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

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. ILV test matrices were the same as those of the ECM. For analyte PBO-acid, method recoveries did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for the surface water at the LOQ in the ILV. In the ILV, the linearity was not satisfactory for PBO-acid; the specificity of the method was not satisfactory for PBO-aldehyde and PBO-acid. The LODs for the analytes were not reported in the ILV.

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EFED Final Reviewer: Richard Shamblen, Biologist
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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 water using HPLC/MS/MS. In water, the method is quantitative for PBO, PBO-alcohol, PBO-aldehyde at the stated LOQ of 0.1 µg/L and for PBO-acid at the stated LOQ of 1.0 µg/L. The LOQs are **less than** the lowest toxicological level of concern in water (MRID 49031801 and 49193301). 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) and sufficient evidence was provided to support the independence of the two laboratories (Appendix A). Characterized ground and surface water were used for the ECM validation; the same matrices 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-acid in surface water at the LOQ. In the ILV, linearity was not satisfactory for PBO-acid. All ILV data regarding specificity were satisfactory for PBO and PBO-alcohol in both matrices; significant matrix interferences (*ca*. 29-50% were noted in representative chromatograms of PBO-aldehyde in both matrices and PBO-acid in ground water. 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]		Water	29/09/2014	CSPA/PBTFH	LC/MS/MS	0.1 µg/L
PBO-alcohol								
PBO-aldehyde								
PBO-acid								1.0 µg/L

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-carbaldehdye; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

2 In the ECM, ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri were characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Figures 3-4, pp. 30-33 of MRID 49480801). The specific water source type of the ground water was not reported.

3 In the ILV, ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio, and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri, were characterized by Agvise

Laboratories, Northwood, North Dakota (pp. 13-14; Appendix C, pp. 74-77 of MRID 49592901). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were the same as those of the ECM. The specific water source type of the ground water was not reported.

I. Principle of the Method

Water (40 mL) in a 50-mL centrifuge tube was fortified with the mixed fortification solution then mixed with 4 mL of 0.1% formic acid in methanol (pp. 14-15; Appendix A, pp. 89, 95 of MRID 49480801). For PBO, an aliquot (0.10 mL) was transferred to an autosampler vial for analysis by LC/MS/MS. For PBO-alcohol, PBO-aldehyde and PBO-acid, a Waters Oasis HLB solid phase extraction (SPE; 6 cc 200 mg) column was conditioned with 3 mL each of methanol then water (the flow rate should be 1-2 drops per second with vacuum). The acidified water sample was applied to the SPE column; the column was dried under high vacuum for 2 min. after elution stopped to remove all water. The analytes were eluted with 4.0 mL of 0.1% formic acid in methanol; vacuum was applied to the column after elution to ensure that all of the analytes had been collected. The volume of the eluates were adjusted to 4 mL with methanol, if necessary. The eluate was mixed and diluted 2-fold with water in an autosampler vial (0.5 mL eluate and 0.5 mL water). The autosampler vial was vortexed and analyzed 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 µL, and MRM with positive Turbo Spray ionization (Collision Energy 19 V). One ion pair transition was monitored: *m/z* 356.2→177.1. Observed retention time was *ca*. 3.4 minutes (Figures 33-56, pp. 62-85).

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 µL, 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→119.2 for PBO-alcohol, *m/z* 193.2→107.0 for PBO-aldehyde and *m/z* 191.0→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 33-56, pp. 62-85).

The ILV performed the ECM methods for each analyte as written, including analytical methods and instrumentation (pp. 16-20 of MRID 49592901). Observed retention times were *ca*. 3.2-3.6, 3.5, 2.5-2.6 and 2.2 minutes for PBO, PBO-alcohol, PBO-aldehyde and PBO-acid, respectively (Appendix D, Figures 11-17, pp. 94-100; Appendix D, Figures 28-34, pp. 111-117; Appendix D, Figures 45-51, pp. 128-134; Appendix D, Figures 62-68, pp. 145-151).

In the ECM and ILV, the Limits of Quantification (LOQ) were 0.1 µg/L for PBO, PBO-alcohol, PBO-aldehyde and 1.0 µg/L for 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 0.00946-0.0309 µg/L for PBO, PBO-alcohol and PBO-aldehyde and 0.179-0.233 µg/L for PBO-acid. 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 ≤20%) for analysis of PBO and its transformation products PBO-alcohol and PBO-aldehyde at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) and for its transformation product PBO-acid at fortification levels of 1.0 µg/L (LOQ) and 10.0 µg/L (10×LOQ) in water matrices (Tables 7-8, pp. 26-27). 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. Ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio, and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Figures 3-4, pp. 30-33). The specific water source type of the ground water was not reported.

ILV (MRID 49592901): Mean recoveries and RSDs were within guidelines for analysis of PBO and its transformation products PBO-alcohol and PBO-aldehyde at fortification levels of 0.1 µg/L (LOQ) and 1.0 µg/L (10×LOQ) and for its transformation product PBO-acid at fortification levels of 1.0 µg/L (LOQ) and 10.0 µg/L (10×LOQ) in water matrices, except for analysis of PBO-acid in surface water at the LOQ (RSD, 25.74%; Tables 3-4, pp. 27-28). 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. Ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio, and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (pp. 13-14; Appendix C, pp. 74-77). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were the same as those of the ECM. The specific water source type of the ground water was not reported. 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 Water

Analyte[1]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water[2]					
PBO	0.1 (LOQ)	5	78.5-85.6	82.1	2.56	3.1
	1.0	5	71.4-81.9	78.0	4.18	5.4
PBO-alcohol	0.1 (LOQ)	5	93.5-101	98.7	3.10	3.1
	1.0	5	100-104	102	1.58	1.6
PBO-aldehyde	0.1 (LOQ)	5	84.9-90.1	87.0	2.01	2.3
	1.0	5	83.4-89.0	86.3	2.63	3.1
PBO-acid	1.0 (LOQ)	5	88.4-101	94.2	5.13	5.4
	10.0	5	88.1-93.3	91.0	2.16	2.4
	Surface Water[2]					
PBO	0.1 (LOQ)	5	81.7-89.7	87.2	3.27	3.7
	1.0	5	78.1-84.8	82.3	2.83	3.4
PBO-alcohol	0.1 (LOQ)	5	98.8-105	102	2.24	2.2
	1.0	5	99.2-103	101	1.55	1.5
PBO-aldehyde	0.1 (LOQ)	5	79.0-93.5	87.8	6.83	7.8
	1.0	5	79.2-93.2	88.6	5.62	6.3
PBO-acid	1.0 (LOQ)	5	83.4-91.8	87.7	3.94	4.5
	10.0	5	85.2-93.3	89.4	3.60	4.0

Data (uncorrected recovery results; pp. 37-38) were obtained from Tables 7-8, pp. 26-27 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-carbaldehdye; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

2 Ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio, and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Figures 3-4, pp. 30-33). The specific water source type of the ground water was not reported.

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 Water

Analyte[1]	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Ground Water[2]					
PBO	0.1 (LOQ)	5	74.60-81.40	76.64	2.83	3.70
	1.0	5	71.80-74.80	73.16	1.11	1.51
PBO-alcohol	0.1 (LOQ)	5	93.90-102.00	99.22	3.15	3.18
	1.0	5	82.80-85.90	84.02	1.06	1.26
PBO-aldehyde	0.1 (LOQ)	5	78.20-100.00	86.66	9.26	10.68
	1.0	5	102.00-109.00	105.40	2.51	2.38
PBO-acid	1.0 (LOQ)	5	104.00-125.00	116.40	10.14	8.71
	10.0	5	97.50-106.00	102.46	4.04	3.95
	Surface Water[2]					
PBO	0.1 (LOQ)	5	75.50-83.50	78.98	3.03	3.84
	1.0	5	68.70-74.00	71.54	2.05	2.87
PBO-alcohol	0.1 (LOQ)	5	95.00-116.00	102.36	8.36	8.16
	1.0	5	78.20-88.10	83.48	3.84	4.60
PBO-aldehyde	0.1 (LOQ)	5	73.10-85.60	81.48	6.14	7.53
	1.0	5	99.00-106.00	103.60	2.70	2.61
PBO-acid	1.0 (LOQ)	5	78.50-153.00	109.32	28.14	**25.74**
	10.0	5	92.70-115.00	100.52	8.46	8.41

Data (uncorrected recovery results; pp. 20-21) were obtained from Tables 3-4, pp. 27-28 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-carbaldehdye; PBO-acid = 6-Propyl-benzo[1,3]dioxol-5-carboxylic acid.

2 Ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio, and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (pp. 13-14; Appendix C, pp. 74-77). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were the same as those of the ECM. The specific water source type of the ground water was not reported.

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 LOQs were 0.1 µg/L for PBO, PBO-alcohol, PBO-aldehyde and 1.0 µg/L for 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 LOQs for each analyte were calculated by multiplying the calculated LODs by three. The calculated LOQs were 0.0351-0.0450 µg/L for PBO, 0.0329-0.0438 µg/L for PBO alcohol, 0.0284-0.0928 µg/L for PBO-aldehyde and 0.538-0.698 µg/L 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:

$LOD = t_{0.99} \times S$

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 were 0.0117-0.0150 µg/L for PBO, 0.0110-0.0146 µg/L for PBO alcohol, 0.00946-0.0309 µg/L for PBO-aldehyde and 0.179-0.233 µg/L 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 Water

Analyte[1]			PBO	PBO-alcohol	PBO-aldehyde	PBO-acid
Limit of Quantitation (LOQ)	ECM (Reported)		0.1 µg/L			
	ECM (Calculated)		0.0351-0.0450 µg/L	0.0329-0.0438 µg/L	0.0284-0.0928 µg/L	0.538-0.698 µg/L
	ILV		0.1 µg/L			
Limit of Detection (LOD)	ECM (Calculated)		0.0117-0.0150 µg/L	0.0110-0.0146 µg/L	0.00946-0.0309 µg/L	0.179-0.233 µg/L
	ILV		Not reported			
Linearity (calibration curve r^2 and concentration range)	ECM	Ground Water:	$r^2 = 0.9992$	$r^2 = 0.9996$	$r^2 = 0.9996$	$r^2 = 0.9980$
		Surface Water:	$r^2 = 0.9994$	$r^2 = 0.9996$	$r^2 = 0.9994$	$r^2 = 0.9982$
		Range:	0.04-5 ng/mL	0.25-10 ng/mL		2.5-100 ng/mL
	ILV[2]		$r^2 = 0.9996$	$r^2 = 0.9992$	$r^2 = 0.9980$[3]	$r^2 =$ **0.9944**
		Range:	0.04-5 ng/mL	0.04-10 ng/mL		0.4-100 ng/mL
Repeatable	ECM[4]	Ground Water:	Yes at LOQ and 10×LOQ.			
		Surface Water:				
	ILV[5]	Ground Water:	Yes at LOQ and 10×LOQ.			
		Surface Water:	Yes at LOQ and 10×LOQ.			Yes at 10×LOQ. **No** at LOQ RSD = **25.74**%.
Reproducible			Yes at LOQ and 10×LOQ in surface and ground water matrices.			Yes at LOQ and 10×LOQ in ground water matrix. Yes at 10×LOQ in surface water matrix; **No** at LOQ in surface water matrix.
Specific	ECM	Ground Water:	No matrix interferences were observed.	Interferences were <5% of LOQ, based on peak height, at analyte retention times.	No matrix interferences were observed. Minor baseline noise interfered with peak integration at the LOQ.	
		Surface Water:	No matrix interferences were observed. Minor baseline noise interfered with peak integration at the LOQ.			
	ILV	Ground Water:	No matrix interferences were observed; however, minor baseline noise interfered with peak integration.	Matrix interferences were observed at <8% of the LOQ (based on peak area).	Matrix interferences were observed at *ca.* **36**% of the LOQ (based on peak area).[6]	Matrix interferences were observed at *ca.* 29% of the LOQ (based on peak area).[8]
		Surface Water:		Matrix interferences were observed at <2% of the LOQ (based on peak area).	Matrix interferences were observed at *ca.* **50**% of the LOQ (based on peak area).[7]	No matrix interferences were observed; however, minor baseline noise interfered with peak integration.

Data were obtained from pp. Tables 7-8, pp. 26-27 (recovery results); Figures 3-4, pp. 22-23 (calibration curves); Figures 33-56, pp. 62-85 (chromatograms) of MRID 49480801; pp. 7, 15-16, 20; Tables 3-4, pp. 27-28 (recovery results); Appendix A, pp. 52-53; Appendix D, Figure 10, p. 93; Appendix D, Figure 27, p. 110; Appendix D, Figure 44, p. 127; Appendix D, Figure 61, p. 144 (calibration curves); Appendix D, Figures 11-17, pp. 94-100; Appendix D, Figures 28-34, pp. 111-117; Appendix D, Figures 45-51, pp. 128-134; Appendix D, Figures 62-68, pp. 145-151 (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-carbaldehdye; 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 10, p. 93; Appendix D, Figure 27, p. 110; Appendix D, Figure 44, p. 127; Appendix D, Figure 61, p. 144 of MRID 49480801; DER Attachment 2).

3 The reviewer noted that the r value appeared to be incomplete in the report reproduction.

4 In the ECM, ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri were characterized by Agvise Laboratories, Northwood, North Dakota (p. 14; Figures 3-4, pp. 30-33 of MRID 49480801). The specific water source type of the ground water was not reported.

5 In the ILV, ground water (EFS-495; pH 6.9, hardness 33 mg equiv. $CaCO_3$/L, total dissolved solids 92 ppm) from Madison, Ohio, and surface water (EFS-471; pH 7.7, hardness 176 mg equiv. $CaCO_3$/L, total suspended solids 62 ppm, total organic carbon 7.8 ppm) from Moniteau creek in Howard County, Missouri, were characterized by Agvise Laboratories, Northwood, North Dakota (pp. 13-14; Appendix C, pp. 74-77 of MRID 49592901). Matrices were provided by the ECM laboratory, Ricerca Biosciences, LLC. The matrices of the ILV were the same as those of the ECM. The specific water source type of the ground water was not reported.

6 Based on Appendix D, Figures 46-47, pp. 129-130 of MRID 49592901.

7 Based on Appendix D, Figures 49-50, pp. 132-133 of MRID 49592901.

8 Based on Appendix D, Figures 63-64, pp. 146-147 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. However, 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 those of the ECM (EFS-495 and EFS-471; p. 14; Figures 3-4, pp. 30-33 of MRID 49480801pp. 14-15; Appendix C, pp. 74-77 of MRID 49592901). 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-acid 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) ≤20%) at the stated LOQ in the surface water matrix (RSD: 25.74%; Tables 3-4, pp. 27-28 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 ($r^2 = 0.9944$; Appendix D, Figure 61, p. 144 of MRID 49592901; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

5. In the ILV, major matrix interferences were observed in representative chromatograms of PBO-aldehyde in ground water (*ca*. 36% of the LOQ) and surface water (*ca*. 50% of the LOQ; Appendix D, Figures 46-47, pp. 129-130; Appendix D, Figures 49-50, pp. 132-133 of MRID 49592901). Significant matrix interferences were observed in representative chromatograms of PBO-acid in ground water (*ca*. 29% of the LOQ; Figures 63-64, pp. 146-147). Minor matrix interferences (<8% of the LOQ) were observed in PBO-alcohol representative chromatograms in both matrices; minor baseline noise interfered with peak integration at the LOQ in PBO chromatograms in both matrices and in PBO-acid in surface water (Appendix D, Figures 11-17, pp. 94-100; Appendix D, Figures 28-34, pp. 111-117; Appendix D, Figures 45-51, pp. 128-134; Appendix D, Figures 62-68, pp. 145-151).

6. In the ECM, minor baseline interferences with peak resolution or integration was observed in the LOQ representative chromatograms for PBO in surface water and for PBO-aldehyde and PBO-acid in both matrices (Figures 33-56, pp. 62-85 of MRID 49480801). Minor matrix interferences (<5% of the LOQ) were observed in PBO-alcohol representative chromatograms in ground water.

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: $LOD = t_{0.99} \times S$, 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.

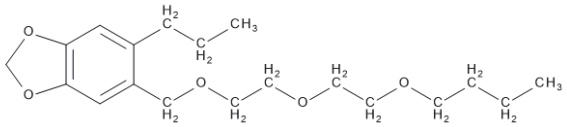
V. References

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.

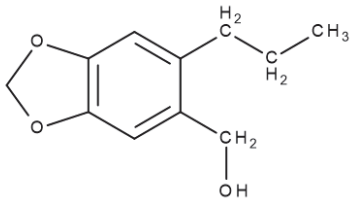
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

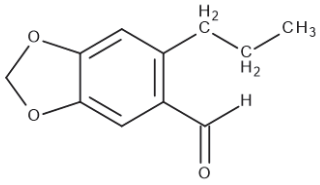
PBO (Piperonyl butoxide)
IUPAC Name: 5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-1,3-benzodioxole
CAS Name: Not reported
CAS Number: 51-03-6
SMILES String: Not reported



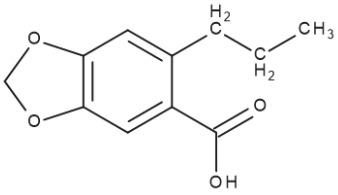
PBO-alcohol
IUPAC Name: (6-Propylbenzo[d][l,3]dioxol-5-yl)methanol
CAS Name: Not reported
CAS Number: 21809-60-9
SMILES String: CCCc1cc2c(cc1CO)OCO2



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