

Analytical method for PBO and its transformation products, PBO-alcohol, PBO-aldehyde and PBO-acid in soil and sediment

Reports: ECM: EPA MRID No.: 49480801. Formanik, J. 2014. Method Validation for the Determination of PBO and Degradates, PBO-alcohol, PBO-aldehyde and PBO-acid in Soil, Sediment, Ground Water and Surface Water. Report prepared by Ag Chem Product Development, Ricerca Biosciences LLC, Concord, Ohio, sponsored and submitted by CSPA/PBTFH, Washington, D.C.; 155 pages. Ricerca Study No: 032384. Ricerca Document No.: 032384-1. Final report issued September 29, 2014.

ILV: EPA MRID No.: 49592901. Fleshman, M.K. 2015. Independent Laboratory Validation (ILV) Study of PBO and Degradates, PBO-alcohol, PBO-aldehyde and PBO-acid in Soil, Sediment, Ground Water and Surface Water. Report prepared by Ag Chem Product Development, Ricerca Biosciences LLC, Concord, Ohio, sponsored and submitted by CSPA/PBTFH, Washington, D.C.; 250 pages. Ricerca Study No: 032385. Ricerca Document No.: 032385-1. Final report issued March 19, 2015.

Document No.: MRIDs 49480801 & 49592901

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards, which are consistent with the OECD Principles of Good Laboratory Practice (p. 3 of MRID 49480801). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was not included.


ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (p. 3 of MRID 49592901). Signed and dated Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Acceptable**. It was determined that the ILV was conducted independently of the ECM (Appendix A). For analyte PBO-alcohol, method recoveries did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for the soil matrix at the LOQ and 10×LOQ in the ILV. In the ILV, the linearity and specificity of the method was not satisfactory for PBO-acid. The LODs for the analytes were not reported in the ILV.

PC Code: 067501

EFED Final Richard Shamblen


Reviewer:

Signature:  2018.12.10
Date: December 10, 2018 15:36:09 -05'00'

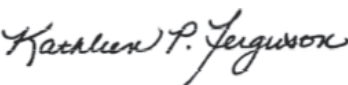
**CDM/CSS-
Dynamac JV**

Reviewers:

Lisa Muto,
Environmental Scientist

Signature: 
Date: 3/28/17

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 
Date: 3/28/17

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

All cited page numbers refer to those listed in the right, bottom-most corner of the documents.

Executive Summary

The analytical method, Ricerca Study No. 032384, is designed for the quantitative determination of PBO and its transformation products, PBO-alcohol, PBO-aldehyde and PBO-acid, in soil and sediment at the stated LOQ of 0.01 µg/g using HPLC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in soil/sediment (MRID 49066502). It was determined that ILV MRID 49592901 was conducted independently of ECM MRID 49480801 since both validations were conducted at the same facility (Ricerca Biosciences LLC) (Appendix A). Characterized sandy loam soil and sand sediment were used for the ECM validation; the same sediment and a similar soil, loamy sand soil, were used for the ILV validation. The ECM method was validated by the ILV with second trial with insignificant modifications to the calibration preparation. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes in both matrices, except for PBO-alcohol in the soil matrix. In the ILV, linearity was not satisfactory for PBO-acid. All ILV data regarding specificity were satisfactory for all analytes in both matrices, except that LOQ representative chromatograms of PBO-acid showed very prominent baseline in the soil and sediment. The LODs for the analytes were not reported in the ILV. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for all analytes in both matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
PBO	49480801 ²	49592901 ³		Soil/ Sediment	29/09/2014	CSPA/PBTFH	LC/MS/MS	0.01 µg/g
PBO- alcohol								
PBO- aldehyde								
PBO-acid								

1 PBO = Piperonyl butoxide; 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole; PBO-alcohol = (6-Propylbenzo[d][1,3]dioxol-5-yl)methanol; PBO-aldehyde = 6-Propylbenzo[d][1,3]dioxol-5-carbaldehyde; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

2 In the ECM, sandy loam soil [EFS-436; 73% sand 23% silt 4% clay; pH 8.0 (1:1 soil:water ratio); 0.44% organic matter] from Potterville, California, and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Figures 1-2, pp. 28-29 of MRID 49480801).

3 In the ILV, loamy sand soil [EFS-473; 78% sand 19% silt 3% clay; pH 7.8 (1:1 soil:water ratio); 0.47% organic matter] from California (collected by Research for Hire) and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization (pp. 14-15; Appendix C, pp. 71-75 of MRID 49592901). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The ILV sediment was the same as the ECM sediment.

I. Principle of the Method

Soil or sediment (20 ± 0.2 g; not dried) in a glass wide mouth jar was fortified with the mixed fortification solution then extracted with 50 mL of water:acetonitrile (10:90, v:v) by shaking on a shaker for at least 4 hours (pp. 14-15; Appendix A, pp. 90, 95 of MRID 49480801). After settling, an aliquot (1-2 mL) was removed and centrifuged to clear the extract. For PBO, dilute the extract 10-fold by combining and mixing 0.1 mL of extract with 0.9 mL of water into an autosampler vial for analysis by LC/MS/MS. For PBO-alcohol, PBO-aldehyde and PBO-acid, dilute the extract 2-fold by combining and mixing 0.5 mL of extract with 0.5 mL of water into an autosampler vial for analysis by LC/MS/MS.

LC/MS/MS for PBO: Samples are analyzed using an AB Sciex API 4000 Series Mass Spectrometer with Thermo Shimadzu LC-10ADvp or Shimadzu LC-10ATvp Liquid Chromatograph (Appendix A, pp. 89, 96-97 of MRID 49480801). The following LC conditions were used: Phenomenex Luna C8(2) column (2.0 mm x 30 mm, 3 μ ; column temperature ambient), Phenomenex Security Guard® C18 guard column (dimensions not reported), mobile phase of (A) ammonium acetate (10 mM, pH 5.5) and (B) acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0 min. 70:30, 3.0-4.0 min. 10:90, 4.1-6.0 min. 70:30], injection volume of 5 μ L, and MRM with positive Turbo Spray ionization (Collision Energy 50 V). One ion pair transition was monitored: m/z 356.2 \rightarrow 177.1. Observed retention time was *ca.* 3.5 minutes (Figures 5-32, pp. 34-61).

LC/MS/MS for PBO-alcohol, PBO-aldehyde and PBO-acid: Samples are analyzed using an AB Sciex API 4000 Series Mass Spectrometer with Thermo Shimadzu LC-10ADvp or Shimadzu LC-10ATvp Liquid Chromatograph (Appendix A, pp. 89, 97-98 of MRID 49480801). The following LC conditions were used: Phenomenex Luna C8(2) column (2.0 mm x 30 mm, 3 μ ; column temperature ambient), Phenomenex Security Guard® C18 guard column (dimensions not reported), mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0 min. 70:30, 3.0-4.0 min. 10:90, 4.1-6.0 min. 70:30], injection volume of 20 μ L, and MRM with positive Turbo Spray ionization (Collision Energy 23-26 V). One ion pair transition was monitored for each analyte: m/z 177.2 \rightarrow 119.2 for PBO-alcohol, m/z 193.2 \rightarrow 107.0 for PBO-aldehyde and m/z 191.0 \rightarrow 133.0 for PBO-acid. Observed retention times were *ca.* 2.3, 2.8 and 2.4 minutes for PBO-alcohol, PBO-aldehyde and PBO-acid, respectively (Figures 5-32, pp. 34-61).

The ILV performed the ECM methods for each analyte as written, including analytical methods and instrumentation, except that the calibration solvent was modified to improve chromatography (pp. 16-20 of MRID 49592901). Observed retention times were *ca.* 3.4, 3.5, 2.4-2.5 and 2.1 minutes for PBO, PBO-alcohol, PBO-aldehyde and PBO-acid, respectively (Appendix D, Figures 78-84, pp. 161-167; Appendix D, Figures 94-100, pp. 177-183; Appendix D, Figures 110-116, pp. 193-199; Appendix D, Figures 126-132, pp. 209-215).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 μ g/g for PBO, PBO-alcohol, PBO-aldehyde and PBO-acid (pp. 6, 16-19 of MRID 49480801; pp. 7, 15, 20; Appendix A, pp. 52-53 of MRID 49592901). In the ECM, the Limits of Detection (LOD) were calculated using the standard deviation of the LOQ samples; the calculated LODs ranged 0.000588-0.00346 μ g/g for all four analytes. The LODs for the analytes were not reported in the ILV.

II. Recovery Findings

ECM (MRID 49480801): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of PBO and its transformation products PBO-alcohol, PBO-aldehyde and PBO-acid at fortification levels of 0.01 $\mu\text{g/g}$ (LOQ) and 0.10 $\mu\text{g/g}$ (10 \times LOQ) in soil matrices (Tables 5-6, pp. 24-25). One ion transition was monitored for each analyte using LC/MS/MS; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Sandy loam soil [EFS-436; 73% sand 23% silt 4% clay; pH 8.0 (1:1 soil:water ratio); 0.44% organic matter] from Potterville, California, and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Figures 1-2, pp. 28-29).

ILV (MRID 49592901): Mean recoveries and RSDs were within guidelines for analysis of PBO and its transformation products PBO-alcohol, PBO-aldehyde and PBO-acid at fortification levels of 0.01 $\mu\text{g/g}$ (LOQ) and 0.10 $\mu\text{g/g}$ (10 \times LOQ) in soil matrices, except for analysis of PBO-alcohol in loamy sand soil (RSD: 23.03% LOQ, 24.15% 10 \times LOQ; Tables 1-2, pp. 25-26). One ion transition was monitored for each analyte using LC/MS/MS; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Loamy sand soil [EFS-473; 78% sand 19% silt 3% clay; pH 7.8 (1:1 soil:water ratio); 0.47% organic matter] from California (collected by Research for Hire) and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization (pp. 14-15; Appendix C, pp. 71-75). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The ILV sediment was the same as the ECM sediment. The method was validated with second trial with insignificant modifications to the calibration preparation (pp. 16-20; Appendix F, p. 249).

Table 2. Initial Validation Method Recoveries for PBO and Its Transformation Products PBO-alcohol, PBO-aldehyde and PBO-acid in Soil/Sediment

Analyte ¹	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil²						
PBO	0.01 (LOQ)	5	97.3-111	105	5.18	4.9
	0.1	5	98.1-113	105	6.90	6.5
PBO-alcohol	0.01 (LOQ)	5	93.3-97.2	95.5	1.41	1.5
	0.1	5	93.0-97.9	95.7	1.98	2.1
PBO-aldehyde	0.01 (LOQ)	5	89.2-100	92.7	4.22	4.6
	0.1	5	90.6-92.1	91.1	1.25	1.4
PBO-acid	0.01 (LOQ)	5	70.8-84.4	77.5	4.94	6.4
	0.1	5	80.5-94.8	86.0	5.57	6.5
Sediment²						
PBO	0.01 (LOQ)	5	111-122	117	4.24	3.6
	0.1	5	104-112	107	3.13	2.9
PBO-alcohol	0.01 (LOQ)	5	96.6-103	100.2	2.38	2.4
	0.1	5	93.7-98.7	96.3	1.95	2.0
PBO-aldehyde	0.01 (LOQ)	5	89.8-94.8	92.3	1.93	2.1
	0.1	5	87.7-90.6	89.1	1.05	1.2
PBO-acid	0.01 (LOQ)	5	70.4-90.6	81.7	8.31	10.2
	0.1	5	88.1-99.8	92.1	4.64	5.0

Data (uncorrected recovery results; pp. 37-38) were obtained from Tables 5-6, pp. 24-25 of MRID 49480801.

1 PBO = Piperonyl butoxide; 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole; PBO-alcohol = (6-Propylbenzo[d][1,3]dioxol-5-yl)methanol; PBO-aldehyde = 6-Propylbenzo[d][1,3]dioxol-5-carbaldehyde; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

2 Sandy loam soil [EFS-436; 73% sand 23% silt 4% clay; pH 8.0 (1:1 soil:water ratio); 0.44% organic matter] from Potterville, California, and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Figures 1-2, pp. 28-29).

3 One ion pair transition was monitored for each analyte: m/z 356.2→177.1 for PBO, m/z 177.2→119.2 for PBO-alcohol, m/z 193.2→107.0 for PBO-aldehyde and m/z 191.0→133.0 for PBO-acid.

Table 3. Independent Validation Method Recoveries for PBO and Its Transformation Products PBO-alcohol, PBO-aldehyde and PBO-acid in Soil/Sediment

Analyte ¹	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Loamy Sand Soil²						
PBO	0.01 (LOQ)	5	72.00-102.00	91.56	11.56	12.63
	0.1	5	88.90-97.20	92.50	3.63	3.92
PBO-alcohol	0.01 (LOQ)	5	58.10-113.00	97.62	22.48	23.03
	0.1	5	65.30-115.00	92.86	22.43	24.15
PBO-aldehyde	0.01 (LOQ)	5	108.00-121.00	116.80	5.12	4.38
	0.1	5	110.00-115.00	113.00	2.00	1.77
PBO-acid	0.01 (LOQ)	5	84.90-132.00	110.78	17.32	15.63
	0.1	5	103.00-114.00	108.60	4.45	4.10
Sand Sediment²						
PBO	0.01 (LOQ)	5	86.40-101.00	95.92	5.74	5.98
	0.1	5	87.50-95.50	92.42	3.02	3.27
PBO-alcohol	0.01 (LOQ)	5	113.00-119.00	117.00	2.45	2.09
	0.1	5	114.00-119.00	115.40	2.07	1.80
PBO-aldehyde	0.01 (LOQ)	5	113.00-121.00	117.60	2.97	2.52
	0.1	5	106.00-113.00	108.80	3.11	2.86
PBO-acid	0.01 (LOQ)	5	84.20-108.00	98.34	11.51	11.71
	0.1	5	103.00-114.00	110.00	4.85	4.41

Data (uncorrected recovery results; pp. 20-21) were obtained from Tables 1-2, pp. 25-26 of MRID 49592901.

1 PBO = Piperonyl butoxide; 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole; PBO-alcohol = (6-Propylbenzo[d][1,3]dioxol-5-yl)methanol; PBO-aldehyde = 6-Propylbenzo[d][1,3]dioxol-5-carbaldehyde; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

2 Loamy sand soil [EFS-473; 78% sand 19% silt 3% clay; pH 7.8 (1:1 soil:water ratio); 0.47% organic matter] from California (collected by Research for Hire) and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization (pp. 14-15; Appendix C, pp. 71-75). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The ILV sediment was the same as the ECM sediment.

3 One ion pair transition was monitored for each analyte: m/z 356.2→177.1 for PBO, m/z 177.2→119.2 for PBO-alcohol, m/z 193.2→107.0 for PBO-aldehyde and m/z 191.0→133.0 for PBO-acid.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 µg/g for PBO, PBO-alcohol, PBO-aldehyde and PBO-acid (pp. 6, 16-19 of MRID 49480801; pp. 7, 15, 20; Appendix A, pp. 52-53 of MRID 49592901). No justification was provided. In the ECM, the LOQs for each analyte were calculated by multiplying the calculated LODs by three. The calculated LOQs were 0.00521-0.00715 µg/g for PBO, 0.00176-0.00287 µg/g for PBO alcohol, 0.00239-0.00581 µg/g for PBO-aldehyde and 0.00667-0.01039 µg/g for PBO-acid. The calculated LOQs support the method LOQ. In the ECM, the LODs were calculated using the standard deviation of the LOQ samples in the following equation:

$$\text{LOD} = t_{0.99} \times S$$

Where t = one-tailed t-statistic at the 99% confidence level for n-1 replicates (where n = 5, $t_{0.99} = 4.541$).

S = standard deviation of n samples spiked at the LOQ.

The calculated LODs were 0.00174-0.00238 µg/g for PBO, 0.000588-0.000958 µg/g for PBO alcohol, 0.000796-0.00194 µg/g for PBO-aldehyde and 0.00222-0.00346 µg/g for PBO-acid. No method LOD was reported in the ECM. The LODs for the analytes were not reported in the ILV.

Table 4. Method Characteristics for PBO and Its Transformation Products PBO-alcohol, PBO-aldehyde and PBO-acid in Soil/Sediment

		PBO	PBO-alcohol	PBO-aldehyde	PBO-acid	
Limit of Quantitation (LOQ)	ECM (Reported)	0.01 µg/g				
	ECM (Calculated)	0.00521-0.00715 µg/g	0.00176-0.00287 µg/g	0.00239-0.00581 µg/g	0.00667-0.01039 µg/g	
	ILV	0.01 µg/g				
Limit of Detection (LOD)	ECM (Calculated)	0.00174-0.00238 µg/g	0.000588-0.000958 µg/g	0.000796-0.00194 µg/g	0.00222-0.00346 µg/g	
	ILV	Not reported				
Linearity (calibration curve r^2 and concentration range)	ECM	Soil:	$r^2 = 0.9950$	$r^2 = 0.9998$	$r^2 = 0.9996$	$r^2 = 0.9988$
		Sediment:	$r^2 = 0.9998$	$r^2 = 0.9998$	$r^2 = 0.9996$	$r^2 = 0.9972$
		Range:	0.20-10 ng/mL	1.0-50 ng/mL		
	ILV ²		$r^2 = 0.9958$	$r^2 = 0.9980$	$r^2 = 0.9954$	$r^2 = 0.9942$
		Range:	0.20-10 ng/mL	0.20-50 ng/mL		
Repeatable	ECM ³	Soil:	Yes at LOQ and 10×LOQ.			
		Sediment:	Yes at LOQ and 10×LOQ.			
	ILV ⁴	Soil:	Yes at LOQ and 10×LOQ. Yes at 10×LOQ.	No at RSD = 23.03% (LOQ), 24.15% (10×LOQ).	Yes at LOQ and 10×LOQ. Yes at 10×LOQ.	
		Sediment:	Yes at LOQ and 10×LOQ.			
Reproducible		Yes at LOQ and 10×LOQ in soil and sediment matrices.	Yes at LOQ and 10×LOQ in sediment matrix. No in soil matrix.	Yes at LOQ and 10×LOQ in soil and sediment matrices.		
Specific	ECM	Soil:	No matrix interferences were observed.		No matrix interferences were observed. Minor baseline noise interfered with peak integration at the LOQ.	
		Sediment:	No matrix interferences were observed.	Interferences were <5% of LOQ, based on peak height, at analyte retention times.		
	ILV	Soil:	No matrix interferences were observed; however, minor baseline noise interfered with peak integration.	Matrix interferences were observed at <3% of the LOQ (based on peak area).	No matrix interferences were observed; however, minor baseline noise interfered with peak integration.	
		Sediment:		No matrix interferences were observed; however, minor baseline noise interfered with peak integration.		No matrix interferences were observed; however, baseline noise was prominent at LOQ. ⁵

Data were obtained from pp. 6, 16-19; Tables 5-6, pp. 24-25 (recovery results); Figures 1-2, pp. 20-21 (calibration curves); Figures 5-32, pp. 34-61 (chromatograms) of MRID 49480801; pp. 7, 15-16, 20; Tables 1-2, pp. 25-26 (recovery results); Appendix D, Figure 77, p. 160; Appendix D, Figure 93, p. 176; Appendix D, Figure 109, p. 192; Appendix D, Figure 125, p. 208 (calibration curves); Appendix D, Figures 78-84, pp. 161-167; Appendix D, Figures 94-100, pp. 177-183; Appendix D, Figures 110-116, pp. 193-199; Appendix D, Figures 126-132, pp. 209-215 (chromatograms) of MRID 49592901.

1 PBO = Piperonyl butoxide; 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole; PBO-alcohol = (6-Propylbenzo[d][1,3]dioxol-5-yl)methanol; PBO-aldehyde = 6-Propylbenzo[d][1,3]dioxol-5-carbaldehyde; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

- 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. 16; Appendix A, pp. 52-53; Appendix D, Figure 77, p. 160; Appendix D, Figure 93, p. 176; Appendix D, Figure 109, p. 192; Appendix D, Figure 125, p. 208 of MRID 49480801; DER Attachment 2).
- 3 In the ECM, sandy loam soil [EFS-436; 73% sand 23% silt 4% clay; pH 8.0 (1:1 soil:water ratio); 0.44% organic matter] from Potterville, California, and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization; p. 14; Figures 1-2, pp. 28-29 of MRID 49480801).
- 4 In the ILV, loamy sand soil [EFS-473; 78% sand 19% silt 3% clay; pH 7.8 (1:1 soil:water ratio); 0.47% organic matter] from California (collected by Research for Hire) and sand sediment [EFS-471; 96% sand 1% silt 3% clay; pH 7.5 (1:1 soil:water ratio); 0.22% organic matter] from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture characterization (pp. 14-15; Appendix C, pp. 71-75 of MRID 49592901). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The ILV sediment was the same as the ECM sediment.
- 5 Based on Appendix D, Figure 128, p. 211 and Appendix D, Figure 131, p. 214 of MRID 49592901. Linearity is satisfactory when $r^2 \geq 0.995$.
A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. Initially, it could not be determined that ILV MRID 49592901 was conducted independently of ECM MRID 49480801 since both validations were conducted at the same facility (Ag Chem Product Development, Ricerca Biosciences LLC, Concord, Ohio) and insufficient evidence was provided to support the independence of the two laboratories (p. 1 of MRID 49480801; p. 1 of MRID 49592901). According to OCSPP guidelines, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been 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, Ricerca showed that the staff working on each validation were different and listed the communication between the staff of the initial and independent validations (p. 12 of MRID 49480801; p. 10; Appendix F, p. 249 of MRID 49592901). However, the communication and equipment lists indicated that both validations used the same API 4000 chromatograph (Appendix A, pp. 89, 97-98 of MRID 49480801; pp. 16-20; Appendix F, p. 249 of MRID 49592901). The ILV study director reportedly changed some of the LC/MS "instruments and columns" after the failure of the first trial, but there's no indication of using a different chromatograph. The registrant provided additional information to confirm no interactions between staff and no sharing of equipment when both validations occur at the same address (Appendix A).
2. The ILV test matrices were the same as or similar to those of the ECM. The ILV sediment was the same as the ECM sediment; both were EFS-471 (p. 14; Figures 1-2, pp. 28-29 of MRID 49480801 pp. 14-15; Appendix C, pp. 71-75 of MRID 49592901). The loamy sand soil (78% sand 19% silt 3% clay) of the ILV was very similar to the sandy loam soil (73% sand 23% silt 4% clay) of the ECM, and both soils originated from California (it could not be determined if they originated from the exact same site since the site location was not further specified in the ILV). As well as the matrix similarities supporting the lack of independence of the ILV from the ECM, it could not be determined if the ILV was provided

with the most difficult matrix with which to validate the method.

3. In the ILV, the analysis of PBO-alcohol did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) $\leq 20\%$) at the stated LOQ and $10\times\text{LOQ}$ in the loamy sand soil matrix (RSDs: 23.03% LOQ, 24.15% $10\times\text{LOQ}$; Tables 1-2, pp. 25-26 of MRID 49592901). The ILV study author reported that “this exceedance of the specifications is due to higher variability of one or two samples within the sample sets but the overall impact is minor and the precision of the method is considered adequate” (p. 24).
4. In the ILV, linearity was not satisfactory for PBO-acid ($r^2 = 0.9942$; Appendix D, Figure 125, p. 208 of MRID 49592901; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.
5. In the ILV, LOQ representative chromatograms of PBO-acid showed very prominent baseline in the soil and sediment (Appendix D, Figure 128, p. 211; Appendix D, Figure 131, p. 214 of MRID 49592901). Minor baseline noise interfered with peak integration at the LOQ in a majority of the analyte chromatograms in both matrices (Appendix D, Figures 78-84, pp. 161-167; Appendix D, Figures 94-100, pp. 177-183; Appendix D, Figures 110-116, pp. 193-199; Appendix D, Figures 126-132, pp. 209-215). Minor matrix interferences ($<3\%$ of the LOQ) were observed in PBO-alcohol representative chromatograms in soil.
6. In the ECM, the LOQ chromatograms for PBO-acid in both matrices showed minor baseline interferences with peak resolution or integration (Figure 19, p. 48; Figure 31, p. 60 of MRID 49480801). Minor matrix interferences ($<5\%$ of the LOQ) were observed in PBO-alcohol representative chromatograms in sediment.
7. 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. 6, 16-19 of MRID 49480801; pp. 7, 15, 20; Appendix A, pp. 52-53 of MRID 49592901). In the ECM, the LOQs for each analyte were calculated by multiplying the calculated LODs by three. The LODs were calculated using the standard deviation of the LOQ samples in the following equation: $\text{LOD} = t_{0.99} \times S$, where $t =$ one-tailed t-statistic at the 99% confidence level for $n-1$ replicates (where $n = 5$, $t_{0.99} = 4.541$) and $S =$ standard deviation of n samples spiked at the LOQ. No method LOD was reported in the ECM. No justification for the LOQ was provided in the ILV. The LODs for the analytes were not reported in the ILV.
8. The communications between the ILV and study developers and sponsors were detailed; communications involved failed trial discussions and suggested modifications (Appendix F, p. 249 of MRID 49592901).
9. The total time required to perform the method was not reported in the ECM or ILV.

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.

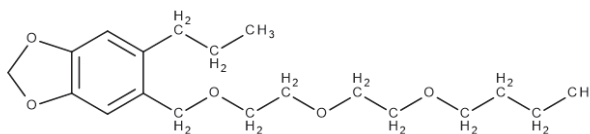
Attachment 1: Chemical Names and Structures**PBO (Piperonyl butoxide)**

IUPAC Name: 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole

CAS Name: Not reported

CAS Number: 51-03-6

SMILES String: Not reported

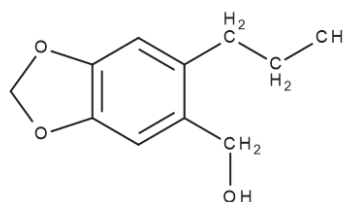
**PBO-alcohol**

IUPAC Name: (6-Propylbenzo[d][1,3]dioxol-5-yl)methanol

CAS Name: Not reported

CAS Number: 21809-60-9

SMILES String: CCCc1cc2c(cc1CO)OCO2

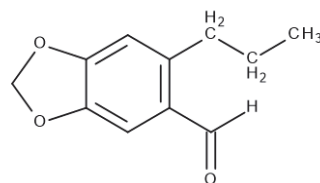
**PBO-aldehyde**

IUPAC Name: 6-Propylbenzo[d][1,3]dioxole-5-carbaldehyde

CAS Name: Not reported

CAS Number: 34827-22-0

SMILES String: [H]C(=O)c1cc2c(cc1CCC)OCO2

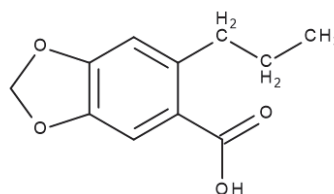
**PBO-acid**

IUPAC Name: 6-Propyl-1,3-benzodioxole-5-carboxylic acid

CAS Name: Not reported

CAS Number: 23505-33-1

SMILES String: CCCc1cc2c(cc1C(=O)O)OCO2



APPENDIX A