

FLONICAMID

FMC CORPORATION  
AGRICULTURAL PRODUCTS GROUP  
Princeton, New Jersey

P-3561M  
Page 1 of 41

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**Report Title**

Analytical Methodology for IKI-220 (F1785) and its Major Metabolites in/on  
Peach, Potato Tuber, and Wheat Straw

**Study Title**

Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on  
Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide

**Data Requirement**

Residue Chemistry Test Guidelines OPPTS 860.1340, Residue Analytical Method

**Author**

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**Study Completion Date**

August 28, 2002

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**Study Number**

178MVL02R1

**Report Number**

P-3561M

**Non-Proprietary Information**  
**ISK Authorizes the Release or Use of This Method**  
**by Federal and State Agencies**

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**STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS**

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Company: Ishihara Sangyo Kaisha, Ltd.

F. Kimura / Dr

Fumio Kimura, Ph.D.  
Board Member & General Manager  
**COMPANY AGENT**

09/20/2002  
Date

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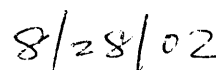
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**GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT**


This report "Analytical Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato Tuber and Wheat Straw", FMC Corporation, Agricultural Products Group, P-3561M) was generated as part of the radiovalidation study (Study number 178MVL02R1, Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide". The analytical methodology was conducted and reported in compliance with the Good Laboratory Practice Standards set forth in Title 40, Part 160 of the Code of Federal Regulations of the United States of America.



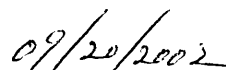
Audrey W. Chen, Ph.D.  
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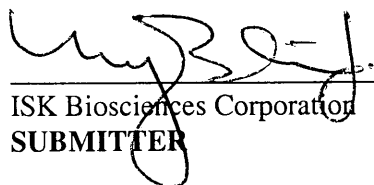
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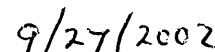
Fumio Kimura, Ph.D.  
Board Member & General Manager  
Ishihara Sangyo Kaisha, Ltd.  
**SPONSOR**



Date



ISK Biosciences Corporation  
**SUBMITTER**



Date

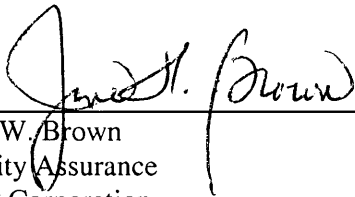
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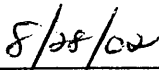
QUALITY ASSURANCE STATEMENT

It is the intent of FMC Corporation that all studies sponsored by or conducted by our facility be of the highest quality in design and performance. Study Number 178MVL02R1, "Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide", was inspected and the findings reviewed and signed by the Study Director and Management of FMC Corporation on the following dates:

<u>Inspection Date</u>	<u>Signed by Study Director</u>	<u>Signed by Management</u>
4/2/02	4/3/02	4/11/02
4/30-5/1/02	5/2/03	5/3/02

This report and all records and raw data were audited and the report was found to be an accurate reflection of the study. All raw data will be maintained by FMC Corporation, PO Box 8, Princeton, NJ 08543 in the Quality Assurance Unit archives.

  
\_\_\_\_\_  
Jane W. Brown  
Quality Assurance  
FMC Corporation

  
\_\_\_\_\_  
Date

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**CERTIFICATION OF AUTHENTICITY**

**Report Title**

Analytical Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato Tuber and Wheat Straw

**Study Title**

Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedures herein described, and that this report provides a true and accurate record of the results obtained.



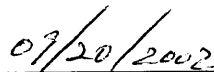
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COMMONLY USED ABBREVIATIONS AND SYMBOLS

<u>Abbreviation</u>	<u>Definition</u>	<u>Abbreviation</u>	<u>Definition</u>
A	Acre(s)	EW	emulsifiable concentrate
ACDS	Agricultural Chemicals Development Services, Inc.	F	flowable
ACN	Acetonitrile	°F	degrees Fahrenheit
ai	active ingredient	FedEx	Federal Express
APG	Agricultural Products Group	FIFRA	Federal Insecticide-Miticide, Fungicide and Rodenticide Act
app	Application	ft	feet
ArCH <sub>4</sub>	argon methane	FPD	flame photometric detector
avg	Average	g	gram(s)
BF <sub>3</sub>	boron trifluoride	gal	gallon(s)
BSTFA	N-O-bis(trimethylsilyl)- trifluoroacetamide	GC	gas chromatograph
C	control	GLPS	Good Laboratory Practice Standards
°C	degrees Celsius or Centigrade	GPA	gallons per acre
ca., ~	approximately	HCl	hydrochloric acid
CAS	Chemical Abstracts Services	He	helium
CFR	Code of Federal Regulations	HP	Hewlett Packard
cm	centimeter (s)	HPLC	high performance liquid chromatography
CI	confidence interval	hr	hour(s)
CO <sub>2</sub>	carbon dioxide	kg	kilogram(s)
CV	column volume, or coefficient of variance	i.d.	internal diameter
DALT	days after last treatment	ISK	Ishihara Sangyo Kaisha, Ltd.
DAT	day after treatment	L	liter(s)
DCI	data call-in	lb	pound(s)
DCM	dichloromethane	LC/MS/MS	Triple Quadrupole Mass Spectrometer
DF	dry flowable	LOD	limit of detection
DI	deionized	LOQ	limit of quantitation
dpm	Disintegrations per minute	LSC	liquid scintillation counting
EC	emulsifiable concentrate	m	meter(s)
ECD	electron capture detector	max	maximum
EPA	Environmental Protection Agency		
EtOAc	ethyl acetate		

COMMONLY USED ABBREVIATIONS AND SYMBOLS (*continued*)

<u>Abbreviation</u>	<u>Definition</u>	<u>Abbreviation</u>	<u>Definition</u>
. ME	microencapsulated	psi	pounds per square inch
MeOH	methanol	pt	pint(s)
mg	milligram(s)	QAU	Quality Assurance Unit
min	minimum	qt	quart(s)
mL	milliliter(s)	RAC	raw agricultural commodity
mm	millimeter(s)	rpm	revolutions per minute
mph	miles per hour	RSD	relative standard deviation
MRID	Master Record Identification	SD	standard deviation
MSD	mass selective detector	Si	silica
mV	millivolt(s)	SIM	single ion mode
n	number	SOP	Standard Operating Procedure
N	normality/normal	SPE	solid phase extraction
NA	not applicable	T	treated
NaCl	sodium chloride	TBA	tetrabutyl ammonium phosphate
NaOH	sodium hydroxide	TD	treated duplicate
ND	not detected	temp	temperature
ng	nanogram(s)	TRR	total radioactive residue
Ni	nickel	USDA	United States Department of Agriculture
NJ	New Jersey	$\mu$	micron(s)
nm	nanometer(s)	$\mu$ g	microgram(s)
NPD	nitrogen phosphorous detector	$\mu$ L	microliter(s)
OPPTS	Office of Prevention, Pesticides and Toxic Substances	$\mu$ m	micrometer(s)
o.d.	outside diameter	$\mu$ v	microvolt(s)
oz	ounce(s)	v	volume
PHI	pre-harvest interval	v:v	volume:volume
pg	picogram(s)	w	weight
ppb	parts per billion	w:v	weight:volume
PPI	pre plant incorporated	w:w	weight:weight
ppm	parts per million	WP	wettable powder
PRE	pre-emergent	WSB	water soluble bag
		XSD	halogen specific detector

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**GENERAL STUDY INFORMATION**

**Study Number**

178MVL02R1

**Report Title**

Analytical Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato Tuber and Wheat Straw

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Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide

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Ming Xiong, Research Technician  
David J. Letinski, Associate Chemist  
Scott Curry, Associate Research Chemist  
David Baffuto, Senior Research Technician

**Test Substance**

IKI-220 (F1785)

**Study Execution Dates**

<u>Study Initiation Date:</u>	March 11, 2002
<u>Study Experimental Start Date:</u>	March 12, 2002
<u>Study Experimental Termination Date:</u>	May 3, 2002
<u>Study Completion Date:</u>	August 28, 2002

**Testing Facility**

Analytical

FMC Corporation  
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USA

## 1.0 SUMMARY

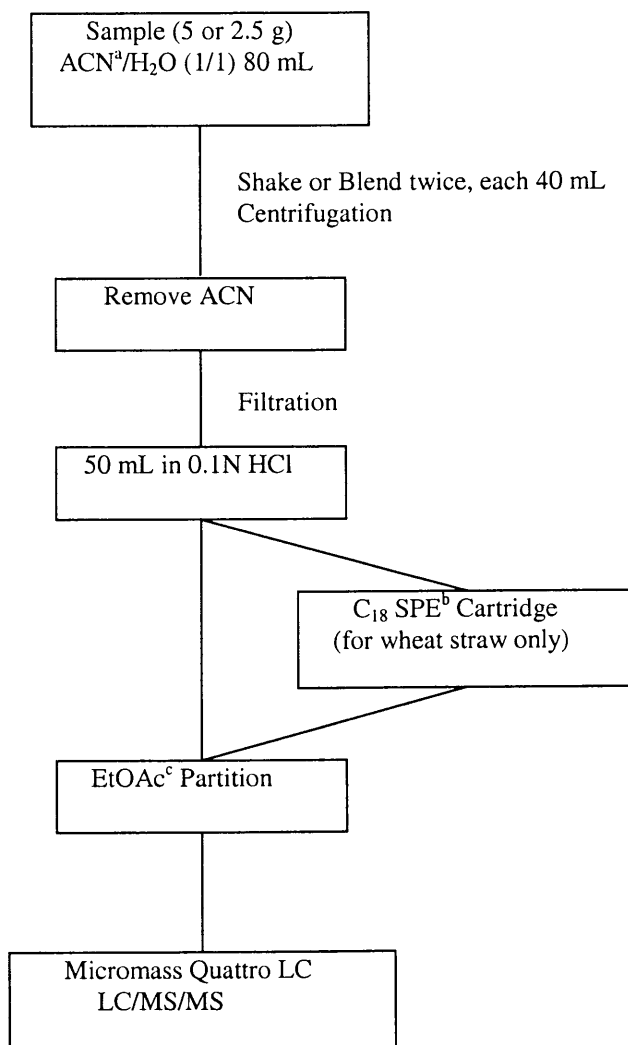
Analytical methodology has been developed to determine the residues of IKI-220 (*N*-cyanomethyl-4-trifluoromethylnicotinamide) and its three major plant metabolites, TFNA-AM (4-trifluoromethylnicotinamide), TFNA (4-trifluoromethylnicotinic acid) and TFNG (*N*-(4-trifluoromethylnicotinoyl)glycine), in peach, potato tuber and wheat straw samples. The residue analytical method for peach and potato tuber includes an initial extraction with acetonitrile (ACN)/deionized (DI) water, followed by a liquid-liquid partition with ethyl acetate. The residue method for wheat straw is similar, except that a C<sub>18</sub> solid phase extraction (SPE) is added prior to the liquid-liquid partition. The final sample solution is quantitated using a liquid chromatograph (LC) equipped with a reverse phase column and a triple quadrupole mass spectrometer (MS/MS).

The analytical methodology has been validated on the following matrices: peach, potato tuber and wheat straw. For wheat straw, the method limit of quantitation (LOQ) was validated at 0.02 ppm and the method limit of detection (LOD) was set at 0.01 ppm. For peach and potato tuber, the LOQ was validated at 0.01 ppm and the LOD was set at 0.005 ppm. For peach, the average method recoveries (n=9) were 105±5, 97±4, 101±6, and 96±11% for IKI-220, TFNA-AM, TFNA and TFNG, respectively. The average method recoveries (n=9) for potato tuber were 99±9, 86±8, 107±9, and 86±10% for IKI-220, TFNA-AM, TFNA and TFNG, respectively. For wheat straw, the average method recoveries (n=9) were 113±14, 72±9, 91±14, and 82±11% for IKI-220, TFNA-AM, TFNA and TFNG, respectively. The average recoveries, range of recoveries, standard deviations, and method flow scheme are summarized on the following pages.

Summary of Method Recovery Values for IKI-220, TFNA-AM, TFNA and TFNG  
From Laboratory Fortified Control Peach, Potato Tuber and Wheat Straw Samples

Matrix/ Compound	Fortification Level (ppm)	Number of Analysis	Recovery Range (%)	Recovery Average (%)	Recovery Std. Dev. (%)
<b><u>Peach</u></b>					
IKI-220	0.01, 0.02, 0.05	9	95-113	105	±5
TFNA-AM	0.01, 0.02, 0.05	9	88-103	97	±4
TFNA	0.01, 0.02, 0.05	9	92-108	101	±6
TFNG	0.01, 0.02, 0.05	9	74-110	96	±11
<b><u>Potato Tuber</u></b>					
IKI-220	0.01, 0.02, 0.05	9	82-109	99	±9
TFNA-AM	0.01, 0.02, 0.05	9	73-98	86	±8
TFNA	0.01, 0.02, 0.05	9	93-122	107	±9
TFNG	0.01, 0.02, 0.05	9	74-107	86	±10
<b><u>Wheat Straw</u></b>					
IKI-220	0.02, 0.04, 0.10	9	83-128	113	±14
TFNA-AM	0.02, 0.04, 0.10	9	61-86	72	±9
TFNA	0.02, 0.04, 0.10	9	63-110	91	±14
TFNG	0.02, 0.04, 0.10	9	66-97	82	±11

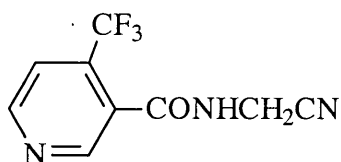
Analytical Method Flow Scheme for IKI-220, TFNA-AM, TFNA and TFNG  
in/on Peach, Potato and Wheat Matrices



- a. ACN - acetonitrile
- b. SPE – solid phase extraction
- c. EtOAc – ethyl acetate

## 2.0. INTRODUCTION

Flonicamid (IKI-220, F1785) is currently being developed by ISK (Ishihara Sangyo Kaisha, Ltd.) and FMC Corporation as an insecticide for use on various crops. The chemical name of the active ingredient in Flonicamid is *N*-cyanomethyl-4-trifluoromethylnicotinamide. The proposed common name for this compound is Flonicamid.



**Flonicamid**

This report was generated as part of the study 178MVL02R1 (Section 7.0, Reference 1) to support the determination of IKI-220, TFNA-AM, TFNA and TFNG in/on peach, potato tuber and wheat straw samples. The analytical methods used solvent extraction, solid phase extraction and/or liquid-liquid partition, and LC/MS/MS detection.

## 3.0 MATERIALS AND METHODS

The protocol and amendments for this study are provided in Appendix IV.

### 3.1 Study Information and Procedures

#### 3.1.1 Test Substance

No test substance was administered during this study.

#### 3.1.2 Test Commodity

The test systems were peach, potato and wheat and the specific crop commodities used for the validation were peach, potato tuber and wheat straw collected from previously completed ISK metabolism study (Section 7.0, Reference 2) and FMC raw agricultural commodity (RAC) studies (Section 7.0, References 3 and 4). Peach and potato matrices were selected as representative of fruit and vegetable crops and wheat straw was selected as representative of cereal grain crops. These samples were analyzed according to the United States Environmental Protection Agency (EPA) Residue Chemistry Guidelines, Office of Prevention, Pesticides and Toxic Substances (OPPTS) 860.1340, Residue Analytical Method (Section 7.0, Reference 5). Dates of sampling, shipment, preparation, extraction and analysis can be found in Table 1.

### 3.1.3 Study Design and Procedures

The residue methods were validated with acceptable and reproducible method recoveries. A method validation set consisted of one control sample and nine fortified control samples. Fortification was accomplished by adding known amounts of IKI-220, TFNA-AM, TFNA and TFNG standards in solvent directly onto the control sample matrix by pipette or syringe. After fortification, the solvent was allowed to evaporate and the fortified samples were analyzed as part of the assay set with the control sample to determine the method recoveries.

### 3.1.4 Reference Substances

Stock solutions of 1000 ng/ $\mu$ L (nanograms per microliter) were prepared by dissolving appropriate amounts of the reference substances in acetonitrile. Working solutions were prepared in volumetric flasks by appropriate dilutions of stock solutions for each analyte or combination of analytes. A working standard solution containing IKI-220, TFNA-AM, TFNA and TFNG was prepared in acetonitrile and used for fortification. All standards were stored in a refrigerator/freezer when not in use. Information for the reference substances and reference solutions is provided in Tables 2 and 3, respectively.

### 3.1.5 Fortification Procedure

The adequacy of the analytical method was determined by the recoveries resulting from the fortified control samples. These control samples were fortified by adding known amounts of non-radiolabeled standard solutions of IKI-220, TFNA-AM, TFNA and TFNG to the surface of peach, potato tuber or wheat straw by pipette or syringe. Fortifications were evaluated at LOQ, 2 x LOQ, and 5 x LOQ levels. These samples were carried through the same analytical procedures as the control sample. The amount of analyte found in the sample extract versus the known amount of standard added before analysis was used to calculate method recovery. The individual recoveries and average recovery are listed in Table 4.

## 3.2 Equipment and Supplies

The following equipment and supplies were used in the analysis:

Adapter, Neoprene  
Adapter, Reducing  
Balance, Analytical PM 2000, Mettler  
Balance, Top Loading, Mettler  
Buchner Filter Funnels, Porcelain, 10.5 cm i.d., Coors  
Centrifuge, IEC HN-SII  
Centrifuge Tube, 13 mL graduated, 0.1 mL, Pyrex®  
Centrifuge Tube, polypropylene, 50 mL, disposable, Corning  
Cylinder, Graduated, 50 mL, 100 mL (mixing)  
Filter Paper, Whatman #1, 11 cm diameter  
Filter Device, polypropylene, 0.2 or 0.45  $\mu$ m, disposable  
Flask, Filter, 250 mL

Flask, Volumetric, 100 mL  
LC, 1100, Agilent  
LC Column, Agilent ZORBAX Eclipse XDB-C8, 250 mm x 4.6 mm id., 5  $\mu$ m, Agilent  
LC Column, XTerra RP8, 150 mm x 4.6 mm id., 5  $\mu$ m, Waters  
Micro Syringe, Hamilton  
Multi - Tube Vortexer, VWR Scientific  
N-EVAP® Evaporator, Organomation  
Pipet, Disposable and volumetric  
Reservoir, Plastic, 75 mL  
Shaker, Eberbach Corp.  
Solid Phase Extraction Cartridge, C<sub>18</sub> (1 g), Varian  
Syringe, disposable  
Triple quadrupole mass spectrometer, Quattro LC, Micromass  
TurboVap® Evaporator, Zymark  
TurboVap Vessel, 200 mL, Zymark  
TurboVap Vessel Support Rack, Zymark  
Visiprep® manifold, Supelco  
Visidry® vacuum manifold drying attachment, Supelco  
Vortex, 37600, Thermolyne

### 3.3 Reagents

The following reagents were used in the analysis:

Acetone, Resi-Analyzed®, JT Baker  
Acetonitrile, Baker Analyzed®, HPLC solvent, JT Baker  
Ethyl Acetate, Resi-Analyzed®, JT Baker  
Hexane, Resi-Analyzed®, JT Baker  
Hydrochloric acid (HCl, 36.5 - 38.0%), JT Baker  
Methanol, HPLC grade, EM Science

Equivalent equipment and reagents may be substituted as appropriate, unless otherwise specified in this method report.

### 3.4 Analytical Procedures

Completely homogenous samples should be prepared prior to analysis. The step-by-step procedures are listed in Appendix I. The detailed analytical procedure follows:

#### 3.4.1 Acetonitrile/DI Water Extraction

Weigh 2.5 (wheat straw) or 5 (peach and potato tuber) grams of the matrix into a 50 mL disposable centrifuge tube. To fortify the control sample, add an accurately measured volume of a standard solution containing IKI-220, TFNA-AM, TFNA and TFNG uniformly to the matrix by syringe. Allow the solvent to evaporate. Add 40 mL of ACN/DI water (1/1, v/v), cap the tube, and extract the sample with a shaker for 30 min at a fast speed. Transfer the tube to a centrifuge for 10 min @ 2000 rpm. Decant the clear

solution into a TurboVap vessel. Add another 40 mL of extraction solvent to the centrifuge tube. Manually mix the centrifuged sample pellet well with the solvent before placing the centrifuge tube on the shaker. Repeat the shaking, centrifugation, and decanting steps. Combine the first and second sample extracts and concentrate the sample extracts to 15-20 mL using a TurboVap evaporator (water bath at ~50°C, increase pressure up to 30 psi as volume decreases) and add 0.5 mL of concentrated HCl. **It is important to remove all traces of ACN.**

#### 3.4.2 Filtration

Filter the sample extract through a Whatman #1 (11 cm) filter paper into a filter flask using a Buchner funnel and vacuum (~15" Hg). Rinse the TurboVap vessel with 2 x 5 mL of DI water and pass the rinsate through the post-extract solid and filter paper. Transfer the filtrate to a 100 mL mixing cylinder. Rinse the filter flask with 2 x 5 mL of DI water and add the rinsate to the mixing cylinder. Bring the volume up to 50 mL with DI water.

#### 3.4.3 C<sub>18</sub> Solid Phase Extraction (SPE)

**C<sub>18</sub> solid phase extraction is needed for wheat straw only. For peach and potato tuber, continue the clean-up procedure in Section 3.4.4.**

Place a C<sub>18</sub> SPE cartridge (1 g, Varian) on a vacuum manifold and condition it with 6 mL of methanol followed by 6 mL of 0.25N HCl. When conditioning the SPE cartridge, allow the first conditioning solvent to reach the top of the cartridge packing before adding the second solvent. Maintain the flow rate through the C<sub>18</sub> cartridge at ~5 mL/min by regulating the vacuum pump (~5" Hg). Attach a 75 mL plastic reservoir with an adapter to the top of the C<sub>18</sub> cartridge. Transfer a 10 mL aqueous sample aliquot to the reservoir. Pass the sample through the C<sub>18</sub> cartridge. Once the entire sample has passed through the cartridge, use full vacuum briefly (~5 sec), and elute the IKI-220 compounds with 6 mL of 20% ACN in DI water into a 13 mL centrifuge tube. Evaporate the eluate under nitrogen stream in a water bath (~45°C) to ~4 mL. Bring the volume up to 5 mL with 0.25N HCl.

#### 3.4.4 Liquid-Liquid Partition

Pipet ~3 mL of sample aliquot (from Section 3.4.2 for peach and potato) or ~2 mL of aliquot (from Section 3.4.3 for wheat straw) using a syringe and filter the sample solution with a syringe filter. Pipet 2 mL (for peach and potato tuber) or 1 mL (for wheat straw) of the filtered sample solution into a 13 mL centrifuge tube. Partition twice, each with 2 mL (for peach and potato tuber) or 1 mL (for wheat straw) of ethyl acetate by vortex. Collect the ethyl acetate fractions in another test tube. Evaporate the sample solution in ethyl acetate under low nitrogen stream (just enough to produce a ripple on the surface) in a water bath (~45°C) to near dryness (**until a thin oily film remains, do not overdry**). Use a syringe filter if the solution is not clear. Dilute the sample solution to the appropriate final volume with 30% ACN in DI water.

### 3.5 Instrumentation and Quantitation

#### 3.5.1 Instrumentation

IKI-220, TFNA-AM, TFNA and TFNG residues were quantitated by an Agilent 1100 LC equipped with a Micromass Quattro LC Triple Quadrupole Mass Spectrometer (LC/MS/MS) and MassLynx™ 3.5 software. An Agilent ZORBAX Eclipse XDB-C8 4.6 mm x 250 mm LC column or Waters Xterra RP8 4.6 mm x 150 mm LC column was used to separate and analyze the analytes of interest. See Appendix II for an example of detailed LC/MS/MS operating conditions. Examples of chromatograms for the fortified samples are presented in Figures 1 to 3.

#### 3.5.2 Quantitation

IKI-220, TFNA-AM, TFNA and TFNG were quantitated by a multiple point external standard calibration standard method. A computer spreadsheet program (Microsoft® Excel 2000) was used for calculation and reporting. A full description of the calculations for quantitation can be found in Appendix III.

### 3.6 Interferences

In general, interference peaks were rarely observed when using the highly selective triple quadrupole mass spectrometer as the detection system. However, low-level interference peaks were found in the more difficult matrix, wheat straw, or when the LC column resolution was not adequate to separate the interference peaks from the analyte of interest. By reducing the LC column flow rate or using a longer column, the interference peaks usually were eliminated or minimized.

### 3.7 Confirmatory Technique

No additional confirmation for the IKI-220 compounds is necessary, since the triple quadrupole mass spectrometer provides tremendous and unique selectivity by detecting both the parent ion and the secondary product ion for each compound. The selected molecular ions used were 229.8/202.8 for IKI-220, 190.8/147.8 for TFNA-AM, 191.8/147.8 for TFNA, and 248.8/202.8 for TFNG.

### 3.8 Time Required for Analysis

For a set of ten samples, the analytical method can be completed from the time of sample weighing to LC injection within 8 hours. Sample extracts can be stored at the end of each step, if the entire method cannot be completed within one day. Samples should be stored in full-volume solvent and refrigerated.

### 3.9 Modifications or Potential Problems

1. Extraction efficiency using a blender or shaker has been compared and both methods gave similar results. A wheat straw sample treated with radioactive IKI-220 and collected from a metabolism study (Section 7.0, Reference 2) was used for comparison.

Extraction using a shaker was selected for the method because multiple samples can be extracted simultaneously.

2. After the initial extraction with ACN and DI water, it is important to remove all traces of ACN using a TurboVap Evaporator. Traces of solvent can lead to analyte loss through the liquid-liquid partition and/or SPE cartridge. Also, over-evaporation of the sample extracts can lead to analyte loss as well, probably due to compound volatility.
3. It is important to determine the proper elution and wash solvent composition, volume and flow rate through the SPE cartridges. The solid phase extraction steps are critical to the separation and clean-up of the sample extract. The listed brand for C<sub>18</sub> cartridges should be used, if possible.
4. Depending on the sample matrix, emulsion may occur during the liquid-liquid partition with ethyl acetate. The emulsion can be eliminated by centrifuging the sample solution, adding a salt (such as NaCl) to the aqueous solutions, and/or double the volume of ethyl acetate for the partition.
5. Optimizing the LC/MS/MS instrument is crucial for the quantitation of IKI-220 and its metabolites, especially at the LOQ levels. The injection standards must have a low coefficient of variation (<15%) and the linearity standards must have a correlation coefficient of at least 0.99. Before injection of the analysis set, it is important to condition the column with sample matrix. This can be done by injecting a matrix sample extract several times before the standard, repeating this “conditioning” until the injection standard gives a reproducible response and provides adequate sensitivity.
6. Ion suppression or enhancement in LC/MS/MS due to matrix effects is possible. The sample clean-up procedure with liquid-liquid partition and/or SPE cartridge is necessary to minimize the matrix effect. Splitting and/or diverting the LC flow are also common practices to reduce matrix effects.

### 3.10 Storage Stability

The analytical reference standards were assayed on a regular basis for percent purity and structural integrity (Table 2). Standard stock solutions (~1000 ng/uL) were prepared from the analytical standards in ACN and were stored in a refrigerator/freezer (ca. -18°C) for up to one year. Standard working solutions were stored in a refrigerator/freezer (ca. -18°C) for up to six months (Table 3). Under these storage conditions, these analytical standards have shown a pattern of stability.

### 3.11 Record of Sample Dates

The dates of sampling, shipment, receipt, preparation, extraction, and analysis can be found in Table 1. Additional detailed sample information can be found in Section 7.0, References 2 to 4.

## 4.0 RESULTS AND DISCUSSION

### 4.1 Accuracy

The accuracy of the method was determined by the average method recovery of the individual results of the fortified-control samples of three different matrices (Table 4). The average method recoveries (n=9) for peach were 105% for IKI-220, 97% for TFNA-AM, 101% for TFNA, and 96% for TFNG. For potato tuber, the average method recoveries (n=9) were 99% for IKI-220, 86% for TFNA-AM, 107% for TFNA, and 86% for TFNG. For wheat straw, the average method recoveries (n=9) were 113% for IKI-220, 72% for TFNA-AM, 91% for TFNA, and 82% for TFNG.

### 4.2. Precision

The precision of the analytical method was determined by the standard deviation of the individual results of the fortified-control samples of three different matrices (Table 4). The standard deviations (n=9) for peach were  $\pm 5\%$  for IKI-220,  $\pm 4\%$  for TFNA-AM,  $\pm 6\%$  for TFNA, and  $\pm 11\%$  for TFNG. For potato tuber, the standard deviations (n=9) were  $\pm 9\%$  for IKI-220,  $\pm 8\%$  for TFNA-AM,  $\pm 9\%$  for TFNA, and  $\pm 10\%$  for TFNG. For wheat straw, the standard deviations (n=9) were  $\pm 14\%$  for IKI-220,  $\pm 9\%$  for TFNA-AM,  $\pm 14\%$  for TFNA, and  $\pm 11\%$  for TFNG.

### 4.3. Limits of Detection and Quantitation

The LOQ for peach and potato tuber was established at 0.01 ppm and the method LOD was set at 0.005 ppm. Due to analytical difficulties with the dried plant matrices, the LOQ for wheat straw was established at 0.02 ppm and the LOD was set at 0.01 ppm.

### 4.4. Ruggedness

The acceptable and reproducible method recoveries for the analytical methods on three different matrices indicate that these methods are reliable and accurate. Considering the potential problems noted above and details within the procedure, analyses for IKI-220, TFNA-AM, TFNA and TFNG should be possible on the various matrices included in this report.

The analytical methodology has been applied to analyze stone and pome fruits, cucurbit, fruiting and leafy vegetables, potato, cotton, turnip and wheat matrices. Analytical method for fruits and vegetables can be applied to potato, turnip and wheat grain and forage samples. The method for the oily crop matrices, such as potato chips and cottonseed was slightly modified with an additional hexane partition from the method for fruits and vegetables. Stepwise procedures for various sample matrices are provided in Appendix I. The additional acceptable and reproducible method recoveries generated have further supported the ruggedness of this methodology.

## 5.0 CONCLUSIONS

The residue analytical methods were developed and successfully employed for the extraction and detection for IKI-220, TFNA-AM, TFNA and TFNG in/on peach, potato tuber and wheat straw. The analytical methods used solvent extraction, SPE and/or liquid-liquid extraction, and LC/MS/MS detection.

All equipment needed to perform the analyses is readily available in most residue analytical laboratories. An experienced residue analyst, following the procedure exactly as written and being aware of the potential problems, can obtain adequate recoveries of IKI-220, TFNA-AM, TFNA and TFNG in/on peach, potato tuber and wheat straw matrices.

## 6.0 RETENTION OF RECORDS

The raw data from the study, including the final report, all analytical data and other study information will be stored in the Quality Assurance archives of the FMC Corporation Agricultural Products Group, Princeton, NJ.

## 7.0 REFERENCES

1. Chen, A.W., "Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide", FMC Corporation, Agricultural Products Group, Princeton, NJ, P-3561, August 2002.
2. Gupta, K.S. and Kaman, R.A., "Metabolism of [<sup>14</sup>C]IKI-220 by Wheat", Ricerca, LLC., Metabolism Division, Concord, OH, No. 010416-1, March 2002.
3. Latorre, L., "Magnitude of the Residue of zeta-Cypermethrin in/on Cherries, Peaches, and Plums and the Processed Products of Plums and Peaches from Fruit Treated with Fury 1.5 EC or Fury 1.5 EW Insecticide", FMC Corporation, Agricultural Products Group, Princeton, NJ, P-3558, June 2002.
4. Arabinick, J. R., "Magnitude of the Residue of Carfentrazone-ethyl and Significant Metabolites in/on Potatoes and Processed Parts from Potatoes Treated with Either F8426 2EC, F8426 2EW or F8426 40DF Herbicide", FMC Corporation, Agricultural Products Group, Princeton, NJ, P-3553, March 2002.
5. United States Environmental Protection Agency, "Residue Chemistry Test Guidelines, OPPTS 860.1340: Residue Analytical Method", EPA 712-C-96-174 August 1996.

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TABLES

Table 1

Record of Sample Dates

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Matrix/ Sample ID	Date of Sampling	Shipment to FMC	Receipt at FMC	Date of Preparation	Date of Extraction	Date of Analysis
Control Wheat Straw WCS	9/14/99	1/14/02	1/15/02	NA <sup>a</sup>	4/4/02	4/17/02
Control Whole Peach 01LJL071C	8/23/01	9/12/01	9/26/01	11/7/01	4/4/02	4/17/02
Control Potato Tuber 01JRA182C	9/8/01	10/13/01	11/5/01	12/11/01	4/12/02	4/18/02

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a. NA – Not-Applicable (no further sample preparation was needed)

Table 2

Reference Substances

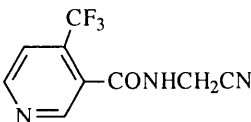
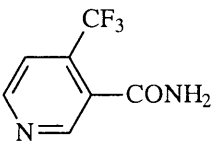
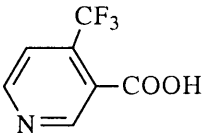
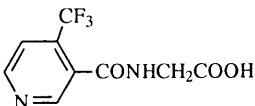
<u>Common Name</u> Chemical Name	Structure	CAS Reference Number	Database Inventory Number	Percent Purity
Fonicamid (IKI-220) <i>N</i> -cyanomethyl-4-trifluoromethylnicotinamide		158062-67-0	TF-2	99.7
<u>TFNA-AM</u> 4-trifluoromethylnicotinamide		158062-71-6	TF-5	100
<u>TFNA</u> 4-trifluoromethylnicotinic acid		158063-66-2	TF-3	99.4
<u>TFNG</u> <i>N</i> -(4-trifluoromethylnicotinoyl)glycine		207502-65-6	TF-4	99.4

Table 3

Reference Solutions

Compound	Solution Solvent	Solution Concentration (ng/ $\mu$ L)	Standard Solution Index Number	Date Prepared
IKI-220	Acetonitrile	1001	1199	12/4/01
TFNA-AM	Acetonitrile	1000	1201	12/4/01
TFNA	Acetonitrile	1000	1202	12/4/01
TFNG	Acetonitrile	1000	1200	12/4/01
IKI-220 + TFNA-AM + TFNA + TFNG	Acetonitrile	10	1203-1	12/5/01
IKI-220 + TFNA-AM + TFNA + TFNG	Acetonitrile	10	1203-8	3/5/02

Table 4

Method Recovery Data for IKI-220, TFNA-AM, TFNA and TFNG  
From Laboratory Fortified Control Peach, Potato Tuber and Wheat Straw

Matrix	Fortification Level (ppb)	Method Recovery (%)			
		IKI-220	TFNA-AM	TFNA	TFNG
<b>Peach</b> (01LJL071C)	10	113	96	95	95
	10	95	88	92	74
	10	108	96	97	85
	20	108	100	94	96
	20	106	97	108	96
	20	105	103	105	99
	50	102	97	106	102
	50	106	98	107	110
	50	104	98	105	106
		<b>Avg. ± Std. Dev.</b>	<b>105 ± 5</b>	<b>97 ± 4</b>	<b>101 ± 6</b>
<b>Potato Tuber</b> (01JRA182C)	10	94	80	107	86
	10	108	98	102	107
	10	92	98	115	82
	20	98	83	107	80
	20	104	87	115	85
	20	109	89	122	95
	50	99	80	96	75
	50	82	73	93	74
	50	106	85	106	87
		<b>Avg. ± Std. Dev.</b>	<b>99 ± 9</b>	<b>86 ± 8</b>	<b>107 ± 9</b>
<b>Wheat Straw</b> (WCS)	20	127	61	84	66
	20	119	70	84	73
	20	83	80	109	96
	40	109	63	63	84
	40	107	64	93	72
	40	128	71	95	88
	100	122	77	96	83
	100	105	78	86	80
	100	115	86	110	97
		<b>Avg. ± Std. Dev.</b>	<b>113 ± 14</b>	<b>72 ± 9</b>	<b>91 ± 14</b>

Figures 1 - 3

Chromatogram Index

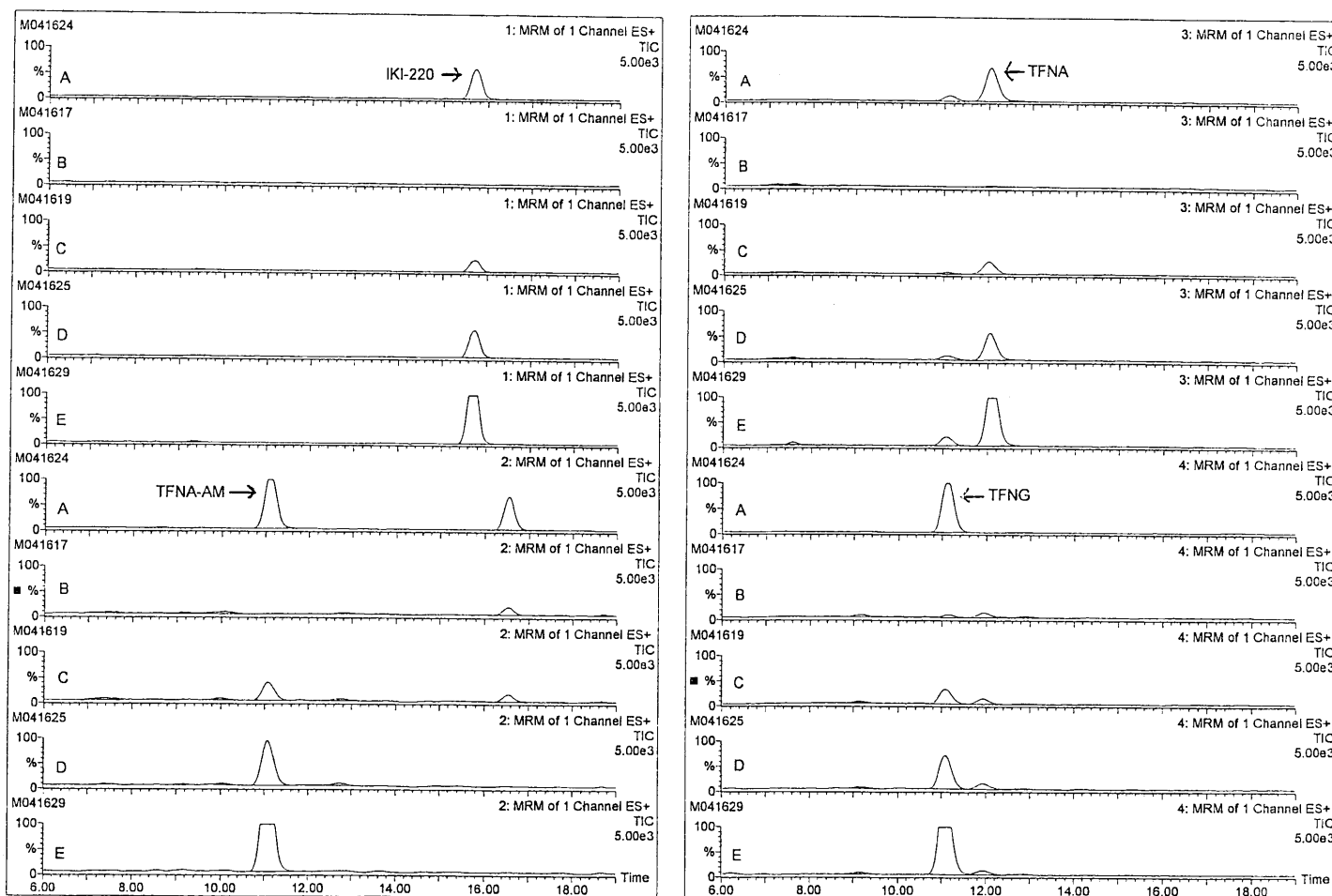
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Figure Number	Chromatogram Description
1	IKI-220, TFNA-AM, TFNA and TFNG in/on Peach Set 4: Standard, Control, and Fortified Samples
2	IKI-220, TFNA-AM, TFNA and TFNG in/on Potato Tuber Set 2: Standard, Control, and Fortified Samples
3	IKI-220, TFNA-AM, TFNA and TFNG in/on Wheat Straw Set 6: Standard, Control, and Fortified Samples

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Figure 1

IKI-220, TFNA-AM, TFNA and TFNG in/on Peach by LC/MS/MS  
Set #: 4 (RT: ~15.7 min for IKI-220, ~11.1 min for TFNA-AM,  
~12.0 min for TFNA, and ~11.1 min for TFNG)

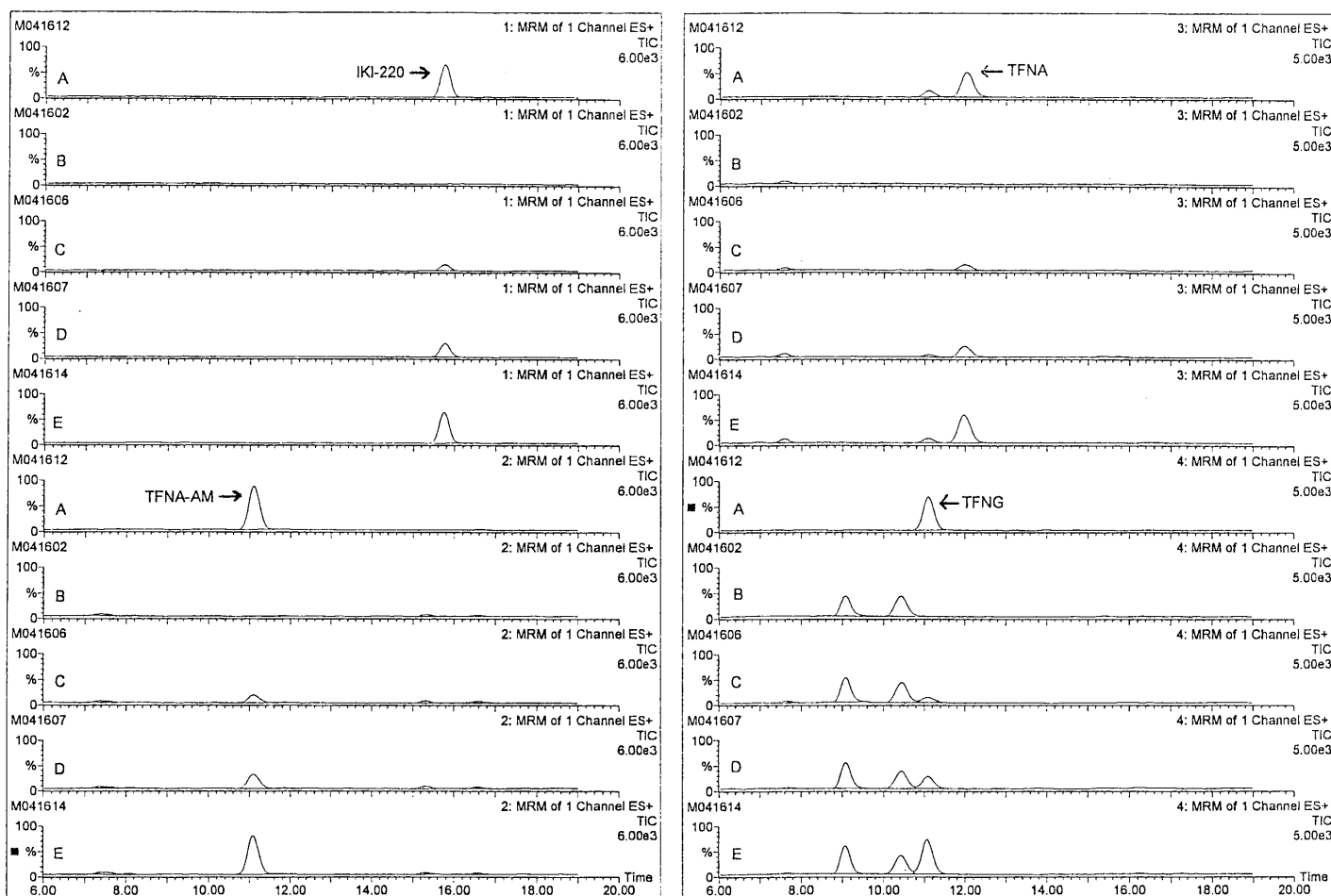


	Standard/ Sample ID	Sample Type/ Assay No.	Amount Injected	IKI-220		TFNA-AM		TFNA		TFNG	
				Peak Area	Amount Detected <sup>b</sup>	Peak Area	Amount Detected	Peak Area	Amount Detected	Peak Area	Amount Detected
A	1203-10 (dil) <sup>a</sup>	Standard, 0.005 ng/uL	0.125 ng	839	0.125 ng	1694	0.125 ng	1109	0.125 ng	1579	0.125 ng
B	01LJL071C	Control, 4-1	5 mg	1	ND <sup>c</sup>	35	ND	40	ND	69	ND
C	01LJL071C	Fortified, 4-3 (10 ppb)	5 mg	330	95%	582	88%	420	92%	478	74%
D	01LJL071C	Fortified, 4-7 (20 ppb)	5 mg	767	105%	1462	103%	880	105%	1085	99%
E	01LJL071C	Fortified, 4-10 (50 ppb)	5 mg	1941	104%	3555	98%	2098	105%	2701	106%

- The standard was prepared by placing 50 uL of standard 1203-10 (0.1 ng/uL) in a test tube and diluting with 250 uL of ACN and 700 uL of H<sub>2</sub>O.
- A multiple point external standard calibration method was used for quantitation; for IKI-220 the slope (y-intercept) was 189 (-29), for TFNA-AM the slope (y-intercept) was 369 (-65), for TFNA the slope (y-intercept) was 193 (66), and for TFNG the slope (y-intercept) was 243 (121).
- ND = Non-Detectable [ $< LOD$  (5 ppb)]

Figure 2

**IKI-220, TFNA-AM, TFNA and TFNG in/on Potato Tuber by LC/MS/MS**  
Set #: 2 (RT: ~15.7 min for IKI-220, ~11.1 min for TFNA-AM,  
~12.0 min for TFNA, and ~11.1 min for TFNG)

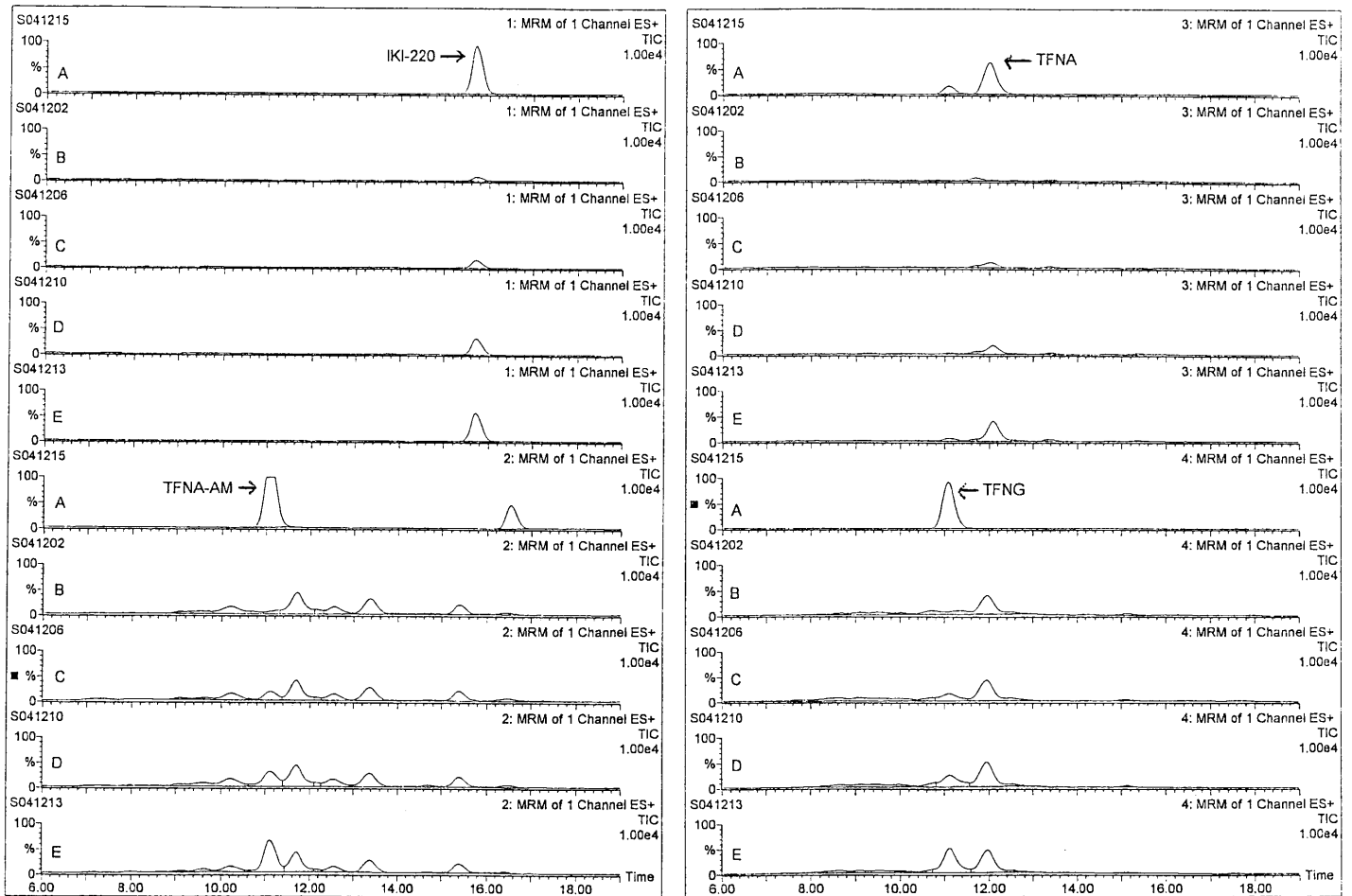


	Standard/ Sample ID	Sample Type/ Assay No.	Amount Injected	IKI-220		TFNA-AM		TFNA		TFNG	
				Peak Area	Amount Detected <sup>b</sup>	Peak Area	Amount Detected	Peak Area	Amount Detected	Peak Area	Amount Detected
A	1203-10 (dil) <sup>a</sup>	Standard, 0.01 ng/uL	0.25 ng	1073	0.25 ng	1598	0.25 ng	846	0.25 ng	1060	0.25 ng
B	01JRA182C	Control, 2-1	5 mg	0	ND <sup>c</sup>	3	ND	8	ND	0	ND
C	01JRA182C	Fortified, 2-4 (10 ppb)	5 mg	201	92%	288	98%	191	115%	135	82%
D	01JRA182C	Fortified, 2-5 (20 ppb)	5 mg	405	98%	553	83%	380	107%	332	80%
E	01JRA182C	Fortified, 2-10 (50 ppb)	5 mg	1056	106%	1433	85%	976	106%	1018	87%

- The standard was prepared by placing 100 uL of standard 1203-10 (0.1 ng/uL) in a test tube and diluting with 200 uL of ACN and 700 uL of H<sub>2</sub>O.
- A multiple point external standard calibration method was used for quantitation; for IKI-220 the slope (y-intercept) was 97 (22), for TFNA-AM the slope (y-intercept) was 170 (-10), for TFNA the slope (y-intercept) was 95 (-28), and for TFNG the slope (y-intercept) was 125 (-71).
- ND = Non-Detectable [( $<$  LOD (5 ppb)]

Figure 3

**IKI-220, TFNA-AM, TFNA and TFNG in/on Wheat Straw by LC/MS/MS**  
**Set #: 6 (RT: ~10.0 min for IKI-220, ~6.7 min for TFNA-AM,**  
**~7.3 min for TFNA, and ~6.7 min for TFNG)**



	Standard/ Sample ID	Sample Type/ Assay No.	Amount Injected	IKI-220		TFNA-AM		TFNA		TFNG	
				Peak Area	Amount Detected <sup>b</sup>	Peak Area	Amount Detected	Peak Area	Amount Detected	Peak Area	Amount Detected
A	1203-10 (dil) <sup>a</sup>	Standard, 0.02 ng/uL	0.5 ng	2648	0.5 ng	4183	0.5 ng	2168	0.5 ng	2989	0.5 ng
B	WCS	Control, 6-1	2.5 mg	211	12.5 ppb	0	ND <sup>c</sup>	78	ND	66	ND
C	WCS	Fortified, 6-4 (20 ppb)	2.5 mg	429	83%	353	80%	309	109%	350	96%
D	WCS	Fortified, 6-7 (40 ppb)	2.5 mg	888	128%	611	71%	487	95%	591	88%
E	WCS	Fortified, 6-9 (100 ppb)	2.5 mg	1596	105%	1649	78%	1006	86%	1254	80%

- The standard was prepared by placing 200 uL of standard 1203-10 (0.1 ng/uL) in a test tube and diluting with 100 uL of ACN and 700 uL of H<sub>2</sub>O.
- A multiple point external standard calibration method was used for quantitation; for IKI-220 the slope (y-intercept) was 132 (45), for TFNA-AM the slope (y-intercept) was 210 (19), for TFNA the slope (y-intercept) was 109 (73), and for TFNG the slope (y-intercept) was 149 (66).
- ND = Non-Detectable [( $<$  LOD (10 ppb)]

## Appendix I

### A. Analytical Method for IKI-220, TFNA-AM, TFNA and TFNG in/on Peach and Potato Matrices

1. Weigh 5 g of sample into a 50 mL polypropylene centrifuge tube.
2. Fortify as appropriate. Allow time for the solvent to evaporate.
3. Add 40 mL of 50% ACN. Shake samples for 30 min.
4. Centrifuge samples for 10 min. @ 2000 rpm.
5. Decant the liquid into a TurboVap tube.
6. Repeat steps 3 through 5.
7. Evaporate the solution in the TurboVap to 20 mL or less and add 0.5 mL of concentrated HCl.
8. Filter through Whatman #1 (11 cm) filter paper. Rinse TurboVap vessel with 2 x 5 mL of DI H<sub>2</sub>O.
9. Transfer the sample solution into a 100 mL mixing cylinder. Rinse with 2 x 5 mL of DI H<sub>2</sub>O and add the rinsate into the mixing cylinder and bring the volume up to 50 ml with DI H<sub>2</sub>O.
10. Pipet a 3 mL aliquot and filter with a 0.45  $\mu$ m syringe filter (or 0.45  $\mu$ m centrifugal microfilter tube). Pipet 2 mL of filtered sample into a 13 mL centrifuge tube.
11. Partition with 2 x 2 mL of ethyl acetate by vortex (If the sample solution is not clear, centrifuge the sample solution after partition). Pipet the ethyl acetate fraction into a 13 mL centrifuge tube.
12. Evaporate the sample solution to near dryness (avoid overdrying) and make up to final volume with 1 mL of 30% ACN in DI H<sub>2</sub>O. Filter with a 0.2  $\mu$ m syringe filter before injection.
13. Inject on LC/MS/MS.

**B. Analytical Method for IKI-220, TFNA-AM, TFNA and TFNG in/on Wheat Straw**

1. Weigh 2.5 g of sample into a 50 mL polypropylene centrifuge tube.
2. Fortify as appropriate. Allow time for the solvent to evaporate.
3. Add 40 mL of 50% ACN. Shake sample for 30 min.
4. Centrifuge sample for 10 min. @ 2000 rpm.
5. Decant the solution into a TurboVap vessel.
6. Repeat steps 3 through 5.
7. Evaporate the combined solution in the TurboVap to 20 mL or less and add 0.5 mL of concentrated HCl.
8. Filter through Whatman #1 (11 cm) filter paper. Rinse TurboVap vessel with 2 x 5 mL of DI H<sub>2</sub>O.
9. Transfer the sample solution into a 100 mL mixing cylinder. Rinse with 2 x 5 mL of DI H<sub>2</sub>O and add the rinsate into the mixing cylinder and bring the volume up to 50 mL with DI H<sub>2</sub>O.
10. Condition a C<sub>18</sub> cartridge with 6 mL of methanol and 6 mL of 0.25 N HCl. Load 10 mL of sample solution. Elute with 6 mL of 20% ACN in DI H<sub>2</sub>O.
11. Evaporate the solution to 4 mL. Make the volume up to 5 mL with 0.25 N HCl.
12. Pipet a 2 mL of aliquot and filter with a 0.45  $\mu$ m syringe filter (or 0.45  $\mu$ m centrifugal microfilter tube). Pipet 1 mL of filtered sample into a 13 mL centrifuge tube.
13. Partition with 2 x 1 mL of ethyl acetate by vortex (if the sample solution is not clear, centrifuge the sample solution after partition). Pipet the ethyl acetate fraction into a 13 mL centrifuge tube.
14. Evaporate the sample solution to near dryness (avoid overdrying) and make up to final volume with 1 mL of 30% ACN in DI H<sub>2</sub>O. Filter with a 0.2  $\mu$ m syringe filter before injection.
15. Inject on LC/MS/MS.

**C. Analytical Method for IKI-220, TFNA-AM, TFNA and TFNG in/on Cotton Matrices and Potato Chips**

1. Weigh 2.5 g of sample into a 50 mL polypropylene centrifuge tube.
2. Fortify as appropriate. Allow time for the solvent to evaporate.
3. Add 40 mL of 50% ACN. Shake samples for 30 min.
4. Centrifuge samples for 10 min. @ 2000 rpm.
5. Decant the liquid into a TurboVap tube.
6. Repeat steps 3 through 5.
7. Evaporate the solution in the TurboVap to 20 mL or less. Transfer the sample solution into a 50 mL polypropylene centrifuge tube. Rinse TurboVap vessel with 2 x 2 mL of DI H<sub>2</sub>O and add the rinsate into the 50 mL polypropylene centrifuge tube.
8. Partition with 20 mL of hexane and discard the hexane portion. Add 0.5 mL of conc. HCl.
9. Filter the sample solution through Whatman #1 (11 cm) filter paper. Rinse polypropylene centrifuge tube with 2 x 2 mL of DI H<sub>2</sub>O.
10. Transfer the sample solution into a 100 mL mixing cylinder. Rinse with 2 x 5 mL of DI H<sub>2</sub>O and add the rinsate into the mixing cylinder and bring the volume up to 50 ml with DI H<sub>2</sub>O.
11. Pipet about 3 mL of aliquot and filter with 0.45 um syringe filter (or 0.45 um centrifugal microfilter tube). Pipet 2 mL of filtered sample into a 13 mL centrifuge test tube.
12. Partition with 2 x 4 mL or of ethyl acetate by vortex (If the sample solution is not clear, centrifuge the sample solution after partition). Pipet the ethyl acetate fraction into a 13 mL centrifuge test tube.
13. Evaporate the sample solution to near dryness (avoid over dry) and make the final volume with 1 mL of 30% ACN in DI H<sub>2</sub>O. Filter with 0.2 um syringe filter before injection.
14. Inject on LC/MS/MS.

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**Appendix II**

**Instrument Parameters for IKI-220, TFNA-AM, TFNA and TFNG**

Instrument: Agilent 1100 Series High Performance Liquid Chromatograph

Column: Agilent ZORBAX Eclipse XDB-C8, 250 mm x 4.6 mm id., 5 µm

Injector: Agilent 1100 Series Autosampler

Injection Volume: 25 µL

Solvents: (A) H<sub>2</sub>O  
(B) 0.2% Acetic Acid in Acetonitrile  
(C) 0.2% Acetic Acid  
(D) Methanol

Program: Gradient

Step No.	Time (min)	%A	%B	%C	%D	Flow Rate (mL/min)
1	0	0	30	70	0	0.3
2	2	0	30	70	0	0.3
3	15	0	95	0	5	0.3
4	16	0	95	0	5	0.3
5	17	0	30	70	0	0.3
6	22	0	30	70	0	0.3

Detector: Micromass Quattro LC MS/MS Detector  
(electrospray positive mode)

Retention Time: ~15.7 min (IKI-220)  
~11.1 min (TFNA-AM)  
~12.0 min (TFNA)  
~11.1 min (TFNG)

Ion Monitored: 229.8 > 202.8 (IKI-220)  
190.8 > 147.8 (TFNA-AM)  
191.8 > 147.8 (TFNA)  
248.8 > 202.8 (TFNG)

### Appendix III

#### Method of Calculation

IKI-220 and its acid metabolites were quantitated by a multiple point external standard calibration method. A computer spreadsheet program (Microsoft® Excel 2000) was used for calculation and reporting.

A multi-point linearity curve was generated by injecting known amounts of various standard concentrations and calculating a response curve from the linear regression analysis of the standard. The nanogram value of the analytes in each sample was calculated by subtracting the y intercept of the line from the area units of the unknown sample and dividing by the slope of the line using the following formula:

$$\text{ng of analyte in sample} = \frac{\text{area units (sample)} - \text{y intercept}}{\text{slope}} \times \text{sample extract injected } (\mu\text{L})$$

The peak area of an interference in a control sample, if necessary, was subtracted from the corresponding fortified control sample peak area if the magnitude of the interference was larger than the limit of detection (LOD).

For this study, the LOD was set at 10 ppb for peach and potato samples and 20 ppb for the wheat straw sample. The LOD was 5 ppb for peach and potato samples and 10 ppb for wheat straw samples.

A 0.2 g aliquot of crop matrix resulted in a final sample extract volume of 1.0 mL. A 25  $\mu\text{L}$  volume of the final sample extract was injected onto the LC/MS/MS yielding a 5 mg sample injection in most cases. The following formula was used to obtain the milligram of sample injected:

$$\text{mg of sample injected} = \frac{\text{sample aliquot weight (mg)}}{\text{final sample extract volume } (\mu\text{L})} \times \text{sample extract injected } (\mu\text{L})$$

The ng of analyte in the sample and the mg of sample injected were used to calculate the uncorrected ppm (ng/mg) by the following formula:

$$\text{uncorrected ppm (ng/mg)} = \frac{\text{ng of analyte in sample}}{\text{mg of sample injected}}$$

The uncorrected ppm of the fortified control samples was divided by the fortification level and multiplied by 100 to calculate the method recovery (%). The following formula was used:

$$\text{method recovery (\%)} = \frac{\text{uncorrected ppm}}{\text{fortification level (ppm)}} \times 100\%$$

Unrounded numbers were used in all of the calculations. An example of how to calculate the recovery of IKI-220 in a fortified peach sample (Figure 1, assay number 4-3) using the multiple point method is given below:

$$\begin{aligned} \text{y intercept} &= -29 \\ \text{slope} &= 189 \\ \text{area units (sample)} &= 330 \\ \text{final sample extract} &= 1000 \\ \text{volume } (\mu\text{L}) \\ \text{ng of IKI-220} &= \frac{330 - (-29)}{189} \times 25 \text{ uL} = 47 \text{ pg} = 0.047 \text{ ng} \\ \text{in sample} \\ \text{mg of sample} &= \frac{200 \text{ mg}}{1000 \text{ uL}} \times 25 \text{ uL} = 5 \text{ mg} \\ \text{injected} \\ \text{ppm (ng/mg)} &= \frac{0.047 \text{ ng}}{5 \text{ mg}} = 0.0095 \text{ ppm} \\ \text{method recovery} &= \frac{0.0095 \text{ ppm}}{0.01 \text{ ppm}} \times 100\% = 95\% \\ \text{(\%)} \end{aligned}$$

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Appendix IV

Protocol and Revision

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RESIDUE STUDY PROTOCOL

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STUDY NUMBER: 178MVL02R1

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**STUDY TITLE:**

Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated With <sup>14</sup>C-labeled IKI-220 Insecticide

**EPA REQUIREMENT:**

OPPTS (Office of Prevention, Pesticides, and Toxic Substances) Residue Chemistry Test Guidelines, 860.1340, Residue Analytical Method – Extraction Efficiency (Reference 1)

**OBJECTIVE:**

Demonstrate that the residue analytical methods for the determination of the residues of IKI-220 (*N*-cyanomethyl-4-trifluoromethylnicotinamide), *N*-(4-trifluoromethylnicotinoyl)glycine (TFNG), 4-trifluoromethylnicotinic acid (TFNA), and 4-trifluoromethylnicotinamide (TFNA-AM) in/on radiolabeled peach, potato and wheat straw samples from the plant metabolism studies (Ricerca study no. 011586, 010424 and 010416 for peach, potato and wheat, respectively) are capable of adequately recovering the analytes of concern.

**STUDY DIRECTOR/TEST FACILITY:**

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**PROPOSED STUDY SCHEDULE:**

Experiment Start Date:	March 2002
Experiment Termination Date:	April 2002
Study Completion Date:	June 2002

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**TEST SUBSTANCE:**

No test substance will be administered in this study.

**TEST SYSTEM:**

The test system will be existing peach, potato and wheat straw samples from the plant metabolism studies (Ricerca study no. 011586, 010424 and 010416 for peach, potato and wheat, respectively) that have been maintained in frozen storage.

**JUSTIFICATION OF TEST SYSTEM:**

Peach and potato were selected as representative of fruit and vegetable crops and wheat straw was selected as representative of cereal grain crops. In addition, the wheat straw is expected to be the most difficult matrix for the analytical method.

**IN-LIFE PHASE AND TREATMENT DESCRIPTION:**

There is no in life phase for this study. All information regarding the growth and treatment of the peach, potato and wheat straw can be found in the respective plant metabolism study reports.

**SAMPLE IDENTIFICATION:**

Control and radiolabeled peach, potato and wheat straw samples from the plant metabolism studies were received from Ricerca on 1/15/02.

**SAMPLE STORAGE:**

Samples were stored frozen from the time of collection until analysis, unless otherwise required by handling and/or preparative procedures. Concurrent analysis and radioactive comparison during this study should minimize concern for sample stability during the period of frozen storage.

**SAMPLE PREPARATION PROCEDURES:**

All samples will be prepared for analysis in accordance with applicable SOPs (Standard Operating Procedures) to provide a homogeneous sample for analysis. The equipment used for sample preparation will be documented on the sample preparation form. Potato and depitted peach samples will be grossly chopped manually, then homogenized using Robot Coupe® RSI 2YI desktop chopper/mixer. The wheat straw samples to be used in this study were previously processed during the metabolism study and no further processing is needed.

**ANALYTICAL PHASE**

**ANALYSIS FACILITY:**

Environmental Sciences  
FMC Corporation  
Princeton, NJ 08543

**PRINCIPAL ANALYST(S)**

The principal analyst(s) will be documented in the study file.

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REFERENCE SUBSTANCES:

Common Name: IKI-220 (F1785)  
Common Name: Flonicamid  
Chemical Name: *N-cyanomethyl-4-trifluoromethylnicotinamide*  
CAS Number: 158062-67-0

Common Name: TFNG  
Chemical Name: *N-(4-trifluoromethylnicotinoyl) glycine*  
CAS Number: 207502-65-6

Common Name: TFNA  
Chemical Name: *4-trifluoromethylnicotinic acid*  
CAS Number: 158063-66-2

Common Name: TFNA-AM  
Chemical Name: *4-trifluoromethylnicotinamide*  
CAS Number: 158062-71-6

PROPOSED ANALYTICAL PLAN:

The total radioactive residues (TRR) of the peach, potato and wheat straw treated with radiolabeled IKI-220 will be determined by oxidizer combustion. The crop samples will be analyzed for analytes of concern by both the current residue methodology and conventional metabolism/radiolabel techniques. A comparison will be made between the analytical results from the residue chromatographic methods (including LC and GC) and the radioactive determinations by HPLC from the plant metabolism studies. This comparison will determine the effectiveness of the residue analytical methods for the determination of the residues of IKI-220, TFNG, TFNA and TFNA-AM in/on peach, potato and wheat straw.

RESIDUE ANALYTICAL METHOD:

The residue analytical methods to be used in this study will be described in detail in the final study report. The residue methods will include an initial extraction of the crops with solvent mixtures and followed by separation of the extract and solids. The TRR in the post-extraction solids may be measured by combustion to determine the percentage of non-extractable radioactivity, if necessary. The extraction efficiency of the total <sup>14</sup>C-labeled residues in the sample extract will be determined using Liquid Scintillation Counting (LSC). The organic solvent will be removed from the extract by evaporation and an aliquot of the aqueous extract will be further extracted by liquid-liquid partition or solid phase extraction (SPE). In addition, the radioactive residues before and after each major analytical procedure will be measured by LSC. Concentrations of IKI-220, TFNG, TFNA and TFNA-AM will be quantitated by LC equipped with a tandem mass selective detector or GC equipped with a mass selective detector. Control and fortified control (fortified with non-radiolabeled standards) samples will also be analyzed to determine residue method recovery based on the "cold" fortified samples. The proposed limit of quantitation (LOQ) and limit of detection (LOD) will be 0.01 ppm and 0.005 ppm for peach and potato and 0.02 ppm and 0.01 ppm for wheat straw, respectively.

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**GENERAL RECORDS AND REPORTING**

**INSTRUMENTATION AND EQUIPMENT:**

Instruments and equipment that generate raw data or significantly impact the validity and results of the study should be maintained in proper working order. A logbook will be used to record maintenance, inspection, cleaning and calibration of such equipment.

**RECORDS TO BE MAINTAINED:**

All records necessary to support the study and to allow for reconstruction of the study will be provided in the Study File. These records will be archived according to FMC Residue Chemistry SOPs and will include, but are not limited to:

- The study protocol and all revisions.
- Sample history records.
- A record of all SOP deviations.
- Laboratory equipment maintenance and calibration records.
- A list of all personnel involved with the study conduct.
- An exact copy or original of all applicable correspondence.
- An exact copy or original of the analytical method.
- Reference substance purity and preparation records.
- All laboratory raw data including laboratory data sheets, chromatograms and spreadsheets.
- A record of problems or protocol deviations and their justification and/or resolution.
- The original final report.

**STATISTICAL TREATMENT OF THE DATA:**

Statistical treatment of data will consist of mean, standard deviation and coefficient of variance determinations where appropriate. Any additional statistical treatment of the data will be documented in the study file. An example of residue analytical calculations will be provided in the final study report.

**GLP COMPLIANCE:**

It is the policy of FMC to conduct all studies in compliance with GLPS and according to relevant SOPs. A GLPS compliance statement will be included in the final study report.

**QUALITY ASSURANCE:**

The FMC QAU will inspect the analytical phase of the study providing the analytical phase is conducted in-house. In addition, FMC QA will review the raw data and final report to verify the integrity of reported results and to assure compliance with existing regulations.

**PROTOCOL AMENDMENTS/DEVIATIONS:**

Any amendments to or deviations from the study protocol will be documented on a Study Protocol Revision Form, according to applicable SOPs. The form must be completed, signed and sent promptly to the Study Director, Study Sponsor and FMC Quality Assurance Unit for authorization. The original protocol amendment/deviation must be maintained in the study file and copies distributed by the Study Director or delegate to all pertinent study personnel.

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STUDY NUMBER: 178MVL02R1

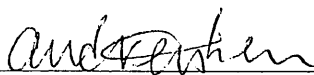
**REPORTING:**

One report will be issued to document the results of this study. The final study report will include a complete description of the residue methods employed.

**REFERENCES:**

1. United States Environmental Protection Agency, "Residue Chemistry Test Guidelines, OPPTS 860.1340 Residue Analytical Method – Extraction Efficiency", EPA 712-C-96-174, August 1996.

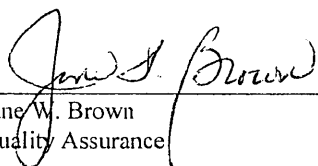
**SIGNATURES:**



Audrey W. Chen, Ph.D.  
Senior Research Associate  
Study Director

3/11/02

Date



Jane W. Brown  
Quality Assurance

3/11/02

Date



Fumio Kimura, Ph.D.  
Board Member & General Manager  
Ishihara Sangyo Kaisha, Ltd.  
Sponsor's Representative

3/15/02

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AGRICULTURAL PRODUCTS GROUP

STUDY PROTOCOL REVISION

Study Number: 178MVL02R1

Revision No. 1

Study Title: Radiovalidation of Residue Methodology for IKI-220 (F1785) and its Major Metabolites in/on Peach, Potato and Wheat Straw Treated with <sup>14</sup>C-labeled IKI-220 Insecticide

Proposed Amendment

Deviation

- Revision:
1. Additional radiolabeled peach and potato samples from the plant metabolism studies were received from Ricerca on 4/25/02.
  2. Radiovalidation was not done for the GC method.
  3. The protocol revision form will not be signed by the Sponsor.

Reason

- for Revision:
1. The same batches of peach and potato samples from the plant metabolism studies were received and analyzed for radiovalidation.
  2. Radiovalidation was done to support the LC method, which was used for all the RAC studies.
  3. The Sponsor's signature on the revision form is not necessary based on the GLPS. The signature is also not needed when the Sponsor is not on-site and the deviation is minor according to the current Environmental Sciences SOPs.

Impact on Study: None

Initiator's

Signature: *audreth*

Date: 8/28/02

APPROVALS

Study Director: *audreth*

Date: 8/28/02

Quality Assurance: *Judith Brown*

Date: 8/28/02