Analytical methods for tau-Fluvalinate and its metabolites ACBA, Diacid, PBA, RCAA, and Haloaniline in soil, thatch and foliage

Reports: ECM: EPA MRID No.: 50552102. Welch, A., and Y.H. Rezenom. 2018. Analytical Method Validation for the Determination of tau-Fluvalinate and its Metabolites in Soils and Turf. Study No.: 4548. Unpublished study performed, sponsored, and submitted by Wellmark International (Central Life Sciences), Dallas, Texas; 377 pages. Final report issued March 23, 2018.

ILV: EPA MRID No. 50552102 (Appendix 2, pp. 137-375). Wu, X. 2016. Independent Laboratory Validation (ILV) of the Analytical Method for Determination of Tau-Fluvalinate and its Degradates in Soil, Thatch and Foliage by LC/MS/MS and GC/MS. Smithers Viscient Study No.: 14081.6103. Report prepared by Smithers Viscient, Wareham, Massachusetts; sponsored and submitted by Central Life Sciences (Wellmark International), Dallas, Texas 239 pages. Final report issued March 17, 2016.

Document No.: MRID 50552102

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practices (GLP) standards (40 CFR Part 160), except that data was not always recorded as specified in Part 160.130 (e; p. 3 of MRID 50552102). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). An Authenticity statement was not included.
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR Part 160; Appendix 2, p. 139 of MRID 50552102). Signed and dated GLP and Quality Assurance statements were provided; however, a Central Life Sciences representative did not sign the GLP statement (Appendix 2, pp. 139-140). A Data Confidentiality statement was included, but not signed (Appendix 2, p. 138). An Authenticity statement was not included.

Classification: This analytical method is classified as supplemental, non-quantifiable. The method could not be validated for ACBA in foliage since the LOQ of the ECM (200 µg/kg) was differed from that of the ILV (20 µg/kg). It could not be determined if the ILV was conducted independently of the ECM. The reproducibility of the method was not supported by ECM and ILV performance data for the following analyses: tau-fluvalinate in soil at the LOQ and in thatch and foliage at 10×LOQ, haloaniline in soil at 10×LOQ, and PBA in thatch at the LOQ due to ECM/ILV performance data. ECM and/or ILV linearity was not satisfactory for tau-fluvalinate and haloaniline in all matrices, RCAA in thatch and foliage, and diacid and PBA in foliage. The specificity of the method was not supported by ILV and ECM representative chromatograms of ACBA in foliage. It could not be determined if the ILV was provided with the most difficult matrix with

	which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. An insufficient number of samples were prepared for ECM analyses of PBA in all matrices.		
PC Code:	109302		
EFED Final Reviewer:	Sheng Lin, Ph.D., Physical Chemist	Signature: Date: 1/15/20	SHENG LIN Digitally signed by SHENG LIN Date: 2021.01.05 13:04:45 -05'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date:	Lisa Muto 04/08/2019
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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Wellmark International Study No. 4548, is designed for the quantitative determination of tau-fluvalinate and its metabolites 2-(2- chloro-4-carboxyl)anilino-3-methylbutanoic acid (diacid), 3-phenoxybenzoic acid (PBA), and 2-(2-chloro-4-trifluoromethyl) anilino-3-methylbutanoic acid (RCAA) in soil at the LOQ of 5 µg/kg, thatch at the LOQ of 10 µg/kg, and foliage at the LOQ of 20 µg/kg using LC/MS/MS, its metabolite 2-chloro-4-trifluoromethylaniline (haloaniline) in soil at the LOQ of 5 µg/kg, thatch at the LOQ of 10 µg/kg, and foliage at the LOQ of 20 µg/kg using GC/MS, and its metabolite 4-amino-3-chlorobenzoic acid (ACBA) in soil at the LOQ of 5 µg/kg, thatch at the LOQ of 10 µg/kg, and foliage at the LOQ of 200 µg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil, thatch, and foliage for all analytes. **The method could not be validated for ACBA in foliage since the LOQ of the ECM (200 µg/kg) differed from that of the ILV (20 µg/kg).** The ECM and ILV each used one soil, thatch, and foliage matrix set; characterization data was reported in the ECM, but not in the ILV. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and that the ILV soil matrix covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the method for all analytes in soil, thatch, and foliage in the first trial. The ECM was performed as written, except for the significant modification of the validation of the method for ACBA at the LOQ of 20 µg/kg in foliage, minor modifications of use of centrifugation and the internal standard for soil, and minor modifications of the LC/MS and GC/MS instrument and parameters also occurred based on available equipment. **It could not be determined if the ILV was conducted independently of the ECM.** The reproducibility for tau-fluvalinate was only acceptable at 10×LOQ in soil and the LOQ in thatch and foliage. The linearity for tau-fluvalinate was only acceptable for the ECM soil and foliage analyses. The reproducibility for haloaniline

was acceptable in soil, thatch and foliage, except for ECM data at 10×LOQ in soil. The linearity for haloaniline was only acceptable for the ILV thatch and foliage analyses. All submitted data for tau-fluvalinate and haloaniline pertaining to specificity was acceptable in soil, thatch and foliage. All submitted data for diacid, PBA, and RCAA pertaining to reproducibility, linearity, and specificity was acceptable in soil and thatch, except for the reproducibility of PBA in thatch at 10×LOQ and the ILV linearity of RCAA in thatch. All submitted data for diacid, PBA, and RCAA pertaining to reproducibility and specificity was acceptable in foliage; ILV linearity was unacceptable. An insufficient number of samples were prepared for ECM analyses of PBA in all matrices. All submitted data for ACBA pertaining to reproducibility, linearity, and specificity was acceptable in soil and thatch; the specificity of the method was not supported by ILV and ECM representative chromatograms of ACBA in foliage due to a significant contaminant near the analyte retention time. Data for precision and repeatability for each analyte/matrix are reported in **Table 4a-c**.

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						
tau-Fluvalinate	50552102[2]	Appendix 2 of 50552102[3]		Soil	23/03/2018	Wellmark International	LC/MS/MS	5 µg/kg
ACBA								
Diacid								
PBA								
RCAA								
Haloaniline							GC/MS	
tau-Fluvalinate				Thatch			LC/MS/MS	10 µg/kg
ACBA								
Diacid								
PBA								
RCAA								
Haloaniline							GC/MS	
tau-Fluvalinate				Foliage			LC/MS/MS	20 µg/kg
ACBA								200 µg/kg (ECM) 20 µg/kg (ILV)
Diacid								20 µg/kg
PBA								
RCAA								
Haloaniline							GC/MS	

1 4-Amino-3-chlorobenzoic acid (ACBA), 2-(2-chloro-4-carboxyl)anilino-3-methylbutanoic acid (Diacid), 3-phenoxybenzoic acid (PBA; 3-PB acid), 2-(2-chloro-4-trifluoromethyl) anilino-3-methylbutanoic acid (RCAA; anilino acid), and 2-chloro-4-trifluoromethylaniline (Haloaniline; Table 1, p. 14 of MRID 50552102).

2 Diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid.

3 In the ECM, the sandy loam or sandy clay loam soil [55% sand, 24% silt, 21% clay; pH 6.4 (method not reported), 1.9% organic matter, taxonomic classification of Marcum – smectitic, thermic, Typic Argixeroll] was obtained from Sutter County, California (EPA Region 10), and used in the study (USDA soil texture classification as sandy clay loam; see Reviewer's Comment #6; p. 15; Appendix 4, p. 377 of MRID 50552102). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil, foliage, and thatch samples were ID No.s S-14-06976, S-14-06977, and S-14-06978, respectively.

4 In the ILV, the soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357 of MRID 50552102). The soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV.

I. Principle of the Method

Soil

Soil samples (10 g) were weighed in a 50 mL QuEChERS type centrifuge tube (VWR, part # 82050-320) and fortified with 25 µL of the 2 µg/mL or 20 µg/mL mixed-spiking solution (pp. 13, 15-17 of MRID 50552102). After one hour to equilibration, 5 mL of acetonitrile:water (90:10, v:v) with 0.5% formic acid and 20 µL internal standard (triphenyl phosphate, TPP) were added. The sample tubes were then mixed thoroughly and centrifuged for 10 minutes at 4000 rpm. The supernatant was filtered using a syringe filter with a pore size of 0.45 µm. The filtrate was assayed twice using LC-MS/MS for diacid, PBA, RCAA, and tau-fluvalinate and using GC/MS for haloaniline. ACBA was fortified separately using a 2.072 µg/mL or 20.72 µg/mL spiking solution in exact same way as the mixed standards to prepare two sets of five replicate samples at LOQ and 10× LOQ. The prepared samples were analyzed as duplicates using LC-MS/MS. Matrix-matched calibration solutions were used for all analyses.

Thatch

Thatch samples (10 g) were weighed into 50 mL QuEChERS type centrifuge tube (VWR, part # 82050-320) and fortified with 50 µL of 2 µg/mL or 20 µg/mL mixed-spiking solution (pp. 13, 15-16, 21 of MRID 50552102). The samples were allowed to sit for 30 minutes to 1 hour before 10 mL of acetonitrile:water (90:10, v:v) with 0.5% formic acid was added. The samples were spiked with 20 µL of internal standard and centrifuged for 10 minutes at 4000 rpm. After filtering the samples using 13 mm syringe filter with a pore size of 0.45 µm, the filtered extract was analyzed twice using LC-MS/MS for diacid, PBA, RCAA, and tau-fluvalinate and using GC/MS for haloaniline. ACBA was fortified separately using a 2.072 µg/mL or 20.72 µg/mL spiking solution in exact same way as the mixed standards to prepare two sets of five replicate samples at LOQ and 10× LOQ. The prepared samples were analyzed as duplicates using LC-MS/MS. Matrix-matched calibration solutions were used for all analyses.

Foliage

Foliage samples (5 g) were weighed into 50 mL QuEChERS type centrifuge tube (VWR, part # 82050-320) and fortified with 50 µL of 2 µg/mL or 20 µg/mL mixed-spiking solution (pp. 13, 16, 21-22 of MRID 50552102). After the samples were let to sit for 1 hour, 10 mL acetonitrile:water (90:10, v:v) with 0.5% formic acid was added. After addition of 20 µL of internal standard, the samples were centrifuged for 10 minutes at 4000 rpm. The upper layer was filtered using a syringe filter with a pore size of 0.45 µm prior to LC-MS/MS and GC/MS. All samples were run as duplicate. ACBA was fortified separately using a 2.072 µg/mL or 20.72 µg/mL spiking solution in exact same way as the mixed standards to prepare two sets of five replicate samples at

LOQ and 10× LOQ. The prepared samples were analyzed as duplicates using LC-MS/MS. Matrix-matched calibration solutions were used for all analyses.

LC/MS/MS

Tau-Fluvalinate and its metabolites ACBA, diacid, PBA, PB aldehyde, and RCAA and the internal standard TPP were identified and quantified by LC/MS using an Agilent series 1200 HPLC system coupled to an Agilent G6410 triple quadrupole mass spectrometer (pp. 13, 22-23 of MRID 50552102). The following conditions were employed for all analytes: Phenomenex Luna C18 (2) column (3.0 × 150 mm, 5 µm; column temperature 35°C) eluted with a gradient mobile phase of A) 0.1% acetic acid in water and B) 0.1% acetic acid in acetonitrile [time, percent A:B; 0.00 min. 55:45, 24.00-26.00 min. 5:95, 28.00-32.00 min. 55:45] and injection volume of 30 µL; and positive ESI ionization MRM scan mode at 350°C sheath gas temperature. ACBA was identified using one ion transition: *m/z* 170→126.0; no additional fragment ion was detected to be used as confirmation ion. Other analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 503.2→180.9 and *m/z* 503.2→208.1 for tau-fluvalinate, *m/z* 270→154.9 and *m/z* 270→146.1 for diacid, *m/z* 213.1→93.1 and *m/z* 213.1→169.1 for PBA, *m/z* 199.1→171.1 and *m/z* 199.1→153.3 for PB aldehyde, *m/z* 294.1→145.1 and *m/z* 294.1→127.2 for RCAA, and *m/z* 327.2→77.1 and *m/z* 327.2→153.1 for TPP. Expected retention times were *ca*. 22.3, 1.9, 3.0, 5.9, 9.2, 11.2, and 12.3 minutes for tau-fluvalinate, ACBA, diacid, PBA, PB aldehyde, RCAA, and TPP, respectively.

PB aldehyde was found to be unstable in soil and thatch, degrading to PBA; therefore, analyses targeted PBA rather than PB aldehyde (p. 17 of MRID 50552102). Similarly, diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid. Based on preliminary experiments, analyses of PB aldehyde in all matrices and diacid in soil were excluded from the study due to instability (pp. 25-27).

GC/MS

Haloaniline identified and quantified by Agilent series 7890B gas chromatograph equipped with an Agilent series 5977A mass selective detector (pp. 13, 23 of MRID 50552102). The following conditions were employed for all analytes: DB-5 MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness), temperature program (70°C for 1 min., 5°C/min. to 200°C for 5 min., 40°C/min. to 300°C for 10 min.), helium carrier gas, and injection volume of 2.0 µL; and positive ESI ionization SIM scan mode at 230°C MS source temperature. Haloaniline was identified using one ion: *m/z* 195. Expected retention time was *ca*. 11.7 minutes for haloaniline.

ILV

In the ILV, the ECM was performed as written, except for the significant modification of the validation of the method for ACBA at the LOQ of 20 µg/kg in foliage, as well as minor modifications of centrifugation speed, addition of second centrifugation (13,000 rpm for 5 minutes) after extraction for thatch and foliage samples, omission of internal standard for soil and thatch matrices, and minor LC/MS (AB Sciex API 5000 MS equipped with am AB Sciex Turbo V ESI Ion Spray source) and GC/MS (Agilent series 6890 gas chromatograph equipped

with an Agilent series 5975 mass selective detector) instrument and parameter modifications (omission of confirmation MS ion transition for PBA; Appendix 2, pp. 160, 164-170 of MRID 50552102). PB aldehyde was also excluded from the study. The LC/MS and GC/MS conditions were the same as the ECM. For LC/MS, ACBA and PBA were identified using one ion transition: *m/z* 170.00→126.00 for ACBA and *m/z* 213.10→93.10 for PBA; other analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 503.20→180.90 and *m/z* 503.20→208.10 for tau-fluvalinate, *m/z* 270.00→154.90 and *m/z* 270.00→146.10 for diacid, *m/z* 294.10→145.10 and *m/z* 294.10→127.20 for RCAA, and *m/z* 327.20→77.10 and *m/z* 327.20→153.10 for TPP. Expected retention times were *ca*. 20.9, 1.8, 2.8, 5.1, 9.8, and 10.8 minutes for tau-fluvalinate, ACBA, diacid, PBA, RCAA, and TPP, respectively. For GC/MS, haloaniline was identified using one ion: *m/z* 195.00; expected retention time was *ca*. 9.8 minutes for haloaniline. TPP was also analyzed via GC/MS: *m/z* 326.10 (SIM) and 34.5 minutes (RT).

LOQ/LOD

In the ECM, the Limits of Quantification (LOQs) for tau-fluvalinate, diacid, PBA, RCAA, and haloaniline were 5 µg/kg in soil, 10 µg/kg in thatch, and 20 µg/kg in foliage (pp. 27-28; Appendix 2, pp. 173-175 of MRID 50552102). The LOQs of the metabolite ACBA were 5 µg/kg in soil, 10 µg/kg in thatch, and 200 µg/kg in foliage. In the ILV, the LOQs for all analytes were 5 µg/kg in soil, 10 µg/kg in thatch, and 20 µg/kg in foliage. In the ECM, the Limits of Detection (LODs) were estimated to be one-third of the LOQ, about 3 µg/L in all matrices, except for ACBA in foliage, where LOD was 30 µg/L. For tau-fluvalinate, diacid, PBA, RCAA, and haloaniline, these values corresponded to 1.5 µg/kg in soil, *ca*. 3.3 µg/kg in thatch, and *ca*. 6.6 µg/kg in foliage. For ACBA, these values corresponded to 1.5 µg/kg in soil, *ca*. 3.3 µg/kg in thatch, and *ca*. 66 µg/kg in foliage. In the ILV, the calculated LODs ranged 0.00500-0.500 µg/kg for soil, 0.0700-4.00 µg/kg for thatch, and 0.0400-8.00 µg/kg for foliage.

II. Recovery Findings

ECM (MRID 50552102): For soil samples, mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of tau-fluvalinate, ACBA, PBA, RCAA, and haloaniline at fortification levels of 5 µg/kg (LOQ) and 50 µg/kg (10×LOQ), except for tau-fluvalinate at the LOQ (RSD 26%) and haloaniline at 10×LOQ (mean 123%, RSD 36%; Tables 14-30, pp. 34-50; DER Attachment 2). Diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid (p. 17). For thatch samples, mean recoveries and RSDs were within guideline requirements for analysis of tau-fluvalinate, ACBA, diacid, PBA, RCAA, and haloaniline at fortification levels of 10 µg/kg (LOQ) and 100 µg/kg (10×LOQ), except for PBA at the LOQ (mean 129%). For foliage samples, mean recoveries and RSDs were within guideline requirements for analysis of tau-fluvalinate, diacid, PBA, RCAA, and haloaniline at fortification levels of 20 µg/kg (LOQ) and 200 µg/kg (10×LOQ), except for tau-fluvalinate at 10×LOQ (mean 66%). Mean recoveries and RSDs for analysis of ACBA in foliage were within guideline requirements at fortification levels of 200 µg/kg (LOQ); no samples were prepared at 2000 µg/kg (10×LOQ). ACBA could not be detected in foliage samples prepared at 20 µg/kg. An insufficient number of samples were prepared for analyses of PBA in all matrices, n = 3. For tau-fluvalinate at the LOQ and haloaniline at 10×LOQ in soil, recovery statistics were reviewer-calculated since the study authors only based the statistics on n = 3 or 4, due to deeming recovery values as outliers which were not included in statistics. The reviewer calculated statistics using all 5 recovery values. One or two ion transitions were used to identify tau-fluvalinate, ACBA, diacid, PBA, and RCAA via LC/MS/MS, but results were only provided for the quantitation ion transition. One ion was used to quantify haloaniline via GC/MS. A confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. The sandy loam or sandy clay loam soil [55% sand, 24% silt, 21% clay; pH 6.4 (method not reported), 1.9% organic matter, taxonomic classification of Marcum – smectitic, thermic, Typic Argixeroll] was obtained from Sutter County, California (EPA Region 10), and used in the study (USDA soil texture classification as sandy clay loam; see Reviewer's Comment #6; p. 15; Appendix 4, p. 377 of MRID 50552102). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil, foliage, and thatch samples were ID No.s S-14-06976, S-14-06977, and S-14-06978, respectively.

ILV (Appendix 2 of MRID 50552102): For soil samples, mean recoveries and RSDs were within guideline requirements for analysis of tau-fluvalinate, ACBA, PBA, RCAA, and haloaniline at fortification levels of 5 µg/kg (LOQ) and 50 µg/kg (10×LOQ), except for tau-fluvalinate at the LOQ [RSD 28% (Q) 29% (C); Appendix 2, pp. 174-175 and Tables 1-25, pp. 179-203; DER Attachment 2]. Diacid analysis was not included, according to the method directives. For thatch samples, mean recoveries and RSDs were within guideline requirements for analysis of tau-fluvalinate, ACBA, diacid, PBA, RCAA, and haloaniline at fortification levels of 10 µg/kg (LOQ) and 100 µg/kg (10×LOQ), except for tau-fluvalinate at 10×LOQ [RSD 21% (Q) 25% (C)]. For foliage samples, mean recoveries and RSDs were within guideline requirements for analysis of tau-fluvalinate, ACBA, diacid, PBA, RCAA, and haloaniline at fortification levels of 20 µg/kg (LOQ) and 200 µg/kg (10×LOQ). For tau-fluvalinate at the LOQ (Q/C) and haloaniline at the LOQ in soil, tau-fluvalinate at 10×LOQ (Q/C) in thatch, and tau-fluvalinate at 10×LOQ (C) in foliage, recovery statistics were reviewer-calculated since the study authors only based the

statistics on n = 3 or 4, due to deeming recovery values as outliers which were not included in statistics. The reviewer calculated statistics using all 5 recovery values. Two ion transitions were used to quantify tau-fluvalinate, diacid, and RCAA via LC/MS/MS, but only one ion transition was used to quantify ACBA and PBA. One ion was used to quantify haloaniline via GC/MS. A confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data. For analytes quantified using two ion transitions, performance data (recovery results) for the quantitation and confirmation ion analyses were comparable, except for the LOQ analyses of tau-fluvalinate in soil, RCAA in thatch, and diacid in foliage. The soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357). The soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV. The ILV validated the method for all analytes in soil, thatch, and foliage in the first trial (Appendix 2, p. 154). The ECM was performed as written, except for the **significant modification of the validation of the method for ACBA at the LOQ of 20 µg/kg** in foliage, as well as minor modifications of use of centrifugation and the internal standard for soil (Appendix 2, pp. 160, 164-170). Minor modifications of the LC/MS and GC/MS instrument and parameters also occurred based on available equipment.

Table 2. Initial Validation Method Recoveries for Tau-Fluvalinate and its Metabolites ACBA, Diacid, PBA, RCAA, and Haloaniline in Soil, Thatch, and Foliage[1,2,3]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sandy Loam or Sandy Clay Loam Soil					
	LC/MS/MS - Quantitation Ion Transition					
tau-Fluvalinate	5 (LOQ)	5[4]	63-138	109	28	**26**
	50	5	86-120	107	16	15
ACBA	5 (LOQ)	5	87-126	114	16	14
	50	5	79-115	97	16	17
Diacid	5 (LOQ)		Not analyzed[5]			
	50					
PBA	5 (LOQ)	**3**	80-83	81	1	2
	50	**3**	88-89	88	1	1
RCAA	5 (LOQ)	5	73-85	79	5	6
	50	5	91-100	95	4	4
	GC/MS - Quantitation Ion					
Haloaniline	5 (LOQ)	5	72-114	97	17	18
	50	5[4]	70-185	**123**	45	**36**
	Sandy Loam Thatch					
	LC/MS/MS - Quantitation Ion Transition					
tau-Fluvalinate	10 (LOQ)	5	67-97	76	12	16
	100	5	75-87	80	4	5
ACBA	10 (LOQ)	5	84-91	87	4	4
	100	5	66-81	72	6	9
Diacid	10 (LOQ)	5	67-73	70	3	4
	100	5	77-83	81	3	4
PBA	10 (LOQ)	**3**	125-134	**129**	5	4
	100	**3**	103-109	106	3	3
RCAA	10 (LOQ)	5	109-112	110	1	1
	100	5	107-109	108	1	1
	GC/MS - Quantitation Ion					
Haloaniline	10 (LOQ)	5	102-124	108	9	9
	100	5	95-119	104	10	9
	Foliage					
	LC/MS/MS - Quantitation Ion Transition					
tau-Fluvalinate	20 (LOQ)	5	76-87	80	4	5
	200	5	63-68	**66**	2	3
ACBA	20	5	ND	--	--	--
	200 (LOQ)	5	93-107	100	5	5
Diacid	20 (LOQ)	5	89-95	92	2	3
	200	5	78-85	81	3	4
PBA	20 (LOQ)	**3**	90-91	90	1	1
	200	**3**	87-91	89	2	2
RCAA	20 (LOQ)	5	102-115	109	5	5
	200	5	110-115	110	4	4

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	GC/MS - Quantitation Ion					
Haloaniline	20 (LOQ)	5	109-131	117	9	7
	200	5	95-123	114	14	12

Data (uncorrected recovery results, pp. 24-25 of MRID 50552102) were obtained from Tables 14-30, pp. 34-50 of MRID 50552102 and DER Attachment 2.

1 The sandy loam or sandy clay loam soil [55% sand, 24% silt, 21% clay; pH 6.4 (method not reported), 1.9% organic matter, taxonomic classification of Marcum – smectitic, thermic, Typic Argixeroll] was obtained from Sutter County, California (EPA Region 10), and used in the study (USDA soil texture classification as sandy clay loam; see Reviewer's Comment #6; p. 15; Appendix 4, p. 377 of MRID 50552102). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil, foliage, and thatch samples were ID No.s S-14-06976, S-14-06977, and S-14-06978, respectively.

2 ACBA was identified using one ion transition: *m/z* 170→126.0; no additional fragment ion was detected to be used as confirmation ion. Other analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 503.2→180.9 and *m/z* 503.2→208.1 for tau-fluvalinate, *m/z* 270→154.9 and *m/z* 270→146.1 for diacid, *m/z* 213.1→93.1 and *m/z* 213.1→169.1 for PBA, *m/z* 199.1→171.1 and *m/z* 199.1→153.3 for PB aldehyde, and *m/z* 294.1→145.1 and *m/z* 294.1→127.2 for RCAA.

3 Only results for the quantitation ion transition were reported.

4 Means, standard deviations, and RSDs were reviewer-calculated since the study authors only based the statistics on n = 3 or 4, due to deeming recovery values as outliers which were not included in statistics. The reviewer calculated statistics using all 5 recovery values. Rules of significant figures were followed.

5 Diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid (p. 17 of MRID 50552102).

Table 3. Independent Validation Method Recoveries for Tau-Fluvalinate and its Metabolites ACBA, Diacid, PBA, RCAA, and Haloaniline in Soil, Thatch, and Foliage

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
	Soil					
	LC/MS/MS - Quantitation Ion Transition					
tau-Fluvalinate	5 (LOQ)	5[3]	52.2-115	88	25	**28**
	50	5	73.9-112	89.8	14.4	16.0
ACBA	5 (LOQ)	5	99.4-111	106	4.47	4.24
	50	5	83.8-95.7	91.7	5.16	5.63
Diacid	5 (LOQ)		Not analyzed[4]			
	50					
PBA	5 (LOQ)	5	90.5-102	96.1	4.52	4.70
	50	5	81.8-90.2	87.3	3.24	3.71
RCAA	5 (LOQ)	5	72.4-78.9	76.7	2.64	3.44
	50	5	75.2-78.9	76.7	1.73	2.26
	GC/MS - Quantitation Ion					
Haloaniline	5 (LOQ)	5[3]	104-160	118	24	20
	50	5	81.4-116	101	17.3	17.1
	LC/MS/MS - Confirmation Ion Transition					
tau-Fluvalinate	5 (LOQ)	5[3]	47.4-108	85	25	**29**
	50	5	75.4-108	89.2	12.5	14.0
Diacid	5 (LOQ)		Not analyzed[4]			
	50					
RCAA	5 (LOQ)	5	73.5-78.8	75.7	2.05	2.70
	50	5	71.8-76.6	74.3	2.06	2.77
	Thatch					
	LC/MS/MS - Quantitation Ion Transition					
tau-Fluvalinate	10 (LOQ)	5	90.1-107	97.4	7.38	7.59
	100	5[3]	55.9-104	85	18	**21**
ACBA	10 (LOQ)	5	78.6-88.4	81.6	3.85	4.71
	100	5	77.8-81.3	79.7	1.37	1.72
Diacid	10 (LOQ)	5	71.5-83.7	75.5	5.02	6.65
	100	5	76.5-87.3	82.9	4.26	5.14
PBA	10 (LOQ)	5	67.4-80.6	74.9	4.84	6.47
	100	5	71.8-78.8	75.0	2.72	3.62
RCAA	10 (LOQ)	5	93.0-99.6	96.6	2.99	3.09
	100	5	91.8-101	97.1	3.94	4.06
	GC/MS - Quantitation Ion					
Haloaniline	10 (LOQ)	5	95.9-120	110	12.0	10.9
	100	5	82.7-120	110	15.9	14.5
	LC/MS/MS - Confirmation Ion Transition					
tau-Fluvalinate	10 (LOQ)	5	94.1-108	101	6.48	6.44
	100	5[3]	56.8-113	92	22	**25**
Diacid	10 (LOQ)	5	73.4-83.2	78.2	4.51	5.76
	100	5	79.0-90.5	85.5	4.67	5.46

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[1]	Relative Standard Deviation (%)
RCAA	10 (LOQ)	5	72.2-97.0	86.5	10.1	11.5
	100	5	92.9-105	98.8	4.71	4.77
	Foliage					
	LC/MS/MS - Quantitation Ion Transition					
tau-Fluvalinate	20 (LOQ)	5	103-119	114	6.59	5.78
	200	5	79.1-87.7	83.5	4.02	4.81
ACBA	20 (LOQ)	5	80.2-90.7	84.5	3.85	4.55
	200	5	74.4-84.4	80.4	3.87	4.82
Diacid	20 (LOQ)	5	88.7-92.3	89.8	1.57	1.74
	200	5	68.8-83.5	76.6	6.02	7.86
PBA	20 (LOQ)	5	90.4-94.3	92.3	1.83	1.98
	200	5	73.7-88.9	81.8	6.07	7.42
RCAA	20 (LOQ)	5	86.1-95.0	89.7	3.34	3.72
	200	5	81.5-103	93.5	8.03	8.59
	GC/MS - Quantitation Ion					
Haloaniline	20 (LOQ)	5	89.3-111	98.2	8.81	8.97
	200	5	90.2-110	101	7.45	7.35
	LC/MS/MS - Confirmation Ion Transition					
tau-Fluvalinate	20 (LOQ)	5	109-120	115	4.30	3.75
	200	5[3]	68.3-96.5	78.7	10.6	13.5
Diacid	20 (LOQ)	5	94.7-107	103	4.96	4.81
	200	5	66.9-84.0	74.7	6.30	8.43
RCAA	20 (LOQ)	5	83.5-104	93.6	8.76	9.37
	200	5	81.5-96.5	88.6	5.42	6.12

Data (uncorrected recovery results, Appendix 2, pp. 171-173 of MRID 50552102) were obtained from Appendix 2, pp. 174-175 and Tables 1-25, pp. 179-203 of MRID 50552102 and DER Attachment 2.

1 The soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357 of MRID 50552102). The soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV.

2 For LC/MS, ACBA and PBA were identified using one ion transition: *m/z* 170.00→126.00 for ACBA and *m/z* 213.10→93.10 for PBA; other analytes were identified using two ion transitions (quantitation and confirmation, respectively): *m/z* 503.20→180.90 and *m/z* 503.20→208.10 for tau-fluvalinate, *m/z* 270.00→154.90 and *m/z* 270.00→146.10 for diacid, and *m/z* 294.10→145.10 and *m/z* 294.10→127.20 for RCAA. For GC/MS, haloaniline was identified using one ion: *m/z* 195.00. These transitions were similar to those of the ECM.

3 Means, standard deviations, and RSDs were reviewer-calculated since the study authors only based the statistics on n = 3 or 4, due to deeming recovery values as outliers which were not included in statistics. The reviewer calculated statistics using all 5 recovery values. Rules of significant figures were followed.

4 Diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid (p. 17 of MRID 50552102).

III. Method Characteristics

In the ECM, the LOQs for tau-fluvalinate, diacid, PBA, RCAA, and haloaniline were 5 µg/kg in soil, 10 µg/kg in thatch, and 20 µg/kg in foliage (pp. 27-28; Appendix 2, pp. 173-175 of MRID 50552102). The LOQs of the metabolite ACBA were 5 µg/kg in soil, 10 µg/kg in thatch, and 200 µg/kg in foliage. In the ILV, the LOQs for all analytes were 5 µg/kg in soil, 10 µg/kg in thatch, and 20 µg/kg in foliage. No justification for the LOQ was provided in the ECM, and the LOQ was reported in the ILV from the ECM without justification. No calculations were reported to support the LOQ. In the ECM, the LODs were estimated to be one-third of the LOQ, about 3 µg/L in all matrices, except for ACBA in foliage, where LOD was 30 µg/L. For tau-fluvalinate, diacid, PBA, RCAA, and haloaniline, these values corresponded to 1.5 µg/kg in soil, *ca*. 3.3 µg/kg in thatch, and *ca*. 6.6 µg/kg in foliage. For ACBA, these values corresponded to 1.5 µg/kg in soil, *ca*. 3.3 µg/kg in thatch, and *ca*. 66 µg/kg in foliage. No further justification or calculation was provided. In the ILV, the LODs were calculated by evaluating the signal-to-noise (S/N) ratio from samples of a known concentration (i.e. the lowest calibration standard) and blank samples (i.e. control samples) to establish the lowest level at which the analyte can reliably be detected. A S/N ratio of 3:1 was used to determine the LOD for each analyte and transition. The calculated LODs ranged 0.00500-0.500 µg/kg for soil, 0.0700-4.00 µg/kg for thatch, and 0.0400-8.00 µg/kg for foliage.

Table 4a. Method Characteristics - Soil

Analyte[1]		**tau-Fluvalinate**	**ACBA**	**Diacid**[2]	**PBA**	**RCAA**	**Haloaniline**
Analysis		**LC/MS/MS**					**GC/MS**
Limit of Quantitation (LOQ)		5 µg/kg					
Limit of Detection (LOD)	ECM	1.5 µg/kg (one-third of the LOQ, *ca*. 3 µg/L)					
	ILV	0.0500 µg/kg (Q) 0.0200 µg/kg (C)	0.0300 µg/kg	--	0.100 µg/kg	0.00500 µg/kg (Q) 0.500 µg/kg (C)	0.300 µg/kg
Linearity (calibration curve r^2 and concentration range)	ECM[3]	r^2 = 0.9977	r^2 = 0.9989[4]	--	r^2 = 0.9988	r^2 = 0.9990	r^2 = **0.938**
		0.0512-0.7161 µg	0.0518-0.5180 µg	--	0.0517-0.7231 µg	0.0521-0.7294 µg	10.0-140 ng/mL
	ILV	r^2 = **0.99057** (Q) r^2 = **0.99042** (C)	r^2 = 0.99587	--	r^2 = 0.99705	r^2 = 0.99561 (Q) r^2 = **0.99050** (C)	r^2 = **0.99318**
		10.0-140 µg/L					
Repeatable	ECM[5]	Yes at 10×LOQ. **No** at LOQ (RSD **26**%).	Yes at LOQ and 10×LOQ.	--	Yes at LOQ and 10×LOQ, but n = **3**.	Yes at LOQ and 10×LOQ.	Yes at LOQ. **No** at 10×LOQ (mean **123**, RSD **36**%).
		one characterized soil matrix					
	ILV[6,7]	Yes at 10×LOQ. **No** at LOQ [RSD **28**% (Q) **29**% (C)]	Yes at LOQ and 10×LOQ.	--	Yes at LOQ and 10×LOQ		
		one uncharacterized soil matrix					
Reproducible		Yes at 10×LOQ. **No** at LOQ.	Yes at LOQ and 10×LOQ.	--	Yes at LOQ and 10×LOQ.		Yes at LOQ. **No** at 10×LOQ.
Specificity	ECM[8]	Yes, matrix interferences were *ca*. 27% of the LOQ (based on peak height).	Yes, matrix interferences were <5% of the LOQ (based on peak height), but the LOQ peak was small compared to baseline noise which interfered with peak integration and attenuation.[9]	--	Yes, matrix interferences were *ca*. 17% of the LOQ (based on peak height).	Yes, matrix interferences were <5% of the LOQ (based on peak height).	Yes, no matrix interferences were observed.

	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area). Minor baseline interference at analyte peak base was observed at 10×LOQ.	Yes, matrix interferences were <5% of the LOQ (based on peak area). Peak tailing was observed.	--	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <5% of the LOQ (based on peak area), but LOQ peak was small. [10]

Data were obtained from pp. 27-28; Appendix 2, pp. 173-175 (ECM/ILV LOQ/LOD); Tables 14-30, pp. 34-50 (ECM recovery data); Figures 1-15, pp. 51-67 (ECM calibration curve); Figures 18-67, pp. 68-122 (ECM chromatograms) of MRID 50552102; Appendix 2, pp. 174-175 and Tables 1-25, pp. 179-203 (ILV recovery data); Appendix 2, Figures 1-25, pp. 204-228 (ILV calibration curves); Appendix 2, Figures 35-134, pp. 238-337 (ILV chromatograms); of MRID 50552102; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition; no designation = Quantitation ion transition or ion.

1 4-Amino-3-chlorobenzoic acid (ACBA), 2-(2-chloro-4-carboxyl)anilino-3-methylbutanoic acid (Diacid), 3-phenoxybenzoic acid (PBA; 3-PB acid), 2-(2-chloro-4-trifluoromethyl) anilino-3-methylbutanoic acid (RCAA; anilino acid), and 2-chloro-4-trifluoromethylaniline (Haloaniline; Table 1, p. 14 of MRID 50552102).

2 Diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid.

3 Only the quantitation ion or ion transition data was provided in the ECM.

4 Only 4 calibration standard concentrations were used for the calibration curve because the highest concentration was not used.

5 In the ECM, the sandy loam or sandy clay loam soil [55% sand, 24% silt, 21% clay; pH 6.4 (method not reported), 1.9% organic matter, taxonomic classification of Marcum – smectitic, thermic, Typic Argixeroll] was obtained from Sutter County, California (EPA Region 10), and used in the study (USDA soil texture classification as sandy clay loam; see Reviewer's Comment #6; p. 15; Appendix 4, p. 377 of MRID 50552102). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil, foliage, and thatch samples were ID No.s S-14-06976, S-14-06977, and S-14-06978, respectively.

6 In the ILV, the soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357 of MRID 50552102). The soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV.

7 The ILV validated the method for all analytes in soil, thatch, and foliage in the first trial (Appendix 2, p. 154). The ECM was performed as written, except for the significant modification of the validation of the method for ACBA at the LOQ of 20 µg/kg in foliage, as well as minor modifications of use of centrifugation and the internal standard for soil (Appendix 2, pp. 160, 164-170). Minor modifications of the LC/MS and GC/MS instrument and parameters also occurred based on available equipment.

8 Peak areas were not reported, so matrix interferences were compared using observed peak heights.

9 Based on Figure 22, p. 72 of MRID 50552102.

10 Based on Appendix 2, Figure 43, p. 246 of MRID 50552102.

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

Table 4b. Method Characteristics - Thatch

Analyte[1]		**tau-Fluvalinate**	**ACBA**	**Diacid**	**PBA**	**RCAA**	**Haloaniline**
Analysis		**LC/MS/MS**					**GC/MS**
Limit of Quantitation (LOQ)		10 µg/kg					
Limit of Detection (LOD)	ECM	*ca*. 3.3 µg/kg (one-third of the LOQ, *ca*. 3 µg/L)					
	ILV	0.100 µg/kg (Q) 0.0400 µg/kg (C)	0.0700 µg/kg	0.100 µg/kg (Q) 0.500 µg/kg (C)	0.200 µg/kg	0.0800 µg/kg (Q) 4.00 µg/kg (C)	0.400 µg/kg
Linearity (calibration curve r^2 and concentration range)	ECM[2]	r^2 = **0.9945**	r^2 = 0.9990[3]	r^2 = 0.9979	r^2 = 0.9990	r^2 = 0.9994	r^2 = **0.991**
		0.1023-1.432 µg	0.1036-1.036 µg	0.1034-1.448 µg	0.1033-1.446 µg	0.1042-1.459 µg	10.0-140 ng/mL
	ILV	r^2 = **0.99129** (Q) r^2 = **0.99359** (C)	r^2 = 0.99689	r^2 = 0.99920 (Q) r^2 = **0.99411** (C)	r^2 = 0.99845	r^2 = **0.99331** (Q) r^2 = **0.99070** (C)	r^2 = 0.99698
		10.0-140 µg/L					
Repeatable	ECM[4]	Yes at LOQ and 10×LOQ.			Yes at 10×LOQ, but n = **3**. **No** at LOQ (mean **129**%), n = **3**.	Yes at LOQ and 10×LOQ.	
		one characterized thatch matrix					
	ILV[5,6]	Yes at LOQ. **No** at 10×LOQ [RSD **21**% (Q) **25**% (C)].	Yes at LOQ and 10×LOQ.				
		one uncharacterized thatch matrix					
Reproducible		Yes at LOQ. **No** at 10×LOQ.	Yes at LOQ and 10×LOQ.		Yes at 10×LOQ. **No** at LOQ.	Yes at LOQ and 10×LOQ.	
Specificity	ECM[7]	Yes, matrix interferences were *ca*. 7% of the LOQ (based on peak height).	Yes, matrix interferences were <5% of the LOQ (based on peak height), but the LOQ peak was small compared to baseline noise which interfered with peak integration and attenuation.[8]	Yes, matrix interferences were <5% of the LOQ (based on peak height), but the LOQ peak was poorly defined.[9]	Yes, matrix interferences were < 5% of the LOQ (based on peak height).		Yes, no matrix interferences were observed.

	ILV	Yes, matrix interferences were <5% of the LOQ (based on peak area). Minor baseline interference at analyte peak base was observed at 10×LOQ.	Yes, matrix interferences were <5% of the LOQ (based on peak area). Peak tailing was observed.	Yes, matrix interferences were <5% of the LOQ (based on peak area).

Data were obtained from pp. 27-28; Appendix 2, pp. 173-175 (ECM/ILV LOQ/LOD); Tables 14-30, pp. 34-50 (ECM recovery data); Figures 1-15, pp. 51-67 (ECM calibration curve); Figures 18-67, pp. 68-122 (ECM chromatograms) of MRID 50552102; Appendix 2, pp. 174-175 and Tables 1-25, pp. 179-203 (ILV recovery data); Appendix 2, Figures 1-25, pp. 204-228 (ILV calibration curves); Appendix 2, Figures 35-134, pp. 238-337 (ILV chromatograms); of MRID 50552102; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition; no designation = Quantitation ion transition or ion.

1 4-Amino-3-chlorobenzoic acid (ACBA), 2-(2-chloro-4-carboxyl)anilino-3-methylbutanoic acid (Diacid), 3-phenoxybenzoic acid (PBA; 3-PB acid), 2-(2-chloro-4-trifluoromethyl) anilino-3-methylbutanoic acid (RCAA; anilino acid), and 2-chloro-4-trifluoromethylaniline (Haloaniline; Table 1, p. 14 of MRID 50552102).

2 Only the quantitation ion or ion transition data was provided in the ECM.

3 Only 4 calibration standard concentrations were used for the calibration curve because the highest concentration was not used.

4 In the ECM, the sandy loam or sandy clay loam soil [55% sand, 24% silt, 21% clay; pH 6.4 (method not reported), 1.9% organic matter, taxonomic classification of Marcum – smectitic, thermic, Typic Argixeroll] was obtained from Sutter County, California (EPA Region 10), and used in the study (USDA soil texture classification as sandy clay loam; see Reviewer's Comment #6; p. 15; Appendix 4, p. 377 of MRID 50552102). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil, foliage, and thatch samples were ID No.s S-14-06976, S-14-06977, and S-14-06978, respectively.

5 In the ILV, the soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357 of MRID 50552102). The soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV.

6 The ILV validated the method for all analytes in soil, thatch, and foliage in the first trial (Appendix 2, p. 154). The ECM was performed as written, except for the significant modification of the validation of the method for ACBA at the LOQ of 20 µg/kg in foliage, as well as minor modifications of use of centrifugation and the internal standard for soil (Appendix 2, pp. 160, 164-170). Minor modifications of the LC/MS and GC/MS instrument and parameters also occurred based on available equipment.

7 Peak areas were not reported, so matrix interferences were compared using observed peak heights.

8 Based on Figure 37, p. 89 of MRID 50552102.

9 Based on Figure 40, p. 92 of MRID 50552102.

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

Table 4c. Method Characteristics - Foliage

Analyte[1]		**tau-Fluvalinate**	**ACBA**	**Diacid**	**PBA**	**RCAA**	**Haloaniline**
Analysis		**LC/MS/MS**					**GC/MS**
Limit of Quantitation (LOQ)	ECM	20 µg/kg	200 µg/kg[2]	20 µg/kg			
	ILV	20 µg/kg					
Limit of Detection (LOD)	ECM	*ca*. 6.6 µg/kg (one-third of the LOQ, *ca*. 3 µg/L)	*ca*. 66 µg/kg (one-third of the LOQ, *ca*. 30 µg/L)	*ca*. 6.6 µg/kg (one-third of the LOQ, *ca*. 3 µg/L)			
	ILV	0.400 µg/kg (Q) 1.00 µg/kg (C)	4.00 µg/kg	0.0400 µg/kg (Q) 0.800 µg/kg (C)	2.00 µg/kg	0.200 µg/kg (Q) 8.00 µg/kg (C)	0.800 µg/kg
Linearity (calibration curve r^2 and concentration range)	ECM[3]	r^2 = 0.9993	r^2 = 0.9987[4]	r^2 = 0.9993	r^2 = 0.9994	r^2 = 0.9999	r^2 = **0.9728**
		0.1023-1.432 µg	0.6216-1.450 µg	0.1034-1.448 µg	0.1033-1.446 µg	0.1042-1.459 µg	10.0-140 ng/mL
	ILV	r^2 = **0.99214** (Q) r^2 = **0.99071** (C)[5]	r^2 = 0.99718	r^2 = **0.99182** (Q) r^2 = 0.99640 (C)	r^2 = **0.99086**	r^2 = **0.99101** (Q) r^2 = 0.99761 (C)	r^2 = 0.99604
		10.0-140 µg/L (Q) 10.0-100 µg/L (C)	10.0-140 µg/L				
Repeatable	ECM[6]	Yes at LOQ. **No** at 10×LOQ (mean **66**%).	Yes at LOQ. **No** at 10×LOQ; no samples prepared at 2000 µg/kg.	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ, but n = **3**.	Yes at LOQ and 10×LOQ.	
		one foliage matrix					
	ILV[7,8]	Yes at LOQ and 10×LOQ. (one foliage matrix)					
Reproducible		Yes at LOQ. **No** at 10×LOQ.	Yes at 200 µg/kg. **No** at 20 µg/kg; only one set of performance data submitted.	Yes at LOQ and 10×LOQ.			
Specificity	ECM[9]	Yes, matrix interferences were *ca*. 11% of the LOQ (based on peak height).	**No**, analyte co-eluted with significant contaminant (peak height *ca.* 3xs LOQ peak height).[10]	Yes, matrix interferences were <5% of the LOQ (based on peak height), but the LOQ peak was poorly defined.[11]	Yes, matrix interferences were <10% of the LOQ (based on peak height).	Yes, matrix interferences were <5% of the LOQ (based on peak height).	Yes, no matrix interferences were observed but some matrix interferences were observed.

	ILV	Yes, no matrix interferences were observed. Minor baseline interference at analyte peak base was observed at 10×LOQ.	**No**, LOQ analyte peak was very small and not defined. A significant contaminant (peak height *ca.* 20xs LOQ peak height) elevated baseline around analyte peak.[12]	Yes, matrix interferences were <5% of the LOQ (based on peak area). Peak tailing was observed.	Yes, matrix interferences were <5% of the LOQ (based on peak area).	Yes, matrix interferences were <5% of the LOQ (based on peak area). A large contaminant in C ion near analyte was observed. [13]	Yes, matrix interferences were <5% of the LOQ (based on peak area), but LOQ peak was small.[14]

Data were obtained from pp. 27-28; Appendix 2, pp. 173-175 (ECM/ILV LOQ/LOD); Tables 14-30, pp. 34-50 (ECM recovery data); Figures 1-15, pp. 51-67 (ECM calibration curve); Figures 18-67, pp. 68-122 (ECM chromatograms) of MRID 50552102; Appendix 2, pp. 174-175 and Tables 1-25, pp. 179-203 (ILV recovery data); Appendix 2, Figures 1-25, pp. 204-228 (ILV calibration curves); Appendix 2, Figures 35-134, pp. 238-337 (ILV chromatograms); of MRID 50552102; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition; no designation = Quantitation ion transition or ion.

1 4-Amino-3-chlorobenzoic acid (ACBA), 2-(2-chloro-4-carboxyl)anilino-3-methylbutanoic acid (Diacid), 3-phenoxybenzoic acid (PBA; 3-PB acid), 2-(2-chloro-4-trifluoromethyl) anilino-3-methylbutanoic acid (RCAA; anilino acid), and 2-chloro-4-trifluoromethylaniline (Haloaniline; Table 1, p. 14 of MRID 50552102).

2 ACBA could not be detected in ECM foliage samples prepared at 20 µg/kg.

3 Only the quantitation ion or ion transition data was provided in the ECM.

4 Only 3 calibration standard concentrations were used for the calibration curve because the analyte was not detected in the two lowest concentrations.

5 Only 4 calibration standard concentrations were used for the calibration curve because the highest concentration was not used.

6 In the ECM, the sandy loam or sandy clay loam soil [55% sand, 24% silt, 21% clay; pH 6.4 (method not reported), 1.9% organic matter, taxonomic classification of Marcum – smectitic, thermic, Typic Argixeroll] was obtained from Sutter County, California (EPA Region 10), and used in the study (USDA soil texture classification as sandy clay loam; see Reviewer's Comment #6; p. 15; Appendix 4, p. 377 of MRID 50552102). Soil characterization was performed by Agvise Laboratories, Northwood, North Dakota. Soil, foliage, and thatch samples were ID No.s S-14-06976, S-14-06977, and S-14-06978, respectively.

7 In the ILV, the soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357 of MRID 50552102). The soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV.

8 The ILV validated the method for all analytes in soil, thatch, and foliage in the first trial (Appendix 2, p. 154). The ECM was performed as written, except for the significant modification of the validation of the method for ACBA at the LOQ of 20 µg/kg in foliage, as well as minor modifications of use of centrifugation and the internal standard for soil (Appendix 2, pp. 160, 164-170). Minor modifications of the LC/MS and GC/MS instrument and parameters also occurred based on available equipment.

9 Peak areas were not reported, so matrix interferences were compared using observed peak heights.

10 Based on Figures 54-55, pp. 108-109 of MRID 50552102. No 10×LOQ chromatograms were provided.

11 Based on Figure 57, p. 111 of MRID 50552102.

12 Based on Appendix 2, Figures 131-132, pp. 334-335 of MRID 50552102.

13 Based on Appendix 2, Figure 118, p. 321 of MRID 50552102. A confirmatory method is not always required when LC/MS is the primary method used to generate study data.

14 Based on Appendix 2, Figure 107, p. 310 of MRID 50552102.
Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies

1. The method could not be validated for ACBA in foliage since the **LOQ of the ECM (200 µg/kg) differed from that of the ILV (20 µg/kg)**. The ECM and ILV prepared samples at 20 µg/kg and 200 µg/kg, but ACBA could not be detected in the ECM at 20 µg/kg (Table 26, p. 46 of MRID 50552102). The ECM study author determined that the LOQ for the method for ACBA in foliage was 200 µg/kg; however, no samples were prepared at 2000 µg/kg (10×LOQ; p. 27). In the ILV, the method was validated for ACBA in foliage at 20 µg/kg and 200 µg/kg. Consequently, two sets of performance data were only provided for ACBA in foliage at 200 µg/kg, and no validated LOQ was clear from the method validation data.

2. It could not be determined if the ILV was conducted independently of the ECM since the ILV study author (Wu, X) communicated directly with Welch, A of Wellmark International (Central Life Sciences) who was the ECM study author, as well as the ILV Study Sponsor Representative (Appendix 2, pp. 139, 171; Appendix 2, Appendix 3, pp. 356-375 of MRID 50552102). These communications included discussion of test materials, method development testing and any applicable method modifications necessary prior to the start of ILV testing, as well as the use of internal standard, confirmation transitions and interpretation of study results upon completion of the ILV testing. OCSPP guidelines state that ILV validations are performed without collusion with the ECM personnel.

3. The reproducibility of the method was not supported by ECM and ILV performance data for the following analyses: tau-fluvalinate in soil at the LOQ and in thatch and foliage at 10×LOQ, haloaniline in soil at 10×LOQ, and PBA in thatch at the LOQ. See below for performance data deficiency details.

   In the ILV, the performance data for the following analyses were outside guideline requirements (mean 70-120%; RSD ≤20%): tau-fluvalinate in soil at the LOQ [RSD 28% (Q) 29% (C)] and in thatch at 10×LOQ [RSD 21% (Q) 25% (C); Appendix 2, pp. 174-175 and Tables 1-25, pp. 179-203; DER Attachment 2].

   For ECM, the performance data for the following analyses were outside guideline requirements: tau-fluvalinate in soil at the LOQ (RSD 26%), haloaniline in soil at 10×LOQ (mean 123%, RSD 36%), PBA in thatch at the LOQ (mean 129%), and tau-fluvalinate in foliage at 10×LOQ (mean 66%; Tables 14-30, pp. 34-50; DER Attachment 2).

   Many of the unacceptable recovery values were reviewer-calculated since the study authors only based the statistics on n = 3 or 4, due to deeming recovery values as outliers which were not included in statistics. The reviewer calculated statistics using all 5 recovery values (See DER Attachment 2).

4. Linearity was not satisfactory for the following analyses in <u>soil</u>:

In the ILV: tau-fluvalinate (Q, $r^2 = 0.99057$; C, $r^2 = 0.99042$) and haloaniline ($r^2 = 0.99318$), as well as RCAA (C, $r^2 = 0.99050$; Appendix 2, Figures 1-25, pp. 204-228 of MRID 50552102). In the ECM: haloaniline ($r^2 = 0.938$; Figures 1-17, pp. 51-67). Linearity is satisfactory when $r^2 \geq 0.995$.

Linearity was not satisfactory for the following analyses in thatch:

In the ILV: tau-fluvalinate (Q, $r^2 = 0.99129$; C, $r^2 = 0.99359$) and RCAA (Q, $r^2 = 0.99331$; C, $r^2 = 0.99070$), as well as diacid (C, $r^2 = 0.99411$; Appendix 2, Figures 1-25, pp. 204-228 of MRID 50552102). In the ECM: tau-fluvalinate ($r^2 = 0.9945$) and haloaniline ($r^2 = 0.991$; Figures 1-15, pp. 51-67). Linearity is satisfactory when $r^2 \geq 0.995$.

Linearity was not satisfactory for the following analyses in foliage:

In the ILV: tau-fluvalinate (Q, $r^2 = 0.99214$; C, $r^2 = 0.99071$), diacid ($r^2 = 0.99182$), PBA ($r^2 = 0.99086$), and RCAA ($r^2 = 0.99101$; Appendix 2, Figures 1-25, pp. 204-228 of MRID 50552102). In the ECM: haloaniline ($r^2 = 0.9728$; Figures 1-17, pp. 51-67). Linearity is satisfactory when $r^2 \geq 0.995$.

The reviewer noted that linearity deviations in the confirmation ion analyses do not affect the validity of the method since a confirmation method is not usually required when LC/MS or GC/MS is used as the primary method to generate study data.

The reviewer noted that many ILV recoveries were footnoted with the fact that the calibration curve was inadequate for accessing the sample response since it was lower than that of the lowest calibration standard (Appendix 2, Tables 1-25, pp. 179-203 of MRID 50552102).

5. The specificity of the method was not supported by ILV and ECM representative chromatograms of ACBA in foliage. In the ILV representative chromatograms, the LOQ analyte peak was very small and not defined. A significant contaminant (peak height *ca.* 20xs LOQ peak height) elevated baseline around analyte peak (Appendix 2, Figures 131-132, pp. 334-335 of MRID 50552102). In the ECM representative chromatograms, the analyte co-eluted with significant contaminant (peak height *ca.* 3xs LOQ peak height; Figures 54-55, pp. 108-109). Additionally, in the ECM, no 10×LOQ chromatograms were provided.

   The specificity of the method was not well-supported by ILV representative chromatograms for haloaniline in soil and foliage since the LOQ peak was small (Appendix 2, Figure 107, p. 310 of MRID 50552102).

   The specificity of the method was not well-supported by ECM representative chromatograms for ACBA in soil and thatch, and diacid in thatch. The ACBA LOQ peak was small compared to baseline noise which interfered with peak integration and attenuation in soil and thatch (Figure 22, p. 72; Figure 37, p. 89 of MRID 50552102). The diacid LOQ peak was poorly defined in thatch (Figure 40, p. 92).

6. It could not be determined that the ILV was provided with the most difficult soil matrix with which to validate the method since only one uncharacterized soil matrix was tested. OCSPP 850.6100 guidance suggests for a given sample matrix, the registrant should select the most difficult analytical sample condition from the study (*e.g.*, high organic content versus low organic content in a soil matrix) to analyze from the study to demonstrate how well the method performs. Even though a certain number of soil matrices is not specified in the OCSPP guidelines, more than one soil/soil matrix would need to be included in an ILV in order to cover the range of soils used in the terrestrial field dissipation studies. The soil, thatch, and foliage were provided by the Sponsor Central Life Sciences (Wellmark International), Dallas, Texas, and not designated as terrestrial field study matrices (Appendix 2, p. 160; Appendix 2, Appendix 3, p. 357 of MRID 50552102). The ILV soil source was Sutter County, California (EPA Region 10), which was the same source as that of the ECM. Soil and thatch classifications were not included in the ILV. A tau-fluvalinate terrestrial field dissipation study (MRID 50552101) was submitted along with the method validation MRID 50552102. One of the soils was sourced from Sutter County, California (EPA Region 10) from which turf and grass clippings were also collected (pp. 330-339 of MRID 50552101). No matrix sample ID could be found in MRID 50552101 to equate to the matrix sample IDs reported in MRID 50552102.

   The reviewer noted that the ECM soil matrix was designated as sandy loam and sandy clay loam soil in the study report (55% sand, 24% silt, 21% clay; USDA soil texture classification as sandy clay loam; p. 15; Appendix 4, p. 377 of MRID 50552102). Using the soil texture calculator based on USDA soil texture particle distributions, the reviewer determined that the soil was sandy clay loam.

   The reviewer assumed that the soil characterization data in Appendix 4 referred to the ECM test soil even though the sample #S-14-06976 was not included in the soil characterization report.

7. An insufficient number of samples were prepared for ECM analyses of PBA in all matrices, n = 3. OCSPP guidelines state that a minimally complete sample set includes a reagent blank, two matrix blanks, five samples spiked at the LOQ, and five samples spiked at 10× LOQ for each matrix.

8. One of the potential metabolites, m-phenoxybenzaldehyde cyanohydrin, was shown to degrade in acetonitrile (p. 27 of MRID 50552102). After 22 hours, cyanohydrin was almost completely converted to the 3- phenoxybenzaldehyde. Cyanohydrin was also seen to be unstable in methanol as well. Due to its instability, cyanohydrin could not be prepared as spiking solution or standard solution for the soil spike recovery study.

9. The estimation of LOQ and LOD in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 27-28; Appendix 2, pp. 173-175 of MRID 50552102). No justification for the LOQ was provided in the ECM, and the LOQ was reported in the ILV from the ECM without justification. No calculations were

reported to support the LOQ. In the ECM, the LODs were estimated to be one-third of the LOQ; no calculations were provided. In the ILV, the LODs were calculated by evaluating the signal-to-noise (S/N) ratio from samples of a known concentration (i.e. the lowest calibration standard) and blank samples (i.e. control samples) to establish the lowest level at which the analyte can reliably be detected. A S/N ratio of 3:1 was used to determine the LOD for each analyte and transition. Detection limits should not be based on arbitrary values.

V. Reviewer's Comments

1. In the ILV, the time required to complete the extraction of one set of 19 samples (a reagent blank, 10 fortified samples, 2 unfortified samples, 1 matr-matched standard blank, and 5 matrix-matched standards) required *ca*. 8 hours of work, with LC/MS/MS and GC/MS performed overnight (Appendix 2, p. 171 of MRID 50552102).

2. The reviewer noted that that the chemical purity of tau-fluvalinate was 91.49% in the ECM (Table 1, p. 14 of MRID 50552102).

3. The ECM reported that the residue levels of tau-Fluvalinate, ACBA, Diacid, PBA, and RCAA were determined using LC-MS/MS and haloaniline was determined using GC/MS (p. 12 of MRID 50552102). PB aldehyde was found to be unstable in soil and thatch, degrading to PBA; therefore, analyses targeted PBA rather than PB aldehyde. Similarly, diacid was observed to degrade rapidly to ACBA in soil thus ACBA was quantified in soil analyses instead of diacid

V. References

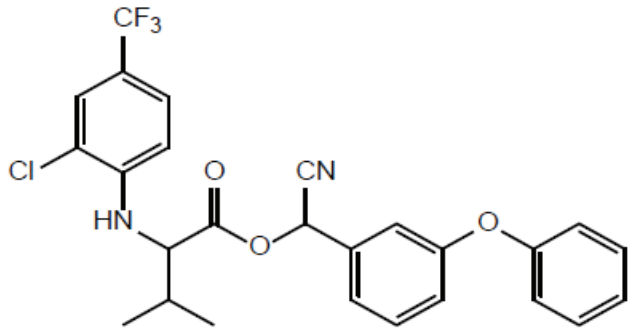
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

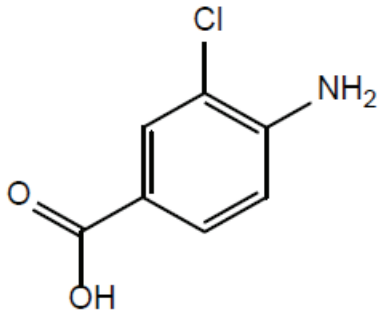
Tau-fluvalinate

IUPAC Name: (RS)-α-cyano-3-phenoxybenzyl-R-2-(2-chloro-4-trifluoromethyl)anilino-3-butanoate
CAS Name: Not reported
CAS Number: 102851-06-9
SMILES String: Not reported



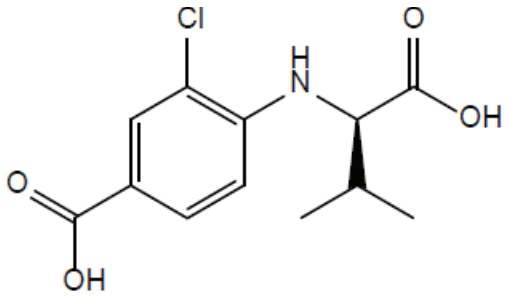
ACBA

IUPAC Name: 4-Amino-3-chlorobenzoic acid
CAS Name: Not reported
CAS Number: 2486-71-7
SMILES String: Not reported



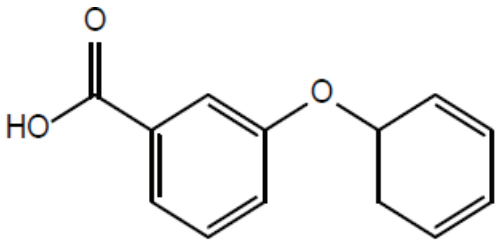
Diacid (FDA fluvalinate diacid)

IUPAC Name: 2-(2-Chloro-4-carboxyl)anilino-3-methylbutanoic acid
CAS Name: Not reported
CAS Number: 85236-41-5
SMILES String: Not reported



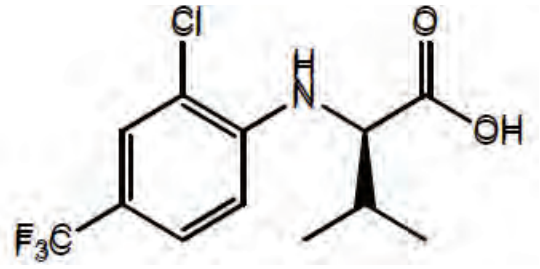
PBA (3-PB acid)

IUPAC Name: 3-Phenoxybenzoic acid
CAS Name: Not reported
CAS Number: 3739-38-6
SMILES String: Not reported



RCAA (Anilino acid)

IUPAC Name: 2-(2-Chloro-4-trifluoromethyl) anilino-3-methylbutanoic acid
CAS Name: Not reported
CAS Number: 76769-07-8
SMILES String: Not reported



Haloaniline

IUPAC Name: 2-chloro-4-trifluoromethylaniline
CAS Name: Not reported
CAS Number: 39885-50-2
SMILES String: Not reported