana, Spain; p. 36). Each water matrix sample was divided into different subsamples which were denoted by sequential alphabetical naming. For triallate validation, drinking water (Sample ID: VAL268/1/C; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/C; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/5/C; pH 8.5, <1.0 mg/L dissolved oxygen content, 700 mg/L hardness, 1356 µS/cm conductivity at 20°C) were used (pp. 36-37, 45, 50, 54, 75, 79, 83; Appendix 2, pp. 106-111). For TCPSA validation, drinking water (Sample ID: VAL268/1/A; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/A; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L

hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/6/A; 8.4, 1.2 mg/L dissolved oxygen content, 834 mg/L hardness, 1554 µS/cm conductivity at 20°C) were used. Water characterization was performed by Eurofins IPROMA Laboratorio y Asesoría, Castellón, Spain. The reviewer noted that water matrices VAL268/2 and VAL268/3 were not used in the validations and different ground water matrices were used for the triallate and TCPSA validations.

ILV (MRID 51130402): Mean recoveries and RSDs were within guideline requirements for analysis of triallate and its metabolite TCPSA in one water matrix at fortification levels of 0.100 µg/L (LOQ) and 1.00 µg/L (10×LOQ; pp. 13, 27). Performance data (recovery results) from primary and confirmatory analyses were comparable. Drinking water (pH 7.66, 0.7 mg/L total organic carbon, 2.42 mmol/L hardness, 423 µS/cm conductivity at 25°C) obtained from a local water supplier in Pforzheim, Germany, was used in the study (p. 16). The ECM method (Gowan Study No. 268-16) for the quantitation and confirmation analyses of triallate and TCPSA in one water matrix was validated with multiple modifications of the analytical parameters, equipment, and instruments (pp. 11-13; 16-22). The LC columns of the ILV differed from those used in the ECM. The ILV LC columns were also longer than those of the ECM, and the mobile phase polarity switches were adjusted because of the longer LC column length. Since the ECM method (Gowan Study No. 268-16) was a dilution then direct injection for triallate and centrifugation/filtration then direct injection for TCPSA, the specifics of the method mainly involved the analytical portion. No ILV communication or discussion was provided which explained the modifications to the analytical portion of the ECM; therefore, it could not be determined if the ECM analytical portion of the method was repeatable as written. Additionally, the number of trials required to validate the ECM was not reported in the ILV.

Table 2. Initial Validation Method Recoveries for Triallate and TCPSA in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Drinking Water Sample IDs: VAL268/1/C (for Triallate) and VAL268/1/A (for TCPSA)					
	Quantitation ion					
Triallate	0.100 (LOQ)	5	89.8-99.2	93.3	3.7	3.9
	1.00	5	84.9-94.9	90.2	3.7	4.0
TCPSA	0.100 (LOQ)	5	106.1-109.5	107.7	1.5	1.4
	1.00	5	87.2-89.9	88.6	1.3	1.4
	Confirmation ion or analysis					
Triallate	0.100 (LOQ)	5	87.3-111.8	98.5	10.9	11.1
	1.00	5	85.2-94.7	91.7	3.9	4.2
TCPSA	0.100 (LOQ)	5	104.5-113.5	109.1	3.3	3.0
	1.00	5	86.4-90.9	88.7	2.0	2.2
	Surface Water Sample IDs: VAL268/4/C (for Triallate) and VAL268/4/A (for TCPSA)					
	Quantitation ion					
Triallate	0.100 (LOQ)	5	80.1-96.7	87.1	6.7	7.7
	1.00	5	82.3-95.1	90.7	5.7	6.3
TCPSA	0.100 (LOQ)	5	98.6-103.1	100.4	1.9	1.9
	1.00	5	98.4-102.4	100.2	1.4	1.5
	Confirmation ion or analysis					
Triallate	0.100 (LOQ)	5	74.5-89.9	84.2	6.3	7.5
	1.00	5	85.3-89.4	87.5	1.9	2.2
TCPSA	0.100 (LOQ)	5	84.5-105.3	94.1	7.6	8.0
	1.00	5	98.9-103.5	101.2	1.9	1.9
	Ground Water Sample IDs: VAL268/5/C (for Triallate) and VAL268/6/A (for TCPSA)					
	Quantitation ion					
Triallate	0.100 (LOQ)	5	80.9-98.0	89.9	7.2	8.0
	1.00	5	76.1-101.5	91.1	10.1	11.1
TCPSA	0.100 (LOQ)	5	99.7-107.2	103.3	3.3	3.2
	1.00	5	93.6-101.0	97.3	3.2	3.3
	Confirmation ion or analysis					
Triallate	0.100 (LOQ)	5	71.3-101.5	83.9	11.0	13.1
	1.00	5	77.0-95.1	88.2	7.5	8.6
TCPSA	0.100 (LOQ)	5	108.3-111.8	110.2	1.5	1.4
	1.00	5	102.9-119.4	110.2	5.5	5.0

Data (uncorrected recovery results, pp. 40, 43; Appendix 4, p. 130) were obtained from pp. 11-14, 45, 50, 54, 75, 79, 83; Tables 14-25, pp. 46-57; Tables 50-61, pp. 75-86 of MRID 51130401; and DER Excel Attachment.

1 Two water matrices per water type were collected for the study: drinking water matrices were VAL268/1 (from a public water supply in Grao de Castellón, Spain) and VAL268/2 (from a public water supply in Castellón, Spain ; surface water matrices were VAL268/13 (from Clot de la Mare de Deu in Burriana, Spain) and VAL268/4 (from Millars River in Castellón, Spain); and ground water matrices were VAL268/5 (from a well in Castellón, Spain and VAL268/6 (from a well in Burriana, Spain; p. 36). Each water matrix sample was divided into different subsamples which were denoted by sequential alphabetical naming. For triallate validation, drinking water (Sample ID: VAL268/1/C; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/C; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/5/C; pH 8.5, <1.0

mg/L dissolved oxygen content, 700 mg/L hardness, 1356 µS/cm conductivity at 20°C) were used (pp. 36-37, 45, 50, 54, 75, 79, 83; Appendix 2, pp. 106-111). For TCPSA validation, drinking water (Sample ID: VAL268/1/A; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/A; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/6/A; 8.4, 1.2 mg/L dissolved oxygen content, 834 mg/L hardness, 1554 µS/cm conductivity at 20°C) were used. Water characterization was performed by Eurofins IPROMA Laboratorio y Asesoría, Castellón, Spain. The reviewer noted that water matrices VAL268/2 and VAL268/3 were not used in the validations and different ground water matrices were used for the triallate and TCPSA validations.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 304→86 and *m/z* 304→128 for triallate. One ion transition was monitored for TCPSA primary and confirmatory analysis: *m/z* 223→80. Different analytical columns were used for primary (Phenomenex Phenyl Hexyl column) and confirmatory (Phenomenex Kinetex C18 column) analyses of TCPSA.

3 Standard deviations were reviewer-calculated based on recovery values in the study report since the study author did not report these values. Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Triallate and TCPSA in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[3]	Relative Standard Deviation (%)
	Drinking Water					
	Quantitation ion					
Triallate	0.100 (LOQ)	5	95-98	96	1	1.4
	1.00	5	101-106	103	2	2.2
TCPSA	0.100 (LOQ)	5	96-100	98	2	1.7
	1.00	5	100-105	102	2	2.0
	Confirmation ion or analysis					
Triallate	0.100 (LOQ)	5	96-100	97	2	1.6
	1.00	5	103-107	105	2	1.4
TCPSA	0.100 (LOQ)	5	93-100	96	3	2.8
	1.00	5	101-114	106	5	4.8

Data (uncorrected recovery results, pp. 22-23) were obtained from pp. 13, 27 of MRID 51130402; DER Excel Attachment.

1 The drinking water (pH 7.66, 0.7 mg/L total organic carbon, 2.42 mmol/L hardness, 423 µS/cm conductivity at 25°C) obtained from a local water supplier in Pforzheim, Germany, was used in the study (p. 16).

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 304→86 and *m/z* 304→128 for triallate. These were the same as those of the ECM. One ion transition was monitored for TCPSA primary and confirmatory analysis: *m/z* 223→80. This was the same as that of the ECM. Different analytical columns were used for primary (Thermo Betasil Phenyl-Hexyl column) and confirmatory (Phenomenex Luna C18(2) column) analyses of TCPSA.

3 Standard deviations were reviewer-calculated based on recovery values in the study report since the study author did not report these values. Rules of significant figures were followed.

III. Method Characteristics

The LOQ was 0.100 µg/L for triallate and TCPSA in water in the ECM and ILV (pp. 10-11, 57-58, 86-87 of MRID 51130401; p. 11 of MRID 51130402). In the ECM, the LOQ was defined as the lowest fortification level yielding an acceptable RSD of <20%; no calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM. In the ILV, the LOQ was reported from the ECM without justification. The LOD in drinking, surface, and ground water was 0.02 µg/L for triallate and 0.005-0.006 µg/L for TCPSA in the ECM. The LOD was reported in the ILV as 30% of the LOQ (0.03 µg/L) for both analytes in drinking water. The LOD was calculated in the ECM using chromatographic software and defined as the concentration corresponding to a chromatographic peak with a signal/noise ratio of 3. ECM calculated LODs in all water matrices were 0.01-0.02 for the primary and confirmatory analyses for triallate and 0.004-0.006 µg/L for the primary and confirmatory analyses for TCPSA**.**

Since the reported method 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.

Table 4. Method Characteristics

Analyte[1]			**Triallate**	**TCPSA**
Limit of Quantitation (LOQ)*	ECM		0.100 µg/L	
	ILV			
Limit of Detection (LOD)	ECM (calc)	Drinking	0.02 µg/L (Q) 0.01 µg/L (C)	0.005 µg/L (Q) 0.004 µg/L (C)
		Surface	0.01 µg/L (Q) 0.02 µg/L (C)	0.004 µg/L (Q) 0.006 µg/L (C)
		Ground	0.01 µg/L (Q) 0.02 µg/L (C)	0.004 µg/L (Q) 0.006 µg/L (C)
	ILV (method, Drinking)		0.03 µg/L (Q & C; 30% of the LOQ)	
Linearity (calibration curve r and concentration range)	ECM[1]	Drinking	r = 0.9984/1.0000 (Q) r = 0.9998/1.0000 (C)	r = 0.9983/0.9994 (Q) r = 0.9988/0.9994 (C)
		Surface	r = 1.0000/0.9996 (Q) r = 0.9996/0.9990 (C)	r = 0.9998/1.0000 (Q) r = 0.9981/0.9989 (C)
		Ground	r = 0.9998/0.9998 (Q) r = 0.9996/0.9998 (C)	r = 0.9999/1.0000 (Q) r = 0.9983/0.9998 (C)
		Range	0.01-2.5 µg/L (corresponding to 0.02-5 µg a.i./L in water)	0.025-2.5 µg/L
	ILV	Drinking	r = 0.99946 (Q) r = 0.99816 (C)	r = 0.99932 (Q) r = 0.99955 (C)
	Range		0.0125-2.5 µg/L	0.025-2.5 µg/L
Repeatable	ECM[2]		Yes at LOQ and 10×LOQ (characterized drinking, surface, and ground water matrices).	
	ILV[3,4]		Yes at LOQ and 10×LOQ (characterized drinking water matrix).	
Reproducible			Yes for 0.100 µg/L (LLMV)* and 1.00 µg/L in tested water matrices with differing LC columns[5]	
Specific	ECM		Yes, matrix interferences were <10% of the LOQ (based on peak height) around analyte RT.	Yes, matrix interferences were <10% of the LOQ (based on peak height) around analyte RT. In ground water, some contaminants (peak height *ca.* 10-80% of LOQ peak) were observed near LOQ analyte peak.[6]
	ILV		Yes, matrix interferences were *ca.* 31% of the LOQ (based on peak area)[7]; however, matrix-match calibration standards were used.	Yes, matrix interferences were <10% of the LOQ (based on peak area). Matrix-match calibration standards were used.

Data were obtained from pp. 10-11, 57-58, 86-87 (LOQ/LOD); Tables 14-25, pp. 46-57; Tables 50-61, pp. 75-86 (recovery data & correlation coefficients); Appendix, 5, Figures 3-18, pp. 131-138 (calibration curves); Appendix 6, Figures 19-70, pp. 139-156 (chromatograms) of MRID 51130401; p. 11 (LOQ); pp. 13, 27 (recovery data); p. 12 (linearity data); Appendix 2, Figures 1-4, pp. 31-34 (calibration curves); Appendix 3, Figures 5-14, pp. 35-39 (chromatograms) of MRID 51130402; and DER Excel Attachment. Q = Quantitation ion transition; C = Confirmation ion transition or analysis.

* 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 Correlation coefficients (r) values are presented for LOQ/10×LOQ analyses.

2 In the ECM, two water matrices per water type were collected for the study: drinking water matrices were VAL268/1 (from a public water supply in Grao de Castellón, Spain) and VAL268/2 (from a public water supply in Castellón, Spain); surface water matrices were VAL268/13 (from Clot de la Mare de Deu in Burriana, Spain) and VAL268/4 (from Millars River in Castellón, Spain ; and ground water matrices were VAL268/5 (from a well in Castellón, Spain) and VAL268/6 (from a well in Burriana, Spain; p. 36 of MRID 51130401). Each water matrix sample was divided into different subsamples which were denoted by sequential alphabetical naming. For triallate validation, drinking water (Sample ID: VAL268/1/C; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/C; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/5/C; pH 8.5, <1.0 mg/L dissolved oxygen content, 700 mg/L hardness, 1356 µS/cm conductivity at 20°C) were used (pp. 36-37, 45, 50, 54, 75, 79, 83; Appendix 2, pp. 106-111). For TCPSA validation, drinking water (Sample ID: VAL268/1/A; pH 7.2, <1.0 mg/L dissolved oxygen content, 8 mg/L hardness, 104 µS/cm conductivity at 20°C), surface water (Sample ID: VAL268/4/A; pH 8.4, <1.0 mg/L dissolved oxygen content, 548 mg/L hardness, 1730 µS/cm conductivity at 20°C), and ground water (Sample ID: VAL268/6/A; 8.4, 1.2 mg/L dissolved oxygen content, 834 mg/L hardness, 1554 µS/cm conductivity at 20°C) were used. Water characterization was performed by Eurofins IPROMA Laboratorio y Asesoría, Castellón, Spain. The reviewer noted that water matrices VAL268/2 and VAL268/3 were not used in the validations and different ground water matrices were used for the triallate and TCPSA validations.

3 In the ILV, drinking water (pH 7.66, 0.7 mg/L total organic carbon, 2.42 mmol/L hardness, 423 µS/cm conductivity at 25°C) obtained from a local water supplier in Pforzheim, Germany, was used in the study (p. 16 of MRID 51130402).

4 The ILV validated the ECM method (Gowan Study No. 268-16) for the quantitation and confirmation analyses of triallate and TCPSA in one water matrix with multiple modifications of the analytical parameters, equipment, and instruments (pp. 11-13; 16-22 of MRID 51130402). The LC columns of the ILV differed from those used in the ECM. The ILV LC columns were also longer than those of the ECM, and the mobile phase polarity switches were adjusted because of the longer LC column length. Since the ECM method (Gowan Study No. 268-16) was a dilution then direct injection for triallate and centrifugation/filtration then direct injection for TCPSA, the specifics of the method mainly involved the analytical portion. No ILV communication or discussion was provided which explained the modifications to the analytical portion of the ECM; therefore, it could not be determined if the ECM analytical portion of the method was repeatable as written. Additionally, the number of trials required to validate the ECM was not reported in the ILV.

5 ECM LC columns were as follows: for triallate [Phenomenex Kinetex XB-C_{18} column (2.1 x 50 mm, 5-µm)]; and for TCPSA [primary analysis- Phenomenex Phenyl Hexyl column (2.1 x 50 mm, 5-µm; column temperature room temperature) or confirmatory analysis - Phenomenex Kinetex C18 column (2.1 x 50 mm, 5-µm); pp. 28, 38-39 of MRID 51130401]. ILV LC columns were as follows: for triallate [Phenomenex Luna C18(2) column (2.0 x 150 mm, 5.0-µm)]; and for TCPSA [primary analysis - Thermo Betasil Phenyl-Hexyl column (2.1 x 150 mm, 3.0-µm) or confirmatory analysis - Phenomenex Luna C18(2) column (2.0 x 150 mm, 5.0-µm; pp. 11-13; 16-22 of MRID 51130402].

6 Based on Appendix 6, Figure 55, p. 151 of MRID 51130401.

7 Based on Appendix 3, Figures 7, p. 37 and Figure 9, p. 38 of MRID 51130402. The reviewer noted that the study author quantified the residues in the controls as <LOQ in Appendix 3, Figure 9, p. 38 (See Reviewer Comment #7).

IV. Method Deficiencies and Reviewer's Comments

1. No ILV communications were summarized or reported for review. Communications between the ECM and ILV should be addressed in order to assess the independence of the ILV from the ECM.

2. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 10-11, 57-58, 86-87 of MRID 51130401; p. 11 of MRID 51130402). Since the reported method 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 (pp. 10-11, 57-58, 86-87 of MRID 51130401; p. 11 of MRID 51130402). The lowest concentration tested with sufficiently accurate and precise recoveries is the LLMV. Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the ECM reported method LOQ for in the tested water matrices (0.100 µg/L).

In the ECM, the LOQ was defined as the lowest fortification level yielding an acceptable RSD of <20%; no calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM. In the ILV, the LOQ was reported from the ECM without justification. The LOD was calculated in the ECM using chromatographic software and defined as the concentration corresponding to a chromatographic peak with a signal/noise ratio of 3. The ECM chromatographic software used for LOD calculation was not specified. The LOD was reported in the ILV as 30% of the LOQ. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

3. The reproducibility of the ECM was difficult to evaluate (Gowan Study No. 268-16) since the ILV modifications mainly involved the analytical portion of the ECM. The ILV validated the method with multiple modifications to the analytical parameters, equipment, and instruments (pp. 11-13; 16-22; Appendix 5, p. 50 of MRID 51130402). The ECM method (Gowan Study No. 268-16) was a dilution then direct injection for triallate and centrifugation/filtration then direct injection for TCPSA. While no sample processing modifications were made by the ILV during validation, the LC columns of the ILV differed from those used in the ECM. The ILV LC columns were also longer than those of the ECM, and the mobile phase polarity switches were adjusted because of the longer LC column length. ECM LC columns were as follows: for triallate [Phenomenex Kinetex XB-C_{18} column (2.1 x **50 mm**, 5-µm)]; and for TCPSA [primary analysis- Phenomenex Phenyl Hexyl column (2.1 x **50 mm**, 5-µm; column temperature room temperature) or confirmatory analysis - Phenomenex Kinetex C18 column (2.1 x **50 mm**, 5-µm); pp. 28, 38-39 of MRID 51130401]. ILV LC columns were as follows: for triallate [Phenomenex Luna C18(2) column (2.0 x **150 mm**, 5.0-µm)]; and for TCPSA [primary analysis - Thermo Betasil Phenyl-Hexyl column (2.1 x **150 mm**, 3.0-µm) or confirmatory analysis - Phenomenex Luna C18(2) column (2.0 x **150 mm**, 5.0-µm; pp. 11-13; 16-22 of MRID 51130402]. No ILV communication or discussion was provided which explained the modifications to the analytical portion of the ECM; therefore, it could not be determined if the ECM analytical portion of the method was repeatable as written.

4. The number of trials required to validate the ECM was not reported in the ILV. Due to the numerous ILV modifications to the ECM the number of trials used to validate the ECM should be reported.

5. In the ILV, only one water matrix was used for validation, whereas three water matrices were used for validation in the ECM (p. 36 of MRID 51130401; p. 16 of MRID 51130402). The ILV used more than one sample set for validation. Although the number of matrices required for independent laboratory validations is not specified in OCSPP Guideline 850.6100, the ILV should be performed with at least as many matrices as that

of the ECM to show equivalent reproducibility.

6. Matrix-matched calibration standards were used in the ECM and ILV for matrices which did not exhibit significant (>20%) matrix effects. Matrix effects were studied in the ECM (pp. 11, 65-70, 92-97 of MRID 51130401). For triallate, no significant (>20%) matrix effects were observed for any matrix, so solvent-based calibration standards were used for quantification. For TCPSA, significant matrix effects were only observed in the ground water matrix; however, matrix-match calibration standards were used for quantification in all systems.

   Matrix effects were studied in the ILV (p. 28 of MRID 51130402). No significant (>20%) matrix effects were observed for either analyte in the drinking water matrix; however, matrix-match calibration standards were used for quantification.

7. The reviewer calculated the matrix interferences for triallate as *ca*. 31% of the LOQ [based on peak areas of 8685/3388 (Q/C) for the control and 28047/11098 (Q/C) for the LOQ fortified sample; Appendix 3, Figures 7, p. 36 and Figure 9, p. 37 of MRID 51130402]. However, the reviewer noted that the study author quantified the residues in the controls as <LOQ (Appendix 3, Figure 9, p. 38). The reviewer considered the use of matrix-matched calibration standards as compensation for the observed matrix interferences.

8. The stability of the water extracts fortified with either triallate at 1 µg/L or TCPSA at 1 µg/L was determined to be up to 7 days when stored in a freezer at <-18°C (pp. 11, 59-65, 88-92 of MRID 51130401). Mean concentrations were 70-110% of the total concentration for all samples after frozen storage.

   In the ILV, it was reported that analyses were performed within 24 hours after sample preparation (p. 29 of MRID 51130402). No further stability testing was performed in the ILV.

9. The reviewer noted that there was a previously submitted water ECM/ILV for triallate and TCPSA (Smithers Viscient Study No. 12791.6265; LOQ of 0.100 µg/L using LC/MS/MS): ECM MRID 50432501 (Wu, X. 2016. Validation of the Analytical Method for the Determination of Triallate and TCPSA in Aqueous Matrices by LC-MS/MS. Smithers Viscient Study No. 12791.6265. Report prepared by Smithers Viscient, Wareham Massachusetts, and sponsored and submitted by Gowan Company, Yuma, Arizona; 115 pages. Final report issued December 19, 2016) and ILV MRID (50352202; MacGregor, J.A., E.S. Bodle, R.L. VanHoven. 2017. Independent Laboratory Validation of a Method for the Determination of Triallate and TCPSA in Aqueous Matrices by LC/MS/MS. Project No. 334C-134. Report prepared by Wildlife International (now doing business as EAG), Easton, Maryland, sponsored and submitted by Gowan Company, Yuma, Arizona; 113 pages. Final report issued July 21, 2017).

10. The time requirement for the method was not reported in the ECM or ILV.

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.

USEPA. 2012. *Environmental Chemistry Method Guidance*. Memorandum From D. Brady to Environmental Fate and Effects Division. December 20, 2012. Environmental Fate and Effects Division. Office of Pesticide Programs. Office of Chemical Safety and Pollution Prevention. U.S. Environmental Protection Agency. Available at: https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/environmental-chemistry-methods-guidance-pesticides.

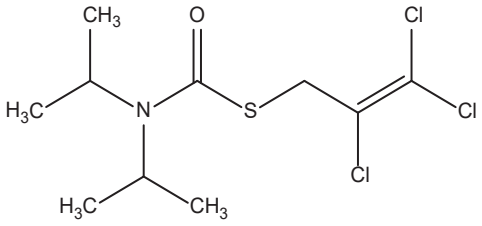
USEPA. 2014. Registration Review Problem Formulation for Triallate. Office of Chemical Safety and Pollution Prevention. Environmental Fate and Effects Division. August 21, 2019. DP 419162.

40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 344-347, and Revision 2; 2015 and 2016.

Attachment 1: Chemical Names and Structures

Triallate

IUPAC Name: S-2,3,3-trichloroallyl diisopropyl(thiocarbamate)
CAS Name: S-(2,3,3-trichloro-2-propen-1-yl) N,N-bis(1-methylethyl)carbamothioate
CAS Number: 2303-17-5
SMILES String: CC(C)N(C(C)C)C(=O)SCC(Cl)=C(Cl)Cl



TCPSA (as sodium salt)

IUPAC Name: 2,3,3-trichloropropen-2-sulfonic acid sodium salt
CAS Name: Not reported
CAS Number: 65600-61-5
SMILES String: Not found