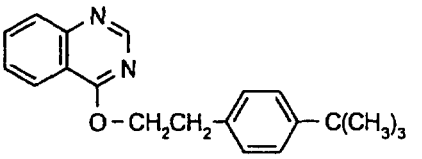
3 MATERIALS AND METHODS

3.1 TEST ITEM

The test item and the information concerning the test item were provided by the Sponsor or were taken from the e-Pesticide Manual (12th Ed.) Ver. 2.0.

3.1.1 FENAZAQUIN

Chemical Name (IUPAC):	4-tert-butylphenethyl quinazolin-4-yl ether
Structural Formula:	
CAS Reg. No.:	*[120928-09-8]*
Molecular Formula:	$C_{20}H_{22}N_2O$
Molecular Weight:	306.4 g/mol
Appearance:	Light Beige Solid
Batch No.:	ACPR 334-106
Purity:	98.1 %
Storage:	Deep freezer
Expiration Date:	March, 2007
Date of receipt:	June 27, 2003
Quantity received:	1 g
Safety Precautions:	Routine hygienic procedures will be sufficient to assure personnel health and safety.

3.2 TEST SYSTEM

3.2.1 DRINKING WATER

The drinking water (regular tab water) was collected from the village Itingen, Switzerland. It was specified as follows.

Type	Drinking Water
Source	RCC Ltd Zelgliweg 1 CH-4452 Itingen/Switzerland
Date of sampling	October 23, 2003
Dissolved organic carbon (DOC) [mg C/L]	2.12
pH	7.77
Residue of evaporation [mg/L]	1050
Silt content [mg/L]	30
Hardness [°dH]	23

During the laboratory phase the untreated control sample was stored at ambient conditions at RCC Ltd.

3.2.2 GROUND WATER

The ground water was collected from a typical rural area in Switzerland. It was specified as follows.

Type	Ground Water
Source	Public fountain Dorfstr., CH-4452 Itingen/Switzerland
Date of sampling	October 24, 2003
Dissolved organic carbon (DOC) [mg C/L]	3.84
pH	7.39
Residue of evaporation [mg/L]	250
Silt content [mg/L]	20
Hardness [°dH]	15

The region the sample was collected is a typically rural area mainly used for farming (animal breeding) with only little industry.

During the laboratory phase the untreated control sample was stored at ambient conditions at RCC Ltd.

3.2.3 SURFACE WATER

The surface water was collected from a typical rural area in Switzerland. It was specified as follows.

Type	Surface Water
Source	Creek "Ergolz" Itingen/Switzerland
Date of sampling	October 24, 2003
Dissolved organic carbon (DOC) [mg C/L]	3.79
pH	7.95
Residue of evaporation [mg/L]	370
Silt content [mg/L]	110
Hardness [°dH]	15

The region the sample was collected is a typically rural area mainly used for farming (animal breeding) with only little industry.

During the laboratory phase the untreated control sample was stored at ambient conditions at RCC Ltd.

3.3 REAGENTS AND APPARATUS

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

REAGENTS & APPARATUS	SUPPLIER	ARTICLE NO.
Dichloromethane min. 99.8%	Baker	9264
Acetone min. 99.4%	Baker	9254
Hexane min. 95% n-hexane	Baker	9262
Ethyl acetate min. 99.6%	Baker	9260
Sodium sulphate	Baker	0313
Bakerbond Amino SPE (500 mg/3 mL)	Baker	7088-03
Glass bottles	various sizes	
Volumetric pipettes	various sizes	
Measuring pipettes	various sizes	
Measuring cylinders	various sizes	
Measuring flasks	various sizes	
Separatory funnel	1200 mL	
Funnels		
Test tubes	ça 12 mL	
Round-bottomed flasks	1000/500/25	
SPE 3.6.1Manifold	Baker	
Hamilton syringes	various sizes	
Rotary evaporator	Büchi	011
Ultra sonic bath	Bender + Hobein 220	
Analytical balance	Sartorius L2200P	111606
Analytical balance	Mettler UMT2	
Folded filter*	S&S	595 1/2
0.45 µm PTFE filter*	BGB	F 2504-2
Aquamerck test kit*	Merck	1.11104.0001
TOC 5000A*	Shimazu	
pH-meter*	WTW	InoLab pH Level 1

* Reagents and Apparatus for the characterising of the test system

Mixed Solution:

Hexane/acetone (99+1, v/v):
99 mL hexane + 1 mL acetone were mixed

3.4 STANDARD SOLUTIONS

3.4.1 STOCK SOLUTION

For measurement: 6.9497 mg of Fenazaquin (98.1%) were dissolved in 6.820 mL acetone to obtain a stock solution of 1000 µg Fenazaquin/mL (100 %).

The stock solution 1000 µg of Fenazaquin/mL was kept at about -20 °C (in a freezer).

3.4.2 FORTIFICATION SOLUTIONS

Defined volumes of the stock solution (see section 3.4.1) were diluted using acetone to obtain further stock solutions with a concentration of 100 µg, 10 µg and 1 µg of Fenazaquin/mL.

This stock solutions were kept at about 4 °C (in a refrigerator).

3.4.3 CALIBRATION SOLUTIONS

Defined volumes of the fortification solutions 100 µg and 10 µg Fenazaquin/mL (see section 3.4.2) were diluted using ethyl acetate to obtain calibration solutions with a concentration of 1.0 µg, 0.5 µg, 0.2 µg, 0.1 µg, 0.05 µg, 0.02 µg and 0.01 µg Fenazaquin/mL.

All solutions were kept at about 4 °C (in a refrigerator).

Quantification was performed using a GC/NPD.

Fenazaquin calibration standards were injected concurrently with the Fenazaquin sample injections for the determination of the retention time and for preparing of the standard calibration curve. Each analytical run was started and ended with a Fenazaquin calibration standard injection. A maximum of two sample injections were made between Fenazaquin calibration standard injections.

3.5 FORTIFICATION

To demonstrate the validity of the method used untreated samples were fortified with different amounts of Fenazaquin.

Fortification levels:

0.05 µg/L: 500 µL of the fortification solution (0.1 µg/mL) were added to 1000 mL of the untreated water sample.

0.50 µg/L: 500 µL of the fortification solution (1 µg/mL) were added to 1000 mL of the untreated water sample.

3.6 ANALYTICAL METHOD

3.6.1 EXTRACTION

1000 mL water sample were transferred into a 1200 mL separatory funnel. Samples were fortified at this stage. Thereafter 100 mL of hexane was added and the sample was shaken for about 1-2 minutes. After phase separation, the lower water was drained into a glass bottle. The hexane phase was filtered through about 20-30 g sodium sulfate into a 500 mL round-bottomed flask. This extraction was repeated twice with 100 mL of hexane and the hexane phase was filtered through the sodium sulfate into the 500 mL round-bottomed flask. The sodium sulphate was washed with 20-30 mL of dichloromethane. The solvent was evaporated to about 2 mL under vacuum at about 30-40 °C using a rotary evaporator. The remaining solvent was blew up at room temperature using a gentle stream of nitrogen. The residue was dissolved in 5 mL of hexane using an ultrasonic bath.

3.6.2 CLEAN-UP

A 500 mg (3 mL) Amino SPE column was attached to a SPE Manifold. The sample (from section 3.6.1) was transferred onto the column with a Pasteur pipette. The column was not allowed to get dry. The round-bottomed flask was rinsed using hexane (5 mL) and the rinsing was transferred onto the column. The column was not allowed to get dry. All eluates obtained so far were discarded.

3.6.3 ELUTION

The flask was rinsed twice with 5 mL of a mixture of hexane/acetone (99+1, v/v). This rinsings were transferred onto the column. The eluates were collected and combined using a 12-mL test tube. The column was not allowed to get dry. The sample was transferred into a 25-mL round-bottomed flask using 5 mL of hexane and evaporated to about 1 mL under reduced pressure at about 30-40 °C. The remaining solvent was blew up using a gentle stream of nitrogen (at room temperature). The residue was dissolved in 1 mL of ethyl acetate using an ultrasonic bath.
The concentrations of Fenazaquin were determined after GC separation using a NPD. Peak confirmatory was performed using GC-MSD.

3.7 GC/NPD CONDITIONS

3.7.1 PRIMARY METHOD

Gas chromatograph:	Agilent 6890
Auto sampler:	Agilent 7683
Data Handling:	Agilent Chemstation +Ver G2071AA
Column:	30 m x 0.25 mm RTX-5 (0.25 µm) (Restek) pre-column: deactivated fused silica (2 m x 0.32 mm) (J & W Scientific)
Carrier Gas:	Helium (column flow: 1.1 mL/min, constant flow)
Temperatures:	Injector: 250 °C Oven: 70 °C (initial time: 1 min rate: 12.5 °C/min to 325 °C for 5 min)
Detector:	325 °C
Injection:	2 µL; Splitless
Detector:	Nitrogene Phosphoric Detector (NPD)
Retention Time:	about 14.7-15 min

3.7.2 CONFIRMATORY METHOD (GC/MS)

Gas chromatograph:	Agilent 6890		
Auto sampler:	Agilent 7683		
Data Handling:	Agilent Chemstation +Ver G2071AA		
Injection:	2 µL; Splitless		
Column:	30 m x 0.25 mm HP5 MS (0.25 µm) (Agilent)		
Carrier Gas:	Helium (column flow: 1mL/min, constant flow)		
Temperatures:	Injector:	275°C	
	Oven:	70 °C	(initial time: 1 min rate: 12.5 °C/min to 325 °C for 5 min)
MS Condition:			
Ionization Mode:	EI		
Ion Source Temp.:	230°C		
Transfer Line Temp:	300°C		
Quad Temp.:	150°C		
Scan Mode:	SIM m/z 145 m/z 160		
Solvent Delay:	5 minutes		
MS off:	-		
Scan Time:	4.26 minutes		
Retention Time:	approx. 15.3 min		

3.8 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 (mean value in counts from duplicate injection) 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).
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 (LOD up to 100 times LOD) of the calibration curve.

$$Y = a \cdot x^b \quad \text{or} \quad x = (Y/a)^{1/b} \qquad (1)$$

where

Y = Peak area of injected sample (mean value in counts from duplicate injection)

x = Fenazaquin in injected sample [µg/mL]

a = Constant factor

b = Exponent

The residue of Fenazaquin in the samples is calculated according to equation 2.

$$R = \frac{X \cdot V_F}{W} \qquad (2)$$

where

R = Residue of Fenazaquin in sample material [µg/L]

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

V_F = Final volume [mL]

W = Sample volume [L]

The recovery of Fenazaquin in the samples is calculated according to equation 3.

$$Rec = \frac{R \cdot 100}{F} \qquad (3)$$

where

Rec = Recovery of Fenazaquin [%]

R = Residue of Fenazaquin in sample material [µg/L]

F = Fortification level [µg/L]

Example for calculation

RCC sample 27 (mean value from duplicate injection: 0.799 area counts)
Recovery = (0.799/22.247) $^{(1\ /\ 1.1036)}$ x 1 (final volume) / 1 (water volume) x 100 (%) / 0.05 (fortification level) = 98.3 % (the result is calculated with rounded numbers).