

Test Material: Quizalofop-p-ethyl

MRID: 49634806

Title: Validation of BASF Analytical Method (D1303/02): "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl (BAS 9152 H) and its two Metabolites Quizlofop-p and 3-OH-Quizalofop-acid in Soil by LC-MS/MS"

MRID: 49634808

Title: Independent Lab Validation of BASF Analytical Method D1303/02: "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl (BAS 9152 H) and its two Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Soil by LC-MS/MS"

EPA PC Code: 128709

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lynne Binari

Signature: 

Date: 8/18/15

Secondary Reviewer: Lisa Muto

Signature: 

Date: 8/18/15

QC/QA Manager: Joan Gaidos

Signature: 

Date: 8/18/15

Analytical method for quizalofop-p-ethyl (BAS 9152 H) and its transformation products quizalofop-p and 3-OH-quizalofop-acid in soil

Reports: ECM: EPA MRID No.: 49634806. Shen, X. 2015. Validation of BASF Analytical Method (D1303/02): "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl (BAS 9152 H) and its two Metabolites Quizlofop-p and 3-OH-Quizalofop-acid in Soil by LC-MS/MS". Report prepared by Primera Analytical Solutions Corporation (PASC), Princeton, New Jersey, sponsored, and submitted by BASF Corporation, Research Triangle Park, North Carolina; 208 pages. PASC Study No.: 053-0977A and Report No.: PASC-REP-0506. BASF Study No.: 437873 and Registration Document No.: 2014/7003590. Final report issued March 13, 2015.
ILV: EPA MRID No. 49634808. Sharp, S.E. 2015. Independent Lab Validation of BASF Analytical Method D1303/02: "Analytical Method for the Determination of Residues of Quizalofop-p-ethyl (BAS 9152 H) and its two Metabolites Quizalofop-p and 3-OH-Quizalofop-acid in Soil by LC-MS/MS". Report prepared by EPL Bio Analytical Services (EPL), Niantic, Illinois, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 123 pages. EPL Study No.: 137G967. BASF Study No.: 437875 and Registration Document No.: 2014/7003592. Final report issued March 4, 2015.

Document No.: MRIDs 49634806 & 49634808


Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 49634806). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).
ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49634808). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

Classification: This analytical method is classified as **acceptable**. The version of the method using a 0.1-g soil sample size was not validated by an independent laboratory. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures.

PC Code: 128709

Reviewer: Stephen P. Wentz, Ph.D
Biologist

Signature: 
Date: October 25, 2016

Executive Summary

The analytical method, BASF Analytical Method D1303/02, is designed for the quantitative determination of quizalofop-p-ethyl (BAS 9152 H) and its transformation products quizalofop-p and 3-OH-quizalofop-acid in soil using LC/MS/MS. The method is quantitative for the analytes at the stated LOQs of 0.005 mg/kg (ppm) for quizalofop-p-ethyl and quizalofop-p and 0.001 mg/kg for 3-OH-quizalofop-acid. The LOQ is less than the lowest toxicological level of concern in soil. The independent laboratory validated the method using a 5-g soil sample size for analysis of quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid at the LOQ and 10x LOQ in a silty clay loam soil matrix after one trial. The version of the method using a 0.1-g soil sample size was not included in the ILV. No major modifications were made by the independent laboratory.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Quizalofop-p-ethyl (BAS 9152 H)	49634806	49634808 ¹		Soil ²	13/03/2015	BASF	LC/MS/MS	0.005 mg/kg (ppm)
Quizalofop-p								0.005 mg/kg
3-OH-Quizalofop-acid								0.001 mg/kg

1 The version of the method using a 0.1-g soil sample size was not included in the ILV.

2 Characterized loamy sand (3% clay, 0.27% organic matter) and silt loam (14% clay, 1.6% organic matter) soils were used for the ECM validation, with silty clay loam soil (34% clay, 0.56% organic matter) used for the ILV (Appendix 5, pp. 137-138 of MRID 49634806; Appendix J, p. 123 of MRID 49634808).

I. Principle of the Method

Soil (5 ± 0.1 g or 0.1 ± 0.01 g) was fortified with a mixed standard solution of quizalofop-p-ethyl (BAS 9152 H) and quizalofop-p in acetonitrile and 3-OH-quizalofop-acid in methanol for procedural recoveries (pp. 27, 30-31 of MRID 49634806). Characterized loamy sand and silt loam soils were used for the validation; source locations were not reported (p. 19; Appendix 5, pp. 137-138). Soil (5 ± 0.1 g) is weighed into a 40-mL Teflon centrifuge tube, combined with 1.5 g of Celite[®], then extracted with 15 mL of acetonitrile:6% phosphoric acid in water (80:20, v:v, S1) by shaking horizontally (mechanical shaker, 300 rpm) for *ca.* 30 minutes (pp. 25, 32; Figure 2, p. 52). Soil and extract are separated by centrifugation (*ca.* 4,000 rpm, *ca.* 5 minutes). A 7.5-mL aliquot of the extract was transferred to a culture tube, and the remaining sample was again extracted as described above. Following centrifugation, 7 mL of the initial extract was added to the second extraction sample (extract plus soil), plus two 4-mL aliquots of rinsate following two 4.5-mL acetonitrile rinses of the culture tube which contained the initial extract: total extract sample volume = 37.5 mL. The sample is manually shaken for 10 seconds, then centrifuged. A 30-mL aliquot of the supernatant is taken and diluted with 20 mL of acetonitrile. For quizalofop-p-ethyl and quizalofop-p, a 0.1-mL aliquot of the sample is diluted with 0.7 mL of acetonitrile:water (90:10, v:v, S2) for LC/MS/MS analysis (pp. 25, 33). For 3-OH-quizalofop-acid, a 0.5-mL aliquot of the sample is diluted with 0.3 mL of water for LC/MS/MS analysis.

Soil (0.1 ± 0.01 g) is weighed into an Alpha Tube in a 96-well plate, combined with 0.03 g of Celite[®], then extracted with 0.3 mL of acetonitrile:6% phosphoric acid in water (80:20, v:v) using a multi-tube vortexer for *ca.* 10 minutes with the 96-well plate upright, followed by 10 minutes with the 96-well plate inverted (pp. 25, 30-32; Figure 1, p. 51). Soil and extract are separated by centrifugation (*ca.* 4,000 rpm, *ca.* 5 minutes). A 0.15-mL aliquot of the extract was transferred to a glass micro tube, and the remaining sample was again extracted as described above. Following centrifugation, 0.13 mL of the initial extract was added to the second extraction sample (extract plus soil), plus two 0.085-mL aliquots of rinsate following two 0.1-mL acetonitrile rinses of the glass micro tube which contained the initial extract: total extract sample volume = 0.75 mL. The 96-well plate is vortexed upright for 15 seconds and inverted for 15 seconds, then centrifuged. A 0.6-mL aliquot of the supernatant is taken and diluted with 0.4 mL of acetonitrile. Samples were diluted for LC/MS/MS analysis as described above (p. 33).

LC/MS/MS of Quizalofop-p-ethyl (BAS 9152 H) and Quizalofop-p: Samples are analyzed using a Waters UPLC Acquity system and an Applied Biosystems Sciex 5000 MS with electrospray ionization (ESI; p. 34 of MRID 49634806). The following LC conditions were used: Acquity UPLC BEH C18 column (2.1 mm x 50 mm, 1.7 μ m, column temperature 50°C), mobile phase of (A) 4mM ammonium formate with 0.1% formic acid in water and (B) 4mM ammonium formate with 0.1% formic acid in methanol [percent A:B (v:v) at 0.0-0.5 min. 95:5, 1.0 min. 50:50, 3.0-3.5 min. 5:95, 3.6-4.0 min. 95:5], and injection volume of 20 μ L. The following MS/MS conditions were used: positive ion mode and ionization temperature 500°C. Analytes are identified using two ion pair transitions; one for quantitation (Q, "primary") and one for confirmation (C, "secondary"). Ion transitions monitored were as follows: m/z 373 \rightarrow 299 (Q) and m/z 375 \rightarrow 301 (C) for quizalofop-p-ethyl, and m/z 345 \rightarrow 299 (Q) and m/z 345 \rightarrow 100 (C) for quizalofop-p. Expected retention times are *ca.* 2.6 and 2.3 minutes for quizalofop-p-ethyl and quizalofop-p, respectively.

LC/MS/MS of 3-OH-Quizalofop-acid: Samples are analyzed using the same UPLC/MS/MS system as describe above (p. 35 of MRID 49634806). The following "primary" LC conditions were used: Acquity UPLC BEH Phenyl column (2.1 mm x 100 mm, 1.7 μ m, column temperature 50°C), mobile phase of (A) 4mM ammonium formate with 0.1% formic acid in water and (B) 4mM ammonium formate with 0.1% formic acid in methanol [percent A:B (v:v) at 0.0-0.5 min. 95:5, 3.0-3.5 min. 5:95, 3.6-4.0 min. 95:5], and injection volume of 30 μ L. The following MS/MS conditions were used: negative ion mode and ionization temperature 550°C. 3-OH-Quizalofop-acid is identified using a single ion pair transition: m/z 359 \rightarrow 166. Expected retention time is *ca.* 3.2 minutes. The following modification was made as a confirmatory "secondary" UPLC/MS/MS method: Acquity UPLC HSS T3 column (2.1 mm x 100 mm, 1.8 μ m), with expected retention time of *ca.* 3.1 minutes (p. 36).

ILV: Test compounds and silty clay loam soil were supplied by BASF (pp. 17-19 of MRID 49634808). The soil matrix was characterized, but the source location was not reported (Appendix J, p. 123). The independent laboratory performed the method for 5-g soil samples as written with the following modifications: centrifugation speeds after the initial centrifugation step were reduced to 2,000 rpm; Celite[®] aliquots (1.45-1.55 g) were pre-weighed into glass scintillation vials prior to use; 10x LOQ fortifications of quizalofop-p-ethyl and quizalofop-p were diluted by a factor of 5 with acetonitrile:water (90:10, v:v, S2) and 10x LOQ fortifications of 3-OH-quizalofop-acid were diluted by a factor of 2 with acetonitrile:water (55:45, v:v, S3) prior to analysis; samples were analyzed using an Agilent 1290 HPLC system and an Applied Biosystems Sciex 6500 Q-Trap MS/MS with ESI; an Acquity BEH C18 column (2.1 mm x 100 mm, 1.7 μ m) was used for quizalofop-p-ethyl and quizalofop-p analyses; and injection volume was reduced to 20 μ L for 3-

OH-quizalofop-acid analyses (pp. 20-24). Mobile phase gradients used were not reported. Expected retention times were *ca.* 3.3, 2.9, and 3.15 minutes for quizalofop-p-ethyl, quizalofop-p, and 3-OH-quizalofop-acid, respectively.

LOQ and LOD: In the ECM and ILV, LOQs were 0.005 mg/kg for quizalofop-p-ethyl and quizalofop-p and 0.001 mg/kg for 3-OH-quizalofop-acid (pp. 7, 45-46 of MRID 49634806; pp. 7, 29 of MRID 49634808). LODs were estimated at 0.001 mg/kg for quizalofop-p-ethyl and quizalofop-p and 0.0002 mg/kg for 3-OH-quizalofop-acid.

II. Recovery Findings

ECM (MRID 49634806): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of quizalofop-p-ethyl (BAS 9152 H) and its transformation product quizalofop-p in loamy sand soil (5-g sample size) and silt loam soil (5- and 0.1-g sample sizes) at fortification levels of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10x LOQ) and for transformation product 3-OH-quizalofop-acid in the two soils at fortification levels of 0.001 mg/kg (LOQ) and 0.01 mg/kg (10x LOQ), except for the 0.001 mg/kg 3-OH-quizalofop-acid confirmation method analysis in the loamy sand soil (RSD 21%; Tables 1-3, pp. 41-43). Quizalofop-p-ethyl and quizalofop-p were identified and quantified using two ion transitions, while 3-OH-quizalofop-acid was identified and quantified using two chromatographic methods; quantitation and confirmation recovery results were comparable. The loamy sand (3% clay, 0.27% organic matter) and silt loam (14% clay, 1.6% organic matter) soil matrices were fully characterized by Agvise Laboratories, Northwood, North Dakota (Appendix 5, pp. 137-138).

ILV (MRID 49634808): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of quizalofop-p-ethyl (BAS 9152 H) and its transformation product quizalofop-p in a silty clay loam soil (5-g sample size) at fortification levels of 0.005 mg/kg (LOQ) and 0.05 mg/kg (10x LOQ) and for transformation product 3-OH-quizalofop-acid at fortification levels of 0.001 mg/kg (LOQ) and 0.01 mg/kg (10x LOQ; Tables 1-3, p. 27). Quizalofop-p-ethyl and quizalofop-p were identified and quantified using two ion transitions, and 3-OH-quizalofop-acid was identified and quantified using two chromatographic methods; quantitation and confirmation recovery results were comparable. The method was validated for the analytes at both fortification levels in the soil matrix after one trial, with minor method modifications and equivalent instrument substitutions (pp. 9, 23, 26). The silt clay loam soil (34% clay, 0.56% organic matter) matrix was fully characterized by Agvise Laboratories, Northwood, North Dakota (Appendix J, p. 123).

Table 2. Initial Validation Method Recoveries for Quizalofop-p-ethyl (BAS 9152 H) and Its Transformation Products Quizalofop-p and 3-OH-Quizalofop-acid in Soil¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Loamy Sand Soil (5-g sample size)						
Quantitation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	89.6-92.6	90.9	1.12	1
	0.05	5	85.0-94.2	90.4	4.05	4
Quizalofop-p	0.005 (LOQ)	5	87.6-108	97.6	7.20	7
	0.05	5	88.0-100	94.2	4.42	5
3-OH-Quizalofop-acid	0.001 (LOQ)	5	83.6-109	95.4	9.29	10
	0.01	5	84.6-93.0	89.2	3.56	4
Confirmation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	80.8-99.4	89.5	8.12	9
	0.05	5	84.8-92.0	88.3	3.47	4
Quizalofop-p	0.005 (LOQ)	5	82.2-120	96.9	14.3	15
	0.05	5	79.6-99.6	92.8	7.84	8
3-OH-Quizalofop-acid	0.001 (LOQ)	5	71.2-117	96.2	19.9	21
	0.01	5	85.2-102	94.3	7.23	8
Silt Loam Soil (5-g sample size)						
Quantitation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	85.6-94.2	88.7	3.57	4
	0.05	5	81.6-86.6	84.2	2.11	3
Quizalofop-p	0.005 (LOQ)	5	67.0-110	82.8	17.0	20
	0.05	5	91.0-98.4	95.2	3.03	3
3-OH-Quizalofop-acid	0.001 (LOQ)	5	83.0-98.0	88.6	5.84	7
	0.01	5	79.6-87.6	84.0	2.88	3
Confirmation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	81.8-101	88.6	7.54	9
	0.05	5	79.8-84.4	82.0	1.75	2
Quizalofop-p	0.005 (LOQ)	5	69.0-108	90.0	17.0	19
	0.05	5	88.0-96.6	92.0	3.28	4
3-OH-Quizalofop-acid	0.001 (LOQ)	5	101-121	113	7.54	7
	0.01	5	88.4-106	96.8	7.79	8
Silt Loam Soil (0.1-g sample size)						
Quantitation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	89.2-93.8	91.1	1.71	2
	0.05	5	88.2-94.8	91.9	2.48	3
Quizalofop-p	0.005 (LOQ)	5	93.4-101	95.7	3.00	3
	0.05	5	93.6-101	97.5	3.14	3
3-OH-Quizalofop-acid	0.001 (LOQ)	5	81.0-103	93.8	7.96	8
	0.01	5	83.2-92.0	86.7	3.69	4
Confirmation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	88.4-97.2	94.0	3.92	4
	0.05	5	91.4-98.4	95.3	3.38	4
Quizalofop-p	0.005 (LOQ)	5	86.0-108	98.4	7.96	8
	0.05	5	96.8-105	102	4.28	4
3-OH-Quizalofop-acid	0.001 (LOQ)	5	84.0-98.8	92.8	6.13	7
	0.01	5	84.8-95.8	89.1	4.84	5

Data (uncorrected recovery results; Appendix 6, pp. 155-172) were obtained from Tables 1-3, pp. 41-43 of MRID 49634806.

1 Soil characterizations were provided, but source locations were not reported (Appendix 5, pp. 137-138).

Table 3. Independent Validation Method Recoveries for Quizalofop-p-ethyl (BAS 9152 H) and Its Transformation Products Quizalofop-p and 3-OH-Quizalofop-acid in Silty Clay Loam Soil (5-g sample size)¹

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	63.20-83.60	70.48	8.00	11.35
	0.05	5	78.60-95.10	86.78	6.10	7.03
Quizalofop-p	0.005 (LOQ)	5	90.60-124.60	109.16	16.00	14.66
	0.05	5	111.80-125.60	117.90	5.36	4.54
3-OH-Quizalofop-acid	0.001 (LOQ)	5	81.64-89.27	85.78	3.13	3.65
	0.01	5	87.74-96.37	92.98	3.36	3.61
Confirmation						
Quizalofop-p-ethyl	0.005 (LOQ)	5	62.40-86.60	71.60	9.03	12.61
	0.05	5	76.00-96.40	87.94	7.72	8.78
Quizalofop-p	0.005 (LOQ)	5	93.40-117.40	108.52	11.35	10.46
	0.05	5	107.60-125.70	118.30	6.58	5.57
3-OH-Quizalofop-acid	0.001 (LOQ)	5	79.44-90.27	84.05	4.03	4.80
	0.01	5	86.10-92.56	90.46	2.73	3.02

Data (uncorrected recovery results; p. 25; Appendix H, pp. 110-115) were obtained from Tables 1-3, p. 27 of MRID 49634808.

1 The soil matrix, supplied by BASF, was characterized, but source location was not reported (p. 17; Appendix J, p. 123).

III. Method Characteristics

In the ECM, the LOQs in soil were 0.005 mg/kg (ppm) for quizalofop-p-ethyl (BAS 9152 H) and its transformation product quizalofop-p and 0.001 mg/kg for transformation product 3-OH-quizalofop-acid, equivalent to 0.05 ng/mL in the final sample extract solutions (pp. 7, 45-46 of MRID 49634806). The ECM defined the LOQ as the lowest fortification level successfully tested. The ECM defined the LOD as 20% of the LOQ, or 0.001 mg/kg for quizalofop-p-ethyl and quizalofop-p and 0.0002 mg/kg for 3-OH-quizalofop-acid, equivalent to the lowest calibrant standard, 0.01 ng/mL, for each analyte. The lowest calibrant standards were reported as having good detectability (signal to noise ratio greater than 3:1; Appendix 4, pp. 77, 80, 83, 86, 89, 92). The ILV reported the same LOQs and LODs as the ECM (pp. 7, 28-29 of MRID 49634808).

Table 4. Method Characteristics for Quizalofop-p-ethyl (BAS 9152 H) and Its Transformation Products Quizalofop-p and 3-OH-Quizalofop-acid in Soil

		Quizalofop-p-ethyl	Quizalofop-p	3-OH-Quizalofop-acid
Limit of Quantitation (LOQ)		0.005 mg/kg (ppm)		0.001 mg/kg
Limit of Detection (LOD)		0.001 mg/kg		0.0002 mg/kg
Linearity (calibration curve r^2 and concentration range) ¹	ECM:	Q ion: $r^2 = 0.9962-0.9998$ C ion: $r^2 = 0.9950-0.9994$	Q ion: $r^2 = \mathbf{0.9934}-0.9986$ C ion: $r^2 = \mathbf{0.9890}-0.9984$	Q LC-1: $r^2 = 0.9968-0.9996$ C LC-2: $r^2 = 0.9966-0.9996$
	ILV:	Q ion: $r^2 = 0.9998$ C ion: $r^2 = 0.9995$	Q ion: $r^2 = 0.9998$ C ion: $r^2 = \mathbf{0.9942}$	Q LC-1: $r^2 = 0.9998$ C LC-2: $r^2 = 0.9997$
	Range:	0.01-1 ng/mL		
Repeatable	ECM:	Yes at LOQ and 10x LOQ, except for 0.001 mg/kg 3-OH-quizalofop-acid confirmation analysis in the loamy sand soil (RSD 21%). [5-g loamy sand (3% clay, 0.27% organic matter) and 5-g and 0.1-g silt loam (14% clay, 1.6% organic matter) soil matrices] ²		
	ILV:	Yes at LOQ and 10x LOQ. [5-g silty clay loam (34% clay, 0.56% organic matter) soil matrix] ²		
Reproducible		Yes.		
Specific	ECM: ³	Interferences (based on peak height) were 42-50% of LOD (11-14% of LOQ) at analyte retention time in matrix controls.	Interferences (based on peak height) were 40-71% of LOD (12-29% of LOQ) at analyte retention time in matrix controls.	Interferences (based on peak height) were 30-63% of LOD (6-13% of LOQ) at analyte retention time in matrix controls.
	ILV:	Interferences (based on peak height) were <i>ca.</i> 10-17% of LOD (2-5% of LOQ) at analyte retention time in reagent blank and matrix controls.	Peak of lowest calibrant (0.01 ng/mL, LOD) barely resolved above baseline. Interferences (based on peak height) were <i>ca.</i> 13-56% of LOQ (0.05 ng/mL calibrant) at analyte retention time in reagent blank and matrix controls.	Interferences (based on peak height) were <i>ca.</i> 9-18% of LOD (3-5% of LOQ) at analyte retention time in reagent blank and matrix controls.

Data were obtained from pp. 7-8, 19, 37, 44-46; Tables 1-3, pp. 41-43; Appendix 2, pp. 63-68; Appendix 3, p. 69-75; Appendix 4, pp. 77-78, 80-81, 83-84, 86-87, 89-90, 95-98, 107-110, 119-122; Appendix 5, pp. 137-138; Appendix 6, pp. 155-172 of MRID 49634806; pp. 7, 28-29; Tables 1-3, p. 27; Appendix A, pp. 34-35, 37, 41-42; Appendix B, pp. 46-47, 49, 53-54; Appendix C, pp. 58-59, 61, 65-66; Appendix D, pp. 70-71, 73, 77-78; Appendix E, pp. 82-83, 85, 89-90; Appendix F, pp. 94-95, 97, 101-102 of MRID 49634808.

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

1 Linear regression with 1/x weighting (Appendix 2, pp. 63-68 of MRID 49634806; Appendix A, p. 34; Appendix B, p. 46; Appendix C, p. 58; Appendix D, p. 70; Appendix E, p. 82; Appendix F, p. 94 of MRID 49634808). ECM coefficient of determination (r^2) values are reviewer-generated from reported correlation coefficient (r) values (Appendix 6, pp. 155-172 of MRID 49634806; DER Attachment 2).

2 Soil characterizations were provided, but source locations were not reported (p. 19; Appendix 5, pp. 137-138 of MRID 49634806; p. 17; Appendix J, p. 123 of MRID 49634808). Soil matrix for ILV supplied by BASF.

3 Soil sample chromatograms were for 5-g sample size; chromatograms from 0.1-g sample size analyses were not provided (Appendix 4, pp. 95-130 of MRID 49634806).

IV. Method Deficiencies and Reviewer's Comments

1. The version of the method using a 0.1-g soil sample size and 96-well plate extraction method was not validated by the independent laboratory.
2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. The ECM defined the LOQ as the lowest fortification level successfully tested (p. 7, 46 of MRID 49634806). The ECM defined the LOD as 20% of the LOQ, equivalent to the lowest calibrant standard, 0.01 ng/mL, for each analyte. The lowest calibrant standards were reported as having good detectability (signal to noise ratio greater than 3:1; Appendix 4, pp. 77, 80, 83, 86, 89, 92). The ILV reported the same LOQs and LODs as the ECM (pp. 7, 28-29 of MRID 49634808). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in soil was not reported. A LOQ above toxicological levels of concern results in an unacceptable method classification.
3. For the ECM validation, the 0.001 mg/kg (LOD) 3-OH-quizalofop-acid confirmation method analysis in the loamy sand soil (RSD 21%) did not meet OCSP 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) $\leq 20\%$].
4. For the ILV, chromatograms for quizalofop-p show the peak of the lowest calibrant (0.01 ng/mL, LOD) is barely resolved above the baseline noise (Appendix A, p. 35; Appendix B, p. 47 of MRID 49634808). For quizalofop-p, interferences (based on peak height) at the analyte retention time were 13-56% of LOQ (0.05 ng/mL calibrant) in reagent blank and matrix controls (Appendix A, pp. 35, 37, 41-42; Appendix B, pp. 47, 49, 53-54).
5. For the quizalofop-p calibration standard curves, linearity was not always satisfactory ($r^2 \geq 0.995$) for both the quantitation ion (ECM validation, ILV) and confirmation ion (ILV) analyses (Appendix B, p. 46 of MRID 49634808; DER Attachment 2).
6. For the ECM validation, chromatograms for reagent blank samples and 0.1-g soil sample analyses were not provided (Appendix 4, pp. 95-130 of MRID 49634806).
7. For both the ECM validation and ILV, the source locations for the soil matrices were not reported (p. 19 of MRID 49634806; p. 17 of MRID 49634808).
8. For the 5-g soil sample analysis, the equipment substitutions and method modifications implemented by the independent laboratory (see section **I. Principle of the Method, ILV**: above for details) are not considered substantial changes to the ECM.
9. As part of the ECM validation, it was determined that under refrigerator storage stock solutions of all three analytes were stable for 95 days (<10% change), fortification and calibration solutions were stable for 29 days (<10% change), and soil extract and final solutions were stable for 7 days (<12% change; p. 8, 22, 39; Appendix 1, pp. 53-58 of MRID 49634806).

10. For the ECM study report, there is a typographical error in the study title; "Quizlofop-p" should read Quizalofop-p (p. 1 of MRID 49634806). Also in section **2.2 Test and Reference Items** (pp. 20-21), "-chloroquinoxanlin-" should read -chloroquinoxalin-.
11. It was reported for the ILV that a set of thirteen samples (one reagent blank, two matrix controls, and ten fortified samples) required *ca.* 14-16 work hours, which included calculation of the results (p. 29 of MRID 49634808).

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

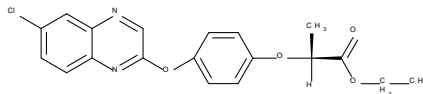
Quizalofop-p-ethyl (QPE, BAS 9152 H, D(+))NC-302)

IUPAC Name: Ethyl (2R)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionate

CAS Name: Ethyl (2R)-2-[4-[(6-chloro-2-quinoxalinyloxy]phenoxy]propanoate

CAS Number: 100646-51-3

SMILES String: n1c2ccc(Cl)cc2ncc1Oc3ccc(OC(C)C(=O)OCC)cc3



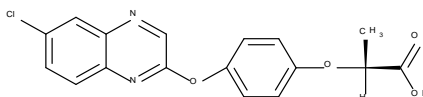
Quizalofop-p (QP, R-QA, 302D-ACID)

IUPAC Name: (R)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionic acid

CAS Name: (2R)-2-[4-[(6-chloro-2-quinoxalinyloxy]phenoxy]propanoic acid

CAS Number: 94051-08-8

SMILES String: n1c2ccc(Cl)cc2ncc1Oc3ccc(OC(C)C(=O)O)cc3



3-OH-Quizalofop-acid (3-OH-QA, R-3-OH-QA, R(+)-3-OH-quizalofop-acid)

IUPAC Name: (2S)-2-[4-(6-chloro-3-hydroxyquinoxalin-2-yl)oxyphenoxy]propanoic acid

CAS Name: (R)-2-[4-(6-Chloro-3-hydroxyquinoxalin-2-yloxy)phenoxy]propionic acid

CAS Number: Not available.

SMILES String: [H]C(C)(C(=O)O)Oc1ccc(cc1)Oc2c(nc3cc(O)ccc3n2)Cl

