

**Analytical method for tembotrione and its transformation products, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336<sup>1</sup>, in soil**

**Reports:** ECM: EPA MRID No.: 46695523. Netzband, D.J., J.M. Wade. 2005. AE 0172747: Analytical Method for the Determination of AE 0172747 and its Metabolites AE 0456148, AE 0968400, AE 1392936, AE 0941989 by LC/MS/MS And For The Determination Of AE 1124336 By GC/MSD In Soil. Report prepared by Bayer CropScience, Stillwell, Kansas, and sponsored and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 90 pages. Bayer Method No.: AE002-S04-03. Final report issued August 8, 2005.

ILV: EPA MRID No.: 46695525. Brumhard, B. 2005. Independent Laboratory Validation of Method AE002-S04-02 for the Determination of AE 0172747 and its Metabolites AE 0456148, AE 0968400, AE 1392936, AE 0941989 by HPLC-MS/MS and for the Determination of AE 1124336 by GC/MSD in Soil. Report prepared and sponsored by Bayer CropScience AG, Monheim am Rhein, Germany, and submitted by Bayer CropScience, Research Triangle Park, North Carolina; 57 pages. Bayer CropScience AG Report No: MR-181/04. Laboratory Project ID: P611040014. Final report issued July 13, 2005.

**Document No.:** MRIDs 46695523 & 46695525

**Guideline:** 850.6100

**Statements:** ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards, since it was not an experimental study (p. 3 of MRID 46695523). Signed and dated Data Confidentiality and GLP and statements were provided (pp. 2-3). The Quality Assurance and Authenticity statements were not included.

ILV: The study was conducted in compliance with OECD, USEPA FIFRA (40 CFR 160), German and Japanese GLP standards, with the exception of the recognized differences between the OECD and German and Japanese GLP principles/standards (p. 3; Appendix 1, pp. 56-57 of MRID 46695525). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5; Appendix 1, pp. 56-57). The statement of authenticity was not included.


**Classification:** This analytical method is classified as **unacceptable, but upgradeable**. In the ILV, linearity was not satisfactory for AE 0456148. In the ECM and ILV, the LOD of the method was not reported, and insufficient chromatographic support was provided for the method validation. In the ECM, no samples were prepared at 10×LOQ, and test soil matrices were not characterized. Also, in the ECM, linear regression curves and correlation coefficients were not provided for all analytes.

**PC Code:** 012801

---


<sup>1</sup> 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 include all the tembotrione ROCs.

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

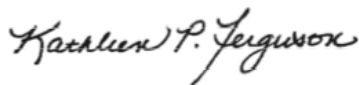
Signature:   
Date: 6/21/2021

**CDM/CSS-  
Dynamac JV**

Lisa Muto,  
Environmental Scientist

Signature:   
Date: 6/14/17

**Reviewers:** 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 AE002-S04-02/AE002-S04-03, is designed for the quantitative determination of tembotrione (AE 0172747) and its transformation products AE 0456148, AE 0968400, AE 1392936 and AE 0941989 using HPLC/MS/MS and its transformation product AE 1124336 using GC/MS in soil at the stated LOQ of 0.01 mg/kg. The LOQ is less than the lowest toxicological level of concern in soil for all analytes. The ILV validated the method with the first trial for AE 0172747, AE 0456148, AE 0968400, AE 1392936 and AE 0941989 with no modifications. The method was validated with the second trial for AE 1124336 with insignificant modifications to the sample processing instrumentation. Characterized silt loam and sandy loam soils from Germany were used in the ILV. All ILV data regarding repeatability, accuracy, and precision were satisfactory for all analytes; linearity was satisfactory for all analytes, except AE 0456148. In the ILV, the specificity of the method was supported by the LOQ representative chromatograms; however, no 10×LOQ chromatograms were provided. In the ECM, no samples were prepared at 10×LOQ, and test soil matrices were not characterized or described. An insufficient number of samples was prepared at 5×LOQ for all analyses. All ECM data regarding repeatability, accuracy and precision were satisfactory for all analytes, except for AE 1124336 in the Nebraska soil at 5×LOQ (RSD 25%). In the ECM, insufficient chromatographic support was provided for the method validation since no NE chromatograms were provided and no GC/MS control chromatograms were provided. Also, linear regression curves and correlation coefficients were not provided for all analytes. The LOD of the method was not reported in the ECM and ILV.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide <sup>1</sup>	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Tembotrione (AE 0172747)	46695523 <sup>2</sup>	46695525 <sup>3</sup>		Soil	08/08/2005 (Revision 3)	Bayer CropScience	LC/MS/MS	0.01 mg/kg
AE 0456148								
AE 0968400								
AE 1392936								
AE 0941989								
AE 1124336							GC/MS	

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 0968400 = 2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]phenyl; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid; AE 0941989 = 6-(Methylsulfonyl)-5-[(2,2,2-trifluoroethoxy)methyl]-3,4-dihydro-1H-xanthene-1,9(2H)-dione; and AE 1124336 = 2-Chloro-4-(methylsulfonyl)-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene.

2 In the ECM, soil types and characterization were not reported; specific sources were not reported (Appendix 3, p. 43 of MRID 46695523).

3 In the ILV, Höfchen Silt Loam Soil [4.3% sand, 76.3% silt 19.4% clay, pH 6.7 (in CaCl<sub>2</sub>), 1.58% organic matter] and Laacher Hof Sandy Loam Soil [69.7% sand 18.3% silt 12.0% clay, pH 6.8 (in CaCl<sub>2</sub>), 2.06% organic matter] were well-characterized (USDA soil texture characterization; p. 15; Tables 11-12, pp. 54-55 of MRID 46695525). Specific sources were not reported, but both soils were from Germany.

## I. Principle of the Method

Soil samples (10 ± 0.05 g) were placed in Milestone Ethos E Teflon pressure reactor vessels and fortified, if necessary, with the mixed fortification solution (pp. 13-15; Table 2, p. 28; Appendix 6, p. 89 of MRID 46695523). The samples were extracted twice with 50 mL of acetonitrile:0.1% acetic acid in water (1:1, v:v) via microwave extraction using a magnetic stirrer (Extraction 1: 5 min. at 80°C temperature with 600 max Watts and 20 stirrer speed; Extraction 2: 45 min. at 130°C temperature with 800 max Watts and 20 stirrer speed) and with temperature monitoring of the control sample. For each extraction, once the samples have cooled, the supernatant was transferred to a 250-mL graduated mixing cylinder via filtration through a Büchner funnel lined with a Whatman GF/A filter paper. For the first extraction after decanting the supernatant, the reaction vessel was rinsed with 50 mL of acetonitrile:0.1% acetic acid in water (1:1, v:v) with gentle swirling for 10-15 seconds, and the rinsate was added to the extract through the same Büchner funnel. For the second extraction after decanting the supernatant, the reaction vessel was rinsed with 50 mL of acetonitrile with gentle swirling for 10-15 seconds, and the rinsate was added to the extract through the same Büchner funnel. The same Büchner funnel was rinsed with additional 20 mL of acetonitrile. The volume of the combined extracts and rinsates was adjusted to 250 mL using acetonitrile. The method noted that this was a stopping point where samples could be capped and stored refrigerated until the procedure can continue. The method also noted that the microwave extraction procedure can cause degradation of the analyte if the two-step extraction procedure is not followed (p. 25).

For AE 0172747, AE 0456148, AE 0968400, AE 1392936, and AE 0941989, an aliquot (typically 100 mL) of the combined extracts and rinsates was transferred to a 200 mL Zymark evaporation

flask (p. 15; Appendix 6, p. 89 of MRID 46695523). After 2.0 mL of the 0.05 µg/mL deuterated internal standard was added, the sample was evaporated to about 25 to 35 mL using a Turbovap® II evaporator set to 50°C. The reduced volume was diluted to 100 mL using 0.1% acetic acid in DI water. After mixing, an aliquot was transferred via Acrodisc® 0.45 µm syringe filter to an autosampler vial for analysis by LC/MS/MS.

For AE 1124336 (incorrectly written as AE 1392936 in the study report), an aliquot (typically 25 mL) of the combined extracts and rinsates was transferred to a 200 mL Zymark evaporation flask (pp. 9, 16, 21; Appendix 6, p. 89 of MRID 46695523). The sample was evaporated to dryness using a Turbovap® II evaporator set to 50°C or a rotary evaporator (based on the ILV results, the ECM method noted that the TurboVap LV was not to be used). The residue was reconstituted in 10 mL of ethyl acetate. After mixing, an aliquot was transferred via Acrodisc® 0.45 µm syringe filter to an GC vial for analysis by GC/MS.

A procedure for analysis of application pads from terrestrial field dissipation studies was provided but not included in the method validation (pp. 16-17 of MRID 46695523).

Samples were analyzed for AE 0172747, AE 0456148, AE 0968400, AE 1392936, and AE 0941989 using a Shimadzu LC-10AD VP HPLC coupled with an Applied Biosystems API 3000 MS/MS (pp. 17-18; Table 2, p. 28; Appendix 1, pp. 30-34 of MRID 46695523). The following LC conditions were used: Waters SymmetryShield RP8 100Å column (150 mm x 3.0 mm, 5 µ; column temperature not reported), mobile phase of (A) acetonitrile and (B) 0.1% acetic acid [mobile gradient phase of percent A:B (v:v) at 0.00 min. 20:80, 14.00-14.99 min. 99:1, 15.00-19.00 min. 20:80], injection volume of 15 µL, and MRM with TurboIon Spray (500°C) in positive mode for AE 0941989 and deuterated AE 0941989 and negative mode for AE 0172747, AE 0456148, AE 0968400, AE 1392936 and their deuterated counterparts. One ion pair transition was monitored for each analyte:  $m/z$  438.97→402.93 for AE 0172747,  $m/z$  344.88→300.74 for AE 0456148,  $m/z$  316.93→139.99 for AE 0968400,  $m/z$  262.78→188.81 for AE 1392936, and  $m/z$  405.0→228.2 for AE 0941989. Observed retention times were 10.7, 14.2, 9.3, 13.1 and 8.2 minutes for AE 0172747, AE 0456148, AE 0968400, AE 1392936 and AE 0941989, respectively.

Samples were analyzed for AE 1124336 using an Agilent 6890 Series II GC coupled to an Agilent 5973 Mass Selective Detector (p. 18; Table 2, p. 28; Appendix 1, pp. 35-37 of MRID 46695523). The following GC conditions were used: HP Ultra 1 Methyl Siloxane column (12.0 m x 200 µm, 0.33 µm film thickness), temperature program (90°C initial temp to 215°C at 10.00°C/min. to 280°C at 60.00°C/min.), MS source temperature 230°C and injection volume of 2 µL. Three product ions were monitored for AE 1124336:  $m/z$  233.0,  $m/z$  249.0 and  $m/z$  251.0, as well as the total ion count (TIC). Observed retention time was 12.2 minutes for AE 1124336.

The ILV performed the ECM methods for each analyte as written, except that a vacuum rotary evaporator was used instead of a TurboVap LV in the sample processing of AE 1124336 (pp. 16-19 of MRID 46695525). The LC/MS/MS analysis was performed using a HP 1100 LC coupled to an IONICS EP 10+ with turbo-ionspray interface mass selective detector (MS/MS). The GC/MS analysis was performed using an Agilent 6890 Plus GC coupled with a 5973N mass selective detector. The columns for the LC and GC were the same as those of the ECM; other chromatographic parameters were also the same as those of the ECM.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 mg/kg for tembotrione and its transformation products, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 (p. 7 of MRID 46695523; pp. 8, 21 of MRID 46695525). In the ECM, the study authors reported that LOQs were calculated in a previous in-house method validation (Netzband, D.J., J.M. Wade. 2004.; pp. 7, 26 of MRID 46695523). The calculated LOQs for tembotrione, AE 0456148, AE 0968400, AE 1392936 and AE 0941989 were reportedly lower than the targeted LOQ, although the exact values were not reported. However, the calculated LOQ for AE 1124336 was 0.016 mg/kg. In the ECM and ILV, the Limit of Detection (LOD) was not reported.

## II. Recovery Findings

ECM (MRID 46695523): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD  $\leq$ 20%) for analysis of tembotrione, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 at fortification levels of 0.01 mg/kg (LOQ) and 0.05 mg/kg (5 $\times$ LOQ) in two soil matrices, except for the analysis of AE 1124336 at 5 $\times$ LOQ in the Nebraska soil (RSD 25%; Appendix 3, p. 43; DER Attachment 2). No samples were prepared at 10 $\times$ LOQ. An insufficient number of samples was prepared at 5 $\times$ LOQ for all analyses, n = 3. Data was from Bayer CropScience Study 03RAAEX022 (Netzband, D.J. and J.M. Wade. 2004) and Bayer CropScience Study 02DO36414 (Wyatt, D.R. 2002; pp. 8, 26; Appendix 3, p. 43). 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. Tembotrione (AE 0172747), AE 0456148, AE 0968400, AE 1392936 and AE 0941989 were identified using LC/MS/MS; one ion pair transition was monitored for each analyte. AE 1124336 was identified using GC/MS; three product ions were monitored for AE 1124336, but quantification was based on the total ion count (TIC). A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The soil types and characterization were not reported; specific sources were not reported (Appendix 3, p. 43).

ILV (MRID 46695525): Mean recoveries and RSDs were within guidelines for analysis of tembotrione, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 at fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 $\times$ LOQ) in two soil matrices (Tables 5-10, pp. 21-24; DER Attachment 2). The same identification methods were used as in the ECM. The Höfchen Silt Loam Soil [4.3% sand, 76.3% silt 19.4% clay, pH 6.7 (in CaCl<sub>2</sub>), 1.58% organic matter] and Laacher Hof Sandy Loam Soil [69.7% sand 18.3% silt 12.0% clay, pH 6.8 (in CaCl<sub>2</sub>), 2.06% organic matter] were well-characterized (USDA soil texture characterization; p. 15; Tables 11-12, pp. 54-55). Specific sources were not reported. The method was validated with the first trial for tembotrione (AE 0172747), AE 0456148, AE 0968400, AE 1392936 and AE 0941989 with no modifications (pp. 8, 19). The method was validated with the second trial for AE 1124336 with insignificant modifications to the sample processing instrumentation.

**Table 2. Initial Validation Method Recoveries for Tembotrione, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 in Soil<sup>1,2,3,4</sup>**

Analyte <sup>1</sup>	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Nebraska Soil</b>						
Tembotrione (AE 0172747)	0.01 (LOQ)	7	71-86	80	5	7
	0.05	<b>3</b>	79-95	89	9	10
AE 0456148	0.01 (LOQ)	7	93-101	96	3	3
	0.05	<b>3</b>	71-104	88	17	19
AE 0968400	0.01 (LOQ)	7	91-111	99	7	7
	0.05	<b>3</b>	100-116	107	8	8
AE 1392936	0.01 (LOQ)	7	84-99	91	4	5
	0.05	<b>3</b>	96-103	100	4	4
AE 0941989	0.01 (LOQ)	7	78-93	86	6	7
	0.05	<b>3</b>	94-101	96	4	4
AE 1124336	0.01 (LOQ)	7	73-98	88	10	11
	0.05	<b>3</b>	68-109	86	21	<b>25</b>
<b>New York Soil</b>						
Tembotrione (AE 0172747)	0.01 (LOQ)	7	73-94	86	7	9
	0.05	<b>3</b>	76-90	83	7	8
AE 0456148	0.01 (LOQ)	7	91-107	99	6	6
	0.05	<b>3</b>	96-102	100	3	3
AE 0968400	0.01 (LOQ)	7	89-99	94	4	5
	0.05	<b>3</b>	92-99	96	4	4
AE 1392936	0.01 (LOQ)	7	85-109	92	8	9
	0.05	<b>3</b>	78-101	91	12	13
AE 0941989	0.01 (LOQ)	7	65-84	75	6	8
	0.05	<b>3</b>	68-98	84	15	18
AE 1124336	0.01 (LOQ)	7	69-111	91	14	16
	0.05	<b>3</b>	88-102	97	8	8

Data (uncorrected recovery results; Appendix 4, pp. 44-45) were obtained from Appendix 3, p. 43 of MRID 46695523 and DER Attachment 2. Data was from Bayer CropScience Study 03RAAEX022 (Netzband, D.J. and J.M. Wade. 2004) and Bayer CropScience Study 02DO36414 (Wyatt, D.R. 2002; pp. 8, 26; Appendix 3, p. 43).

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 0968400 = 2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]phenyl; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid; AE 0941989 = 6-(Methylsulfonyl)-5-[(2,2,2-trifluoroethoxy)methyl]-3,4-dihydro-1H-xanthene-1,9(2H)-dione; and AE 1124336 = 2-Chloro-4-(methylsulfonyl)-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene.

2 The soil types and characterization were not reported; specific sources were not reported (Appendix 3, p. 43).

3 One ion pair transition was monitored for each analyte:  $m/z$  438.97→402.93 for AE 0172747,  $m/z$  344.88→300.74 for AE 0456148,  $m/z$  316.93→139.99 for AE 0968400  $m/z$  262.78→188.81 for AE 1392936, and  $m/z$  405.0→228.2 for AE 0941989. Three product ions were monitored for AE 1124336:  $m/z$  233.0,  $m/z$  249.0 and  $m/z$  251.0, as well as the total ion count (TIC).

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, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 in Soil<sup>1,2,3</sup>**

Analyte <sup>1</sup>	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) <sup>4</sup>	Relative Standard Deviation (%)
<b>Höfchen Silt Loam Soil</b>						
Tembotrione (AE 0172747)	0.01 (LOQ)	5	71-106	94	14	14.9
	0.1	5	95-100	96	2	2.2
AE 0456148	0.01 (LOQ)	5	93-114	106	8	8.0
	0.1	5	99-114	107	6	5.5
AE 0968400	0.01 (LOQ)	5	96-114	106	7	7.0
	0.1	5	95-103	100	3	3.2
AE 1392936	0.01 (LOQ)	5	97-107	103	4	3.9
	0.1	5	95-103	99	3	2.0
AE 0941989	0.01 (LOQ)	5	84-102	96	7	7.4
	0.1	5	84-97	93	5	5.7
AE 1124336	0.01 (LOQ)	5	69-82	77	5	6.7
	0.1	5	100-111	107	4	4.0
<b>Laacher Hof Sandy Loam Soil</b>						
Tembotrione (AE 0172747)	0.01 (LOQ)	5	96-114	106	7	6.4
	0.1	5	96-101	98	2	2.2
AE 0456148	0.01 (LOQ)	5	85-113	104	12	11.1
	0.1	5	97-109	102	5	4.8
AE 0968400	0.01 (LOQ)	5	97-115	104	8	7.6
	0.1	5	97-105	102	4	3.6
AE 1392936	0.01 (LOQ)	5	93-113	103	8	7.5
	0.1	5	95-103	99	3	3.1
AE 0941989	0.01 (LOQ)	5	100-107	102	3	3.0
	0.1	5	95-100	97	2	2.0
AE 1124336	0.01 (LOQ)	5	81-92	87	5	5.9
	0.1	5	101-117	107	6	5.9

Data (uncorrected recovery results; pp. 18-19) were obtained from Tables 5-10, pp. 21-24 of MRID 46695525 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 0968400 = 2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]phenyl; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid; AE 0941989 = 6-(Methylsulfonyl)-5-[(2,2,2-trifluoroethoxy)methyl]-3,4-dihydro-1H-xanthene-1,9(2H)-dione; and AE 1124336 = 2-Chloro-4-(methylsulfonyl)-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene.

2 The Höfchen Silt Loam Soil [4.3% sand, 76.3% silt 19.4% clay, pH 6.7 (in CaCl<sub>2</sub>), 1.58% organic matter] and Laacher Hof Sandy Loam Soil [69.7% sand 18.3% silt 12.0% clay, pH 6.8 (in CaCl<sub>2</sub>), 2.06% organic matter] were well-characterized (USDA soil texture characterization; p. 15; Tables 11-12, pp. 54-55). Specific sources were not reported, but both soils were from Germany.

3 One ion pair transition was monitored for each analyte: *m/z* 438.97→402.93 for AE 0172747, *m/z* 344.88→300.74 for AE 0456148, *m/z* 316.93→139.99 for AE 0968400 *m/z* 262.78→188.81 for AE 1392936, and *m/z* 405.0→228.2 for AE 0941989. Three product ions were monitored for AE 1124336: *m/z* 233.0, *m/z* 249.0 and *m/z* 251.0, as well as the total ion count (TIC).

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



### III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 mg/kg for tembotrione and its transformation products, AE 0456148, AE 0968400, AE 1392936, AE 0941989 and AE 1124336 (p. 7 of MRID 46695523; pp. 8, 21 of MRID 46695525). In the ECM, the study authors reported that LOQs were calculated in a previous in-house method validation (Netzband, D.J., J.M. Wade. 2004.; pp. 7, 26 of MRID 46695523). The calculated LOQs for tembotrione, AE 0456148, AE 0968400, AE 1392936 and AE 0941989 were reportedly lower than the targeted LOQ, although the exact values were not reported. However, the calculated LOQ for AE 1124336 was 0.016 mg/kg. No justifications, calculations or comparisons to background levels were reported to support the method LOQ; the calculation method for the calculated LOQs was not reported in this study. In the ECM and ILV, the LOD for the method was not reported.

**Table 4. Method Characteristics**

Analyte <sup>1</sup>	Tembotrione (AE 0172747)	AE 0456148	AE 0968400	AE 1392936	AE 0941989	AE 1124336
Limit of Quantitation (LOQ)	0.01 mg/kg					
Limit of Detection (LOD)	<b>Not reported</b>					
Linearity (calibration curve $r^2$ and concentration range)	ECM <sup>2</sup>	$r^2 = 0.9995$	<b>Not reported</b>			$r^2 = 0.9966$
	Range:	0.0-2.0 ng/mL	Not applicable			0.25-5.00 ng/mL
	ILV <sup>3</sup>	$r^2 = 0.9990$	$r^2 = 0.9947$	$r^2 = 0.9982$	$r^2 = 0.9996$	$r^2 = 0.9998$
	Range:	0.05-5.0 ng/mL				0.5-5.0 ng/mL
Repeatable	ECM <sup>4,5</sup>	No sample were prepared at 10×LOQ.				Yes at LOQ. Yes at 5×LOQ in NY soil, but n = <b>3</b> . <b>No</b> at 5×LOQ in NE soil.
		Yes at LOQ. Yes at 5×LOQ, but n = <b>3</b> .				
	ILV <sup>4,6,7</sup>	Yes at LOQ and 10×LOQ.				
Reproducible	Yes at LOQ. <b>Could not be determined</b> for 10×LOQ; only one set of performance data was submitted.					
Specific	ECM	Minor baseline noise interfered with peak integration at the LOQ.	A nearby minor contaminant peak (5-10% of the LOQ, based on peak height) interfered with peak integration at the LOQ. <sup>8</sup>	No matrix interferences were observed.	Minor baseline noise interfered with peak integration at the LOQ.	No control chromatogram was provided. No matrix interferences were observed at LOQ.
	ILV	Only chromatograms for the NY soil were provided. Yes, matrix interferences were <5% of the LOQ (based on peak area). No 10×LOQ chromatograms were provided.				

Data were obtained from pp. 7, 12-13; Appendix 3, p. 43 (recovery results); Appendix 4, pp. 46-47 (calibration curves); Appendix 5, Figures 7-9, pp. 73-88 (chromatograms) of MRID 46695523; pp. 8, 20; Tables 5-10, pp. 21-24 (recovery results); Figures 1-6, pp. 29-30 (calibration curves); Figures 8-16, pp. 35-53 (chromatograms) of MRID 46695525; 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 0968400 = 2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]phenyl; AE 1392936 = 2-Chloro-3-hydroxyethyl-4-mesylbenzoic acid; AE 0941989 = 6-(Methylsulfonyl)-5-[(2,2,2-trifluoroethoxy)methyl]-3,4-dihydro-1H-xanthene-1,9(2H)-dione; and AE 1124336 = 2-Chloro-4-(methylsulfonyl)-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene.
- 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 (pp. 12-13; Appendix 5, Figures 7-9, pp. 73-88 of MRID 46695523; 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. 20; Figures 1-6, pp. 29-30 of MRID 46695525; DER Attachment 2).
- 4 Tembotrione (AE 0172747), AE 0456148, AE 0968400, AE 1392936 and AE 0941989 were identified using LC/MS/MS; one ion pair transition was monitored for each analyte. AE 1124336 was identified using GC/MS; three product ions were monitored for AE 1124336, but quantification was based on the total ion count (TIC). A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.
- 5 In the ECM, soil types and characterization were not reported; specific sources were not reported (Appendix 3, p. 43 of MRID 46695523).
- 6 In the ILV, Höfchen Silt Loam Soil [4.3% sand, 76.3% silt 19.4% clay, pH 6.7 (in CaCl<sub>2</sub>), 1.58% organic matter] and Laacher Hof Sandy Loam Soil [69.7% sand 18.3% silt 12.0% clay, pH 6.8 (in CaCl<sub>2</sub>), 2.06% organic matter] were well-characterized (USDA soil texture characterization; p. 15; Tables 11-12, pp. 54-55 of MRID 46695525). Specific sources were not reported, but both soils were from Germany.
- 7 The method was validated with the first trial for tembotrione (AE 0172747), AE 0456148, AE 0968400, AE 1392936 and AE 0941989 with no modifications (pp. 8, 19 of MRID 46695525). The method was validated with the second trial for AE 1124336 with insignificant modifications to the sample processing instrumentation.
- 8 Based on Figure 8, p. 79 of MRID 46695523.  
Linearity is satisfactory when  $r^2 \geq 0.995$ .

#### IV. Method Deficiencies and Reviewer's Comments

1. The following deficiencies were noted in the ILV MRID 46695525:

Linearity was not satisfactory for AE 0456148 ( $r^2 = 0.9947$ ); linearity is satisfactory when  $r^2 \geq 0.995$  (Figures 1-6, pp. 29-30).

Insufficient chromatographic support was provided for the method validation since no 10×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.

2. The following deficiencies were noted in the ECM MRID 46695523:

No samples were prepared at 10×LOQ, and an insufficient number of samples was prepared at 5×LOQ for all analyses, n = 3 (Appendix 3, p. 43; DER Attachment 2). OCSPP Guideline 850.6100 criteria states that a minimum of five spiked replicates were analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

Performance data for AE 1124336 in the Nebraska soil did not meet OCSPP guidelines requirements for repeatability at 5×LOQ (RSD was 25%; Appendix 3, p. 43; DER

Attachment 2). OCSPP Guideline 850.6100 criteria for precision states that RSDs for replicates at each spiking level are  $\leq 20\%$ .

The specific type and characterization of the ECM test soil matrices were not reported (Appendix 3, p. 43 of MRID 46695523).

Representative linear regression curves and correlation coefficients were not provided for AE 0456148, AE 0968400, AE 1392936 and AE 0941989.

Insufficient chromatographic support was provided for the method validation since no NE chromatograms were provided and no GC/MS control 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.

A nearby minor contaminant peak (5-10% of the LOQ, based on peak height) interfered with peak integration of AE 0456148 at the LOQ (Figure 8, p. 79 of MRID 46695523).

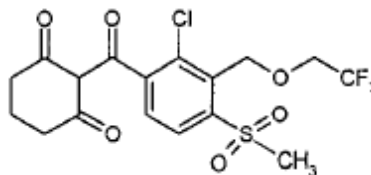
3. The determination of the LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 7 of MRID 46695523; pp. 8, 21 of MRID 46695525). In the ECM, the study authors reported that LOQs were calculated in a previous in-house method validation (Netzband, D.J., J.M. Wade. 2004.; pp. 7, 26 of MRID 46695523). The calculated LOQs for tembotrione, AE 0456148, AE 0968400, AE 1392936 and AE 0941989 were reportedly lower than the targeted LOQ, although the exact values were not reported. No justifications, calculations or comparisons to background levels were reported to support the method LOQ; the calculation method for the calculated LOQs was not reported in this study. No method LOD was reported in the ECM or ILV.
4. The ILV was provided Bayer Method AE002-S04-02 as the ECM (p. 9 of MRID 46695525). Bayer revised the original method to reflect the findings of the ILV in Bayer Method AE002-S04-03 (MRID 46695523; p. 21 of MRID 46695523).
5. The reviewer noted the following typographical error in the ECM: AE 1124336 was incorrectly written as AE 1392936 in the analytical procedure of the study report (pp. 16-18 of MRID 46695523).
6. The communications between the ILV and validation laboratory Study Director and Study Monitor were reported (pp. 25-26 of MRID 46695525). The ILV reported that emails were exchanged regarding study progress and clarification of some details of the equipment used (HPLC column and syringe filter); no contact relative to the performance of the study occurred.
7. In the ILV, the total time required to complete one set of 12 samples was reported as *ca.* 2 calendar days to complete (p. 24 of MRID 46695525). Sample preparation required *ca.* 4 hours plus 2 hours for preparation of GC/MS subsamples; LC/MS/MS and GC/MS analyses were run overnight.

## **V. References**

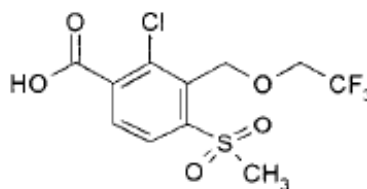
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition 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)**

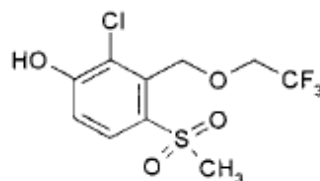
**IUPAC Name:** Not reported  
**CAS Name:** 2-[2-Chloro-4-mesyl-3-((2,2,2-trifluoroethoxy)methyl)benzoyl]cyclohexane-1,3-dione  
**CAS Number:** 335104-84-2  
**SMILES String:** Not found

**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

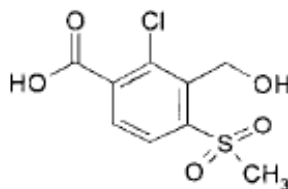
**AE 0968400 (M1)**

**IUPAC Name:** Not reported  
**CAS Name:** 2-Chloro-4-(methylsulfonyl)-3-[(2,2,2-trifluoroethoxy)methyl]phenyl  
2-Chloro-4-(mesyl)-3-[(2,2,2-trifluoroethoxy)methyl]phenol  
**CAS Number:** Not reported  
**SMILES String:** Not found

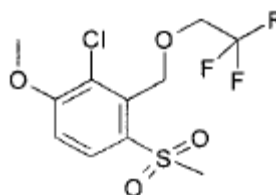


**AE 1392936 (M2)**

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

**AE 1124336 (M7)**

**IUPAC Name:** Not reported  
 2-Chloro-4-(methylsulfonyl)-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene  
**CAS Name:** 2-Chloro-4-mesyl-1-methoxy-3-[(2,2,2-trifluoroethoxy)methyl]benzene  
**CAS Number:** Not reported  
**SMILES String:** Not found

**AE 0941989 (M3)**

**IUPAC Name:** Not reported  
 6-(Methylsulfonyl)-5-[(2,2,2-trifluoroethoxy)methyl]-3,4-dihydro-1H-xanthene-1,9(2H)-dione  
**CAS Name:** 6-Mesyl-5-(2,2,2-trifluoro-ethoxymethyl)-3,4-dihydro-2H-xanthene-1,9-dione  
**CAS Number:** Not reported  
**SMILES String:** Not found

