

**Analytical method for cyazofamid (IKF-916) and its four metabolites CCIM, CCIM-AM, CTCA, and CCBA in water**

**Reports:** ECM: EPA MRID No. 50396409. Jutson, J.I. 2017. Method Validation – Determination of Residues of Cyazofamid (IKF-916), and Four Metabolites (CCIM, CCIM-AM, CTCA, and CCBA) in Surface Water and Ground Water Using LC-MS/MS. Concord Biosciences Study No.: 035868 and Document No.: 035868-1. Report prepared by AgChem Product Development, Concord Biosciences, LLC, Concord, Ohio; sponsored and submitted by ISK Biosciences Corporation, Concord, Ohio; 156 pages. Final report issued September 29, 2017.

ILV: EPA MRID No. 50396410. Formanik, J. 2017. Independent Laboratory Validation - Determination of Residues of Cyazofamid (IKF-916), and Four Metabolites (CCIM, CCIM-AM, CTCA, and CCBA), in Surface Water and Ground Water Using LC-MS/MS. Concord Biosciences Study No.: 035869 and Document No.: 035869-1. Report prepared by AgChem Product Development, Concord Biosciences, LLC, Concord, Ohio; sponsored and submitted by ISK Biosciences Corporation, Concord, Ohio; 139 pages. Final report issued October 4, 2017.

**Document No.:** MRIDs 50396409 & 50396410

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practices (GLP; 40 CFR Part 160; p. 3 of MRID 50396409). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). A certification of the authenticity of the report was not included.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP, except that the analyst software version 1.6.3, which was used during the study, was not validated (analyst software version 1.6.2 was validated; p. 3 of MRID 50396410). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). A certification of the authenticity of the report was not included.

**Classification:** This analytical method is classified as Unacceptable. The ILV was not conducted independently from the ECM. An updated ECM was not submitted incorporating the ILV modification for CTCA. The specificity of the method for CTCA was insufficiently supported by ECM and ILV representative chromatograms. Some ECM linearity was not satisfactory for CCBA. ILV matrices were the same as the matrices used in the ECM. The LOD was not reported in the ILV. The LOQ for CTCA is greater than the lowest toxicological level of concern.

**PC Code:** 085651


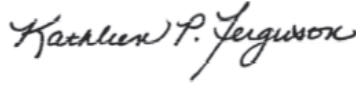
**EFED Final** Patricia Engel

**Reviewer:**

Signature:  
Date: 4/21/2020



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<b>CDM/CSS- Dynamac JV Reviewers:</b>	Lisa Muto, M.S., Environmental Scientist	Signature: 
		Date: 4/19/18
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature: 
		Date: 4/19/18

*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, Concord Biosciences Study No. 035868, is designed for the quantitative determination of cyazofamid (IKF-916) and its transformation products CCIM, CCIM-AM, CTCA, and CCBA in water using LC/MS/MS. The method is quantitative for cyazofamid (IKF-916) at the stated LOQ of 0.10 µg/L and for CCIM, CCIM-AM, CTCA, and CCBA at the stated LOQ of 0.50 µg/L. The LOQs are less than the lowest toxicological level of concern in water for cyazofamid, CCIM, and CCIM-AM. The LOQ is greater than the current lowest toxicological level of concern in water for CTCA. The lowest toxicological level of concern for CCBA have not been established at this time. The ILV was not conducted independently from the ECM; the ECM and ILV were performed at the same laboratory company, and the MV study director was advising and recommending laboratory work to the ILV study director which was subsequently performed. The ECM used one characterized surface water and one characterized ground water matrices; ILV matrices were the same as the matrices used in the ECM. The ILV validated the ECM in the first trial without modifications for analysis of cyazofamid, CCIM, and CCIM-AM. The ILV validated the ECM for analysis of CCBA and CTCA in the second trial of surface water and the first trial of ground water with the modification that the 500 ng/mL and 50 ng/mL CTCA/CCBA mixed fortification solutions were prepared in water:acetonitrile (50:50, v:v), instead of acetonitrile (of MRID 50396410). The ILV found that the CTCA significantly binds to glass vial surfaces in acetonitrile, but not in water:acetonitrile (50:50, v:v). An updated ECM was not submitted incorporating the ILV modification and observation. All ECM and ILV data was satisfactory regarding accuracy and precision for all analytes. All ECM and ILV data was satisfactory regarding linearity and specificity for all analytes, except CTCA and CCBA. The specificity of the method for CTCA was insufficiently supported by ECM and ILV representative chromatograms since several significant contaminant peaks which were present in the standards, controls and fortified samples were extremely close to the analyte peak/retention time. Some ECM linearity was not satisfactory for CCBA. The LOD was not reported in the ILV.

**Table 1. Analytical Method Summary**

Analyte(s) by Pesticide <sup>1</sup>	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Cyazofamid (IKF-916)	50396409	50396410		Water <sup>2,3</sup>	29/09/2017	ISK Biosciences Corporation	LC/MS/MS	0.10 µg/L
CCIM			0.50 µg/L					
CCIM-AM								
CTCA								
CCBA								

1 Cyazofamid = IKF-916; 4-Chloro-2-cyano-N,N-dimethyl-5-p-tolylimidazole-1-sulfonamide; CCIM = 4-Chloro-5-p-tolylimidazole-2-carbonitrile; CCIM-AM = 4-Chloro-5-p-tolylimidazole-2-carboxamide; CTCA = 4-Chloro-5-p-tolylimidazole-2-carboxylic acid; and CCBA = 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid.

2 In the ECM, surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv. CaCO<sub>3</sub>/L, 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv. CaCO<sub>3</sub>/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 18; Appendix C, pp. 131-132 of MRID 50396409). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The pond water was filtered (Corning 0.22 µM filter) prior to use..

3 In the ILV, surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv. CaCO<sub>3</sub>/L, 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv. CaCO<sub>3</sub>/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 17; Appendix C, pp. 117-118 of MRID 50396410). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The ILV water matrices were the same as those of the ECM.

## I. Principle of the Method

### Cyazofamid (IKF-916), CCIM, and CCIM-AM

Samples (20 mL) were transferred to a separatory funnel and fortified as necessary with a mixed fortification solution containing cyazofamid (IKF-916), CCIM, and CCIM-AM (pp. 23-24 of MRID 50396409). The water samples were extracted twice with 10 mL of dichloromethane. For each extraction, the mixture was shaken for about 2 minutes, then the dichloromethane layer was removed, placed in a 15-mL glass tube, and evaporated using a TurboVap under nitrogen set at 40°C. The combined residue was reconstituted with 2 mL of acetonitrile:water (50:50, v:v) and analyzed via LC/MS/MS. For 10×LOQ fortifications, an 0.5-mL aliquot of the extract was mixed with 0.5 mL of acetonitrile:water (50:50, v:v) before analysis via LC/MS/MS.

### CCBA and CTCA

Samples (10 mL) were transferred into 50-mL centrifuge tubes and fortified as necessary with a mixed fortification solution containing CCBA and CTCA (pp. 24-25 of MRID 50396409). The sample was mixed with 10 mL of acetonitrile. The mixture was extracted with QuEChERS method (Chromabond; 1 packet of 6.5 g salt mixture). The mixture was shaken by hand for about 1 minute then the mixture was centrifuged (3500 rpm for 10 minutes at room temperature). An 8-mL aliquot of the top layer was transferred into a graduated glass tube. The solvent was evaporated to *ca.* 1 mL using a TurboVap under nitrogen set at 40°C. The residue was

reconstituted with 2 mL of water and analyzed via LC/MS/MS. Matrix-matched standards were used for calibration curves.

### LC/MS/MS

Samples were analyzed by Shimadzu Nexera UPLC system (System V) coupled with an AB-Sciex API 6500 MS (Version 1.6.2; Phenomenex Synergi Hydro-RP column, 2.0 mm x 50 mm, 4  $\mu$ m column; column temperature 30°C) using a mobile phase gradient of (A) 5 mM ammonium acetate + 0.1% formic acid in water and (B) 0.1% formic acid in methanol [percent A:B at 0.5 min. 80:20, 2.5-3.5 min. 0:100, 3.6-4.2 min. 80:20] with MS/MS detection and Multiple Reaction Monitoring (MRM; TEM 500°C; pp. 25-27 of MRID 50396409). Ion source was Turbo Spray in positive polarity for IKF-916, CCIM, and CCIM-AM and in negative polarity for CCBA and CTCA. Two ion transitions were monitored, as follows (quantitation and confirmation, respectively):  $m/z$  325 $\rightarrow$ 108 and  $m/z$  327 $\rightarrow$ 108 for cyazofamid (IKF-916),  $m/z$  218 $\rightarrow$ 183 and  $m/z$  218 $\rightarrow$ 139 for CCIM,  $m/z$  236 $\rightarrow$ 219 and  $m/z$  236 $\rightarrow$ 164 for CCIM-AM,  $m/z$  234.9 $\rightarrow$ 154.9 and  $m/z$  234.9 $\rightarrow$ 190.9 for CTCA, and  $m/z$  245.9 $\rightarrow$ 201.9 and  $m/z$  245.9 $\rightarrow$ 166.0 for CCBA. Injection volume was 5-10  $\mu$ L.

### ILV

The ECM was performed as written, except that the 500 ng/mL and 50 ng/mL CTCA/CCBA mixed fortification solutions were prepared in water:acetonitrile (50:50, v:v), instead of acetonitrile (pp. 21-27, 32-33 of MRID 50396410). The ILV found that the CTCA significantly binds to glass vial surfaces in acetonitrile, but not in water:acetonitrile (50:50, v:v). The ILV reported that Shimadzu Nexera UPLC system (System X) coupled with an AB-Sciex API 6500 MS (Version 1.6.2) was used. All monitored ion transitions were the same as those of the ECM.

### LOQ/LOD

In the ECM and ILV, the method Limits of Quantification (LOQs) in water were 0.10  $\mu$ g/L for cyazofamid and 0.50  $\mu$ g/L for all metabolites, CCIM, CCIM-AM, CCBA, and CTCA (pp. 28-29, 32; Appendix D, pp. 134-135 of MRID 50396409; pp. 31-32 of MRID 50396410). In the ECM, the method Limits of Detection (LOD) were calculated as 0.0117-0.0171  $\mu$ g/L for cyazofamid and 0.0146-0.269  $\mu$ g/L for the four metabolites. In the ILV, the LODs were not reported or calculated.

## **II. Recovery Findings**

ECM (MRID 50396409): Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of cyazofamid (IKF-916) at 0.10  $\mu$ g/L (LOQ) and 1.0  $\mu$ g/L (10 $\times$ LOQ) and for analysis of CCIM, CCIM-AM, CCBA, and CTCA at 0.50  $\mu$ g/L (LOQ), 5.00  $\mu$ g/L (10 $\times$ LOQ) in water matrices (Tables 2-11, pp. 35-44). LC/MS/MS Analysis in positive or negative ion mode was used for all analytes. Analytes were identified using two ion transitions; performance data (recoveries) of the quantitation and confirmation analyses were comparable. Surface (pond) water (Concord Inventory No. EFS-615;

pH 8.2, 157 mg equiv. CaCO<sub>3</sub>/L, 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv. CaCO<sub>3</sub>/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 18; Appendix C, pp. 131-132). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The pond water was filtered (Corning 0.22 µM filter) prior to use.

ILV (MRID 50396410): Mean recoveries and RSDs were within guideline requirements for analysis of cyazofamid (IKF-916) at 0.10 µg/L (LOQ) and 1.0 µg/L (10×LOQ) and for analysis of CCIM, CCIM-AM, CCBA, and CTCA at 0.50 µg/L (LOQ), 5.00 µg/L (10×LOQ) in water matrices (Tables 2-11, pp. 36-45). LC/MS/MS Analysis in positive or negative ion mode was used for all analytes. Analytes were identified using two ion transitions; performance data (recoveries) of the quantitation and confirmation analyses were comparable. Surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv. CaCO<sub>3</sub>/L, 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv. CaCO<sub>3</sub>/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 17; Appendix C, pp. 117-118). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The ILV water matrices were the same as those of the ECM. The ILV validated the ECM in the first trial without modifications for analysis of cyazofamid, CCIM, and CCIM-AM (pp. 21-27, 32-34). The ILV validated the ECM for analysis of CCBA and CTCA in the second trial of surface water and the first trial of ground water with the modification that the 500 ng/mL and 50 ng/mL CTCA/CCBA mixed fortification solutions were prepared in water:acetonitrile (50:50, v:v), instead of acetonitrile. The ILV found that the CTCA significantly binds to glass vial surfaces in acetonitrile, but not in water:acetonitrile (50:50, v:v). An updated ECM should be submitted incorporating the ILV modification and observation.

**Table 2. Initial Validation Method Recoveries for Cyazofamid (IKF-916) and Its Transformation Products CCIM, CCIM-AM, CCBA, and CTCA in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Surface (Pond) Water</b>						
Quantitation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	7	86.1-96.8	89.9	3.7	4.1
	1.0	5	79.6-91.2	87.0	5.0	5.8
CCIM	0.50 (LOQ)	7	84.8-87.4	86.0	0.9	1.1
	5.0	5	78.0-90.0	86.3	4.8	5.5
CCIM-AM	0.50 (LOQ)	7	95.2-101	97.9	1.8	1.9
	5.0	5	92.8-102	98.9	3.7	3.7
CCBA	0.50 (LOQ)	7	92.5-136	111	17.1	15.4
	5.0	5	101-108	104	2.6	2.5
CTCA	0.50 (LOQ)	7	66.5-99.0	82.5	13.4	16.2
	5.0	5	92.5-98.0	95.3	2.8	2.9
Confirmation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	7	83.6-89.6	86.7	2.0	2.3
	1.0	5	85.0-90.8	87.6	2.2	2.6
CCIM	0.50 (LOQ)	7	83.8-87.4	85.9	1.4	1.6
	5.0	5	76.8-90.8	86.3	5.6	6.4
CCIM-AM	0.50 (LOQ)	7	95.4-100	98.2	1.8	1.8
	5.0	5	92.8-103	99.8	4.2	4.2
CCBA	0.50 (LOQ)	7	94.5-134	112	16.2	14.2
	5.0	5	100-107	104	2.7	2.6
CTCA	0.50 (LOQ)	7	71.5-97.5	87.4	7.9	9.0
	5.0	5	90.0-96.5	94.3	2.9	3.1
<b>Ground (Creek) Water</b>						
Quantitation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	7	85.5-102	95.8	5.4	5.7
	1.0	5	86.6-97.2	91.8	4.5	4.9
CCIM	0.50 (LOQ)	7	61.4-85.2	76.8	10.6	13.9
	5.0	5	87.2-93.2	89.7	2.3	2.5
CCIM-AM	0.50 (LOQ)	7	93.0-102	98.1	3.4	3.4
	5.0	5	92.0-102	95.4	3.9	4.1
CCBA	0.50 (LOQ)	7	99.0-119	106	6.4	6.0
	5.0	5	86.0-128	112	17.4	15.6
CTCA	0.50 (LOQ)	7	70.0-78.0	73.7	3.4	4.6
	5.0	5	77.0-92.5	86.2	5.7	6.6
Confirmation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	7	90.1-107	100	6.4	6.4
	1.0	5	86.4-97.2	91.7	5.0	5.5
CCIM	0.50 (LOQ)	7	60.0-88.4	77.2	11.5	14.9
	5.0	5	85.2-93.6	88.9	3.0	3.4
CCIM-AM	0.50 (LOQ)	7	89.4-102	98.3	4.8	4.9
	5.0	5	94.4-103	97.6	3.3	3.4
CCBA	0.50 (LOQ)	7	99.0-121	105	7.5	7.1
	5.0	5	85.5-125	112	17.9	16.0

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CTCA	0.50 (LOQ)	7	77.5-84.5	81.1	2.3	2.9
	5.0	5	74.0-88.5	83.3	5.5	6.6

Data (uncorrected recovery results, pp. 29-31 and Appendix E, pp. 136-156) were obtained from Tables 2-11, pp. 35-44 of MRID 50396409.

- Two ion transitions were monitored, as follows (quantitation and confirmation, respectively):  $m/z$  325→108 and  $m/z$  327→108 for cyazofamid (IKF-916),  $m/z$  218→183 and  $m/z$  218→139 for CCIM,  $m/z$  236→219 and  $m/z$  236→164 for CCIM-AM,  $m/z$  234.9→154.9 and  $m/z$  234.9→190.9 for CTCA, and  $m/z$  245.9→201.9 and  $m/z$  245.9→166.0 for CCBA.
- Surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv. CaCO<sub>3</sub>/L, 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv. CaCO<sub>3</sub>/L, 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 18; Appendix C, pp. 131-132). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The pond water was filtered (Corning 0.22 µM filter) prior to use.

**Table 3. Independent Validation Method Recoveries for Cyazofamid (IKF-916) and Its Transformation Products CCIM, CCIM-AM, CCBA, and CTCA in Water<sup>1,2</sup>**

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Surface (Pond) Water</b>						
Quantitation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	5	97.2-102	99.2	2.6	2.6
	1.0	5	95.6-101	99.2	2.4	2.4
CCIM	0.50 (LOQ)	5	82.2-87.0	84.7	2.0	2.4
	5.0	5	81.6-87.2	85.7	2.4	2.8
CCIM-AM	0.50 (LOQ)	5	95.2-99.8	96.9	1.7	1.8
	5.0	5	94.8-100	97.4	2.4	2.5
CCBA	0.50 (LOQ)	5	102-123	114	8.4	7.4
	5.0	5	99.0-114	107	6.4	6.0
CTCA	0.50 (LOQ)	5	84.5-97.5	91.5	5.3	5.8
	5.0	5	90.5-102	97.4	4.4	4.5
Confirmation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	5	97.5-102	99.3	1.7	1.7
	1.0	5	96.4-102	99.4	2.1	2.1
CCIM	0.50 (LOQ)	5	79.6-86.4	84.4	2.8	3.3
	5.0	5	81.6-87.6	85.4	2.4	2.8
CCIM-AM	0.50 (LOQ)	5	95.2-98.6	96.7	1.4	1.4
	5.0	5	93.6-98.4	96.5	1.9	2.0
CCBA	0.50 (LOQ)	5	102-121	113	7.9	7.0
	5.0	5	101-111	106	5.0	4.7
CTCA	0.50 (LOQ)	5	82.0-93.5	86.8	4.4	5.1
	5.0	5	93.0-102	97.6	3.4	3.4
<b>Ground (Creek) Water</b>						
Quantitation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	5	85.7-99.3	92.4	5.1	5.5
	1.0	5	67.0-93.2	82.5	11.0	13.3
CCIM	0.50 (LOQ)	5	83.4-90.2	86.3	3.0	3.5
	5.0	5	78.0-88.0	83.7	4.0	4.8
CCIM-AM	0.50 (LOQ)	5	94.0-107	98.8	5.4	5.5
	5.0	5	86.8-102	95.8	6.2	6.5
CCBA	0.50 (LOQ)	5	96.0-104	99.3	2.9	2.9
	5.0	5	95.0-111	103	6.1	5.9
CTCA	0.50 (LOQ)	5	97.0-106	103	3.6	3.5
	5.0	5	93.0-108	101	5.7	5.7
Confirmation Ion Transition						
Cyazofamid (IKF-916)	0.10 (LOQ)	5	86.9-99.3	91.3	5.0	5.5
	1.0	5	67.2-95.4	83.1	11.7	14.1
CCIM	0.50 (LOQ)	5	83.8-82.0	86.9	3.3	3.8
	5.0	5	78.0-86.8	83.4	3.8	4.6
CCIM-AM	0.50 (LOQ)	5	94.4-107	98.6	5.2	5.3
	5.0	5	86.0-101	95.0	6.6	6.9
CCBA	0.50 (LOQ)	5	97.0-104	98.6	2.8	2.8
	5.0	5	97.0-109	103	5.2	5.0



Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
CTCA	0.50 (LOQ)	5	101-109	105	3.2	3.0
	5.0	5	95.5-111	103	6.3	6.2

Data (uncorrected recovery results, pp. 27-30 and Appendix D, pp. 119-139) were obtained from Tables 2-11, pp. 36-45 of MRID 50396410.

- Two ion transitions were monitored, as follows (quantitation and confirmation, respectively):  $m/z$  325→108 and  $m/z$  327→108 for cyazofamid (IKF-916),  $m/z$  218→183 and  $m/z$  218→139 for CCIM,  $m/z$  236→219 and  $m/z$  236→164 for CCIM-AM,  $m/z$  234.9→154.9 and  $m/z$  234.9→190.9 for CTCA, and  $m/z$  245.9→201.9 and  $m/z$  245.9→166.0 for CCBA.
- Surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv.  $\text{CaCO}_3/\text{L}$ , 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv.  $\text{CaCO}_3/\text{L}$ , 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 17; Appendix C, pp. 117-118). Water characterization was performed by Agvise Laboratories, Northwood, North Dakota. The ILV water matrices were the same as those of the ECM.

### III. Method Characteristics

In the ECM and ILV, the method LOQs in water were 0.10 µg/L for cyazofamid and 0.50 µg/L for all metabolites, CCIM, CCIM-AM, CCBA, and CTCA (pp. 28-29, 32; Appendix D, pp. 134-135 of MRID 50396409; pp. 31-32 of MRID 50396410). In the ECM, the method LODs were calculated as 0.0117-0.0171 µg/L for cyazofamid and 0.0146-0.269 µg/L for the four metabolites. The LOD was calculated as the standard deviation multiplied by  $t_{0.99}$ , where  $t_{0.99}$  equalled 3.143 for  $n-1$  degrees of freedom where  $n = 7$ . In the ILV, the LODs were not reported or calculated.

**Table 4. Method Characteristics for Cyzofamid (IKF-916), CCIM, CCIM-AM, CCBA, and CTCA in Water**

Analyte <sup>1</sup>		Cyzofamid (IKF-916)	CCIM	CCIM-AM	CCBA	CTCA
Limit of Quantitation (LOQ)	ECM	0.10 µg/L	0.50 µg/L			
	ILV		0.50 µg/L			
Limit of Detection (LOD)	ECM	Not reported				
	ILV	Calculated <sup>2</sup>	0.0146-0.167 µg/L	0.0287-0.0527 µg/L	0.101-0.269 µg/L	0.0533-0.210 µg/L
		Nominal	Not reported			
Linearity (calibration curve $r^2$ and concentration range) <sup>3</sup>	ECM	Surface water	Not calculated			$r^2 = 0.9976$ (Q) $r^2 = 0.9994$ (C)
			Ground water	$r^2 = 0.9996$ (Q & C)	$r^2 = 0.9908$ (Q) $r^2 = 0.9918$ (C) <sup>4</sup>	$r^2 = 0.9976$ (Q) $r^2 = 0.9994$ (C)
	ILV	Surface water		$r^2 = 0.9998$ (Q) $r^2 = 0.9996$ (C)	$r^2 = 0.9988$ (Q) $r^2 = 0.9998$ (C)	$r^2 = 0.9966$ (Q) $r^2 = 0.9970$ (C)
			Ground water	$r^2 = 0.9998$ (Q) $r^2 = 1.0000$ (C)	$r^2 = 1.0000$ (Q & C)	$r^2 = 0.9992$ (Q & C)
	Range	0.1-10 ng/mL		0.5-50 ng/mL		
			Yes at LOQ and 10×LOQ in one surface water and one ground water matrices (characterized).			
Repeatable	ECM <sup>5</sup>	Yes at LOQ and 10×LOQ in one surface water and one ground water matrices (characterized).				
	ILV <sup>6,7</sup>	Yes at LOQ and 10×LOQ				
Reproducible	Yes at LOQ and 10×LOQ					
	ECM	Yes, matrix interferences were < 1% of the LOQ (based on peak area). Some minor peak tailing was observed.				No, no matrix interferences were observed at analyte peak retention time, but several significant contaminant peaks (50-200% of the Q ion LOQ height) which were present in the standards, controls and fortified samples were extremely close to the analyte peak/RT. <sup>8,9</sup>

Analyte <sup>1</sup>	Cyzofamid (IKF-916)	CCIM	CCIM-AM	CCBA	CTCA
Specific	ILV  Yes, no matrix interferences were observed.	Yes, no matrix interferences were observed; however, analyte peak splitting from shouldering was observed.	Yes, no matrix interferences were observed.	Yes, matrix interferences were < 2% of the LOQ (based on peak area).	No, no matrix interferences were observed at analyte peak retention time, but several significant contaminant peaks (40% of the Q ion LOQ height) which were present in the standards, controls and fortified samples were extremely close to the analyte peak/RT. <sup>8,10</sup>

Data were obtained from pp. 28-29, 32; Appendix D, pp. 134-135 (LOD/LOQ); Tables 2-11, pp. 35-44 (recovery results); Figures 1-50, pp. 45-94 (chromatograms); Figures 51-60, pp. 95-104 (calibration curves) of MRID 50396409; pp. 31-32 (LOQ); Tables 2-11, pp. 36-45 (recovery results); Figures 1-40, pp. 46-85 (chromatograms); Figures 41-50, pp. 86-95 (calibration curves) of MRID 50396410; DER Attachment 2. Q = Quantitation ion transition; C =

Confirmation ion transition.

1 Cyzofamid = IKF-916; 4-Chloro-2-cyano-N,N-dimethyl-5-p-tolylimidazole-1-sulfonamide; CCIM = 4-Chloro-5-p-tolylimidazole-2-carbonitrile; CCIM-AM = 4-Chloro-5-p-tolylimidazole-2-carboxamide; CTCA = 4-Chloro-5-p-tolylimidazole-2-carboxylic acid; and CCBA = 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid.

2 Matrices combined.

3 ECM and ILV coefficient of determination ( $r^2$ ) values are reviewer-generated from reported correlation coefficient (r) values (1/x weighting; Figures 51-60, pp. 95-104 of MRID 50396409; Figures 41-50, pp. 86-95 of MRID 50396410; DER Attachment 2). The reviewer assumed that solvent-based calibration standards were used for IKF-916, CCIM, and CCIM-AM, but calibration curves were provided for all sample sets (pp. 21-25 of MRID 50396409; pp. 20-24 of MRID 50396410). Matrix-matched calibration standards were used for CCBA and CTCA.

4 Deviation of linearity does not affect the validity of the method since a confirmation method is usually not required when LC/MS or GC/MS is used as the primary method for quantifying residues.

5 In the ECM, surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv.  $\text{CaCO}_3/\text{L}$ , 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv.  $\text{CaCO}_3/\text{L}$ , 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 18; Appendix C, pp. 131-132 of MRID 50396409). Water characterization was performed by Agyise Laboratories, Northwood, North Dakota. The pond water was filtered (Corning 0.22  $\mu\text{m}$  filter) prior to use.

6 In the ILV, surface (pond) water (Concord Inventory No. EFS-615; pH 8.2, 157 mg equiv.  $\text{CaCO}_3/\text{L}$ , 366 ppm total dissolved solids) obtained from Smokey Oaks Pond, and ground (creek) water (Concord Inventory No. EFS-625; pH 8.0, 242 mg equiv.  $\text{CaCO}_3/\text{L}$ , 348 ppm total dissolved solids) obtained from Rock Creek, Ohio, were used (p. 17; Appendix C, pp. 117-118 of MRID 50396410). Water characterization was performed by Agyise Laboratories, Northwood, North Dakota. The ILV water matrices were the same as those of the ECM.

7 The ILV validated the ECM in the first trial without modifications for analysis of cyazofamid, CCIM, and CCIM-AM (pp. 21-27, 32-34 of MRID 50396410). The ILV validated the ECM for analysis of CCBA and CTCA in the second trial of surface water and the first trial of ground water with the modification that the 500 ng/mL and 50 ng/mL CTCA/CCBA mixed fortification solutions were prepared in water:acetonitrile (50:50, v:v), instead of acetonitrile. The ILV

found that the CTCA significantly binds to glass vial surfaces in acetonitrile, but not in water:acetonitrile (50:50, v:v). An updated ECM should be submitted incorporating the ILV modification and observation.

8 The significant baseline noise and contaminant peaks which were observed near the analyte peak in all LOQ CTCA C ion chromatograms caused the confirmation chromatograms to be inadequate for supporting the method for CTCA. A confirmation method is usually not required when LC/MS or GC/MS is used as the primary method for quantifying residues.

9 Based on Figures 41-50, pp. 85-94 of MRID 50396409.

10 Based on Figures 33-40, pp. 78-85 of MRID 50396410.  
Linearity is satisfactory when  $r^2 \geq 0.995$ .

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

1. The ILV was not conducted independently from the ECM. The laboratory which performed the ILV was the same as that which performed the ECM, AgChem Product Development, Concord Biosciences, LLC, Concord, Ohio (p. 1 of MRID 50396409; pp. 1, 31, 33-34 of MRID 50396410). The ILV specified that separate labs and different personnel were used for each study; however, communication between the two labs was mediated by the Sponsor. Although no direct communication occurred between the two groups, the Sponsor "acquired" responses from the MV study director and "directed the response to the ILV study director" (p. 33 of MRID 50396410). Additionally, in the communications from the MV study director, the MV study director was advising and recommending laboratory work to the ILV study director which was subsequently performed. With these details of the Sponsor's role, the reviewer believed that the mediated communication was no different from direct communication. The ILV report cannot be considered independent as defined by the OCSPP guidelines since, if the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

The reviewer noted that the study authors and LC/MS/MS systems differed between the ECM and ILV (pp. 1, 26 of MRID 50396409; pp. 1, 25, 31, 33-34 of MRID 50396410). Shimadzu Nexera UPLC system (System V) was used in the ECM, and Shimadzu Nexera UPLC system (System X) was used in the ILV. However, the water matrices of the ECM were the same as those of the ILV (p. 18; Appendix C, pp. 131-132 of MRID 50396409; p. 17; Appendix C, pp. 117-118 of MRID 50396410).

Additionally, the reviewer noted that the Study Sponsor listed in the lists of laboratory personnel (Jason A. MacDonald) in the ECM and ILV did not match the Sponsor/Agent (Michael F. Leggett) or Sponsor/Submitter (Michael A. Peplowski) listed in the GLP Statements (pp. 3, 13 of MRID 50396409; pp. 3, 12 of MRID 50396410).

2. An updated ECM was not submitted incorporating the ILV modification and observation for CTCA. The ILV validated the ECM for analysis of CCBA and CTCA in the second trial of surface water and the first trial of ground water with the modification that the 500 ng/mL and 50 ng/mL CTCA/CCBA mixed fortification solutions were prepared in water:acetonitrile (50:50, v:v), instead of acetonitrile (pp. 21-27, 32-34 of MRID 50396410). The ILV found that the CTCA significantly binds to glass vial surfaces in acetonitrile, but not in water:acetonitrile (50:50, v:v). The ILV modification was necessary for the successful validation of the ECM method.
3. The specificity of the method for CTCA was insufficiently supported by ECM and ILV representative chromatograms (Figures 41-50, pp. 85-94 of MRID 50396409; Figures 33-40, pp. 78-85 of MRID 50396410). In the ECM and ILV representative quantitation chromatograms, no matrix interferences were observed at analyte peak retention time, but

several significant contaminant peaks which were present in the standards, controls and fortified samples were extremely close to the analyte peak/RT. In the ECM, the significant contaminant peaks were 50-200% of the LOQ height; in the ILV, the significant contaminant peaks were 40% of the LOQ height. Consistent quantification would be difficult with this level of baseline/baseline noise. The reviewer did not know if the use of additional SPE clean-up processes had been attempted to reduce contaminants or the use of different LC/MS/MS parameters had been explored to increase the separation of the analyte signal from contaminants.

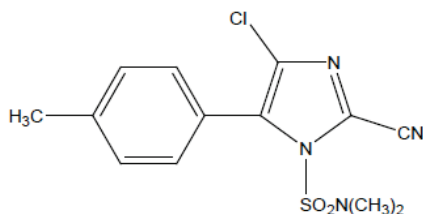
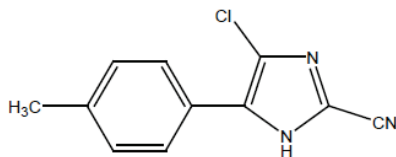
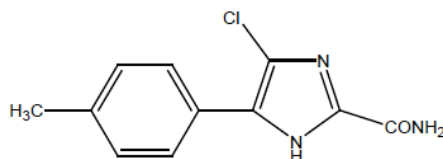
4. ECM linearity was not satisfactory for CCBA in surface water calibration curves,  $r^2 = 0.9908$  (Q) and  $0.9918$  (C; Figures 51-60, pp. 95-104 of MRID 50396409; DER Attachment 2). Linearity is satisfactory when  $r^2 \geq 0.995$ .
5. The ILV matrices were the same as the matrices used in the ECM. The ILV should provide a more challenging and comprehensive test of the ECM method than the ECM.
6. The LODs of the method were not reported or calculated by the ILV. Method LODs were not reported by the ECM; calculated LODs were provided. The estimations of the LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 28-29, 32; Appendix D, pp. 134-135 of MRID 50396409; pp. 31-32 of MRID 50396410). No justifications or calculations of the LOQ were provided in the ILV or ECM. In the ECM, the LOD was calculated as the standard deviation multiplied by  $t_{0.99}$ , where  $t_{0.99}$  equalled 3.143 for n-1 degrees of freedom where  $n = 7$ . In the ILV, the LODs were not reported or calculated. Further work could have been done to explore the actual LOQ and LOD.
7. The LOQ for CTCA exceeds the current lowest toxicological level of concern.
8. The reviewer noted that the ILV results should have specified from which trial number the results of each analyte were produced.
9. The matrix effects were not addressed in the ECM and ILV. The reviewer assumed that solvent-based calibration standards were used for IKF-916, CCIM, and CCIM-AM, but calibration curves were provided for all sample sets (pp. 21-25 of MRID 50396409; pp. 20-24 of MRID 50396410). Matrix-matched calibration standards were used for CCBA and CTCA.
10. In the ECM and ILV, the concentrations of the fortification solutions were verified (Table 1, p. 34 of MRID 50396409; Table 1, p. 35 of MRID 50396410).
11. The reviewer noted that the ILV GLP Statement and Report Statistics Section stated that the analyst software version 1.6.3, which was used during the study, was not validated, and that the previous analyst software version 1.6.2 was validated (pp. 3, 27 of MRID 50396410). However, the reviewer noted that only analyst software version 1.6.2 was specified in the analytical instrument, parameters and information section (p. 25).

12. It was reported for the ILV that one sample set required 8 hours to prepare and analyse by LC/MS/MS (including standard, sample preparation and analysis; p. 27 of MRID 50396410).

## 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.

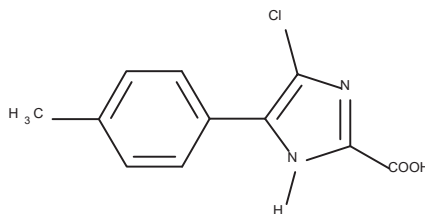
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

**DER Attachment 1: Chemical Names and Structures****Cyazofamid (IKF-916)****IUPAC Name:** 4-Chloro-2-cyano-N,N-dimethyl-5-p-tolylimidazole-1-sulfonamide**CAS Name:** Not reported**CAS Number:** 120116-88-3**SMILES String:** Not found**CCIM****IUPAC Name:** 4-Chloro-5-p-tolylimidazole-2-carbonitrile**CAS Name:** Not reported**CAS Number:** Not reported**SMILES String:** Not found**CCIM-AM****IUPAC Name:** 4-Chloro-5-p-tolylimidazole-2-carboxamide**CAS Name:** Not reported**CAS Number:** Not reported**SMILES String:** Not found



**CTCA**

**IUPAC Name:** 4-Chloro-5-(4-methylphenyl)imidazole-2-carboxylic acid  
**CAS Name:** 4-Chloro-5-(4-methylphenyl)-1H-imidazole-2-carboxylic acid  
**CAS Number:** Not reported  
**SMILES String:** [H]n1c(c(nc1C(=O)O)Cl)c2ccc(cc2)C

**CCBA**

**IUPAC Name:** 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid  
**CAS Name:** Not reported  
**CAS Number:** Not reported  
**SMILES String:** Not found

