Analytical method for the diastereomeric forms of BAS 311 I [Cis I, Cis II (BAS 310 I; alpha-cypermethrin), Trans III and Trans IV] and its metabolites, 3-phenoxybenzoic acid (3-PBA) and DCVA (cis and trans isomers), in soil

Reports: ECM: EPA MRID No. 50339901. Carter, M.L., and S. Perez. 2014. Validation of BASF Analytical Method R0034/01: "Method for the Quantitation of the Diastereomeric Forms of BAS 311 I (Reg. 127266) and Its Metabolites 3-Phenoxybenzoic Acid (Reg. No. 130213) and DCVA (Cis and Trans Isomers, Reg. No. 180011) in Soil by LC-MS/MS". BASF Study No.: 405215. ADPEN Study No.: 2K13-903-405215. BASF Registration Document No.: 2017/7008352. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 441 pages. Final original report issued October 17, 2013; Amended report dated September 9, 2014; Second amended report dated January 19, 2015; Third amended report dated June 28, 2017.

ILV: EPA MRID No. 50339902. Shen, X. 2014. INDEPENDENT LABORATORY VALIDATION OF METHOD R0034/01: Method for the Quantitation of the Diastereomeric Forms of BAS 311 I (Reg. 127266) and Its Metabolites 3-Phenoxybenzoic Acid (Reg. No. 130213) and DCVA (Cis and Trans Isomers, Reg. No. 180011) in Soil by LC-MS/MS. BASF Study No.: 405216. PASC Study No.: 053-0894. PASC Report No.: PASC-REP-0416. BASF Registration Document No.: 2017/7008353. Report prepared by Primera Analytical Solutions Corp. (PASC), Princeton, New Jersey; sponsored and submitted by BASF Crop Protection, Research Triangle Park, North Carolina; 169 pages. Final original report issued October 16, 2013; Amended report dated September 9, 2014; Second amended report dated June 28, 2017.

Document No.: MRIDs 50339901 (ECM) & 50339902 (ILV)

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with USEPA GLP (Title 40, Part 160 of CFR; p. 3 of MRID 50339901). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). A signatures page for the Amended Final Report was also included (p. 6).

ILV: The study was conducted in accordance with USEPA GLP (p. 3 of MRID 50339902). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-5). A signatures page for the Amended Final Report was also included (p. 6).

Classification: This analytical method is classified as **unacceptable**. It could not be determined if the independent laboratory was provided with the most difficult matrix with which to validate the method. Based on the provided representative chromatograms in the ECM and ILV, the specificity of the ECM method was not demonstrated for

Cis II (alpha-cypermethrin), Trans III and Trans IV. In the ECM, sample recoveries were corrected for residues in the matrix for cis and trans DCVA.

PC Code: 209600

EFED Final Reviewer: Kristy Crews, PhD, Chemist Signature: Kristy Crews

Date: 4/1/19

CDM/CSS-Dynamac JV Reviewers: Lisa Muto, M.S. Environmental Scientist Signature: Lisa Muto

Date:

Joan Gaidos, Ph.D., Environmental Scientist Signature:

Date:

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

This analytical method, BASF Analytical Method R0034/01, is designed for the quantitative determination of diastereomeric forms of BAS 311 I [Reg. 127266; including Cis I, Cis II (BAS 310 I; alpha-cypermethrin), Trans III and Trans IV] and its metabolites 3-phenoxybenzoic acid (3-PBA; Reg. No. 130213) and DCVA (cis and trans isomers, Reg. No. 180011) in soil at the LOQ of 0.001 mg/kg. The LOQ is **less than** the lowest toxicological level of concern in soil (PPDB 3/27/19[1]). A successful ECM validation was conducted using sandy loam (7% clay, 1.2% organic matter) and clay loam (37% clay, 0.92% organic matter) soils; the ILV soil matrix was the same clay loam as the ECM. The method was validated by the ILV with the first trial with insignificant modifications to the analytical parameters, as well as the modification of Method G of the ECM [used for 3-PBA and DCVA (cis and trans)] to Method I to improve peak shape. The ILV modifications of the extraction and analytical ECM procedure did not warrant an updated ECM since the advantage of Method I versus Method G was not evident. A quantification and confirmation transition or method was monitored for both UPLC/MS/MS and HPLC/MS/MS analysis for all analytes. Based on the provided representative chromatograms in the ECM and ILV, the specificity of BASF Analytical Method R0034/01 was not demonstrated for Cis II (alpha-cypermethrin), Trans III and Trans IV due to incomplete separation of distereomers/isomers; however, recovery data for the quantitative and confirmatory ions/methods were acceptable and comparable in the UPLC and HPLC Methods (comparable between methods depending on soil). Some quantitative linearity was unacceptable for Trans III, 3-PB A and Cis-DCVA.

[1] http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/24.htm

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
BAS 311 I Cis I	50339901	50339902		Soil[1,2]	17/10/2013 (Original Report) 09/09/2014 1st Amended Report) 19/01/2015 (2nd Amended Report) 28/06/2017 (3rd Amended Report)	BASF Corporation	LC/MS/MS (UPLC and HPLC Methods)	0.001 mg/kg
BAS 311 I Cis II (alpha-cypermethrin)								
BAS 311 I Trans III								
BAS 311 I Trans IV								
3-PBA								
Cis-DCVA								
Trans-DCVA								

1 In the ECM, sandy loam soil matrix (CHAR 0-6"; 69% sand 24% silt 7% clay, pH 6.8 in saturated paste, 0.68% organic carbon, 1.2% organic matter) from California and the clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) from Louisiana were fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; Figures 9.5.1-9.5.2, pp. 299-307 of MRID 50339901).

2 In the ILV, clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) was provided by the sponsor and fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 16; Appendix B, p. 168 of MRID 50339902). This soil matrix was the same as one of the ECM soil matrices.

I. Principle of the Method

Procedure for BAS 311 I [Cis I, Cis II (BAS 310 I; alpha-cypermethrin), Trans III and Trans IV]
Soil samples (5 g) were measured into 150 mL centrifuge bottles and fortified, as necessary, using mixed fortification solutions of the diastereomers of BAS 311 I (pp. 25-27, 29-30; Appendix 9.3, p. 118 of MRID 50339901). The samples were extracted with 50 mL of S1 (0.1% formic acid in acetonitrile, v:v) via shaking for *ca*. 30 minutes via a mechanical shaker (speed not reported). A 20-mL aliquot was centrifuged (4000 rpm) for 5 minutes. A 10-mL aliquot of the supernatant was transferred to a culture tube. The extract was evaporated to dryness using nitrogen at *ca*. 50°C (water bath). The residue was reconstituted using S3 (water:0.1% formic aid in acetonitrile; 50:50, v:v). For LOQ and control samples, 2 mL of S3 was used. For 10×LOQ samples, the volume of S3 was not specified; however, the study authors reported that dilution should be performed, as necessary, to ensure that the residue response fit into the calibration curve. After reconstitution of the sample with S3, the sample was sonicated for *ca*. 1 minute then vortexed for *ca*. 15 seconds. A 1-mL aliquot was transferred to a HPLC vial using a primed syringe filter (0.45 µm Nylon, primed by discarding the first 200 µL of filtered sample).

Procedure for 3-PBA and DCVA (cis and trans isomers)
Soil samples (5 g) were measured into disposable centrifuge tubes and fortified, as necessary, using mixed fortification solutions of 3-PBA and DCVA (cis and trans isomers; pp. 25-30; Appendix 9.3, p. 119 of MRID 50339901). The samples were extracted twice with 25 mL of S2 (acetonitrile:water; 70:30, v:v) via shaking for *ca*. 30 minutes via a mechanical shaker (speed not reported). After centrifugation (4000 rpm) for 5 minutes at *ca.* 0°C, the supernatant was transferred to a 50-mL disposable centrifuge tube. The combined extracts were vortexed thoroughly and centrifuged (4000 rpm) for 5 minutes at *ca*. 0°C. A 5-mL of the extract was transferred to a culture tube and evaporated to dryness using nitrogen at *ca*. 50°C (water bath). The residue was reconstituted using S4 (methanol:0.1% formic aid in water; 20:80, v:v). For LOQ and control samples, 1 mL of S4 was used. For 10×LOQ samples, the volume of S4 was not specified. After reconstitution of the sample with S4, the sample was sonicated for *ca*. 1-2 minutes then vortexed for *ca*. 15 seconds. A 1-mL aliquot was transferred to a HPLC vial using a syringe filter (0.45 µm PTFE).

Instrumental analysis for BAS 311 I [Cis I, Cis II (BAS 310 I; alpha-cypermethrin), Trans III and Trans IV]

Method B (UPLC Mode) - for primary and confirmatory quantitation:
Samples were analyzed using an Agilent 1200 SL HPLC System coupled to an AB Sciex 5500 Mass Spectrometer with Electrospray ionization (ESI; p. 29 of MRID 50339901). The instrumental conditions consisted of an Acquity UPLC HSS T3 column (2.1 x 150 mm, 1.8-µm; column temperature 60°C), a mobile phase gradient of (A) water containing 0.1% formic acid (4mM ammonium formate) and (B) methanol containing 0.1% formic acid (4mM ammonium formate) [percent A:B (v:v) at 0.00-1.00 min. 90.0:10.0, 6.00-18.00 min. 22.0:78.0, 18.10-19.00 min. 2.0:98.0, 19.10-22.10 min. 90.0:10.0] and MS/MS detection in positive ion mode (ionization temperature 600°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 433 → 191 (Q) and *m/z* 435 → 193 (C) for alpha-cypermethrin (Cis II isomer), Cis I isomer, Trans III isomer and Trans IV isomer. Expected retention times were *ca*. 13.0 min., *ca*. 13.1 min., *ca*. 12.9 min. and *ca*. 12.7 min. for alpha-cypermethrin (Cis II isomer), Cis I isomer, Trans III isomer and Trans IV isomer, respectively. Injection volume was 30 µL.

Method C (HPLC Mode) - for primary and confirmatory quantitation:
Samples were analyzed using an Agilent 1200 SL HPLC System coupled to an AB Sciex QTRAP 4000 Mass Spectrometer with Electrospray ionization (ESI; p. 30 of MRID 50339901). The instrumental conditions consisted of an XSelect HSS T3 column (2.1 x 150 mm, 2.5-µm; column temperature 60°C), a mobile phase gradient of (A) water containing 0.1% formic acid (4 mM ammonium formate) and (B) methanol containing 0.1% formic acid (4 mM ammonium formate) [percent A:B (v:v) at 0.0-1.0 min. 95:5, 6.0-27.0 min. 22:78, 27.1-28.1 min. 2:98, 28.2-31.2 min. 95:5] and MS/MS detection in positive ion mode (ionization temperature 500°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored: *m/z* 433 → 191 (Q) and *m/z* 435 → 193 (C) for alpha-cypermethrin (Cis II isomer), Cis I isomer, Trans III isomer and Trans IV isomer. Expected retention times were *ca*. 24.0 min., *ca*. 25.0 min., *ca*. 23.6 min. and *ca*. 23.1 min. for alpha-cypermethrin (Cis II isomer), Cis I isomer, Trans III isomer and Trans IV isomer, respectively. Injection volume was 20 µL.

Instrumental analysis for 3-PBA and DCVA (cis and trans isomers)

Method E (HPLC Mode) - for primary and confirmatory quantitation of 3-PBA, primary quantitation in HPLC mode of DCVA (cis and trans) and confirmatory quantitation for Method G:
Samples were analyzed using an Agilent 1200 SL HPLC System coupled to an AB Sciex QTRAP 5500 Mass Spectrometer with Electrospray ionization (ESI; p. 31 of MRID 50339901). The instrumental conditions consisted of an BEH Phenyl column (2.1 x 100 mm, 2.5-µm; column temperature 60°C), a mobile phase gradient of (A) water and (B) acetonitrile [percent A:B (v:v) at 0.00-0.50 min. 100.0:0.0, 14.00 min. 25.0:75.0, 14.10-17.00 min. 100.0:0.0] and MS/MS detection in negative ion mode (ionization temperature 600°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored for 3-PBA: *m/z* 213 → 93 (Q) and *m/z* 213 → 169 (C). Ions monitored for Trans-DCVA and Cis-DCVA were *m/z* 207 → 207 (Q) and *m/z* 209 → 209 (C; both ions for each isomer; these were not transitions – an alternative method is required for confirmation). Expected retention times were *ca*. 8.8 min., *ca*. 9.7 min. and *ca*. 10.4 min. for 3-PBA, Trans-DCVA and Cis-DCVA, respectively. Injection volume was 50 µL.

Method F (HPLC Mode) – for confirmatory quantitation of DCVA (cis and trans) in HPLC mode and for Method E:
Samples were analyzed using an Agilent 1200 SL HPLC System coupled to an AB Sciex QTRAP 5500 Mass Spectrometer with Electrospray ionization (ESI; p. 32 of MRID 50339901). The instrumental conditions consisted of an XSelect HSS T3 column (2.1 x 150 mm, 2.5-µm; column temperature 60°C), a mobile phase gradient of (A) water and (B) acetonitrile [percent A:B (v:v) at 0.00 min. 100.0:0.0, 15.00 min. 30.0:70.0, 15.10-17.00 min. 5.0:95.0, 17.10-20.00 min. 100.0:0.0] and MS/MS detection in negative ion mode (ionization temperature 600°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored for 3-PBA: *m/z* 213 → 93 (Q) and *m/z* 213 → 169 (C). Ions monitored for Trans-DCVA and Cis-DCVA were *m/z* 207 → 207 (Q) and *m/z* 209 → 209 (C; both ions for each isomer; these were not transitions – an alternative method is required for confirmation). Expected retention times were *ca*. 10.5 min., *ca*. 11.2 min. and *ca*. 12.2 min. for 3-PBA, Trans-DCVA and Cis-DCVA, respectively. Injection volume was 50 µL.

Method G (UPLC Mode) – for primary and confirmatory quantitation of 3-PBA and primary quantitation of DCVA (cis and trans):

Samples were analyzed using an Agilent 1200 SL HPLC System coupled to an AB Sciex 5500 Triple Quad Mass Spectrometer with Electrospray ionization (ESI; p. 33 of MRID 50339901). The instrumental conditions consisted of an Acquity UPLC HSS T3 column (2.1 x 50 mm, 1.8-µm; column temperature 60°C), a mobile phase gradient of (A) water and (B) acetonitrile [percent A:B (v:v) at 0.00 min. 85.0:15.0, 8.00 min. 45.0:55.0, 8.10-9.00 min. 5.0:95.0, 9.10-13.00 min. 85.0:15.0] and MS/MS detection in negative ion mode (ionization temperature 600°C). Two parent-daughter ion transitions (quantitative = Q, confirmatory = C) were monitored for 3-PBA: *m/z* 213 → 93 (Q) and *m/z* 213 → 169 (C). Ions monitored for Trans-DCVA and Cis-DCVA were *m/z* 207 → 207 (Q) and *m/z* 209 → 209 (C; both ions for each isomer; these were not transitions – an alternative method is required for confirmation). Expected retention times were *ca*. 5.3 min., *ca*. 6.1 min. and *ca*. 6.7 min. for 3-PBA, Trans-DCVA and Cis-DCVA, respectively. Injection volume was 50 µL.

Reported Potential Problems with the Method

The ECM study authors reported that five potential problems were known about the method: 1) alpha-cypermethrin (Cis II) can be unstable in methanol, and all BAS 311 I standard solutions should be stored in amber glassware when possible to protect from the light; 2) glassware should be rinsed with acetonitrile when possible to avoid contamination; 3) nylon filters should be primed prior to vialing for analysis since all diastereomers of BAS 311 I are absorbed to the nylon filters during filtration; 4) some soil types can cause interferences with 3-PBA and DCVA (cis and trans) LC/MS/MS analysis; therefore, LC gradient modifications might be necessary; and 5) the retention times of the diastereomers of BAS 311 I shift depending upon the instrument (Appendix 9.3, p. 120 of MRID 50339901).

Amendments to the original report did not include any changes to the extraction or analytical procedure (Appendix 9.7, p. 441 of MRID 50339901).

ILV

In the ILV, the extraction procedure for all analytes was the same as the ECM, except that the mechanical shaker speed was specified as 300 rpm and the centrifugation was performed at 3600 rpm (pp. 16-17; Table 11.9, pp. 45-49 of MRID 50339902). The extracts were analyzed for the diastereomers of BAS 311 I using identical or similar instruments and instrumental conditions, except that the injection volume of Method B was reduced to 20 µL and two temperatures were changed (column temperature, Method B; MS/MS temperature, Method C). Retention times of the analytes were ±0.8 min. for Method B and *ca*. 8 min. less for Method C compared to those listed in the ECM. Method G of the ECM [used for 3-PBA and DCVA (cis and trans)] was modified to Method I to improve peak shape. For Method I, the mobile phase gradient of Method G was modified to [(A) water and (B) acetonitrile [percent A:B (v:v) at 0.0-0.5 min. 90:10, 8.5 min. 35:65, 8.6-11.0 min. 95:5, 11.1-14.0 min. 90.0:10]; also, the MS/MS temperature was changed. Otherwise, identical or similar instruments and instrumental conditions were used. For 3-PBA, only the primary transition was monitored for all methods; Method F was used as a confirmatory method for Method I (UPLC) and Method E (HPLC). For DCVA (cis and trans), the primary/confirmatory quantification methods were Method I/Method E for UPLC and Method E/Method F for HPLC. Methods E and F were performed using identical or similar instruments and instrumental conditions, except that the flow rate was reduced for the 15.1-17.0 min. gradient and two temperatures were changed (MS/MS temperature, Methods E and F). Retention times of the

analytes were ±0.1 min. for Method E, ±0.5 min. for Method F and *ca*. 1 min. less for Method I compared to those listed in the ECM.

LOQ/LOD

For all four diastereomers of BAS 311 I, 3-PBA and DCVA (cis and trans), the LOQ and LOD were reported as 0.001 mg/kg and 0.0002 mg/kg (20% of the LOQ), respectively, in both the ECM and ILV (pp. 7, 51-52 of MRID 50339901; p. 18 of MRID 50339902).

II. Recovery Findings

ECM (MRID 50339901): Mean recoveries and relative standard deviations (RSD) were within guidelines (mean 70-120%; RSD ≤20%) in sandy loam and clay loam soils at the LOQ (0.001 ppm) and 10×LOQ (0.01 ppm) for all analytes (pp. 9-15; Tables 8-14, pp. 38-44; Appendix 9.6, pp. 316-367). The analytes included the diastereomeric forms of BAS 311 I [Cis I, Cis II (alpha-cypermethrin), Trans III and Trans IV], 3-PBA and the cis and trans isomers of DCVA. LC/MS/MS analysis was employed for identification of the residues, with at least one primary method and one confirmatory method for each analyte (pp. 29-33; Tables 8-14, pp. 38-44). For the diastereomeric forms of BAS 311 I and 3-PBA, two parent-daughter ion transitions were monitored in each LC/MS/MS method which was used. For cis- and trans-DCVA, one of the two ions was monitored for quantification (*m/z* 207); the study authors reported that an additional LC/MS/MS method was necessary for confirmation of analyte identification. For the diastereomeric forms of BAS 311 I, the primary and confirmatory LC/MS/MS methods were Method B (UPLC) and Method C (HPLC), respectively. For 3-PBA and DCVA (cis and trans), the primary and confirmatory LC/MS/MS methods were Method G (UPLC) and Method E (HPLC), respectively; Method E could also be used as a primary method for those analytes. Additionally, for cis- and trans-DCVA, Method F (HPLC) was used as a confirmatory LC/MS/MS method for Method E. Quantitative ion and confirmatory ion results were comparable. For the diastereomeric forms of BAS 311 I, mean recoveries using Method B were *ca.* 15-30% lower than those of Method C in the sandy loam soil (those of the clay loam soil were comparable); also, RSDs in the clay loam soil were generally less using Method B than Method C (those of the sandy loam soil were comparable). For 3-PBA and DCVA (cis and trans), mean recoveries and RSDs of Methods G, E and F were generally comparable. A few of the recovery results for DCVA (cis and trans) in the clay loam soil with Methods G and F were corrected for residues found in the controls (0.0003-0.0005 ppm; p. 34; Appendix 9.6, pp. 316-367). The sandy loam soil matrix (CHAR 0-6"; 69% sand 24% silt 7% clay, pH 6.8 in saturated paste, 0.68% organic carbon, 1.2% organic matter) from California and the clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) from Louisiana were fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; Figures 9.5.1-9.5.2, pp. 299-307).

ILV (MRID 50339902): Mean recoveries and RSDs were within guidelines in clay loam soil at the LOQ (0.001 ppm) and 10×LOQ (0.01 ppm) for all analytes (pp. 11-13, 20-23; Tables 11.1-11.7, pp. 27-40 9; Appendix A, pp. pp. 141-167). The analytes included the diastereomeric forms of BAS 311 I [Cis I, Cis II (alpha-cypermethrin), Trans III and Trans IV], 3-PBA and the cis and trans isomers of DCVA. LC/MS/MS analysis was employed for identification of the residues, with one primary method and one confirmatory method for each analyte (p. 17). For the diastereomeric forms of BAS 311 I, two parent-daughter ion transitions were monitored in each LC/MS/MS method which was

used. For 3-PBA, one parent-daughter ion transition was monitored. For cis- and trans-DCVA, one or two ions were monitored. For the diastereomeric forms of BAS 311 I, the primary and confirmatory LC/MS/MS methods were Method B (UPLC) and Method C (HPLC), respectively. For 3-PBA, the primary and confirmatory LC/MS/MS methods were Method I and Method F, respectively, for UPLC analysis and Method E and Method F, respectively, for HPLC analysis. For cis- and trans-DCVA, the primary and confirmatory LC/MS/MS methods were Method I (*m/z* 207) and Method E (*m/z* 209), respectively, for UPLC analysis and Method E and Method F (*m/z* 207 for both), respectively, for HPLC analysis. Quantitative ion and confirmatory ion results were comparable. Mean recoveries and RSDs of the different LC/MS/MS methods were generally comparable. Recoveries were not corrected for residues found in the controls (pp. 18-19 and Tables 11.1-11.7, pp. 27-40). The clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) was provided by the sponsor and fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 16; Appendix B, p. 168). This soil matrix was the same as one of the ECM soil matrices. The method was validated with the first trial with insignificant modifications to the analytical parameters, as well as the modification of Method G of the ECM [used for 3-PBA and DCVA (cis and trans)] to Method I to improve peak shape (pp. 17, 19).

Table 2. Initial Validation Method Recoveries for the Diastereomeric Forms of BAS 311 I [Cis I, Cis II (alpha-cypermethrin), Trans III and Trans IV] and Its Metabolites 3-Phenoxybenzoic acid (3-PBA) and DCVA (Cis and Trans Isomers) in Soil [1,2]

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Quantitative ion						
	Method B (UPLC Mode)					
Cis I	0.001 (LOQ)	5	68-74	71	2.4	3.4
	0.01	5	69-76	72	2.9	4.0
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	67-79	73	4.7	6.5
	0.01	5	69-78	74	3.9	5.3
Trans III	0.001 (LOQ)	5	72-90	83	7.7	9.3
	0.01	5	76-85	78	3.7	4.7
Trans IV	0.001 (LOQ)	5	70-86	77	6.0	7.9
	0.01	5	65-85	74	7.7	10.4
	Method C (HPLC Mode)					
Cis I	0.001 (LOQ)	5	85-105	93	8.0	8.6
	0.01	5	86-94	91	3.0	3.4
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	96-107	100	4.2	4.2
	0.01	5	94-101	98	3.0	3.1
Trans III	0.001 (LOQ)	5	84-112	100	10.8	10.9
	0.01	5	97-118	103	9.0	8.7

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Trans IV	0.001 (LOQ)	5	93-114	101	8.1	8.1
	0.01	5	94-119	105	9.6	9.2
	Method G (UPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	88-101	94	5.8	6.2
	0.01	5	92-100	96	3.7	3.9
Trans-DCVA	0.001 (LOQ)	5	100-112	105	4.5	4.3
	0.01	5	92-105	99	5.3	5.4
3-PBA	0.001 (LOQ)	5	93-105	99	5.1	5.2
	0.01	5	88-103	96	5.7	6.0
	Method E (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	81-106	92	11.7	12.8
	0.01	5	74-92	82	7.2	8.7
Trans-DCVA	0.001 (LOQ)	5	97-113	103	6.5	6.4
	0.01	5	77-97	90	7.9	8.8
3-PBA	0.001 (LOQ)	5	88-110	97	9.4	9.7
	0.01	5	85-107	98	8.2	8.4
	Method F (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	76-118	101	16.2	16.0
	0.01	5	87-100	94	5.1	5.4
Trans-DCVA	0.001 (LOQ)	5	93-105	97	4.7	4.9
	0.01	5	93-106	98	4.9	4.9
Confirmatory ion						
	Method B (UPLC Mode)					
Cis I	0.001 (LOQ)	5	70-79	74	4.1	5.5
	0.01	5	71-79	75	3.5	4.6
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	68-77	72	3.7	5.1
	0.01	5	69-76	73	3.1	4.3
Trans III	0.001 (LOQ)	5	71-94	82	10.3	12.6
	0.01	5	72-91	81	7.4	9.1
Trans IV	0.001 (LOQ)	5	68-83	76	6.1	7.9
	0.01	5	64-86	74	8.4	11.4
	Method C (HPLC Mode)					
Cis I	0.001 (LOQ)	5	86-102	94	7.5	7.9
	0.01	5	85-100	93	5.4	5.8
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	86-111	98	9.0	9.1
	0.01	5	92-101	97	4.6	4.7
Trans III	0.001 (LOQ)	5	83-108	99	9.5	9.7

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.01	5	93-119	103	9.9	9.6
Trans IV	0.001 (LOQ)	5	105-113	108	3.1	2.9
	0.01	5	93-116	103	9.0	8.7
	Method G (UPLC Mode)					
3-PBA	0.001 (LOQ)	5	91-103	96	4.2	4.4
	0.01	5	89-104	98	5.3	5.4
	Method E (HPLC Mode)					
3-PBA	0.001 (LOQ)	5	90-109	98	6.7	6.8
	0.01	5	83-110	98	9.8	10.0
Clay Loam Soil						
Quantitative ion						
	Method B (UPLC Mode)					
Cis I	0.001 (LOQ)	5	80-82	81	0.6	0.7
	0.01	5	88-91	88	2.0	2.3
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	85-87	86	0.9	1.1
	0.01	5	90-95	91	2.2	2.4
Trans III	0.001 (LOQ)	5	92-102	97	4.0	4.1
	0.01	5	86-94	90	3.1	3.4
Trans IV	0.001 (LOQ)	5	86-94	91	3.2	3.5
	0.01	5	81-95	88	5.7	6.4
	Method C (HPLC Mode)					
Cis I	0.001 (LOQ)	5	74-91	83	6.0	7.2
	0.01	5	77-109	92	13.6	14.9
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	84-98	94	5.8	6.2
	0.01	5	82-112	95	12.8	13.4
Trans III	0.001 (LOQ)	5	89-93	91	1.7	1.9
	0.01	5	88-108	97	9.2	9.5
Trans IV	0.001 (LOQ)	5	86-105	94	7.3	7.8
	0.01	5	89-110	97	8.3	8.5
	Method G (UPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	88-104	96	6.1	6.4
	0.01	5	94-106	100	4.9	4.9
Trans-DCVA	0.001 (LOQ)	5	91-115	102	9.5	9.3
	0.01	5	96-108	104	4.8	4.6
3-PBA	0.001 (LOQ)	5	100-108	105	3.4	3.3
	0.01	5	98-110	105	4.6	4.3
	Method E (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	88-100	94	4.9	5.2
	0.01	5	89-101	96	5.2	5.4

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Trans-DCVA	0.001 (LOQ)	5	82-101	90	9.5	10.6
	0.01	5	89-104	98	6.4	6.5
3-PBA	0.001 (LOQ)	5	89-115	100	13.4	13.4
	0.01	5	94-110	101	6.4	6.3
	Method F (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	75-93	87	7.2	8.3
	0.01	5	93-103	97	4.4	4.5
Trans-DCVA	0.001 (LOQ)	5	87-115	100	12.3	12.3
	0.01	5	93-102	97	3.8	3.9
Confirmatory ion						
	Method B (UPLC Mode)					
Cis I	0.001 (LOQ)	5	85-87	86	0.9	1.0
	0.01	5	87-92	89	2.0	2.2
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	86-90	88	1.6	1.8
	0.01	5	88-95	90	2.6	2.9
Trans III	0.001 (LOQ)	5	90-100	95	3.4	3.6
	0.01	5	89-103	93	6.1	6.5
Trans IV	0.001 (LOQ)	5	86-96	91	3.8	4.2
	0.01	5	82-94	88	5.7	6.5
	Method C (HPLC Mode)					
Cis I	0.001 (LOQ)	5	94-120	104	9.9	9.5
	0.01	5	91-131	110	17.2	15.6
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	81-103	93	8.3	8.9
	0.01	5	83-108	95	11.3	11.9
Trans III	0.001 (LOQ)	5	73-97	84	9.5	11.3
	0.01	5	88-106	96	7.9	8.2
Trans IV	0.001 (LOQ)	5	88-102	97	5.7	5.9
	0.01	5	89-109	98	9.3	9.5
	Method G (UPLC Mode)					
3-PBA	0.001 (LOQ)	5	92-111	102	8.1	8.0
	0.01	5	103-110	107	2.7	2.5
	Method E (HPLC Mode)					
3-PBA	0.001 (LOQ)	5	83-109	95	10.7	11.2
	0.01	5	95-105	102	4.3	4.2

Data (uncorrected recovery results, except for cis/trans DVCA in clay loam soil; p. 34; Appendix 9.6, pp. 316-367) were obtained from pp. 9-15; Tables 8-14, pp. 38-44 of MRID 50339901. Individual and mean percent recovery values were rounded in the Abstract (pp. 9-15) and Tables 8-14 (pp. 38-44); unrounded values showing data to a tenth of a percent were reported in Appendix 9.6 (pp. 316-367).

1 The sandy loam soil matrix (CHAR 0-6"; 69% sand 24% silt 7% clay, pH 6.8 in saturated paste, 0.68% organic carbon, 1.2% organic matter) from California and the clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) from Louisiana were fully

characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; Figures 9.5.1-9.5.2, pp. 299-307).

2 Ion transitions monitored were as follows (quantitative ion and confirmatory ion, respectively): *m/z* 433 → 191 (Q) and *m/z* 435 → 193 (C) for alpha-cypermethrin (Cis II isomer), Cis I isomer, Trans III isomer and Trans IV isomer; and *m/z* 213 → 93 (Q) and *m/z* 213 → 169 (C) for 3-PBA. The ion monitored for Trans-DCVA and Cis-DCVA was *m/z* 207 → 207.

Table 3. Independent Validation Method Recoveries for the Diastereomeric Forms of BAS 311 I [Cis I, Cis II (alpha-cypermethrin), Trans III and Trans IV] and Its Metabolites 3-Phenoxybenzoic acid (3-PBA) and DCVA (Cis and Trans Isomers) in Soil[1,2]

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Clay Loam Soil						
Primary Quantification						
	Method B (UPLC Mode)					
Cis I	0.001 (LOQ)	5	88-100	95	5	5
	0.01	5	89-97	93	4	4
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	88-97	93	4	4
	0.01	5	85-92	89	3	3
Trans III	0.001 (LOQ)	5	84-103	91	8	9
	0.01	5	90-111	97	9	9
Trans IV	0.001 (LOQ)	5	89-92	91	2	2
	0.01	5	93-96	95	1	2
	Method C (HPLC Mode)					
Cis I	0.001 (LOQ)	5	79-97	91	7	8
	0.01	5	83-93	87	4	5
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	82-94	88	5	6
	0.01	5	83-93	87	4	4
Trans III	0.001 (LOQ)	5	74-94	86	8	9
	0.01	5	78-92	86	5	6
Trans IV	0.001 (LOQ)	5	81-101	89	8	9
	0.01	5	79-91	86	4	6
	Method I (UPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	107-125	119	7	6
	0.01	5	91-107	99	6	6
Trans-DCVA	0.001 (LOQ)	5	82-123	99	18	19
	0.01	5	93-105	100	5	5
3-PBA	0.001 (LOQ)	5	96-98	97	1	1
	0.01	5	97-104	100	3	3
	Method E (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	82-100	96	8	8
	0.01	5	97-106	101	3	3
Trans-DCVA	0.001 (LOQ)	5	72-85	82	5	7
	0.01	5	94-100	96	2	3
3-PBA	0.001 (LOQ)	5	94-107	103	5	5
	0.01	5	98-110	104	4	4

Analyte	Fortification Level (ppm)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Confirmatory Quantification						
	Method B (UPLC Mode)					
Cis I	0.001 (LOQ)	5	87-100	94	5	5
	0.01	5	91-98	94	3	3
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	71-82	76	5	6
	0.01	5	93-102	96	3	4
Trans III	0.001 (LOQ)	5	83-96	90	5	6
	0.01	5	70-87	81	6	8
Trans IV	0.001 (LOQ)	5	91-101	97	4	4
	0.01	5	91-97	94	2	2
	Method C (HPLC Mode)					
Cis I	0.001 (LOQ)	5	70-97	87	10	12
	0.01	5	81-94	88	6	6
Cis II (alpha-cypermethrin)	0.001 (LOQ)	5	85-116	100	15	15
	0.01	5	81-93	87	5	6
Trans III	0.001 (LOQ)	5	78-103	87	11	12
	0.01	5	87-91	88	5	5
Trans IV	0.001 (LOQ)	5	86-113	100	12	12
	0.01	5	80-92	86	6	6
	Method F (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	84-98	92	5	6
	0.01	5	71-98	93	12	13
Trans-DCVA	0.001 (LOQ)	5	86-98	94	5	5
	0.01	5	87-100	97	6	6
3-PBA	0.001 (LOQ)	5	79-98	91	7	8
	0.01	5	64-93	86	12	14
	Method E (HPLC Mode)					
Cis-DCVA	0.001 (LOQ)	5	78-113	94	14	14
	0.01	5	97-105	102	3	3
Trans-DCVA	0.001 (LOQ)	5	87-99	90	7	8
	0.01	5	92-98	95	2	2

Data (uncorrected recovery results, pp. 18-19 and Tables 11.1-11.7, pp. 27-40) were obtained from pp. 11-13, 20-23; Tables 11.1-11.7, pp. 27-40 of MRID 50339902. Individual and mean percent recovery values, as well as RSD and s.d. values, were rounded in the Abstract (pp. 11-13) and Results (pp. 20-23); unrounded values showing data to a tenth and hundredth of a percent were reported in Tables 11.1-11.7 (pp. 27-40) and Appendix A (pp. 141-167).

1 The clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) was provided by the sponsor and fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 16; Appendix B, p. 168). This soil matrix was the same as one of the ECM soil matrices.

2 Ion transitions monitored were *m/z* 433 → 191 (Q) and *m/z* 435 → 193 (C) for alpha-cypermethrin (Cis II isomer), Cis I isomer, Trans III isomer and Trans IV isomer (quantitative ion and confirmatory ion, respectively); only the

quantitative ion, *m/z* 213 → 93 (Q), was monitored for 3-PBA. The ions monitored for Trans-DCVA and Cis-DCVA were *m/z* 207 → 207 and *m/z* 209 → 209.

III. Method Characteristics

For all four diastereomers of BAS 311 I, 3-PBA and DCVA (cis and trans), the LOQ and LOD were reported as 0.001 mg/kg and 0.0002 mg/kg (20% of the LOQ), respectively, in both the ECM and ILV (pp. 7, 51-52; Appendix 9.3, p. 121 of MRID 50339901; p. 18 of MRID 50339902). The LOQ corresponded to a concentration of 0.5 ng/mL in the final extract; the LOD corresponded to a concentration of 0.1 ng/mL in the final extract. In the ECM, the LOQs for all analytes were supported by being the lowest fortification level tested for each analyte and being significantly lower than the relevant endpoint in soil ecotoxicology (i.e. the NOEC of 0.4 mg/kg dry soil from the nitrogen transfer study). The LOD was further defined as the absolute amount of analyte injected (0.005 ng on column for the least sensitive analyte, PBA) which yielded an acceptable signal to noise ratio (S/N is <3:1). In the ILV, the LOQ and LOD were reported from the ECM; no justification was provided.

Table 4. Method Characteristics

		Cis I	Cis II (alpha-cypermethrin)	Trans III	Trans IV	3-PBA	Cis-DCVA	Trans-DCVA
Limit of Quantitation (LOQ)		0.001 ppm (0.001 mg/kg)						
Limit of Detection (LOD)		0.0002 ppm (0.0002 mg/kg) (20% of the LOQ)						
Linearity (calibration curve r^2 and concentration range)	ECM (Method)[1]	**Quantification ion**						
		r^2 = 0.9996 (B) r^2 = 0.9970 (C)	r^2 = 0.9996 (B) r^2 = 0.9978 (C)	r^2 = 0.9978 (B) r^2 = 0.9970 (C)	r^2 = 0.9970 (B) r^2 = 0.9954 (C)	r^2 = 0.9966 (G) r^2 = **0.9920** (E)	r^2 = 0.9980 (G) r^2 = 0.9960 (E) r^2 = **0.9930** (F)	r^2 = 0.9956 (G) r^2 = 0.9952 (E) r^2 = 0.9960 (F)
		Confirmation ion						
		r^2 = 0.9998 (B) r^2 = **0.9944** (C)	r^2 = 0.9996 (B) r^2 = 0.9990 (C)	r^2 = **0.9936** (B) r^2 = **0.9948** (C)	r^2 = 0.9976 (B) r^2 = 0.9958 (C)	r^2 = 0.9960 (G) r^2 = **0.9902** (E)	NA	NA
		(0.05-10.0 ng/mL)						
	ILV (Method)[2]	**Quantification ion**						
		r^2 = 0.9988 (B) r^2 = 0.9958 (C)	r^2 = 0.9956 (B) r^2 = 0.9958 (C)	r^2 = **0.9900** (B)[3] r^2 = 0.9980 (C)	r^2 = 0.9960 (B)[3] r^2 = 0.9962 (C)	r^2 = 0.9978 (E) r^2 = 0.9992 (I)	r^2 = 0.9974 (E) r^2 = 0.9962 (F) r^2 = 0.9966 (I)	r^2 = 0.9974 (E) r^2 = 0.9988 (F) r^2 = 0.9974 (I)
		(0.10-10.0 ng/mL)						
		Confirmation ion						
		r^2 = 0.9984 (B) r^2 = 0.9956 (C)	r^2 = 0.9968 (B) r^2 = 0.9952 (C)	r^2 = 0.9988 (B) r^2 = 0.9968 (C)	r^2 = 0.9954 (B) r^2 = **0.9922** (C)	r^2 = **0.9888** (F)	r^2 = 0.9976 (E)	r^2 = 0.9968 (E)
Repeatable	ECM[4]	Yes at LOQ and 10×LOQ [sandy loam (7% clay, 1.2% organic matter) and clay loam (37% clay, 0.92% organic matter) soils].						
	ILV[5,6]	Yes at LOQ and 10×LOQ [clay loam (37% clay, 0.92% organic matter) soils].						
		No modifications to ECM.				No modifications to Methods E and F of the ECM. Method G of ECM was optimized to Method I of the ILV (gradient of mobile phase changed).		
Reproducible	ECM	Yes at LOQ and 10×LOQ.						
	ILV							
Specific	ECM[7]	The separation of the diastereomers varied slightly among the two soils and two methods; however, complete isolation was only achieved for Cis I isomer with Method C. Matrix interferences at analyte retention times were <LOD.				Yes, matrix interferences were <LOD.	Yes, matrix interferences were <LOD for Methods G and E (both soils) and Method F (sandy loam soil). In clay	Yes, matrix interferences were <LOD for Method E (both soils) and Methods G and F (sandy loam soil). In clay loam soil,
		Minor interference with attenuation due to	Major interference with attenuation due to	Major interference with attenuation due to	Some interference with attenuation due to Trans III			

		Cis II peak with Method B. In sandy loam soil, minor interference with attenuation due to contaminant (RT 13.05) in the Q ion/Method B at the LOQ.	Trans III peak. Minor interference with attenuation due to Cis I peak with Method B.	Cis II peak. Some interference with attenuation due to Trans IV peak; more interference with Method B.	peak; more interference with Method B.		loam soil, contaminant (*ca.* 20-40% LOQ) at the retention time of the analyte in Method F.[8]	contaminant (*ca.* 40-50% LOQ) at the retention time of the analyte in Methods G and F.
	ILV[3]	The separation of the diastereomers varied slightly among the two methods; however, complete isolation was only achieved for Cis I isomer. Matrix interferences at analyte retention times were <LOD.				Yes, matrix interferences were <LOD.	Yes, matrix interferences were <LOD for Methods E and F.	
		No interference with attenuation due to Cis II peak.	Major interference with attenuation due to Trans III peak.	Major interference with attenuation due to Cis II peak. Some interference with attenuation due to Trans IV peak.	Some interference with attenuation due to Trans III peak.		Peak shape was not well-resolved in Method I, and matrix interferences (*ca.* 40-50% LOQ) interfered with the attenuation of the peak.	Peak shape was not well-resolved in Method I; however, matrix interferences were <LOD.

Data were obtained from pp. 7, 51-52 and Appendix 9.3, p. 121 (LOD/LOQ); pp. 9-15 and Tables 8-14, pp. 38-44 (recovery results); Figures 9.1.8-9.1.14, pp. 62-75 (calibration curves); Figures 9.4.8-9.4.43, pp. 200-297 (chromatograms) of MRID 50339901; p. 18 (LOD/LOQ); pp. 11-13, 20-23 and Tables 11.1-11.7, pp. 27-40 (recovery results); Figures 12.1-12.7, pp. 50-63 (calibration curves); Figures 12.15-12.42, pp. 85-139 (chromatograms) of MRID 50339902; DER Attachment 2.

1 ECM r^2 values are reviewer-generated from reported r values (Figures 9.1.8-9.1.14, pp. 62-75 of MRID 50339901; DER Attachment 2).

2 ILV r^2 values are reviewer-generated from reported r values (Figures 12.1-12.7, pp. 50-63 of MRID 50339902; DER Attachment 2).

3 Only 3 significant figures were reported for r value.

4 In the ECM, sandy loam soil matrix (CHAR 0-6"; 69% sand 24% silt 7% clay, pH 6.8 in saturated paste, 0.68% organic carbon, 1.2% organic matter) from California and the clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) from Louisiana were fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; Figures 9.5.1-9.5.2, pp. 299-307 of MRID 50339901).

5 In the ILV, clay loam soil matrix (R120066.LA.T.CHAR.18-24"; 20% sand 43% silt 37% clay, pH 5.4 in saturated paste, 0.54% organic carbon, 0.92% organic matter) was provided by the sponsor and fully characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil characterization; p. 16; Appendix B, p. 168 of MRID 50339902). This soil matrix was the same as one of the ECM soil matrices.

6 The ILV validated the method with the first trial with insignificant modifications to the analytical parameters, as well as the modification of Method G of the ECM [used for 3-PBA and DCVA (cis and trans)] to Method I to improve peak shape (pp. 17, 19 of MRID 50339902).

7 Comments refer to both ions/both soils/all methods used, unless noted otherwise. Interfering peaks were quantified by the reviewer based on the comparison of the peak area counts reported by the study authors.

8 Quantification based on Figure 9.4.18, p. 231; Figure 9.4.19, p. 234 and Appendix 9.3, p. 364 of MRID 50339901.

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

IV. Method Deficiencies and Reviewer's Comments

1. ECM MRID 50339901 replaces ECM MRID 49501701; ILV MRID 50339902 replaces ILV MRID 49501702. MRIDs 49501701 & 49501702 were previously reviewed by CDM/CSS-Dynamac: primary reviewer Lisa Muto, M.S., secondary reviewer Kathleen Ferguson, Ph.D. The updated MRIDs were provided in response to deficiencies/comments cited due their review. The changes were reported on last pages of amended study reports. The following deficiencies were corrected:

   A. The LOQ justification was added to the ECM.
   B. ILV soil matrix characterization data was included in the amended ILV report.
   C. ECM and ILV reagent blank chromatograms were added.
   D. Correlation coefficients were provided for all methods/analytes in the ILV.
   E. ILV fixed all typographical errors which were noted by the reviewer.

2. The ILV soil matrix, clay loam soil matrix, was the same as one of the ECM soil matrices; therefore, it could not be determined if the ILV was provided with the most difficult analytical sample condition with which to validate the method (p. 16; Appendix B, p. 168). Also, since no terrestrial field dissipation (TFD) studies were provided for review, it could not be determined if the ILV soil matrices covered the range of soils used in the TFD studies.

3. Based on the provided representative chromatograms in the ECM and ILV, the specificity of BASF Analytical Method R0034/01 was not demonstrated for Cis II (alpha-cypermethrin), Trans III and Trans IV (Figures 9.4.8-9.4.43, pp. 200-297 of MRID 50339901; Figures 12.15-12.42, pp. 85-139 of of MRID 50339902). The analyte peaks for Cis II (alpha-cypermethrin) and Trans III were so close that the merged area was generally *ca*. 50% of the peak height of Trans III and *ca.* 20% of the peak height of Cis II, although more extreme overlap was observed (see Figure 9.4.9, p. 203 of MRID 50339901). For Trans IV, some interference with attenuation was observed due to the Trans III peak in the ECM (more pronounced with Method B) and ILV.

   In the provided representative chromatograms of the ECM and ILV, the specificity of BASF Analytical Method R0034/01 was demonstrated for Cis I (Method C, ECM; Methods B and C, ILV), 3-PBA (Methods G and E, ECM; Methods I, E and F, ILV), DCVA (cis and trans; Method E, ECM; Methods E and F, ILV), DCVA (cis; Method G, ECM), and DCVA (trans, Method I, ILV; Figures 9.4.8-9.4.43, pp. 200-297 of MRID 50339901; Figures 12.15-12.42, pp. 85-139 of of MRID 50339902). In representative ECM chromatograms of Cis I with Method B, minor interferences with attenuation were observed: due to the Cis II peak in the clay loam and sandy loam soils (more pronounced with 10×LOQ samples) and due to contaminant (RT 13.05) in the Q ion at the LOQ in the sandy loam soil. In representative ECM chromatograms of clay loam soil fortified with DCVA, contaminants (*ca*. 20-50% LOQ) were observed at the retention time of the analyte with Method F (cis and trans) and Method G (trans only); DCVA recoveries were corrected for these residues (p. 34; Appendix 9.6, pp. 316-367 of MRID 50339901). In representative ILV chromatograms of DCVA (cis and trans) with Method I, the analyte peak was not well-resolved (multi-peaked; Figures 12.35-12.41, pp. 125-138 of MRID 50339902); however, the peak for trans-DVCA was

isolated for accurate integration [matrix interferences (*ca*. 40-50% LOQ) interfered with the attenuation of the peak for cis-DCVA; Figures 12.40, p. 135 and Figure 12.41, p. 137].

A confirmation method is not necessarily required when LC/MS/MS is used as the primary identification method. Based on representative chromatograms, one method, which was shown to be specific in the ECM, was validated by the ILV for Cis I, 3-PBA and DCVA (cis and trans). The analyte peaks for Cis II and Trans III were merged in all chromatograms of the ECM and ILV, and the analyte peak for Trans IV was not isolated in any chromatogram of the ECM and ILV.

4. The sample recoveries were corrected in the ECM [DCVA (cis and trans) in clay loam soil with Methods G and F; 0.0003-0.0005 ppm], but not corrected in the ILV (p. 34; Appendix 9.6, pp. 364-365 of MRID 50339901; pp. 18-19 and Tables 11.1-11.7, pp. 27-40 of MRID 50339902). Per the OSCPP 850.6100 guideline, for the ECM, reported sample values should not be corrected for recoveries.

5. The reported limit of quantitation (LOQ) was determined as the lowest level of method validation (LLMV). Further work could have been done to explore the actual LOQ. This means that concentrations can be reliably quantified at the LOQ (i.e., LLMV), but whether lower concentrations may also be reliably quantified is uncertain.

6. The ILV performed the ECM as written, except for a few minor changes to the extraction procedure and a few changes to the analytical procedure (pp. 16-17; Table 11.9, pp. 45-49 of MRID 50339902). Changes to the analytical procedure included: the modification of the gradient of the mobile phase and MS/MS temperature of Method G to perform Method I for primary quantification of 3-PBA and DCVA (cis and trans); the monitoring of only the primary transition for 3-PBA (all methods); and the use of Method F as a confirmation method for 3-PBA. The reviewer did not observe the advantage of Method I versus Method G since the ILV report did not provide chromatograms of Method G; in general, ECM chromatograms of Method G showed better specificity for DCVA (cis and trans in clay loam soil; Figure 9.4.19, p. 232; Figure 9.4.21, p. 237 of MRID 50339901; Figure 12.41, p. 137; Figure 12.38, p. 131 of MRID 50339902). The ILV also reported the following recommendations for the ECM: calculations for all analytes should be listed and the aliquotation factor should be specified for all analytes (p. 24). None of the modifications or recommendations of the ILV were significant enough to require an updated ECM.

7. The ECM study authors monitored the stability of the analytes in solvent and final extracts from soil (pp. 25-26, 35-36; Appendix 9.6, pp. 315-439 of MRID 50339901). The calibration and fortification solutions of BAS 311 I were stable up to 64-65 days of storage under refrigeration; the calibration and fortification solutions of metabolites of BAS 311 I were stable up to 38 days of storage under refrigeration. The final extracts from the clay loam soil were stable under refrigerated storage for 14 days with BAS 311 I analytes and for 25 days with metabolites of BAS 311 I analytes.

   The extractability in soil data using the residue and metabolism extraction procedures were found to be comparable between two uncharacterized soils from New York (p. 37; Appendix 9.6, pp. 315-439 of MRID 50339901).

8. Matrix effects were studied as part of the ECM validation (pp. 44-50; Tables 15-21, pp. 44-50; Appendix 9.6, pp. 315-439 of MRID 50339901). Sandy loam and clay loam were used for studying matrix effects of BAS 311 I; only clay loam was used for studying matrix effects of the metabolites of BAS 311 I. Matrix effects (suppression or enhancement) were less than 20% for all analyte/soil/method combinations which were evaluated.

9. In the ECM and ILV, it was reported that a set consisting of 13 samples required approximately 12 work hours, including calculation of the results (p. 51 of MRID 50339901; p. 17 of MRID 50339902).

10. In the ILV, the communications between the ILV study author and study sponsor/ECM study authors was briefly reported (p. 24 of MRID 50339902). Three protocol amendments were created from these communications, including the change of the confirmatory UPLC method for 3-PBA, the change of the LC/MS/MS methods (Method G to Method I), and the change of the study title. The communication and protocol amendments were unchanged from the previously submitted ILV MRID 49501702 (p. 23 of MRID 49501702).

V. References

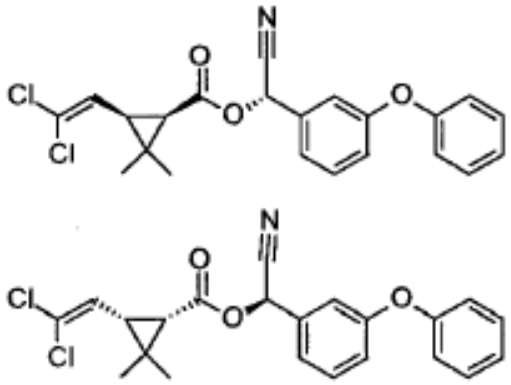
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

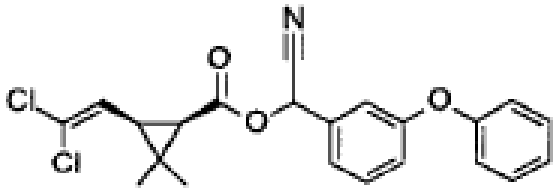
Alpha-cypermethrin (Cis II; BAS 301 I)

IUPAC Name:	Racemate of (R)-cyano-3-phenoxybenzyl (1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (S)-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Name:	(R)-cyano(3-phenoxyphenyl)methyl (1S,3S)-rel-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	67375-30-8
SMILES String:	ClC(Cl)=CC1C(C)(C)C1C(=O)OC(C#N)c2cccc(Oc3ccccc3)c2



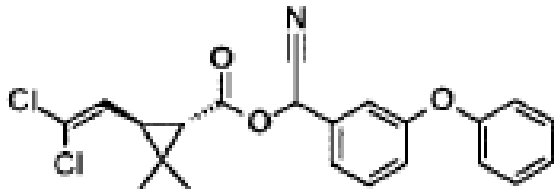
Cis I/Cis II Isomers (45:55)

IUPAC Name:	(RS)-α-cyano-3-phenoxybenzyl (1RS,3RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Name:	Not reported
CAS Number:	211504-93-7
SMILES String:	ClC(Cl)=CC1C(C)(C)C1C(=O)OC(C#N)c2cccc(Oc3ccccc3)c2



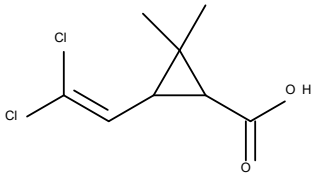
Trans III/Trans IV (43.5:56.5)

IUPAC Name:	(RS)-α-cyano-3-phenoxybenzyl (1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Name:	Not reported
CAS Number:	211504-94-8
SMILES String:	ClC(Cl)=CC1C(C)(C)C1C(=O)OC(C#N)c2cccc(Oc3ccccc3)c2



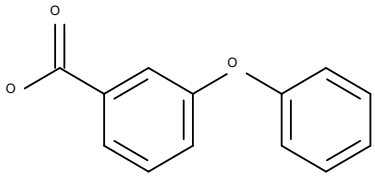
DCVA (Cis/Trans isomers, 51.5:48.5; Reg. No. 180011)

IUPAC Name:	3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid
CAS Name:	Not reported
CAS Number:	55701-05-8
SMILES String:	CC1(C(C1C(=O)O)C=C(Cl)Cl)C



3-PBA (Reg. No. 130213)

IUPAC Name:	3-Phenoxybenzoic acid
CAS Name:	Not reported
CAS Number:	3739-38-6
SMILES String:	OC(=O)c2cccc(Oc1ccccc1)c2



Cypermethrin (BAS 311 I, Reg. No. 127266)

IUPAC Name:	(RS)-α-cyano-3-phenoxybenzyl (1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate
CAS Name:	Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate
CAS Number:	52315-07-8
SMILES String:	ClC(Cl)=CC1C(C)(C)C1C(=O)OC(C#N)c2cccc(Oc3ccccc3)c2