

Analytical method for diuron, DCPMU, and mCPDMU in water

Reports: ECM: EPA MRID No.: 47033303. Reichert, N. 2006. Analytical Method for the Determination of Diuron, Linuron, and Metabolites in Water. Report prepared by SGS Institut Fresenius GmbH, Taunusstein, Germany, and sponsored and submitted by E.I. du Pont de Nemours and Company, Newark, Delaware; 94 pages. DuPont Study No.: DuPont-19220. SGS Institut Fresenius Study No.: IF-06/00588606. Final report issued August 4, 2006.

ILV: EPA MRID No.: 50677002. DeVellis, S. 2018. Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Diuron and Metabolites in Water by LC-MS/MS. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored and submitted by ADAMA, Raleigh, North Carolina, and Tessenderlo Kerley, Inc., Phoenix, Arizona; 107 pages. Smithers Viscient Study No.: 14134.6112. Final report issued August 31, 2018.

Document No.: MRIDs 47033303 & 50677002

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with German and OECD Good Laboratory Practices (GLP; OECD-DOC.ENV/MC/CHEM(98)17; 1998; pp. 3-4 of MRID 47033303). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Certification of Authenticity statements were provided (pp. 2-6).

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, as accepted by OECD GLP (1998; p. 3 of MRID 50677002). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was included with the Quality Assurance statement.

Classification: This analytical method is classified as **acceptable**. The method generally satisfied the repeatability and reproducibility criteria, with RSDs < 20% and mean recoveries in the range of 70 – 120%; however, the specificity of the method for diuron in ground water was not well-supported by the ILV representative chromatograms of the quantitation ion transition due to matrix interferences.


PC Code: 035505

EFED Final William Gardner, Ph.D.,

Reviewer: Environmental Scientist

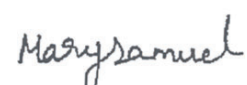
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Lisa Muto, M.S.,
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Signature: 
Date: 05/15/2020

**CDM/CSS-
Dynamac JV**

Reviewers: Mary Samuel, M.S.,
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Date: 05/15/2020

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Executive Summary

The analytical method, DuPont-19220, is designed for the quantitative determination of diuron, linuron, DCPMU, DCPU, mCPDMU and demethylated linuron in water at the stated LOQ of 0.05 µg/L using LC/MS/MS; however, only diuron, DCPMU, and mCPDMU were evaluated in this method validation. The LOQ is less than the lowest toxicological level of concern of 3.08, 7.1 and 107 µg/L in water for diuron, DCPMU, and mCPDMU, respectively (USEPA 2015, USEPA 2019a, USEPA 2019b). Based on the performance data submitted by the ILV and ECM, the LLMV was equivalent to the reported method LOQ for the three analytes in the tested water matrices. The ECM validated the method using drinking/tap/ground and surface water matrices; the ILV validated the method using ground and surface water matrices, but the ground water was not well-characterized. The method for diuron, DCPMU, and mCPDMU in water was validated in the first trial for surface water and the second trial for ground water with insignificant modifications to the analytical parameters, the temperature of the final nitrogen evaporation was lowered, and omission of filtering prior to LC/MS/MS analysis. The ILV noted that an initial ILV attempt of the method using ground water was unacceptable due to execution of the validation. No ILV modifications were made based on the failed first ILV trial in ground water but a more sensitive LC/MS/MS instrument was employed. All ILV and ECM data regarding repeatability, accuracy, and, precision were satisfactory for diuron, DCPMU and mCPDMU. All ILV data regarding linearity and specificity were satisfactory for the three analytes; however, the specificity of the method for diuron in ground water was not well-supported by the ILV representative chromatograms of the quantitation ion transition due to matrix interferences. Also, a nearby significant contaminant was observed in all diuron chromatograms of the quantitation ion transition. The ILV calculated LOD for diuron (Q) in ground water (0.04 µg/L) was higher than the Method Detection Limit and 80% of the method LOQ. All ECM data regarding linearity and specificity were satisfactory for the three analytes, but only one set of calibration curves were provided.

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						
Diuron	47033303 ²	50677002 ³		Water	04/08/2006	ADAMA and Tessenderlo Kerley, Inc. ⁴	LC/MS/MS	0.05 µg/L
DCPMU								
mCPDMU								

1 Diuron = 3-(3,4-Dichlorophenyl)-1,1-dimethylurea; DCPMU = 3-(3,4-Dichlorophenyl)-1-methylurea; mCPDMU = 3-(3-Chlorophenyl)-1,1-dimethylurea (pp. 19-21 of MRID 47033303, and pp. 14-15 of MRID 50677002).

2 In the ECM, drinking water (Specimen Code: 010/6202451; pH 7.75, conductivity 503 µS/cm, total organic carbon 2.3 mg/L) was tap water generated from ground water taken at SGS Institut Fresenius, Taunusstein (p. 25 of MRID 47033303). The surface water (Specimen Code: 010/6202452; pH 8.03, conductivity 759 µS/cm, total organic carbon 5.4 mg/L) was collected from the Großbach river from a ditch in Limburg. Water characterization was performed at the ECM.

3 In the ILV, ground water was in-house well water, unadulterated water from a 100-meter bedrock well which is considered soft with a typical hardness of <160 mg (as CaCO₃; p. 16 of MRID 50677002). The surface water (Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L) was collected from Weweantic River, West Wareham, Massachusetts. The surface water was characterized by Agvise Laboratories, Northwood, North Dakota.

4 The reviewer noted that the ECM was Sponsored by E.I. DuPont de Nemours and Company.

I. Principle of the Method

Paper-filtered (185 mm, Schleicher and Schuell Part No. 10311647) water samples (20 mL) were fortified (0.05 mL of 0.02 µg/mL or 0.10 mL of 0.10 µg/mL fortification solution) and treated with 0.2 mL of methanol and applied to a Supelco solid phase extraction (SPE) cartridge (reverse-phase, C-18) which was pre-conditioned with 5 mL of methanol and 2 x 5 mL of deionized water (pp. 15, 18-19, 22-23, 25-26 of MRID 47033303). After the sample was eluted through the cartridge (cartridge not allowed to go to dryness), the cartridge was rinsed twice with 2 mL of deionized water then dried using vacuum for *ca.* 10 minutes. An aliquot (0.5 mL) of methanol:deionized water (8:2, v:v) was added to the cartridge. After 2 minutes of incubation, the analytes were eluted with 2 mL of methanol:deionized water (8:2, v:v). The eluate was evaporated to <1 mL using a gentle stream of nitrogen at 40°C (water bath). The extract was diluted with methanol to a final volume of 1 mL then filtered (0.2 µm PTFE) prior to LC/MS/MS analysis.

The method cautioned that labware is clean, test materials are handled properly, and LC/MS/MS instrument is maintained to prevent contamination and cross-over (p. 37 of MRID 47033303).

Samples were analyzed for diuron using an Agilent G1316A/G1311A HPLC coupled with an Applied Biosystems API 3000 mass spectrometer using electrospray ionization (ESI) operated in the positive ion mode with multiple reaction monitoring (MRM; pp. 19, 26-30 of MRID 47033303). The following LC conditions were used: Hypurity C8 column (3.0 mm x 150 mm, 5.0 µ; column temperature 40°C), mobile phase of (A) methanol and (B) 0.2% formic acid [mobile gradient phase of percent A:B (v:v) at 0.00 min. 30:70, 10.00 min. 90:10, 15.00-15.10 min. 99:1, 17.10-23.00 min. 30:70] and injection volume of 20 µL. MS temperature was 350°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): m/z 233→72 and m/z 233→46 for diuron; m/z 219→127 and m/z 219→162 for DCPMU; and m/z 199→72 and m/z 199→46 for mCPDMU. Reported retention times were *ca.* 8.7, 8.6, and 6.9 minutes for diuron, DCPMU, and mCPDMU, respectively.

The ILV performed the ECM methods for each analyte as written, except for insignificant modifications to the analytical parameters, the final evaporation to <1 mL using a gentle stream of nitrogen was conducted at 25°C, and omission of filtering (0.2 µm PTFE) prior to LC/MS/MS analysis (pp. 16, 19-23 of MRID 50677002). A Supelchem LC-18 SPE column (500 mg, 3 mL) was used. Samples were analyzed for diuron using a Shimadzu LC-20AD HPLC coupled with an AB Sciex API 4000 LC/MS/MS equipped with an AB MDS Sciex ESI Turbo V source. The LC/MS/MS parameters were the same as those of the ECM, except that the injection volume was decreased to 10 µL for surface water and 15 µL for ground water and the MS temperature was 500°C. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): m/z 233.12→72.10 and m/z 233.12→46.10 for diuron; m/z 219.02→161.96 and m/z 219.02→127.06 for DCPMU; and m/z 199.04→72.10 and m/z 199.04→46.19 for mCPDMU. These were essentially the same as those of the ECM, except that the quantitation and confirmation ion transitions for DCPMU were reversed. Reported retention times were *ca.* 7.7-7.8, 7.7, and 6.1 minutes for diuron, DCPMU, and mCPDMU, respectively. The ILV also noted that the calibration curve range was truncated for ground water by removing the highest two standards. The ILV modifications did not warrant an updated ECM.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.05 µg/L for diuron, DCPMU, and mCPDMU in water (p. 37 of MRID 47033303; pp. 24, 26-28 of MRID 50677002). In the ECM, the

Limit of Detection (LOD) for diuron, DCPMU, and mCPDMU was 0.01 µg/L. The LOD in water was calculated in the ILV as 0.04 µg/L (ground water) and 0.003 µg/L (surface water) for diuron, 0.007-0.008 µg/L (ground water) and 0.001-0.002 µg/L (surface water) for DCPMU, and 0.0004-0.0006 µg/L (ground water) and 0.0005-0.001 µg/L (surface water) for mCPDMU. 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.

II. Recovery Findings

ECM (MRID 47033303): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of diuron, DCPMU, and mCPDMU at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/L (10×LOQ) in two water matrices (Table 1, p. 40; Table 3, p. 42; Tables 6-7, pp. 45-46; Table 9, p. 48; and Table 12, p. 51). Two ion pair transitions were monitored for the analytes; performance data was fairly comparable between the quantitation and confirmation analyses. The recoveries were reportedly corrected for residues quantified in the controls, but residues (<30% of the LOQ) were only quantified in the surface water controls for diuron and DCPMU (p. 30; Figures 19-30, pp. 68-73; Figures 49-60, pp. 83-88). The drinking water (Specimen Code: 010/6202451; pH 7.75, conductivity 503 µS/cm, total organic carbon 2.3 mg/L) was tap water generated from ground water taken at SGS Institut Fresenius, Taunusstein (p. 25 of MRID 47033303). The surface water (Specimen Code: 010/6202452; pH 8.03, conductivity 759 µS/cm, total organic carbon 5.4 mg/L) was collected from the Großbach river from a ditch in Limburg. Water characterization was performed at the ECM.

ILV (MRID 50677002): Mean recoveries and RSDs were within guidelines for analysis of diuron, DCPMU, and mCPDMU at fortification levels of 0.05 µg/L (LOQ) and 0.50 µg/L (10×LOQ) in two water matrices (Tables 1-12, pp. 34-45). Two ion pair transitions were monitored for the analytes; performance data was comparable between the quantitation and confirmation analyses. The quantitation and confirmation ion transitions for DCPMU were reversed. The ground water was in-house well water, unadulterated water from a 100-meter bedrock well which is considered soft with a typical hardness of <160 mg (as CaCO₃; p. 16 of MRID 50677002). The surface water (Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L) was collected from Weweantic River, West Wareham, Massachusetts. The surface water was characterized by Agvise Laboratories, Northwood, North Dakota. The method for diuron, DCPMU, and mCPDMU in water was validated in the first trial for surface water and the second trial for ground water with insignificant modifications to the analytical parameters, the final evaporation to <1 mL using a gentle stream of nitrogen was conducted at 25°C, and omission of filtering (0.2 µm PTFE) prior to LC/MS/MS analysis (see Reviewer's Comment #3; pp. 16, 19-23, 27). The ILV noted that an initial ILV attempt of the method using ground water was unacceptable due to execution of the validation. No ILV modifications were made based on the failed first ILV trial in ground water but a more sensitive LC/MS/MS instrument was employed (p. 27; Appendix 3, p. 105).

Table 2. Initial Validation Method Recoveries for Diuron, DCPMU, and mCPDMU 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 transition						
Diuron	0.05 (LOQ)	5	94-108	100	7	7
	0.50	5	91-109	100	7	7
DCPMU	0.05 (LOQ)	5	85-97	91	5	5
	0.50	5	89-100	95	4	4
mCPDMU	0.05 (LOQ)	5	90-106	96	6	7
	0.50	5	91-110	102	7	7
Confirmation ion transition						
Diuron	0.05 (LOQ)	5	86-98	92	5	5
	0.50	5	84-97	92	6	6
DCPMU	0.05 (LOQ)	5	90-105	94	6	7
	0.50	5	94-110	104	7	7
mCPDMU	0.05 (LOQ)	5	80-91	85	4	5
	0.50	5	89-109	98	8	9
Surface Water						
Quantitation ion transition						
Diuron	0.05 (LOQ)	5	92-107	99	6	6
	0.50	5	93-105	100	5	6
DCPMU	0.05 (LOQ)	5	85-101	91	6	7
	0.50	5	89-97	93	3	3
mCPDMU	0.05 (LOQ)	5	82-95	89	5	5
	0.50	5	82-87	84	2	3
Confirmation ion transition						
Diuron	0.05 (LOQ)	5	96-105	101	4	4
	0.50	5	94-104	99	4	4
DCPMU	0.05 (LOQ)	5	94-107	99	5	5
	0.50	5	91-106	99	7	7
mCPDMU	0.05 (LOQ)	5	85-103	92	7	8
	0.50	5	82-91	86	3	4

Data (recovery results were corrected for residues quantified in the controls; p. 30) were obtained from Table 1, p. 40; Table 3, p. 42; Tables 6-7, pp. 45-46; Table 9, p. 48; and Table 12, p. 51 of MRID 47033303; DER Attachment 2.

1 The drinking water (Specimen Code: 010/6202451; pH 7.75, conductivity 503 µS/cm, total organic carbon 2.3 mg/L) was tap water generated from ground water taken at SGS Institut Fresenius, Taunusstein (p. 25). The surface water (Specimen Code: 010/6202452; pH 8.03, conductivity 759 µS/cm, total organic carbon 5.4 mg/L) was collected from the Großbach river from a ditch in Limburg. Water characterization was performed at the ECM.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): m/z 233→72 and m/z 233→46 for diuron; m/z 219→127 and m/z 219→162 for DCPMU; and m/z 199→72 and m/z 199→46 for mCPDMU.

3 Standard deviations were reviewer-calculated since these values were not calculated in the study report (see DER Attachment 2). The rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Diuron, DCPMU, and mCPDMU in Water^{1,2,3}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitation ion transition						
Diuron	0.05 (LOQ)	5	109-115	111	2.58	2.32
	0.50	5	92.3-97.1	95.4	1.91	2.00
DCPMU	0.05 (LOQ)	5	86.8-90.3	88.4	1.25	1.41
	0.50	5	91.2-97.6	94.5	2.47	2.62
mCPDMU	0.05 (LOQ)	5	88.5-93.8	90.2	2.15	2.38
	0.50	5	88.9-94.4	92.1	2.14	2.32
Confirmation ion transition						
Diuron	0.05 (LOQ)	5	107-115	111	3.35	3.01
	0.50	5	92.1-98.5	94.8	2.41	2.54
DCPMU	0.05 (LOQ)	5	84.9-89.7	87.6	2.05	2.34
	0.50	5	90.4-94.8	92.5	2.02	2.18
mCPDMU	0.05 (LOQ)	5	84.8-91.0	88.0	2.63	2.99
	0.50	5	89.8-95.8	92.8	2.44	2.63
Surface Water						
Quantitation ion transition						
Diuron	0.05 (LOQ)	5	73.9-83.4	78.8	4.28	5.43
	0.50	5	82.0-93.9	90.4	4.76	5.27
DCPMU	0.05 (LOQ)	5	74.2-80.7	77.7	2.42	3.12
	0.50	5	86.6-92.4	90.4	2.28	2.52
mCPDMU	0.05 (LOQ)	5	75.3-78.5	76.9	1.33	1.73
	0.50	5	83.3-94.2	91.2	4.48	4.91
Confirmation ion transition						
Diuron	0.05 (LOQ)	5	72.7-80.5	77.3	3.58	4.63
	0.50	5	89.2-108	97.5	7.43	7.62
DCPMU	0.05 (LOQ)	5	79.6-82.8	81.0	1.42	1.75
	0.50	5	86.1-99.9	93.7	4.98	5.32
mCPDMU	0.05 (LOQ)	5	74.9-83.0	78.7	2.96	3.76
	0.50	5	83.4-98.6	91.7	5.49	5.99

Data (uncorrected recovery results; pp. 25-26) were obtained from Tables 1-12, pp. 34-45 of MRID 50677002.

1 The ground water was in-house well water, unadulterated water from a 100-meter bedrock well which is considered soft with a typical hardness of <160 mg (as CaCO₃; p. 16). The surface water (Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L) was collected from Weweantic River, West Wareham, Massachusetts. The surface water was characterized by Agvise Laboratories, Northwood, North Dakota.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 233.12→72.10 and *m/z* 233.12→46.10 for diuron; *m/z* 219.02→161.96 and *m/z* 219.02→127.06 for DCPMU; and *m/z* 199.04→72.10 and *m/z* 199.04→46.19 for mCPDMU. These were essentially the same as those of the ECM, except that the quantitation and confirmation ion transitions for DCPMU were reversed.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.05 µg/L for diuron, DCPMU, and mCPDMU in water (p. 37 of MRID 47033303; pp. 24, 26-28 of MRID 50677002). In the ECM, the LOQ was defined as the lowest fortification level at which average recoveries of 70-110% and a RSD <20% are achieved. Also, at the LOQ fortification level, the analyte peak consistently represents a signal-to-noise ratio of *ca.* 11-32 to 1 for all analytes. In the ECM, the LOD for the analytes was estimated to be 0.01 µg/L, based on the limiting response analyte, DCPU (analyte not included in this DER; see Reviewer's Comment #1). The LOD was defined as the analyte concentration in matrix with a response equivalent to a signal-to-noise ratio of approximately 3 to 1. The LOD was estimated from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

$$\begin{aligned}\text{LOD} &= \{[\text{LOD signal-to-noise response (3/1)}/\text{Observed LOQ signal-to-noise response}]\} \times \text{LOQ} \\ &= (3/1)/(11/1) \times 0.05 \text{ } \mu\text{g/L} \\ &= 0.01 \text{ } \mu\text{g/L}\end{aligned}$$

No further justification of the LOQ or LOD was reported in the ECM. No justification of the LOQ was reported in the ILV.

The LOD was calculated in the ILV using the following equation:

$$\text{LOD} = (3 \times N_{\text{ctl}}) / (\text{Res}_{\text{LS}}) \times \text{Conc}_{\text{LS}}$$

Where, LOD is the limit of detection of the analysis, N_{ctl} is the mean signal to noise in height of the control samples (or blanks), Res_{LS} is the mean response in height of the two low calibration standards (0.300 µg/L), and Conc_{LS} is the concentration of the low calibration standard.

The LOD in water was calculated in the ILV as 0.04 µg/L (ground water) and 0.003 µg/L (surface water) for diuron, 0.007-0.008 µg/L (ground water) and 0.001-0.002 µg/L (surface water) for DCPMU, and 0.0004-0.0006 µg/L (ground water) and 0.0005-0.001 µg/L (surface water) for mCPDMU.

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.

Table 4. Method Characteristics in Water

		Diuron	DCPMU	mCPDMU
Limit of Quantitation (LOQ)*	ECM	0.05 µg/L		
	ILV			
Limit of Detection (LOD)	ECM (estimated method)	0.01 µg/L		
	ILV (calc)	0.04 µg/L (GW) ¹ 0.003 µg/L (SW)	0.007-0.008 µg/L (GW) 0.001-0.002 µg/L (SW)	0.0004-0.0006 µg/L (GW) 0.0005-0.001 µg/L (SW)
Linearity (calibration curve r and concentration range)	ECM ^{2,3}	r = 0.99989	r = 0.99992	r = 0.99986
		0.3-15 ng/mL		
	ILV ^{2,4}	r = 1.0000 (GW, Q & C) r = 0.9995 (SW, Q & C)	r = 1.0000 (GW, Q & C) r = 0.9995 (SW, Q & C)	r = 1.0000 (GW, Q & C) r = 1.0000 (SW, Q) r = 0.9995 (SW, C)
		0.3-5.0 ng/mL (GW) 0.3-15 ng/mL (SW)		
Repeatable	ECM ⁵	Yes at LOQ and 10×LOQ (two characterized water matrices – drinking/tap/ground and surface)		
	ILV ^{6,7}	Yes at LOQ and 10×LOQ (two partly characterized water matrices – ground and surface)		
Reproducible		Yes for 0.05 µg/L (LLMV)* and 0.50 µg/L in ground and surface water matrices		
Specific	ECM	Yes, matrix interferences were <5% of the LOQ (reviewer-estimate) ⁸ in SW. No matrix interferences were observed in DW. Baseline noise interfered with peak integration and attenuation. Nearby significant peak (RT <i>ca.</i> 7.8 min.) was observed.	Yes, matrix interferences were <9% of the LOQ (based on peak area) in SW. No matrix interferences were observed in DW.	Yes, no matrix interferences were observed.
	ILV	Yes, matrix interferences were <i>ca.</i> 22% (GW) ⁹ and <5% (SW) of the LOQ (based on peak area) in GW. Baseline noise interfered with peak integration and attenuation. Nearby significant peak (RT <i>ca.</i> 6.75 min.) was observed in all Q chromatograms.	Yes, matrix interferences were <5% of the LOQ (based on peak height) ¹⁰ in GW. Yes, matrix interferences were <1% of the LOQ (based on peak area) in SW.	Yes, matrix interferences were <1% of the LOQ (based on peak area).

Data were obtained from p. 37 (LOQ/LOD); Table 1, p. 40; Table 3, p. 42; Tables 6-7, pp. 45-46; Table 9, p. 48; and Table 12, p. 51 (recovery results); Figures 7-11, pp. 59-63 (calibration curves); Figures 13-72, pp. 65-94 (chromatograms) of MRID 47033303; pp. 24, 26-28 (LOQ/LOD); Tables 1-12, pp. 34-45 (recovery results); p. 29; Figures 31-42, pp. 76-87 (calibration curves); Figures 1-30, pp. 46-75 (chromatograms) of MRID 50677002. GW = ground water; SW = surface water; 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 The ILV noted that the calculated LOD for diuron in ground water was higher than the Method Detection Limit (MDL, 0.0150 µg/L; p. 11 of MRID 50677002).
- 2 Solvent standards were also used for calibration in the ECM and ILV (pp. 24, 38 of MRID 47033303; p. 18 of MRID 50677002).
- 3 Only one set of calibration curves were provided. Ion transition was not specified.
- 4 Correlation coefficients (r) were reviewer-calculated based on r² values reported in the study report; solvent standards were used (pp. 17-18; Figures 31-42, pp. 76-87 of MRID 50677002; DER Attachment 2). The two highest calibration standards were not used for the ground water analysis due to the fact that their use skews the calibration curve and adversely affects the recovery of lower concentration calibration standards causing them to fail acceptance criteria. The reviewer noted that the 5.0 ng/mL calibration standard (instead of the 15 ng/mL calibration standard) was erroneously noted as an omitted one in Figure 35, p. 80.
- 5 In the ECM, drinking water (Specimen Code: 010/6202451; pH 7.75, conductivity 503 µS/cm, total organic carbon 2.3 mg/L) was tap water generated from ground water taken at SGS Institut Fresenius, Taunusstein (p. 25 of MRID 47033303). The surface water (Specimen Code: 010/6202452; pH 8.03, conductivity 759 µS/cm, total organic carbon 5.4 mg/L) was collected from the Großbach river from a ditch in Limburg. Water characterization was performed at the ECM.
- 6 In the ILV, ground water was in-house well water, unadulterated water from a 100-meter bedrock well which is considered soft with a typical hardness of <160 mg (as CaCO₃; p. 16 of MRID 50677002). The surface water (Lot No. 17Oct16Wat-A-3; pH 6.9, dissolved oxygen concentration 9.3 mg/L) was collected from Weweantic River, West Wareham, Massachusetts. The surface water was characterized by Agvise Laboratories, Northwood, North Dakota.
- 7 The ILV validated the method for diuron, DCPMU, and mCPDMU in water in the first trial for surface water and the second trial for ground water with insignificant modifications to the analytical parameters, the final evaporation to <1 mL using a gentle stream of nitrogen was conducted at 25°C, and omission of filtering (0.2 µm PTFE) prior to LC/MS/MS analysis (see Reviewer's Comment #3; pp. 16, 19-23, 27 of MRID 50677002). The ILV noted that an initial ILV attempt of the method using ground water was unacceptable due to execution of the validation. No ILV modifications were made based on the failed first ILV trial in ground water but a more sensitive LC/MS/MS instrument was employed (p. 27; Appendix 3, p. 105).
- 8 Based on Figure 55, p. 86, and Figure 61, p. 89 of MRID 47033303. No peak area reported for diuron in control sample.
- 9 Based on Figure 2, p. 47, and Figure 4, p. 49 of MRID 50677002.
- 10 Based on Figure 7, p. 52, and Figure 9, p. 54 of MRID 50677002. Peak height was used instead of peak area since the LOQ peak area was difficult to read.

IV. Method Deficiencies and Reviewer's Comments

1. The ECM MRID 47033303 was DuPont-19220 which is an analytical method designed for the quantitative determination of diuron, linuron, DCPMU, DCPU, mCPDMU and desmethylated linuron in water at the stated LOQ of 0.05 µg/L using LC/MS/MS (p. 15 of MRID 47033303). The ILV MRID 50677002 only validated DuPont-19220 for diuron and its metabolites DCPMU and mCPDMU. Based on EFED directives accompanying this method validation, the DER only included validation data for diuron, DCPMU, and mCPDMU.
2. 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 (p. 37 of MRID 47033303; pp. 24, 26-28 of MRID 50677002). 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 reported method LOQ for the three analytes in the tested water matrices.
3. The ILV reported that DuPont-19220 was validated for diuron, DCPMU, and mCPDMU in water in the first trial for both water matrices; however, the ILV also reported that an "ILV in ground water was initially attempted on 19 and 26 March, 2018" (p. 27 of MRID 50677002). The ILV noted that the initial ILV attempt of the method using ground water was

unacceptable due to execution of the validation, but no details about the failed ILV attempts were reported. No ILV modifications were made based on the failed first ILV attempts in ground water. The reviewer considered the first ILV attempts in ground water to be the first ILV trial; therefore, the reviewer reported that the ILV trial for the analytes in ground water was successful in the second trial.

The reviewer noted that the ILV stated that they “had to move to a more sensitive instrument in order to run the method” when they reported a successful trial for ground water and the elimination of the two highest calibration standards for the ground water calibration curve (Appendix 3, p. 105 of MRID 50677002). The former analytical instrument was not reported.

4. The communications between the ILV study author (Stephen DeVellis), ILV personnel (Paul Reibach and Peter Stchur), and ILV Study Monitor (Jennifer Gates, Waterborne Environmental, Inc.) were summarized, and some email communication was also provided (pp. 5, 25; Appendix 1, p. 89; Appendix 3, pp. 105-107 of MRID 50677002). Reported communications included: approval of the protocol and method, modification of the calibration curve range for ground water, and the results of the first attempt of the ILV. No discussion of the failed ILV attempts in ground water was reported.
5. The ILV noted that the calculated quantitation ion transition LOD for diuron in ground water (0.04 µg/L) was higher than the Method Detection Limit (MDL, 0.0150 µg/L; p. 11 of MRID 50677002). The reviewer also noted that this calculated LOD was 80% of the LOQ.
6. The ILV ground water was not well-characterized (p. 16 of MRID 50677002).
7. The specificity of the method for diuron in ground water was not well-supported by the ILV representative chromatograms of the quantitation ion transition due to matrix interferences which were *ca.* 22% of the LOQ (based on peak area) and a nearby significant contaminant (RT *ca.* 6.75 min.; p. 32; Figure 2, p. 47, and Figures 4-5, pp. 49-50 of MRID 50677002). This contaminant was also observed in diuron chromatograms of the quantitation ion transition in the surface water (Figures 16-20, pp. 61-65). Solvent standards were used for calibration in the ILV; matrix effects were not investigated (p. 18 of MRID 50677002).
8. In the ECM, one set of calibration curves were provided, and the ion transition was not specified (Figures 7-11, pp. 59-63 of MRID 47033303).
9. The ECM recoveries were reportedly corrected for residues quantified in the controls, but residues (<10% of the LOQ) were only quantified in the surface water controls for diuron and DCPMU (p. 30; Figures 19-30, pp. 68-73; Figures 49-60, pp. 83-88 of MRID 47033303).
10. The ILV reversed the monitored quantitation and confirmation ion transitions for DCPMU (m/z 219.02→161.96 and m/z 219.02→127.06) which were reported in the ECM (m/z 219→127 and m/z 219→162; p. 29 of MRID 47033303; p. 22 of MRID 50677002). Recoveries of the m/z 219→162 ion transition were slightly higher in both water matrices in the ECM and in the ground water in the ILV. No reason for the ILV switch was reported, and the ILV switch was not reported in the ILV method differences (p. 23 of MRID

50677002).

11. 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 (p. 37 of MRID 47033303; pp. 24, 26-28 of MRID 50677002). In the ECM, the LOQ was defined as the lowest fortification level at which average recoveries of 70-110% and a RSD <20% are achieved. Also, at the LOQ fortification level, the analyte peak consistently represents a signal-to-noise ratio of *ca.* 11-32 to 1 for all analytes. In the ECM, the LOD for the analytes was estimated to be 0.01 µg/L, based on the limiting response analyte, DCPU (analyte not included in this DER; see Reviewer's Comment #1). The LOD was defined as the analyte concentration in matrix with a response equivalent to a signal-to-noise ratio of approximately 3 to 1. The LOD was estimated from the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation: $LOD = \{[LOD \text{ signal-to-noise response } (3/1)] / \text{Observed LOQ signal-to-noise response}\} \times LOQ$, which equaled $(3/1)/(11/1) \times 0.05 \text{ } \mu\text{g/L}$. No further justification of the LOQ or LOD was reported in the ECM. No justification of the LOQ was reported in the ILV. The LOD was calculated in the ILV using the following equation: $LOD = (3 \times N_{ctl}) / (Resp_{LS}) \times Conc_{LS}$, where, LOD is the limit of detection of the analysis, N_{ctl} is the mean noise in height of the control samples (or blanks), $Resp_{LS}$ is the mean response in height of the two low calibration standards (0.300 µg/L), and $Conc_{LS}$ is the concentration of the low calibration standard. The LOD was calculated in the ILV using the following equation: $LOD (\mu\text{g/L}) = 3 \times \text{height of control baseline noise} \times \text{control dilution factor} \times \text{calibration standard concentration } (\mu\text{g/L}) / \text{height of calibration standard peak}$. 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 the lowest level of method validation (LLMV) rather than an LOQ.

The method detection limit (MDL) was calculated in the ILV as dependent upon the lowest concentration calibration standard and the dilution factor of the controls (i.e., $0.300 \text{ } \mu\text{g/L} \times 0.0500 = 0.0150 \text{ } \mu\text{g/L}$; pp. 24, 26-28 of MRID 50677002).

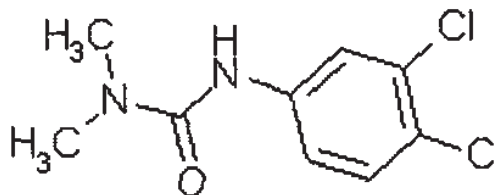
12. The matrix interferences were not studied in the ECM but assumed to be insignificant; solvent standards were used for calibration (pp. 24, 38 of MRID 47033303). Solvent standards were also used for calibration in the ILV (p. 18 of MRID 50677002).
13. In the ILV, the total time required to complete one set of 20 samples (one reagent blank, two matrix controls, ten fortified samples and seven calibration samples) was reported as 16 hours to complete (p. 25 of MRID 50677002). The sample processing required one working day (8 hours), and the LC/MS/MS was performed overnight (16 hours).

V. References

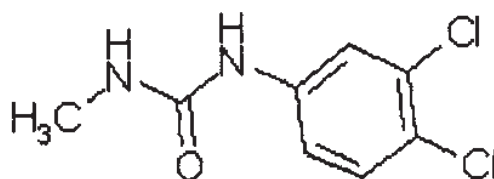
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- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**Diuron (DPX-14740-235; Diuron TC, DCMU, Karmex, Direx, Diuron II Technical)**

IUPAC Name: 3-(3,4-Dichlorophenyl)-1,1-dimethylurea
CAS Name: N'-(3,4-Dichlorophenyl)-N,N-dimethylurea
CAS Number: 330-54-1
SMILES String: Not found

**DCPMU (IN-15654-012)**

IUPAC Name: 3-(3,4-Dichlorophenyl)-1-methylurea
CAS Name: N'-(3,4-Dichlorophenyl)-N'-methylurea
CAS Number: 3567-62-2
SMILES String: [H]N(C)C(=O)N([H])c1ccc(c(c1)Cl)Cl

**mCPDMU (IN-12894-007)**

IUPAC Name: 3-(3-Chlorophenyl)-1,1-dimethylurea
CAS Name: N'-(3-Chlorophenyl)-N,N-dimethylurea
CAS Number: 587-34-8
SMILES String: Not found

