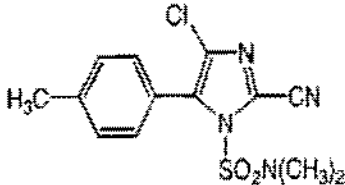
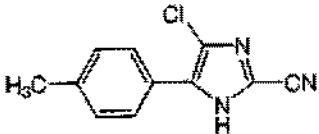
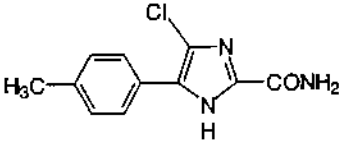


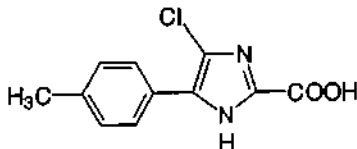
III. TEST SUBSTANCES

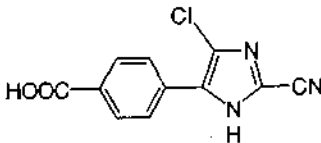
The test substances used in this study were received from Ricerca, LLC on March 28, 2002 with the exception of CTCA which was received on November 28, 2000. The test substances are described as follows:

TEST SUBSTANCES	IKF-916
Chemical Name (IUPAC):	4-chloro-2-cyano- <i>N,N</i> -dimethyl-5- <i>p</i> -tolylimidazole-1-sulfonamide
Purity:	99.5%
Date of Expiry:	July 11, 2004
Cas No.:	120116-88-3
Lot No.:	9704-1
Structure:	

TEST SUBSTANCES	CCIM
Chemical Name (IUPAC):	4-chloro-5- <i>p</i> -tolylimidazole-2-carbonitrile
Purity:	99.7%
Date of Expiry:	June 2005
Cas No.:	120118-14-1
Lot No.:	950308
Structure:	

TEST SUBSTANCES	CCIM -AM
Chemical Name (IUPAC):	4-chloro-5- <i>p</i> -tolylimidazole-2-carboxamide
Purity:	99.6%
Date of Expiry:	April 2004
Cas No.:	Not Available
Lot No.:	9804
Structure:	

TEST SUBSTANCES	CTCA
Chemical Name (IUPAC):	4-chloro-5- <i>p</i> -tolylimidazole-2-carboxylic acid
Purity:	99.3%
Date of Expiry:	June 2005
Cas No.:	Not Available
Lot No.:	9804
Structure:	

TEST SUBSTANCES	CCBA
Chemical Name (IUPAC):	4-(4-chloro-2-cyanoimidazol-5-yl) benzoic acid
Purity:	99.2%
Date of Expiry:	January 2006
Cas No.:	Not Available
Lot No.:	9907
Structure:	

TEST SUBSTANCES RECEIPT AND STORAGE INFORMATION

The analytical standards were shipped frozen by Ricerca, LLC in Concord, OH via Federal Express on March 28, 2002. The standards were received frozen at Pyxant Labs Inc on March 29, 2002. The standards were stored in Freezer #1 at $\leq -20^{\circ}\text{C}$ when not in use.

The CTCA analytical standard was shipped frozen by Ricerca, LLC in Painesville, OH via Federal Express on November 27, 2000. The standard was received frozen at Pyxant Labs Inc on November 28, 2000. The standard was stored in Freezer #1 at $\leq -20^{\circ}\text{C}$ when not in use.

Fresh standard solutions were prepared as specified in the method. The stock solutions for all compounds were prepared using acetonitrile. The calibration and fortification standards for IKF-916 and CCIM were prepared using acetonitrile/water (50:50 (v/v)). The stability period of the standards in acetonitrile/water (50:50 (v/v)) has previously been demonstrated to be one month. Standard solutions for IKF-916 and CCIM were assigned an expiration date of one month from the preparation date. The calibration and fortification standards for CCIM-AM, CTCA, and CCBA were prepared using acetonitrile/water (30:70 (v/v)). The stability period of the standards in acetonitrile/water (30:70 (v/v)) has previously been demonstrated to be three months. Standard solutions for CCIM-AM, CTCA, and CCBA were assigned an expiration date of three months from the preparation date. Analytical standards were stored in Freezer #1 at $\leq -20^{\circ}\text{C}$ when not in use.

The certificates of analysis are attached as Appendix 1. The standards were logged into the master logbook as follows:

Master Logbook Number	Description	Date Received	Receipt Condition	Storage upon Receipt
14102133	IKF-916 reference standard CAS No. 120116-88-3 Lot No. 9704-1 Purity 99.5% Expiration Date: July 11, 2004	3/29/02	Frozen	≤-20°C
14102134	CCIM reference standard CAS No. 120118-14-1 Lot No. 950308 Purity 99.7% Expiration Date: June 2005	3/29/02	Frozen	≤-20°C
14102135	CCIM-AM reference standard Lot No. 9804 Purity 99.6% Expiration Date: April 2004	3/29/02	Frozen	≤-20°C
13751963	CTCA reference standard Lot No. 9804 Purity 99.3% Expiration Date: June 2005	11/28/00	Frozen	≤-20°C
14102136	CCBA reference standard Lot No. 9907 Purity 99.2% Expiration Date: January 2006	3/29/02	Frozen	≤-20°C

IV. METHOD SUMMARY

The samples were analyzed using the analytical method "Analytical Procedure for the Determination of Residues of IKF-916 and its Metabolites (CCIM, CCIM-AM, CTCA, and CCBA) in Soil (Ricerca Method 011141-2)" as presented in the Pyxant Labs Inc document number Ricerca-1375. The standard curve range for IKF-916 and CCIM was 0.025 µg/mL to 1.0 µg/mL. The standard curve range for CCIM-AM, CTCA, and CCBA was 0.025 µg/mL to 1.0 µg/mL. The content of the compound of interest was determined by comparing the manual peak height or integrator peak height of the sample with that of a standard curve of the compound of interest.

The samples were weighed out and fortified. The compounds of interest were extracted from the samples with a solution of 80/20 acetonitrile/0.1N HCl. A 125 mL aliquot (fraction "A") of the extract was buffered to pH 3, concentrated, and partitioned with 2%

aqueous sodium sulfate and methylene chloride to isolate IKF-916 and CCIM. The organic phase was evaporated to dryness and taken through a Florisil[®] cleanup. The eluate from the cleanup column was evaporated to dryness, and the residue was then diluted with 50/50 acetonitrile/water for HPLC quantitation of IKF-916 and CCIM. A second 125 mL aliquot (fraction "B") of the extract was concentrated, buffered to pH 3, and then taken through a C₁₈ solid phase cleanup to isolate CCIM-AM, CTCA, and CCBA. The eluate from the SPE was concentrated to a volume of about 0.5 mL. The sample volume was brought to 2 mL with 30/70 acetonitrile/water. All samples were quantitated by reverse-phase HPLC with UV absorbance detection at 280 nm. Typical liquid chromatographic conditions are listed in Table II.

V. METHOD MODIFICATIONS

Two minor modifications were made to the analytical method "Analytical Procedure for the Determination of Residues of IKF-916 and its Metabolites (CCIM, CCIM-AM, CTCA, and CCBA) in Soil (Ricerca Method 011141-2)." No vacuum manifold was used for any of the cleanup steps. Positive pressure was applied to the top of the cleanup cartridges by use of gas-tight syringes. This change had no effect on the method. An alternative chromatographic column and HPLC mobile phase was used for the analysis of the polar metabolites, CCIM-AM, CTCA, and CCBA. This modification improved the chromatographic quality of the analyses.

Table II
(page 1 of 2)

Typical High Performance Liquid Chromatographic Conditions

For IKF-916 and CCIM

<u>Instrument:</u>	Hewlett-Packard Model 1090 Series II Liquid Chromatograph
<u>Mobile Phase:</u>	Acetonitrile/Water/Acetic Acid (50/50/0.5% (v/v/v))
<u>Mobile Phase Flow Rate:</u>	0.5 mL/min
<u>Column:</u>	Phenomenex Luna RP18, 3.0 mm ID x 150 mm
<u>Guard Column:</u>	Phenomenex Security Guard C18
<u>Injection Volume:</u>	100 µL
<u>Stop Time:</u>	35 minutes
<u>Detector:</u>	UV
<u>Wavelength:</u>	280 nm
<u>Retention time:</u>	18.05 minutes for IKF-916 7.94 minutes for CCIM

Table II
(page 2 of 2)

Typical High Performance Liquid Chromatographic Conditions

For CCIM-AM, CTCA, CCBA

<u>Instrument:</u>	Hewlett-Packard Model 1090 Series II Liquid Chromatograph
<u>Mobile Phase:</u>	Acetonitrile/Water/Acetic Acid (35/65/0.5% v/v/v)
<u>Mobile Phase Flow Rate:</u>	0.5 mL/min
<u>Column:</u>	Phenomenex Luna RP18, 3.0 mm ID x 150 mm
<u>Guard Column:</u>	Phenomenex Security Guard C18
<u>Injection Volume:</u>	100 μ L
<u>Stop Time:</u>	40 minutes
<u>Detector:</u>	UV
<u>Wavelength:</u>	280 nm
<u>Retention time:</u>	9.10 minutes for CCIM-AM 7.73 minutes for CTCA 5.99 minutes for CCBA