

Analytical method for cyazofamid (IKF-916) and its transformation products, CCIM, CCIM-AM, CTCA, and CCBA in soil and sediment

Reports: ECM: EPA MRID No.: 50143502. Jablonski, J.E. 2000. Analytical Procedure for the Determination of Residues of IKF-916 and its Metabolites (CCIM, CCIM-AM, CTCA and CCBA) in Soil. Report prepared by Department of Residue Analysis, Ricerca, LLC (formerly Ricerca, Inc.), Painesville, Ohio, sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and submitted by ISK Biosciences Corporation, Concord, Ohio; 142 pages. Ricerca Document No.: 011141-2. Ricerca Project Identification No.: 011141. Final report issued October 2, 2000.

ILV: EPA MRID No.: 50143503. Lala, M. 2002. Validation of a Method for IKF-916 and its Soil Metabolites CCIM, CCIM-AM, CTCA and CCBA in Sediment. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, sponsored and submitted by ISK Biosciences Corporation, Concord, Ohio; 148 pages. Pyxant Study No: ISK-1410. Sponsor Study No.: IB-2002-JLW-005-00-00. Final report issued July 11, 2002.

Document No.: MRIDs 50143502 & 50143503

Guideline: 850.6100


Statements: ECM: The study was conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160), except that reference substances CCIM, CTCA, and CCBA were characterized under GLP after the study started (p. 3 of MRID 50143502). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 6). The statement of authenticity was not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (p. 3 of MRID 50143503). Signed and dated Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as Unacceptable. The ILV was not conducted independently of the ECM. The ECM should be updated with the significant method precautions noted by the ILV. Only one set of performance data was provided for the 10×LOQ fortification. For CCIM-AM and CTCA, method recoveries did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy for the one and two ECM soil matrices at the LOQ or 10×LOQ. The specificity of the method at the LOQ was not supported by the ILV HPLC/UV chromatograms. Only HPLC/UV analysis was used to identify and quantify the analytes. ILV performance data for the 20×LOQ analysis of CCIM was unacceptable. LODs for the analytes were not reported in the ILV.


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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, Ricerca Document No. 011141-2, is designed for the quantitative determination of cyazofamid (IKF-916) and its transformation products, CCIM, CCIM-AM, CTCA and CCBA, in soil and sediment at the stated LOQ of 0.01 mg/kg using HPLC/UV. The LOQ is less than the lowest toxicological level of concern in soil/sediment. ILV MRID 50143503 was not conducted independently of ECM MRID 50143502 since the ECM and ILV shared laboratory personnel and ILV personnel communicated directly with ECM personnel regarding technical method issues. Insufficient evidence was provided to define the roles of the laboratory personnel and support the independence of the two laboratories. One characterized sediment was used for the ILV validation; four characterized soil matrices were used for the ECM validation. USDA soil texture classification not specified for the ECM or ILV test matrices. The ILV validated the method with second trial with insignificant modifications to the HPLC/UV parameters and equipment; however, the ILV noted a few significant method precautions. **The ECM should be updated with cautions regarding the use of amber glassware and the instability of CTCA in the final extract, as well as the need to optimize the chromatographic conditions for the test soil/sediment matrix.** Analytes were identified using HPLC/UV; no confirmatory method was used. A confirmatory method is usually required when LC/MS and GC/MS is not the primary method to generate study data. The reproducibility of the method could not be determined for the 10×LOQ fortification since only one set of performance data was provided, that of the ECM. All ILV data regarding repeatability, accuracy, precision, and linearity were satisfactory for all analytes in the sediment matrix, except for the 20×LOQ analysis of CCIM. The specificity of the method at the LOQ was not supported by the ILV HPLC/UV chromatograms due to small analyte peaks and excessive baseline noise; the analyte peak could only be differentiated from baseline noise by retention time. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for all analytes in the four soil matrices, except for the LOQ analyses of CTCA in two soils and the 10×LOQ analysis of CCIM-AM in one soil. The LODs for the analytes were not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide ¹	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Cyazofamid (IKF-916)	50143502 ²	50143503 ³		Soil/ Sediment	02/10/2000	ISK Biosciences Corporation	HPLC/UV	0.01 mg/kg
CCIM								
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; CCBA = 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid (pp. 11-13 of MRID 50143502).

2 In the ECM, NY Loamy sand/Sandy loam [0-12"; 74.0-78.0% sand, 16.4-20.4% silt, 5.6% clay; pH 5.8-6.1 (not specified); 2.13-3.33% organic matter], NY Sandy loam [18-24"; 72.0% sand, 22.4% silt, 5.6% clay; pH 5.5 (not specified); 0.82% organic matter], WA Loamy sand [0-12"; 76.8-80.8% sand, 14.0-18.0% silt, 5.2% clay; pH 6.5-6.8 (not specified); 0.38-0.60% organic matter], and WA Loamy sand [18-24"; 80.8% sand, 16.0% silt, 3.2% clay; pH 7.4 (not specified); 0.22% organic matter] were used in the study (USDA soil texture classification not specified; p. 15; Table 1, p. 26 of MRID 50143502). The soil characterization location was not reported. Soil sources were not further specified.

3 In the ILV, loamy sand sediment [from Ricerca Document No. 014188-1; 83% sand, 14% silt, 3% clay; pH 6.8 (not specified); 2.1% organic matter] from a river/creek bed located in Madison, Ohio (locally obtained 01-192) was used in the study (USDA soil texture classification not specified; p. 9 of MRID 50143503). The sediment characterization location was not reported.

I. Principle of the Method

Soil or sediment samples (20 g) in 250-mL Nalgene centrifuge tubes were weighed and fortified with the mixed fortification solutions (10 µg/mL solution of cyazofamid /CCIM or CCBA/CTCA/CCIM-AM) then extracted with 200 mL of acetonitrile:0.1N HCl (80:20, v:v) by shaking on a mechanical shaker for *ca.* 15 minutes (pp. 16-17; Figure 1, p. 25; Appendix B, pp. 53-59 of MRID 50143502). After settling, extract was removed via filtration (934-AH paper/Büchner funnel, leaving most of the soil in the flask. Extraction solvent (25 mL then 10 mL) was added to the soil in the flask and passed through the filter. The volume was adjusted to 250 mL with acetonitrile. An aliquot (125 mL) was removed for cyazofamid and CCIM analysis. The remainder of the extract was used for CCBA, CTCA, and CCIM-AM analysis.

For cyazofamid and CCIM analysis, pH 4 sodium acetate buffer (1M, 15 mL), and acetonitrile (50 mL) were added to the sample, and the sample was reduced to *ca.* 15 to 20 mL with a rotary evaporator in a water bath ($\leq 40^{\circ}\text{C}$) with vacuum (*ca.* 27" Hg; pp. 16-17; Figure 1, p. 25; Appendix B, pp. 53-59 of MRID 50143502). The solution was transferred to a 250-mL separatory funnel. Methylene chloride and 2% sodium sulfate in water (50 mL each) were added to the separatory flask via the sample flask. After shaking the separatory funnel for *ca.* one minute, the lower (organic layer) was drained through anhydrous sodium sulfate contained in a funnel plugged with cotton, and into a 250-mL flask. The extraction was repeated with 50 mL of methylene chloride. The method noted that analysis of IKF-916 and CCIM must be completed to this step on the same day the samples are extracted. The sample was reduced to dryness with a rotary evaporator in a water bath ($\leq 40^{\circ}\text{C}$) with vacuum (*ca.* 27" Hg; proceed to next step as soon as flask has cooled to room temperature - do not let sample sit 'dry' for very long). The residue was reconstituted with 1 mL of ethyl acetate then 20 mL of hexane. Waters Sep-Pak Florisil™ solid phase extraction cartridges were pre-conditioned with 10 mL of acetone then 10 mL of hexane. After the sample was added to the cartridge reservoir, the cartridge was washed with 10 mL of diethyl ether:hexane (20:80, v:v). The analytes were eluted with 50 mL of acetone into a 100-mL flask. The eluate was reduced to dryness with a rotary evaporator in a water bath ($\leq 40^{\circ}\text{C}$) with vacuum (*ca.* 27" Hg; proceed to next step as soon as flask has cooled to room temperature (do not let sample sit 'dry' for very long). The residue was reconstituted with 2 mL of acetonitrile:water (50:50, v:v). An aliquot was taken for HPLC analysis via filtration through a PTFE disk, if necessary.

For CCBA, CTCA, and CCIM-AM analysis, the 125-mL of solution to a volume of *ca.* 5 to 10 mL with a rotary evaporator in a water bath ($\leq 40^{\circ}\text{C}$ with vacuum (*ca.* 27" Hg; pp. 16-17; Figure 1, p. 25; Appendix B, pp. 53-59 of MRID 50143502). The pH was adjusted to *ca.* pH 3 using 15 mL of pH 4 sodium acetate buffer (1M). Waters tC₁₈ Sep-Pak solid phase extraction columns (on a vacuum manifold) were pre-conditioned with 5 mL of acetonitrile then 5 mL of acetonitrile:water + 0.5% acetic acid (1:9, v:v; do not let packing go dry). After the sample was added to the cartridge reservoir, the cartridge was washed with 5 mL of acetonitrile:water + 0.5% acetic acid (1:9, v:v) and 15 mL of acetonitrile/water + 0.5% acetic acid (25:75, v:v). The analytes were eluted with 30 mL of acetonitrile/water + 0.5% acetic acid (1:1, v:v) into a 100-mL flask. After 70 mL of acetonitrile was added to the eluate, the eluate was reduced to 5 to 10 mL with a rotary evaporator in a water bath ($\leq 40^{\circ}\text{C}$) with vacuum (*ca.* 27" Hg). The residue was reconstituted with 10 mL of acetonitrile and reduced to 0.5 to 1 mL with the water bath. In a 5 mL graduated centrifuge tube, the volume to 2 mL with acetonitrile:water (30:70, v:v) carefully by first adding 1 mL of acetonitrile:water (30:70, v:v), then bringing to 2 mL. An aliquot was taken for HPLC analysis via filtration through a PTFE disk, if necessary.

Samples were analyzed using HPLC/UV with Waters 600 E controller and quaternary pump coupled with an Applied Biosystems 785A Programmable Absorbance Detector (p. 16; Appendix B, pp. 52, 59-60 of MRID 50143502). The following LC conditions were used: Luna C₁₈ (2) column (3.0 x 150 mm, 3 μm; column temperature 40°C), Security Guard C₁₈ column, injection volume of 100 μL, and UV detection (280 nm). For polar fraction (CCBA, CTCA, and CCIM-AM), isocratic mobile phase was acetonitrile:water + 0.5% acetic acid (32:68, v:v); for CCIM/cyazofamid, isocratic mobile phase was acetonitrile:water + 0.5% acetic acid (48:52, v:v). Expected retention times were *ca.* 17 minutes for cyazofamid (IKF-916), *ca.* 8 minutes for CCIM, *ca.* 13 minutes for CCIM-AM, *ca.* 9 minutes for CTCA, and *ca.* 8 minutes for CCBA. No confirmation method was used.

The ILV performed the ECM methods for each analyte as written, including analytical methods, except that no vacuum manifold was used for solid phase extraction clean-up and HPLC/UV parameter and equipment modifications (pp. 17-19; Table II, pp. 27-28 of MRID 50143503). Samples were analyzed using a Hewlett-Packard Model 1090 Series II LC equipped with Phenomenex Luna RP18 column (3.0 x 150 mm; column temperature not reported) and Phenomenex Security Guard C18 column. For polar fraction (CCBA, CTCA, and CCIM-AM), isocratic mobile phase was acetonitrile:water + 0.5% acetic acid (35:65, v:v); for CCIM/cyazofamid, isocratic mobile phase was acetonitrile:water + 0.5% acetic acid (50:50, v:v). Expected retention times were *ca.* 18.05 minutes for cyazofamid (IKF-916), *ca.* 7.94 minutes for CCIM, *ca.* 9.10 minutes for CCIM-AM, *ca.* 7.73 minutes for CTCA, and *ca.* 5.99 minutes for CCBA. No confirmation method was used.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 mg/kg in soil and sediment for cyazofamid (IKF-916), CCIM, CCIM-AM, CTCA, and CCBA (pp. 17, 21-23 of MRID 50143502; p. 10 of MRID 50143503). In the ECM, the Limit of Detection (LOD) was 0.0025 mg/kg in soil and sediment for all five analytes. In the ILV, the LOD was not reported.

II. Recovery Findings

ECM (MRID 50143502): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD \leq 20%) for analysis of cyazofamid (IKF-916) and its transformation products, CCIM, CCIM-AM, CTCA, and CCBA, at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10 \times LOQ) in the four soil matrices, except for the LOQ analysis of CTCA in the NY sandy loam (18-24") soil (mean 66%) and WA loamy sand (18-24") soil (mean 134%) and the 10 \times LOQ analysis of CCIM-AM in the NY sandy loam (18-24") soil (mean 68%; Tables 4-7, pp. 29-32; DER Attachment 2). Five replicates were prepared for all soils/fortification levels, but data from only four replicates was used for the LOQ analysis in the NY sandy loam (18-24") soil due to the fact that the solid phase extraction eluate was spilled during transfer or the sample extract was over-evaporated (Table 4, p. 29). Recovery results were corrected when residues were quantified in the controls; residues were observed in the control for CCIM (<2% of the LOQ; Appendix C, pp. 66-67 and Figure C-43, p. 110). Analytes were quantified with HPLC/UV; no confirmation method was reported. A confirmation method is usually required when the primary method to generate study data is not HPLC/MS/MS or GC/MS. NY Loamy sand/Sandy loam [0-12"; 74.0-78.0% sand, 16.4-20.4% silt, 5.6% clay; pH 5.8-6.1 (not specified); 2.13-3.33% organic matter], NY Sandy loam [18-24"; 72.0% sand, 22.4% silt, 5.6% clay; pH 5.5 (not specified); 0.82% organic matter], WA Loamy sand [0-12"; 76.8-80.8% sand, 14.0-18.0% silt, 5.2% clay; pH 6.5-6.8 (not specified); 0.38-0.60% organic matter], and WA Loamy sand [18-24"; 80.8% sand, 16.0% silt, 3.2% clay; pH 7.4 (not specified); 0.22% organic matter] were used in the study (USDA soil texture classification not specified; p. 15; Table 1, p. 26). The soil characterization location was not reported. Soil sources were not further specified.

ILV (MRID 50143503): Mean recoveries and RSDs were within for analysis of cyazofamid (IKF-916) and its transformation products, CCIM, CCIM-AM, CTCA, and CCBA, at fortification levels of 0.01 mg/kg (LOQ), 0.05 mg/kg (5 \times LOQ), and 0.20 mg/kg (20 \times LOQ) in the one sediment matrix, except for the 20 \times LOQ analysis of CCIM (RSD 23.7%; Tables III-VII, pp. 29-33). No samples were prepared at 0.10 mg/kg (10 \times LOQ). Analytes were quantified with HPLC/UV; no confirmation method was reported. A confirmation method is usually required when the primary method to generate study data is not HPLC/MS/MS or GC/MS. Loamy sand sediment [from Ricerca Document No. 014188-1; 83% sand, 14% silt, 3% clay; pH 6.8 (not specified); 2.1% organic matter] from a river/creek bed located in Madison, Ohio (locally obtained 01-192) was used in the study (USDA soil texture classification not specified; p. 9). The sediment characterization location was not reported. The method was validated with second trial with insignificant modifications to the HPLC/UV parameters and equipment; however, the ILV noted a few significant method precautions (pp. 11, 17-19). In the first trial, the cyazofamid and CCIM analysis portion failed due to the photodegradation of the calibration curve because the calibration solutions were inadvertently prepared in clear glassware, and the CCIM-AM, CTCA, and CCBA analysis failed due to chromatographic issues which were solved with modifications of the HPLC column and mobile phase. The ILV also noted that CTCA had stability issues and needed to be processed and analyzed immediately; losses of 40-50% were observed after *ca.* 10 days in final extract form. **The ECM should be updated with ILV precautions regarding the use of amber glassware and the instability of CTCA in the final extract, as well as the need to optimize the chromatographic conditions for the test soil/sediment matrix.**

Table 2. Initial Validation Method Recoveries for Cyazofamid (IKF-916) and Its Transformation Products, CCIM, CCIM-AM, CTCA, and CCBA, in Soil/Sediment

Analyte ¹	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
HPLC/UV						
NY Loamy sand/Sandy loam (0-12") Soil²						
Cyazofamid (IKF-916)	0.01 (LOQ)	5	88-93	90	2	2
	0.10	5	92-102	97	4	4
CCIM	0.01 (LOQ)	5	88-100	92	5	5
	0.10	5	106-117	114	4	4
CCIM-AM	0.01 (LOQ)	5	82-112	99	12	12
	0.10	5	88-95	91	3	3
CTCA	0.01 (LOQ)	5	76-99	87	9	10
	0.10	5	65-82	74	7	9
CCBA	0.01 (LOQ)	5	83-109	97	9	10
	0.10	5	84-99	92	6	7
NY Sandy loam (18-24") Soil²						
Cyazofamid (IKF-916)	0.01 (LOQ)	4 ³	88-98	93	4	5
	0.10	5	83-90	86	3	3
CCIM	0.01 (LOQ)	4 ³	80-98	93	9	9
	0.10	5	84-94	88	4	4
CCIM-AM	0.01 (LOQ)	4 ³	91-125	101	16	16
	0.10	5	60-82	68	8	12
CTCA	0.01 (LOQ)	4 ³	63-67	66	2	3
	0.10	5	74-86	80	5	7
CCBA	0.01 (LOQ)	4 ³	94-99	96	2	2
	0.10	5	87-95	90	3	4
WA Loamy sand (0-12") Soil²						
Cyazofamid (IKF-916)	0.01 (LOQ)	5	78-104	92	11	11
	0.10	5	75-78	76	1	1
CCIM	0.01 (LOQ)	5	77-91	86	6	7
	0.10	5	107-112	110	2	2
CCIM-AM	0.01 (LOQ)	5	92-110	101	7	7
	0.10	5	86-102	94	7	7
CTCA	0.01 (LOQ)	5	87-104	96	6	6
	0.10	5	86-87	87	0	1
CCBA	0.01 (LOQ)	5	74-95	85	8	10
	0.10	5	88-95	92	3	3
WA Loamy sand (18-24") Soil²						
Cyazofamid (IKF-916)	0.01 (LOQ)	5	92-98	96	3	3
	0.10	5	78-93	88	7	7
CCIM	0.01 (LOQ)	5	89-97	94	4	4
	0.10	5	77-93	89	7	8
CCIM-AM	0.01 (LOQ)	5	71-112	86	16	18
	0.10	5	104-114	109	4	3
CTCA	0.01 (LOQ)	5	129-139	134	4	3
	0.10	5	92-101	96	4	4
CCBA	0.01 (LOQ)	5	74-105	86	13	15
	0.10	5	111-118	114	4	3

Data (recovery results were corrected when residues were quantified in the controls; Appendix C, pp. 66-67) were obtained from Tables 4-7, pp. 29-32 of MRID 50143502 and DER Attachment 2.

- 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; CCBA = 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid (pp. 11-13 of MRID 50143502).
- 2 NY Loamy sand/Sandy loam [0-12"; 74.0-78.0% sand, 16.4-20.4% silt, 5.6% clay; pH 5.8-6.1 (not specified); 2.13-3.33% organic matter], NY Sandy loam [18-24"; 72.0% sand, 22.4% silt, 5.6% clay; pH 5.5 (not specified); 0.82% organic matter], WA Loamy sand [0-12"; 76.8-80.8% sand, 14.0-18.0% silt, 5.2% clay; pH 6.5-6.8 (not specified); 0.38-0.60% organic matter], and WA Loamy sand [18-24"; 80.8% sand, 16.0% silt, 3.2% clay; pH 7.4 (not specified); 0.22% organic matter] were used in the study (USDA soil texture classification not specified; p. 15; Table 1, p. 26). The soil characterization location was not reported. Soil sources were not further specified.
- 3 Data from one replicate of the five was not reported due to the fact that the solid phase extraction eluate was spilled during transfer or the sample extract was over-evaporated (Table 4, p. 29).

Table 3. Independent Validation Method Recoveries for Cyazofamid (IKF-916) and Its Transformation Products, CCIM, CCIM-AM, CTCA, and CCBA, in Soil/Sediment

Analyte ¹	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Loamy Sand Sediment²						
HPLC/UV						
Cyazofamid (IKF-916)	0.01 (LOQ)	3	96.9-103.2	101.1	3.6	3.6
	0.05	3	73.0-84.8	80.1	6.3	7.8
	0.20	3	80.4-84.4	82.3	2.0	2.4
CCIM	0.01 (LOQ)	3	78.4-86.9	83.1	4.3	5.2
	0.05	3	66.3-96.4	85.4	16.6	19.5
	0.20	3	63.1-101.0	86.6	20.5	23.7
CCIM-AM	0.01 (LOQ)	3	76.0-86.1	82.1	5.3	6.5
	0.05	3	78.2-89.5	84.7	5.8	6.9
	0.20	3	85.4-92.3	88.4	3.6	4.0
CTCA	0.01 (LOQ)	3	75.0-84.8	80.7	5.1	6.3
	0.05	3	88.5-98.3	94.0	5.0	5.3
	0.20	3	84.2-91.5	87.6	3.7	4.2
CCBA	0.01 (LOQ)	3	104.8-113.3	107.6	4.9	4.6
	0.05	3	94.4-108.0	102.1	7.0	6.8
	0.20	3	91.5-99.5	96.6	4.5	4.6

Data (uncorrected recovery results; pp. 19-21; Tables III-VII, pp. 29-33) were obtained from Tables III-VII, pp. 29-33 of MRID 50143503.

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; CCBA = 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid (pp. 11-13 of MRID 50143502).

2 Loamy sand sediment [from Ricerca Document No. 014188-1; 83% sand, 14% silt, 3% clay; pH 6.8 (not specified); 2.1% organic matter] from a river/creek bed located in Madison, Ohio (locally obtained 01-192) was used in the study (USDA soil texture classification not specified; p. 9). The sediment characterization location was not reported.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 mg/kg in soil and sediment for cyazofamid (IKF-916), CCIM, CCIM-AM, CTCA, and CCBA (pp. 17, 21-23 of MRID 50143502; p. 10 of MRID 50143503). In the ECM, the LOQ was supported by the acceptable validation results at that fortification level (mean 70-120%, RSD <20%); the LOQ was reported in the ILV from the ECM without justification. No calculations were used to support the LOQ. In the ECM, the LOD was 0.0025 mg/kg in soil and sediment for all five analytes, based on half of the response of the 0.025 µg/mL calibration standard. In the ILV, the LOD was not reported.

Table 4. Method Characteristics for Cyazofamid (IKF-916) and Its Transformation Products, CCIM, CCIM-AM, CTCA, and CCBA, in Soil/Sediment

Parameter		Cyazofamid (IKF-916)	CCIM	CCIM-AM	CTCA	CCBA
Limit of Quantitation (LOQ)	ECM			0.01 mg/kg		
	ILV					
Limit of Detection (LOD)	ECM			0.0025 mg/kg		
	ILV			Not reported		
Linearity (calibration curve r^2 and concentration range)	ECM	$r^2 = 0.999925$	$r^2 = 0.99988$	$r^2 = 0.999717$	$r^2 = 0.999954$	$r^2 = 0.999985$
	ILV	$r^2 = 0.99976$	$r^2 = 1.0000$	$r^2 = 0.9998$	$r^2 = 0.9983$	$r^2 = 0.9998$
Repeatable	ECM ²	Range:		0.025-1.00 µg/mL		
		NY 0-12” Soil	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.
		NY 18-24” Soil	Yes at LOQ; No at 10×LOQ (mean 68%).	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	
		WA 0-12” Soil	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	
		WA 18-24” Soil	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	Yes at LOQ and 10×LOQ.	
		OH Sediment	Yes at LOQ and 5×LOQ.	Yes at LOQ and 5×LOQ.	Yes at 20×LOQ.	
ILV ^{3,4}	Yes at 20×LOQ.	No at 20×LOQ (RSD 23.7%).	No at 10×LOQ; no samples were prepared.			
Reproducible	ECM/ILV	Yes at LOQ in four soil and one sediment matrices.	Yes at LOQ in three soil and one sediment matrices.	Yes at LOQ in two soil and one sediment matrices.	Yes at LOQ in four soil and one sediment matrices.	
		Could not be determined at 10×LOQ – only one set of performance data was submitted. Could not be determined at 5×LOQ – only one set of performance data was submitted. Could not be determined at 20×LOQ – only one set of performance data was submitted.				
Specific	ECM/ILV	HPLC/UV analysis only; no confirmation analysis was performed.				

ECM		Yes at LOQ and 10×LOQ. No matrix interferences were observed at retention time, but analyte LOQ peak was broad and very small. Some nearby peaks were noted.	Yes at LOQ and 10×LOQ. Matrix interferences were <2% of the LOQ at analyte retention time, but analyte LOQ peak was broad and small. Some nearby peaks were noted.	Yes at LOQ and 10×LOQ. No matrix interferences were observed at analyte retention time, but analyte LOQ peak was broad and small in some chromatograms. Some nearby peaks were noted.
ILV		No at LOQ due to small analyte peaks and excessive baseline noise ⁵ . Analyte peak could only be differentiated from baseline noise by retention time. Significant baseline noise interfered with analyte attenuation and integration. Yes at 5×LOQ and 20×LOQ; however, baseline noise interfered with analyte attenuation and integration.	No at 10×LOQ – no samples prepared.	Yes at LOQ and 10×LOQ. No matrix interferences were observed at analyte retention time, but analyte LOQ peak was broad and small in some chromatograms. Some nearby peaks were noted.

Data were obtained from pp. 17, 21-23 (LOQ/LOD); Tables 4-7, pp. 29-32 of MRID 50143502 (recovery results); Appendix C, Figures C-1 to C-8, pp. 68-75 and Figures C-16 to C-24, pp. 83-91 (calibration curves); Appendix C, Figures C-9 to C-15, pp. 76-82 and Figures C-25 to C-71, pp. 92-138 (chromatograms) of MRID 50143502; p. 10 (LOQ/LOD); Tables III-VII, pp. 29-33 (recovery results); Table VIII, pp. 34-38 (calibration curves); Appendix 4, pp. 96-148 (chromatograms) of MRID 50143503; and DER Attachment 2.

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; CCBA = 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid (pp. 11-13 of MRID 50143502).

2 In the ECM, NY Loamy sand/Sandy loam [0-12"; 74.0-78.0% sand, 16.4-20.4% silt, 5.6% clay; pH 5.8-6.1 (not specified); 2.13-3.33% organic matter], NY Sandy loam [18-24"; 72.0% sand, 22.4% silt, 5.6% clay; pH 5.5 (not specified); 0.82% organic matter], WA Loamy sand [0-12"; 76.8-80.8% sand, 14.0-18.0% silt, 5.2% clay; pH 6.5-6.8 (not specified); 0.38-0.60% organic matter], and WA Loamy sand [18-24"; 80.8% sand, 16.0% silt, 3.2% clay; pH 7.4 (not specified); 0.22% organic matter] were used in the study (USDA soil texture classification not specified; p. 15; Table 1, p. 26 of MRID 50143502). The soil characterization location was not reported. Soil sources were not further specified.

3 In the ILV, loamy sand sediment [from Ricerca Document No. 014188-1; 83% sand, 14% silt, 3% clay; pH 6.8 (not specified); 2.1% organic matter] from a river/creek bed located in Madison, Ohio (locally obtained 01-192) was used in the study (USDA soil texture classification not specified; p. 9 of MRID 50143503). The sediment characterization location was not reported.

4 The ILV validated the method with second trial with insignificant modifications to the HPLC/UV parameters and equipment; however, the ILV noted a few significant method cautions (pp. 11, 17-19 of MRID 50143503). In the first trial, the cyazofamid and CCIM analysis portion failed due to the photodegradation of the calibration curve because the calibration solutions were inadvertently prepared in clear glassware, and the CCIM-AM, CTCA, and CCBA analysis failed due to chromatographic issues which were solved with modifications of the HPLC column and mobile phase. The ILV also noted that CTCA had stability issues and needed to be processed and analyzed immediately; losses of 40-50% were observed after *ca.* 10 days in final extract form. **The ECM should be updated with ILV precautions regarding the use of amber glassware and the instability of CTCA in the final extract, as well as the need to optimize the chromatographic conditions for the test soil/sediment matrix.**

5 Based on Appendix 4, pp. 101, 103, 119-120, 122 of MRID 50143503.

A confirmatory method is usually required when LC/MS and GC/MS is not the primary method to generate study data.

IV. Method Deficiencies and Reviewer's Comments

1. ILV MRID 50143503 was not conducted independently of ECM MRID 50143502 since the ECM Section Head of the Department of Residue Analysis, Jerome L. Wiedmann, was the ILV Study Director, as well (pp. 5, 15 of MRID 50143502; p. 7 of MRID 50143503). Also, the ILV Principal Analytical Investigator, Susan E. Kane, communicated directly with Jerome L. Wiedmann regarding technical method issues (Table IX, p. 39 of MRID 50143503). Insufficient evidence was provided to define the roles of the laboratory personnel and support the independence of the two laboratories. According to OCSPP guidelines, 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. Furthermore, 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.
2. The ECM should be updated with significant method precautions noted by the ILV regarding the use of amber glassware and the instability of CTCA in the final extract, as well as the need to optimize the chromatographic conditions for the test soil/sediment matrix (pp. 11, 17-19 of MRID 50143503). The first ILV trial failed, and the second ILV trial would not have passed without the ILV modifications/cautions.
3. The reproducibility of the method could not be determined for the 10×LOQ fortification since only one set of performance data was provided, that of the ECM. OCSPP guidelines state that a minimum of five spiked replicates should be analyzed at each concentration (*i.e.*, minimally, the LOQ and 10× LOQ) for each analyte.

In the ILV, fortifications were prepared at 5×LOQ and 20×LOQ. The reproducibility of the method could not be determined for these fortifications since no ECM samples were prepared.

4. In the ECM, the analysis of CCIM-AM and CTCA did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD) ≤20%) in one or two soils: the LOQ analysis of CTCA in the NY sandy loam (18-24") soil (mean 66%) and WA loamy sand (18-24") soil (mean 134%) and the 10×LOQ analysis of CCIM-AM in the NY sandy loam (18-24") soil (mean 68%; Tables 4-7, pp. 29-32 of MRID 50143502; DER Attachment 2).
5. The specificity of the method at the LOQ was not supported by the ILV HPLC/UV chromatograms due to small analyte peaks and excessive baseline noise (Appendix 4, pp. 101, 103, 119-120, 122 of MRID 50143503). Analyte peak could only be differentiated from baseline noise by retention time. Significant baseline noise interfered with analyte attenuation and integration. The results indicated that the test matrix of the ILV could require a different sample processing in order to enhance the distinction of the analyte peaks.
6. In the ILV and ECM, only HPLC/UV analysis was used to identify and quantify the analytes. A confirmatory method is usually required when LC/MS and GC/MS is not the primary method to generate study data.

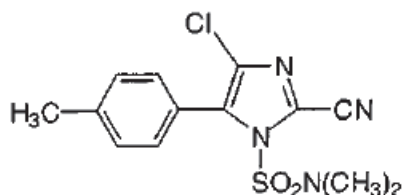
7. In the ILV, performance data for the 20×LOQ analysis of CCIM (RSD 23.7%) did not meet OCSPP Guideline 850.6100 criteria for precision and accuracy (mean recoveries for replicates at each spiking level between 70% and 120% and relative standard deviations (RSD \leq 20%; Tables III-VII, pp. 29-33 of MRID 50143503).
8. In the ECM, no reagent blank was included.
9. In the ECM, recovery results were corrected when residues were quantified in the controls; residues were observed in the control for CCIM (<2% of the LOQ; Appendix C, pp. 66-67 and Figure C-43, p. 110 of MRID 50143502).
10. Two of the four ECM matrices were homogenized 0-12" soils from 0-6" and 6-12" soil samples; therefore, these homogenized test soils had a range of values for the soil characterization data (p. 15; Table 1, p. 26 of MRID 50143502). USDA soil texture classification not specified for the ECM or ILV test matrices.
11. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136. The LOQ and LOD were not adequately supported by calculations or comparison to background levels in the ECM (pp. 17, 21-23 of MRID 50143502; p. 10 of MRID 50143503). In the ECM, the LOQ was supported by the acceptable validation results at that fortification level (mean 70-120%, RSD <20%); the LOQ was reported in the ILV from the ECM without justification. No calculations were used to support the LOQ. In the ECM, the LOD was 0.0025 mg/kg in soil and sediment for all five analytes, based on half of the response of the 0.025 μ g/mL calibration standard. In the ILV, the LOD was not reported. Further work could have been done to explore the actual LOQ and LOD.
12. The total time required to perform the method for one set of twelve soil/sediment samples was reported as sixteen hours, or two calendar days, in the ILV (p. 22 of MRID 50143503).

V. References

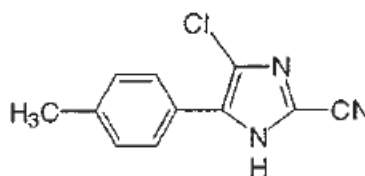
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition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

Attachment 1: Chemical Names and Structures**Cyazofamid (IKF-916)**

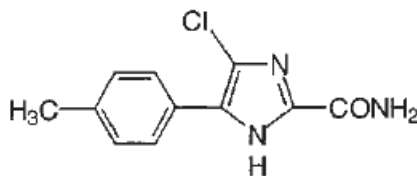
IUPAC Name: 4-Chloro-2-cyano-*N,N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide
CAS Name: 4-Chloro-2-cyano-*N,N*-dirnethyl-5-(4-methylphenyl)-1*H*-imidazole-1-sulfonamide
CAS Number: 120116-88-3
SMILES String: Not reported

**CCIM**

IUPAC Name: 4-Chloro-5-*p*-tolylimidazole-2-carbonitrile
CAS Name: 4-Chloro-5-(4-methylphenyl)-1*H*-imidazole-2-carbonitrile
CAS Number: 120118-14-1
SMILES String: Not reported

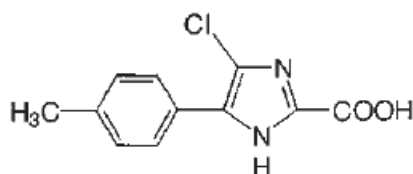
**CCIM-AM**

IUPAC Name: 4-Chloro-5-*p*-tolylimidazole-2-carboxamide
CAS Name: 4-Chloro-5-(4-methylphenyl)-1*H*-imidazole-2-carboxamide
CAS Number: Not reported
SMILES String: Not reported



CTCA

IUPAC Name: 4-Chloro-5-*p*-tolylimidazole-2-carboxylic acid
CAS Name: 4-Chloro-5-(4-methylphenyl)-1*H*-imidazole-2-carboxylic acid
CAS Number: Not reported
SMILES String: Not reported

**CCBA**

IUPAC Name: 4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid
CAS Name: 4-(4-Chloro-2-cyano-1*H*-imidazol-5-yl)benzoic acid
CAS Number: Not reported
SMILES String: Not reported

