458548-16

STUDY TITLE

VALIDATION OF THE RESIDUE ANALYTICAL METHOD FOR IKI-220 AND METABOLITES (TFNA, TFNG, TFNA-AM, TFNA-OH AND TFNG-AM) IN SURFACE WATER AND DRINKING WATER

NOTE: FLONICAMID (CAS #158062-67-0) is *N*-(cyanomethyl)-4-(trifluoromethyl)-3-pyridinecarboxamide. Other identifiers are IKI-220 and F-1785.

DOCUMENT NO.

834131

DATA REQUIREMENT

Pesticide Assessment Guidelines
161-General

1 PURPOSE

The purpose of the study was to validate a residue analytical method for IKI-220 and its metabolites TFNA, TFNG, TFNA-AM, TFNA-OH, TFNA and TFNG-AM in surface and drinking water.

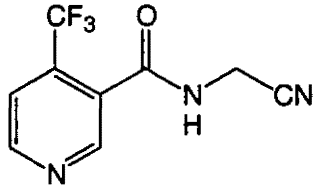
A limit of quantitation (LOQ) of 0.1 µg/L and a working range from 0.1 to 1.0 µg/L were successfully validated for IKI-220 and its metabolites TFNA, TFNG, TFNA-AM, TFNA-OH, TFNA and TFNG-AM.

2 MATERIALS AND METHODS

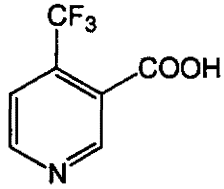
2.1 TEST ITEMS

The test items and the information concerning the test items were provided by the sponsor.
All test items are stable at room temperature but are stored at about -20 °C, except for IKI-220, batch 9803.

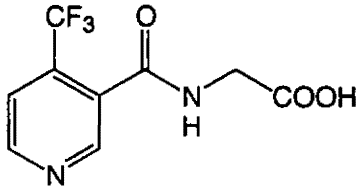
2.1.1 IKI-220

Identity:	IKI-220 PAI
Chemical name [IUPAC]:	*N*-cyanomethyl-4-trifluoromethyl-nicotinamide
Chemical name [CA]:	*N*-(cyanomethyl)-4-(trifluoromethyl)-3-pyridine-carboxamide
CAS registry no.:	158062-67-0
Chemical structure:	
Molecular formula:	$C_9H_6F_3N_3O$
Molecular mass:	229.2
Lot no.:	9803
Purity:	99.7 %
Expiry date:	November 2003
Appearance:	White, odourless, crystalline powder
Storage conditions:	Refrigerate

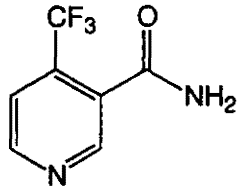
2.1.2 TFNA

Identity:	TFNA
Chemical name:	4-trifluoromethylnicotinic acid
Chemical structure:	
Molecular formula:	$C_7H_4F_3NO_2$
Molecular mass:	191.1
Lot no.:	#0006
Purity:	99.4 %
Expiry date:	January 2006
Storage conditions:	freezer at - 20 °C

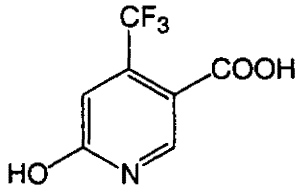
2.1.3 TFNG

Identity:	TFNG
Chemical name [IUPAC]:	*N*-(4-trifluoromethylnicotinoyl)glycine
Chemical structure:	
Molecular formula:	$C_9H_7F_3N_2O_3$
Molecular mass:	248.2
Lot no.:	#0006
Purity:	99.4 %
Expiry date:	January 2006
Storage conditions:	freezer at - 20 °C

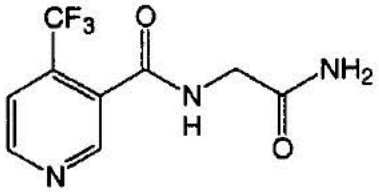
2.1.4 TFNA-AM

Identity:	TFNA-AM
Chemical name:	4-trifluoromethylnicotinamide
Chemical structure:	
Molecular formula:	$C_7H_5F_3N_2O$
Molecular mass:	190.1
Lot no.:	#0006
Purity:	100 %
Expiry date:	January 2006
Storage conditions:	freezer at - 20 °C

2.1.5 TFNA-OH

Identity:	TFNA-OH
Chemical name:	6-hydroxy-4-trifluoromethylnicotinic acid
Chemical structure:	
Molecular formula:	$C_7H_4F_3NO_3$
Molecular mass:	207.1
Lot no.:	#0010
Purity:	100 %
Expiry date:	January 2006
Storage conditions:	freezer at - 20 °C

2.1.6 TFNG-AM

Identity:	TFNG-AM
Chemical name [IUPAC]:	*N*-(4-trifluoromethylnicotinoyl)glycinamide
Chemical structure:	
Molecular formula:	$C_9H_8F_3N_3O_2$
Molecular mass:	247.2
Lot no.:	#0006
Purity:	99.5 %
Expiry date:	January 2006
Storage conditions:	freezer at - 20 °C

2.2 TEST SYSTEM

The test system drinking water was sampled from tab water at RCC on May 14, 2002. The sample was at room temperature with the following physical parameters:

Dry residue:	600 mg/L
silt content:	40 mg/L
pH-value:	7.42
dissolved organic carbon:	2.1 mg/L
Hardness:	23° DH

The test system surface water was sampled at Ergolz in Lausen (Switzerland) on May 13, 2002. The sample was at room temperature with the following physical parameters:

Dry residue:	820 mg/L
silt content:	20 mg/L
pH-value:	8.12
dissolved organic carbon:	2.4 mg/L
Hardness:	26° DH

2.3 REAGENTS AND APPARATUS

All reagents were of analytical, residue analytical or HPLC grade.

REAGENTS & APPARATUS	SUPPLIER	ARTICLE NO.
Acetonitrile	Baker	9017
Ethanol	Baker	8006
Dichlormethane	Baker	9264
Hydrochloric acid (32%)	Baker	6070
Methanol	Baker	8402
Water, distilled	in-house prepared	
Analytical balance	Mettler	PM 4600
Analytical balance	Mettler	UMT 2
Folded filter	S + S	595 1/2
0.45 µm filter	BGB	F 2503-2
Round bottom flasks	1000 mL	
Aquamerck test kit	Merck	1.11104.0001
Shimazu TOC 500		
Isolute ENV+ (500 mg/6mL)	IST	915-0050-C
Ultrasonic bath		
Hamilton syringes	various sizes	
Measuring flasks	various sizes	
Measuring cylinders	various sizes	
Bulb pipettes	various sizes	
Measuring pipettes	various sizes	
Suction flasks	500 mL	
Rotary evaporator	Büchi	011
Vacuum manifold	Supelco	

Solutions:

Hydrochloric acid (about 0.1 mol/L):

1 mL hydrochloric acid (32 %) was dissolved with 1000 mL water.

2.4 STOCK AND STANDARD SOLUTIONS

2.4.1 Stock solution

2.4.1.1 IKI 220

For example: 5.8587 mg of IKI-220 (99.7 %) were dissolved in 5.8411 mL acetonitrile to obtain a stock solution of 1000 µg/mL IKI-220 (100 %) using an ultrasonic bath for about 5 min.
The solution was stored in a freezer at about -20 °C.

Defined volumes of this stock solution were diluted using acetonitrile to obtain further stock solutions of 100, 10 and 1 µg/mL of IKI-220.
These solutions were stored in a refrigerator at about 4 °C.

2.4.1.2 TFNA

For example: 5.7642 mg of TFNA (99.4 %) were dissolved in 5.730 mL acetonitrile to obtain a stock solution of 1000 µg/mL TFNA (100 %) using an ultrasonic bath for about 5 min.
The solution was stored in a freezer at about -20 °C.

Defined volumes of this stock solution were diluted using acetonitrile to obtain further stock solutions of 100, 10 and 1 µg/mL of TFNA.
These solutions were stored in a refrigerator at about 4 °C.

2.4.1.3 TFNG

For example: 5.2425 mg of TFNG (99.4 %) were dissolved in 5.211 mL acetonitrile to obtain a stock solution of 1000 µg/mL TFNG (100 %) using an ultrasonic bath for about 5 min.
The solution was stored in a freezer at about -20 °C.

Defined volumes of this stock solution were diluted using acetonitrile to obtain further stock solutions of 100, 10 and 1 µg/mL of TFNG.
These solutions were stored in a refrigerator at about 4 °C.

2.4.1.4 TFNA-AM

For example: 6.9651 mg of TFNA-AM (100 %) were dissolved in 6.965 mL acetonitrile to obtain a stock solution of 1000 µg/mL TFNA-AM using an ultrasonic bath for about 5 min.
The solution was stored in a freezer at about -20 °C.

Defined volumes of this stock solution were diluted using acetonitrile to obtain further stock solutions of 100, 10 and 1 µg/mL of TFNA-AM.
These solutions were stored in a refrigerator at about 4 °C.

2.4.1.5 TFNA-OH

For example: 5.7000 mg of TFNA-OH (100 %) were dissolved in 5.700 mL methanol to obtain a stock solution of 1000 µg/mL TFNA-OH using an ultrasonic bath for about 5 min.
The solution was stored in a freezer at about -20 °C.

Defined volumes of this stock solution were diluted using acetonitrile to obtain further stock solutions of 100, 10 and 1 µg/mL of TFNA-OH.
These solutions were stored in a refrigerator at about 4 °C.

2.4.1.6 TFNG-AM

For example: 6.6730 mg of TFNG-AM (99.5 %) were dissolved in 6.640 mL acetonitrile to obtain a stock solution of 1000 µg/mL TFNG-AM using an ultrasonic bath for about 5 min.
The solution was stored in a freezer at about -20 °C.

Defined volumes of this stock solution were diluted using acetonitrile to obtain further stock solutions of 100, 10 and 1 µg/mL of TFNG-AM.
These solutions were stored in a refrigerator at about 4 °C.

2.4.1.7 Mixed stock solution of IKI-220 and its metabolites

Defined volumes of these stock solutions of each compound with the same stock concentration were combined and diluted using acetonitril to obtain mixed standard solutions in the range of 100, 10 and 1 µg/mL containing IKI-220 and its metabolites.

These solutions were stored in a refrigerator at about 4 °C.

2.4.2 Fortification solutions

The 10 and 1 µg/mL mixed stock solutions are used for fortification.

2.4.3 Standard solutions

Defined volumes of the mixed 10 µg/mL stock solution were diluted using control water to obtain mixed standard solutions in the range of 1.0, 0.5 and 0.2 µg/mL containing IKI-220 and its metabolites.

Defined volumes of the mixed 1.0 µg/mL stock solution were diluted using control water to obtain mixed standard solutions in the range of 0.1, 0.05, 0.02 and 0.01 µg/mL containing IKI-220 and its metabolites.

These solutions were stored in a refrigerator at about 4 °C.

2.5 FORTIFICATION

To demonstrate the validity of the method, drinking and surface water samples were fortified with different concentrations using fortification solutions containing IKI-220 and its metabolites.

Fortification levels:

0.1 µg/L: 100 µL of the fortification solution 1.0 µg/mL IKI-220 and its metabolites were added to 1000 mL of drinking or surface water.

1.0 µg/L : 100 µL of the fortification solution 10.0 µg/mL IKI-220 and its metabolites were added to 1000 mL of drinking or surface water.

2.6 ANALYTICAL METHOD

The analytical method was developed and validated during the course of this study.

An ENV+ column was conditioned by passing 5 mL dichlormethane, 5 mL methanol, 5 mL water (distilled) and 5 mL hydrochloric acid (about 0.1 mol/L) one after another using a vacuum manifold.
The ENV+ column was filled with 5 mL hydrochloric acid (32 %) and a 70 mL reservoir was installed.
1000 mL water sample were transferred into a 1000 mL laboratory flask. Samples were fortified at this stage and 2 mL of hydrochloric acid (about 0.1 mol/L) was added. After shaking, the sample was sucked through the ENV+ column at a flow rate of < 10 mL/minute. Then the column was sucked to dryness for about 10 sec.

Thereafter, the ENV+ column was filled with 5 mL of methanol and let drip without vacuum. Then the methanol was sucked with a few vaccum. The same process was repeated twice. The methanol solutions were combined in a 100 mL round-bottomed flask and evaporated to dryness under vacuum at about 50-60 °C using a rotary evaporator. The residue was dissolved in 10 mL of ethanol and evaporated again to dryness. The residue was dissolved in 2.0 mL of water/acetonitril (9+1 v/v) using an ultrasonic bath.

The concentrations of IKI-220 and its metabolites were determined after LC separation using a MS/MS Detector.

2.7 HPLC CONDITIONS

Auto Sampler: Merck-L-7200

Pump: Merck L-7100

Column: Phenomenex Luna C8, 3 μm (150 mm x 3.0 mm)

Pre Column: Phenomenex Security Guard C8 (4 mm x 3.0 mm)

Column Temp.: 40 °C

Solvent Systems: Solvent A: 0.05 % formic acid in water
Solvent B: methanol

Time (min)	0	2	4	12	15	20	20.1	28
% solvent A	90	90	78	30	10	10	90	90
% solvent B	10	10	22	70	90	90	10	10
Flow (mL/min)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Injection Volume: 40 μL

Washing Solution: Water/methanol (90/10, v/v)

Detector: TSQ, *Xcalibur* 1.2 for Windows NT, Finnigan MAT

Time (min)	0	4	4	15	15	28
Divert Valve switched to:	Waste	Waste	MS	MS	Waste	Waste

Ionization Mode: APCI; Positive; Centroid

MS Conditions:

Vaporizer Temp:	500 °C
Capillary Temp:	200 °C
Sheath:	80 psi N_2
Capillary Voltage:	62.1 V
Discharge Current:	4.0 μA
Spray Voltage:	approx. 4.0 kV

Scan Mode: SRM

	Mass exact	Parent Mass	Centre Mass	Width	Scan Time (sec)
1 IKI-220	229.0	229.8	203.1	0.6	0.5
2 TFNA	191.1	192.1	147.9	0.6	0.5
3 TFNA-AM	190.0	191.2	148.1	0.6	0.5
4 TFNA- OH	207.0	208.1	145.9	0.6	0.5
5 TFNG-AM	190.0	248.0	174.0	0.6	0.5
6 TFNG	248.0	249.0	202.9	0.6	0.5

Retention Time:
About 9.5 min for IKI-220
About 11.6 min for TFNA
About 7.5 min for TFNA-AM
About 10.0 min for TFNA- OH
About 9.3 min for TFNG
About 7.1 min for TFNG-AM

2.8 EVALUATION OF RESULTS

Concentration of Residues

Injected samples were quantified by peak area with reference to the calibration curve. The latter was obtained by correlation of the peak area (in counts) of the analytical standards with their corresponding concentration in µg/mL.
The correlation is performed using a least squares fit of a non-linear function (equation 1).

$$y = a \cdot x^b \qquad \text{or} \qquad x = \left(\frac{y}{a}\right)^{1/b} \tag{1}$$

where

y = Peak area of injected sample [counts]

x = IKI-220 or metabolite in injected sample [µg/mL]

a = Constant factor

b = Exponent

The residue of IKI-220 or metabolite in the samples is calculated according to equation 2.

$$R = \frac{x \cdot V_F \cdot d}{V_{sample}} \tag{2}$$

where

R = Residue of IKI-220 or metabolite in sample material [mg/L]

x = Concentration of injected sample [µg/mL] calculated from equation 1

V_F = Final volume [mL]

d = Dilution factor (here: 1)

V_{sample} = Volume of sample [mL]

The recovery of IKI-220 or metabolite in the samples is calculated according to equation 3.

$$\text{Rec} = \frac{R \cdot 100\%}{F} \tag{3}$$

where

Rec = Recovery of IKI-220 or metabolite [%]

R = Residue of IKI-220 or metabolite in sample material [mg/L]

F = Fortification level [mg/L]

3 RESULTS

The detailed results of the determination of IKI-220 and its metabolites TFNA, TFNA-AM, OH-TFNA-AM, TFNG and TFNA are summarized in Tables 1 - 12.

The tabulated values represent rounded results obtained by calculation using the exact raw data.

The limit of quantification was found to be 0.1 µg/L deriving from the lowest fortification level.

A non-linear function was chosen for calculation of the calibration curve as the relative deviation between $counts_{measured}$ and $counts_{calculated}$ was found to be higher than 20 % for a linear function due to the large range (0.01 mg/L – 1.0 mg/L) of the calibration curve.

3.3 SPECIFICITY

The method allows the determination of IKI-220 and its metabolites TFNA, TFNG, TFNA-AM, TFNA- OH and TFNG-AM in drinking and surface water. There was no interference with other substances observed at the retention times of IKI-220 and its metabolites above 30 % of the limit of quantification as well as above the limit of detection (see Figure 3 and 6).

A further proof of specificity (confirmatory method) is not required as the performed MS-MS (TSQ) is the most highly specific method for detection of residues in this low concentration range

3.4 LIMIT OF DETECTION (LOD)

The limit of detection was found to be about 0.02 µg/L. The LOD was estimated from the lowest calibration standard concentration used (0.01 µg/mL).

3.5 ANALYTICAL SAMPLE STORAGE PERIOD

The maximum time between starting the extraction of surface and drinking water samples and measurement was three days. Extracts were stored in a refrigerator (at about 4 °C) before HPLC/MS-MS measurement. The recovery results show that there is no impact due to this storage period.

APPENDIX I

COPY OF THE STUDY PLAN

1 PURPOSE

The purpose of the study is to validate the residue analytical method for IKI-220 and metabolites (TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM) in surface water and drinking water as indicated in the experimental section of this study plan.

A limit of quantitation (LOQ) of 0.1 µg/L and a working range from 0.1 µg/L to 1.0 µg/L will be validated.

2 MATERIALS AND METHODS

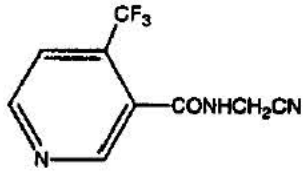
2.1 TEST ITEMS

All data are provided by the Sponsor, unless stated otherwise, and will be included in the final report.
All test items are stable at room temperature but are stored at about -20 °C, except for IKI-220, batch 9803.

2.1.1 IKI-220

Chemical name: *N*-cyanomethyl-4-trifluoromethylnicotinamide

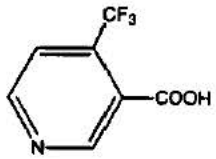
Structural formula:



Lot number:	9803 (RCC no. 115407/A)
Chemical purity:	99.7 %
Storage conditions:	Store in a refrigerator (5 °C)
Expiration date:	November 2003

2.1.2 TFNA

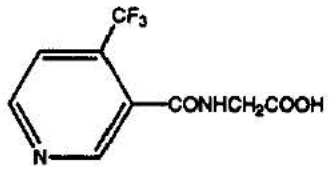
Structural formula:



Chemical name:	4-trifluoromethylnicotinic acid
Storage conditions:	At about – 20°C
Lot number:	#0006 (RCC no. 115893/A)
Chemical purity:	99.4 %
Expiration date:	January 2006

2.1.3 TFNG

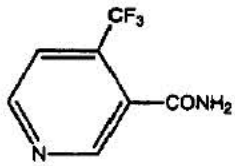
Structural formula:



Chemical name:	*N*-(4-trifluoromethylnicotinoyl)glycine
Lot number:	#0006 (RCC no. 115893/B)
Chemical purity:	99.4 %
Storage conditions:	At about -20 °C
Expiration date:	January 2006
Safety precautions:	Routine hygienic procedures are sufficient to ensure personnel health and safety.

2.1.4 TFNA-AM

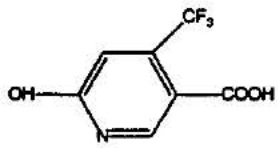
Structural formula:



Chemical name:	4-trifluoromethylnicotinamide
Storage conditions:	At about – 20°C
Lot number:	#0006 (RCC no. 115893/D)
Chemical purity:	100 %
Expiration date:	January 2006

2.1.5 TFNA-OH

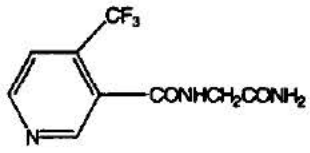
Structural formula:



Chemical name:	6-hydroxy-4-trifluoromethylnicotinic acid
Storage conditions:	At about – 20°C
Lot number:	#0010
Chemical purity:	100 %
Expiration date:	January 2006

2.1.6 TFNG-AM

Structural formula:



Chemical name:	*N*-(4-trifluoromethylnicotinoyl)glycinamide
Storage conditions:	At about – 20°C
Lot number:	#0006
Chemical purity:	99.5 %
Expiration date:	January 2006

2.2 TEST SYSTEM

The test system surface water was selected by the sponsor in accordance with the quoted guidelines. It will sampled from a typical, rural area.
The origin, date of sampling, the pH-value, the dissolved organic carbon content (DOC), hardness and the silt content will be determined and described in the final report to characterize the test system.

The test system drinking water will be sampled from tab water.
The origin, date of sampling, the pH-value, the dissolved organic carbon content (DOC), hardness and the silt content will be determined and described in the final report to characterize the test system.

2.3 REAGENTS

All reagents will be of analytical or residue analytical grade. Details will be given in the final report.

2.4 ANALYTICAL METHOD

The analytical method to be validated was developed during the course of this study. Details will be given in the final report.

2.5 CALIBRATION PROCEDURE

The instrument calibration standards for this study will be prepared by making appropriate dilutions of stock standard solutions of IKI-220 and its TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM. The standard solutions should bracket the working range. The lowest standard of the working range should be at least half the concentration of the lowest sample concentration expected.

Calibration standards will be injected in single concurrently with the sample injections for the determination of the retention time and of the standard calibration curve. Each analytical run will begin and end with a standard injection. A maximum of two injections will be made between standard injections.

2.6 EXPERIMENTAL DESIGN

The following procedure will be undertaken for each matrices
Known amounts of fortification solutions of IKI-220 and its TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM will be added to untreated surface water to achieve the following fortification levels:

0	µg/L	Control
0.1	µg/L	Limit of Quantitation (LOQ)
1	µg/L	10 times of LOQ (upper working range)

At least two control samples and five samples of each fortification level will be analyzed during this study.

Quantification will be performed using e.g. HPLC/MS/MS.
Peak identification will not be performed as it is not required for HPLC/MS/MS, as this technique is agreed to be highly specific.

The percent recovery of IKI-220 and its metabolites its TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM will be determined as follows:

$$\% \text{ Recovery} = \left[\frac{\text{Found } (\mu g/L)}{\text{Fortification Amount } (\mu g/L)} \right] * 100\ (\%)$$

The results will be interpreted as follows:

The mean recovery should be within 70 and 110% of the nominal concentration. Its relative standard deviation should be ≤ 20%.
Control values at the retention time of IKI-220 and its TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM should be less than 30 % of the LOQ.

2.7 EVALUATION OF RESULTS

The quantity of the residues in each sample shall be determined by comparison to a calibration curve consisting at least 5 points (for single injections) or 4 points (for duplicate injections), one of these shall be at the quantification limit of the method. The calibration curves should bracket the working range. If the concentration of a sample is beyond the range of the calibration curve it will be diluted appropriately and re-run.

Calibration curves will be generated along with every sample series. Peak heights or peak areas are plotted versus µg/mL test item. The concentration of IKI-220 and its metabolites TFNA, TFNG, TFNA-AM, TFNA-OH and TFNG-AM in surface water samples is then determined from the standard curve.

2.8 STATISTICAL METHODS

The overall average and standard deviation found for the fortified samples will be calculated and included in the final report.

2.9 SAMPLE IDENTIFICATION

All samples and/or vessels containing sample aliquots will be labelled individually with RCC study number and further information regarding the test item and/or sample to assure an unmistakable identification.