

Analytical method for dichlobenil and its metabolite 2,6-dichlorobenzamide in soil

Reports: ECM: EPA MRID No.: 49948601. Shen, H., L. Mannella. 2015. Method Validation for the Determination of Dichlobenil and its Metabolite 2,6-Dichlorobenzamide in Soil. PTRL Study No.: 2792W. Report No. GCDI-027401-1245. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by MacDermid Agricultural Solutions Inc., Waterbury, Connecticut; 79 pages. Final report issued December 16, 2015.

ILV: EPA MRID No. 49948602. Smith, R. 2016. Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of Dichlobenil and its Metabolite 2,6-Dichlorobenzamide in Soil. Smithers Viscient Study No.: 14088.6106. Report No. GCDI-027401-1245. Report prepared by PTRL West (a division of EAG, Inc.), Hercules, California, sponsored and submitted by MacDermid Agricultural Solutions Inc., Waterbury, Connecticut; 79 pages. Final report issued June 9, 2016.

Document No.: MRIDs 49948601 & 49948602

Guideline: 850.6100


Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, with the exception that the certification of the internal standard, 4-chlorobenzonitrile, provided by Sigma-Aldrich does not specify whether the analysis was conducted under the requirements of GLP (p. 3 of MRID 49948601). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49948602). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was not included.

Classification: This analytical method is classified as supplemental. An updated ECM should be submitted incorporating the ILV modifications for DBN validation without the internal standard. Most of the mean recoveries of DBN did not meet guideline requirements for precision and accuracy in the ILV validation with the internal standard. The ILV soil matrix was poorly characterized.


PC Code: 027401

Final EPA Reviewer: James Lin
Environmental Engineer

Signature: 

Date: 2/27/17

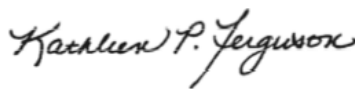
CDM/CSS-Dynamac JV Reviewers: Lisa Muto,
Environmental Scientist

Signature: 

Date: 12/6/16

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature:



Date: 12/6/16

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

This analytical method, PTRL Study No.: 2792W (MacDermid Agricultural Solutions Inc.), is designed for the quantitative determination of dichlobenil (DBN) at the LOQ of 0.01 mg/kg and its metabolite 2,6-dichlorobenzamide (BAM) at the LOQ of 0.005 mg/kg in soil using GC/MS/SIM (selective ion monitoring mode). The LOQs are less than the lowest toxicological levels of concern in soil. The ECM used characterized sandy loam soil and incorporated an internal standard for analysis. The ILV validated the method for BAM with the internal standard on the first trial with no modifications. The ILV validated the method for DBN on the third trial with the minor modifications of the elimination of the internal standard and repetition of an extraction step. The method could not be validated by the ILV for DBN without these modifications; therefore, an updated ECM should be submitted incorporating the ILV modifications as alternative procedures. Most of the mean recoveries did not meet guideline requirements for precision and accuracy in the ILV validation of DBN with the internal standard. The ILV sandy loam soil matrix was poorly characterized; it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

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						
Dichlobenil (DBN)	49948601 ¹	49948602 ²		Soil	16/12/2015	MacDermid Agricultural Solutions Inc.	GC/MS/SIM	0.01 mg/kg
2,6-Dichlorobenzamide (BAM)			0.005 mg/kg					

1 In the ECM, sandy loam soil (2705W-015; 62% sand 30% silt 8% clay; 1.01% organic matter; pH 5.3 in 1:1, soil:water) matrix was collected from Hickman, California and well characterized (USDA soil texture classification; p. 17; Appendix C, pp. 75-76 of MRID 49948601). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by Hulst Research Farm Service, Inc.; collection procedures were reported. The soil was sieved (6-mm) prior to use.

2 In the ILV, Rochester sandy loam soil (SMV Lot No. 093015, moisture content 8.61%; SMV Lot No. 011116, moisture content 9.82%) was poorly characterized (soil texture classification not reported; p. 13 of MRID 49948602). The source was not further specified.

I. Principle of the Method

Soil (10.0 g) was fortified (0.100 mL) with the appropriate fortification solution, as necessary, in a 50-mL plastic centrifuge tube (pp. 18, 22-24; Figure 1, p. 39 of MRID 49948601). The sample was extracted with 30 mL of acetone:hexanes (1:1, v:v), 3.4 mL of 0.2% freshly prepared NH₄Cl solution and four 4-mm SS grinding balls. The sample was placed on a SPEX GenoGrinder at 1500 rpm for 3 minutes. After filtration (Whatman glass fiber GF8 filter) in a Büchner funnel into a 125 mL filtering flask, the filter cake was rinsed with approximately 6 mL of acetone:hexanes (1:1, v:v). The extract was transferred to a 125-mL separatory funnel and further extracted with 60 mL of water and 2 mL of saturated NaCl solution via shaking for 1 minute. After the phases separate, the hexanes layer was collected into a 125-mL round bottom flask via fluted filter containing 4 g of anhydrous Na₂SO₄. The aqueous layer was extracted three times with 20 mL of hexanes via shaking for 1 minute and passing through the same fluted filter containing 4 g of anhydrous Na₂SO₄. The combined extracts were reduced to a volume of *ca.* 5 mL using a rotary evaporator (temperature not specified). The residue was transferred to a 10-mL volumetric flask with a 5-mL rinse of the round bottom flask. The volume was adjusted to 10 mL with hexanes (this is DBN extract A). 5.0 mL of DBN extract A was applied to an alumina SPE cartridge (pre-conditions with 5 mL x 2 hexanes) and eluted with 2 x 4 mL of 2% acetone:hexanes into a 15-mL graduated glass centrifuge tube. The solvent was evaporated to *ca.* 2 mL at room temperature under a gentle stream of nitrogen. After 0.5 mL of the internal standard working solution (0.5 g/mL) was added via pipet, the sample was sonicated and analyzed by GC/MS/SIM for DBN. The pH of the aqueous layer from the separatory funnel was adjusted to >9 with NH₄OH, dropwise. The basified aqueous phase was extracted three times with 20 mL of ethyl acetate via shaking for 1 minute. After the phases separate, the ethyl acetate layer was collected into a 125-mL round bottom flask via fluted filter containing 4 g of anhydrous Na₂SO₄. The combined extracts were reduced to a volume of *ca.* 2 mL using a rotary evaporator at room temperature. The residue was sonicated and filtered (0.2 µm nylon filter) into a 15-mL graduated glass centrifuge tube. 2 mL of ethyl acetate was added to the tube, then the extract was sonicated and filtered (0.2 µm nylon filter) into the same 15-mL graduated glass centrifuge tube (BAM extract B). The filtered extract was evaporated at 40°C to 2 mL. After 0.5 mL of the internal standard working solution (0.5 g/mL) was added via pipet, the sample was sonicated and analyzed by GC/MS/SIM for BAM.

Samples were analyzed for dichlobenil (DBN) and 2,6-dichlorobenzamide (BAM) by Agilent 6890 GC equipped with Agilent 5973 Mass Selective Detector (MSD) using Agilent J & W DB-17 capillary column (30 m x 0.25 mm i.d. x 0.5 µm film thickness), a temperature gradient of 250°C injector, 60°C initial temperature, 15°C/minute ramp rate and 270°C final temperature, and scan mode SIM (selective ion monitoring; pp. 24-25 of MRID 49948601). Injection volume was 1 µL. DBN was identified using three ions; one for quantitation (Q) and two for confirmation (C): *m/z* 171 (Q), *m/z* 173 (C1) and *m/z* 136 (C2). BAM was identified using two ions; one for quantitation (Q) and one for confirmation (C): *m/z* 173 (Q) and *m/z* 175 (C). Approximate retention times were *ca.* 11.8 and 15.3 minutes for DBN and BAM, respectively.

In the ILV, the ECM was performed as written, except that, for DBN only, the initial liquid-liquid extraction was performed twice instead of once in order to improve extraction efficiency and the internal standard was removed from the samples for one validation set (pp. 13, 19-24, 28

of MRID 49948602). The same analytical instrument and conditions were used as were used in the ECM. DBN was identified using the same three ions as the ECM. BAM was identified using three ions; one for quantitation (Q) and two for confirmation (C): m/z 173 (Q), m/z 175 (C1) and m/z 189 (C2). Approximate retention times were *ca.* 12.0 and 15.6 minutes for DBN and BAM, respectively.

In the ECM and ILV, the Limit of Quantitation (LOQ) for soil was 0.01 mg/kg for dichlobenil (DBN) and 0.005 mg/kg for 2,6-dichlorobenzamide (BAM; pp. 6, 28 of MRID 49948601; p. 9 of MRID 49948602). In the ECM, the Limit of Detection (LOD) for soil was 0.002 mg/kg for dichlobenil (DBN) and 0.001 mg/kg for 2,6-dichlorobenzamide (BAM). The LOD values were not specifically reported in the ILV, but the reviewer assumed that they were the same values as reported in the ECM based on the information in Tables 1-6, pp. 32-37 of MRID 49948602.

II. Recovery Findings

ECM (MRID 49948601): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD \leq 20%) for analysis of dichlobenil (DBN) in soil at fortification levels of 0.01 mg/g (LOQ) and 0.1 mg/kg (10 \times LOQ) and 2,6-dichlorobenzamide (BAM) in soil at fortification levels of 0.005 mg/g (LOQ) and 0.05 mg/kg (10 \times LOQ); quantitation and confirmatory ions; pp. 12-13; Tables I-II, pp. 34-35). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. Three ions were monitored for DBN; two ions were monitored for BAM. The sandy loam soil (2705W-015; 62% sand 30% silt 8% clay; 1.01% organic matter; pH 5.3 in 1:1, soil:water) matrix was collected from Hickman, California and well characterized (USDA soil texture classification; p. 17; Appendix C, pp. 75-76). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by Hulst Research Farm Service, Inc.; collection procedures were reported. The soil was sieved (6-mm) prior to use.

ILV (MRID 49948602): With the internal standard, mean recoveries and RSDs were within guideline requirements for analysis of 2,6-dichlorobenzamide (BAM) in soil at fortification levels of 0.005 mg/g (LOQ) and 0.05 mg/kg (10 \times LOQ); quantitation and confirmatory ions; p. 28; Tables 1-6, pp. 32-37). With the internal standard, RSDs were within guideline requirements for analysis of dichlobenil (DBN) in soil at fortification levels of 0.01 mg/g (LOQ) and 0.1 mg/kg (10 \times LOQ); however, only the mean recovery of the confirmatory ion 2 at the LOQ was within guideline requirements. Mean recoveries for the quantitation ion (66.8% LOQ, 67.6% 10 \times LOQ), confirmatory ion 1 (67.6% LOQ, 67.9% 10 \times LOQ) and confirmatory ion 2 (69.2% 10 \times LOQ) did not meet guideline requirements for precision and accuracy. Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable for DBN, but not as much in the case of BAM. Without the internal standard, mean recoveries and RSDs were within guideline requirements for analysis of dichlobenil (DBN) in soil at fortification levels of 0.01 mg/g (LOQ) and 0.1 mg/kg (10 \times LOQ). Performance data (recovery results) from quantitation ion analyses and confirmation ion analyses were comparable. For all analyses, three ions were monitored for DBN and BAM. The Rochester sandy loam soil (SMV Lot No. 093015, moisture content 8.61%; SMV Lot No. 011116, moisture content 9.82%) was poorly characterized (soil texture classification not reported; p. 13 of MRID 49948602). The source was not further specified. The method was validated for BAM on the first trial with the

internal standard with no modifications and for DBN on the third trial with the minor modifications of the elimination of the internal standard and repetition of an extraction step (p. 9).

Table 2. Initial Validation Method Recoveries for Dichlobenil (DBN) and 2,6-dichlorobenzamide (BAM) in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitation ion						
Dichlobenil	0.01 (LOQ)	5	74-83	79	3	4
	0.1	5	62-78	72	6	8
2,6-Dichlorobenzamide	0.005 (LOQ)	5	76-90	84	6	7
	0.05	5	57-80	72	9	13
Confirmatory ion 1						
Dichlobenil	0.01 (LOQ)	5	70-79	75	3	4
	0.1	5	61-78	72	7	10
2,6-Dichlorobenzamide	0.005 (LOQ)	5	77-87	83	5	6
	0.05	5	58-81	73	9	12
Confirmatory ion 2						
Dichlobenil	0.01 (LOQ)	5	68-76	72	3	4
	0.1	5	61-77	72	6	8

Data (uncorrected recovery results, pp. 26-27; Appendix E, pp. 77-79) were obtained from pp. 12-13; Tables I-II, pp. 34-35 of MRID 49948601.

1 The sandy loam soil (2705W-015; 62% sand 30% silt 8% clay; 1.01% organic matter; pH 5.3 in 1:1, soil:water) matrix was collected from Hickman, California and well characterized (USDA soil texture classification; p. 17; Appendix C, pp. 75-76 of MRID 49948601). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by Hulst Research Farm Service, Inc.; collection procedures were reported. The soil was sieved (6-mm) prior to use.

2 DBN was identified using three ions; one for quantitation (Q) and two for confirmation (C): m/z 171 (Q), m/z 173 (C1) and m/z 136 (C2). BAM was identified using two ions; one for quantitation (Q) and one for confirmation (C): m/z 173 (Q) and m/z 175 (C).

Table 3. Independent Validation Method Recoveries for Dichlobenil (DBN) and 2,6-dichlorobenzamide (BAM) in Soil^{1,2}

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Analytical Results With Internal Standard						
Sandy Loam Soil						
Quantitation ion						
Dichlobenil ³	0.01 (LOQ)	5	63.1-71.6	66.8	3.08	4.61
	0.1	5	62.2-70.8	67.6	3.21	4.76
2,6-Dichlorobenzamide ⁴	0.005 (LOQ)	5	68.1-87.5	79.6	9.97	12.5
	0.05	5	76.3-93.1	86.4	6.76	7.82
Confirmatory ion 1						
Dichlobenil ³	0.01 (LOQ)	5	61.4-71.2	67.6	4.27	6.33
	0.1	5	63.3-71.5	67.9	2.94	4.33
2,6-Dichlorobenzamide ⁴	0.005 (LOQ)	5	68.2-101	84.3	12.4	14.7
	0.05	5	81.5-99.8	91.5	7.27	7.95
Confirmatory ion 2						
Dichlobenil ³	0.01 (LOQ)	5	64.0-78.8	72.0	5.32	7.39
	0.1	5	63.4-72.5	69.2	3.45	4.98
2,6-Dichlorobenzamide ⁴	0.005 (LOQ)	5	83.4-109	97.2	10.0	10.3
	0.05	5	85.1-105	95.5	8.53	8.93
Analytical Results Without Internal Standard						
Sandy Loam Soil						
Quantitation ion						
Dichlobenil ³	0.01 (LOQ)	5	77.1-82.2	80.0	2.03	2.54
	0.1	5	68.7-95.4	86.3	11.1	12.8
Confirmatory ion 1						
Dichlobenil ³	0.01 (LOQ)	5	75.1-87.6	81.1	5.83	7.19
	0.1	5	70.0-95.2	86.8	10.8	12.4
Confirmatory ion 2						
Dichlobenil ³	0.01 (LOQ)	5	82.2-91.0	88.6	3.63	4.10
	0.1	5	70.5-98.8	88.9	11.6	13.1

Data (uncorrected recovery results, pp. 26-27) were obtained from p. 28; Tables 1-6, pp. 32-37 of MRID 49948602.

1 The Rochester sandy loam soil (SMV Lot No. 093015, moisture content 8.61%; SMV Lot No. 011116, moisture content 9.82%) was poorly characterized (soil texture classification not reported; p. 13 of MRID 49948602). The source was not further specified.

2 DBN was identified using three ions; one for quantitation (Q) and two for confirmation (C): *m/z* 171 (Q), *m/z* 173 (C1) and *m/z* 136 (C2). BAM was identified using three ions; one for quantitation (Q) and two for confirmation (C): *m/z* 173 (Q), *m/z* 175 (C1) and *m/z* 189 (C2).

3 Results were reported from the third trial (p. 9 of MRID 49948602).

4 Results were reported from the first trial; no procedure was performed without the internal standard (p. 9 of MRID 49948602).

III. Method Characteristics

In the ECM and ILV, the Limit of Quantitation (LOQ) for soil was 0.01 mg/kg for dichlobenil (DBN) and 0.005 mg/kg for 2,6-dichlorobenzamide (BAM; pp. 6, 28 of MRID 49948601; pp. 9, 27 of MRID 49948602). In the ECM, the LOQ was defined by the lowest fortification level successfully tested. The LOQ values represented 20 ng/mL of each analyte in calibration standard solution using current methodology. No justification of the LOQ was provided in the ILV. In the ECM, the Limit of Detection (LOD) for soil was 0.002 mg/kg for dichlobenil (DBN) and 0.001 mg/kg for 2,6-dichlorobenzamide (BAM). In the ECM, the LOD values represented 4 ng/mL of each analyte in calibration standard solution using current methodology. In the ILV, the LOD was reportedly calculated by evaluating the signal-to-noise (S/N) ratio from samples of the lowest calibration standard and control samples to establish the lowest level at which the analyte can reliably be detected. A S/N ratio of 3:1 was used to determine the LOD for each analyte and each ion monitored. The LOD values were not specifically reported in the ILV, but the reviewer assumed that they were the same values as reported in the ECM based on the information in Tables 1-6, pp. 32-37 of MRID 49948602. No other calculated values were reported.

Table 4. Method Characteristics*

Analyte		Dichlobenil (DBN)	2,6-Dichlorobenzamide (BAM)	
Limit of Quantitation (LOQ)		0.01 mg/kg	0.005 mg/kg	
Limit of Detection (LOD) ¹		0.002 mg/kg	0.001 mg/kg	
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.9998$ (Q) $r^2 = 0.9997$ (C1 & C2)	$r^2 = 0.9979-0.9988$ (Q) $r^2 = 0.9978-0.9984$ (C1)	
		(4.00-400 ng/mL)		
	ILV	$r^2 = 0.99958$ (Q) $r^2 = 0.99952$ (C1) $r^2 = 0.99914$ (C2)	$r^2 = 0.99707$ (Q) $r^2 = 0.99812$ (C1) $r^2 = 0.99643$ (C2)	
		(4.00-400 µg/L)		
Repeatable	ECM ²		Yes at LOQ and 10×LOQ (with IS)	
	ILV ³	With IS	Yes at LOQ (C2); No at LOQ (Q 66.8% , C1 67.6%) and 10×LOQ (Q 67.6% , C1 67.9% , C2 69.2%)	Yes at LOQ and 10×LOQ ⁴
		Without IS	Yes at LOQ and 10×LOQ ⁵	Not performed
Reproducible		Yes at LOQ and 10×LOQ (without IS)	Yes at LOQ and 10×LOQ (with IS)	
Specific	ECM ⁶		Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, no matrix interferences were noted.
	ILV		Yes, no matrix interferences were noted. Nearby peaks were noted in the C2 LOQ chromatogram.	Yes, no matrix interferences were noted. Analyte peak was very small at LOQ in C1 & C2 chromatogram and at 10×LOQ in C1 chromatogram.

Data were obtained from pp. 6, 12-13, 28; Tables I-II, pp. 34-35 (recovery data); Tables III-IV, pp. 36-37 (calibration coefficients); Figures 5-6, pp. 43-47 (calibration curves); Figures 9-18, pp. 50-61 (chromatograms) of MRID 49948601; pp. 9, 27-28; Tables 1-6, pp. 32-37 (recovery data); Figures 1-7, pp. 38-47 (DBN chromatograms); Figures 6-8, pp. 48-50 (DBN calibration curves); Figures 9-13, pp. 51-60 (BAM chromatograms); Figures 14-16, pp. 61-63 (BAM calibration curves) of MRID 49948602; DER Attachment 2. Q = quantitation ion; C1 = confirmatory ion; C2 = confirmatory ion 2. IS = Internal Standard.

* Results are for all ions monitored unless stated otherwise.

- 1 The LOD values were not specifically reported in the ILV, but the reviewer assumed that they were the same values as reported in the ECM based on the information in Tables 1-6, pp. 32-37 of MRID 49948602.
- 2 In the ECM, sandy loam soil (2705W-015; 62% sand 30% silt 8% clay; 1.01% organic matter; pH 5.3 in 1:1, soil:water) matrix was collected from Hickman, California and well characterized (USDA soil texture classification; p. 17; Appendix C, pp. 75-76 of MRID 49948601). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. The soil matrix was provided by Hulst Research Farm Service, Inc.; collection procedures were reported. The soil was sieved (6-mm) prior to use.
- 3 In the ILV, Rochester sandy loam soil (SMV Lot No. 093015, moisture content 8.61%; SMV Lot No. 011116, moisture content 9.82%) was poorly characterized (soil texture classification not reported; p. 13 of MRID 49948602). The source was not further specified.
- 4 Results were reported from the first trial; no procedure was performed without the internal standard (p. 9 of MRID 49948602). No ECM modifications were reported.
- 5 Results were reported from the third trial (p. 9 of MRID 49948602). Minor ECM modifications included the elimination of the internal standard and repetition of an extraction step (p. 24).
- 6 In the ECM, chromatograms were only provided for the quantitation ion for DBN and BAM.

IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, two modifications were made to the method for DBN only: the initial liquid-liquid extraction was performed twice instead of once in order to improve extraction efficiency and the internal standard was removed from the samples for one validation set (pp. 13, 19-24, 28 of MRID 49948602). The method could not be validated by the ILV for DBN without these modifications; therefore, an updated ECM should be submitted incorporating these modifications as alternative procedures.

For the ILV validation with the internal standard, mean recoveries of DBN for the quantitation ion (66.8% LOQ, 67.6% 10×LOQ), confirmatory ion 1 (67.6% LOQ, 67.9% 10×LOQ) and confirmatory ion 2 (69.2% 10×LOQ) did not meet guideline requirements for precision and accuracy (Tables 1-6, pp. 32-37 of MRID 49948602). OCSPP Guideline 850.6100 criteria for precision and accuracy at the stated LOQ and at higher concentrations is mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%.

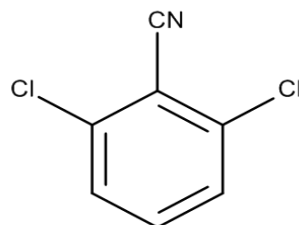
2. In the ILV, the Rochester sandy loam soil (SMV Lot No. 093015, moisture content 8.61%; SMV Lot No. 011116, moisture content 9.82%) was poorly characterized (soil texture classification not reported; p. 13 of MRID 49948602). It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.
3. In the ECM, chromatograms were only provided for the quantitation ion for DBN and BAM (Figures 9-18, pp. 50-61 of MRID 49948601). Representative chromatograms from all fortification levels should be provided for review of method specificity. The reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
4. 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. 6, 28 of MRID 49948601; pp. 9, 27 of MRID 49948602). In the ECM, the LOQ was defined by the lowest fortification level successfully tested. No justification of the LOQ was provided in the ILV. In the ECM, no justification of the LOD was provided. In the ILV, the LOD was reportedly calculated by evaluating the signal-to-noise (S/N) ratio from samples of the lowest calibration standard and control samples to establish the lowest level at which the analyte can reliably be detected. A S/N ratio of 3:1 was used to determine the LOD for each analyte and each ion monitored. The LOD values were not specifically reported in the ILV, but the reviewer assumed that they were the same values as reported in the ECM based on the information in Tables 1-6, pp. 32-37 of MRID 49948602. No other calculated values were reported.

Additionally, the lowest toxicological levels of concern in soil for dichlobenil and 2,6-dichlorobenzamide were not reported in the ECM and ILV. An LOQ above toxicological levels of concern results in an unacceptable method classification.

5. In the ILV, communications between the ILV, study sponsor and study monitor were detailed in the study (p. 25; Appendix 3, pp. 76-79 of MRID 49948602).
6. In the ECM, no significant matrix effects were observed ($< \pm 20\%$), and non-matrix-matched calibration standards were used (pp. 32; Table V, p. 38; Figures 19-20, pp. 62-65 of MRID 49948601).
7. It was reported for the ILV that one batch of twenty samples (one reagent blank, two controls, five samples fortified at the LOQ and $10\times$ LOQ, and seven calibration standards) required one working day for preparation and sample processing (p. 26 of MRID 49948602). The GC/MS analysis was performed overnight. Overall, the data set required two working days to complete.

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**Dichlobenil; DBN****IUPAC Name:** 2,6-Dichlorobenzonitrile**CAS Name:** 2,6-Dichlorobenzonitrile**CAS Number:** 1194-65-6**SMILES String:** c1cc(c(c1)Cl)C#N)Cl**2,6-Dichlorobenzamide; BAM****IUPAC Name:** 2,6-Dichlorobenzamide**CAS Name:** 2,6-Dichlorobenzamide**CAS Number:** 2008-58-4**SMILES String:** [H]N([H])C(=O)c1c(ccc1Cl)Cl