

Analytical method for pyrimethanil and its transformation product, AE F132593, in soil and water

Reports: ECM: EPA MRID No.: 50145801. Gould, T.J. 2016. An Analytical Method for the Determination of Residues of Pyrimethanil (AE B100309) and its Metabolites AE F132593 in Soil and Water Using LC/MS/MS. Report prepared, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 21 pages. Bayer Method PI-003-S16-02. Final report issued December 16, 2016.

ILV: EPA MRID No.: 50145802. Li, F. 2016. Independent Laboratory Validation of Bayer Method PI-003-S16-01: An Analytical Method for the Determination of Residues of (AE B100309) and its Metabolites AE F132593 in Soil and Water Using LC/MS/MS – Final Report. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 120 pages. Bayer Study No: RAPIN005. CPS Study No.: 16-CPS-051. Final report issued December 19, 2016.

Document No.: MRIDs 50145801 & 50145802

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards, since it was not an experimental study (p. 3 of MRID 50145801). Signed and dated Data Confidentiality and GLP and statements were provided (pp. 2-3). The Quality Assurance and Authenticity statements were not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR 160; p. 3 of MRID 50145802). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was not included.

Classification: This analytical method is classified as supplemental but upgradeable. Only one set of performance data was submitted for the soil portion of the method. Insufficient ECM data was provided to validate the water portion of the method. The ILV soil and water matrices were undescribed and uncharacterized. The LOD of the method was not reported in the ECM.

PC Code: 288201

EFED Final Reviewer: Sheng Lin, Ph.D.,
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Signature:
Date: 4/29/19

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CDM/CSS-Dynamac JV Reviewers: Lisa Muto,
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Date: 5/11/17

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

Executive Summary

The analytical method, Bayer Method PI-003-S16-01/ PI-003-S16-02, is designed for the quantitative determination of pyrimethanil and its transformation product, AE F132593, in soil at the stated LOQ of 5 ng/g and in water at the stated LOQ of 0.5 ng/g using HPLC/MS/MS. The LOQ is [less than] the lowest toxicological level of concern in soil. The LOQ is [less than] the lowest toxicological level of concern in water. Only one set of performance data was submitted for the soil portion of the method; ECM 50145801 only provided a method for soil analysis. Insufficient ECM data was provided to validate the water portion of the method; the reviewer was able to calculate ECM recovery values for one LOQ sample for each analyte based on peak areas and calibration curve data, but this amount of data was insufficient to assess the repeatability and reproducibility of the method. Two ion pair transitions were monitored for each analyte. The ILV validated the method in soil and water with first trial with insignificant modifications to the analytical methods and instrumentation; however, specific type and characterization of the soil and water matrices used for validation were not reported. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes. In the ILV, the specificity of the method was not well supported by the LOQ representative chromatograms for AE F132593 due to interference to peak resolution and integration caused by baseline noise and a nearby contaminant, especially in the water analysis. In the ECM, insufficient representative chromatograms were provided to support the specificity of the water method; no soil chromatograms were provided. The LOD of the method was not reported in the ECM.

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						
Pyrimethanil	50145801 ²	50145802 ³		Soil	16/12/2016	Bayer CropScience	LC/MS/MS	5 ng/g
AE F132593				Water				0.5 ng/g
Pyrimethanil								
AE F132593								

¹ Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

² In the ECM, no soil or water matrix was described.

⁴ In the ILV, the soil matrix (CPS ID GS-16-179-1) and the water matrix (CPS ID GS-16-179-2) were provided by the sponsor, Bayer CropScience (p. 14 of MRID 50145802). The specific soil or water type and characterization were not reported.

I. Principle of the Method

Water: Water (10 ± 0.10 g) in a 20x150 mm glass culture tube was fortified, if necessary, with the fortification solution and 0.100 mL of the 5.000 $\mu\text{g/mL}$ internal standard solution (p. 9 of MRID 49846001). The sample was mixed thoroughly via vortex before being transferred to an autosampler vial for LC/MS/MS analysis.

Soil: Soil samples (10 ± 0.10 g) were placed in Soxhlet extraction thimble and fortified, if necessary with the fortification solution (p. 10 of MRID 50145801). After sitting for at least 10 minutes, the Soxhlet extraction thimble was lightly plugged with glass wool. The extraction assembly was prepared by attaching a 250-mL boiling flask containing 125-mL of acetonitrile:water (9:1, v:v) to a Soxhlet extractor containing the extraction thimble with the sample, followed by a condenser. The boiling flask was refluxed for 6 hours. After cooling the apparatus, the condenser was rinsed with *ca.* 10-20 mL of acetonitrile. The rinsate and the extract in the 250-mL boiling flask were mixed with 0.100 mL of the 5.000 $\mu\text{g/mL}$ internal standard solution. An aliquot (12-15 mL) of the supernatant was transferred to a 20x150 mm culture tube. The solvent was evaporated to near dryness on a TurboVap (bath temperature 45-50°C). The residue was reconstituted with 1.0 mL of water then the sample was transferred to an autosampler vial for LC/MS/MS analysis.

Samples were analyzed for the analytes using a Triple Quadrupole LC/MS/MS equipped with electrospray ionization (ESI) interface (pp. 6, 10-12 of MRID 50145801). The following LC conditions were used: Phenomenex Kinetex XB-C18 column (2.1 mm x 100 mm, 2.6 μm ; column temperature 50°C), mobile phase of (A) methanol:water (1:9, v:v) with 10 mM ammonium formate and 0.12 mL/L formic acid and (B) methanol:water (9:1, v:v) with 10 mM ammonium formate and 0.12 mL/L formic acid [mobile gradient phase of percent A:B (v:v) at 0.00-0.30 min. 70:30, 0.31-2.50 min. 5:95, 2.51-5.00 min. 70:30], injection volume of 10 μL , and MRM with positive ESI. Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 200.15 \rightarrow 107.05 and m/z 200.15 \rightarrow 182.1 for pyrimethanil and m/z 124.20 \rightarrow 107.05 and m/z 124.20 \rightarrow 82.2 for AE F132593. Reported retention times were *ca.* 2.8 and 1.5 minutes for pyrimethanil and AE F132593, respectively.

The ECM method noted that 1) if an HPLC pump with a smaller dead volume than the one used for method development (1.0 min. dead volume at a flow rate of 0.25 mL/min.), the gradient step time should be increased accordingly; and 2) improved AE F132593 peak shape was observed when the analysis of samples and quantitation standards contained less than 5% organic solvent and use of a 10-L injection loop (p. 13 of MRID 50145801).

The ILV performed the ECM methods for each analyte as written, except for insignificant modifications of the analytical methods and instrumentation (pp. 15-16, 18, 20; Tables 5-6, pp. 26-27 of MRID 50145802). LC/MS/MS analysis was performed using a Shimadzu Nexera X2 Modular UHPLC system coupled with a Sciex® Triple Quad™ 6500 LC/MS/MS. The analytical method was the same as the ECM, except that the HPLC gradient was adjusted for the UPLC used and the best separation. Mobile phase was (A) methanol:water (1:9, v:v) with 10 mM ammonium formate and 0.12 mL/L formic acid and (B) methanol:water (9:1, v:v) with 10 mM ammonium formate and 0.12 mL/L formic acid [mobile gradient phase of percent A:B (v:v) at 0.00-0.10 min. 95:5, 1.00-2.50 min. 5:95, 2.51-5.00 min. 95:5]; injection volume was 10 μL for water and 4 μL for soil. Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 200 \rightarrow 107 and m/z 200 \rightarrow 76.9 for pyrimethanil and m/z 124 \rightarrow 107 and m/z 124 \rightarrow 66.9 for AE

F132593 (the confirmatory ion transitions differed from those of the ECM). Reported retention times were *ca.* 3.12 and 2.41 minutes for pyrimethanil and AE F132593, respectively (Figures 2-13, pp. 30-41; Figures 15-26, pp. 43-54; Figures 28-39, pp. 56-67; Figures 41-52, pp. 69-80). The only other modification was that the lowest calibration standard was 0.150 ppb instead of 0.250 ppb (p. 20).

In the ECM and ILV, the Limits of Quantification (LOQs) for pyrimethanil and AE F132593 were 5 ng/g (5.00 ppb) in soil and 0.5 ng/g (0.500 ppb) in water (p. 6 of MRID 50145801; pp. 12, 20; Tables 1-4, pp. 22-25 of MRID 50145802). No justifications, calculations or comparisons to background levels were reported to support the method LOQ. In the ECM, the Limit of Detection (LOD) for the method was not reported. In the ILV, the LOD was reported as 1.50 ppb for soil and 0.250 ppb for water.

II. Recovery Findings

ECM (MRID 50145801) - Water: Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of pyrimethanil and AE F132593 at fortification levels of 0.5 ng/g (LOQ) in one water matrix, but only one sample was reported ($n = 1$) for each analyte (Appendix 2, p. 18; Appendix 4, p. 20; DER Attachment 2). No samples were prepared at 10 \times LOQ (5 ng/g). Recovery values were reviewer-calculated based on peak area data and calibration equations provided in the study report. Two ion pair transitions were monitored for each analyte, but results were only provided for the quantitation ion; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. No water matrix was described.

ECM (MRID 50145801) - Soil: Mean recoveries and relative standard deviations (RSDs) were not reported. No soil matrix was described. No samples were prepared; only the method was described.

ILV (MRID 50145802) - Water: Mean recoveries and RSDs were within guidelines for analysis of pyrimethanil and AE F132593 at fortification levels of 0.5 ng/g (LOQ) and 5 ng/g (10 \times LOQ) in one water matrix (Tables 1-2, pp. 22-23). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The water matrix (CPS ID GS-16-179-2) was provided by the sponsor, Bayer CropScience (p. 14). The specific soil type and characterization were not reported. The method was validated with first trial with insignificant modifications to the analytical methods and instrumentation (pp. 15-16, 18-20; Tables 5-6, pp. 26-27).

ILV (MRID 50145802) - Soil: Mean recoveries and RSDs were within guidelines for analysis of pyrimethanil and AE F132593 at fortification levels of 5 ng/g (LOQ) and 50 ng/g (10 \times LOQ) in one soil matrix (Tables 3-4, pp. 24-25). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The soil matrix (CPS ID GS-16-179-1) was provided by the sponsor, Bayer CropScience (p. 14). The specific soil type and characterization were not reported. The method was validated with first trial with insignificant modifications to the analytical methods and instrumentation (pp. 15-16, 18-20; Tables 5-6, pp. 26-27).

Table 2a. Initial Validation Method Recoveries for Pyrimethanil and Its Transformation Product, AE F132593, in Water

Analyte ¹	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water²						
Quantitation ion³						
Pyrimethanil (AE B100309)	0.5 (LOQ)	1	94 ⁴	-- ⁵	--	--
	5.0		No data was reported			
AE F132593	0.5 (LOQ)	1	98 ⁴	--	--	--
	5.0		No data was reported			
Confirmatory ion¹						
Pyrimethanil (AE B100309)	0.5 (LOQ)		No data was reported			
	5.0					
AE F132593	0.5 (LOQ)					
	5.0					

Data (uncorrected recovery values, p. 12 and DER Attachment 2) were obtained from Appendix 2, p. 18 and Appendix 4, p. 20 of MRID 50145801 and DER Attachment 2.

1 Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

2 No water matrix was described.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 200.15→107.05 and m/z 200.15→182.1 for pyrimethanil and m/z 124.20→107.05 and m/z 124.20→82.2 for AE F132593.

4 Recovery values were reviewer-calculated based on peak area data and calibration equations provided in the study report (see DER Attachment 2).

5 Could not be determined because $n = 1$.

Table 2b. Initial Validation Method Recoveries for Pyrimethanil and Its Transformation Product, AE F132593, in Soil

Analyte ¹	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil (Quantitation and Confirmation Ions)²						
Pyrimethanil (AE B100309)	5 (LOQ)		No data was reported			
	50					
AE F132593	5 (LOQ)					
	50					

Data were obtained from MRID 50145801.

1 Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

2 No soil matrix was described.

Table 3a. Independent Validation Method Recoveries for Pyrimethanil and Its Transformation Product, AE F132593, in Water

Analyte ¹	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Water²						
Quantitation ion ³						
Pyrimethanil (AE B100309)	0.5 (LOQ)	5	94.8-98.0	96.0	1.3	1.4
	5.0	5	97.4-101	99.6	1.3	1.3
AE F132593	0.5 (LOQ)	5	92.8-105	98.1	5.0	5.1
	5.0	5	94.8-99.0	96.3	1.7	1.8
Confirmatory ion ³						
Pyrimethanil (AE B100309)	0.5 (LOQ)	5	93.2-101	96.2	2.9	3.0
	5.0	5	97.4-102	100	1.8	1.8
AE F132593	0.5 (LOQ)	5	95.4-101	98.2	2.3	2.3
	5.0	5	95.2-98.4	96.4	1.2	1.2

Data (uncorrected recovery results) were obtained from Tables 1-2, pp. 22-23 of MRID 50145802.

1 Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

2 The water matrix (CPS ID GS-16-179-2) was provided by the sponsor, Bayer CropScience (p. 14). The specific water type and characterization were not reported.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 200→107 and m/z 200→76.9 for pyrimethanil and m/z 124→107 and m/z 124→66.9 for AE F132593 (the confirmatory ion transitions differed from those of the ECM).

Table 3b. Independent Validation Method Recoveries for Pyrimethanil and Its Transformation Product, AE F132593, in Soil

Analyte ¹	Fortification Level (ng/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Soil²						
Quantitation ion ³						
Pyrimethanil (AE B100309)	5 (LOQ)	5	78.8-85.6	81.8	2.9	3.5
	50	5	94.0-99.0	96.6	2.0	2.1
AE F132593	5 (LOQ)	5	85.3-99.6	92.3	5.4	5.9
	50	5	88.0-98.2	92.9	4.5	4.8
Confirmatory ion ³						
Pyrimethanil (AE B100309)	5 (LOQ)	5	81.1-89.9	85.8	3.1	3.6
	50	5	94.4-101	97.7	2.4	2.5
AE F132593	5 (LOQ)	5	95.0-108	103	5.5	5.3
	50	5	85.4-95.2	90.8	4.0	4.4

Data (uncorrected recovery results) were obtained from Tables 3-4, pp. 24-25 of MRID 50145802.

1 Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

2 The soil matrix (CPS ID GS-16-179-1) was provided by the sponsor, Bayer CropScience (p. 14). The specific soil type and characterization were not reported.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): m/z 200→107 and m/z 200→76.9 for pyrimethanil and m/z 124→107 and m/z 124→66.9 for AE F132593 (the confirmatory ion transitions differed from those of the ECM).

III. Method Characteristics

In the ECM and ILV, the LOQs for pyrimethanil and AE F132593 were 5 ng/g (5.00 ppb) in soil and 0.5 ng/g (0.500 ppb) in water (p. 6 of MRID 50145801; pp. 12, 20; Tables 1-4, pp. 22-25 of MRID 50145802). No justifications, calculations or comparisons to background levels were reported to support the method LOQ. In the ECM, the LOD for the method was not reported. In the ILV, the LOD was reported as 1.50 ppb for soil and 0.250 ppb for water.

Table 4a. Method Characteristics for Pyrimethanil and Its Transformation Product, AE F132593, in Water

Analyte ¹		Pyrimethanil (AE B100309)	AE F132593
Limit of Quantitation (LOQ)		0.5 ng/g	
Limit of Detection (LOD)	ECM	Not reported	
	ILV	0.250 ng/g	
Linearity (calibration curve r ² and concentration range)	ECM ²	r ² = 0.9999	r ² = 0.9979
	Range:	0.25-50 ppb	
	ILV	r ² = 1.0000 (Q) r ² = 0.9999 (C)	r ² = 0.9999 (Q) r ² = 0.9999 (C)
	Range:	0.150-50 ng/mL	
Repeatable	ECM ³	Could not be determined. Yes at LOQ, but n = 1 (quantitation ion data only). No samples prepared at 10×LOQ.	
	ILV ⁴	Yes at LOQ and 10×LOQ.	
Reproducible		Could not be determined; only one set of performance data was submitted.	
Specific	ECM	Yes, matrix interferences were <i>ca.</i> 3% of the LOQ (based on peak area).	Yes, matrix interferences were <i>ca.</i> 8% of the LOQ (based on peak area).
	ILV	No matrix interferences were observed. Minor baseline noise near analyte peak of the C ion at the LOQ.	A nearby contaminant (peak size, <i>ca.</i> 25-50% of the LOQ) and baseline noise interfered with peak integration at the LOQ.

Data were obtained from pp. 6, 9; Appendix 4, p. 20 (calibration curves); Appendix 2, pp. 17-18 (chromatograms) of MRID 50145801; pp. 12, 20; Tables 1-2, pp. 22-23 (recovery results); Appendix 1, pp. 82-85 (calibration curves); Figures 2-13, pp. 30-41; Figures 15-26, pp. 43-54; Figures 28-39, pp. 56-67; Figures 41-52, pp. 69-80 (chromatograms) of MRID 50145802. Q = quantitation ion transition; C = confirmation ion transition.

1 Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

2 Only one calibration curve was reported. The reviewer assumed that it was for the quantitation ion since only quantitation ion representative chromatograms were provided. The reviewer assumed that the calibration standards were prepared in solvent, not matrix-matched.

3 In the ECM, no water matrix was described.

4 In the ILV, the water matrix (CPS ID GS-16-179-2) was provided by the sponsor, Bayer CropScience (p. 14 of MRID 50145802). The specific water type and characterization were not reported.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

Table 4b. Method Characteristics for Pyrimethanil and Its Transformation Product, AE F132593, in Soil

Analyte ¹		Pyrimethanil (AE B100309)	AE F132593
Limit of Quantitation (LOQ)		5 ng/g	
Limit of Detection (LOD)	ECM	Not reported	
	ILV	1.50 ng/g	
Linearity (calibration curve r ² and concentration range)	ECM ²	r ² = 0.9999	r ² = 0.9979
	ILV	r ² = 0.9999 (Q & C)	r ² = 0.9992 (Q) r ² = 0.9991 (C)
	Range:	1.5-1500 ppb	
Repeatable	ECM ³	Could not be determined ; only method was reported.	
	ILV ⁴	Yes at LOQ and 10×LOQ.	
Reproducible		Could not be determined ; only one set of performance data was submitted.	
Specific	ECM	Could not be determined ; no chromatograms were provided.	
	ILV	Yes, matrix interferences were <i>ca.</i> 4% of the LOQ (based on peak area).	Significant baseline noise and a nearby contaminant (peak size, <i>ca.</i> 10% of the LOQ) interfered with peak integration at the LOQ.

Data were obtained from pp. 6, 10; Appendix 4, p. 20 (calibration curves); Appendix 2, pp. 17-18 (chromatograms) of MRID 50145801; pp. 12, 20; Tables 3-4, pp. 24-25 (recovery results); Appendix 1, pp. 86-89 (calibration curves); Figures 2-13, pp. 30-41; Figures 15-26, pp. 43-54; Figures 28-39, pp. 56-67; Figures 41-52, pp. 69-80 (chromatograms) of MRID 50145802. Q = quantitation ion transition; C = confirmation ion transition.

1 Pyrimethanil = 4,6-Dimethyl-N-phenyl-2-pyrimidinamine; AE F132593) = 4-Dimethyl-2-pyrimidinamine.

2 Only one calibration curve was reported. The reviewer assumed that it was for the quantitation ion since only quantitation ion representative chromatograms were provided. The reviewer assumed that the calibration standards were prepared in solvent, not matrix-matched.

3 In the ECM, no soil matrix was described.

4 In the ILV, the soil matrix (CPS ID GS-16-179-1) was provided by the sponsor, Bayer CropScience (p. 14 of MRID 50145802). The specific soil type and characterization were not reported.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies

- For the soil portion of the method, only one set of performance data was submitted. For the water portion of the method, the reviewer was able to calculate ECM recovery values for one LOQ sample for each analyte based on peak areas and calibration curve data, but this amount of data was insufficient to assess the repeatability and reproducibility of the method. Generally, ECM 50145801 was a method only, including one set of representative solvent-based calibration curves and control and LOQ chromatograms of an undescribed water matrix. For the soil portion of the method, no samples were prepared to validate the method with an internal validation. For the water portion of the method, no recovery data was presented by the study author and only one LOQ sample was prepared per analyte. OCSPP guidelines state that sets of performance data should be submitted, one for the initial or other internal validation and one for the ILV, with the following exception: if the initial validation was performed by a governmental agency, a reference to the agency’s documentation of the ECM will serve as the ECM report.

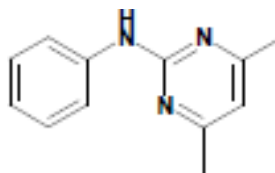
2. The specific type and characterization of the ILV test water and soil matrices were not reported; however, it was reported that the water and soil matrices were provided by the sponsor, Bayer CropScience (p. 14 of MRID 50145802). It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.
3. In the ILV, the LOQ representative chromatograms for AE F132593 showed significant baseline noise and a nearby contaminant interfered with peak integration at the LOQ (p. 19; Figures 28-39, pp. 56-67; Figures 41-52, pp. 69-80 of MRID 50145802). The peak size of the nearby contaminant was *ca.* 10% of the LOQ in soil and *ca.* 25-50% of the LOQ in water. The ILV study author commented on the interfering peak, noting that it was found in the water analysis only. The ILV study author reported that the “HPLC gradient was modified slightly to separate the interfering peak from AE F132593 peak as suggested in the method” (p. 19). The reviewer noted that the interference of the baseline noise was more prominent in the soil analysis than the water analysis.
4. In the ECM, no 10×LOQ representative chromatograms were provided (Appendix 2, pp. 17-18 of MRID 50145801). Only quantitative chromatograms were provided for the LOQ fortification in water; the reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

V. Reviewer’s Comments

1. The ILV was provided Bayer Method PI-003-S16-01 (method date November 8, 2016) as the ECM (Appendix 4, pp. 97-117 of MRID 50145802). After the successful completion of the ILV, Bayer submitted Bayer Method PI-003-S16-02 (MRID 50145801; method date December 16, 2016; Appendix 5, p. 21 of MRID 50145801).
2. The communications between the ILV and validation laboratory Study Director and Study Monitor were briefly described (p. 20 of MRID 50145802). The ILV reported that emails were exchanged regarding study progress only; no technical or procedural aspects of the analytical method were discussed.
3. In the ILV, the total time required to complete one set of 13 samples (one reagent blank, two matrix controls and ten fortified samples) was reported as *ca.* 1 day to complete for water and as *ca.* 1.5 day to complete for soil, where sample preparation required *ca.* 2 hours for water and *ca.* 8 hours for soil and LC/MS/MS analysis and data processing required *ca.* 3 hours (p. 19 of MRID 50145802).
4. The determination of the LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 6 of MRID 50145801; pp. 12, 20; Tables 1-4, pp. 22-25 of MRID 50145802). No justifications, calculations or comparisons to background levels were reported to support the method LOQ. No method LOD was reported in the ECM.

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**Pyrimethanil (AE B100309)****IUPAC Name:** Not found**CAS Name:** 4,6-Dimethyl-N-phenyl-2-pyrimidinamine**CAS Number:** 53112-28-0**SMILES String:** Not found**AE F132593****IUPAC Name:** Not found**CAS Name:** 4-Dimethyl-2-pyrimidinamine**CAS Number:** 767-15-7**SMILES String:** Not found