

Analytical method for tembotrione and its transformation products, AE 0456148 and AE 1392936¹, in water

Reports: ECM: EPA MRID No.: 46695522. Seymour, R.J., D. Beck. 2005. In House Laboratory Validation of an Analytical Method for the Determination of Residues of AE 0172747 and its Metabolites AE 0456148 and AE 1392936 in Water Using LC/MS/MS. Report prepared by Bayer CropScience, Stillwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 66 pages. Bayer CropScience Study No.: RAAEX021. Final report issued October 6, 2005.

ILV: EPA MRID No.: 46695526. Krebber, R. 2005. Independent Laboratory Validation of Method AE-005-W05-02 for the Determination of AE 0172747 and its Metabolites AE 0456148 and AE 1392936 in Water. Report prepared and sponsored by Bayer CropScience AG, Monheim am Rhein, Germany, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 38 pages. Bayer CropScience AG Report No: MR-091/05. Laboratory Project ID: P614 057033. Final report issued September 30, 2005.

Document No.: MRIDs 46695522 & 46695526

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards, except that some of the instrument-printed raw data was not initialed and dated on the day printed (p. 3 of MRID 46695522). Signed and dated Data Confidentiality, GLP, Authenticity and Quality Assurance statements were provided (pp. 2-5).


ILV: The study was conducted in compliance with OECD, USEPA FIFRA (40 CFR 160), German and Japanese GLP standards, with the exception of the recognized differences between the OECD and FIFRA and Japanese GLP principles/standards (p. 3; Appendix 1, pp. 37-38 of MRID 46695526). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5; Appendix 1, pp. 37-38). The statement of authenticity was not included.

Classification: This analytical method is classified as **unacceptable, but upgradeable**. An updated ECM was not submitted in response to the ILV findings. Performance data at 10×LOQ was not reported to validate the method. In the ILV, the number of trials performed to validate the method was not reported, the LOD of the method was not reported, and insufficient chromatographic support was provided for the method validation. In the ECM, an insufficient number of samples was prepared for all analyses, and the test water matrices were not characterized.

PC Code: 012801


¹ The Residues of Concern (ROCs) for tembotrione include parent tembotrione and the degradates AE0456148 and AE0968400 when considering potential risks to aquatic invertebrates. Parent tembotrione is the only ROC for all other taxa. This ECM/ILV does not include AE0968400, but does include AE1392936, which is not an ROC.

Final EPA Reviewer: **Stephen P. Wentz, Ph.D., Senior Scientist**

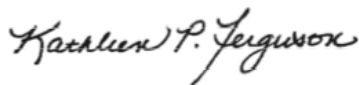
Signature: 
Date: 6/21/2021

CDM/CSS-Dynamac JV Reviewers:

Lisa Muto,
Environmental Scientist

Signature: 
Date: 6/14/17

Kathleen Ferguson, Ph.D.,
Environmental Scientist

Signature: 
Date: 6/14/17

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

Executive Summary

The analytical method, Bayer Method AE-005-W05-02, is designed for the quantitative determination of tembotrione (AE 0172747) and its transformation products AE 0456148 and AE 1392936 using HPLC/MS/MS in water at the stated LOQ of 0.05 ng/mL (0.05 µg/L). The LOQ is less than the lowest toxicological level of concern in water for all analytes. The ECM used uncharacterized surface, ground and tap water matrices; the ILV used characterized surface and tap water matrices. The number of trials performed by the ILV to validate the method was not reported, but the method was validated for AE 0172747, AE 0456148 and AE 1392936 with significant modifications to the HPLC parameters analytical column, flow rate, gradient program and the MS/MS acquisition method. The ILV reported the necessity for an updated ECM to address the identified analytical issues, but an updated ECM was not submitted in response to the ILV findings. Performance data at 10×LOQ was not reported in the ECM or ILV to validate the method; only LOQ and 5×LOQ fortifications were prepared. All ILV data regarding repeatability, accuracy, precision and linearity were satisfactory for all analytes. In the ILV, the specificity of the method was supported by the LOQ representative chromatograms; however, no 5×LOQ chromatograms were provided. In the ECM, an insufficient number of samples was prepared for all analyses, and the test water matrices were not characterized. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for all analytes. The LOD of the method was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Tembotrione (AE 0172747)	46695522 ²	46695526 ³		Water	06/10/2005	Bayer CropScience	LC/MS/MS	0.05 ng/mL
AE 0456148								
AE 1392936								

¹ Tembotrione = AE 0172747; 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione; AE 0456148 = 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoic acid; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid.

² In the ECM, the surface water (Sample ID 19458-CA0097) was obtained from Lodi, California and characterized in Study No. 00M19458 (pp. 11, 17 of MRID 46695522). The ground water (Sample ID MK-2-9/10/04-A) was well

water obtained from Biglerville, Pennsylvania, and characterized in Study No. MEGUY003. The treated tap water (Sample ID BRP002) was obtained from Bayer Research Park, Stilwell, Kansas, and reportedly characterized in Study No. RAAEX021 (current study). No characterization data for any water matrix was found in the study report. The specific source of the surface water was not described.

3 In the ILV, surface water (pH 7.3, hardness 11.2 °dH, dissolved organic carbon and total organic carbon 3 mg/L) from the River Rhine at Leverkusen-Hitdorf, Germany, and tap water (pH 7.6, hardness 14.8 °dH, dissolved organic carbon and total organic carbon <2 mg/L) from Monheim am Rhein were well-characterized by Bayer Industry Services (p. 13; Tables 12-13, p. 36 of MRID 46695526).

I. Principle of the Method

Water samples (10 mL) were placed in disposable glass vials or plastic bottles and acidified with 250 µL of formic acid (p. 12; Attachment, pp. 52, 54 of MRID 46695522). The samples were fortified, if necessary, with the mixed fortification solution. The deuterated internal standard solution was added (200 µL of the 50 ng/mL solution), and the sample was diluted, if necessary, to make sure that the analyte response was within the calibration curve. An aliquot (*ca.* 1-1.5 mL) was transferred to an autosampler vial for analysis by LC/MS/MS.

The method noted that tembotrione (AE 0172747) degrades readily to metabolite AE 0456148 in water containing free chlorine, sodium thiosulfate must be added at the time of sampling before the water sample is fortified with AE 0172747 (Attachment, pp. 54-55 of MRID 46695522). 10 ppm of sodium thiosulfate can remove 2 ppm of chlorine, so sample bottles for collecting 100 mL of treated water should contain 1 mL of 1000 ppm solution of sodium thiosulfate.

Samples were analyzed for AE 0172747, AE 0456148 and AE 1392936 using a Shimadzu LC-10AVP HPLC coupled to a Perkin Elmer Sciex API 4000 LC/MS/MS (Attachment, pp. 55-58 of MRID 46695522). The following LC conditions were used: Waters SymmetryShield RP8 100Å column [(50 mm x 2.1 mm, 5 µ; column temperature ambient); two columns in series], mobile phase of (A) 1.5% acetic acid in DI water and (B) acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.00-0.50 min. 90:10, 2.50-5.00 min. 5:95, 5.01-7 min. 90:10], injection volume of 90 µL, and MRM with TurboIon Spray (500°C) in negative mode. One ion pair transition was monitored for each analyte: m/z 439.00→403.00 for AE 0172747, m/z 345.00→217.00 for AE 0456148 and m/z 263.00→189.00 for AE 1392936. Observed retention times were 4.58, 5.44 and 4.63 minutes for AE 0172747, AE 0456148 and AE 1392936, respectively.

The ILV performed the ECM method as written, except for modifications of the analytical method (pp. 14-19 of MRID 46695526). The LC/MS/MS analysis was performed using a HP 1100 LC coupled to an Applied Biosystems API 4000 LC/MS/MS with Turbo Ion Electrospray interface (MRM). SymmetryShield RP8 100Å column (150 mm x 3 mm, 5 µ) and mobile phase of (A) 1.5% acetic acid in DI water and (B) acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.0-3.0 min. 90:10, 8.0-10.0 min. 10:90, 10.1-15.0 min. 90:10]. One ion pair transition was monitored for each analyte: m/z 439.03→403.00 for AE 0172747, m/z 344.91→217.10 for AE 0456148 and m/z 262.90→189.00 for AE 1392936. Observed retention times were *ca.* 8.7, 9.5 and 3.4 minutes for AE 0172747, AE 0456148 and AE 1392936, respectively. Other chromatographic parameters were the same as those of the ECM.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.05 ng/mL (0.05 µg/L) for tembotrione, and its transformation products, AE 0456148 and AE 1392936 (pp. 8, 14-16 of MRID 46695522; pp. 9, 20 of MRID 46695526). In the ECM, the LOD was reported as 0.012 ng/mL. In

the ECM, the calculated LOQs for tembotrione, AE 0456148 and AE 1392936 were reported as 0.024, 0.015 and 0.017 ng/mL, respectively. In the ECM, the Limits of Detection (LODs) were calculated as 0.007, 0.004 and 0.005 ng/mL for AE 0172747, AE 0456148 and AE 1392936, respectively. In the ILV, the LOD was not reported.

II. Recovery Findings

ECM (MRID 46695522): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of tembotrione, AE 0456148 and AE 1392936 at fortification levels of 0.05 ng/mL (LOQ) and 0.25 ng/mL (5 \times LOQ) in three water matrices using LC/MS/MS (Appendix 1, Tables 1a-1c, pp. 18-20; DER Attachment 2). No samples were prepared at 10 \times LOQ. An insufficient number of samples was prepared for all analyses, n = 3. Means, s.d.s and RSDs were reviewer-calculated based on data provided in the study report since these values were not provided by the study authors. One ion pair transition was monitored for each analyte; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The surface water (Sample ID 19458-CA0097) was obtained from Lodi, California and characterized in Study No. 00M19458 (pp. 11, 17). The ground water (Sample ID MK-2-9/10/04-A) was well water obtained from Biglerville, Pennsylvania, and characterized in Study No. MEGUY003. The treated tap water (Sample ID BRP002) was obtained from Bayer Research Park, Stilwell, Kansas, and reportedly characterized in Study No. RAAEX021 (current study). No characterization data for any water matrix was found in the study report. The specific source of the surface water was not described.

ILV (MRID 46695526): Mean recoveries and RSDs were within guidelines for analysis of tembotrione, AE 0456148 and AE 1392936 at fortification levels of 0.05 ng/mL (LOQ) and 0.25 ng/mL (5 \times LOQ) in two water matrices (Tables 5-10, pp. 21-24; DER Attachment 2). No samples were prepared at 10 \times LOQ. The same identification methods were used as the ECM. The surface water (pH 7.3, hardness 11.2 $^{\circ}$ dH, dissolved organic carbon and total organic carbon 3 mg/L) from the River Rhine at Leverkusen-Hitdorf, Germany, and tap water (pH 7.6, hardness 14.8 $^{\circ}$ dH, dissolved organic carbon and total organic carbon <2 mg/L) from Monheim am Rhein were well-characterized by Bayer Industry Services (p. 13; Tables 12-13, p. 36). The number of trials performed to validate the method was not reported, but the method was validated for tembotrione (AE 0172747), AE 0456148 and AE 1392936 with significant modifications to the HPLC parameters analytical column, flow rate, gradient program and the MS/MS acquisition method (pp. 9, 18-19, 23). The ILV reported the necessity for an updated ECM to address the identified analytical issues.

Table 2. Initial Validation Method Recoveries for Tembotrione, AE 0456148 and AE 1392936 in Water^{1,2,3,4}

Analyte ¹	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Surface Water						
Tembotrione (AE 0172747)	0.05 (LOQ)	3	99.6-108.6	104.8	4.7	4.4
	0.25	3	98.5-102.0	100.2	1.8	1.8
AE 0456148	0.05 (LOQ)	3	102.2-109.0	106.0	3.5	3.3
	0.25	3	100.0-103.2	101.5	1.6	1.6
AE 1392936	0.05 (LOQ)	3	93.2-97.6	95.5	2.2	2.3
	0.25	3	100.8-105.6	103.2	2.4	2.3
Ground (Well) Water						
Tembotrione (AE 0172747)	0.05 (LOQ)	3	98.2-110.8	103.8	6.4	6.2
	0.25	3	88.8-111.6	101.9	11.8	11.5
AE 0456148	0.05 (LOQ)	3	101.0-105.2	103.6	2.3	2.2
	0.25	3	100.4-106.4	103.9	3.1	3.0
AE 1392936	0.05 (LOQ)	3	100.2-102.2	101.2	1.0	1.0
	0.25	3	99.6-106.0	103.9	3.7	3.6
Treated Tap Water						
Tembotrione (AE 0172747)	0.05 (LOQ)	3	98.2-99.8	98.7	0.9	0.9
	0.25	3	98.0-105.2	100.8	3.9	3.8
AE 0456148	0.05 (LOQ)	3	103.0-109.6	106.0	3.3	3.2
	0.25	3	102.8-104.8	103.5	1.2	1.1
AE 1392936	0.05 (LOQ)	3	97.0-103.8	100.3	3.4	3.4
	0.25	3	101.2-103.6	102.1	1.3	1.3

Data (uncorrected recovery results; pp. 12-14) were obtained from Appendix 1, Tables 1a-1c, pp. 18-20 of MRID 46695522 and DER Attachment 2.

1 Tembotrione = AE 0172747; 2-[2-Chloro-4-mesy-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione; AE 0456148 = 2-[2-Chloro-4-mesy-3-((2,2,2-trifluoroethoxy)methyl)benzoic acid; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesybenzoic acid.

2 The surface water (Sample ID 19458-CA0097) was obtained from Lodi, California and characterized in Study No. 00M19458 (pp. 11, 17). The ground water (Sample ID MK-2-9/10/04-A) was well water obtained from Biglerville, Pennsylvania, and characterized in Study No. MEGUY003. The treated tap water (Sample ID BRP002) was obtained from Bayer Research Park, Stilwell, Kansas, and reportedly characterized in Study No. RAAEX021 (current study). No characterization data for any water matrix was found in the study report. The specific source of the surface water was not described.

3 One ion pair transition was monitored for each analyte: m/z 439.03→403.00 for AE 0172747, m/z 344.91→217.10 for AE 0456148 and m/z 262.90→189.00 for AE 1392936.

4 Means, s.d.s and RSDs were reviewer-calculated based on data provided in the study report since these values were not provided by the study authors.

Table 3. Independent Validation Method Recoveries for Tembotrione, AE 0456148 and AE 1392936 in Water^{1,2,3}

Analyte ¹	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ⁴	Relative Standard Deviation (%)
Rhine River (Surface) Water						
Tembotrione (AE 0172747)	0.05 (LOQ)	10	98-105	101	3	2.7
	0.25	10	96-106	100	3	3.1
AE 0456148	0.05 (LOQ)	10	101-106	103	2	1.9
	0.25	10	98-105	102	2	2.4
AE 1392936	0.05 (LOQ)	10	101-111	107	3	2.5
	0.25	10	100-107	104	2	2.0
Monheim Tap Water						
Tembotrione (AE 0172747)	0.05 (LOQ)	10	92-104	98	4	3.9
	0.25	10	95-101	98	2	2.3
AE 0456148	0.05 (LOQ)	10	95-106	101	4	3.4
	0.25	10	98-106	101	2	2.3
AE 1392936	0.05 (LOQ)	10	104-113	107	3	2.6
	0.25	10	102-111	107	3	2.4

Data (uncorrected recovery results; p. 18) were obtained from Tables 9-11, pp. 20-21 of MRID 46695526 and DER Attachment 2.

1 Tembotrione = AE 0172747; 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione; AE 0456148 = 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoic acid; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid.

2 The surface water (pH 7.3, hardness 11.2 °dH, dissolved organic carbon and total organic carbon 3 mg/L) from the River Rhine at Leverkusen-Hitdorf, Germany, and tap water (pH 7.6, hardness 14.8 °dH, dissolved organic carbon and total organic carbon <2 mg/L) from Monheim am Rhein were well-characterized by Bayer Industry Services (p. 13; Tables 12-13, p. 36).

3 One ion pair transition was monitored for each analyte: m/z 439.03→403.00 for AE 0172747, m/z 344.91→217.10 for AE 0456148 and m/z 262.90→189.00 for AE 1392936.

4 Standard deviations were reviewer-calculated based on data provided in the study report since these values were not provided by the study authors. Rules of significant figures were followed.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.05 ng/mL for tembotrione and its transformation products, AE 0456148 and AE 1392936 (pp. 8, 14-16; Appendix 1, Table 1d, p. 21 of MRID 46695522; pp. 9, 20 of MRID 46695526). In the ECM, the LOD was reported as 0.012 ng/mL. In the ECM, the LOD and LOQ for determination in water were calculated using the standard deviation from the combined 0.05 ng/mL recovery results from the three water matrices. The LOD was calculated as three times the standard deviation ($3s$), and the LOQ was calculated as ten times the standard deviation ($10s$) of the recovery results. The calculated LOQs for tembotrione, AE 0456148 and AE 1392936 were reported as 0.024, 0.015 and 0.017 ng/mL, respectively. In the ECM, LODs were calculated as 0.007, 0.004 and 0.005 ng/mL for AE 0172747, AE 0456148 and AE 1392936, respectively. The calculated values support the LOQ and LOD established for the study. In the ILV, the LOD was not reported.

Table 4. Method Characteristics

Analyte ¹		Tembotrione (AE 0172747)	AE 0456148	AE 1392936
Limit of Quantitation (LOQ)	ECM	0.05 ng/mL		
		0.024 ng/mL (calculated)	0.015 ng/mL (calculated)	0.017 ng/mL (calculated)
	ILV	0.05 ng/mL		
Limit of Detection (LOD)	ECM	0.012 ng/mL		
		0.007 ng/mL (calculated)	0.004 ng/mL (calculated)	0.005 ng/mL (calculated)
	ILV	Not reported		
Linearity (calibration curve r^2 and concentration range)	ECM ²	$r^2 = 0.9989$	$r^2 = 0.9996$	$r^2 = 0.9992$
	ILV ³	$r^2 = 0.9992$	$r^2 = 0.9990$	$r^2 = 0.9992$
	Range:	0.02-0.5 ng/mL		
Repeatable		No sample were prepared at $10\times$ LOQ.		
	ECM ^{4,5}	Yes at LOQ and $5\times$ LOQ, but $n = 3$.		
	ILV ^{4,6,7}	Yes at LOQ and $5\times$ LOQ.		
Reproducible		Yes at LOQ. Could not be determined for $10\times$ LOQ; no performance data was submitted.		
Specific	ECM	No matrix interferences were observed.	Minor baseline noise interfered with peak integration at the LOQ.	Minor baseline noise interfered with peak integration at the LOQ in all three matrices. A nearby minor contaminant peak (5-10% of the LOQ, based on peak height) interfered with peak integration at the LOQ in the tap water. ⁸
		Matrix interferences were <5% of the LOQ (based on peak height).	No matrix interferences were observed.	No matrix interferences were observed, but the baseline was significantly irregular/elevated.
	ILV	No $5\times$ LOQ chromatograms were provided.		

Data were obtained from pp. 8, 14-16; Appendix 1, Tables 1a-1c, pp. 18-20 (recovery results); Appendix 1, p. 20 and Appendix 2, Figures 1-3, pp. 22-24 (calibration curves); Appendix 4, Figures 3-11, pp. 34-42 (chromatograms) of MRID 46695522; pp. 9, 18-20; Tables 9-11, pp. 20-21 (recovery results); Figures 1-3, pp. 25-26 (calibration curves); Figures 5-21, pp. 27-35 (chromatograms) of MRID 46695526; DER Attachment 2.

- 1 Tembotrione = AE 0172747; 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione; AE 0456148 = 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoic acid; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid.
- 2 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (Appendix 1, p. 20; Attachment, p. 53 of MRID 46695522; DER Attachment 2).
- 3 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (p. 13; Figures 1-3, pp. 25-26 of MRID 46695526; DER Attachment 2).
- 4 Using LC/MS/MS, one ion pair transition was monitored for each analyte. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
- 5 In the ECM, the surface water (Sample ID 19458-CA0097) was obtained from Lodi, California and characterized in Study No. 00M19458 (pp. 11, 17 of MRID 46695522). The ground water (Sample ID MK-2-9/10/04-A) was well water obtained from Biglerville, Pennsylvania, and characterized in Study No. MEGUY003. The treated tap water (Sample ID BRP002) was obtained from Bayer Research Park, Stilwell, Kansas, and reportedly characterized in Study No. RAAEX021 (current study). No characterization data for any water matrix was found in the study report. The specific source of the surface water was not described.
- 6 In the ILV, surface water (pH 7.3, hardness 11.2 °dH, dissolved organic carbon and total organic carbon 3 mg/L) from the River Rhine at Leverkusen-Hitdorf, Germany, and tap water (pH 7.6, hardness 14.8 °dH, dissolved organic carbon and total organic carbon <2 mg/L) from Monheim am Rhein were well-characterized by Bayer Industry Services (p. 13; Tables 12-13, p. 36 of MRID 46695526).
- 7 The number of trials performed to validate the method was not reported, but the method was validated for tembotrione (AE 0172747), AE 0456148 and AE 1392936 with significant modifications to the HPLC parameters analytical column, flow rate, gradient program and the MS/MS acquisition method (pp. 9, 18-19, 23 of MRID 46695526). The ILV reported the necessity for an updated ECM to address the identified analytical issues.
- 8 Based on Figure 10, p. 41 of MRID 46695522.

IV. Method Deficiencies and Reviewer's Comments

1. An updated ECM was not submitted in response to the ILV findings. The ILV reported the necessity for an updated ECM to address the identified analytical issues (pp. 9, 18-19, 23 of MRID 46695526). The ILV validated the method for tembotrione (AE 0172747), AE 0456148 and AE 1392936 with significant modifications to the HPLC parameters analytical column, flow rate, gradient program and the MS/MS acquisition method. The ILV could not reproduce the reported retention times and resolution of the analytes using the parameters in the ECM method.
2. Performance data at 10×LOQ was not reported to validate the method in either the ECM or ILV. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix. The reproducibility of the method at 10×LOQ could not be determined.
3. The following additional deficiencies were noted in the ILV MRID 46695526:

The number of trials performed by the ILV to validate the method was not reported (pp. 9, 18-19, 23).

The LOD was not reported.

Insufficient chromatographic support was provided for the method validation since no 5×LOQ chromatograms were provided. Representative chromatograms for the controls and each fortification level should be provided for all matrices tested so that the specificity of the method can be fully evaluated.

4. The following additional deficiencies were noted in the ECM MRID 46695522:

An insufficient number of samples was prepared for all analyses, $n = 3$ (Appendix 1, Tables 1a-1c, pp. 18-20). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.

The characterization of the ECM test water matrices was not provided in the study report (pp. 11, 17). The surface, ground and treated tap water matrices were reportedly characterized in either other cited studies or the current study Study No. RAAEX021; however, no characterization data for any water matrix was found in the study report. Also, the specific source of the surface water was not described.

A nearby minor contaminant peak (5-10% of the LOQ) interfered with peak integration of AE 1392936 at the LOQ in the tap water (Figure 10, p. 41).

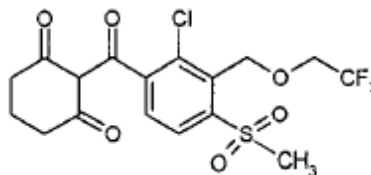
5. The communications between the ILV and validation laboratory Study Director and Study Monitor were reported (pp. 22-23 of MRID 46695526). The ILV reported that emails were exchanged regarding study progress, the AE 1392936 MRM monitored ion and modifications to the analytical method; no contact relative to the performance of the study occurred.
6. In the ECM, the study authors discussed that the fragment ion for AE 0456148 at m/z 301 was more intense than the chosen quantitation fragment at m/z 217; however, there was less background interference and a better signal-to-noise ratio observed at m/z 217 (MRID 46695522). The reviewer noted that m/z 301 was used as the quantitation fragment ion for AE 0456148 in the tembotrione soil method validation by Bayer CropScience (MRID 46695523).
7. Bayer Method AE-005-W05-02 (issued May 24, 2005) was included in the ECM as the Attachment (pp. 44-66 of MRID 46695522). Bayer Method AE-005-W05-02 was a method only without performance data.
8. The reviewer noted the following typographical error in Appendix 1, Tables 1a-1c where 10×LOQ was written instead of 5×LOQ (Appendix 1, Tables 1a-1c, pp. 18-20 of MRID 46695522).
9. In the ILV, the total time required to complete one set of 15 samples, two matrix blank fortifications, a matrix blank (untreated control water) and a reagent blank (HPLC water) was reported as *ca.* 2 calendar days to complete (p. 16 of MRID 46695526). Sample preparation required *ca.* 5 hours; LC/MS/MS analyses were run overnight.

V. References

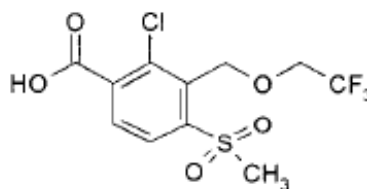
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**Tembotrione (AE 0172747)**

IUPAC Name: Not reported
CAS Name: 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione
CAS Number: 335104-84-2
SMILES String: Not found

**AE 0456148 (M6)**

IUPAC Name: Not reported
CAS Name: 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoic acid
CAS Number: 120100-77-8
SMILES String: Not found

**AE 1392936 (M2)**

IUPAC Name: Not reported
CAS Name: 2-Chloro-3-hydroxymethyl-4-mesylbenzoic acid
CAS Number: 120100-47-2
SMILES String: Not found

