Analytical method for diuron, linuron, DCPMU and DCPU in soil

Reports:	ECM: EPA MRID No.: 47033301. Pentz, A.M., F.Q. Bramble, Jr. 2005. Analytical Method for the Determination of Linuron, Diuron, and Relevant Metabolites in Soils Using LC/MS/MS. Report prepared, sponsored and submitted by E.I. du Pont de Nemours and Company, Newark, Delaware; 63 pages. Project ID No.: DuPont-16919. Final report issued August 2, 2005. ILV: EPA MRID No.: 47033302. McCracken, B. 2006. Independent Laboratory Validation of the Analytical Method DuPont-16919, "Analytical Method for the Determination of Linuron, Diuron, and Relevant Metabolites in Soils Using LC/MS/MS". Report prepared by Exygen Research, State College, Pennsylvania, sponsored and submitted by E.I. du Pont de Nemours and Company, Newark, Delaware; 30 pages. Exygen Project No.: P0002038. DuPont Study No.: DuPont-16865. ABC Laboratories Study No.: 49992. Final report issued May 25, 2006.
Document No.:	MRIDs 47033301 & 47033302
Guideline:	850.6100
Statements:	ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR 160) and OECD GLP, but the work was conducted in a GLP facility (p. 3 of MRID 47033301). Signed and dated Data Confidentiality, GLP and Authenticity statements were provided (pp. 2-4 of MRID 47033301). The Quality Assurance statement was not included. ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (p. 3 of MRID 47033302). Signed and dated Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as Supplemental. The independence of the ILV was poorly documented. The LOQ (0.010 mg/kg) is greater than the lowest toxicological level of concern (0.0023 mg/kg).
PC Code:	035505 (diuron), 035506 (linuron)
EFED Final Reviewer:	Lewis Ross Brown, Env. Biologist — Signature: Lewis Ross Brown, III — Date: May 18, 2017
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist — Signature: Lisa Muto — Date: 4/26/17 Kathleen Ferguson, Ph.D., Environmental Scientist — Signature: Kathleen P. Ferguson — Date: 4/26/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, DuPont-16919, is designed for the quantitative determination of diuron, linuron, DCPMU and DCPU in soil at the stated LOQ of 0.010 mg/kg (0.01 ppm) using LC/MS/MS. The LOQ is greater than the lowest toxicological level of concern in soil for all four analytes (0.0023 mg/kg; US EPA, 2015, DP Barcode 423230). The independence of the ILV, MRID 47033302, was not properly documented since the DuPont Study Monitor for the ILV was the ECM Study Director and one of the ECM study authors and the communications (email and phone logs) between the Study Monitor and ILV staff were not provided. Characterized silt loam, silty clay loam, sand, sandy loam, clay loam and loam soil matrices were used for the ECM validation. Uncharacterized sand soil was used for the ILV validation and the ILV was not provided with the most difficult matrix used to validate the method. In the ECM and ILV, diuron, linuron, DCPMU and DCPU were identified using two ion transitions in the ILV, but only one ion transition was quantified; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The ECM method for diuron was validated in the re-injection of the first trial with insignificant modifications to the analytical parameters. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for diuron, linuron, DCPMU and DCPU, based on the data from the re-injection of the first trial. The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Diuron	47033301[1]	47033302[2]		Soil	02/08/2005	E.I. DuPont de Nemours and Company	LC/MS/MS	0.01 ppm
Linuron								
DCPMU (Desmethoxy linuron)								
DCPU (Norlinuron)								

1 In the ECM, Cajun silt loam soil (38.0% sand, 58.0% silt, 4.0% clay; pH 8.1; 0.8% organic matter) obtained from Porterville, California, Baldwin silty clay loam soil (19.2% sand, 47.8% silt, 33.0% clay; pH 5.5; 2.3% organic matter) obtained from Washington, Louisiana, Eau Gallie sand soil (97.0% sand, 3.0% silt, 0.0% clay; pH 8.0; 1.0% organic matter) obtained from Bradenton, Florida, Sassafras #16 sandy loam soil (58% sand, 35% silt, 7% clay; pH 5.9; 1% organic matter) obtained from Chesapeake Farms, Maryland, Drummer #7 clay loam soil (24% sand, 43% silt, 33% clay; pH 6.1; 4.8% organic matter) obtained from Rochelle, Illinois, and Nambsheim loam soil (51% sand, 38% silt, 10% clay; pH 7.9; 1.4% organic matter) obtained from Nambsheim, France, were characterized and used in the study (USDA textural classification not specified; pp. 16-17 of MRID 47033301). The soil characterization data for Louisiana Baldwin silty clay loam soil were representative properties for Baldwin soil (0-12") provided by the Missouri Cooperative Soil Survey; actual characterization data can be found in DuPont-16918 study report. The California and Louisiana soils were collected from test sites used in the DuPont-16918 study report, the linuron field dissipation study.

2 In the ILV, sand soil (L0007386-0001) was provided by the sponsor (DuPont Crop Protection) and used in the study, but not characterized (USDA textural classification not specified; p. 11 of MRID 47033302). The source of the soil was not described further.

I. Principle of the Method

For each sample, 22-mL ASE extraction vessels were fitted with an encap + 10 µm steel frits (pp. 17-18 of MRID 47033301). Two cellulose filters (19.1 mm diam., type D28) were inserted into the vessels and pressed to the bottom using a plunger from a 10-mL disposable syringe, if necessary. Sand (*ca*. 1 g) and test soil (5.0 ± 0.05 g) were weighed into the vessel. The sample was fortified and allowed to stand for at least 15 minutes in fume hood. After each vessel was filled with sand and capped, the sample was extracted in the ASE extraction system using methanol:0.38% formic acid-0.1% Triton X 100® (9:1, v:v) and the following conditions: heat for 5 minutes, static for 3 minutes, flush 100%, purge 60 seconds. Cycle 3 times, temperature 100°C, pressure 1000 psi. Remove vessels and glass collection tubes containing extracts from the ASE extractor. The method noted that *ca*. 40 mL or 8 cm of extract solution is to be expected and that the ASE extracts are stable for at least four days when stored under refrigeration. with 15 mL of acetonitrile and 5 mL of HPLC water via sonicating for fifteen minutes. After extraction, the extract was transferred to a 50-mL graduated cylinder and diluted to a final volume of 50.0 mL with methanol rinses of the extract collection vial. An aliquot (5.0 mL) of the final extract was transferred to a 15-mL polypropylene centrifuge tube. After 1 mL of water was added to the centrifuge tube, the solvent was evaporated to *ca*. 1 mL in a gentle stream of nitrogen at *ca*. 50°C. After 3 mL of methanol was added to the residue, the final volume was adjusted to 10.0 mL using the gradation on the tube with aqueous 0.01M formic acid solution. For samples fortified at 5.0 ppm, extracts were diluted within the calibration range. The aliquots of extracts were filtered (0.2 µm AcroDisc PTFE 13 mm) prior to LC/MS/MS analysis or stored refrigerated if not analyzed immediately.

Samples were analyzed for diuron using an Agilent HP1100 HPLC coupled with a Waters Quattro Premier triple quadrupole mass spectrometer using electrospray ionization (ESI) operated in the positive ion mode with multiple reaction monitoring (MRM; pp. 19-20 of MRID 47033301). The following LC conditions were used: Varian Pursuit C8 column (4.6 mm x 150 mm, 3 µ; column temperature 40°C), mobile phase of (A) aqueous 0.01M formic acid and (B) methanol [mobile gradient phase of percent A:B (v:v) at 0.0 min. 70:30, 10.0 min. 10:90, 15.0-15.1 min. 1:99, 17.1-23.0 min. 70:30] and injection volume of 25 µL. Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 248.90→159.60 and *m/z* 248.90→181.60 for linuron; *m/z* 232.80→45.60 and *m/z* 232.80→71.40 for diuron; *m/z* 219.00→126.60 and *m/z* 219.00→161.50 for DCPMU; and *m/z* 204.80→126.70 and *m/z* 204.80→161.70 for DCPU. Reported retention times were *ca*. 11.9, 11.1, 11.1 and 10.7 minutes for linuron, diuron, DCPMU and DCPU, respectively.

The method cautioned that reusable glassware should be clean and free from contamination and that the LC/MS/MS should be backflushed with high percentage organic solvent if peak shapes deteriorate (p. 25 of MRID 47033301).

The ILV performed the ECM methods for each analyte as written, except for insignificant modifications to the analytical parameters (pp. 14-18, 20 of MRID 47033302). Samples were analyzed for diuron using an Agilent HP1100 HPLC coupled with a PE Sciex API 3000 Triple Quadrupole LC/MS/MS (trial 1) or MDS Sciex API 5000 Triple Quadrupole LC/MS/MS (re-injection of trial 1) using ESI operated in the positive ion mode with MRM. The LC/MS/MS parameters were the same as those of the ECM, except that the injection volume was increased to 50 µL. Two ion pair transitions were monitored for the four analytes. For trial 1, the same ion pairs

were used as the ECM. For the re-injection of trial 1, slightly different ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 249.0→160.2 and *m/z* 249.0→182.2 for linuron; *m/z* 233.0→46.3 and *m/z* 233.0→72.3 for diuron; *m/z* 219.0→127.1 and *m/z* 219.0→162.1 for DCPMU; and *m/z* 205.0→127.1 and *m/z* 205.0→162.1 for DCPU. Observed retention times were *ca*. 13.9, 13.2, 13.2 and 12.8 minutes for linuron, diuron, DCPMU and DCPU, respectively (Figures 2-5, pp. 23-26).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.010 mg/kg (0.01 ppm) for diuron, linuron, DCPMU and DCPU in soil (pp. 9, 24-25 of MRID 47033301; pp. 10-11, 20 of MRID 47033302). In the ECM, the Limit of Detection (LOD) for diuron, linuron, DCPMU and DCPU was 0.002 mg/kg. No LOD was reported in the ILV.

II. Recovery Findings

ECM (MRID 47033301): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of diuron and linuron at fortification levels of 0.010 mg/kg (0.010 ppm; LOQ), 0.10 mg/kg (0.10 ppm; 10×LOQ) and 5.0 mg/kg (5.0 ppm; 500×LOQ) in the six soil matrices (n = 5 for LOQ and 10×LOQ; n = 5 or 10 for 500×LOQ; Tables 1-6, pp. 28-33). Mean recoveries and RSDs were within guidelines for analysis of DCPMU and DCPU at fortification levels of 0.010 mg/kg (0.010 ppm; LOQ) and 0.10 mg/kg (0.10 ppm; 10×LOQ) in the six soil matrices (n = 5). Two ion pair transitions were monitored for the analytes using LC/MS/MS in positive ESI mode; however, only one ion transition was quantified. Confirmation of analyte identification/quantification was performed by calculating the confirmation ion transition peak ratio; acceptable response ratios were achieved (p. 26; Appendix 3, p. 58). The recoveries of diuron and DCPMU at the LOQ and 10×LOQ fortifications in California silt loam soil were corrected for residues quantified in the controls; no other recoveries were corrected. For the 5.0 ppm fortification results, approximately half of the recovery values of each set were normalized to 5 ppm fortification standard recovery values because fortification standard and calibration standard results were inconsistent, except for analysis of diuron in the California and Louisiana soils. The Cajun silt loam soil (38.0% sand, 58.0% silt, 4.0% clay; pH 8.1; 0.8% organic matter) obtained from Porterville, California, Baldwin silty clay loam soil (19.2% sand, 47.8% silt, 33.0% clay; pH 5.5; 2.3% organic matter) obtained from Washington, Louisiana, Eau Gallie sand soil (97.0% sand, 3.0% silt, 0.0% clay; pH 8.0; 1.0% organic matter) obtained from Bradenton, Florida, Sassafras #16 sandy loam soil (58% sand, 35% silt, 7% clay; pH 5.9; 1% organic matter) obtained from Chesapeake Farms, Maryland, Drummer #7 clay loam soil (24% sand, 43% silt, 33% clay; pH 6.1; 4.8% organic matter) obtained from Rochelle, Illinois, and Nambsheim loam soil (51% sand, 38% silt, 10% clay; pH 7.9; 1.4% organic matter) obtained from Nambsheim, France, were characterized and used in the study (USDA textural classification not specified; pp. 16-17 of MRID 47033301). The soil characterization data for Louisiana Baldwin silty clay loam soil were representative properties for Baldwin soil (0-12”) provided by the Missouri Cooperative Soil Survey; actual characterization data can be found in DuPont-16918 study report. The California and Louisiana soils were collected from test sites used in the DuPont-16918 study report, the linuron field dissipation study.

ILV (MRID 47033302): For Trial 1 and the Re-injection of Trial 1, mean recoveries and RSDs were within guidelines for analysis of diuron, linuron, DCPMU and DCPU at fortification levels of 0.010

mg/kg (0.010 ppm; LOQ), 0.10 mg/kg (0.10 ppm; 10×LOQ) in one soil matrix (p. 18; Appendix 1, pp. 29-30). Two ion pair transitions were monitored for the analytes using LC/MS/MS in positive ESI mode; however, only one ion transition was quantified. No confirmation method was described; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Trial 1 used a Sciex API 3000 LC/MS/MS for analysis and the same ion transitions as the ECM. Due to the unsatisfactory signal-to-noise ratio for diuron, the Re-injection of Trial 1 was performed using an API 5000 system. For the Re-injection of Trial 1, slightly different ion pair transitions were monitored for each analyte, and acceptable signal-to noise ratios were achieved. The sand soil (L0007386-0001) was provided by the sponsor (DuPont Crop Protection) and used in the study, but not characterized (USDA textural classification not specified; p. 11). The source of the soil was not described further. The method for diuron was validated in the first trial and the re-injection of the first trial with insignificant modifications to the analytical parameters (pp. 10-11, 18, 20).

Table 2. Initial Validation Method Recoveries for Diuron, Linuron, DCPMU and DCPU in Soil[1,2]

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation ion					
	California Cajun Silt Loam Soil					
Diuron	0.010 (LOQ)[3]	5	104-86	94	6	7
	0.10[3]	5	93-100	97	3	3
	5.0	5	100-105	102	2	2
Linuron	0.010 (LOQ)	5	102-108	105	2	2
	0.10	5	96-109	101	6	6
	5.0[4]	5	99-127	108	11	10
DCPMU (Desmethoxy linuron)	0.010 (LOQ)[3]	5	91-107	99	6	6
	0.10[3]	5	99-108	103	4	4
DCPU (Norlinuron)	0.010 (LOQ)	5	94-104	101	4	4
	0.10	5	98-104	100	3	3
	Louisiana Baldwin Silty Clay Loam Soil					
Diuron	0.010 (LOQ)	5	86-105	95	7	8
	0.10	5	86-94	91	4	4
	5.0	5	86-93	90	3	3
Linuron	0.010 (LOQ)	5	94-109	100	6	6
	0.10	5	88-100	93	5	5
	5.0[4]	5	86-97	90	4	5
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	98-106	102	3	3
	0.10	5	97-105	100	4	4
DCPU (Norlinuron)	0.010 (LOQ)	5	98-105	102	3	3
	0.10	5	92-102	97	4	4
	Florida Eau Gallie Sand Soil					
Diuron	0.010 (LOQ)	5	89-108	98	8	8
	0.10	5	81-89	85	3	4
	5.0	10[4]	89-117	101	8	8
Linuron	0.010 (LOQ)	5	92-113	104	7	7
	0.10	5	91-100	96	3	4
	5.0	10[4]	93-109	100	6	6

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	96-112	100	7	7
	0.10	5	94-97	96	1	1
DCPU (Norlinuron)	0.010 (LOQ)	5	97-18	103	6	6
	0.10	5	96-100	98	1	1
	Maryland Sassafras #16 Sandy Loam Soil					
Diuron	0.010 (LOQ)	5	77-90	84	6	7
	0.10	5	80-94	86	6	7
	5.0	$10^{4,5}$	87-139	109	15	14
Linuron	0.010 (LOQ)	5	86-95	89	4	4
	0.10	5	90-94	93	2	2
	5.0	$10^{4,5}$	88-127	104	11	11
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	88-101	94	6	6
	0.10	5	93-101	97	4	4
DCPU (Norlinuron)	0.010 (LOQ)	5	91-97	94	3	3
	0.10	5	91-101	97	4	5
	Illinois Drummer #7 Clay Loam Soil					
Diuron	0.010 (LOQ)	5	84-109	96	12	12
	0.10	5	80-86	82	3	3
	5.0	10^{4}	80-116	96	13	13
Linuron	0.010 (LOQ)	5	81-86	84	2	2
	0.10	5	77-88	84	4	5
	5.0	10^{4}	86-120	97	10	10
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	94-102	97	4	4
	0.10	5	85-94	90	4	5
DCPU (Norlinuron)	0.010 (LOQ)	5	82-93	89	6	7
	0.10	5	82-92	88	5	5
	French Nambsheim Loam Soil					
Diuron	0.010 (LOQ)	5	76-98	90	9	10
	0.10	5	83-89	86	3	3
	5.0	10^{4}	84-127	101	14	14
Linuron	0.010 (LOQ)	5	86-95	90	4	4
	0.10	5	86-96	91	4	5
	5.0	10^{4}	85-122	97	11	12
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	95-105	99	4	4
	0.10	5	95-102	99	2	2
DCPU (Norlinuron)	0.010 (LOQ)	5	79-104	95	9	10
	0.10	5	94-103	100	4	4

Data (uncorrected recovery results, unless noted otherwise; pp. 21-22; Tables 1-6, pp. 28-33) were obtained from Tables 1-6, pp. 28-33 of MRID 47033301.

1 The Cajun silt loam soil (38.0% sand, 58.0% silt, 4.0% clay; pH 8.1; 0.8% organic matter) obtained from Porterville, California, Baldwin silty clay loam soil (19.2% sand, 47.8% silt, 33.0% clay; pH 5.5; 2.3% organic matter) obtained from Washington, Louisiana, Eau Gallie sand soil (97.0% sand, 3.0% silt, 0.0% clay; pH 8.0; 1.0% organic matter) obtained from Bradenton, Florida, Sassafras #16 sandy loam soil (58% sand, 35% silt, 7% clay; pH 5.9; 1% organic matter) obtained from Chesapeake Farms, Maryland, Drummer #7 clay loam soil (24% sand, 43% silt, 33% clay; pH 6.1; 4.8% organic matter) obtained from Rochelle, Illinois, and Nambsheim loam soil (51% sand, 38% silt, 10% clay;

pH 7.9; 1.4% organic matter) obtained from Nambsheim, France, were characterized and used in the study (USDA textural classification not specified; pp. 16-17). The soil characterization data for Louisiana Baldwin silty clay loam soil were representative properties for Baldwin soil (0-12") provided by the Missouri Cooperative Soil Survey; actual characterization data can be found in DuPont-16918 study report. The California and Louisiana soils were collected from test sites used in the DuPont-16918 study report, the linuron field dissipation study.

2 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 248.90→159.60 and *m/z* 248.90→181.60 for linuron; *m/z* 232.80→45.60 and *m/z* 232.80→71.40 for diuron; *m/z* 219.00→126.60 and *m/z* 219.00→161.50 for DCPMU; and *m/z* 204.80→126.70 and *m/z* 204.80→161.70 for DCPU; however, only one ion transition was quantified.

3 Recovery values were corrected for analyte quantified in the control samples.

4 Approximately half of the recovery values were normalized to 5 ppm fortification standard recovery values because fortification standard and calibration standard results were inconsistent.

5 Table 4, p. 31 reported that n = 12 for the 5.0 ppm fortification; however, the reviewer only found 10 samples listed in the table.

Table 3. Independent Validation Method Recoveries for Diuron, Linuron, DCPMU and DCPU in Soil[1,2,3]

Analyte[1]	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation ion					
	Sand Soil (Trial 1)					
Diuron	0.010 (LOQ)	5	96-101	99	2	1
	0.10	5	87-95	91	3	3
Linuron	0.010 (LOQ)	5	99-102	101	1	2
	0.10	5	90-96	92	3	3
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	89-92	91	1	1
	0.10	5	93-95	95	1	1
DCPU (Norlinuron)	0.010 (LOQ)	5	98-112	103	5	5
	0.10	5	94-104	99	4	4
	Sand Soil (Re-injection of Trial 1)					
Diuron	0.010 (LOQ)	5	101-111	107	4	3
	0.10	5	99-108	103	3	3
Linuron	0.010 (LOQ)	5	86-95	92	4	4
	0.10	5	88-99	94	4	4
DCPMU (Desmethoxy linuron)	0.010 (LOQ)	5	101-116	110	6	6
	0.10	5	104-112	109	3	3
DCPU (Norlinuron)	0.010 (LOQ)	5	93-110	105	7	6
	0.10	5	101-108	104	3	3

Data (uncorrected recovery results; Figures 2-5, pp. 23-26) were obtained from p. 18; Appendix 1, pp. 29-30 of MRID 47033302.

1 The sand soil (L0007386-0001) was provided by the sponsor (DuPont Crop Protection), but not characterized (USDA textural classification not specified; p. 11 of MRID 47033302). The source of the soil was not described further.

2 Two ion pair transitions were monitored for the four analytes for each injection; however, only one ion transition was quantified. For trial 1, the following ion transitions were monitored (quantitation and confirmation, respectively): *m/z* 248.90→159.60 and *m/z* 248.90→181.60 for linuron; *m/z* 232.80→45.60 and *m/z* 232.80→71.40 for diuron; *m/z* 219.00→126.60 and *m/z* 219.00→161.50 for DCPMU; and *m/z* 204.80→126.70 and *m/z* 204.80→161.70 for DCPU. For the re-injection of trial 1, slightly different ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 249.0→160.2 and *m/z* 249.0→182.2 for linuron; *m/z* 233.0→46.3 and *m/z* 233.0→72.3 for diuron; *m/z* 219.0→127.1 and *m/z* 219.0→162.1 for DCPMU; and *m/z* 205.0→127.1 and *m/z* 205.0→162.1 for DCPU.

3 Means and RSDs were reported from p. 18 of the study report, but individual values and standard deviations were not reported. The reviewer provided the recovery range and calculated the standard deviations by using the recovery raw data provided in Appendix 1 of the study report (see DER Attachment 2). The resolution of the raw data was poor so errors in the reviewer-calculated values is possible; however, the reviewer-calculated means and RSDs agreed with the values reported by the study report within ±1%.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.010 mg/kg (0.01 ppm) for diuron, linuron, DCPMU and DCPU in soil (pp. 9, 24-25 of MRID 47033301; pp. 10-11, 20 of MRID 47033302). In the ECM, the LOQ was defined as the lowest fortification level at which average recoveries of 70-120% and a RSD <20% are achieved. Also, at the LOQ fortification level, the analyte peak consistently represents a signal-to-noise ratio of approximately 5-20 to 1 for the least responsive analyte, diuron. In the ECM, the LOD for the four analytes was estimated to be 0.002 mg/kg, based on the limiting response analyte, diuron. 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 form the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation:

LOD = {[LOD signal-to-noise response (3/1)]/Observed LOQ signal-to-noise response} x LOQ
= (3/1)/(12/1) x 0.010 mg/kg
= 0.002 mg/kg

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

Table 4. Method Characteristics in Soil

		Diuron	Linuron	DCPMU	DCPU
Limit of Quantitation (LOQ)	ECM	0.010 mg/kg (0.01 ppm)			
	ILV				
Limit of Detection (LOD)	ECM	0.002 mg/kg			
	ILV	Not reported			
Linearity (calibration curve r^2 and concentration range)[1]	ECM	r^2 = 0.9998	r^2 = 0.9999	r^2 = 1	r^2 = 1
	ILV[2]	r^2 = 0.9996 (T1) r^2 = 0.9966 (RT1)	r^2 = 0.9976 (T1) r^2 = 0.9992 (RT1)	r^2 = 0.9960 (T1) r^2 = 0.9978 (RT1)	r^2 = **0.9930** (T1) r^2 = 0.9996 (RT1)
	Range:	0.25-50 ng/mL			
Repeatable[1]	ECM[3]	Yes at LOQ, 10×LOQ and 500×LOQ (six characterized soil matrices)		Yes at LOQ and 10×LOQ (six characterized soil matrices)	
	ILV[4,5,6]	Yes at LOQ and 10×LOQ (T1 & RT1; one uncharacterized soil matrix)			
Reproducible[1]		Yes at LOQ and 10×LOQ			
Specific	ECM[7]	Yes, matrix interferences were <5% of the LOQ (based on peak area) in the CA soil. No matrix interferences were observed in the other soils.	Yes, matrix interferences were <8% of the LOQ (based on peak area) in the MD soil. No matrix interferences were observed in the other soils.	Yes, matrix interferences were <17% of the LOQ (based on peak area) in the CA soil. No matrix interferences were observed in the other soils.	Yes, matrix interferences were <8% of the LOQ (based on peak area) in the FL and IL soils. No matrix interferences were observed in the other soils.
	ILV[8]	Yes, no matrix interferences were observed.			

Data were obtained from pp. 9, 15-16, 24-25; Tables 1-6, pp. 28-33 (recovery results); Figure 5, p. 38 (calibration curves); Figures 8-19, pp. 41-52 (chromatograms) of MRID 47033301; pp. 10-11, 18, 20; Appendix 1, pp. 29-30 (recovery results and calibration coefficients); Figures 2-5, pp. 23-26 (chromatograms) of MRID 47033302. T1 = Trial 1; RT1 = Re-injection of Trial 1. All ILV results reported for T1 and RT1 unless specified otherwise.

1 Although two ion transitions were monitored in the ECM and ILV; only the quantitation ion transition was quantified for recovery results and calibration data.

2 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (p. 17; Appendix 1, pp. 29-30 of MRID 47033302; DER Attachment 2).

3 In the ECM, Cajun silt loam soil (38.0% sand, 58.0% silt, 4.0% clay; pH 8.1; 0.8% organic matter) obtained from Porterville, California, Baldwin silty clay loam soil (19.2% sand, 47.8% silt, 33.0% clay; pH 5.5; 2.3% organic matter) obtained from Washington, Louisiana, Eau Gallie sand soil (97.0% sand, 3.0% silt, 0.0% clay; pH 8.0; 1.0% organic matter) obtained from Bradenton, Florida, Sassafras #16 sandy loam soil (58% sand, 35% silt, 7% clay; pH 5.9; 1% organic matter) obtained from Chesapeake Farms, Maryland, Drummer #7 clay loam soil (24% sand, 43% silt, 33% clay; pH 6.1; 4.8% organic matter) obtained from Rochelle, Illinois, and Nambsheim loam soil (51% sand, 38% silt, 10% clay; pH 7.9; 1.4% organic matter) obtained from Nambsheim, France, were characterized and used in the study (USDA textural classification not specified; pp. 16-17 of MRID 47033301). The soil characterization data for Louisiana Baldwin silty clay loam soil were representative properties for Baldwin soil (0-12") provided by the Missouri Cooperative Soil Survey; actual characterization data can be found in DuPont-16918 study report. The California and Louisiana soils were collected from test sites used in the DuPont-16918 study report, the linuron field dissipation study.

4 In the ILV, sand soil (L0007386-0001) was provided by the sponsor (DuPont Crop Protection) and used in the study, but not characterized (USDA textural classification not specified; p. 11 of MRID 47033302). The source of the soil was not described further.

5 The method for diuron was validated in the first trial and the re-injection of the first trial with insignificant modifications to the analytical parameters (pp. 10-11, 18, 20 of MRID 47033302).

6 Trial 1 used a Sciex API 3000 LC/MS/MS for analysis and the same ion transitions as the ECM (pp. 10-11, 15-18, 20 of MRID 47033302). Due to the unsatisfactory signal-to-noise ratio for diuron, the Re-injection of Trial 1 was performed using an API 5000 system. For the re-injection of trial 1, slightly different ion pair transitions were monitored for each analyte, and acceptable signal-to noise ratios were achieved.
7 Confirmation of analyte identification/quantification was performed by calculating the confirmation ion transition peak ratio; acceptable response ratios were achieved (p. 26; Appendix 3, p. 58 of MRID 47033301).
8 No confirmation method was described; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The independence of the ILV was poorly documented. The ILV reported that communications between the ILV staff and the "Study Monitor" involved discussion and approval of trial results and suggestions of modifications to improve results; however, the actual communications (email and phone logs) were not provided (pp. 18, 20 of MRID 47033302). The reviewer noted that the "Study Monitor" was not specified as the DuPont Study Monitor or the ABC Study Monitor (see below) and, without the actual correspondence, the reviewer could not determine if the ILV communication took place with the DuPont Study Monitor. According to OCSPP guidelines, the analysts, study director, equipment, instruments, and supplies of the two laboratories must be distinct and operated separately and without collusion. Furthermore, the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies. In order to support their independence claim, Exygen Research should have showed that no communication occurred between the staff of the initial and independent validations.

   The reviewer noted that an ABC Study Monitor was also listed in the ILV (p. 6 of MRID 47033302). No data regarding results produced by ABC Laboratories was found in the ILV; however, method validation results from ABC Laboratories with some of the analytes was found in the ECM (see Reviewer' Comment #8).

2. The ILV soil was not characterized (p. 11 of MRID 47033302). The reviewer assumed that the soil was sand because the study author described the validation set as consisting of "one sand blank", but this soil description was not specified as USDA soil texture classification (p. 11). This soil was provided to the ILV by the study sponsor, DuPont Crop Protection. Without soil characterization data, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. Additionally, the ECM validated the method using six soil matrices while the ILV only used one soil matrix. The ILV should present a more vigorous test of the method than the ECM, and therefore, should have included more than one soil matrix.

3. The reviewer included data from both Trial 1 (T1) and the Re-injection of Trial 1 (RT1), even though RT1 was reported as the definitive results of the ILV since acceptable signal-to-noise ratios were achieved (pp. 10-11 of MRID 47033302).

4. 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. The LOQ and LOD were not adequately supported by calculations or comparison to background levels in the ECM

(pp. 9, 24-25 of MRID 47033301; pp. 10-11, 20 of MRID 47033302). In the ECM, the LOQ was defined as the lowest fortification level at which average recoveries of 70-120% and a RSD <20% are achieved. Also, at the LOQ fortification level, the analyte peak consistently represents a signal-to-noise ratio of approximately 5-20 to 1 for the least responsive analyte, diuron. In the ECM, the LOD for the four analytes was estimated to be 0.002 mg/kg, based on the limiting response analyte, diuron. 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 form the signal-to-noise response of each analyte in matrix at the LOQ level using the following equation: LOD = {[LOD signal-to-noise response (3/1)]/Observed LOQ signal-to-noise response} x LOQ, which equaled (3/1)/(12/1) x 0.010 mg/kg. The reviewer noted that this LOD calculation equalled 0.0025 mg/kg. No further justification of the LOQ or LOD was reported in the ECM. No justification of the LOQ was reported in the ILV. No LOD was reported in the ILV.

5. The linearity of the calibration curve of DCPU for Trial 1 was unsatisfactory ($r^2 = 0.9930$; Appendix 1, p. 29 of MRID 47033302; see DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$; however, the reviewer noted that the linearity of the Re-injection of Trial 1 was satisfactory.

6. The matrix interferences were determined to be insignificant in the ECM based on matrix interferences in the controls; solvent standards were used for calibration (pp. 15-16, 23 of MRID 47033301).

7. The ECM study author reported that DCPMU and diuron were detected *ca*. 20% and 10% of the LOQ in the CA soil control samples (p. 23 of MRID 47033301). The reviewer considered this to be minor matrix interference which did not affect the method specificity.

8. In the ECM, a Second Lab Tryout of the method for linuron, DCPMU and DCPU was performed by ABC Laboratories using the California and Louisiana control soils (p. 26; Appendix 4, pp. 59-60; Appendix 5, pp. 61-63 of MRID 47033301). The ABC Laboratories used a Sciex API-3000 MS and an injection volume of 50 µL. Acceptable results were reported for the initial trial where n = 3 for LOQ fortifications and n = 1 for 10×LOQ fortifications. Linuron was also fortified at 500×LOQ (5 ppm; n = 1). Only the recovery results and instrumental conditions were reported from the ABC Laboratories trial of the method; no personnel, experimental dates or study report was included.

9. 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 20 hours over 3 days to complete (p. 20 of MRID 47033302). The ASE extraction was performed overnight then sample processing prior to LC/MS/MS required *ca*. 2-3 hours. Finally, the LC/MS/MS analysis required *ca*. 8 hours (run overnight). In the ECM, the time requirements were similar to those reported in the ILV (p. 25 of MRID 47033301).

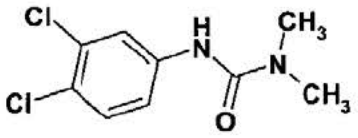
V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

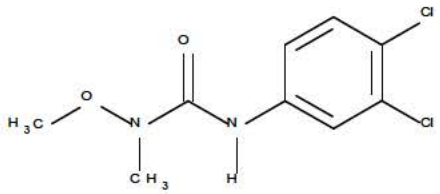
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

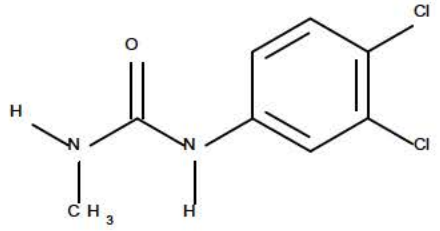
Diuron (DPX-14740)
IUPAC Name: 3-(3,4-Dichlorophenyl)-1,1-dimethylurea
CAS Name: N'-(3,4-Dichlorophenyl)-N,N-dimethylurea
CAS Number: 330-54-1
SMILES String:



Linuron (DPX-Z0326)
IUPAC Name: 3-(3,4-Dichlorophenyl)-1-methoxy-1-methylurea
CAS Name: N′-(3,4-Dichlorophenyl)-N-methoxy-N-methylurea
CAS Number: 330-55-2
SMILES String: O=C(N(OC)C)Nc(ccc(c1Cl)Cl)c1



DCPMU (Desmethoxy linuron; IN-1564-012; IN-15654)
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



DCPU (Norlinuron; IN-RD915-008)

IUPAC Name:	(1-3,4-Dichlorophenyl)urea N'-(3,4-Dichlorophenyl)urea
CAS Name:	3,4-Dichlorophenyl urea
CAS Number:	2327-02-8
SMILES String:	[H]N([H])C(=O)N([H])c1ccc(c(c1)Cl)Cl