Test Material:	Pendimethalin
MRID:	49397802
Title:	Adaptation/Development and Validation of Residue Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and its Metabolites M455H001, Reg. No. 4061757 and Reg. No. 4982164 (P36) in Soil (Including Amendment No. 1)
MRID:	49397803
Title:	Independent Laboratory Validation of the method determining the residues of Pendimethalin in soil
EPA PC Code:	108501
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 3/12/15

Secondary Reviewer: Lynne Binari **Signature:** 

Date: 3/12/15

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 3/12/15

Analytical method for pendimethalin and its transformation products, M455H001, Reg. No. 4061757 and Reg. No. 4982164 (P36), in soil

Reports: ECM: EPA MRID No. 49397802. Class, T., and N. Heinz. 2013. Adaptation/Development and Validation of Residue Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and its Metabolites M455H001, Reg. No. 4061757 and Reg. No. 4982164 (P36) in Soil (Including Amendment No. 1). BASF ID: 364239_1. PTRL Europe ID: P 2768 G. BASF Registration Document No.: 2013/7004790. Report prepared by PTRL Europe, Ulm, Germany; sponsored by BASF SE, Ludwigshafen, Germany; and submitted by BASF Corporation, Research Triangle Park, North Carolina; 114 pages. Final original report issued April 18, 2013; amendment completion date, August 8, 2013.
ILV: EPA MRID No. 49397803. Shen, X., and P. Michener. 2014. Independent Laboratory Validation of the method determining the residues of Pendimethalin in soil. BASF Study ID: 438119. PASC Study No.: 053-0963. PASC Report No.: PASC-REP-0466. BASF Registration Document No.: 2014/7001462. Report prepared by Primera Analytical Solutions Corp. (PASC), Princeton, New Jersey; sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 47 pages. Final original report issued April 24, 2014; amendment completion date, May 19, 2014.

Document No.: MRIDs 49397802 & 49397803

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with current German Principles of Good Laboratory Practice (GLP; 2011) which are based on the OECD Principles of GLP, as well as in accordance with EC Guidance (SANCO/825/00 and SANCO/3029/99; pp. 3-4, 6; Appendix 5, p. 92; Amendment No. 1, p. 94; Amendment No. 1, Appendix 1, p. 114 of MRID 49397802). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-6; Appendix 5, p. 92; Amendment No. 1, pp. 94-95; Amendment No. 1, Appendix 1, p. 114).
ILV: The study was conducted in accordance with USEPA GLP (Title 40, Part 160 of CFR; p. 3 of MRID 49397803). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). A signatures page was also provided (p. 6).

Classification: This analytical method is classified as acceptable The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Linearity coefficients were <0.995 for ILV calibrations.

PC Code: 108501

EPA Reviewer:

Ibrahim Abdel-saheb **Signature:**
Environmental Scientist **Date: 11-23-2015**

All cited page numbers for ECM MRID 49397802 refer to those in the bottom right-hand corner of the document. All cited page numbers for ILV MRID 49397803 refer to those in the upper right-hand corner of the document.

Executive Summary

This analytical method, BASF Document No. 2013/7004790, is designed for the quantitative determination of pendimethalin in soil at the LOQ of 1 µg/kg, as well as its transformation products M455H001 and Reg. No. 4061757 in soil at the LOQ of 10 µg/kg and its transformation product Reg. No. 4982164 (P36) in soil and sediment at the LOQ of 10 µg/kg. The LOQs are [less than/equal to/greater than] the lowest toxicological level of concern in soil for all analytes. Detection was performed with LC/MS/MS for pendimethalin, M455H001 and P36 and GC/MS/MS (NCI) for Reg. No. 4061757. Only pendimethalin was evaluated in the ILV; M455H01, P36 and Reg. No. 4061757 were not included as analytes. Sandy loam soil and loamy sand soil and sediment were used in the ECM, while loam and loamy sand soil were used in the ILV. No major issues were discovered by the independent laboratory; the method was validated for pendimethalin with the first trial.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Pendimethalin (BAS 455 H; Reg. No. 900072)	49397802	49397803		Soil	4/18/2013 (Original Method) 8/8/2013 (Amendment 1)	BASF Corporation	LC/MS/MS	1 µg/kg
M455H001 (Reg. No. 4108474)								0.010 mg/kg (10 µg/kg)
P36 (Reg. No. 4982164)				Soil and Sediment				
Reg. No. 4061757				Soil			GC/MS/MS	

I. Principle of the Method

Procedure for BAS 455 H (Pendimethalin), M455H001 and P36 (Reg. No. 4982164)
Samples (10.0 g) were measured into 50 mL centrifuge tubes and fortified, as necessary (p. 18 of MRID 49397802). The soil/sediment samples were extracted sequentially with 2% HCl in methanol, methanol:water (7:3, v:v) and methanol:water (1:1, v:v; 20 mL each). For each extraction, the samples were extracted via shaking for 30 minutes with a mechanical shaker (225 rpm) and sonication for 10 minutes. After centrifugation at 4000 rpm for 5 minutes, the supernatant was decanted. The combined supernatants were brought to volume (60 mL for soil and 65 mL for sediment) using methanol:water (7:3, v:v). Prior to LC/MS/MS analysis, the soil extracts were diluted (typically DF = 10) with acetonitrile:water (50:50, v:v) + 0.1% formic acid.

Procedure for Reg. No. 4061757
Samples (10.0 g) were measured into 50 mL centrifuge tubes and fortified, as necessary (p. 18 of MRID 49397802). The soil/sediment samples were extracted twice with acetone (10 mL each). For each extraction, the samples were extracted via shaking for 30 minutes via a mechanical shaker (225 rpm) and sonication for 10 minutes. After centrifugation at 4000 rpm for 5 minutes, the supernatant was decanted. The combined supernatants were brought to volume (20 mL) using acetone and analyzed by GC/MS/MS.

Samples were analyzed for pendimethalin, M455H001 and P36 (Reg. No. 4982164) using an Agilent 1200 SL HPLC System coupled to an AB MDS Sciex 4000 Triple Quad mass spectrometer with TurboIonspray (ESI; pp. 19-20 of MRID 49397802). The HPLC/MS conditions consisted of a Thermo Betasil C18 column (2.1 x 100 mm, 5-µm; column temperature 35°C), a mobile phase gradient of (A) water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid [percent A:B (v:v) at 0.00 min. 66:34, 2.00 min. 26:74, 4.00-6.50 min. 10:90, 6.60-10.00 min. 0:100, 10.10-12.00 min. 66:34] and MS/MS detection in positive (pendimethalin and P36) or negative (M455H001) ion mode. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 282 → 194 (Q) and *m/z* 282 → 212 (C) for pendimethalin; *m/z* 262 → 146 (Q) and *m/z* 262 → 119 (C) for P36; and *m/z* 310 → 266 (Q) and *m/z* 310 → 236 (C) for M455H001. Approximate retention times were 5.4 min. for pendimethalin, 4.5 min. for P36 and 5.0 min. for M455H001. Injection volume was 50 µL.

Samples were analyzed for Reg. No. 4061757 using a Thermo TSQ Quantum GC/MS System equipped with a Trace Ultra GC gas chromatograph in negative chemical ionization mode (NCI; p. 21 of MRID 49397802). The GC/MS conditions consisted of an Agilent VF-5MS column (0.32 mm x 30 m, 0.25-µm; isothermal injection temperature 250°C), splitless injection and helium carrier gas. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 211 → 194 (Q) and *m/z* 211 → 193 (C) for Reg. No. 4061757. Approximate retention time was 10 min. for Reg. No. 4061757. Injection volume was 3.0 µL.

In Amendment No. 1, the same analytical method and instrumentation were used as the primary study (Amendment No. 1, pp. 99-101). The only difference was the improved LOQ of 1 µg/kg versus 10 µg/kg for pendimethalin; only pendimethalin was employed. A complete set of calibration standards were prepared for quantification. The LC/MS/MS monitored ion transitions (quantitative = Q, confirmatory = C) were *m/z* 282 → 194 (Q) and *m/z* 282 → 212 (C). The approximate retention time was 4.5-4.6 min. for pendimethalin (Amendment No. 1, Figures 5-10, pp. 108-113).

The ILV was performed in support of the Amendment to the ECM (Amendment No. 1) with the improved LOQ for pendimethalin (pp. 11, 19 of MRID 49397803). In the ILV, samples were processed in the same manner as the ECM (pp. 14-15; Table 11.3, p. 22 of MRID 49397803). Only pendimethalin was included as a test material. The extracts were analyzed for pendimethalin using identical or similar instruments and instrumental conditions; the approximate retention time was *ca*. 4.24 min. for pendimethalin. The monitored mass transitions were inverted (quantitative = Q, confirmatory = C): *m/z* 282 → 212 (Q) and *m/z* 282 → 194 (C).

For pendimethalin, the LOQ and LOD were reported as 1 µg/kg and 0.3 µg/kg (30% of the LOQ), respectively, in both the ECM and ILV (Amendment No. 1, pp. 100-101 of MRID 49397802; pp. 15, 19 of MRID 49397803). For M455H01, P36 and Reg. No. 4061757, the LOQ and LOD were reported as 0.010 mg/kg (10 µg/kg) and 0.003 mg/kg (30% of the LOQ), respectively, in the ECM only; these analytes were not included in the ILV (pp. 24-25 of MRID 49397802).

II. Recovery Findings

ECM (MRID 49397802): For pendimethalin, mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD ≤20%) in Li10 loamy sand soil and Bruch West sandy loam soil at the LOQ (1 µg/kg), 10×LOQ (10 µg/kg) and 100×LOQ (100 µg/kg; pp. 26-27; Tables 1-6, pp. 31-36; Amendment No. 1, Table 1, p. 102). For M455H001 and Reg. No. 4061757, mean recoveries and relative standard deviations (RSD) were within guidelines for analysis in Li10 loamy sand soil and Bruch West sandy loam soil at the LOQ (10 µg/kg) and 10×LOQ (100 µg/kg). For P36, mean recoveries and relative standard deviations (RSD) were within guidelines for analysis of P36 in Li10 loamy sand soil and Ranschgraben loamy sand sediment at the LOQ (10 µg/kg) and 10×LOQ (100 µg/kg). LC/MS/MS analysis was employed for identification of residues of pendimethalin, M455H01 and P36; GC/MS/MS (NCI) analysis was employed for identification of residues of Reg. No. 4061757. Two parent-daughter ion transitions were monitored. Quantitative ion and confirmatory ion results were comparable. The number of samples was five for all analytes/matrix/ion results, with the first sample of each fortification/matrix/ion injected twice and the mean of the two injections reported for that sample. The soil/sediment matrices were fully characterized by BioChem agrar (USDA soil characterization; p. 14; Appendices 2-4, pp. 87-91). The same soil matrices were used in Amendment No. 1 as the primary study (Amendment No. 1, p. 99).

ILV (MRID 49397803): Mean recoveries and RSDs were within guidelines for analysis of pendimethalin in New Jersey loam soil and California loamy sand soil at the LOQ (1 µg/kg) and 10×LOQ (10 µg/kg; pp. 17-18; Tables 11.1-11.2, pp. 20-21). LC/MS/MS analysis was employed for identification of residues of pendimethalin (pp. 14-15; Table 11.3, p. 22). The ILV was in support of the Amendment to the ECM; M455H01, P36 and Reg. No. 4061757 were not included as analytes. Two parent-daughter ion transitions were monitored. Quantitative ion and confirmatory ion results were comparable. The method was validated with the first trial (p. 17). The soil matrices were provided by the sponsor and fully characterized by Agvise Laboratories, North Dakota (USDA soil characterization; p. 14; Appendix C, pp. 40-41).

Table 2. Initial Validation Method Recoveries for Pendimethalin and Its Transformation Products, M455H001, P36 (Reg. No. 4982164) and Reg. No. 4061757, in Soil/Sediment[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Loamy Sand Soil (Li10)						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	97-110	106	5	5
	10	5	104-110	107	3	2
	100	5	91-97	95	3	3
M455H001 (Reg. No. 4108474)	10 (LOQ)	5	85-97	92	5	5
	100	5	102-108	104	2	2
P36 (Reg. No. 4982164)	10 (LOQ)	5	91-101	95	5	5
	100	5	96-98	98	1	0.9
Reg. No. 4061757	10 (LOQ)	5	70-98	86	10	12
	100	5	76-86	81	4	5
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	103-109	105	3	2
	10	5	105-110	108	2	2
	100	5	89-97	94	3	3
M455H001 (Reg. No. 4108474)	10 (LOQ)	5	93-106	99	5	5
	100	5	101-105	103	1	1
P36 (Reg. No. 4982164)	10 (LOQ)	5	91-102	96	4	4
	100	5	95-100	98	2	2
Reg. No. 4061757	10 (LOQ)	5	70-88	83	7	9
	100	5	74-88	82	6	7
Sandy Loam Soil (Bruch West)						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	77-101	90	10	11
	10	5	92-105	99	5	5
	100	5	95-102	98	3	3
M455H001 (Reg. No. 4108474)	10 (LOQ)	5	90-94	92	2	2
	100	5	97-105	101	3	3
Reg. No. 4061757	10 (LOQ)	5	78-96	85	7	8
	100	5	87-99	94	5	5
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	88-109	95	8	8
	10	5	96-105	99	4	4
	100	5	95-100	97	2	2
M455H001 (Reg. No. 4108474)	10 (LOQ)	5	88-95	91	3	3
	100	5	96-101	99	2	2

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Reg. No. 4061757	10 (LOQ)	5	72-95	81	9	11
	100	5	91-105	97	5	5
Loamy Sand Sediment (Ranschgraben)						
Quantitative ion						
P36 (Reg. No. 4982164)	10 (LOQ)	5	83-85	83	1	0.9
	100	5	90-92	91	1	1
Confirmatory ion						
P36 (Reg. No. 4982164)	10 (LOQ)	5	81-87	85	2	3
	100	5	90-93	91	1	1

Data (uncorrected recovery results) were obtained from pp. 26-27; Tables 1-6, pp. 31-36; Amendment No. 1, p. 101; Amendment No. 1, Table 1, p. 102 of MRID 49397802. Standard deviations were reviewer-calculated based on data provided in the study report. The first sample of each fortification/matrix/ion was injected twice, and the mean of the two injections was reported for that sample. LC/MS/MS analysis was employed for identification of residues of pendimethalin, M455H01 and P36; GC/MS/MS (NCI) analysis was employed for identification of residues of Reg. No. 4061757 (pp. 19-21).

1 The soil/sediment matrices were fully characterized by BioChem agrar (USDA soil characterization; p. 14; Appendices 2-4, pp. 87-91 of MRID 49397802).

2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): *m/z* 282 → 194 (Q) and *m/z* 282 → 212 (C) for pendimethalin; *m/z* 310 → 266 (Q) and *m/z* 310 → 236 (C) for M455H001; *m/z* 262 → 146 (Q) and *m/z* 262 → 119 (C) for P36; and *m/z* 211 → 194 (Q) and *m/z* 211 → 193 (C) for Reg. No. 4061757 (pp. 19-21).

Table 3. Independent Validation Method Recoveries for Pendimethalin in Soil[1,2]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
New Jersey Loam Soil						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	94.8-97.2	96.0	1.1	1.2
	10	5	101-104	103	1.1	1.1
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	100-110	105	4.2	4.0
	10	5	104-109	106	1.7	1.6
California Loamy Sand Soil						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	96.0-104	100	2.9	2.9
	10	5	92.4-101	97.0	3.4	3.5
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	1 (LOQ)	5	90.0-112	99.2	8.2	8.3
	10	5	91.8-99.0	96.1	2.8	2.9

Data (uncorrected recovery results) were obtained from pp. 17-18; Tables 11.1-11.2, pp. 20-21 of MRID 49397803.
LC/MS/MS analysis was employed for identification of residues of pendimethalin (pp. 14-15; Table 11.3, p. 22).

1 The soil matrices were provided by the sponsor and fully characterized by Agvise Laboratories, North Dakota (USDA soil characterization; p. 14; Appendix C, pp. 40-41).

2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): *m/z* 282 → 212 (Q) and *m/z* 282 → 194 (C) for pendimethalin (pp. 14-15; Table 11.3, p. 22).

III. Method Characteristics

For pendimethalin, the LOQ and LOD were reported as 1 µg/kg and 0.3 µg/kg (30% of the LOQ), respectively, in both the ECM and ILV (Amendment No. 1, pp. 100-101 of MRID 49397802; pp. 15, 19 of MRID 49397803). For M455H01, P36 and Reg. No. 4061757, the LOQ and LOD were reported as 0.010 mg/kg (10 µg/kg) and 0.003 mg/kg (30% of the LOQ), respectively, in the ECM only; these analytes were not included in the ILV (pp. 24-25 of MRID 49397802). In the ECM, the LOQs for all analytes were supported by their respective acceptable method validation results. No other justification was provided. No calculations or comparison to background levels were provided for the LOQ or LOD. In the ILV, the LOQ and LOD were reported from the ECM. No justification was provided.

Table 4. Method Characteristics

		Pendimethalin	M455H01	P36	Reg. No. 4061757
Limit of Quantitation (LOQ)		1 µg/kg	0.010 mg/kg (10 µg/kg)		
Limit of Detection (LOD)		0.3 µg/kg (30% of the LOQ)	0.003 mg/kg (30% of the LOQ)		
Linearity (calibration curve r^2 and concentration range)	ECM[1]	**Quantification ion**			
		r^2 = 0.9976-0.9980 r^2 = 0.9996*	r^2 = 0.9994	r^2 = 0.9998	r^2 = 0.9984
		Confirmation ion			
		r^2 = 0.9976-0.9986 r^2 = 0.9996*	r^2 = 0.9992	r^2 = 0.9996	r^2 = 0.9971
		(0.025-5.0 ng/mL)			(1-100 ng/mL)
	ILV[2]	**Quantification ion**			
		r^2 = **0.9898**	Not included in ILV as analytes.		
		Confirmation ion			
		r^2 = **0.9876**	Not included in ILV as analytes.		
		(0.025-5.0 ng/mL)			
Repeatable		Yes for soil using LC/MS/MS.		Yes for soil and sediment using LC/MS/MS.	Yes for soil using GC/MS/MS (NCI).
Reproducible		Yes for soil using LC/MS/MS.	Could not be determined; not included in ILV as analytes.		
Specific	ECM	Yes, matrix interferences were <LOD.			
	ILV	Yes, matrix interferences were <LOD.	Not included in ILV as analytes.		

Data were obtained from pp. 19-21, 24-27; Tables 1-6, pp. 31-36; Figures 1-8, pp. 39-46; Figures 11-16, pp. 49-54; Figures 19-24, pp. 57-62; Figures 27-32, pp. 65-70; Figures 35-40, pp. 73-78 of MRID 49397802; pp. 100- 101; Table 1, p. 102; Figures 1-10, pp. 104-113 of Amendment No. 1 of MRID 49397802; and pp. 14-15, 17-18; Tables 11.1-11.2, pp. 20-21; Figures 12.1-12.4, pp. 23-34 of MRID 49397803. Data for pendimethalin was taken from the Amendment No. 1 of the ECM unless otherwise noted.

* Data taken from ECM original study (LOQ for pendimethalin = 10 µg/kg).

1 ECM Amendment No. 1 r^2 values are reviewer-generated for pendimethalin from reported r values of 0.9988-0.9990 (Q) and 0.9988-0.9993 (C; Figure 1, p. 104; Figure 3, p. 106 of Amendment No. 1 of MRID 49397802; DER Attachment 2). Linearity of these Amendment No. 1 ECM calibration curves was verified by the reviewer [r^2 values of 0.9994-0.9996 (Q) and 0.9996-0.9997 (C); calibration data for each soil provided; DER Attachment 2]. ECM original study r^2 values are reviewer-generated for pendimethalin, M455H01 and P36 from reported r values of 0.9997-0.9999 (Q) and 0.9996-0.9998 (C; Figure 1, p. 39; Figure 3, p. 41; Figure 5, p. 43 of MRID 49397802; DER Attachment 2). The linearity coefficients for Reg. No. 4061757 in the study report were not 1/x weighted. Linearity of the these ECM calibration curves was verified by the reviewer [r^2 values of 0.9997-0.9999 (Q) and 0.9981-0.9998 (C) for pendimethalin, M455H01, P36 and Reg. No. 4061757; solvent-based standards; DER Attachment 2].

2 ILV r^2 values are reviewer-generated for pendimethalin from reported r values of 0.9949 (Q) and 0.9938 (C; Figure 12.1, p. 23 of MRID 49397803; DER Attachment 2). Linearity of the ILV calibration curve was calculated by the reviewer [r^2 value of 0.9999 (C) for matrix-matched standards; quantitative peak area results for the calibration curve were not reported so the reviewer could not validate the quantitative linearity data; DER Attachment 2].

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV was performed in support of the Amendment to the ECM (Amendment No. 1) with the improved LOQ for pendimethalin (pp. 11, 19 of MRID 49397803). Amendment No. 1 of the ECM reported the validation data for pendimethalin at the improved LOQ of 1 µg/kg using the same sample processing procedure and analytical procedure as the original ECM report. No samples dosed at 10×LOQ were included in Amendment No. 1 of MRID 49397802 since the improved 10×LOQ fortification rate was equivalent to the LOQ of the original ECM report. M455H01, P36 and Reg. No. 4061757 were not included as analytes in Amendment No. 1 of the ECM or the ILV.

   The main difference between the sample processing and analysis procedure of ILV and that of the Amendment No. 1 of the ECM was that the monitored mass transitions for pendimethalin were inverted (quantitative = Q, confirmatory = C): *m/z* 282 → 212 (Q) and *m/z* 282 → 194 (C). The quantitative and confirmatory transitions were switched from those monitored in the ECM. This was not considered to be a significant modification.

2. The ECM was not accompanied by an ILV employing M455H01, P36 and Reg. No. 4061757 as analytes; therefore, that portion of the ECM was not validated. The only ILV which accompanied the ECM was the one which supported the Amendment to the ECM.

3. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. For pendimethalin, the LOQ and LOD were reported as 1 µg/kg and 0.3 µg/kg (30% of the LOQ), respectively, in both the ECM and ILV (Amendment No. 1, pp. 100-101 of MRID 49397802; pp. 15, 19 of MRID 49397803). For M455H01, P36 and Reg. No. 4061757, the LOQ and LOD were reported as 0.010 mg/kg (10 µg/kg) and 0.003 mg/kg (30% of the LOQ), respectively, in the ECM only; these analytes were not included in the ILV (pp. 24-25 of MRID 49397802). In the ECM, the LOQs were supported by acceptable method validation results. No other justification was provided. No calculations or comparison to background levels were provided for the LOQ or LOD. In the ILV, the LOQ and LOD for pendimethalin were reported from the ECM (Amendment No. 1). No justification was provided.

   Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

4. In the ILV, the linearity coefficients were <0.995 for calibration curves of pendimethalin (Figure 12.1, p. 23 of MRID 49397803).

5. The ILV included a reagent blank in the sample set, but no chromatograms of reagent blanks were included in the study report (Appendix A, pp. 35-38 of MRID 49397803). A reagent blank was not included in the ECM or Amendment No. 1 of the ECM.

6. The New Jersey loam soil (25% clay) of the ILV was a more difficult soil matrix than those of the ECM (loamy sand soil and sediment and sandy loam soil, 4.6-11.0% clay; p. 14; Appendices 2-4, pp. 87-91; Amendment No. 1, p. 99 of MRID 49397802; p. 14; Appendix

C, pp. 40-41 of MRID 49397803). The ECM soil/sediment matrices were fully characterized by BioChem agrar (USDA soil characterization). The ILV soil matrices were provided by the sponsor and fully characterized by Agvise Laboratories, North Dakota (USDA soil characterization).

7. In the Amendment No. 1 of the ECM MRID 49397802, a full set of chromatograms was provided, except a reagent blank. Chromatograms for representative calibration standards, matrix blanks and samples fortified at the improved LOQ were provided for pendimethalin in loamy sand soil and sandy loam soil (Figures 5-10, pp. 108-113). The reviewer noted that small amounts of residue were observed in most of the matrix blanks, but these amounts were <LOD. Representative calibration spectra were also included (Figures 1-4, pp. 104-107). Product ion spectra were not included.

8. In the ECM MRID 49397802, a full set of chromatograms was provided, except a reagent blank. Chromatograms for matrix blanks and samples fortified at 0.01 mg/kg and 0.1 mg/kg were provided for pendimethalin, M455H01, P36 and Reg. No. 4061757 in loamy sand soil and sandy loam soil or loamy sand sediment (Figures 11-16, pp. 49-54; Figures 19-24, pp. 57-62; Figures 27-32, pp. 65-70; Figures 35-40, pp. 73-78). The reviewer noted that small amounts of residue were observed in most of the matrix blanks, but these amounts were <LOD. Representative calibration spectra were also included (Figures 9-10, pp. 47-48; Figures 17-18, pp. 55-56; Figures 25-26, pp. 63-64; Figures 33-34, pp. 71-72). Product ion spectra were also included (Figures 41-44, pp. 79-82).

9. In the ILV MRID 49397803, a full set of chromatograms was provided, except a reagent blank. Chromatograms for representative calibration standards, matrix blanks and samples fortified at the improved LOQ and 10×LOQ in loam and loamy sand soils were provided for pendimethalin (Figures 12.2-12.4, pp. 24-34). The reviewer noted that small amounts of residue were observed in most of the matrix blanks, but these amounts were <LOD. Product ion spectra were not included.

   In the communications between the ILV study authors and the ECM study director, the ECM study director reported that “it’s difficult to find agricultural soil that is completely free of Pendi due to it’s widespread use” (Appendix D, p. 43 of MRID 49397803).

10. The ECM study authors monitored the stability of the analytes in final extracts of the soil/sediment (p. 17; Tables 7-8, pp. 37-38 of MRID 49397802). After 4-7 days of storage under refrigeration or freezer, recoveries ranged 83-107% for pendimethalin, M455H01 and P36 and 81-108% for Reg. No. 4061757.

11. Matrix effects were studied by the ECM in the original report and Amendment No. 1. In the original report, the ECM study authors reported that no significant matrix effects were observed for pendimethalin, P36 and M455H01 (pp. 27-28 of MRID 49397802). Significant suppression was observed for Reg. No. 4061757 in the Bruch West sandy loam soil at the LOQ. The ECM study authors proposed that matrix components of the soil caused adsorption of a portion of the analyte in the GC injector. In Amendment No. 1, the ECM study authors reported significant matrix effects (enhancement) were observed for pendimethalin (p. 101; Table 2, p. 103 of Amendment No. 1 of MRID 49397802). The ILV

study authors did not study matrix effects, but matrix-matched standards were used for quantification of analytes.

12. It was reported in the ILV that the analysis of two sets of validation samples required 4 days (p. 18 of MRID 49397803).

    In the ECM, it was reported that a set of pendimethalin, M455H01 or P48 consisting of 24 samples required approximately 8-person hours for processing, approximately 8 hours for LC/MS/MS analysis and approximately 4 hours for evaluation of LC/MS/MS results (*ca*. 1.5 calendar days total per set; p. 29 of MRID 49397802). For Reg. No. 4061757, a set of 24 samples required approximately 8-person hours for processing, approximately 15 hours for LC/MS/MS analysis and approximately 4 hours for evaluation of LC/MS/MS results (*ca*. 2 calendar days total per set).

13. The ECM study authors noted that the P36 metabolite "occurred only in the sediment of an aquatic metabolism study" (p. 14 of MRID 49397802).

14. In the ILV, the communications between the ILV study authors and study sponsor/ECM study authors were reported (p. 18; Appendix D, pp. 42-46 of MRID 49397803). An important component of the communications was the background values of pendimethalin and M455H01 in the control soil of the "NC soil". This soil was deemed inappropriate for the ILV, and the California soil was sent to replace it.

15. Changes to the original ILV report were listed; the most significant of these changes was the Study Director change (pp. 18-19; Appendix E, p. 47 of MRID 49397803).

16. In the ILV, the reviewer noted a typographical error: Appendix C was entitled "Water Characterization Reports", not "Soil Characterization Reports" (Appendix C, pp. 40-41 of MRID 49397803).

V. References

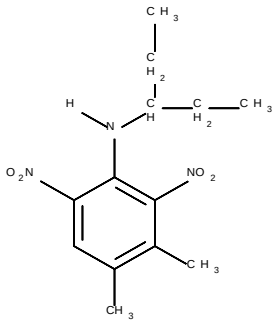
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

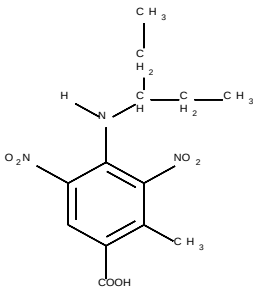
Pendimethalin (AC 92553, CL 92553, BAS 455 H; Reg. No. 900072)

IUPAC Name: N-(1-ethylpropyl)-2,6-dinitro-3,4-xylidine
CAS Name: N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine
CAS Number: 40487-42-1
SMILES String: CCC(CC)Nc1c(cc(C)c(C)c1N(=O)=O)N(=O)=O



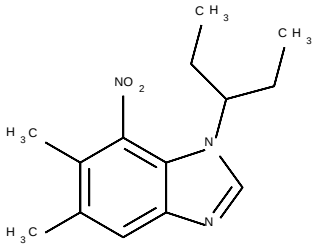
M455H01 (Reg No. 4108474; CL 99900; P44)

IUPAC Name: 4-[(1-Ethylpropyl)amino]-2-methyl-3,5-dinitrobenzoic acid
CAS Name: 4-[(1-Ethyl-propyl)amino]-3,5-dinitro-o-toluic acid
CAS Number: 127971-53-3
SMILES String: CCC(CC)Nc1c(cc(c(c1[N+](=O)[O-])C)C(=O)O)[N+](=O)[O-]



P36 (Reg No. 4982164)

IUPAC Name: 1-(1-Ethylpropyl)-5,6-dimethyl-7-nitro-1H-benzimidazole
CAS Name: Not reported
CAS Number: Not reported
SMILES String: CCC(CC)n1cnc2c1c(c(c(c2)C)C)[N+](=O)[O-]



Reg No. 4061757 (CL 84846)

IUPAC Name: 2,6-Dinitro-3,4-dimethylaniline
2,6-Dinitro-3,4-xylidine
CAS Name: Not reported
CAS Number: 40318-31-8
SMILES String: Cc1cc(c(c(c1C)[N+](=O)[O-])N)[N+](=O)[O-]