Analytical method for etoxazole and its metabolites R-8 and R-13 in sediment

Reports:	ECM: EPA MRID No.: 50539904. Perez, R. 2018. Method Validation of Analytical Method Number RM-37S-3: The Determination of Residues of Etoxazole and its Metabolites, R-8 and R-13 in Sediment and Water Matrices using LC-MS/MS. Laboratory Project ID: VP-41072. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by Valent USA, LLC, Dublin, California; 200 pages. Final report issued January 22, 2018. ILV: EPA MRID No. 50539906. Perez, S. 2018. Independent Laboratory Validation of Valent Analytical Method RM-37S-3: "The Determination of Residues of Etoxazole and its Metabolites, R-8 and R-13 in Sediment and Water Matrices Using LC-MS/MS". Laboratory Project ID: V-17-200100. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, and sponsored and submitted by Valent USA, LLC, Dublin, California; 135 pages. Final report issued January 30, 2018. See Reviewer's Comment #1 regarding study author citation.
Document No.:	MRIDs 50539904 & 50539906
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p. 3 of MRID 50539904). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5). ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR Part 160; p. 3 of MRID 50539906). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).
Classification:	This analytical method is classified as unacceptable. It could not be determined that ILV MRID 50539906 was conducted independently of ECM MRID 50539904. The specificity of the method for R-8 was not supported by ILV and ECM chromatograms. ILV linearity was not satisfactory for etoxazole. It could not be determined that the ILV were provided with the most difficult matrix with which to validate the method and that ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. This study may be reevaluated if the missing information can be supplied and the aforementioned issues addressed.
PC Code:	107091

EFED Final Reviewers:	Zoe Ruge, M.S., Physical Scientist	Signature: Zoe Ruge Date: 7/3/19
	Dena Barrett, PhD., Chemist	Signature: Dena Barrett Date: 7/3/19
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Lisa Muto Date: 10/11/2018
	Mary Samuel, M.S., Environmental Scientist	Signature: Marysamuel Date: 10/24/2018

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Valent Analytical Method RM-37S-3, is designed for the quantitative determination of etoxazole and its metabolites R-8 and R-13 in sediment at the LOQ of 0.01 mg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in sediment for etoxazole and its metabolites R-8 and R-13 (0.87 mg/kg). The ECM and ILV validated the method using characterized clay loam sediment; ILV sediment matrix was the same one which was used in the ECM. It could not be determined that the ILV were provided with the most difficult matrix with which to validate the method and that ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the method in the first trial with insignificant modifications to the final dilution ratio and LC/MS injection volume; however, it could not be determined that ILV MRID 50539906 was conducted independently of ECM MRID 50539904 since both validations were conducted at the same facility (ADPEN Laboratories, Inc., Jacksonville, Florida) and insufficient evidence was provided to support the independence of the two laboratories. The communication between the staff of the initial and independent validations was not addressed. The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM. **The registrant needs to provide additional information to confirm no interactions between staff and no sharing of equipment when both validations occur at the same address.** All ILV and ECM data regarding repeatability, accuracy, precision, and linearity were satisfactory for etoxazole and its metabolites R-8 and R-13, except for the ILV linearity for etoxazole. The specificity of the method for etoxazole and R-13 was supported by ILV and ECM representative chromatograms, but specificity of the method for R-8 was not supported due to one or more nearby contaminants which were 20xs-75xs larger (peak ht. > LOQ peak ht.) in R-8 chromatograms compared to etoxazole and R-13 chromatograms.

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						
Etoxazole	50539904[1]	50539906[2]	Unacceptable	Sediment	22/01/2018	Valent USA, LLC	LC/MS/MS	0.01 mg/kg
R-8								
R-13								

[1] In the ECM, the clay loam sediment (Lab Code #170110002-018 0-2"; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; pp. 14-15; Appendix A, p. 41 of MRID 50539904). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota.

[2] In the ILV, the clay loam sediment (Lab Code #170110002-018 0-2"; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; p. 14; Appendix A, p. 94 of MRID 50539906). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota. The sediment matrix was the same one which was used in the ECM.

I. Principle of the Method

Samples (5 ± 0.01 g) were fortified, as necessary, and extracted twice with 20 mL of methanol:water, 90:10 (v:v), containing 10 mM ammonium bicarbonate (pp. 19-21 of MRID 50539904). Each extraction was performed by sonicating for five minutes then shaking twice for 30 seconds on the Omni Bead Ruptor Homogenizer (speed 4 m/s) at room temperature (beads not required). After centrifugation for 15 minutes at 3500 rpm, the supernatant was decanted into a clean 50 mL graduated polypropylene test tube. After the combined extract was sonicated for one minute and centrifuged for 5 minutes at 3500 rpm, a 1 mL aliquot of the supernatant was diluted with 1 mL of methanol:water, 10:90 (v:v). The extract was further diluted by transferring 1 mL aliquot to a 25-mL graduated centrifuge tube and adjusting the total volume to 10 mL with methanol:water, 50:50 (v:v). After sonication and vortexing for 30 seconds, a 1.5 mL aliquot was transferred to an autosampler vial, and the analytes residues was analyzed by LC-MS/MS. A flow chart was provided (Appendix C, p. 66).

Samples were analyzed for etoxazole and metabolites R-8 and R-13 by Agilent 1290 UPLC system coupled with an AB SCIEX 5500 QTrap MS (Acquity UPLC BEH C18 column, 2.1 mm x 50 mm, 1.7 µm column; column temperature 60°C) using a gradient mobile phase of A) 0.1% formic acid + 5 mM ammonium formate in HPLC water and B) 0.1% formic acid + 5 mM ammonium formate in methanol [percent A:B; 0.0-0.2 min. 90:10, 0.7-2.0 min. 5:95, 2.1-3.0 min. 90:10] with MS/MS-ESI (electrospray ionization; temperature 450°C) detection in positive ion mode and multiple reaction monitoring (MRM; pp. 19, 21-23 of MRID 50539904). Injection volume was 5.0 µL. Etoxazole and metabolites R-8 and R-13 was identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were *m/z* 360.203→141.0 (Q) and *m/z* 360.203→177.1 (C) for etoxazole, *m/z* 238.087→165.1 (Q) and *m/z* 238.087→147.1 (C) for R-8, and *m/z* 358.145→140.8 (Q) and *m/z* 358.145→274.0 (C) for R-13. Approximate retention times were 1.3, 1.1, and 1.4 minutes for etoxazole, R-8 and R-13, respectively.

In the ECM, the following experimental precautions were listed: 1) the use of HPLC grade sediment for mobile phase solutions was important to lower background noise; 2) carry-over in the LC/MS analysis can be avoided by diluting high-recovery samples and including blank injections after high-level samples; and 3) additional needle washes and valve washes with organic solvent may help eliminate carry-over of the analytes (p. 24 of MRID 50539904).

In the ILV, the ECM was performed as written, except the final dilution ratio differed (0.2 mL aliquot diluted to 15 mL) and increase of the injection volume to 25.0 µL (pp. 16, 19, 24; Table 14, p. 40 of MRID 50539906). The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM; monitored ion transitions matched those reported in the ECM. Approximate retention times were 1.7, 1.6, and 1.8 minutes for etoxazole, R-8 and R-13, respectively.

The Limit of Quantitation (LOQ) for etoxazole and its metabolites R-8 and R-13 in sediment was 0.01 mg/kg in the ECM and ILV (pp. 26-27, 29 of MRID 50539904; pp. 20-22 of MRID 50539906). In the ECM and ILV, the Limit of Detection (LOD) for sediment was set at 0.005 mg/kg (50% of the LOQ) for all three analytes. In the ILV, the LOD was calculated as 0.002 mg/kg for the quantification and confirmation analyses for all three analytes.

II. Recovery Findings

ECM (MRID 50539904): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of etoxazole and its metabolites R-8 and R-13 in one sediment matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; p. 25; Table 1, p. 33). Etoxazole and its metabolites R-8 and R-13 were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable, except for the LOQ analysis of R-8. The clay loam sediment (Lab Code #170110002-018 0-2”; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; pp. 14-15; Appendix A, p. 41). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota.

ILV (MRID 50539906): Mean recoveries and RSDs were within guideline requirements for analysis of etoxazole and its metabolites R-8 and R-13 in one sediment matrix at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10×LOQ; p. 18; Tables 7-12, pp. 33-38). Etoxazole and metabolites R-8 and R-13 were identified using two ion transitions; performance data (recovery results) from primary and confirmatory analyses were comparable. The clay loam sediment (Lab Code #170110002-018 0-2”; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; p. 14; Appendix A, p. 94). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota. The sediment matrix was the same one which was used in the ECM. The method was validated for the surface sediment matrix in the first trial with insignificant modifications to the final dilution ratio and LC/MS injection volume (pp. 16, 19, 24; Table 14, p. 40). The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM.

Table 2. Initial Validation Method Recoveries for Etoxazole and Its Metabolites R-8 and R-13 in Sediment[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sediment					
	Quantitation ion					
Etoxazole	0.01 (LOQ)	5	97-105	100	3.1	3.1
	0.10	5	89-103	98	5.7	5.9
R-8	0.01 (LOQ)	5	80-86	83	2.5	3.0
	0.10	5	68-85	78	6.5	8.3
R-13	0.01 (LOQ)	5	88-101	94	5.2	5.5
	0.10	5	85-99	91	5.0	5.5
	Confirmatory ion					
Etoxazole	0.01 (LOQ)	5	92-106	99	6.0	6.1
	0.10	5	91-102	97	4.2	4.4
R-8	0.01 (LOQ)	5	82-120	100	13.7	13.7
	0.10	5	70-91	80	9.3	11.6
R-13	0.01 (LOQ)	5	89-100	96	4.4	4.5
	0.10	5	83-101	93	6.4	6.9

Data (uncorrected recovery results, Appendices D-E, pp. 68, 78-83) were obtained from p. 25; Table 1, p. 33 of MRID 50539904.

1 The clay loam sediment (Lab Code #170110002-018 0-2”; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; pp. 14-15; Appendix A, p. 41). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 360.203→141.0 (Q) and *m/z* 360.203→177.1 (C) for etoxazole, *m/z* 238.087→165.1 (Q) and *m/z* 238.087→147.1 (C) for R-8, and *m/z* 358.145→140.8 (Q) and *m/z* 358.145→274.0 (C) for R-13.

Table 3. Independent Validation Method Recoveries for Etoxazole and Its Metabolites R-8 and R-13 in Sediment[1,2]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Clay Loam Sediment					
	Quantitation ion					
Etoxazole	0.01 (LOQ)	7	73-89	82	6.1	7.5
	0.10	7	92-119	107	8.5	8.0
R-8	0.01 (LOQ)	7	68-82	72	4.6	6.3
	0.10	7	64-80	71	5.3	7.4
R-13	0.01 (LOQ)	7	79-93	88	5.6	6.4
	0.10	7	81-104	94	8.1	8.6
	Confirmatory ion					
Etoxazole	0.01 (LOQ)	7	76-91	83	5.4	6.5
	0.10	7	91-119	105	9.8	9.3
R-8	0.01 (LOQ)	7	61-85	73	7.2	9.9
	0.10	7	67-76	71	3.0	4.3
R-13	0.01 (LOQ)	7	80-96	89	6.0	6.8
	0.10	7	89-105	96	6.6	6.9

Data (uncorrected recovery results, Figure 30, p. 92) were obtained from p. 17; Tables 1-6, pp. 27-32 of MRID 50539906.

[1] The clay loam sediment (Lab Code #170110002-018 0-2"; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; p. 14; Appendix A, p. 94). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota. The sediment matrix was the same one which was used in the ECM.

[2] Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 360.203→141.0 (Q) and *m/z* 360.203→177.1 (C) for etoxazole, *m/z* 238.087→165.1 (Q) and *m/z* 238.087→147.1 (C) for R-8, and *m/z* 358.145→140.8 (Q) and *m/z* 358.145→274.0 (C) for R-13.

III. Method Characteristics

The LOQ for etoxazole and its metabolites R-8 and R-13 in sediment was 0.01 mg/kg in the ECM and ILV (pp. 26-27, 29 of MRID 50539904; pp. 20-22 of MRID 50539906). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. In the ECM and ILV, the LOD for sediment was set at 0.005 mg/kg (50% of the LOQ) for all three analytes. In the ECM and ILV, the LOD was set as the lowest calibration standard with an acceptable signal-to-noise ratio (S:N, >3:1). In the ILV, the LOD was calculated as 0.002 mg/kg for the quantification and confirmation analyses for all three analytes using the data of the seven LOQ recovery samples. The LOD was calculated for each analyte using the following equation:

$\text{LOD} = (t_{0.99} \text{ x SD})$

Where, $t_{0.99}$ is the one-tailed t statistic for n = 5 (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The calculated LODs supported the method LOD. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations were reported to justify the LOD for the method in the ECM.

Table 4. Method Characteristics

Analyte[1]			**Etoxazole**	**R-8**	**R-13**
Limit of Quantitation (LOQ)	ECM		0.01 mg/kg		
	ILV				
Limit of Detection (LOD)	ECM	Method	0.005 mg/kg		
		Calculated	Not calculated		
	ILV	Method	0.005 mg/kg		
		Calculated	0.002 mg/kg		
Linearity (calibration curve r^2 and concentration range)[1]	ECM		r^2 = 0.9982 (Q) r^2 = 0.9986 (C)	r^2 = 0.9976 (Q) r^2 = 0.9986 (C)	r^2 = 0.9980 (Q) r^2 = 0.9990 (C)
			0.00005-0.005 ng (equivalent to 0.01-1.0 ng/mL on column)		
	ILV		r^2 = **0.9927** (Q) r^2 = 0.9987 (C)	r^2 = 0.9990 (Q)[2] r^2 = 0.9983 (C)[2]	r^2 = 0.9992 (Q) r^2 = 0.9997 (C)
			0.00025-0.025 ng	0.00025-0.015 ng (Q) 0.00025-0.025 ng (C)	0.00025-0.025 ng
Repeatable	ECM[3]		Yes at LOQ and 10×LOQ (characterized clay loam sediment matrix)		
	ILV[4,5]				
Reproducible			Yes at LOQ and 10×LOQ		
Specific	ECM		Yes, matrix interferences were <1% of the LOQ (based on peak area). More baseline noise was noted in the C ion.	**No**, no matrix interferences were quantified; however, elevated baseline and nearby contaminants [(RT 1.22-1.28) peak ht. > LOQ (RT 1.09) peak ht.] were noted.[6]	Yes, no matrix interferences were quantified.
	ILV		Yes, matrix interferences were <1% of the LOQ (based on peak area).	**No**, matrix interferences were *ca*. 10% (Q) and *ca*. 14% (C) of the LOQ (based on peak area); however, nearby contaminant [(RT 1.58-1.67) peak ht. > LOQ (RT 1.42) peak ht.] was noted.[6]	Yes, matrix interferences were 2-4% of the LOQ (based on peak area).

Data were obtained from pp. 26-27, 29 (LOQ/LOD); p. 25; Table 1, p. 33 (recovery data); Appendix E, pp. 71-76 (calibration coefficients); Appendix J, Figures 1-3, pp. 154-156 (calibration curves); Appendix J, Figures 7-16, pp. 178-187 (chromatograms) of MRID 50539904; pp. 20-22 (LOQ); p. 17; Tables 1-6, pp. 27-32 (recovery data); Figure 1, pp. 42-44 (calibration curves); Appendix D, pp. 120-125 (calibration coefficients); Figures 5-16, pp. 65-76 (chromatograms) of MRID 50539906; DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition. **Red values** indicate items not in compliance with the guidelines.

1 Correlation coefficients (r^2) values were reviewer-calculated from r values provided in the study report (Appendix E, pp. 71-76 of MRID 50539904; Appendix D, pp. 120-125 of MRID 50539906; DER Attachment 2). Solvent-based calibration standards were used (p. 18 of MRID 50539904; p. 20 of MRID 50539906). For the ILV correlation coefficients, the reviewer limited the value to four significant figures, even though seven significant figures were provided in the study report.

2 Quadratic equations were used for calibration.

3 In the ECM, the clay loam sediment (Lab Code #170110002-018 0-2"; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from

an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; pp. 14-15; Appendix A, p. 41 of MRID 50539904). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota.

4 In the ILV, the clay loam sediment (Lab Code #170110002-018 0-2”; sand/silt/clay, 40/20/40; pH 7.4 in soil:water (1:1); 1.5% organic matter – Walkey Black; 30.8 meq/100 g Cation Exchange Capacity) obtained from an unassociated terrestrial field dissipation, was used in the study (USDA soil texture classification; p. 14; Appendix A, p. 94 of MRID 50539906). The sediment sample was characterized by Agvise Laboratories, Northwood, North Dakota. The sediment matrix was the same one which was used in the ECM.

5 The ILV validated the method for the surface sediment matrix in the first trial with insignificant modifications to the final dilution ratio and LC/MS injection volume (pp. 16, 19, 24; Table 14, p. 40 of MRID 50539906). The analytical instrumentation and all other analytical parameters were the exact same as those in the ECM.

6 Based on Appendix J, Figures 11-13, pp. 182-184 of MRID 50539904; and Figures 9-12, pp. 69-72 of MRID 50539906.

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

IV. Method Deficiencies and Reviewer’s Comments

1. It could not be determined that ILV MRID 50539906 was conducted independently of ECM MRID 50539904 since both validations were conducted at the same facility (ADPEN Laboratories, Inc., Jacksonville, Florida) and insufficient evidence was provided to support the independence of the two laboratories (p. 1 of MRID 50539904; p. 1 of MRID 50539906). 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, ADPEN Laboratories, Inc., showed that the study director and validation performers (chemists and other technical staff) of each validation was different; however, some laboratory personnel were the same, including the Project Coordinator, Sample Custodian, and Laboratory Coordinator (pp. 1-6, 13 of MRID 50539904; pp. 1-6, 11 of MRID 50539906). The communication summary reported that the ILV was conducted independently of the Study Sponsor (Valent USA, LLC) and that “laboratory personnel performing the tests were not familiar with the method” (p. 24 of MRID 50539906). The communication between the staff of the initial and independent validations was not addressed. Additionally, the reported analytical equipment was the same in the ECM and ILV, although retention times differed slightly (pp. 22-23 of MRID 50539904; Table 14, p. 40 of MRID 50539906). Analytical instrument numbers were not reported in the ECM or ILV. Note that the ILV sediment matrix was the same one which was used in the ECM (pp. 14-15; Appendix A, p. 41 of MRID 50539904; p. 14; Appendix A, p. 94 of MRID 50539906). The registrant needs to provide additional information to confirm no interactions between staff and no sharing of equipment when both validations occur at the same address.

   The following significant typographical error was found in the ILV: the study author was reported as “Perez R” when the signature pages reported the study director as Steven Perez (pp. 1-6 of MRID 50539906). Additionally, the ILV was cited in the reference section of the ECM, and "Perez, S." was reported as the author (p. 31 of MRID 50539904). This reviewer concludes that the study director of the ECM and ILV were two different people and corrected the typographical error when reporting the citations of the DER.

2. The specificity of the method for R-8 was not supported by ILV and ECM representative chromatograms. Q LOQ chromatograms of metabolite R-8 showed one or more nearby significant contaminants: ILV [(RT 1.58-1.67) peak ht. > LOQ (RT 1.42) peak ht.] (Figures 9-12, pp. 69-72 of MRID 50539906); and ECM [(RT 1.22-1.28) peak ht. > LOQ (RT 1.09) peak ht.] (Appendix J, Figures 11-13, pp. 182-184 of MRID 50539904). The Certificate of Analysis reported a purity of 98.9% for R-8, so the contaminants did not originate from the test material (Appendix B, p. 45 of MRID 50539904; Appendix B, p. 98 of MRID 50539906). These contaminants were seen in the chromatograms of other analytes, but the peak heights of the contaminants were *ca*. 1000 cps compared to 20000-75000 cps in the R-8 chromatograms (Appendix J, Figures 7-16, pp. 178-187 of MRID 50539904; Figures 5-16, pp. 65-76 of MRID 50539906). Also, there was no apparent loss of R-8 since recoveries were *ca*. 100%. These contaminants should be identified and/or the method should be modified to eliminate these contaminants for R-8 identification and quantification. This reviewer also notes that baseline interferences were most significant in the ECM R-8 confirmatory chromatograms (Appendix J, Figures 11-13, pp. 182-184 of MRID 50539904).

3. ILV linearity was slightly below the satisfactory range for the quantitation ion analysis of etoxazole in surface sediment, $r^2 = 0.9927$ (Appendix D, p. 120 of MRID 50539906; DER Attachment 2). Linearity is satisfactory when $r^2 \geq 0.995$.

4. It could not be determined that the ILV were provided with the most difficult matrix with which to validate the method since only one sediment matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Additionally, since no terrestrial field dissipation studies were submitted, it not be determined if the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/sediment matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies.

5. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 26-27, 29 of MRID 50539904; pp. 20-22 of MRID 50539906). In the ECM and ILV, the LOQ was defined as the lowest fortification level successfully tested. In the ECM and ILV, the LOD for sediment was set at 0.005 mg/kg (50% of the LOQ) for all three analytes. In the ECM and ILV, the LOD was set as the lowest calibration standard with an acceptable signal-to-noise ratio (S:N, >3:1). In the ILV, the LOD was calculated as 0.002 mg/kg for the quantification and confirmation analyses for all three analytes using the data of the seven LOQ recovery

samples. The LOD was calculated for each analyte using the following equation: LOD = ($t_{0.99}$ x SD), where, $t_{0.99}$ is the one-tailed t statistic for n = 5 (3.747) and SD is the standard deviation of the analyte recovery measurements at the target LOQ. The calculated LODs supported the method LOD. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations were reported to justify the LOD for the method in the ECM. Detection limits should not be based on arbitrary values.

6. The ILV and ECM reported that no significant matrix suppression or enhancement was observed (p. 24; Table 4, pp. 37-38 of MRID 50539904; p. 20 of MRID 50539906).

7. In the ECM, the stability of the sample extracts was determined to be acceptable after 7 days of storage in a refrigerator (p. 28; Table 5, p. 39 of MRID 50539904).

8. The time required to complete the method for a validation set (13 samples) was reported as *ca*. 4-6 hours in the ILV, not including the instrument time and the calculation of results (p. 20 of MRID 50539906).

V. References

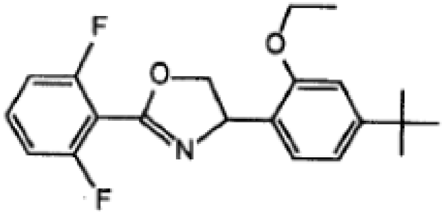
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

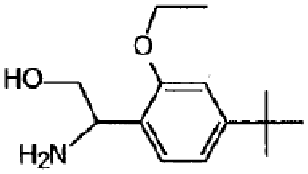
Etoxazole

IUPAC Name:	Not reported
CAS Name:	2-(2,6-difluorophenyl)-4-[4-(1,1-dimethylethyl)-2-ethoxyphenyl]-4,5-dihydrooxazole
CAS Number:	153233-91-1
SMILES String:	Not found



R-8

IUPAC Name:	Not reported
CAS Name:	2-amino-2-(4-tert-butyl-2-ethoxyphenyl)ethanol
CAS Number:	153281-81-3
SMILES String:	Not found



R-13

IUPAC Name:	Not reported
CAS Name:	4-(4-tert-butyl-2-ethoxyphenyl)-2-(2,6-difluorophenyl)oxazol
CAS Number:	Not reported
SMILES String:	Not found