Test Material: Pendimethalin

MRID: 49221401

Title: Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and its Metabolites M455H001, P48 and Reg. No. 4061757 in Surface and Ground Water

MRID: 49207706

Title: Independent Laboratory Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and its Metabolites M455H001, Reg. No. 4295966 (P48) and Reg. No. 4061757 in Surface and Ground Water

EPA PC Code: 108501

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 3/12/15

Secondary Reviewer: Lynne Binari **Signature:** Lynne Binari

Date: 3/12/15

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

Date: 3/12/15

Analytical method for pendimethalin and its transformation products, M455H001, P48 and Reg. No. 4061757, in water

Reports: ECM: EPA MRID No. 49221401. Class, T., and N. Heinz. 2013. Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and its Metabolites M455H001, P48 and Reg. No. 4061757 in Surface and Ground Water. BASF ID: 364241. PTRL Europe ID: P 2708 G. BASF Registration Document No.: 2013/1113495. Report prepared by PTRL Europe, Ulm, Germany; sponsored by BASF SE, Ludwigshafen, Germany; and submitted by BASF Corporation, Research Triangle Park, North Carolina; 95 pages. Final report issued April 22, 2013.

ILV: EPA MRID No. 49207706. Wiesner, F., and N. Breyer. 2013. Independent Laboratory Validation of Analytical Methods for the Determination of BAS 455 H (Pendimethalin) and its Metabolites M455H001, Reg. No. 4295966 (P48) and Reg. No. 4061757 in Surface and Ground Water. BASF Study ID: 438461. Internal Code: BAS-1312V. Eurofins Study No.: S13-02451. BASF Registration Document No.: 2013/1134955. Report prepared by Eurofins Agroscience Services Chem GmbH, Hamburg, Germany; sponsored by BASF SE, Ludwigshafen, Germany; and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 92 pages. Final report issued June 28, 2013.

Document No.: MRIDs 49221401 & 49207706

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 (pp. 3, 6; Appendix 3, p. 95 of MRID 49221401). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-3, 6-8; Appendix 3, p. 95).

ILV: The study was conducted in accordance with the OECD and German GLP (1997 and 2008, respectively; pp. 3, 6, 9 of MRID 49207706). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 6, 9-10). A Certification of Authenticity was not provided, but a signatures page was provided (p. 5).

Classification: This analytical method is classified as acceptable. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The number of samples was inadequate (n = 4) for the ILV analysis of pendimethalin in surface water at the LOQ. Linearity coefficients were <0.995 for ECM calibrations of Reg. No. 4061757.

PC Code: 108501

EPA Reviewer:

Ibrahim Abdel-saheb **Signature:**
Environmental Scientist **Date: 11-22-2015**

All cited page numbers for ECM MRID 49221401 refer to those in the bottom right-hand corner of the document. All cited page numbers for ILV MRID 49207706 refer to those in the bottom center of the document.

Executive Summary

This analytical method, BASF Document No. 2013/1113495, is designed for the quantitative determination of pendimethalin and its transformation products, M455H001, Reg. No. 4295966 (P48) and Reg. No. 4061757 in water at the LOQ of 0.02 µg/L. Detection was performed with LC/MS/MS for pendimethalin, M455H001 and P48 and GC/MS/MS for Reg. No. 4061757. GC/MS/MS (NCI) was employed in the ECM, while GC/MS/MS (EI) was employed in the ILV. The ECM and ILV laboratories used ground and surface water. The LOQ is less than the lowest toxicological level of concern in water for all analytes. No major issues were discovered by the independent laboratory; the method appeared to be validated with the first trial, although the number of trials was not specifically reported. The number of samples was inadequate (n = 4) for the ILV analysis of pendimethalin in surface water at the LOQ.

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)	49221401	49207706		Water	4/22/2013	BASF Corporation	LC/MS/MS	0.02 µg/L
M455H001 (Reg. No. 4108474)								
P48 (Reg. No. 4295966)								
Reg. No. 4061757							GC/MS/MS*	

*GC/MS/MS (NCI) was employed in the ECM, while GC/MS/MS (EI) was employed in the ILV.

I. Principle of the Method

Procedure for BAS 455 H (Pendimethalin), M455H001 and P48

Samples (25.0 mL) were measured into 50 mL centrifuge tubes and fortified, as necessary (pp. 19-21; Appendix 2, pp. 91-93 of MRID 49221401). At this point, samples were either acidified (0.50 mL of acetic acid added) for M455H001 extraction, alkalized (1 mL of 25% ammonia solution added) for P48 extraction, or kept neutral (nothing added) for pendimethalin extraction. The water was extracted three times with pentane (3 x 5 mL) via shaking for 2 minutes. The combined pentane extracts were evaporated to less than 1.0 mL using rotary evaporation at *ca*. 40°C (do not evaporate to dryness). The residue was evaporated to dryness under a stream of nitrogen. Immediately, the residue was dissolved with 1 mL of acetonitrile. The sample was mixed with 1 mL of water containing 0.2% formic acid (V_{End} = 2.0 mL) and analyzed by LC/MS/MS.

Procedure for Reg. No. 4061757

Samples (250 mL) were measured into 500 mL separatory funnel and fortified, as necessary (p. 21; Appendix 2, p. 94 of MRID 49221401). The water was extracted three times with methylene chloride (3 x 25 mL) via shaking for 2 minutes. The combined methylene chloride extracts were filtered through a bed of 25 g anhydrous sodium sulphate (pre-wetted with methylene chloride). The sodium sulphate was rinsed twice with 10 mL of methylene chloride. The combined methylene chloride extract and rinses were evaporated to less than 5 mL using rotary evaporation at *ca*. 40°C. The residue was transferred to a tapered flask using 3 x 5 mL methylene chloride. After 1 mL of toluene was added to the extract as a keeper, the extract was evaporated to less than 1.0 mL using rotary evaporation at *ca*. 40°C (do not evaporate to dryness). The final volume of the residue was adjusted using toluene (V_{End} - 1.0 mL). After sonication, the sample was analyzed by GC/MS/MS.

Samples were analyzed for pendimethalin and M455H001 using an Agilent 1200 SL HPLC System coupled to an AB MDS Sciex 4000 Triple Quad mass spectrometer with TurboIonspray (ESI; pp. 21-22 of MRID 49221401). The HPLC 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) 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; and *m/z* 310 → 266 (Q) and *m/z* 310 → 236 (C) for M455H001. Approximate retention times were 5.1 min. for pendimethalin and 5.0 min. for M455H001. Injection volume was 50 µL.

Samples were analyzed for P48 using an Agilent 1200 SL HPLC System coupled to an AB MDS Sciex 4000 Triple Quad mass spectrometer with TurboIonspray (ESI; p. 23 of MRID 49221401). The HPLC conditions consisted of a Phenomenex Luna C_{18} column (2 x 50 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 0.00-2.00 min. 60:40, 2.10-6.00 min. 0:100, 6.10-8.00 min. 60:40] and MS/MS detection in positive ion mode. Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 252 → 119 (Q) and *m/z* 252 → 146 (C) for P48. Approximate retention time was 3.9 min. for P48. 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. 24 of MRID 49221401). 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 → 193 (Q) and *m/z* 211 → 194 (C) for Reg. No. 4061757. Approximate retention time was 7.9 min. for Reg. No. 4061757. Injection volume was 2.0 µL.

In the ILV, the water samples were processed in the same manner as the ECM (pp. 21-22 of MRID 49207706). The extracts were analyzed for pendimethalin, M455H01 and P48 using identical or similar instruments and instrumental conditions (pp. 22-24). Approximate retention times differed slightly in the ILV and were 5.0 min. for pendimethalin, 4.6 min. for M455H001, and 3.3 min. for P48. The instrumentation for the GC/MS/MS analysis of Reg. No. 4061757 differed from that of the ECM: Agilent GC 7890A coupled to an Agilent 7000 GC/MS Triple Quad (p. 25). The electron ionization (EI) mode was employed, instead of negative chemical ionization (NCI). The monitored mass transitions differed (quantitative = Q, confirmatory = C): *m/z* 211 → 194 (Q), *m/z* 211 → 164 (C) and *m/z* 211 → 118 (C) for Reg. No. 4061757. Approximate retention time was 8.5 min. for Reg. No. 4061757. Injection volume was 2 µL (splitless). The ILV study authors reported that the analysis of Reg. No. 4061757 was changed from GC/MS/MS in NCI mode to GC/MS/MS in EI mode since it is more commonly available (p. 35).

In both the ECM and ILV, the LOQ and LOD were reported as 0.02 µg/L and 0.004 µg/L (20% of the LOQ), respectively, for all analytes in both water matrices (pp. 27-28 of MRID 49221401; pp. 20, 35 of MRID 49207706).

II. Recovery Findings

ECM (MRID 49221401): Mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of pendimethalin, M455H001, P48 and Reg. No. 4061757 in surface (river) and ground (well) water at the LOQ (0.02 µg/L) and 10×LOQ (0.2 µg/L; pp. 21-24; Tables 1-8, pp. 32-39). LC/MS/MS analysis was employed for identification of residues of pendimethalin, M455H01 and P48; 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 ground water source was a local well in Herbrechtingen, located in Southern Germany; the surface water source was River Brenz in Herbrechtingen, located in Southern Germany (p. 15). Waters were fully characterized by Institute Alpha, Ulm, Germany.

ILV (MRID 49207706): Mean recoveries and RSDs were within guidelines for analysis of pendimethalin, M455H001, P48 and Reg. No. 4061757 in surface (river) and ground (well) water at the LOQ (0.02 µg/L) and 10×LOQ (0.2 µg/L; pp. 22-26, 37-39; Tables 1-22, pp. 42-63). LC/MS/MS analysis was employed for identification of residues of pendimethalin, M455H01 and P48; GC/MS/MS (EI) 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, except for pendimethalin in surface water at the LOQ (n = 4); one recovery value was determined to be an outlier according to the Grubbs test and was not considered for statistical analysis (155%, quantitative; 147%, confirmatory). The number of trials was not specified, but the reviewer assumed that the method was validated with the first trial (pp. 13, 35, 39). The ground and surface water sources were not specifically reported (German characterization report with English translation; p. 19; Appendix 5, p.89). The ground water was reported as Förderbrunnen (“wells”), and the surface water was reported as Alsterwasser (“with the addition of 100 mg mud per liter of water”). The locations were not further specified. The waters were fully characterized by Society for Bioanalysis MBH, Germany.

Table 2. Initial Validation Method Recoveries for Pendimethalin and Its Transformation Products, M455H001, P48 and Reg. No. 4061757, in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	5	88-98	91	4	4
	0.2	5	72-89	79	6	8
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	99-105	103	2	2
	0.2	5	86-110	101	11	11
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	77-90	84	6	7
	0.2	5	81-85	83	2	2
Reg. No. 4061757	0.02 (LOQ)	5	82-92	87	4	4
	0.2	5	84-96	91	6	6
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	5	87-100	94	5	5
	0.2	5	77-95	85	7	8
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	101-105	102	2	2
	0.2	5	88-110	102	9	9
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	77-93	85	7	8
	0.2	5	80-87	84	3	3
Reg. No. 4061757	0.02 (LOQ)	5	85-101	94	7	7
	0.2	5	82-94	90	5	6
Surface Water						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	5	104-116	108	5	5
	0.2	5	92-110	97	7	7
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	108-109	109	1	0.8
	0.2	5	94-109	106	7	6
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	83-93	87	4	4
	0.2	5	87-101	93	6	6
Reg. No. 4061757	0.02 (LOQ)	5	74-82	79	3	5
	0.2	5	77-110	94	12	13
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	5	89-111	103	9	9
	0.2	5	87-114	97	10	11
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	109-110	110	0	0.7
	0.2	5	96-109	106	5	5
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	83-90	87	3	3
	0.2	5	88-101	93	5	5
Reg. No. 4061757	0.02 (LOQ)	5	69-88	78	8	10
	0.2	5	79-109	92	11	12

Data (uncorrected recovery results) were obtained from Tables 1-8, pp. 32-39 of MRID 49221401. 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 P48; GC/MS/MS (NCI) analysis was employed for identification of residues of Reg. No. 4061757 (pp. 21-24).

[1] The ground water source was a local well in Herbrechtingen, located in Southern Germany; the surface water source was River Brenz in Herbrechtingen, located in Southern Germany (p. 15). Waters were fully characterized by Institute Alpha, Ulm, Germany.

[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* 252 → 119 (Q) and *m/z* 252 → 146 (C) for P48; and *m/z* 211 → 193 (Q) and *m/z* 211 → 194 (C) for Reg. No. 4061757 (pp. 21-24).

Table 3. Independent Validation Method Recoveries for Pendimethalin and Its Transformation Products, M455H001, P48 and Reg. No. 4061757, in Water[1,2]

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	5	87-105	96	7.1	7.4
	0.2	5	72-101	84	13	16
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	82-93	87	4.3	5.0
	0.2	5	83-89	87	2.5	2.9
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	76-89	80	5.3	6.6
	0.2	5	68-98	89	12	14
Reg. No. 4061757	0.02 (LOQ)	5	84-116	100	11	11
	0.2	5	84-119	104	16	15
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	5	88-95	91	3.6	3.6
	0.2	5	69-94	80	13	16
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	81-92	85	4.4	4.9
	0.2	5	83-89	86	2.3	2.7
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	72-87	79	5.6	7.1
	0.2	5	68-100	89	12	14
Reg. No. 4061757 (*m/z* 211 → 164)	0.02 (LOQ)	5	87-116	102	10	10
	0.2	5	85-123	106	17	16
Reg. No. 4061757 (*m/z* 211 → 118)	0.02 (LOQ)	5	82-115	94	13	14
	0.2	5	82-115	100	15	15
Surface Water						
Quantitative ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	4[3]	92-106	96	9.0	9.3
	0.2	5	79-86	83	3.3	4.0
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	89-93	91	1.6	1.8
	0.2	5	87-91	89	1.6	1.8
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	62-81	72	6.8	9.4
	0.2	5	77-87	82	5.0	6.1
Reg. No. 4061757	0.02 (LOQ)	5	64-81	74	6.7	9.1
	0.2	5	60-88	76	11	14
Confirmatory ion						
Pendimethalin (BAS 455 H; Reg. No. 900072)	0.02 (LOQ)	4[3]	93-102	97	3.7	3.8
	0.2	5	74-80	77	2.3	3.0
M455H001 (Reg. No. 4108474)	0.02 (LOQ)	5	83-93	90	4.0	4.4
	0.2	5	85-90	87	2.3	2.6
P48 (Reg. No. 4295966)	0.02 (LOQ)	5	60-78	72	7.0	9.8
	0.2	5	79-89	85	4.7	5.6

Analyte	Fortification Level (ppb)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Reg. No. 4061757 (*m/z* 211 → 164)	0.02 (LOQ)	5	68-82	75	5.0	6.7
	0.2	5	59-86	74	10	14
Reg. No. 4061757 (*m/z* 211 → 118)	0.02 (LOQ)	5	66-83	73	7.5	10
	0.2	5	59-84	74	9.4	13

Data (uncorrected recovery results) were obtained from pp. 26, 37-39; Tables 1-22, pp. 42-63 of MRID 49207706. LC/MS/MS analysis was employed for identification of residues of pendimethalin, M455H01 and P48; GC/MS/MS (EI) analysis was employed for identification of residues of Reg. No. 4061757 (pp. 22-25).

1 The ground and surface water sources were not specifically reported (German characterization report with English translation; p. 19; Appendix 5, p.89). The ground water was reported as Förderbrunnen (“wells”), and the surface water was reported as Alsterwasser (“with the addition of 100 mg mud per liter of water”). The locations were not further specified. The waters were fully characterized by Society for Bioanalysis MBH, Germany.

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* 252 → 119 (Q) and *m/z* 252 → 146 (C) for P48; and *m/z* 211 → 194 (Q), *m/z* 211 → 164 (C) and *m/z* 211 → 118 (C) for Reg. No. 4061757 (pp. 22-25).

3 One value was determined to be an outlier according to the Grubbs test and was not considered for statistical analysis (155%, quantitative; 147%, confirmatory).

III. Method Characteristics

In both the ECM and ILV, the LOQ and LOD were reported as 0.02 µg/L and 0.004 µg/L (20% of the LOQ), respectively, for all analytes in both water matrices (pp. 27-28 of MRID 49221401; pp. 20, 35 of MRID 49207706). In the ECM, the LOQ was 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 were reported from the ECM. No justification was provided for the LOQ, but the ILV study authors justified the LOD by the fact that no chromatographic peaks were observed at greater than three times the background noise.

Table 4. Method Characteristics

		Pendimethalin	**M455H01**	**P48**	**Reg. No. 4061757**
Limit of Quantitation (LOQ)		0.02 µg/L			
Limit of Detection (LOD)		0.004 µg/L			
Linearity (calibration curve r^2 and concentration range)	ECM[1] (surface and ground tested)	**Quantification ion**			
		r^2 = 0.9996 (Surface)	r^2 = 0.9986 (Solvent)	r^2 = 0.9982 (Ground)	r^2 = **0.9924** (Ground)
		Confirmation ion			
		r^2 = 0.9986 (Surface)	r^2 = 0.9984 (Solvent)	r^2 = 0.9984 (Ground)	r^2 = **0.9910** (Ground)
		(0.050-10.0 ng/mL)			(1-100 ng/mL)
	ILV[2] (surface and ground tested)	**Quantification ion**			
		r^2 = 0.9975 (Ground) r^2 = 0.9988 (Surface)	r^2 = 0.9998 (Ground) r^2 = 0.9997 (Surface)	r^2 = 0.9999 (Ground, LOQ) r^2 = 0.998 (Ground, 10xLOQ) r^2 = 0.9999 (Surface, LOQ) r^2 = 0.998 (Surface, 10xLOQ)	r^2 = 0.9972 (Ground) r^2 = 0.9966 (Surface)
		Confirmation ion			
		r^2 = 0.9983 (Ground) r^2 = 0.9982 (Surface)	r^2 = 0.9989 (Ground) r^2 = 0.9995 (Surface)	r^2 = 0.9998 (Ground, LOQ) r^2 = 0.9987 (Ground, 10xLOQ) r^2 = 0.9998 (Surface, LOQ) r^2 = 0.9987 (Surface, 10xLOQ)	r^2 = 0.998 (Ground, 164 *m/z*) r^2 = 0.9966 (Ground, 118 *m/z*) r^2 = 0.9973 (Surface, 164 *m/z*) r^2 = 0.9953 (Surface, 118 *m/z*)
		(0.050-10.0 ng/mL)			(1-100 ng/mL)
Repeatable		Yes for surface and ground water using LC/MS/MS.			Yes for surface and ground water using GC/MS/MS (NCI).
Reproducible		Yes for ground water using LC/MS/MS. **No** for surface water using LC/MS/MS, n = 4 at LOQ[3]	Yes for surface and ground water using LC/MS/MS.		**No** for surface and ground water using GC/MS/MS (NCI). Yes for surface and ground water using GC/MS/MS (EI).
Specific	ECM	Yes, matrix interferences were <LOD in ground water and <30% of the LOQ in surface water.	Yes, matrix interferences were <LOD in surface and ground water.		
	ILV	Yes, matrix interferences were <LOD in surface and ground water.			

Data were obtained from pp. 21-24, 27-28; Tables 1-8, pp. 32-39; Figures 1-8, pp. 43-50; Figures 15-32, pp. 57-74; Figures 35-40, pp. 77-82 of MRID 49221401; and pp. 20, 22-26, 35, 37-39; Tables 1-22, pp. 42-63; Appendix 3, Figures 1-17, pp. 67-83 of MRID 49207706.

1 ECM r^2 values are reviewer-generated for pendimethalin, M455H01 and P48 from reported r values of 0.9991-0.9998 (Q) and 0.9992-0.9993 (C; Figure 1, p. 43; Figure 3, p. 45; Figure 5, p. 47 of MRID 49221401; DER Attachment 2). The linearity coefficients for Reg. No. 4061757 in the study report were not 1/x weighted. Linearity of the ECM calibration curves was verified by the reviewer [r^2 values of 0.9989-0.9998 (Q) and 0.9989-0.9999 (C) for pendimethalin, M455H01 and P48, and r^2 values of 0.9929 (Q) and 0.9914 (C) for Reg. No. 4061757; DER Attachment 2]. In the ECM, linearity data was identified as that of surface water for pendimethalin, solvent-based for M455H01 and ground water for P48 and Reg. No. 4061757. No other data was provided.

2 Linearity of the ILV calibration curves was verified by the reviewer [r^2 values of 0.9972-0.9999 (Q) and 0.996-0.9998 (C) for Ground Water and 0.9967-0.9999 (Q) and 0.9953-0.9998 (C) for Surface Water; DER Attachment 2].

3 Mean recoveries and RSDs were satisfactory for both matrices at the LOQ and 10×LOQ; however, one value of the LOQ sample set was determined to be an outlier according to the Grubbs test and was not considered for statistical analysis (155%, quantitative; 147%, confirmatory).

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

IV. Method Deficiencies and Reviewer's Comments

1. For Reg. No. 4061757, the analytical method of the ILV differed from that of the ECM. The ILV study authors reported that the analysis of Reg. No. 4061757 was changed from GC/MS/MS in NCI mode to GC/MS/MS in EI mode since it is more commonly available (p. 35 of MRID 49207706). The ILV study authors also reported that GC/MS/MS (EI) analysis provided "equivalent results, with satisfactory accuracy, precision and repeatability" (p. 35). The GC/MS/MS (EI) analysis of the ILV utilized different MRM transitions for quantification (*m/z* 211 → 193 for ECM; *m/z* 211 → 194 for ILV) and confirmation (*m/z* 211 → 194 for ECM; *m/z* 211 → 164 and *m/z* 211 → 118 for ILV; p. 24 of MRID 49221401; p. 25 of MRID 49207706). Additionally, a second confirmation ion transition was monitored in the ILV. These modifications of the ECM Method by the ILV were considered substantial changes to the ECM method; however, no ECM internal validation was conducted using GC/MS/MS (EI) analysis to identify and quantify Reg. No. 4061757. The reviewer noted that all of the analytes were processed in the same manner as the ECM; it was only the analytical method for Reg. No. 4061757 which differed from the ECM (pp. 21-22 of MRID 49207706).

2. The estimations of the LOQ and LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. In the ECM, the LOQ was supported by acceptable method validation results (pp. 27-28 of MRID 49221401). 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 (pp. 20, 35 of MRID 49207706). No justification was provided for the LOQ, but the ILV study authors justified the LOD by the fact that no chromatographic peaks were observed at greater than three times the background noise.

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

3. In the ILV, the number of samples was inadequate (n = 4) for the analysis of pendimethalin in surface water at the LOQ (p. 37; Tables 3-4, pp. 44-45 of MRID 49207706). One value of the LOQ sample set was determined to be an outlier according to the Grubbs test and was not considered for statistical analysis (155%, quantitative; 147%, confirmatory).

4. In the ECM, the linearity coefficients were <0.995 for calibrations of Reg. No. 4061757 in ground water (Figure 7, p. 49 of MRID 49221401).

5. In the ECM MRID 49221401, a full set of chromatograms was provided, except a reagent blank. Chromatograms for matrix blanks and samples fortified at the LOQ and 10×LOQ were provided for pendimethalin, M455H01 and P48 in ground water (10×LOQ & LOQ, Figures 15-20, pp. 57-62; matrix blank, Figures 21-23, pp. 63-65) and surface water (10×LOQ & LOQ Figures 24-29, pp. 66-71; matrix blank, Figures 30-32, pp. 72-74). Chromatograms for matrix blanks and samples fortified at the LOQ and 10×LOQ were provided for Reg. No. 4061757 in ground water (10×LOQ & LOQ, Figures 35-36, pp. 77-78; matrix blank, Figure 37, p. 79) and surface water (10×LOQ & LOQ, Figures 38-39, pp.

80-81; matrix blank, Figure 40, p. 82). Representative calibration spectra were also included (Figures 9-14, pp. 51-56; Figures 33-34, pp. 75-76). Product ion spectra were also included (Figures 41-44, pp. 83-86).

6. In the ILV MRID 49207706, a full set of chromatograms was provided, except a reagent blank. Chromatograms for representative calibration standards, matrix blanks and samples fortified at the LOQ and 10×LOQ in surface and ground water were provided for all analytes: pendimethalin (Appendix 3, Figures 1-4, pp. 67-70); M455H01 (Appendix 3, Figures 5-8, pp. 71-74); P48 (Appendix 3, Figures 9-13, pp. 75-79); and Reg. No. 4061757 (Appendix 3, Figures 14-17, pp. 80-83). Product ion spectra were also included (Appendix 2, Figures A-D, pp. 64-65).

7. The ILV included a reagent blank in the sample set, but no chromatograms of reagent blanks were included in the study report (p. 19; Tables 1-22, pp. 42-63 of MRID 49207706). A reagent blank was not included in the ECM.

8. The ECM study authors monitored the stability of the analytes in solvent and final extracts (p. 19; Tables 9-10, pp. 40-42 of MRID 49221401). Following refrigerator storage of the analytes in solvent, deviations from freshly prepared solutions were ≤2% for pendimethalin and M455H001 after *ca*. 3 months and P48 after *ca*. 5 months (p. 19; Table 9, p. 40). However, for Reg No. 4061757, deviation from the freshly prepared solution was -16 to -18% after *ca*. 4 months of refrigerator storage.

   After 4-5 days of storage of surface and ground water extracts under refrigeration or freezer, recoveries ranged 80-108% for pendimethalin, M455H01 and P48, and 64-86% for Reg. No. 4061757 (Table 10, pp. 41-42).

9. Matrix effects were studied by the ECM and ILV. The ECM study authors reported that significant matrix effects were observed for pendimethalin (suppression), P48 (suppression) and Reg. No. 4061757 (enhancement), but not for M455H01 (p. 29 of MRID 49221401). The ILV study authors reported that significant matrix effects were observed for pendimethalin (suppression) and M455H01 (enhancement), but not for P48; Reg. No. 4061757 was not discussed (pp. 35-36 of MRID 49207706). Matrix-matched standards were used for quantification of analytes when matrix effects were observed.

10. It was reported in the ECM that a set of pendimethalin, M455H01 or P48 consisting of 12 samples required approximately 6-person hours for processing, approximately 7 hours for LC/MS/MS analysis and approximately 2 hours for evaluation of LC/MS/MS results (p. 30 of MRID 49221401). For Reg. No. 4061757, a set of 12 samples required approximately 6-person hours for processing, approximately 9 hours for LC/MS/MS analysis and approximately 3 hours for evaluation of LC/MS/MS results. Each set of 12 samples required a total of *ca*. 2 calendar days for complete analysis. No time requirement was reported in the ILV.

11. In the ILV, it was reported that no communications between the ILV study authors and study sponsor/ECM study authors occurred (p. 35 of MRID 49207706).

V. References

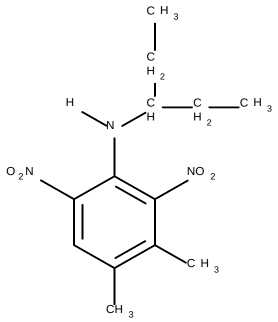
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

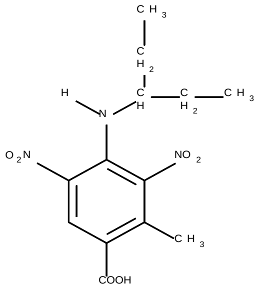
Pendimethalin (AC 92553, CL 92553, BAS 455 H; Reg. No. 900072)

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
None (19-JUN-97)
SMILES String: CCC(CC)Nc1c(cc(c(c1[N+](=O)[O-])C)C(=O)O)[N+](=O)[O-]



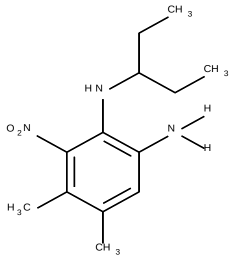
P48 (Reg No. 4295966)

IUPAC Name: 4,5-Dimethyl-3-nitro-N2-(pentan-3-yl)benzene-1,2-diamine

CAS Name: Not reported

CAS Number: Not reported

SMILES String: CCC(CC)Nc1c(cc(c(c1[N+](=O)[O-])C)C)N



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-]