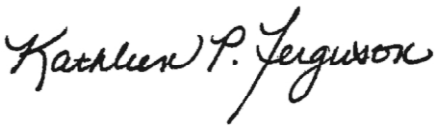
Test Material:	XDE-848 Benzyl Ester
MRID:	49677722
Title:	Aquatic Dissipation of XDE-848 Benzyl Ester (SX-1552) in Pond Systems
MRID:	49677803
Title:	Independent Laboratory Validation of EPL Bio Analytical Services Method 477G696A-1 for the Determination of XDE-848 Benzyl Ester (SX-1552) and Five Metabolites (1552-Acid, 1552-OHBE, 1552-OHA, 1552-DBE and 1552-DA) in Water
EPA PC Code:	030093
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 2/26/16

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 2/26/16

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 2/26/16

Analytical method for XDE-848 Benzyl Ester and its transformation products, 1552-Acid, 1552-OHBE, 1552-OHA, 1552-DBE and 1552-DA, in Water

Reports: ECM: EPA MRID No.: 49677722 (Appendix A and Appendix B, pp. 123 and 448). Lester, L. 2015. Aquatic Dissipation of SX-1552 in Pond Systems. Laboratory Study ID: 477G696. Report prepared by EPL BAS, Bio Analytical Services (EPL BAS), Niantic, Illinois, and sponsored and submitted by SePRO Corporation, Carmel, Indiana; 325 pages (MRID 49677722, 710 pages). Final report issued May 27, 2015.

ILV: EPA MRID No. 49677803. Austin, R. 2015. Independent Laboratory Validation of EPL Bio Analytical Services Method 477G696A-1 for the Determination of XDE-848 Benzyl Ester (SX-1552) and Five Metabolites (1552-Acid, 1552-OHBE, 1552-OHA, 1552-DBE and 1552-DA) in Water. Battelle Study No.: YR/15/010. Report prepared by Battelle UK Ltd., Essex, United Kingdom, and sponsored and submitted by SePRO Corporation, Carmel, Indiana; 229 pages. Final report issued August 5, 2015.

Document No.: MRIDs 49677722 & 49677803

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP; Appendix A, p. 124 of MRID 49677722). Signed and dated GLP and Quality Assurance statements were provided (Appendix A, pp. 124-125). The No Data Confidentiality and Authenticity statements were not included.

ILV: The study was conducted in accordance with USEPA (1989) and OECD GLP standards (1998), as well as the UK Department of Health (Directive 2004/9/EC; p. 3; Appendix 4, p. 229 of MRID 49677803). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-4; Appendix 4, p. 229). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4).

Classification: This analytical method is considered supplemental. In the ECM, the reproducibility of analyses of SX-1552, 1552-DA and 1552-Acid did not meet guidelines at fortifications of LOQ or 10×LOQ in one or both pond waters. In the ECM, representative chromatograms did not support the specificity of the method for all analytes in both matrices. In the ILV, representative chromatograms were not provided for all fortifications. Sample recoveries were corrected in the ECM. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. It was noted that the same laboratory (Agvise Laboratories), provided the water characterization for both, the ECM and ILV.

PC Code: 030093

Reviewer: José Meléndez, U.S. EPA **Date:** November 14, 2016

Signature: JOSE MELENDEZ Digitally signed by JOSE MELENDEZ DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=JOSE MELENDEZ, dnQualifier=0000008590 Date: 2016.11.14 08:33:55 -05'00'

All cited page numbers for MRID 49677722 refer to those written in the bottom right-hand corner of the document pages.

Executive Summary

The analytical method, EPL Bio Analytical Services Method 477G696A-1, is designed for the quantitative determination of XDE-848 (SX-1552) in water matrices at the LOQ of 0.02 µg/L using LC/MS/MS and the five metabolites 1552-Acid, 1552-OHBE, 1552-OHA, 1552-DBE and 1552-DA in water matrices at the LOQ of 0.05 µg/L using LC/MS/MS. The LOQ is equal to the lowest toxicological level of concern in water for XDE-848 (SX-1552)[1]; the LOQs are less than the lowest toxicological level of concern in water for the five metabolites[2]. The original ECM, EPL Bio Analytical Services Method 477G696A-1, was not submitted for review; however, the submitted ECM was performed using EPL Bio Analytical Services Method 477G696A-1. Characterized pond waters from two sites were used in the ECM; the Florida (FL) and North Carolina (NC) ponds were sourced by a well and a source reservoir pond, respectively. The ECM was validated by the ILV in the first trial for all six analytes with insignificant modifications to the analytical parameters using characterized drinking, surface and ground water matrices. In the ILV, representative chromatograms were not provided for the reagent blank and fortifications at the LOD or 10×LOQ, only calibrants, controls and LOQ. In the ECM, the reproducibility of analyses of SX-1552, 1552-DA and 1552-Acid did not meet guidelines at fortifications of LOQ or 10×LOQ in one or both pond waters; recovery results and representative chromatograms were only provided for the quantitation ion. Sample recoveries were corrected in the ECM. Additionally, due to significant interference in the controls at or near the retention times of the analytes, representative ECM chromatograms did not support the specificity of the method for SX-1552 in FL and NC pond waters and for 1552-OHA, 1552-DBE and 1552-Acid in NC pond water.

[1] The lowest toxicological level of concern is IC_{50} = 0.0162 µg a.i./L ~ 0.02 µg/L, for XDE-848 benzyl ester, for Eurasian Watermilfoil (MRID 49677805).

[2] The lowest toxicological level of concern for the degradates appears to be an IC_{50} = 0.497 µg a.i./L ~ 0.5 µg/L, for XDE-848 acid, for Eurasian Watermilfoil (MRID 49677806).

Table 1. Analytical Method Summary[1,2,3]

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						
Florpyrauxifen-benzyl	49677722, Appendix A	49677803		Water	27/05/2015[4]	SePRO Corporation	LC/MS/MS	0.02 µg/L
1552-OHA								0.05 µg/L
1552-DBE								
1552-DA								
1552-OHBE								
1552-Acid								

1 Florpyrauxifen-benzyl = [XDE-848, XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-OHA = [XDE-848 hydroxy acid; TSN305649; X11966341; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-DBE = [Dechlorinated XDE-848 benzyl ester; TSN305649; X12131932; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-DA = [Dechlorinated XDE-848 acid; TSN304479; X12393505; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-OHBE = [XDE-848 hydroxy benzyl ester; TSN305650; X12300837; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and 1552-Acid = [XDE-848 acid; TSN301691; X11438848; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

2 For the ECM, Florida pond water (FL; "very hard" USGS classification; redox potential -124.2 mV at 18°C pH 8.5, bicarbonates 2.53 meq/L) and North Carolina pond water (NC; "soft" USGS classification; redox potential 154.5 mV at 18°C pH 8.5, bicarbonates 0.6 meq/L) were used (pp. 24-25, 28, 36; Tables 7-8, pp. 58-59 of MRID 49677722). The Florida pond was sourced by a well; the North Carolina pond was sourced by a source reservoir pond.

3 For the ILV, surface water (15/003 Surface H_2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (12/045 BATTELLE UK; pH 8.2, dissolved organic carbon 1.0 ppm) were used (p. 20; Appendix 3, pp. 225-228 of MRID 49677803).

4 Date based on EPL Bio Analytical Services Method 477G696 since the original report of EPL Bio Analytical Services Method 477G696A-1 was not provided (See Reviewer's Comment #1).

I. Principle of the Method

During the entire procedure, only glass laboratory equipment was used (Appendix A, pp. 154-155 of MRID 49677722). Samples (5 mL) of water in 15-mL glass culture tubes were mixed with 5 µL of formic acid, 225 L methanol, 25 L of the mixed internal standard (100 ng/mL) and fortified, as necessary. After mixing thoroughly via vortex, an aliquot of the sample was transferred via glass Pasteur pipet to a glass LC/MS/MS vial for analysis.

The method detailed an additional "methanol rinse preparation" which was performed with the remainder of the remaining original water, but this portion of the method appeared to be an auxiliary method and was not performed by the ILV (see Reviewer Comment #8; Appendix A, p. 155 of MRID 49677722; Appendix 1, pp. 221-222 of MRID 49677803).

Samples were analyzed for XDE-848 (SX-1552) and its metabolites using an Agilent 1290 Infinity LC system coupled to an AB Sciex QTRAP 6500 LC/MS/MS (Appendix A, pp. 155-156 of MRID 49677722). The instrumental conditions consisted of a Phenomenex Kinetex PFP column (100 x 2.10 mm, 1.7-µm; column temperature, 35°C), a gradient mobile phase of (A) DI water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid [percent A:B (v:v) at 0.0 min. 90:10, 7.00-8.50 min. 0:100, 8.60-11.00 min. 90:10], MS/MS detection in positive electrospray mode MS (MRM; temperature, 650°C), and injection volume 15 µL. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 441.0 → 65.0 and *m/z* 441.0 → 91.0 for XDE-848 (SX-1552); *m/z* 334.8 → 253.9 and *m/z* 336.8 → 255.9 for 1552-OHA; *m/z* 404.9 → 65.0 and *m/z* 406.9 → 91.0 for 1552-DBE; *m/z* 314.8 → 234.0 and *m/z* 314.8 → 124.0 for 1552-DA; *m/z* 424.9 → 91.0 and *m/z* 426.8 → 91.0 for 1552-OHBE; and *m/z* 348.8 → 267.9 and *m/z* 348.8 → 224.9 for 1552-Acid. Retention times were observed at *ca*. 7.0, 4.6-4.65, 6.8-7.0, 4.85-5.0, 6.6, and 5.5-5.7 min. for XDE-848 (SX-1552), 1552-OHA, 1552-DBE, 1552-DA, 1552-OHBE, and 1552-Acid, respectively (retention times were reviewer-assigned based observed and expected; Appendix A, Figures 103-108, pp. 369-371).

In the ILV, the sample processing of the ECM was performed exactly as written (p. 25; Appendix 1, pp. 217, 221-222 of MRID 49677803). Samples were analyzed for XDE-848 (SX-1552) and its metabolites using an Agilent 1290 Binary Pump LC system coupled to an AB Sciex QTRAP 6500 LC/MS/MS. All instrumental parameters were the same, except for the following: MS/MS detection in positive Turbo Ion Spray mode MS (MRM; temperature, 650°C), and injection volume 40 µL. Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 441.1 → 65.1 and *m/z* 441.1 → 91.0 for XDE-848 (SX-1552); *m/z* 334.9 → 254.0 and *m/z* 336.9 → 256.0 for 1552-OHA; *m/z* 404.8 → 65.1 and *m/z* 407.0 → 91.0 for 1552-DBE; *m/z* 315.0 → 234.0 and *m/z* 315.0 → 124.0 for 1552-DA; *m/z* 425.0 → 91.0 and *m/z* 427.0 → 91.0 for 1552-OHBE; and *m/z* 349.0 → 268.0 and *m/z* 349.0 → 225.0 for 1552-Acid (a majority of the ions differed from those reported for the ECM by +0.0-0.2 *m/z*). Retention times were observed at *ca*. 7.36, 4.95, 7.28, 5.33, 6.95, and 5.95 min. for XDE-848 (SX-1552), 1552-OHA, 1552-DBE, 1552-DA, 1552-OHBE, and 1552-Acid, respectively (retention times were reviewer-assigned based observed; Figures 54-125, pp. 144-215). The ILV study author noted that the increase in the injection volume was due to poor

sensitivity at the lower injection volume (p. 25). None of the minor ILV modifications to the instrumental parameters had an effect on the outcome of the study.

LOQ/LOD

The LOQ and LOD in the ECM and ILV were 0.02 µg/L and 0.006 µg/L, respectively, for XDE-848 (SX-1552) and 0.05 µg/L and 0.015 µg/L, respectively, for the five metabolites of XDE-848 (SX-1552; p. 34; Appendix A, pp. 142, 164 of MRID 49677722; pp. 19, 25 of MRID 49677803).

II. Recovery Findings

ECM [49677722 (Appendix A, pp. 123-447)]: Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of XDE-848 (SX-1552) in the two pond water matrices at the fortification level of 0.2 µg/L (10×LOQ); however, the fortifications at the LOQ (0.02 µg/L) did not meet guidelines since RSDs were 22.241% and 54.282% for the Florida and North Carolina sites, respectively (Appendix A, pp. 164-165; Appendix A, Tables 41-52, pp. 227-250; DER Attachment 2). Mean recoveries and RSDs were within guidelines for analysis of the five metabolites, 1552-OHA, 1552-DBE, 1552-DA, 1552-OHBE, and 1552-Acid, in the two pond water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ), except for analyses for the Florida site of 1552-DA at 10×LOQ (RSD was slightly above the margin, at 20.057%) and 1552-Acid at the LOQ (RSD 30.898%). For all analytes, two ion transitions were monitored using LC/MS/MS; however, performance data (recovery results) were only evaluated and reported for the quantitative ion (see Reviewer's Comment #7). The recovery statistics for all analyses which did not meet guideline requirements, except for 1552-DA, were reviewer-calculated based on all reported data (Appendix A, Tables 41-42, pp. 227, 229, Table 47, p. 239; DER Attachment 2). One of the recovery values for each set was not accepted by the study author; *no justification or calculation was provided for the omission*. The study author calculated recovery statistics for n = 22 (FL) or 21 (NC). The reviewer calculated the recovery of the unaccepted values based on the amount of analyte found without correction (recovery calculations included corrections for residues found in controls). The reported mean, s.d. and RSD were reviewer-calculated based on n = 23 (FL) or 22 (NC). The ECM calculations allowed for recovery data to be corrected for residues found in the control samples (Appendix A, pp. 160-161). For the Florida site, minor residues (<15% of the LOQ) were quantified for five of the six analytes in the representative chromatograms of the control samples (no residues in the other analyte; Appendix A, Figures 109-114, pp. 372-374). For the North Carolina site, residues were quantified for all six analytes in the representative chromatograms of the control samples (Appendix A, Figures 163-168, pp. 399-401). Significant residues (*ca*. 35-95% of the LOQ) were observed in control chromatograms for SX-1552, 1552-Acid and 1552-DBE; minor residues (<5% of the LOQ) were observed in control chromatograms for 1552-OHBE, 1552-OHA and 1552-DA. Both water matrices were pond waters, which were well characterized by Agvise Laboratories, Northwood, North Dakota (pp. 24-25, 28, 36; Tables 7-8, pp. 58-59). The Florida pond was located in Seminole County, north or the town of Oviedo, and sourced by a well. The North Carolina pond was located in Nash County, northwest of the town of Whitakers; the pond was a constructed pond which was sourced by a source reservoir pond. Neither pond had a history of prior pesticide use for 3 years. The water samples which

were used for the method validation study were untreated and taken from either the ponds (prior to field study initiation) or the pond sources (after field study initiation). The Florida pond water (FL) was reported as "very hard" according to USGS classification system (redox potential -124.2 mV at 18°C pH 8.5, bicarbonates 2.53 meq/L). The North Carolina pond water (NC) was reported as "soft" according to USGS classification system (redox potential 154.5 mV at 18°C pH 8.5, bicarbonates 0.6 meq/L).

ILV (MRID 49677803): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of XDE-848 (SX-1552) in drinking, ground and surface water matrices at fortification levels of 0.02 µg/L (LOQ) and 0.2 µg/L (10×LOQ) and the five metabolites, 1552-OHA, 1552-DBE, 1552-DA, 1552-OHBE, and 1552-Acid, in drinking, ground and surface water matrices at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ; uncorrected recovery results; Tables 50-61, pp. 79-84; Figure 47, p. 137). For all analytes, two ion transitions were monitored using LC/MS/MS; performance data (recovery results) of the quantitative and confirmatory results were comparable. Recoveries from samples fortified at 0.006/0.015 µg/L (LOD) ranged (ions/matrices combined) from 16-89% for XDE-848 (SX-1552), 83-113% for 1552-OHA, 89-107% for 1552-DBE, 52-107% for 1552-DA, 58-106% for 1552-OHBE and 82-118% for 1552-Acid (n = 1 for each matrix/analyte; Tables 14-49, pp. 43-78; DER Attachment 2). The water matrices were well characterized by Agvise Laboratories, Northwood, North Dakota[3] (sources not further specified; p. 20; Appendix 3, pp. 225-228). Surface water (15/003 Surface H_2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (12/045 BATTELLE UK; pH 8.2, dissolved organic carbon 1.0 ppm) were used in the study. The method was validated in the first trial for all analytes in drinking, surface and ground water matrices with insignificant modifications to the analytical parameters (p. 25).

[3] The same laboratory provided the water characterization for both, the ECM and ILV.

Table 2. Initial Validation Method Recoveries for Florpyrauxifen-benzyl (XDE-848; XDE-848 BE; SX-1552) and Its Five Metabolites, 1552-OHA, 1552-DBE, 1552-DA, 1552-OHBE, and 1552-Acid, in Surface Water from Two Sites[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Florida (FL) Pond Water						
	Quantitation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.02 (LOQ)	23[4]	35.000-142.000	101.543	22.584	**22.241**
	0.2	23	58.750-133.200	106.052	14.361	13.542
1552-OHA	0.05 (LOQ)	23	76.122-116.735	98.554	11.662	11.833
	0.5	23	53.776-125.633	98.350	15.818	16.083
1552-DBE	0.05 (LOQ)	23	85.361-160.825	112.326	13.903	12.377
	0.5	23	79.258-142.392	107.476	15.728	14.634
1552-DA	0.05 (LOQ)	23	68.367-126.122	95.954	14.436	15.045
	0.5	23	63.653-163.224	103.916	20.842	20.057
1552-OHBE	0.05 (LOQ)	23	78.800-137.400	102.470	15.660	15.283
	0.5	23	66.500-139.280	103.281	20.154	19.514
1552-Acid	0.05 (LOQ)	23[4]	73.800-243.400	105.739	32.672	**30.898**
	0.5	23	62.360-124.320	101.285	13.448	13.277
North Carolina (NC) Pond Water						
	Quantitation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.02 (LOQ)	22[4]	65.000-365.000	108.955	59.142	**54.282**
	0.2	22	87.400-123.750	103.448	9.617	9.297
1552-OHA	0.05 (LOQ)	22	76.735-117.755	94.388	10.305	10.918
	0.5	22	84.143-104.857	95.164	6.263	6.581
1552-DBE	0.05 (LOQ)	22	68.866-117.938	99.410	12.173	12.245
	0.5	22	69.196-118.619	96.905	10.686	11.027

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
1552-DA	0.05 (LOQ)	22	76.531-106.531	90.705	9.525	10.501
	0.5	22	84.449-112.837	96.247	7.373	7.661
1552-OHBE	0.05 (LOQ)	22	83.400-118.600	98.164	10.355	10.548
	0.5	22	87.420-121.720	102.542	8.429	8.220
1552-Acid	0.05 (LOQ)	22	83.400-114.600	95.573	9.114	9.536
	0.5	22	82.800-106.220	95.643	7.697	8.048

Data (recovery results corrected for residues found in the controls; Appendix A, pp. 160-161) were obtained from Appendix A, pp. 164-165; Appendix A, Tables 41-52, pp. 227-250 of MRID 49677722 and DER Attachment 2. Only results from the quantitation ion were reported (see Reviewer's Comment #7).

1 Florpyrauxifen-benzyl = [XDE-848; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-OHA = [XDE-848 hydroxy acid; TSN305649; X11966341; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-DBE = [Dechlorinated XDE-848 benzyl ester; TSN305649; X12131932; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-DA = [Dechlorinated XDE-848 acid; TSN304479; X12393505; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-OHBE = [XDE-848 hydroxy benzyl ester; TSN305650; X12300837; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and 1552-Acid = [XDE-848 acid; TSN301691; X11438848; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

2 Both water matrices were well characterized pond waters (pp. 24-25, 28, 36; Tables 7-8, pp. 58-59). The Florida pond was located in Seminole County, north or the town of Oviedo, and sourced by a well. The North Carolina pond was located in Nash County, northwest of the town of Whitakers; the pond was a constructed pond which was sourced by a source reservoir pond. Neither pond had a history of prior pesticide use for 3 years. The water samples which were used for the method validation study were untreated and taken from either the ponds (prior to field study initiation) or the pond sources (after field study initiation). The Florida pond water (FL) was reported as "very hard" according to USGS classification system (redox potential -124.2 mV at 18°C pH 8.5, bicarbonates 2.53 meq/L). The North Carolina pond water (NC) was reported as "soft" according to USGS classification system (redox potential 154.5 mV at 18°C pH 8.5, bicarbonates 0.6 meq/L).

3 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 441.0 → 65.0 and *m/z* 441.0 → 91.0 for XDE-848 (SX-1552); *m/z* 334.8 → 253.9 and *m/z* 336.8 → 255.9 for 1552-OHA; *m/z* 404.9 → 65.0 and *m/z* 406.9 → 91.0 for 1552-DBE; *m/z* 314.8 → 234.0 and *m/z* 314.8 → 124.0 for 1552-DA; *m/z* 424.9 → 91.0 and *m/z* 426.8 → 91.0 for 1552-OHBE; and *m/z* 348.8 → 267.9 and *m/z* 348.8 → 224.9 for 1552-Acid. However, only the quantification ion was evaluated for residue recovery.

4 One of the recovery values was not accepted by the study author; no justification or calculation was provided for the omission. The reviewer calculated the recovery based on the amount of analyte found without correction (recovery calculations included corrections for residues found in controls). The reported mean, s.d. and RSD were reviewer-calculated (see DER Attachment 2).

Table 3. Independent Validation Method Recoveries for Florpyrauxifen-benzyl (XDE-848; XDE-848 BE; SX-1552) and Its Five Metabolites, 1552-OHA, 1552-DBE, 1552-DA, 1552-OHBE, and 1552-Acid, in Drinking, Ground and Surface Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
	Quantitation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.006 (LOD)	**1**	**16**	--	--	--
	0.02 (LOQ)	5	92-106	100	7.2	7.2
	0.2	5	97-109	103	4.5	4.4
1552-OHA	0.015 (LOD)	1	97	--	--	--
	0.05 (LOQ)	5	89-110	99	7.8	7.9
	0.5	5	94-101	97	2.7	2.8
1552-DBE	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	104-119	112	6.7	6.0
	0.5	5	95-112	106	6.6	6.2
1552-DA	0.015 (LOD)	**1**	87	--	--	--
	0.05 (LOQ)	5	93-105	98	4.6	4.7
	0.5	5	91-97	94	2.6	2.8
1552-OHBE	0.015 (LOD)	**1**	85	--	--	--
	0.05 (LOQ)	5	98-106	102	3.0	3.0
	0.5	5	97-104	102	2.8	2.7
1552-Acid	0.015 (LOD)	**1**	99	--	--	--
	0.05 (LOQ)	5	104-113	109	3.3	3.0
	0.5	5	91-106	100	5.7	5.7
	Confirmation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.006 (LOD)	**1**	81	--	--	--
	0.02 (LOQ)	5	90-105	97	6.1	6.3
	0.2	5	98-105	102	2.9	2.9
1552-OHA	0.015 (LOD)	1	112	--	--	--
	0.05 (LOQ)	5	93-112	103	8.6	8.4
	0.5	5	92-101	96	3.6	3.8
1552-DBE	0.015 (LOD)	**1**	103	--	--	--
	0.05 (LOQ)	5	98-110	104	4.4	4.2
	0.5	5	93-107	104	5.9	5.7
1552-DA	0.015 (LOD)	**1**	97	--	--	--
	0.05 (LOQ)	5	88-99	95	4.2	4.4
	0.5	5	90-97	94	3.4	3.6
1552-OHBE	0.015 (LOD)	**1**	74	--	--	--
	0.05 (LOQ)	5	93-107	100	5.6	5.6
	0.5	5	90-100	97	4.1	4.3
1552-Acid	0.015 (LOD)	**1**	100	--	--	--
	0.05 (LOQ)	5	92-112	105	7.9	7.5
	0.5	5	88-105	99	6.3	6.4

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Ground Water						
	Quantitation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.006 (LOD)	**1**	78	--	--	--
	0.02 (LOQ)	5	78-101	91	10.2	11.2
	0.2	5	103-110	106	2.7	2.6
1552-OHA	0.015 (LOD)	**1**	113	--	--	--
	0.05 (LOQ)	5	93-108	99	5.7	5.7
	0.5	5	94-101	98	2.9	3.0
1552-DBE	0.015 (LOD)	**1**	89	--	--	--
	0.05 (LOQ)	5	91-106	99	5.4	5.4
	0.5	5	94-104	99	4.3	4.4
1552-DA	0.015 (LOD)	**1**	76	--	--	--
	0.05 (LOQ)	5	82-95	88	4.7	5.3
	0.5	5	92-99	96	2.9	3.0
1552-OHBE	0.015 (LOD)	**1**	72	--	--	--
	0.05 (LOQ)	5	83-89	86	2.6	3.0
	0.5	5	95-100	98	2.3	2.4
1552-Acid	0.015 (LOD)	**1**	82	--	--	--
	0.05 (LOQ)	5	93-104	97	4.3	4.4
	0.5	5	99-105	103	2.5	2.4
	Confirmation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.006 (LOD)	**1**	**64**	--	--	--
	0.02 (LOQ)	5	88-97	94	3.8	4.1
	0.2	5	103-105	104	1.1	1.1
1552-OHA	0.015 (LOD)	**1**	85	--	--	--
	0.05 (LOQ)	5	100-106	103	2.8	2.7
	0.5	5	96-102	100	2.4	2.4
1552-DBE	0.015 (LOD)	**1**	107	--	--	--
	0.05 (LOQ)	5	92-100	96	3.5	3.6
	0.5	5	93-103	98	4.6	4.7
1552-DA	0.015 (LOD)	**1**	**52**	--	--	--
	0.05 (LOQ)	5	79-89	83	4.7	5.7
	0.5	5	91-97	95	2.7	2.8
1552-OHBE	0.015 (LOD)	**1**	**58**	--	--	--
	0.05 (LOQ)	5	83-90	87	2.5	2.9
	0.5	5	93-99	96	2.7	2.8
1552-Acid	0.015 (LOD)	**1**	102	--	--	--
	0.05 (LOQ)	5	94-105	99	4.3	4.4
	0.5	5	100-104	102	1.5	1.5
Drinking Water						
	Quantitation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.006 (LOD)	**1**	**48**	--	--	--
	0.02 (LOQ)	5	90-116	106	10.1	9.5
	0.2	5	102-113	108	4.4	4.1

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
1552-OHA	0.015 (LOD)	**1**	83	--	--	--
	0.05 (LOQ)	5	99-109	103	4.0	3.9
	0.5	5	98-102	100	1.8	1.8
1552-DBE	0.015 (LOD)	**1**	101	--	--	--
	0.05 (LOQ)	5	91-101	96	3.7	3.9
	0.5	5	92-101	95	3.4	3.6
1552-DA	0.015 (LOD)	**1**	107	--	--	--
	0.05 (LOQ)	5	102-112	108	3.8	3.5
	0.5	5	98-106	101	3.2	3.2
1552-OHBE	0.015 (LOD)	**1**	106	--	--	--
	0.05 (LOQ)	5	98-109	104	4.3	4.1
	0.5	5	100-108	104	3.0	2.9
1552-Acid	0.015 (LOD)	**1**	98	--	--	--
	0.05 (LOQ)	5	111-113	112	0.9	0.8
	0.5	5	102-115	109	5.2	4.8
	Confirmation ion transition					
XDE-848 (XDE-848 BE; SX-1552)	0.006 (LOD)	**1**	89	--	--	--
	0.02 (LOQ)	5	103-120	113	7.5	6.6
	0.2	5	105-109	107	1.8	1.7
1552-OHA	0.015 (LOD)	**1**	101	--	--	--
	0.05 (LOQ)	5	86-109	102	9.6	9.4
	0.5	5	95-104	99	3.3	3.3
1552-DBE	0.015 (LOD)	**1**	93	--	--	--
	0.05 (LOQ)	5	88-102	94	5.1	5.5
	0.5	5	89-97	93	2.9	3.1
1552-DA	0.015 (LOD)	**1**	89	--	--	--
	0.05 (LOQ)	5	100-111	105	4.2	4.0
	0.5	5	94-100	98	2.5	2.5
1552-OHBE	0.015 (LOD)	**1**	93	--	--	--
	0.05 (LOQ)	5	94-109	103	5.8	5.6
	0.5	5	98-105	101	3.3	3.2
1552-Acid	0.015 (LOD)	**1**	118	--	--	--
	0.05 (LOQ)	5	110-119	114	3.5	3.1
	0.5	5	105-113	109	3.3	3.0

Data (uncorrected recovery results; Figure 47, p. 137) were obtained from Tables 14-49, pp. 43-78 (LOD results) and Tables 50-61, pp. 79-84 of MRID 49677803 and DER Attachment 2 (LOD calculations).

1 Florpyrauxifen-benzyl = [XDE-848; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-OHA = [XDE-848 hydroxy acid; TSN305649; X11966341; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-DBE = [Dechlorinated XDE-848 benzyl ester; TSN305649; X12131932; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-DA = [Dechlorinated XDE-848 acid; TSN304479; X12393505; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-OHBE = [XDE-848 hydroxy benzyl ester; TSN305650; X12300837; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and 1552-Acid = [XDE-848 acid; TSN301691; X11438848; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

2 The water matrices were well characterized (p. 20; Appendix 3, pp. 225-228). Surface water (15/003 Surface H2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (12/045 BATTELLE UK; pH 8.2, dissolved organic carbon 1.0 ppm) were used in the study.

3 Two parent-daughter ion transitions were monitored per analyte (quantification and confirmation, respectively): *m/z* 441.1 → 65.1 and *m/z* 441.1 → 91.0 for XDE-848 (SX-1552); *m/z* 334.9 → 254.0 and *m/z* 336.9 → 256.0 for 1552-OHA; *m/z* 404.8 → 65.1 and *m/z* 407.0 → 91.0 for 1552-DBE; *m/z* 315.0 → 234.0 and *m/z* 315.0 → 124.0 for 1552-DA; *m/z* 425.0 → 91.0 and *m/z* 427.0 → 91.0 for 1552-OHBE; and *m/z* 349.0 → 268.0 and *m/z* 349.0 → 225.0 for 1552-Acid (a majority of the ions differed from those reported for the ECM by +0.0-0.2 *m/z*).

III. Method Characteristics

In the ECM and ILV, the established LOQ and LOD were 0.02 µg/L and 0.006 µg/L, respectively, for florpyrauxifen-benzyl (XDE-848; SX-1552) and 0.05 µg/L and 0.015 µg/L, respectively, for the five metabolites of florpyrauxifen-benzyl (SX-1552; p. 34; Appendix A, pp. 142, 164 of MRID 49677722; pp. 19, 25 of MRID 49677803). In the ECM, no justification or calculation was provided to support the LOQ; the LOD was defined as the concentration which was *ca*. 30% of the LOQ. In the ILV, the LOQ and LOD were cited from the ECM without justification or calculation.

Table 4. Method Characteristics

			Florpyrauxifen-benzyl (SX-1552)	**1552-OHA**	**1552-DBE**	**1552-DA**	**1552-OHBE**	**1552-Acid**
Limit of Quantitation (LOQ)			0.02 µg/L	0.05 µg/L				
Limit of Detection (LOD)			0.006 µg/L	0.015 µg/L				
Linearity (Least squares calibration curve r and concentration range)	ECM[1]		r^2 = 0.9999 (Q)	r^2 = 1.0000 (Q)	r^2 = 0.9997 (Q)	r^2 = 1.0000 (Q)	r^2 = 0.9999 (Q)	r^2 = 0.9999 (Q)
			0.005-10 ng/mL	0.0049-49 ng/mL	0.0049-9.7 ng/mL	0.0049-49 ng/mL	0.005-10 ng/mL	0.005-50 ng/mL
	ILV[2]		r^2 = 0.9984-0.9998 (Q) r^2 = 0.9978-0.9996 (C)	r^2 = 0.9998-1.0000 (Q) r^2 = 0.9998 (C)	r^2 = 0.9980-0.9996 (Q) r^2 = 0.9980-0.9998 (C)	r^2 = 0.9996-1.0000 (Q) r^2 = 0.9994-0.9998 (C)	r^2 = 0.9998-1.0000 (Q & C)	r^2 = 0.9998-1.0000 (Q & C)
			0.005-50 ng/mL					
Repeatable	ECM[3,4]		Only the quantification ion was evaluated for recovery.					
			No at LOQ (RSDs **22.241%** FL and **54.282%** NC); Yes at 10×LOQ (n = 22-23).	Yes at LOQ and 10×LOQ (n = 22-23).		Yes at LOQ; **No** at 10×LOQ in one matrix (RSD 20.057% FL; n = 22-23).	Yes at LOQ and 10×LOQ (n = 22-23).	**No** at LOQ in one matrix (RSDs **30.898%** FL); Yes at 10×LOQ (n = 22-23).
	ILV[5]		Yes at LOQ and 10×LOQ (n = 5; quantification and confirmation ions).					
Reproducible			Yes at LOQ and 10×LOQ (n = 5).					
Specific	ECM	FL	Yes, only minor interferences (<15% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as <LOD. Only chromatograms of the quantification ion were provided.					
			Significant interference was observed due to a peak (>LOQ) which eluted near the retention time of the analyte and overlapped a portion of the analyte peak.	Peaks were well defined and distinct from the baseline at LOQ and 10×LOQ fortifications.				

			Florpyrauxifen-benzyl (SX-1552)	1552-OHA	1552-DBE	1552-DA	1552-OHBE	1552-Acid
		NC	Peaks were well defined and distinct from the baseline at LOQ and 10×LOQ fortifications.					
			Significant interferences (*ca.* 95% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as LOD<LOQ.	Yes, only minor interferences (<5% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as <LOD.	Significant interferences (*ca.* 35% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as LOD<LOQ.	Yes, only minor interferences (<5% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as <LOD.		Significant interferences (*ca.* 50% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as LOD<LOQ.
	ILV		Yes, only minor interferences (<10% of the LOQ) at the retention time of the analytes were observed in the matrix controls. Residues in the matrix controls were quantified as <LOD. No representative chromatograms were provided for the fortifications at the LOD or 10×LOQ, only calibrants, controls and LOQ. Minor baseline noise was observed which disrupted peak attenuation for a few of the analytes, including SX-1552.					

Data were obtained from p. 34; Appendix A, pp. 141-145, 164-165; Appendix A, Tables 41-52, pp. 227-250 (Recovery Results); Appendix A, Figures 97-102, pp. 363-368 (Linear Regressions); Appendix A, Figures 109-126, pp. 372-380 (FL Chromatograms); Appendix A, Figures 163-180, pp. 399-407 (NC Chromatograms) of MRID 49677722 ; pp. 19, 25; Tables 2-13, pp. 31-42 (Correlation Coefficients); Tables 14-49, pp. 43-78 (Control residues and LOD results); Tables 50-61, pp. 79-84 (Summary Recovery Results); Figures 11-46, pp. 101-136 (Linear regressions); Figures 54-125, pp. 144-215 (Chromatograms) of MRID 49677803 and DER Attachment 2. Q = Quantitative HPLC analysis; C = Confirmatory HPLC analysis. FL = Florida pond water matrix; NC = North Carolina pond water matrix.

* XDE-848 = [Florpyrauxifen-benzyl; XDE-848 BE; XDE-848 benzyl ester; TSN301734; X11959130; SX-1552; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-OHA = [XDE-848 hydroxy acid; TSN305649; X11966341; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-DBE = [Dechlorinated XDE-848 benzyl ester; TSN305649; X12131932; benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate]; 1552-DA = [Dechlorinated XDE-848 acid; TSN304479; X12393505; 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid]; 1552-OHBE = [XDE-848 hydroxy benzyl ester; TSN305650; X12300837; benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate]; and 1552-Acid = [XDE-848 acid; TSN301691; X11438848; 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid].

1 ECM standard curves were reviewer-calculated based on data provided in Appendix A, Figures 97-102, pp. 363-368 of MRID 49677722 (see DER Attachment 2). Some calibrant results were excluded by the study author due to high response. The reviewer also excluded these results for linear regression analysis in order to have an accurate linear coefficient for the linear regression used by the study author for recovery calculations.

2 ILV standard curves were weighted 1/x for all analytes. ILV r^2 values are reviewer-generated for the analytes from reported r values of 0.9992-1.0000 (Q) and 0.9989-1.0000 (C; analytes/ions combined; calculated from data in Tables 2-13, pp. 31-42 and Figures 11-46, pp. 101-136 of MRID 49677803; see DER Attachment 2).

3 For the ECM, Florida pond water (FL; "very hard" USGS classification; redox potential -124.2 mV at 18°C pH 8.5, bicarbonates 2.53 meq/L) and North Carolina pond water (NC; "soft" USGS classification; redox potential 154.5 mV at 18°C pH 8.5, bicarbonates 0.6 meq/L) were used (pp. 24-25, 28, 36; Tables 7-8, pp. 58-59 of MRID 49677722). The Florida pond was sourced by a well**; t**he North Carolina pond was sourced by a source reservoir pond.

4 The recovery statistics for all analyses which did not meet guideline requirements, except for 1552-DA, were reviewer-calculated based on all reported data (Appendix A, Tables 41-42, pp. 227, 229, Table 47, p. 239 of MRID 49677722; DER Attachment 2). One of the recovery values for each set was not accepted by the study author; no justification or calculation was provided for the omission. The study author calculated recovery statistics for n = 22 (FL) or 21 (NC). The reviewer calculated the recovery of the unaccepted values based on the amount of analyte found without correction (recovery calculations included corrections for residues found in controls). The reported mean, s.d. and RSD were reviewer-calculated based on n = 23 (FL) or 22 (NC).

5 For the ILV, surface water (15/003 Surface H2O Res; pH 8.0, dissolved organic carbon 3.1 ppm), ground water (12/044 Highland Spring; pH 8.2, dissolved organic carbon 0.1 ppm), and drinking water (12/045 BATTELLE UK; pH 8.2, dissolved organic carbon 1.0 ppm) were used (p. 20; Appendix 3, pp. 225-228 of MRID 49677803).

IV. Method Deficiencies and Reviewer's Comments

1. The submitted ECM which was contained in Appendix A of MRID 49677722 (pp. 123-447) was not the original ECM for the submitted ILV MRID 49677803 (pp. 19, 27 of MRID 49677803). ILV MRID 49677803 was performed to validate EPL Bio Analytical Services Method 477G696A-1 "Determination of XDE-848 Benzyl Ester (SX-1552) and Five Metabolites (1552-Acid, 1552-OHBE, 1552-OHA, 1552-DBE and 1552-DA) in Water". EPL Bio Analytical Services Method 477G696A-1 was authored by F. Claussen in 2014 (incomplete reference detail; Appendix A, p. 166 (Ref. 3) of MRID 49677722). However, the submitted ECM in Appendix A of MRID 49677722 was performed using EPL Bio Analytical Services Method 477G696A-1 and referenced this original ECM [p. 34; p. 48 (Ref. 11); Appendix A, pp. 152, 166 (Ref. 3) of MRID 49677722]. No deviations to the original ECM were reported in the submitted ECM; however, the original method document was not provided for review.

   Note from EPA reviewer: The reviewer noted that Appendix B, p. 448 of MRID 49677722, appears to be the original ECM; however, it was not fully reviewed by the primary reviewer. The following is a brief report of Appendix B: In this part of the study, the FL and NC waters were tested at LOD (n = 1), LOQ (n = 7 for all six chemicals), 10xLOQ (n = 2), and 50 µg/L (n = 2). Mean values and relative standard deviations were within guideline criteria, *but the number of samples tested at 10*x*LOQ was <7.*

   For sediments from FL and NC, samples were tested at LOD (n = 1), LOQ (n = 7 for all six chemicals), 10xLOQ (n = 2), and 0.15 mg/kg (n = 2). Mean values and relative standard deviations were within guideline criteria, with one exception, *but the number of samples tested at 10*x*LOQ was <7.* The only exception is that the mean value at 10xLOQ was 122% for des-chloro XDE-848 benzyl ester for the FL water samples (n=2).

   In the provided data set of the study (Appendix B), example chromatograms at 10xLOQ were provided. Only two samples were tested at 10xLOQ.

2. The estimations of the LOQ and LOD in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. In the ECM, no justification or calculation was provided to support the LOQ; the LOD was defined as the concentration which was *ca*. 30% of the LOQ (p. 34; Appendix A, pp. 142, 164 of MRID 49677722; pp. 19, 25 of MRID 49677803). In the ILV, the LOQ and LOD were reported from the ECM without justification or calculation. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological levels of concern in water were not reported. An LOQ above toxicological level of concern results in an unacceptable method classification.

3. Several relative standard deviations (RSDs) in the ECM did not meet OCSPP guidelines (RSD ≤20%): XDE-848 (SX-1552) at the LOQ in the Florida (RSD 22.241%) and North Carolina (RSD 54.282%) ponds; 1552-DA at 10×LOQ in the Florida pond water (RSD was slightly above the limit, at 20.057%); and 1552-Acid at the LOQ in the Florida pond water (RSD 30.898%; Appendix A, pp. 164-165; Appendix A, Tables 41-52, pp. 227-250 of MRID 49677722; DER Attachment 2).

   The recovery statistics for all analyses which did not meet guideline requirements, except for 1552-DA, were reviewer-calculated based on all reported data (Appendix A, Tables 41-42, pp. 227, 229, Table 47, p. 239 of MRID 49677722; DER Attachment 2). One of the recovery values for each set was not accepted by the study author; *no justification or calculation was provided for the omission*. The study author calculated recovery statistics for n = 22 (FL) or 21 (NC). The study author's means (RSDs) were calculated as 104.591% (16.553%) and 96.762% (15.580%) for SX-1552 in FL and NC, respectively, and 99.482% (12.986%) for 1552-Acid in FL. The recovery calculations of the study author included corrections for residues found in controls; however, the reviewer calculated the recovery of the unaccepted values based on the amount of analyte found without correction since the correction appeared to be variable between samples in the same set. The reported mean, standard deviation and RSD were reviewer-calculated based on n = 23 (FL) or 22 (NC).

4. In the ECM, due to significant interference in the controls at or near the retention times of the analytes, representative ECM chromatograms did not support the specificity of the method for SX-1552 in FL and NC pond waters and for 1552-OHA, 1552-DBE and 1552-Acid in NC pond water (Appendix A, Figures 109-126, pp. 372-380; Appendix A, Figures 163-180, pp. 399-407 of MRID 49677722). For the Florida site, significant interference was observed in the control, LOQ and 10×LOQ chromatograms due to a peak (>LOQ) which eluted near the retention time of the analyte and overlapped a portion of the SX-1552 peak. This caused significant interference with peak integration at the LOQ and some interference at 10×LOQ. For the North Carolina site, significant residues were observed in chromatograms for SX-1552 (*ca*. 95% of the LOQ), 1552-Acid (*ca*. 35% of the LOQ) and 1552-DBE (*ca*. 50% of the LOQ). These residues were quantified as LOD<LOQ by the study author.

5. In the ILV, representative chromatograms were not complete. Representative chromatograms were not provided for the reagent blank and fortifications at the LOD or 10×LOQ, only calibrants, controls and LOQ (Figures 54-125, pp. 144-215 of MRID 49677803). A reagent blank was included in the validation (p. 22).

   In the ECM, representative chromatograms were not complete, only chromatograms of the quantification ion were included. Additionally, representative chromatograms were not provided for the reagent blank [Appendix A, Figures 103-126, pp. 369-380 (FL Chromatograms); Appendix A, Figures 163-180, pp. 399-407 (NC Chromatograms) of MRID 49677722]. A reagent blank was included in the validation (Appendix A, p. 154).

6. The ECM calculations allowed for recovery data to be corrected for residues found in the control samples (Appendix A, pp. 160-161). Residues found in the controls were minor residues (<15% of the LOQ) for the Florida site and ranged from minor (<5% of the LOQ) to major (*ca*. 35-95% of the LOQ) residues for the North Carolina site (Appendix A, Figures 109-114, pp. 372-374; Appendix A, Figures 163-168, pp. 399-401 of MRID 49677722). An example of correction and major residues can be seen in Appendix A, Figure 169 (p. 402) where the SX-1552 recovery in NC was 76.000%, which was calculated from amount found of 0.034 ng/mL and fortification level of 0.02 ng/mL (LOQ).

7. In the ECM, recovery results and representative chromatograms were only provided for the quantitation ion (Appendix A, Tables 41-52, pp. 227-250; Appendix A, Figures 109-126, pp. 372-380; Appendix A, Figures 163-180, pp. 399-407 of MRID 49677722). In the tables, the ion transition was not reported, but the recovery values matched those reported in the chromatograms, where the ion transition was noted (in the raw chromatogram). Nonetheless, a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

8. The ECM method detailed an additional "methanol rinse preparation" which was performed with the remainder of the remaining original water, but this portion of the method appeared to be auxiliary and was not performed by the ILV (Appendix A, p. 155 of MRID 49677722; Appendix 1, pp. 221-222 of MRID 49677803). In the ECM calculations, the example calculation was provided for sample ID 696-X015-S6 (NC747) Set W04 1552-Acid, yielding a "Fortification Recovery" of 101.340% (Appendix A, p. 160 of MRID 49677722). This value was found in Table 48 (p. 242) for 1552-Acid in NC at 10×LOQ. The further example calculations which contain the "MeOH Rinse Concentration" correction (Appendix A, pp. 160-161) employ the use of a new sample ID 696-W233 (NC438) Set W046 1552-OHBE which was a sample from the water field dissipation study (Appendix A, p. 165; Table 61, p. 291). The methanol rinse was used to capture analytes which adsorbed to the glass vessels during storage and transfer. Also, the methanol rinse had a different LOQ (0.008 ng/mL for SX-1552/0.02 ng/mL for metabolites) than EPL Bio Analytical Services Method 477G696A-1 (Appendix A, p. 164).

9. Although the water matrices were well characterized in the ILV, the specific water source of each of the matrices was not reported (p. 20; Appendix 3, pp. 225-228 of MRID 49677803).

10. The reviewer noted a typographical error in the ILV: the higher fortification level was reported as "100×LOQ", instead of "10×LOQ", in the Sample Description (p. 222 of MRID 49677803).

11. The results from the water travel spikes and water field dissipation studies were included in the ECM, but not addressed in this method validation review (Appendix A, pp. 163, 165 of MRID 49677722). Tank mix analyses were also studied for the Florida and North Carolina sites (Appendix A, p. 161).

12. Isotope internal standards were used facilitate analysis (Appendix A, pp. 156-157 of MRID 49677722; p. 21; Appendix 1, p. 219; Appendix 2, p. 224 of MRID 49677803).

13. The ILV reported that communications occurred between the ILV laboratory and the study director of EPL Bio Analytical Services Method 477G696A-1 (F. Claussen; Appendix A, p. 166 of MRID 49677722; p. 25; Appendix 2, p. 224 of MRID 49677803). The communications involved the explanation of the internal standard calculations for 1552-DBE, need for use of matrix-matched standards and the question about the suitability of the ILV analytical instrument.

14. In the ILV, matrix effects were studied (pp. 24-25; Tables 62-67, pp. 85-90; Appendix 2, p. 224 of MRID 49677803). In the ILV, matrix effects were determined to be insignificant in the matrices (±20%) for all analytes but 1552-DBE; however, the use of internal standards were considered necessary to reduce matrix effects. For 1552-DBE, the ILV study author determined that the significant matrix effects with internal standards were due to the different ratio of solvents in the samples and standards, not matrix. Matrix-matched standards were used in the ILV.

15. It was reported for the ILV that the analytical procedure for one set of 19 samples (five calibration standards, two controls, one LOD sample, five LOQ samples, five 10×LOQ samples and one reagent blank) required approximately 4 hours for laboratory preparation (p. 22 of MRID 49677803). The LC/MS/MS was conducted unattended (*ca.* 8 hours or overnight). The interpretation of data required approximately 4 hours. The overall time to complete a set of samples (14 samples, not including calibration standards) was *ca.* 1.5 calendar days.

V. References

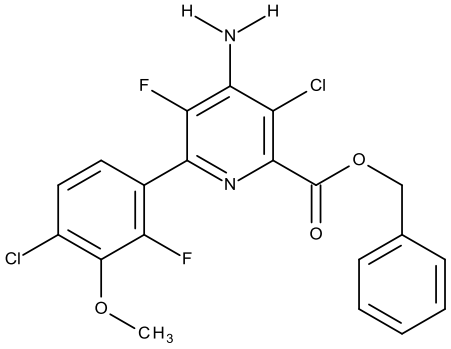
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

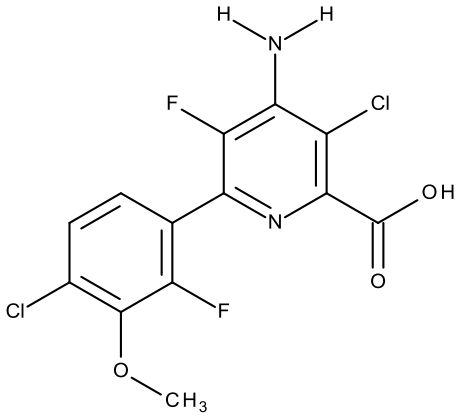
XDE-848 Benzyl Ester (Rinskor, XR-848-BE, XR-848 Benzyl, X11959130, TSN301734)

IUPAC Name:	Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate
CAS Name:	Phenylmethyl ester 3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoro-2-pyridinecarboxylic acid
CAS Number:	1390661-72-9
SMILES String:	[H]N([H])c1c(c(nc(c1Cl)C(=O)OCc2ccccc2)c3ccc(c(c3F)OC)Cl)F



XDE-848 acid (X11433848, TSN304667)

IUPAC Name:	4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid
CAS Name:	--
CAS Number:	--
SMILES String:	[H]N([H])c1c(c(nc(c1Cl)C(=O)O)c2ccc(c(c2F)OC)Cl)F



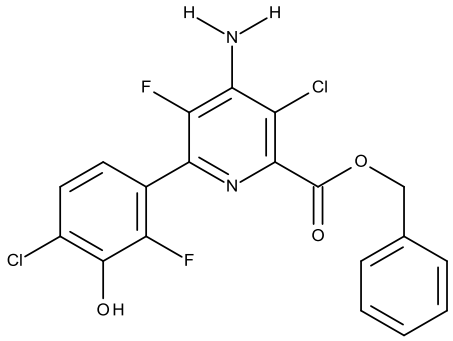
XDE-848 Hydroxy Benzyl Ester (X12300837; TSN305650; XDE-848 BH; Benzyl hydroxyl; 1552-OHBE; OHBE)

IUPAC Name: Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-hydroxyphenyl)-5-fluoropyridine-2-carboxylate

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1c(c(nc(c1Cl)C(=O)OCc2ccccc2)c3ccc(c(c3F)O)Cl)F



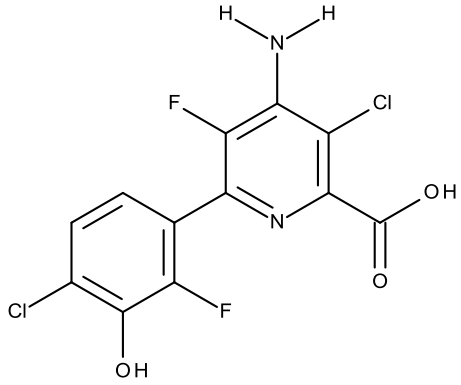
XDE-848 Hydroxy Acid (X11966341; TSN305649; XDE-848 HA; Hydroxy acid; 1552-OHA; OHA)

IUPAC Name: 4-Amino-3-chloro-6-(4-chloro-2-fluoro-3-hydoxyphenyl)-5-fluoropyridine-2-carboxylic acid

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1c(c(nc(c1Cl)C(=O)O)c2ccc(c(c2F)O)Cl)F



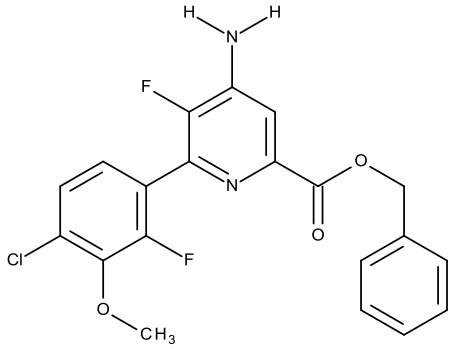
Dechlorinated XDE-848 Benzyl Ester (X12131932; TSN304497; De-chloro BE; Dechlorinated 848 BE; 1552-DBE; DBE)

IUPAC Name: Benzyl 4-amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylate

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1cc(nc(c1F)c2ccc(c(c2F)OC)Cl)C(=O)OCc3ccccc3



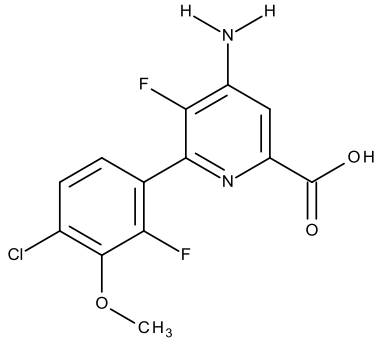
Dechlorinated XDE-848 Acid (X12393505; TSN304479; De-chloro acid; Dechlorinated 848 BE; 1552-DA; DA)

IUPAC Name: 4-Amino-6-(4-chloro-2-fluoro-3-methoxyphenyl)-5-fluoropyridine-2-carboxylic acid

CAS Name: --

CAS Number: --

SMILES String: [H]N([H])c1cc(nc(c1F)c2ccc(c(c2F)OC)Cl)C(=O)O



Attachment 2: Calculations

Chemical: XDE-848
PC: 030093
MRIDs: 49677722/49677803
Guideline: 850.6100

ECM Validation for Determination in Surface Water
Quantiation ion

Fortified (µg/L)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Max	Min	n =
SX-1552 - Florida Water							
0.020	35.000						
LOQ	86.500						
	104.000						
	92.000						
	123.000						
	94.500						
	93.000						
	113.500						
	61.000						
	92.500						
	121.500						
	113.000						
	99.500						
	98.000						
	125.500						
	80.000						
	104.000						
	106.000						
	105.000						
	126.000						
	113.000						
	107.000						
	142.000	101.543	22.584	22.241	142.000	35.000	23.000
SX-1552 - North Carolina Water							
0.020	76.000						
LOQ	84.500						
	102.500						
	120.500						
	106.500						
	95.500						
	100.000						
	117.000						
	88.500						
	102.000						
	70.000						
	105.000						
	122.000						
	365.000						
	105.500						
	91.000						
	65.000						
	105.000						
	106.000						
	80.000						
	94.000						

	95.500	108.955	59.142	54.282	365.000	65.000	22.000
1552-Acid - Florida Water							
0.050	73.800						
LOQ	87.600						
	110.000						
	243.400						
	77.400						
	87.400						
	101.600						
	106.800						
	76.400						
	86.200						
	111.200						
	113.000						
	101.200						
	101.600						
	111.200						
	108.200						
	103.400						
	88.000						
	108.800						
	108.600						
	107.400						
	120.400						
	98.400	105.739	32.672	30.898	243.400	73.800	23.000

Results from Appendix A, Table 41, p. 227, Table 42, p. 229 and Table 47, p. 239 of MRID 49677722.
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and = STDEV(A1:A2).
Any discrepancies between reviewer calculated values and reported results most likely due to rounding.
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

Chemical: XDE-848
PC: 030093
MRIDs: 49677722/49677803
Guideline: 850.6100

ECM Recoveries at LOD of XDE-848 (SX-1552) and its Products

	SX-1552		
Fortified (µg a.i./L)	Found (µg/L)	Recovery (%)	
0.0060	Quantiation ion		
	0.00099	16	Pond Water
	0.00467	78	Well Water
	0.00285	48	Tap Water
	Confirmation ion		
	0.00486	81	Pond Water
	0.00386	64	Well Water
	0.00532	89	Tap Water

Results from Tables 14-49, pp. 43-78 of MRID 49677803.

	1552-OHA		1552-DBE		1552-DA		1552-OHBE		1552-Acid		
Fortified (µg a.i./L)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	Found (µg/L)	Recovery (%)	
0.0150	Quantiation ion										
	0.0146	97	0.0145	97	0.0130	87	0.0128	85	0.0149	99	Pond Water
	0.0169	113	0.0133	89	0.0114	76	0.0108	72	0.0123	82	Well Water
	0.0125	83	0.0151	101	0.0161	107	0.0159	106	0.0147	98	Tap Water
	Confirmation ion										
	0.0168	112	0.0155	103	0.0146	97	0.0111	74	0.0150	100	Pond Water
	0.0127	85	0.0160	107	0.0078	52	0.0087	58	0.0153	102	Well Water
	0.0151	101	0.0140	93	0.0134	89	0.0140	93	0.0177	118	Tap Water

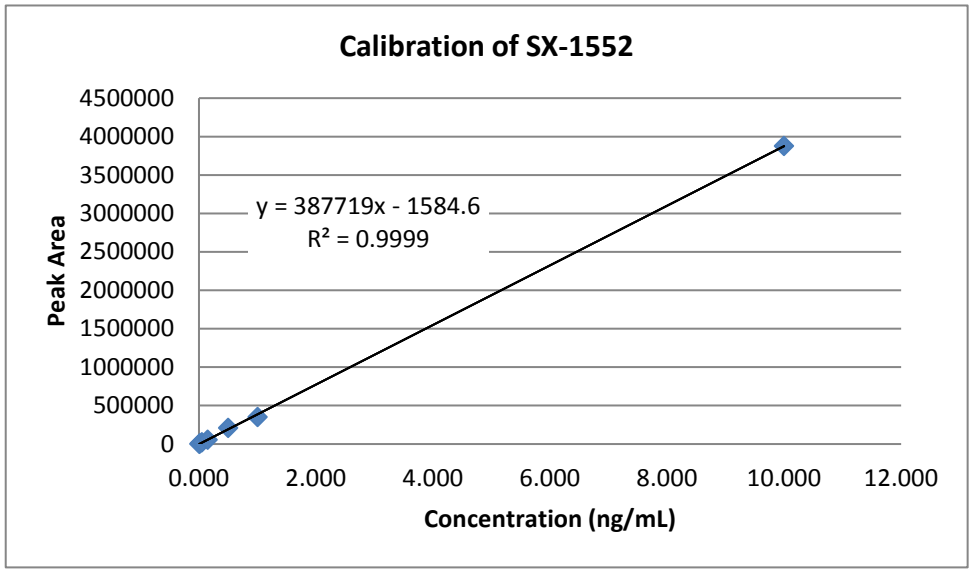
Results from Tables 14-49, pp. 43-78 of MRID 49677803.

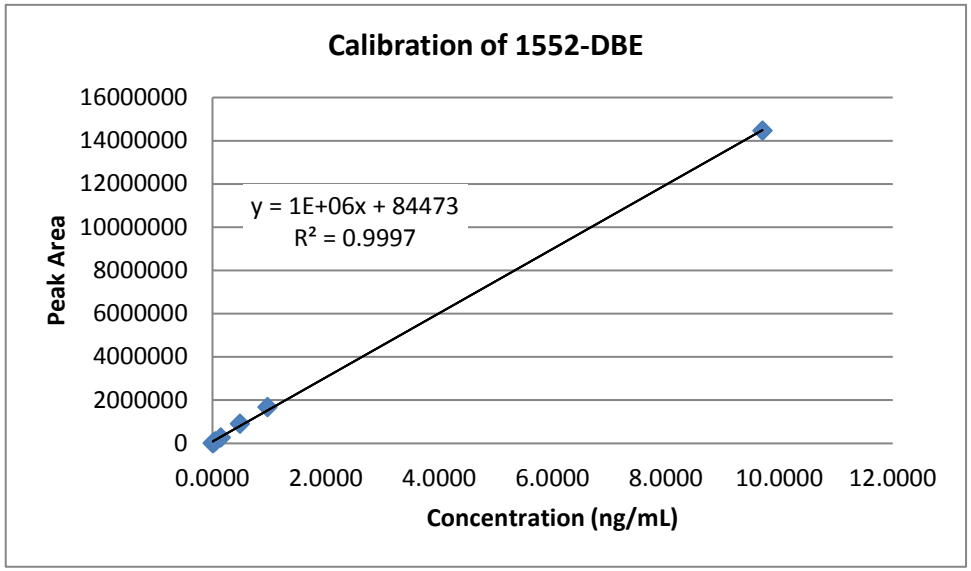
Chemical: XDE-848
PC: 030093
MRIDs: 49677722/49677803
Guideline: 850.6100

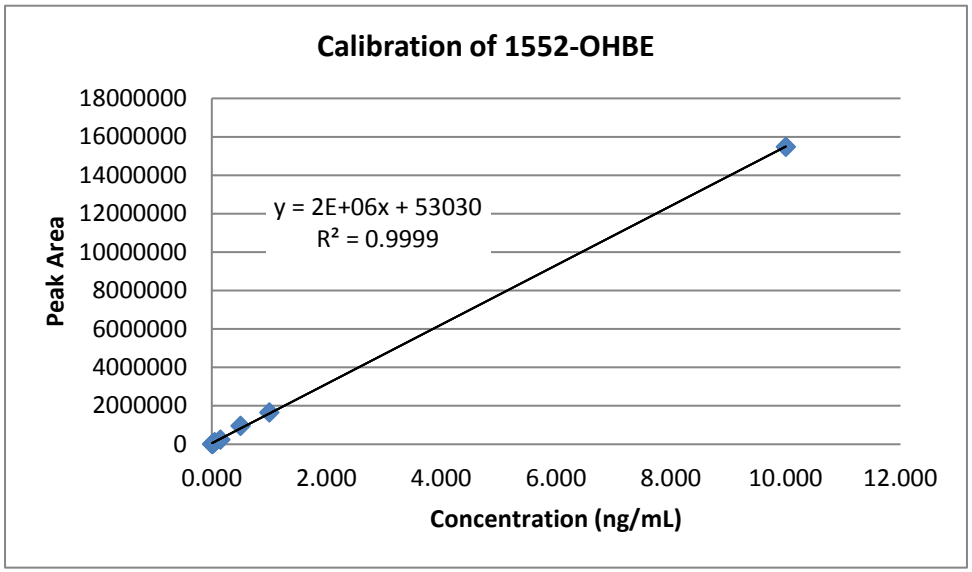
ECM Calibration Curves

Calibration Curves	SX-1552		1552-OHA		1552-DBE		1552-DA		1552-OHBE		1552-Acid	
	Amount ng/mL	Peak Area counts	Amount ng/mL	Peak Area counts	Amount ng/mL	Peak Area counts	Amount ng/mL	Peak Area counts	Amount ng/mL	Peak Area counts	Amount ng/mL	Peak Area counts
	0.005	3106	0.0049	27363	0.0049	11092	0.0049	17172	0.005	8695	0.005	16679
	0.015	10345	0.0147	36879	0.0146	31190			0.015	28306	0.015	31318
	0.050	21557	0.049	69614	0.0485	90923	0.049	131449	0.050	102592	0.050	87618
	0.150	55670	0.147	138177	0.146	272499	0.147	366305	0.150	235413	0.150	196780
	0.500	211901	0.490	454976	0.485	912898	0.490	1221925	0.500	951137	0.500	656455
	1.00	352415	0.98	817399	0.97	1687582	0.98	2304090	1.00	1659077	1.00	1122128
	10.00	3877979	9.80	7658608	9.70	14468975	9.80	22089531	10.00	15480377	10.00	11107938
			49.00	37874620			49.00	109481939			50.00	53374893

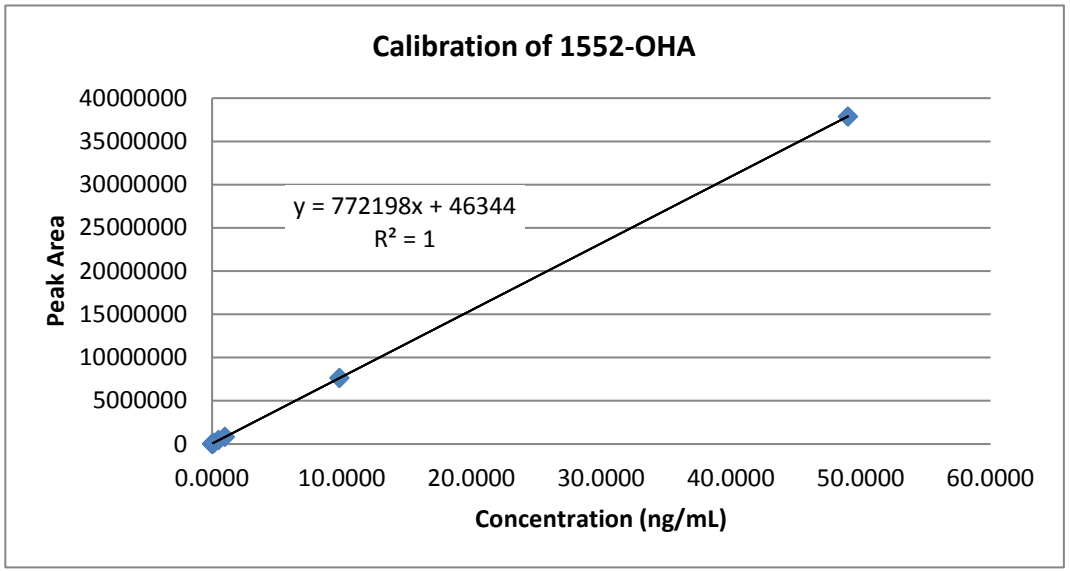
Results (Peak Areas) from Appendix A, Figures 97-102, pp. 363-368 of MRID 49677722.
*Some results were excluded by the study author due to high response; the reviewer also excluded these results from the linear regressions.

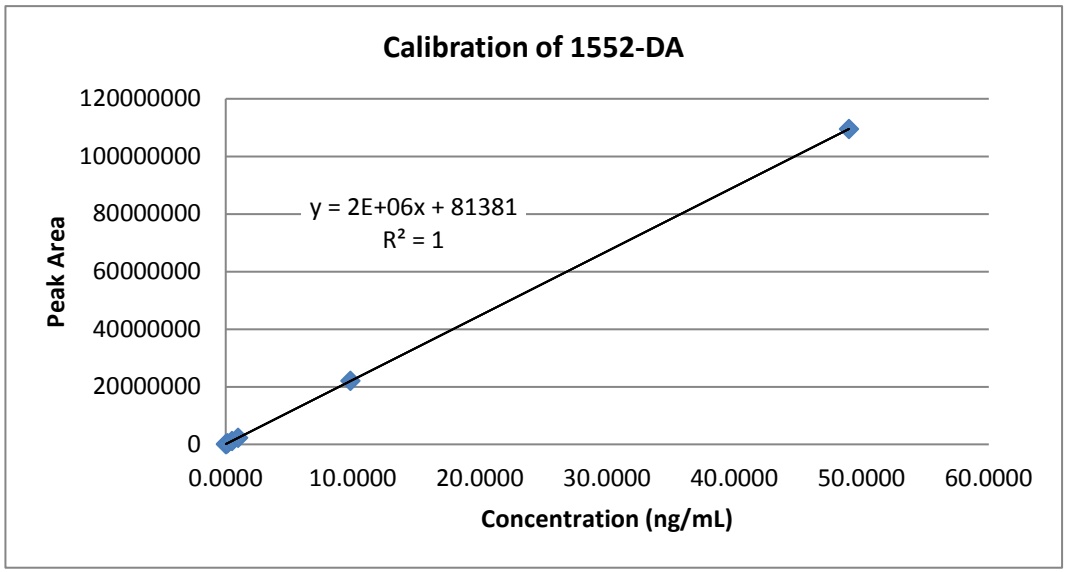


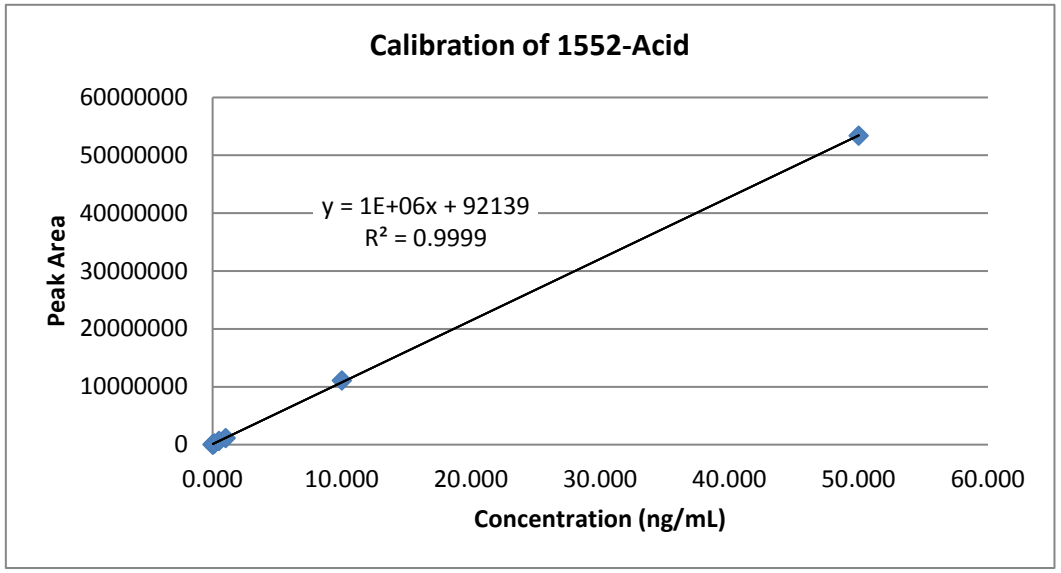

Calibration of 1552-DBE
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Peak Area
y = 1E+06x + 84473
R² = 0.9997
0.0000
2.0000
4.0000
6.0000
8.0000
10.0000
12.0000
Concentration (ng/mL)


Calibration of 1552-OHBE
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
Peak Area
y = 2E+06x + 53030
R² = 0.9999
0.000
2.000
4.000
6.000
8.000
10.000
12.000
Concentration (ng/mL)

Chemical: XDE-848
PC: 030093
MRIDs: 49677722/49677803
Guideline: 850.6100




Calibration of 1552-DA
120000000
100000000
80000000
60000000
40000000
20000000
0
Peak Area
y = 2E+06x + 81381
R² = 1
0.0000
10.0000
20.0000
30.0000
40.0000
50.0000
60.0000
Concentration (ng/mL)


Calibration of 1552-Acid
60000000
50000000
40000000
30000000
20000000
10000000
0
Peak Area
y = 1E+06x + 92139
R² = 0.9999
0.000
10.000
20.000
30.000
40.000
50.000
60.000
Concentration (ng/mL)

Chemical: XDE-848
PC: 030093
MRIDs: 49677722/49677803
Guideline: 850.6100

ILV Calibration Curve Correlation Coefficients r (1/x weighting) converted to r2

	Water				
	First Ion Transition (Q)		Second Ion Transition (C)		
Analyte	Reported r	Calculated r2	Reported r	Calculated r2	
SX-1552	0.9999	0.9998	0.9998	0.9996	High
	0.9992	0.9984	0.9989	0.9978	Low
1552-OHA	1.0000	1.0000	0.9999	0.9998	High
	0.9999	0.9998	0.9999	0.9998	Low
1552-DBE	0.9998	0.9996	0.9999	0.9998	High
	0.9990	0.9980	0.9990	0.9980	Low
1552-DA	1.0000	1.0000	0.9999	0.9998	High
	0.9998	0.9996	0.9997	0.9994	Low
1552-OHBE	1.0000	1.0000	1.0000	1.0000	High
	0.9999	0.9998	0.9999	0.9998	Low
1552-Acid	1.0000	1.0000	1.0000	1.0000	High
	0.9999	0.9998	0.9999	0.9998	Low

Results (r values) from Tables 2-13, pp. 31-42 and Figures 11-46, pp. 101-136 of MRID 49677803.