FENAZAQUIN (297)

The first draft was prepared by Ms M Thomas, Pest Management Regulatory Agency, Canada

EXPLANATION

Fenazaquin is a quinazoline insecticide/acaricide which exhibits contact and ovicidal activity against a broad spectrum of mites in grapes, pome fruit, citrus, peaches, cucurbits, tomatoes, cotton and ornamentals. At the 48th Session of the CCPR (2016), it was scheduled for evaluation as a new compound by 2017 JMPR.

The Meeting received information on the metabolism of fenazaquin in apples, oranges, grapes and maize, lactating goats and laying hens, methods of residue analysis, freezer storage stability, GAP information, supervised field trials on stone fruits, pineapples, tree nuts (almonds and pecans), hops and processing studies.

IDENTITY

ISO common name: Fenazaquin

Chemical name:

IUPAC: 4-*tert*-butylphenethyl quinazolin-4-yl ether

CAS: 4-[2-[4-(1,1-dimethylethyl)phenyl]ethoxy]quinazoline

CAS Registry. No.: 120928-09-8

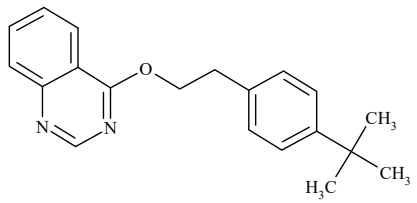
CIPAC No.: 693

Code Name: XDE 436

Molecular formula: $C_{20}H_{22}N_2O$

Molecular weight: 306.4 g/mol

Structural formula:



Specifications

Specifications for fenazaquin have not been developed by the FAO.

PHYSICAL AND CHEMICAL PROPERTIES

Table 1 Physical chemical properties of the technical substance (99.2%)

Parameters	Value	Reference
Appearance	White to tan, crystalline solid	Brian 1995
Melting point	77.5-80.0 °C	Niemtus 1992
Relative Density	1.16	Niemtus 1992
Vapour pressure (25 °C)	$< 6 \times 10^{-6}$ Pa	Lopez 2007
Henry's Law Constant (25 °C)	2.65×10^{-2} Pa m^3 mol^{-1}	Briant 1994

Parameters	Value		Reference
Solubility in water	pH	Solubility (μg/L)	Niemtus 1992
	5	102	
	7	102	
	9	135	
Solubility in organic solvents (room temperature)	Solvent	Solubility g/100 mL	Handy 1992
	Acetonitrile	3.3-5.0	
	Acetone	40-50	
	n-Butyl chloride	>50	
	Chloroform	>50	
	Dimethyl formamide	30-40	
	Ethyl acetate	40-50	
	Ethylene glycol	<0.5	
	Hexane	3.3-5.0	
	Isopropanol	5-10	
	Methanol	5-10	
	Methylene chloride	>60	
	N-methyl-2-pyrolidone	>50	
	Toluene	>50	
Partition coefficient n-octanol/water (25 ºC)	log Kow = 5.71		Saunders 1989
Dissociation constant (pKa at 22 ºC)	2.44		Boothroyd 1993
Hydrolysis	Fenazaquin degrades rapidly to 4-hydroxyquinazoline and TBPE in aqueous solutions under acid conditions whereas the degradation is slower at pH 7 and pH 9.		Saunders 1990, 1991
Photolysis	Photolysis is of importance in its degradation in the environment.		Saunders 1991, Knowles 1999, Knowles 1999, Diehl 2003

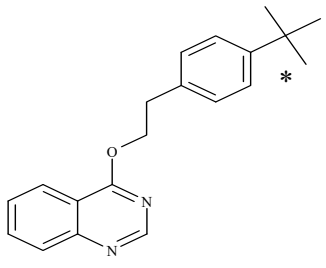
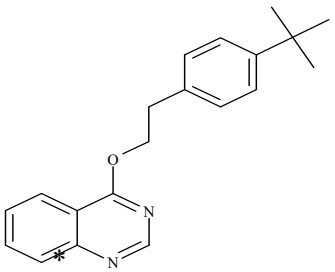
Formulations

Fenazaquin is available in emulsifiable concentrate (EC) and suspension concentrate (SC) formulations.

Formulations	Active ingredient content
EC	100 g/L
SC	200 g/L

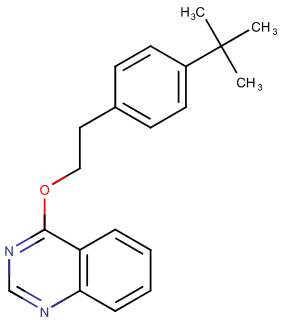
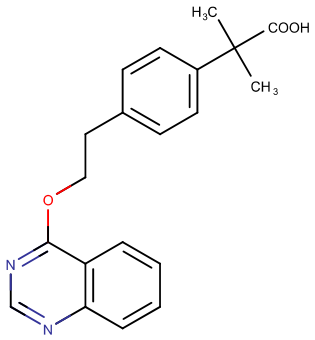
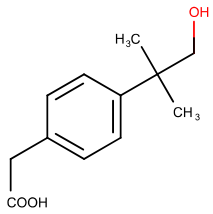
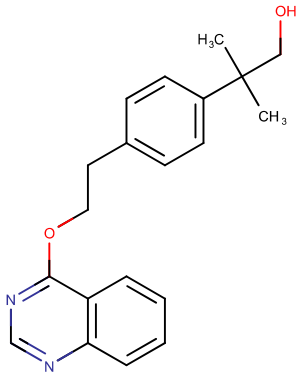
METABOLISM AND ENVIRONMENTAL FATE

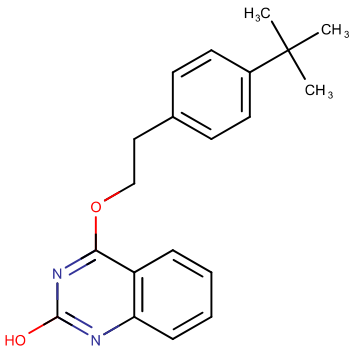
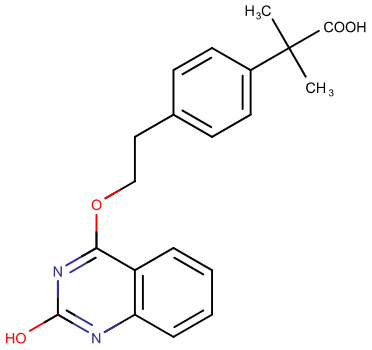
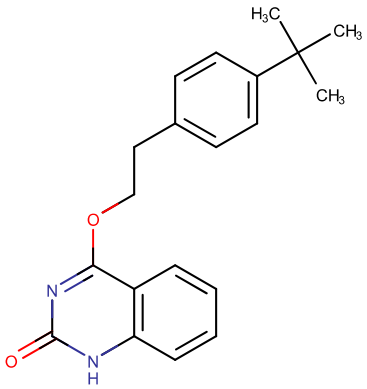
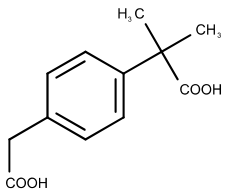
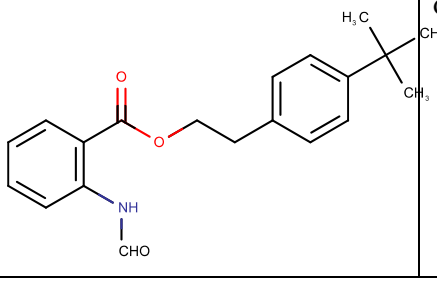
The metabolism and distribution of fenazaquin in plants and animals was investigated using ^{14}C-labelled test material as shown below:

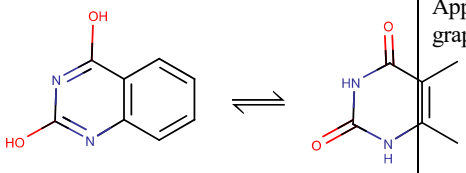
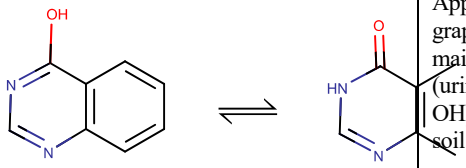
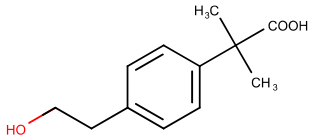
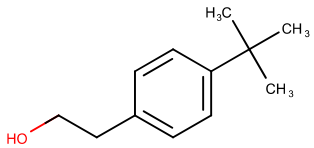
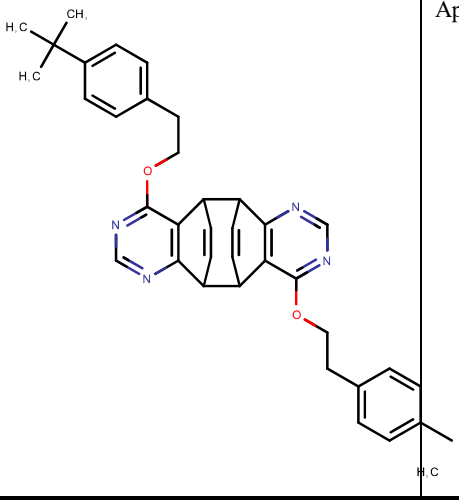
tert-butyl phenyl (P-label)	phenyl ring of quinazoline (Q-label)
	
* indicates position of radiolabel	

Chemical names, structures and code names of metabolites and degradation products of fenazaquin are summarized in Table 2.

Table 2 Common names, chemical names and structures of fenazaquin related substances

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
Fenazaquin	4-[2-(4-tert-Butyl-phenyl)-ethoxy]-quinazoline		Apples, oranges, grapes, maize, rat, goats, hens, soil
Fenazaquin acid	2-methyl-2-{4-[2-(quinazolin-4-yloxy)ethyl]phenyl}propanoic acid		Maize, grapes, rat (faeces, F-2), hen, soil
Metabolite B	2-[4-(1-hydroxy-2-methylpropan-2-yl)phenyl]acetic acid		Rat (urine, AN-1)
Metabolite C	2-Methyl-2-{4-[2-(quinazolin-4-yloxy)-ethyl]-phenyl}-propan-1-ol		Apples, grapes, rat (faeces, F-1)

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
2-Hydroxy-fenazaquin	4-[2-(4-tert-butylphenyl)ethoxy]quinazolin-2-ol/ Exists in tautomeric equilibrium with 2-oxy-fenazaquin		Maize, goats, soil
2-Hydroxy-fenazaquin acid	2-(4-{2-[(2-hydroxyquinazolin-4-yl)oxy]ethyl}phenyl)-2-methylpropanoic acid		Apples, oranges, goats, rats (faeces, F-3)
2-Oxy-fenazaquin	4-[2-(4-tert-butylphenyl)ethoxy]-1,2-Dihydroquinazolin-2-one Exists in tautomeric equilibrium with 2-hydroxy-fenazaquin		Maize, soil
Metabolite G	2-[4-(carboxymethyl)phenyl]-2-methylpropanoic acid		Goats, soil
Metabolite H	2-(4-tert-butylphenyl)ethyl 2-formamidobenzoate		Grapes, soil

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
Dihydroxyquinazoline	Equilibrium exists between the two tautomeric forms: quinazoline-2,4-diol and 1,2,3,4-tetrahydroquinazoline-2,4-dione		Apples, grapes
4-Hydroxyquinazoline	Equilibrium exists between the two tautomeric forms: quinazoline-4-ol and 3,4-dihydroquinazolin-4-one		Apples, grapes, maize, rats (urine, 4-OH), goats, soil
Metabolite K	2-[4-(2-hydroxyethyl)phenyl]-2-methylpropanoic acid		Grapes
Tertiarybutylphenylethanol (TBPE)	2-(4-tert-butylphenyl)ethan-1-ol		Apples, grapes, maize, soil
Fenazaquin Dimer	7,15-bis[2-(4-tert-butylphenyl)ethoxy]-4,6,12,14-tetraazapentacyclo[8.6.2.2^{2,9}.0^{3,8}.0^{11,16}]icosa-3(8),4,6,11(16),12,14,17,19-octaene		Apples

Plant metabolism

The Meeting received information on the fate of fenazaquin labelled in the butyl phenyl ring and the quinazoline ring following foliar application to apples, oranges, grapes and maize.

Apples

Study 1

Four semi-dwarf *Golden Delicious* apple trees, grown outdoors, were treated with a single foliar application of ^{14}C-fenazaquin at a rate of 0.45 kg/ha (Magnussen, 1992, 611-05 and Denis, 1998, ABC 0455-SUPPL). Two trees were sprayed in late June when the apples were 2–3 cm in diameter (early season application), while the remaining two trees were sprayed approximately 4–5 weeks prior to harvest when the apples were 6–7 cm in size (late season application). At each application time point, one tree was sprayed with ^{14}C-fenazaquin labelled in the phenyl ring of the tert-butyl phenyl portion of the molecule (P-Label; specific activity: 26.6 µCi/mg), and the other tree with ^{14}C-fenazaquin

uniformly labelled in the phenyl portion of the quinazoline ring (Q-Label; specific activity: 19.8 μCi/mg).

In order to study the effect of photolysis on the decline of the fenazaquin residues, six apples on the tree receiving the late season application of the P-label were covered shortly after application with bags made of a white muslin cloth.

Apples from trees receiving the early season application were sampled at 0, 4, 7, 14, 29, 57, and 92 days after treatment (DAT), while apples from trees receiving the late season application were sampled at 0, 7, 14, 28, and 42 DAT. The wrapped apples from the photolysis study were sampled at 7 and 14 DAT.

Apples collected at harvest (42- and 92-DAT samples) were peeled and the peel and pulp fractions assayed for total radioactivity by combustion. Radioactivity in the surface washes and in all other liquid fractions was determined by direct liquid scintillation counting (LSC).

The ^{14}C levels in the peel, pulp, and whole fruit of apples from the late season application were approximately 3 times greater than the levels in the corresponding fractions of apples receiving the early season application. The radioactivity in peel was consistently higher than that in pulp, demonstrating limited penetration of the radioactivity.

Table 3 Total Radioactive Residues (mg eq/kg) in the peel, pulp and whole fruits at harvest

Fraction	Early Season Application (92-DAT)		Late Season Application (42-DAT)	
	P-Label	Q-Label	P-Label	Q-Label
Peel	0.653	0.802	1.919	2.473
Pulp	0.026	0.029	0.050	0.063
Whole fruit	0.136	0.161	0.367	0.489

Fruits were sequentially washed with hexane, chloroform and methanol. Following the surface washes, the apples were peeled and the peels were frozen and ground using a mortar and pestle while the pulp was blended.

Peel samples were initially extracted using dichloromethane whereby the extracted peel was subsequently refluxed for one hour with acetonitrile:water (75:25, v:v). The acetonitrile was removed using a rotary vacuum evaporator and the remaining aqueous phase was sequentially partitioned twice with dichloromethane at pH 7 and twice with ethyl acetate at pH 2. The aqueous phase was subjected to enzymatic hydrolysis using β-glucosidase (overnight at 37 °C). Following the incubation period, the hydrolysed extract was partitioned twice with ethyl acetate at pH 5 and pH 2. To further characterize the residues remaining in the post extraction solids (PES) of peel, these were refluxed for one hour in 3 N HCl. Following the reflux period, the remaining peel solids were removed by vacuum filtration and the filter cake was washed once with methanol. The remaining solids were allowed to air dry and then assayed for total radioactivity. The filtrate from the reflux was extracted with ethyl acetate prior to LSC.

Pulp samples were extracted using acetonitrile:water (75:25, v:v) and refluxed for one hour. The resulting solids were removed by vacuum filtration, air dried and submitted for combustion analysis. After removal of the acetonitrile from the reflux filtrate, the aqueous fraction was partitioned twice with dichloromethane at pH 7 and twice with ethyl acetate at pH 2 (combined = organosoluble). The extracted aqueous phase was then subjected to enzyme hydrolysis using β-glucosidase in a similar manner described for the peel. Following the incubation period, the solutions were partitioned twice with ethyl acetate prior to analysis (combined = aglycones).

Initial characterization of sample extracts was accomplished by direct TLC analysis using available reference standards. In some cases, initial characterization was achieved using the silica gel column technique. Pooled fractions from the silica gel columns were then subjected to additional characterization by TLC.

Table 4 Percent distribution of the total radioactive residues in whole apples

	Surface wash (% of the TRR)				Washed fruit (%TRR)	
DAT	Hexane	Chloroform	Methanol	Fenazaquin	Peel	Pulp
			Early Season Application			
			P-Label			
0	90.7	3.8	1.2	89.7	4.3	-
4	79.5	8.7	2.7	81.4	8.0	1.0
7	43.3	15.4	5.9	43.3	32.2	3.3
14	38.3	12.8	6.5	37.4	37.0	5.5
29	30.6	15.9	8.1	30.8	36.2	9.2
57	11.4	15.3	9.7	12.1	49.3	14.2
92	5.7	18.1	8.7	6.1	52.5	15.0
			Q-Label			
0	85.1	7.2	1.7	85.3	6.1	-
4	85.4	12.3	4.1	68.5	15.7	2.5
7	52.6	12.8	4.5	52.3	27.5	2.6
14	37.3	11.4	5.3	36.1	40.4	5.7
29	26.8	15.5	6.9	26.1	50.8	6.9
57	6.7	19.7	7.0	5.8	53.4	13.3
92	2.6	16.9	9.9	1.5	55.9	14.7
			Late Season Application			
			P-Label			
DAT	Hexane	Chloroform	Methanol	Fenazaquin	Peel	Pulp
0	95.3	3.2	0.6	92.2	0.8	<0.1
7	57.4	19.4	4.6	62.4	16.8	1.9
14	40.0	21.2	8.7	40.6	25.4	4.8
28	19.9	26.3	6.8	25.9	37.3	9.7
42	18.2	24.3	6.8	23.3	40.1	10.6
			Q-Label			
0	93.3	4.8	0.7	92.4	1.1	0.1
7	45.3	23.9	4.6	56.1	22.4	3.8
14	29.3	23.8	6.9	31.7	32.9	7.0
28	17.4	24.5	5.9	21.2	39.7	12.5
42	9.3	25.1	5.3	17.3	50.3	9.7
			P-Label / Photolysis Study			
0	95.3	3.2	0.6	92.2	0.8	<0.1
7	67.4	28.4	2.2	93.6	1.6	0.4
14	67.2	25.3	3.6	86.5	2.6	1.3

Note: The percent fenazaquin was determined by analysis of the organic solvent washes

The levels of fenazaquin declined relatively rapidly within the first 7 days following treatment with a slower decline thereafter. The decline of fenazaquin appeared to be associated with a corresponding increase in peel residues. At harvest (42 and 92 DAT), 40–56% of the TRR were found in the peels. Residues in the pulp also increased steadily with time following application. In apples that were covered from the sun, the decline in fenazaquin residues was insignificant as was the accumulation of residues in pulp and peel. Photolysis appears to be a major route of degradation of fenazaquin.

Table 5 Distribution and characterisation of residues in the peel and pulp of mature washed apples

	% ^{14}C residues							
Fraction Identification	P-Label				Q-Label			
	Pulp	Whole Fruit	Peel	Whole Fruit	Pulp	Whole Fruit	Peel	Whole Fruit
	Early Season Application							
Organosoluble [a]	21.7	3.5	36.5	30.7	14.3	2.1	30.4	25.9
Aglycones [b]	18.8	3.0	5.7	4.8	21.6	3.2	7.2	6.1

Fraction Identification	% ^{14}C residues							
	P-Label				Q-Label			
	Pulp	Whole Fruit	Peel	Whole Fruit	Pulp	Whole Fruit	Peel	Whole Fruit
Non hydrolysed and other polar metabolites [c]	53.5	8.6	10.2	8.6	51.0	7.5	5.9	5.0
Unextracted [d]	5.9	0.9	47.7	40.1	13.2	1.9	56.5	48.2
	Late Season Application							
Organosoluble extracts [a]	37.7	4.3	53.0	47.0	39.8	4.3	39.5	35.3
Aglycones [b]	14.9	1.7	4.2	3.7	20.9	2.2	5.1	4.6
Non hydrolysed and other polar metabolites [c]	43.0	4.9	5.2	4.6	30.3	3.2	4.5	4.0
Unextracted [d]	4.5	0.5	37.6	33.4	9.0	1.0	50.8	45.4

[a] Extracted residues remaining in the organosoluble phase following partitioning

[b] Extracted residues following enzyme hydrolysis

[c] Unextracted residues following enzyme hydrolysis

[d] Unextracted residues following initial extraction with acetonitrile:water (75:25, v:v)

Characterization of the residues in peel of washed mature fruit showed 30–53% of the sample radioactivity to be organosoluble, 10–15% as conjugates, and the remainder as residues which were bound or unextracted. Analysis of the extracted radioactivity both before and after enzyme hydrolysis showed fenazaquin to be the major component as it represented up to 20% of the total peel residue at harvest. No metabolites with the intact ether bridge were present at measurable levels in the peel. All of the metabolites found in the extracted peel were confirmed as being cleavage products of fenazaquin. Since cleavage of the fenazaquin ether bridge is known to occur as a result of photolysis, it was assumed that most of these metabolites were photoproducts. A total of six or more cleavage products were observed in the peel extracts, with 4-hydroxyquinazoline and TBPE tentatively identified as the two major cleavage constituents. Both of these metabolites represented 2–5% of the TRR. Collectively, all of the other observed cleavage metabolites were present at < 3% of the TRR. Due to the low levels at which each of the observed metabolites were present, no metabolites other than 4-hydroxyquinazoline and TBPE were identified. The unextracted residues in peel were confirmed as being cleavage products which had been incorporated into natural products.

Characterization of the residues in apple pulp showed 14–40% of the TRR to be present as organosolubles, ~70% of the TRR as aglycones, and 513% of the TRR to be unextracted. Analysis of the extracted residues both before and after enzyme hydrolysis showed there to be no fenazaquin present in the pulp. All of the observed metabolites were confirmed as being cleavage products, most of which were different from those observed in the peels. No individual metabolite in the pulp represented more than 1–3% of the whole fruit residue. As with the peel metabolites, none of the pulp metabolites were present at levels high enough to facilitate isolation and identification.

Study 2

Fenazaquin, labelled in the phenyl ring (P-Label; specific activity: 23.87 µCi/mg) or in the quinazoline ring (Q-Label; specific activity: 88.89 µCi/mg), and formulated as a suspension concentrate, was applied to *Golden Delicious* apple trees, maintained outdoor, at total seasonal application rates of 3.3 g ai/hL or 13.3 g ai/hL (Caley *et al*., 1998, 611-001). The first application was made to apple trees when fruits had reached an average size of 2 cm in diameter. Fruits were harvested on the day of application, 7, 14, 28 days after treatment (DAT) and at maturity (105 DAT). Five weeks later, a separate cluster of apple trees were treated at the low and high application rates. Apples were harvested immediately after treatment (0-DAT) and at maturity (70 DAT). In a separate experiment, a single application of P-labelled fenazaquin was made to one tree at the low rate. Following treatment, the fruit were covered with aluminium foil-lined plastic plant pots, the open end being covered with mesh to exclude light but allow air exchange. These fruit acted as a dark control.

Apples were peeled and the peel and pulp were cut into small pieces, frozen in dry ice, homogenised and subsequently assayed for total radioactivity by combustion. Radioactivity in the surface washes and in all other liquid fractions was determined by direct liquid scintillation counting (LSC).

The total radioactivity in fruits declined relatively rapidly over the duration of the study.

Fruits were sequentially washed with hexane:chloroform (1:1, v:v) and methanol and the washed fruit was separated into peel and pulp. Each was individually homogenized and extracted with hot acetonitrile:water (1:1, v:v) for 3 minutes at room temperature and then incubated at 60ºC in a water bath for 80 minutes. After removal from the water bath, the sample was allowed to cool and centrifuged. The nature of the radioactivity in all fractions was determined by both normal phase TLC and reverse phase HPLC.

Table 6 Distribution and identification of radioactive residues in apples following early application

	% TRR									
	P-Label					Q-Label				
DAT	0	7	14	28	105	0	7	14	28	105
	3.3 g ai/hL									
TRRs (mg eq/kg)	**0.364**	**0.145**	**0.082**	**0.033**	**0.005**	**0.369**	**0.158**	**0.122**	**0.045**	**0.010**
Surface wash	**99.2**	**87.9**	**75.3**	**50.8**	**39.7**	**98.4**	**80.4**	**73.3**	**50.0**	**20.4**
Hexane: Chloroform	94.6	81.0	71.3	48.3	36.9	92.7	73.5	69.6	46.1	17.7
Fenazaquin	*94.6*	*49.4*	*40.7*	*28.5*	*20.8*	*92.7*	*64.2*	*52.0*	*35.4*	*9.7*
Dimer	*N/A*	*31.6*	*30.6*	*19.8*	*16.1*	*N/A*	*9.3*	*17.6*	*10.7*	*8.0*
Methanol	4.6	6.9	4.0	2.5	2.8	5.7	6.9	3.7	3.9	2.7
Fenazaquin	*4.6*	*6.9*	*N/A*	*N/A*	*N/A*	*5.3*	*6.5*	*3.7*	*3.9*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.4*	*N/A*	*N/A*	*N/A*	*N/A*
Dimer	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.3*	*N/A*	*N/A*	*N/A*
Unknown	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.1*	*N/A*	*N/A*	*N/A*
Peel extracted	**N/A**	**2.7**	**3.5**	**8.0**	**5.5**	**N/A**	**2.6**	**4.3**	**4.3**	**5.5**
Fenazaquin	*N/A*	*1.5*	*N/A*	*N/A*	*N/A*	*N/A*	*1.4*	*1.7*	*2.7*	*N/A*
Metabolite C/TBPE	*N/A*	*1.3*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.5*	*N/A*	*N/A*	*N/A*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.6*	*2.6*	*1.6*	*N/A*
Peel unextracted	**1.2**	**6.7**	**12.4**	**19.7**	**36.1**	**1.4**	**12.8**	**27.0**	**32.2**	**53.7**
Pulp extracted	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
Pulp unextracted	**0.2**	**2.4**	**3.7**	**8.3**	**17.2**	**0.4**	**3.2**	**4.0**	**8.4**	**16.4**
Fenazaquin	*99.2*	*57.8*	*40.7*	*28.5*	*20.8*	*98*	*70.7*	*57.4*	*42.0*	*9.7*
Metabolite C/TBPE	*N/A*	*1.3*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
2-Hydroxy-fenazaquin	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.4*	*N/A*	*2.6*	*N/A*	*N/A*
Dimer	*N/A*	*31.6*	*30.6*	*19.8*	*16.1*	*N/A*	*9.6*	*17.6*	*10.7*	*8.0*
Total Identified	*99.2*	*90.7*	*71.3*	*53.4*	*36.9*	*98.4*	*80.3*	*87.6*	*42.2*	*17.4*
Total Characterized	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.1*	*2.6*	*1.6*	*13.3*
Total Extracted	99.2	90.6	78.8	58.8	45.2	98.4	83.0	77.6	54.3	25.9
Total Unextracted	1.4	9.1	16.1	28.0	53.3	1.8	16.0	31.0	40.6	70.1
Accountability	101	100	95	97	99	100	99	109	95	96
	13.3 g ai/hL									
TRRs (mg eq/kg)	**1.16**	**0.547**	**0.433**	**0.146**	**0.048**	**1.026**	**0.607**	**0.434**	**0.214**	**0.040**
Surface wash	**98.4**	**81.7**	**89.5**	**74.4**	**34.6**	**98.4**	**84.0**	**75.5**	**53.9**	**26.5**
Hexane: Chloroform	94.8	74.3	85.6	67.7	31.9	92.4	76.1	71.2	51.2	23.8
Fenazaquin	*94.8*	*52.9*	*53.8*	*39.3*	*14.0*	*92.4*	*66.0*	*52.5*	*33.5*	*11.3*
Dimer	*N/A*	*21.4*	*31.8*	*28.4*	*17.9*	*N/A*	*10.1*	*18.7*	*17.7*	*12.5*
Methanol	3.6	7.4	3.9	6.7	2.7	6.0	7.9	4.3	2.7	2.7
Fenazaquin	*3.6*	*6.4*	*3.9*	*4.4*	*N/A*	*6.0*	*7.9*	*N/A*	*2.3*	*N/A*
Metabolite C/TBPE	*N/A*	*N/A*	*N/A*	*2.3*	*N/A*	*N/A*	*N/A*	*N/A*	*0.4* [a]	*N/A*
Dimer	*N/A*	*1.0*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Peel extracted	**1.3**	**3.0**	**2.2**	**4.8**	**8.7**	**1.5**	**2.9**	**2.8**	**4.8**	**6.9**
Fenazaquin	*N/A*	*1.3*	*0.9*	*2.5*	*2.7*	*1.5*	*1.5*	*1.5*	*0.1*	*0.9*
Metabolite C/TBPE	*N/A*	*0.7*	*0.5*	*1.1*	*1.9*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
2-Hydroxy-fenazaquin	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.8*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*1.0*	*0.3*	*1.8*	*N/A*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.4*	*0.7*	*2.0*	*5.2*
Dimer	*N/A*	*N/A*	*0.3*	*N/A*	*N/A*	*N/A*	*N/A*	*0.2*	*N/A*	*N/A*
Unknowns (no more than 2)	*N/A*	*N/A*	*0.6*	*1.2*	*4.1*	*N/A*	*N/A*	*N/A*	*0.8*	*N/A*
Peel unextracted	**N/A**	**6.0**	**8.3**	**14.5**	**25.0**	**0.2**	**11.4**	**16.3**	**26.6**	**56.5**
Pulp extracted	**N/A**	**0.8**	**1.1**	**3.5**	**8.5**	**N/A**	**1.2**	**1.9**	**5.1**	**8.2**
Fenazaquin	*N/A*	*0.4*	*0.5*	*3.5*	*N/A*	*N/A*	*0.3*	*0.6*	*0.4*	*N/A*

	% TRR									
	P-Label					Q-Label				
DAT	0	7	14	28	105	0	7	14	28	105
Metabolite C/TBPE	*N/A*	*0.2*	*0.6*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.9*	*0.6*	*2.5*	*N/A*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.4*	*1.2*	*N/A*
Dimer	*N/A*	*0.2*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.3*	*N/A*	*N/A*
Unknown	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.9*	*N/A*
Pulp unextracted	**0.2**	**0.8**	**0.8**	**2.9**	**10.0**	**0.4**	**0.7**	**N/A**	**2.9**	**7.4**
Fenazaquin	*98.4*	*61.0*	*59.1*	*49.7*	*16.7*	*99.9*	*75.4*	*54.6*	*34.0*	*12.2*
Metabolite C/TBPE	*N/A*	*0.9*	*1.1*	*3.4*	*1.9*	*N/A*	*N/A*	*N/A*	*0.4*	*N/A*
2-Hydroxy-fenazaquin	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.8*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*1.9*	*0.9*	*4.3*	*N/A*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.4*	*1.1*	*3.2*	*5.2*
Dimer	*N/A*	*22.6*	*32.1*	*28.4*	*17.9*	*N/A*	*10.1*	*10.1*	*17.7*	*12.5*
Total Identified	*98.4*	*74.5*	*92.3*	*81.5*	*36.5*	*99.9*	*67.8*	*66.7*	*59.6*	*30.7*
Total Characterized	*N/A*	*0.6*	*0.6*	*1.2*	*4.1*	*N/A*	*N/A*	*N/A*	*0.8*	*N/A*
Total Extracted	99.7	85.5	92.8	82.7	51.8	99.9	88.1	80.2	60.4	41.6
Total Unextracted	0.2	6.8	9.1	17.4	35.0	0.6	12.1	16.3	29.5	63.9
Accountability	100	92	102	100	87	100	100	96	93	106

N/A: Not analysed as radioactivity was too low

[a] metabolite L

Table 7 Distribution of radioactive residues in apples following late season application

	% TRR							
	P-Label				Q-Label			
	3.3 g ai/hL		13.3 g ai/hL		3.3 g ai/hL		13.3 g ai/hL	
DAT	0	70	0	70	0	70	0	70
TRRs (mg eq/kg)	**0.210**	**0.030**	**0.925**	**0.120**	**0.166**	**0.040**	**0.823**	**0.168**
Surface wash	**103.9**	**46.9**	**96.6**	**58.1**	**96.7**	**41.5**	**96.3**	**47.0**
Hexane: Chloroform	101.9	44.7	94.5	55.2	95.1	39.3	94.1	44.5
Fenazaquin	*101.9*	*26.3*	*94.5*	*21.6*	*95.1*	*32.6*	*94.1*	*30.8*
Metabolite C/TBPE	*N/A*	*N/A*	*N/A*	*1.2*	*N/A*	*N/A*	*N/A*	*N/A*
Dimer	*N/A*	*18.4*	*N/A*	*32.5*	*N/A*	*6.7*	*N/A*	*13.7*
Methanol	2.0	2.2	2.1	2.9	1.6	2.3	2.1	2.5
Fenazaquin	*2.0*	*N/A*	*2.1*	*N/A*	*1.6*	*N/A*	*2.0*	*N/A*
Peel extracted	**1.1**	**8.9**	**1.6**	**9.4**	**1.4**	**8.5**	**1.5**	**8.0**
Fenazaquin	*N/A*	*N/A*	*1.0*	*1.6*	*1.3*	*N/A*	*1.3*	*2.6*
Metabolite C/TBPE	*N/A*	*N/A*	*0.6*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*1.0*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*0.1*	*N/A*	*0.1*	*3.5*
Unknowns (no more than 3)	*N/A*	*N/A*	*N/A*	*7.8*	*0.1*	*N/A*	*N/A*	*0.9*
Peel unextracted	**0.5**	**30.9**	**0.2**	**21.7**	**0.3**	**40.8**	**0.2**	**32.6**
Pulp extracted	**N/A**	**N/A**	**0.6**	**4.9**	**N/A**	**N/A**	**0.8**	**5.4**
Fenazaquin	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.6*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*0.2*	*3.0*
Unknown	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*2.4*
Pulp unextracted	**1.1**	**9.9**	**0.2**	**3.6**	**1.0**	**10.7**	**0.1**	**7.9**
Fenazaquin	*103.9*	*26.3*	*97.6*	*23.2*	*98.0*	*32.6*	*98.0*	*33.4*
Metabolite C/TBPE	*N/A*	*N/A*	*0.6*	*1.2*	*N/A*	*N/A*	*N/A*	*N/A*
Dihydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*N/A*	*1.0*
4-Hydroxyquinazoline	*N/A*	*N/A*	*N/A*	*N/A*	*0.1*	*N/A*	*0.3*	*6.5*
Dimer	*N/A*	*18.4*	*N/A*	*32.5*	*N/A*	*6.7*	*N/A*	*13.7*
Total Identified	*103.9*	*44.7*	*98.2*	*56.9*	*98.1*	*39.3*	*98.3*	*54.6*
Total Characterized	***N/A***	*N/A*	*N/A*	*7.8*	*0.1*	*N/A*	*N/A*	*0.9*
Total Extracted	105	55.8	98.8	72.4	98.1	50.1	98.5	60.4
Total Unextracted	1.6	40.8	0.4	25.3	1.3	51.5	0.3	40.5
Accountability	106.6	96.6	99.2	97.7	99.4	101.6	98.8	100.9

N/A: Not analysed as radioactivity was too low

At least 98% of the TRRs were removed by surface washing of the 0-DAT fruits, the majority of which was in the hexane:chloroform wash (> 92% of the TRR). Following the early season application, the TRRs removed by surface washing declined with increased DAT but remained greater than 50% of the TRR up to and including 28 DAT. At 105-DAT, surface washing removed 20–40% of the TRR from fruit treated at the low rate. Corresponding values for the higher application rate were 42–58% of the TRR.

After the surface washes, most of the radioactivity remaining in the fruit was found in the peel. On 0-DAT, the peel contained 1.2–1.7% of the TRR with 0.2–0.4% of the TRR remaining in the pulp. The radioactivity present in both the peel and the pulp increased with increased DAT for both the low and high rates following early season applications. A similar trend was observed in the late season experiment. The distribution of radioactivity was similar for both the P and the Q labels at the low and high rates of application.

Fenazaquin accounted for greater than 98% of the TRR in samples collected 0-DAT and declined rapidly thereafter following low and high rates of application made early season and late season. The fenazaquin dimer accounted for 16–32% of the TRR of the P-label samples and 8–18% TRR of the Q-labelled samples. This compound, which was only present after at least 7 DAT, appeared to remain stable or decline slightly by the time the fruits reached maturity. Metabolite C/TBPE was present only in the 7-DAT P-label samples from the low rate early season application regime, but following the high rate early season application regime, Metabolite C/TBPE was present at 0.9–3.4% of the TRR at 7-DAT onwards. For the Q-label samples, Metabolite C/TBPE was only present at 0.4% of the TRR at the 28-DAT interval, following the high rate early season application regime. As Metabolites C and TBPE could not be separated either by HPLC or TLC, and were present predominantly in the P-label samples, it likely represents TBPE. Fenazaquin metabolic profiles were qualitatively similar for both the P- and Q- labels at both the high and low application rates. Several other unknown metabolites were characterized, none of which exceeded 6% of the TRR.

For the covered samples harvested at 14 DAT, surface residues were comprised solely of fenazaquin, and peel extracts were fenazaquin and Metabolite C/TBPE. The absence of the dimer on these samples tends to confirm that it is a product of photochemistry.

Oranges

^{14}C-Phenyl-fenazaquin (P-label; specific activity: 2.1 µCi/mg) and ^{14}C-quinazoline-fenazaquin (Q-label; specific activity: 2.1 µCi/mg) were formulated as emulsifiable concentrates (EC) and were separately applied at 0.45 kg ai/ha to orange trees, variety *Valencia*, grown outdoors (Berard,1992, 611-007; Dennis, 1998, ABC-0454SUPPL). Applications were made approximately 6 months prior to fruit maturity (early season application, when fruit were 3 cm in diameter) and approximately 2 months prior to fruit maturity (late season application when fruit were 6 cm in diameter). Fruit was collected at 0, 28, 112 and 191 days after treatment (DAT) following the early season application and at 0, 19 and 63 DAT following the late season application.

In an effort to examine the effect of photolysis on the nature of the residues, some of the fruit from the late season application were covered with muslin cloth shortly after the application of fenazaquin. Samples of covered fruit were collected at 9, 19 and 63 DAT.

For determination of TRRs and characterization of surface residues, orange samples from each radiolabel and harvest interval were solvent washed sequentially with 10% methanol in water, dichloromethane, and 100% methanol. The washes were conducted by submerging whole fruits in each solvent for 10–20 minutes. Radioactivity in each rinse fraction was then determined by liquid scintillation counting (LSC). The dichloromethane and 100% methanol washes were concentrated and directly analysed by 1D-TLC. The 10% methanol washes were concentrated to remove the methanol, and the ^{14}C-residues were then partitioned into ethyl acetate, concentrated and analysed by 1D-TLC. The remaining fruit sample was chopped into small pieces, frozen in liquid nitrogen and ground, and radioactivity in the washed fruits was determined by ^{14}C-combustion with LSC. The total TRR in whole fruit samples were then calculated by summing the radioactivity in the solvent washes and the radioactivity remaining in the washed fruit.

Following the early season application, the whole fruit TRRs were 2.05–2.60 mg eq/kg at 0 DAT and declined rapidly to 0.70–0.84 mg eq/kg by 28 DAT, and then declined more slowly to 0.32–0.36 mg eq/kg by fruit maturity (191 DAT). Following the late-season application, TRRs in whole fruit were 0.50–0.55 mg eq/kg at 0 DAT and remained relatively unchanged (P label), or even appeared to increase (Q label), until maturity, at 63 DAT (0.45–0.90 mg eq/kg). Regardless of the application timing or sampling interval, the two radiolabels yielded similar TRRs. There was no apparent decline in TRRs in the covered fruit from the late season application.

TRRs from both ^{14}C-labels were also distributed similarly in the solvent washes and between the peel and pulp fractions. For the early-season application, the majority of the TRR at 0 DAT was recovered in the solvent washes (90–94% of the TRR), but by 28 DAT and at all subsequent intervals, the majority of TRRs remained in/on the washed fruit (77–92% of the TRR). For the late-season application, the portion of TRRs in the surface washes declined more slowly, from 97–96% of the TRR to 66–76% of the TRR, and in the washed fruits TRRs increased from 3–4% at 0 DAT to 24–34% by 63 DAT. For the covered fruits, the percent TRRs associated with the washed fruits also increased over time, but to a smaller extent. By 63 DAT, the solvent washes from the covered fruits still accounted for 89–92% of the TRRs, and the washed wrapped fruit accounted for only 8–11% of the TRR.

Table 8 Total Radioactive Residues in oranges harvested following different application regimes

Application and Timing	DAT (days)	Matrix	[^{14}C-P]-Label		[^{14}C-Q]-Label	
			mg eq/kg [a]	% TRR	mg eq/kg [a]	% TRR
Exposed Fruits						
Single, early-season, foliar application	0	**Unwashed fruit**	**2.049**	**100**	**2.603**	**100**
		Surface washes [c]	1.854	90.4	2.444	93.9
		washed fruit	0.197	9.6	0.158	6.1
	28	**Unwashed fruit**	**0.700**	**100**	**0.835**	**100**
		Surface washes [c]	0.163	23.3	0.182	21.8
		washed fruit	0.537	76.7	0.653	78.2
	112	**Unwashed fruit**	**0.381**	**100**	**0.331**	**100**
		Surface washes [c]	0.055	14.4	0.026	7.8
		washed fruit	0.326	85.6	0.305	92.2
	191	**Unwashed fruit**	**0.361**	**100**	**0.323**	**100**
		Surface washes [c]	0.078	21.5	0.039	12.0
		washed fruit	0.283	78.5	0.284	88.0
Single, late-season, foliar application	0	**Unwashed fruit**	**0.504**	**100**	**0.547**	**100**
		Surface washes [c]	0.491	97.4	0.528	96.5
		washed fruit	0.014	2.7	0.019	3.5
	19	**Unwashed fruit**	**0.531**	**100**	**0.757**	**100**
		Surface washes [c]	0.476	89.6	0.659	87.1
		washed fruit	0.055	10.4	0.098	12.9
	63	**Unwashed fruit**	**0.451**	**100**	**0.903**	**100**
		Surface washes [c]	0.344	76.1	0.592	65.6
		washed fruit	0.107	23.8	0.311	34.4
Covered Fruits [b]						
Single, late-season, foliar application	9	**Unwashed fruit**	**0.480**	**100**	**0.839**	**100**
		Surface washes [c]	0.456	95.1	0.816	97.3
		washed fruit	0.024	5.0	0.023	2.7
	19	**Unwashed fruit**	**0.617**	**100**	**0.894**	**100**
		Surface washes [c]	0.584	94.7	0.830	92.1
		washed fruit	0.033	5.3	0.064	7.2
	63	**Unwashed fruit**	**0.178**	**100**	**0.566**	**100**
		Surface washes [c]	0.163	91.6	0.503	88.9
		washed fruit	0.015	8.4	0.063	11.1

[a] mg eq/kg values are expressed on a whole fruit basis.

[b] Selected fruits from the late season application were wrapped with muslin cloth after the application dried.

[c] The majority of ^{14}C-residues in the solvent washes were recovered in the dichloromethane fraction.

The distribution of the TRR between peel and pulp were also determined in mature samples of fruit from both the early-season application (191 DAT) and the late season application (63 DAT). Subsamples of whole fruit were separated into peel and pulp, and the fractions were chopped, frozen and ground. Radioactivity in each fraction was then determined by combustion/LSC.

For characterization of ^{14}C-residues in whole fruit at maturity (191 or 63 DAT samples for early season and late season applications, respectively), the solvent washes were analysed by TLC as indicated above, and the remaining washed fruit samples were extracted. The washed, ground fruit was extracted twice with acetonitrile, first at room temperature for 2 hours and then for 1 hour under reflux. The resulting acetonitrile fractions were combined, concentrated and partitioned twice with acetonitrile, first at pH 7.5 and then at pH 3.8 to yield the acetonitrile -2 and -3 fractions, which were analysed by TLC. The remaining aqueous fraction (AQ-2) from early season application fruits was further treated by incubation with β-glucosidase (pH 5, overnight at 37 °C). The enzyme hydrolysate was partitioned with acetonitrile, first at pH 7 and then at pH 2.5, to yield the acetonitrile -4 and -5 fractions which were also analysed by TLC. The remaining aqueous fraction was then retreated with β-glucosidase and again partitioned with acetonitrile at pH 7 and 2.5, combining the two acetonitrile fractions into one fraction (acetonitrile -6), and this was analysed by TLC. The remaining aqueous fraction (AQ-3) was not further analysed.

For the mature fruit sample from the early seaspn application regime (191 DAT), the residual solids were extracted by refluxing in 1N HCl:acetonitrile (1:1, v:v) for 2.5 hours, and the sample was cooled and filtered. The residual solids were radioassayed by combustion/LSC, and ^{14}C-residues in the filtrate were adjusted to pH 7.8 and partitioned sequentially with dichloromethane and acetonitrile. The remaining aqueous fraction was adjusted to pH 2 and repartitioned with acetonitrile. The resulting three organic fractions were combined, concentrated and then fractionated using a silica gel column that was eluted using a step gradient of increasingly polar solvents, from toluene to methanol containing 5% acetic acid. The remaining acidic aqueous fraction (AQ-4) was also fractionated using a resin column that was eluted with a step gradient of water to 60% methanol, followed by acetone.

In addition to the extraction of the whole fruit samples, ^{14}C-residues in the separated peel and pulp fractions of mature fruits from the early season application (191 DAT) were also extracted for analysis. The ground peel and pulp fractions were separately extracted twice with acetonitrile, first at room temperature for 1 hour and then for 1 hour under reflux. The resulting acetonitrile fractions were combined, diluted with water (peel only), and concentrated to remove the acetonitrile. The resulting aqueous fractions were then combined and partitioned twice with acetonitrile, first at pH 7.5 and then at pH 3.8, and the resulting acetonitrile fractions were analysed by TLC.

The distribution of the TRRs between peel and pulp fractions was similar for mature fruits for both ^{14}C-labels. For the early season application, the peel accounted for 86–95% of the TRR in mature fruits at 191 DAT, and the pulp accounted for 5–14% of the TRR. For the late-season application, the peel accounted for 97–99% of the TRRs in mature fruits at 63 DAT, and the pulp accounted for ≤ 3% of the TRR. These findings confirmed that there was limited penetration from the peel into the pulp.

Table 9 Distribution of ^{14}C in oranges following a single early-season foliar application of [^{14}C]fenazaquin

	0 DAT		191 DAT	
	P-Label	Q-Label	P-Label	Q-Label
TRR (mg eq/kg)	2.049	2.603	0.361	0.323
	%TRR			
Surface wash	**90.5**	**93.9**	**21.6**	**12.0**
10% Methanol [a]	9.8	15.1	1.0	1.0
Fenazaquin	*8.0*	*11.7*	*0.3*	*0.1*
2-Hydroxy-fenazaquin	*N/D*	*N/D*	*0.1*	*N/D*
Unknowns	*1.5*	*2.5*	*0.6*	*0.9*
Dichloromethane	50.7	53.2	11.5	4.9
Fenazaquin	*49.1*	*51.0*	*9.2*	*3.6*
2-Hydroxy-fenazaquin	*N/D*	*N/D*	*1.3*	*0.5*
Unknowns	*1.6*	*2.2*	*1.0*	*0.8*
Methanol	30.0	25.6	9.1	6.1

	0 DAT		191 DAT	
	P-Label	Q-Label	P-Label	Q-Label
TRR (mg eq/kg)	2.049	2.603	0.361	0.323
		%TRR		
Fenazaquin	*28.0*	*23.6*	*2.7*	*2.4*
2-Hydroxy-fenazaquin	*N/D*	*N/D*	*1.5*	*0.9*
Unknowns	*2.0*	*2.0*	*4.9*	*2.8*
Washed Fruit	**9.6**	**6.1**	**78.5**	**88.0**
Acetonitrile extract			66.3	61.3
Ethyl acetate-2 (pH 7.5)			47.6	38.5
Fenazaquin			*37.7*	*30.8*
2-Hydroxy-fenazaquin			*5.1*	*3.4*
Unknowns (each @ <2.6% TRR)			*4.7*	*4.3*
Ethyl acetate-3 (pH 3.8)			7.3	4.8
Fenazaquin			*2.1*	*2.0*
2-Hydroxy-fenazaquin			*0.1*	*0.1*
Unknowns			*5.1*	*2.7*
AQ-2 (β-glucosidase)			11.4	18.0
Ethyl acetate-4			0.5	2.4
Radioactivity at or near TLC origin			*N/A* [b]	*2.3*
Ethyl acetate-5			2.1	2.5
Radioactivity at or near TLC origin			*2.1*	*2.2*
Ethyl acetate-6			2.8	1.3
Radioactivity at or near TLC origin			*2.1*	*1.2*
AQ-3 (not further analysed)			6.0	11.8
Post-extraction solids			**12.0**	**26.7**
Acetonitrile:HCl Reflux			6.4	13.7
Combined organic fractions (dichloromethane, Ethyl acetate-7, Ethyl acetate-8)			6.0	5.8
Silica gel fractionations (each ≤5% TRR)			*5.7*	*5.8*
Aqueous-4			0.4	7.9
XAD-4 fractionations (each ≤3.8% TRR)			*N/A*	*7.9*
Total Extracted			94.4	87.0
Fenazaquin	*85.1*	*86.3*	*52.0*	*38.9*
2-Hydroxy-fenazaquin	*N/D*	*N/D*	*8.1*	*4.9*
Total Identfiied	*85.1*	*86.3*	*60.1*	*43.8*
Total Characterized	*5.1*	*6.7*	*32.6*	*42.4*
Remaining Solids			5.6	13.0
Accountability			100	100

[a] The 10% methanol washes were concentrated and extracted with ethyl acetate prior to TLC analysis; ≥95% of the radioactivity in the fraction was recovered in the ethyl acetate fraction.

[b] For the [^{14}C-P]-label, the ethyl acetate-4 and -5 fractions were combined for TLC analysis.

N/A – not applicable; N/D – not detected.

For the early-season application (0-DAT), the majority of the radioactivity was identified by TLC as parent fenazaquin (85–86% of the TRR). At maturity (191 DAT), parent was the major residue (39–52% of the TRR), along with minor amounts of 2-hydroxy-fenazaquin (5–8% of the TRR). The remaining radioactivity was comprised largely of minor unknowns, each present at < 5% TRR.

Table 10 Distribution of ^{14}C-Residues in/on exposed and covered oranges at 63 days following a single late-season application of [^{14}C]fenazaquin

	% TRR			
	P-Label		Q-Label	
	Exposed fruit	Covered fruit	Exposed fruit	Covered fruit
TRR	0.451 mg eq/kg	0.178 mg eq/kg	0.903 mg eq/kg	0.566 mg eq/kg
Surface wash	**76.1**	**91.6**	**65.6**	**88.8**
10% Methanol	2.6	2.1	2.7	2.3
Dichloromethane	59.9	84.5	53.2	82.5
Methanol	13.6	5.0	9.7	4.0
Washed Fruit	**23.8**	**8.4**	**34.4**	**11.1**
Acetonitrile extract	13.9	6.6	10.7	6.9
Ethyl acetate-2	6.7	1.6	-5.9	2.8
Ethyl acetate -3	2.8	1.9	0.9	0.5
Aqueous	4.4	3.1	3.9	3.6
Total Identified [a]				
Fenazaquin	*65.5*	*83.7*	*55.4*	*80.9*
2-Hydroxy-fenazaquin	*0.8*	*0.8*	*0.9*	*1.1*
Accountability	99.9	100	100	99.9

[a] The levels of parent, Metabolite D and unknowns in each fraction were not provided.

For the late-season application, TLC analyses of solvent washes and fruit extracts of mature fruit (63 DAT), identified parent as the primary residue (55–66% of the TRR), along with minor amounts of 2-hydroxy-fenazaquin (≤ 1% of the TRR).

For the wrapped fruits (late season application), the levels of parent in mature fruit (63 DAT) were considerably higher (81–84% TRR) than in the unwrapped fruit. 2-Hydroxy-fenazaquin in the mature wrapped fruit was found at ≤ 1.1% TRR.

These data indicate that parent fenazaquin is the major residue in/on citrus fruits, and that residues are largely confined to the peel. Hydroxylation of fenazaquin was the major pathway, yielding 2-hydroxy-fenazaquin. The minimal amount of degradation of parent that occurred in/on wrapped fruits suggests that photolysis of surface residues plays an important role in the degradation of fenazaquin residues on the surface of these fruits.

Grapes

Field grown grapes, variety *Cabernet Sauvignon,* were treated with single applications of an EC formulation of [^{14}C]fenazaquin labelled in the phenyl ring of the molecule (P-label, specific activity 26.6 µCi/mg) or in the quinazoline portion of the molecule (Q-label; specific activity 64.3 µCi/mg) at 15 g ai/hL (Haq, 1994, 611-009). An early application was made to grape bunches approximately two to three weeks after the end of flowering (BBCH 68). A late application was made to separate grape bunches approximately 7 weeks later (nine to ten weeks after the end of flowering). To aid in the characterisation of any fenazaquin metabolites, applications at 150 g ai/hL were also made to 10 grape bunches spread over three to five vines at the late application time point with both P- and Q-labelled fenazaquin. Grape bunches from the early season application were sampled at 0, 49 and 76 days after treatment (DAT) while grape bunches from the late season application were sampled at 0 and 28 DAT.

To determine the potential for fenazaquin and its metabolites to translocate from the site of application, individual branches on two separate vines were treated with ^{14}C-fenazaquin at the time of the early season application.

Grapes were washed sequentially with 10% methanol:water followed by dichloromethane and methanol. The samples of washed grapes were homogenised, extracted with acetonitrile:water (9:1, v/v) and further partitioned with ethyl acetate. Radioactivity in the surface washes and extracts were determined by liquid scintillation counter (LSC). The residues were analysed by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC) methods. To further release

the unextracted radioactivity, acid (0.1M and 2.0 M HCl)/base (2.0 M NaOH) and enzyme (β-glucosidase) hydrolysis was performed.

Following the early season application regime, the levels of radioactivity removed by the surface washes decreased with time. Conversely, the amount of extracted and unextracted residues increased with increased duration (DAT). No distinct differences between P- and Q-labelled fenazaquin were observed.

Table 11 Percent radioactivity [% TRR] in washes and tissue fractions of grapes following early season application

Radiolabel	Days after treatment (DAT)	Surface washes (%TRR)				Washed fruit (%TRR)	
		10% Methanol	Dichloromethane	100% Methanol	Total	Extracted	Unextracted
P-Label	0	0.7	21.4	55.4	77.5	17.5	5
	49	13.4	25.6	21.3	60.3	34.3	5.4
	76	6.6	14	13.1	33.7	44.6	21.7
Q-Label	0	0.9	25.2	54.8	80.9	15.9	3.2
	49	7.9	18.1	17.5	43.5	37.7	18.8
	76	5.4	11	12.9	29.3	39.1	31.6

Table 12 Distribution [a] of radioactive residues in grapes harvested 76 days following application

	P-Label		Q-Label	
	[%TRR]	mg eq/kg	[%TRR]	mg eq/kg
Surface wash	33.7		29.3	
10% Methanol	6.6		5.4	
Dichloromethane	14.0	0.147	11.0	0.116
Fenazaquin	13.1	0.138	10.0	0.094
Fenazaquin acid	0.4	0.004	0.6	0.006
Dihydroxyquinazoline	N/D	N/D	0.4	0.004
Unknown	0.5	0.005	N/D	N/D
Methanol	13.1	0.140	12.9	0.135
Fenazaquin	7.0	0.074	5.2	0.049
Fenazaquin acid	0.6	0.006	1.7	0.016
Metabolite C	2.1	0.022	1.7	0.016
4-Hydroxyquinazoline	N/D	N/D	4.3	0.041
Metabolite K	0.9	0.010	N/D	N/D
Unknowns (No more than 2)	2.4	0.025	N/D	N/D
Extracted	44.6		39.1	
Ethyl acetate partition	21.0	0.221	24.2	0.228
Fenazaquin	9.3	0.098	8.3	0.078
Metabolite C	1.3	0.014	1.9	0.018
TBPE	2.2	0.023	N/D	N/D
Metabolite H	1.2	0.013	1.1	0.010
Dihydroxyquinazoline	N/D	N/D	5.4	0.051
Unknowns (No more than 4)	5.9	0.062	6.4	0.061
Total extracted	78.3		68.4	
Fenazaquin	29.4	0.310	23.5	0.221
Fenazaquin acid	N/D	N/D	2.3	0.022
Dihydroxyquinazoline	N/D	N/D	5.8	0.055
4-hydroxyquinazoline	N/D	N/D	4.3	0.041
TBPE	2.2	0.023	N/D	N/D
Metabolite C	3.4	0.036	3.6	0.034
Metabolite H	1.2	0.013	1.1	0.010
Metabolite K	0.9	0.010	N/D	N/D
Total identified	37.1	0.392	40.6	0.383
Total characterized	8.8	0.093	6.4	0.061
Unextracted	21.7		31.6	
Accountability	100		100	

N/D Not detected

[a] Since TLC was generally more sensitive than HPLC, only TLC results are reported.

Characterisation of the residues in the surface washes of the fruit samples following the early season application (76 DAT) showed the major component was fenazaquin, ranging from 24 to 29% of TRR. Analysis of the solvent extracted fraction showed the presence of three metabolites derived from the intact fenazaquin molecule: fenazaquin acid, Metabolite C and Metabolite H. The remaining metabolites were products formed as a result of the cleavage of the ether bridge linking the quinazoline and the ethylphenylbutyl portions of the molecule. These metabolites were identified as dihydroxyquinazoline, 4-hydroxyquinazoline, Metabolite K and TBPE. These minor metabolites each accounted for 0.9–5.8% of the TRR (0.010 to 0.055 mg eq/kg). A total of 8 unknown metabolites were characterized, two of which contain both the P- and Q-labels. Four P-labelled and two Q-labelled unknown metabolites were also detected. In total, unknowns accounted for 6–9% of the TRR (0.061–0.093 mg eq/kg).

The ^{14}C remaining in solids after solvent extraction from both P- and Q-labelled samples (22% and 31% of TRR, respectively) were subjected to acid followed by alkali hydrolysis. A maximum of 16% of the unextracted residue was released by acid hydrolysis while base hydrolysis released 5% and 16% of TRR, from the P- and Q-labelled samples, respectively. Analysis of these hydrolysates showed that radioactive residue was either tightly associated with, or perhaps even incorporated into, some natural constituents in the grape tissue.

The translocation experiment showed that following application of fenazaquin to branches, no radioactivity was found in grape bunches despite the measurable TRRs in the sprayed branches (10 mg eq/kg), thus confirming that translocation of fenazaquin and/or any degradation products beyond the site of application did not occur.

Results of the late season application (DAT 28) showed proportionally higher levels (71% of the TRR for the P-label and 61% of the TRR for the Q-label) of radioactivity recovered in the surface washes than in samples taken after the early season application. Unextracted residues accounted for 8-12% of the TRR.

Table 13 Percent radioactivity [% TRR] in washes and tissue fractions of grapes following late season application

Radiolabel	10% Methanol wash [% TRR]	100% Dichloromethane wash [% TRR]	100% Methanol wash [% TRR]	Total surface washes [% TRR]	Extracted tissue residue [% TRR]	Unextracted residue [% TRR]
P-Label	2.7	56.8	11.9	71.4	21.0	7.6
Q-Label	5.6	38.2	17.5	61.3	26.6	12.1

Table 14 Distribution [a] of radioactive residues in grapes harvested 28 days following application

	P-Label		Q-Label	
	[%TRR]	mg eq/kg	[%TRR]	mg eq/kg
Surface washes	71.4	1.035	61.3	1.576
Dichloromethane	56.8	0.826	38.2	0.982
Fenazaquin	55.5	0.829	34.1	0.890
Metabolite C	1.1	0.016	0.4	0.010
Metabolite H	N/D	N/D	2.2	0.057
4-Hydroxyquinazoline	N/D	N/D	1.4	0.036
Unknown	0.2	0.003	N/D	N/D
Methanol	11.9	0.173	17.5	0.450
Fenazaquin	9.0	0.134	11.5	0.300
Metabolite C	0.5	0.008	2.8	0.073
Metabolite H	N/D	N/D	0.4	0.010
Dihydroxyquinazoline	N/D	N/D	1.3	0.034
4-Hydroxyquinazoline	N/D	N/D	1.5	0.039
TBPE	1.1	0.016	N/D	N/D
Unknown	1.4	0.021	N/D	N/D
Washed fruit	21.0	0.351	26.6	0.614
Ethyl acetate partition	9.9	0.148	11.0	0.288

	P-Label		Q-Label	
	[%TRR]	mg eq/kg	[%TRR]	mg eq/kg
Fenazaquin	4.2	0.063	2.9	0.076
Dihydroxyquinazoline	N/D	N/D	0.8	0.021
4-Hydroxyquinazoline	N/D	N/D	6.0	0.157
TBPE	3.3	0.049	N/D	N/D
Unknowns (No more than 3)	2.4	0.036	1.3	0.034
Total extracted	92.4	1.386	87.9	2.190
Fenazaquin	68.7	1.026	48.5	1.266
Dihydroxyquinazoline	N/D	N/D	2.1	0.034
4-Hydroxyquinazoline	N/D	N/D	2.9	0.075
TBPE	4.4	0.065	N/D	N/D
Metabolite C	1.6	0.024	3.2	0.073
Metabolite H	N/D	N/D	2.6	0.067
Total identified	74.7	1.115	59.3	1.515
Total characterized	17.8	0.271	1.3	0.034
Total unextracted	7.6	0.114	12.1	0.310
Accountability	100		100	

N/D – Not Detected

[a]. Since TLC was generally more sensitive than HPLC, only TLC results are reported.

Considering the 10% methanol wash contained less than 6% of the TRR, this fraction was not further analysed. The dichloromethane and 100% methanol washes were analysed by TLC. In the dichloromethane wash, fenazaquin was the predominant residue (0.83 mg eq/kg and 0.89 mg eq/kg for the P- and Q-labelled fenazaquin, respectively). Three metabolites were also identified: Metabolite C, Metabolite H and 4-hydroxyquinazoline in amounts ranging from 0.4 to 2.2% of TRR (0.01 to 0.06 mg eq/kg). In the 100% methanol wash, the main compound was fenazaquin (0.13 mg eq/kg and 0.30 mg eq/kg for the P- and Q-labelled fenazaquin, respectively). One unknown (retention similar to dihydroxyquinazoline) and five metabolites (Metabolite C, Metabolite H, dihydroxyquinazoline, 4-hydroxyquinazoline and TBPE) were also detected in amounts of 0.4 to 2.8% of TRR (0.01 to 0.073 mg eq/kg).

In the ethyl acetate partition of the P-labelled fenazaquin, the parent fenazaquin, TBPE and up to three unknown components were detected. One of the unknowns was very polar, one had a similar retention to 4-hydroxyquinazoline and the third had a retention time similar to dihydroxyquinazoline. The ethyl acetate partition of the Q-labelled samples also contained the parent compound, dihydroxyquinazoline, 4-hydroxyquinazoline and three unknowns: one was characterised as very polar and the others were more polar than 4-hydroxyquinazoline.

Treatment of grape bunches at the higher application rate (150 g ai/hL) were included in the experiment to assist in the identification of metabolites. The distribution of radioactivity in the high level samples was similar to that observed with the late season application samples with only a very small portion (< 2% of the TRRs) washed off with the 10% methanol wash. The dichloromethane wash removed the majority of the radioactivity in both the P- and Q-labelled samples with 40% of the TRR remaining on the fruit. Analysis of the dichloromethane washes showed the presence of the parent compound, fenazaquin which represented 38% of TRR. In the 100% methanol washes a number of other components were observed but each accounted for less than 4% of the TRR. Analysis of the ethyl acetate partitions showed the presence of more components than in the washes but fenazaquin was still the predominant analyte, accounting for an average of 14% of the TRR.

An additional experiment was conducted to assess whether the radioactivity was mainly associated with the grape skin. Grapes were peeled and the peel and pulp analysed separately. The results showed that 68% of the TRR was associated with the grape skin confirming that the major route of fenazaquin degradation is due to photolytic breakdown as the majority of the radioactivity had not yet reached the pulp tissue where metabolic processes are more likely to occur.

Based on the results presented, photolysis is likely to be a key process by which residues of fenazaquin may be broken down on grapes. The cleavage products formed either remain on the surface or penetrate into the tissue where further metabolic transformations may occur. A large fraction of these cleavage products may become associated with the natural constituents of the grapes resulting in the bound residue fraction. The presence in the washes of some metabolites of fenazaquin with the ether bridge intact (e.g. Metabolite C) indicates that cleavage of the ether bridge is not the only transformation occurring on the surface of the grapes. These other transformations may be due to photolytic processes but may also be a result of microbiological or chemical activity on the surface of the grape berries. Some fenazaquin was found in the ethyl acetate partitions of the extracts indicating that the parent compound penetrates into the grape tissue where it may be exposed to metabolic transformation.

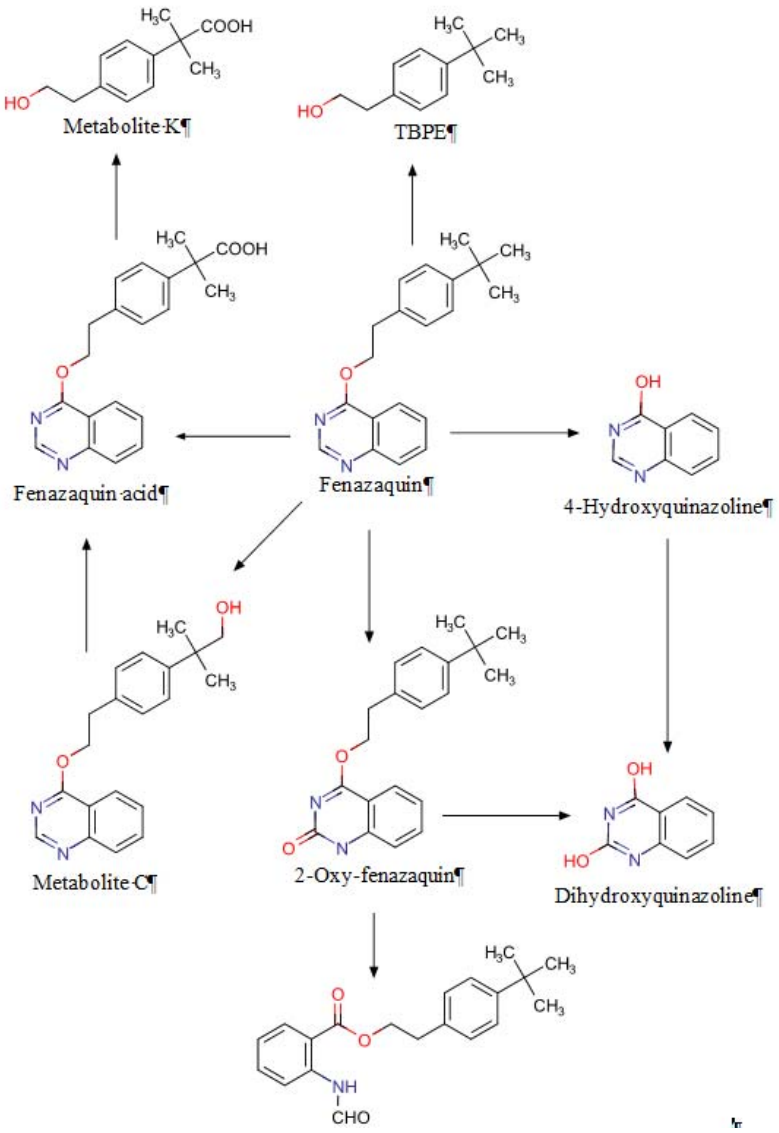


Figure 1 Proposed Metabolic Pathway of Fenazaquin on Grapes

Maize

[^{14}C]Fenazaquin labelled in the phenyl ring (P-label; specific activity 2.48 MBq/mg) and in the quinazoline portion (Q-label; specific activity 2.50 MBq/mg) were applied as aqueous suspensions at rates of 0.556 kg ai/ha and 0.549 kg ai/ha, respectively, by foliar application to field corn (*Zea Mays*) plants at the soft dough stage of development (Sugiyama *et al*., 2010, 1816W). The plants were grown in above ground wooden boxes located outdoors. Maize grain (corn-on-the-cob) and stover were harvested 20 DAT.

The total radioactive residue concentrations (TRR) of the homogenized samples were determined by combustion and liquid scintillation counting (LSC). The TRR values were relatively similar between radiolabels.

Table 15 TRRs in Maize RACs

Raw Agricultural Commodity	P-Label (mg eq/kg)	Q-Label (mg eq/kg)
Maize grain	0.003	0.013
Maize cobs	0.010	0.012
Maize stover	6.43	6.54

The nature of the ^{14}C-residues in P-label grain and P- and Q-label cobs was not further elucidated due to low levels of radioactivity. Therefore, Q-label grain and stover samples from both radiolabels were extracted sequentially with acetonitrile/water (1:1, v:v) and acetonitrile. Separate grain samples were extracted with 100% acetonitrile, as the extraction using acetonitrile/water was not successful in releasing sufficient radioactivity. For stover, the 100% acetonitrile extract was relied upon to elucidate the nature of the residues. The stover unextracted residues were subsequently refluxed for 4 hours using tetrahydrofuran (THF). The released radioactivity was analysed by reverse phase high performance liquid chromatography (HPLC) and normal phase one-dimensional thin layer chromatography (TLC). LC-MS was used to confirm the presence of the metabolites.

Table 16 Summary of characterization and identification of radioactive residues in maize harvested 20 days following application

TRRs [b] [mg eq/kg]	Q-Label maize grain [a]		P-Label maize stover		Q-Label maize stover	
	0.013		6.43		6.54	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Extracted						
Acetonitrile:water	46.2	0.006	88.0	5.851	86.6	5.236
Fenazaquin	23.1	0.003	27.0	1.798	47.4	2.866
Dimer	7.7	0.001	49.4	3.288	18.7	1.130
Fenazaquin acid	N/D	N/D	N/D	N/D	0.5	0.033
TBPE	N/D	N/D	1.8	0.119	N/D	N/D
2-hydroxy-fenazaquin	N/D	N/D	0.5	0.030	1.2	0.073
4-Hydroxyquinazoline	N/D	N/D	N/D	N/D	6.9	0.416
Unknowns (No more than 21)[c]	15.4	0.002	9.3	0.616	11.9	0.718
Tetrahydrofuran			9.9	0.658	5.7	0.342
Fenazaquin			2.8	0.185	1.4	0.087
Dimer			4.9	0.324	1.1	0.066
4-Hydroxyquinazoline			N/D	N/D	< 0.0	0.003
Unknowns (No more than 9) [d]			0.3	0.017	0.3	0.018
Bound to column			2.0	0.132	2.8	0.168
Total Extracted			97.9	6.509	92.3	5.578
Fenazaquin			29.8	1.983	48.8	2.953
Dimer			54.3	3.612	19.8	1.196
Fenazaquin acid			N/D	N/D	0.5	0.033
TBPE			1.8	0.119	N/D	N/D
2-hydroxy-fenazaquin			0.5	0.030	1.2	0.073
4-Hydroxyquinazoline			N/D	N/D	6.9	0.419
Total Identified	30.8	0.004	86.4	5.744	77.2	4.674
Total Characterized	15.4	0.002	9.6	0.633	12.2	0.736
Unextracted [d]	53.8	0.007	2.1	0.141	7.7	0.471
Accountability	100		100		100	

N/D – Not Detected

[a] From 100% acetonitrile extracts

[b] Values from combustion analysis of five replicate sub-samples

[c] Each at ≤0.07 mg eq/kg (≤ 1.1% of the TRR)

[d]. Each at ≤0.006 mg eq/kg (≤ 0.1% of the TRR)

The major component identified in the Q-label residue of maize grain was fenazaquin (23% TRR, 0.003 mg eq/kg). Fenazaquin dimer was found at 7.7% TRR (0.001 mg eq/kg). The major components identified in the acetonitrile:water extract of the P-label stover were fenazaquin (27% TRR) and fenazaquin dimer (49% TRR). Minor components identified included 2-hydroxyfenazaquin (0.5% TRR, 0.03 mg eq/kg) and TBPE (1.8% TRR, 0.12 mg eq/kg). The major components identified in the acetonitrile:water extract of the Q-label stover were fenazaquin (47% TRR, 2.87 mg eq/kg) and fenazaquin dimer (19% TRR, 1.13 mg eq/kg). Minor components identified were 2-hydroxy fenazaquin (1.2% TRR, 0.07 mg eq/kg), fenazaquin acid (0.5% TRR, 0.03 mg eq/kg), and 4-hydroxyquinazoline (6.9% TRR, 0. 42 mg eq/kg).

The TRRs released following extraction with tetrahydrofuran (THF) were 0.66 mg eq/kg (10% TRR), for the phenyl-label stover. The HPLC analyses of the extract demonstrated the presence of fenazaquin (2% of the TRR; 0.18 mg eq/kg) and fenazaquin dimer (5% of the TRR; 0.32 mg eq/kg). Approximately 2.0% TRR (0.13 mg eq/kg) did not elute from the HPLC column. This radioactivity is likely to be the residues bound to polymeric material that was solubilized by the THF.

For the quinazoline-label stover, the total residues released following hydrolysis were (5.7% TRR; 0.34 mg eq/kg). The HPLC analyses of the THF extracts demonstrated the presence of fenazaquin (1.4% of the TRR; 0.09 mg eq/kg), fenazaquin dimer (1.1% of the TRR; 0.07 mg eq/kg), and 4-hydroxyquinazoline (0.003 mg eq/kg). Approximately 50% of the residue (0.17 mg eq/kg) extracted by THF did not elute from the HPLC column. Similarly to the phenyl-label maize stover, these residues were likely bound to polymeric material solubilized by the THF.

Fenazaquin did not readily penetrate, as evidenced by the low levels of radioactive residue in the grain as compared to the much higher levels in stover. The major route of transformation is conversion to the fenazaquin dimer. The presence of the minor identified metabolites 4-hydroxyquinazoline and TBPE suggest cleavage of the ether linkage.. The intact fenazaquin appears to have also been oxidized on the quinazoline ring to yield an alcohol or oxidized on the tert-butyl group to yield a carboxylic acid.

In summary, the metabolism of fenazaquin in fruits and cereals is well understood. The majority of the radioactive residues were located on the surface of the crops with limited penetration from the peel to the pulp. Furthermore, there was no evidence of translocation from the site of application to the untreated parts of the plant. While photolysis was the major route of degradation, the primary metabolic pathways of fenazaquin include: 1) formation of the dimer; 2) cleavage of the ether linkage to form 4-hydroxyquinazoline and TBPE; 3) oxidation of the quinazoline ring to yield 2-hydroxy-fenazaquin or oxidation on the tert-butyl group to yield fenazaquin acid followed by subsequent hydroxylation of these various metabolites. The metabolites, fenazaquin acid and 2-hydroxy-fenazaquin acid were identified as major metabolites in rats while 4-hydroxyquinazoline was a minor metabolite.

Farm animal metabolism

The Meeting received information on the fate of ^{14}C- labelled fenazaquin in lactating goat and laying hens. Metabolism in laboratory animals (rat) was summarised and evaluated by the WHO panel of the 2017 JMPR.

Lactating goat

The metabolism of fenazaquin was investigated in two lactating goats (*Capra hircus,* Alpine breed), weighing 41 kg and 43 kg, dosed orally once daily for 5 consecutive days, by a balling gun, with [^{14}C-quinazoline]fenazaquin (specific activity: 74.25 µCi/mg) or [^{14}C-phenyl]fenazaquin (specific activity: 64.26 µCi/mg) at a dose level of 34 mg/day equivalent to 14 ppm feed (Dohn *et al*., 2009, 1594W-1). Milk production ranged from 1.55–2.06 L/day. During the treatment period, milk was collected twice daily while urine and faeces were collected once daily. Blood was drawn once daily and separated into plasma and blood cells. Only the plasma was retained for analysis. At sacrifice (within 22 hours after the final dose) samples of liver, kidney, muscle (loin and leg), fat (omental and perirenal), blood, bile and GI tract were collected.

The total radioactive residues (TRRs) were determined by liquid scintillation counting (LSC) for liquid samples and by combustion/LSC or tissue solubilisation/LSC for solid samples.

The major route of elimination of the radioactivity was via the feces which accounted for 64–91% of the total administered radioactivity (AD), while urine accounted for approximately 5% of the AD and milk accounted for 0.1% of the AD. The tissue burden was low (<1% of the AD). The overall recovery of administered radioactivity ranged considerably between radiolabels: 108% for the P-label goat and 80% for the Q-label goat.

The total radioactive residues (TRRs) were highest in liver (0.41–0.79 mg eq/kg) followed by fat (0.09 mg eq/kg for Q-label composite fat and 0.07–0.15 mg eq/kg for renal, omental and subcutaneous fat), kidney (0.04-0.09 mg eq/kg), muscle (0.005 mg eq/kg for Q-label composite muscle and 0.007–0.03 mg eq/kg for flank, loin or composite muscle). The tissues from goats administered the [^{14}C-phenyl]fenazaquin had consistently higher concentrations of radioactive residues. The concentrations of ^{14}C-residues in plasma samples, taken approximately 24 hours after individual dose administration, were relatively constant for each test animal (means of 0.012 mg eq/kg for the quinazoline label and 0.026 mg eq/kg for the phenyl label). The plasma ^{14}C-residues were consistently higher for the phenyl treatment.

Table 17 Balance of radioactivity in goats following oral administration of [^{14}C-quinazoline]fenazaquin and [^{14}C-phenyl]fenazaquin for 6 consecutive days

Sample	P-Label		Q-Label	
	%AD	mg eq/kg	%AD	mg eq/kg
Milk	0.1		0.1	
Liver	0.39	0.789	0.21	0.413
Kidney	0.01	0.092	0.00	0.038
Composite fat		0.120		0.089
Renal fat		0.147		
Omental fat		0.146		
Subcutaneous fat		0.068		
Composite muscle				0.005
Flank muscle		0.026		
Loin muscle		0.007		
GI tract/Rumen	10.95		10.12	
Blood/Plasma		0.026		0.012
Bile	0.44		0.02	
Urine	5.24		5.64	
Faeces	90.90		64.15	
Cage wash	0.02		0.04	
Total Recovery	108		80	

While TRRs in milk seemed to peak by the 3rd day of dosing, there was a periodic variation in the concentrations, with the evening samples having consistently higher TRRs than the morning samples.

Table 18 TRRs in goat milk following oral administration of [^{14}C-quinazoline]fenazaquin and [^{14}C-phenyl]fenazaquin for 6 days

Collection Day	P-Label	Q-Label
	mg eq/kg	mg eq/kg
Day 1 AM (prior to milking)	N/A	N/A
Day 1 PM	0.019	0.038
Day 2 AM	0.010	0.015
Day 2 PM	0.020	0.028
Day 3 AM	0.012	0.014
Day 3 PM	0.038	0.030
Day 4 AM	0.011	0.014
Day 4 PM	0.038	0.027
Day 5 AM	0.011	0.012
Day 5 PM	0.020	0.016
Day 6 AM	0.013	0.015

N/A - Not applicable

The milk samples from the Day 4 (PM) collection were selected for characterization. Tissue samples with TRR values > 0.01 mg eq/kg were subjected to extraction and chromatographic analyses. Moreover, the composite quinazoline-label muscle sample and the phenyl label loin muscle contained ^{14}C-residues < 0.01 mg eq/kg, and were not subjected to further analysis. However, the phenyl-label flank muscle was characterized. Samples of fat, liver, and kidney from both test animals were subjected to extraction and chromatographic analysis. The phenyl-label fat sample was a composite of equal amounts of renal, omental, and subcutaneous fat.

Radioactive components were characterized by reverse phase HPLC using authentic reference standards. The identity of metabolites matching available reference standards were confirmed by normal phase TLC. Selected unknown metabolites were identified/characterized by mass spectroscopy.

Table 19 Characterization and identification of radioactivity in goat milk and liver

Component	Milk				Liver			
	P-Label [0.034 mg eq/kg]		Q-Label [0.026 mg eq/kg]		P-Label [0.789 mg eq/kg]		Q-Label [0.413 mg eq/kg]	
	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
Extracted [a]	**88.2**	**0.030**	**92.3**	**0.024**	**60.8**	**0.433**	**42.2**	**0.166**
Fenazaquin	*47.1*	*0.016*	*15.4*	*0.004*	*N/D*	*N/D*	*N/D*	*N/D*
2-hydroxy-fenazaquin acid	*2.9*	*0.001*	*3.8*	*0.001*	*14.9*	*0.106*	*13.7*	*0.054*
4-Hydroxyquinazoline	*N/D*	*N/D*	*23.1*	*0.006*	*N/D*	*N/D*	*9.4*	*0.037*
Metabolite G	*N/D*	*N/D*	*N/D*	*N/D*	*18.8*	*0.134*	*N/D*	*N/D*
Metabolite H	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*1.5*	*0.006*
Unknowns	*14.7*	*0.005*	*11.5*	*0.004*	*17.4*	*0.124*	*11.7*	*0.046*
Total Identified [b]	*50.0*	*0.017*	*42.3*	*0.011*	*33.7*	*0.240*	*24.6*	*0.097*
Total Characterized [c]	*38.2*	*0.013*	*50.0*	*0.013*	*28.6*	*0.190*	*15.8*	*0.062*
Unextracted	**11.8**	**0.004**	**7.7**	**0.002**	**35.7**	**0.253**	**57.8**	**0.227**
Accountability	100		100		97		99	

N/D- Not detected

[a] milk, acetone hexane (1:4, v:v) followed by acetone, liver, acetonitrile: water (1:1, v:v) followed by acetonitrile;

[b] Total Identified = fenazaquin + 2-hydroxy-fenazaquin acid +4-hydroxyquinazoline + Metabolites G and H

[c] Total Characterized = Extractable components observed on HPLC that did not match available reference standards + Unknowns + Methanol:water extracts

Table 20 Characterization and identification of radioactivity in goat kidney, flank muscle and composite fat

Component	Kidney				Flank Muscle		Composite Fat			
	P-Label [0.092 mg eq/kg)		Q-Label [0.038 mg eq/kg]		P-Label [0.026 mg eq/kg]		P-Label [0.120 mg eq/kg]		Q-Label [0.089 mg eq/kg]	
	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
Extracted	**87.9**	**0.080**	**88.6**	**0.031**	**84.0**	**0.021**	**92.8**	**0.103**	**97.7**	**0.086**
Fenazaquin	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*77.3*	*0.085*	*83.0*	*0.073*
4-Hydroxyquinazoline	*N/D*	*N/D*	*5.7*	*0.002*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*
2-Hydroxy-fenazaquin	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*0.9*	*0.001*	*1.1*	*0.001*
2-Hydroxy-fenazaquin acid	*25.3*	*0.023*	*28.6*	*0.010*	*20.0*	*0.005*	*0.9*	*0.001*	*N/D*	*N/D*
Metabolite G	*8.8*	*0.008*	*ND*	*ND*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*
Unknowns	*14.3*	*0.013*	*11.4*	*0.004*	*12.0*	*0.003*	*1.8*	*0.002*	*4.5*	*0.004*
Total identified [a]	*34.1*	*0.031*	*34.3*	*0.012*	*20.0*	*0.005*	*79.1*	*0.087*	*84.1*	*0.074*
Total Characterized [b]	*53.9*	*0.049*	*54.2*	*0.019*	*64.0*	*0.016*	*13.7*	*0.016*	*13.6*	*0.012*
Unextracted	**12.1**	**0.011**	**11.4**	**0.004**	**16.0**	**0.004**	**7.2**	**0.008**	**2.3**	**0.002**
Accountability	100		100		100		100		100	

N/D- Not detected

[a] Total Identified = fenazaquin + 2-hydroxy-fenazaquin + 2-hydroxy-fenazaquin acid + 4-hydroxyquinazoline + Metabolite G

[b] Total Characterized = Extractable components observed on HPLC that did not match available reference standards + Unknowns + Methanol:water extracts

Greater than 88% of the TRR in milk was extracted following sequential extraction with acetonitrile and acetonitrile:water (1:1, v:v). Fenazaquin was detected as a predominant component of the residue in both P-label and Q-label milk samples (15–47% of the TRR; 0.004 to 0.016 mg eq/kg). 4-Hydroxyquinazoline (23% of the TRR; 0.006 mg eq/kg) was found in the Q-label milk sample only. The phenyl label sample was separated into milk fat and skim milk before analysis. The fenazaquin in this sample was found almost entirely in the milk fat portion of the whole milk sample (73% of the TRR; 0.016 mg eq/kg).

Liver samples from both goats were extracted twice with acetonitrile:water (1:1), and once with acetonitrile. The solids remaining after the acetonitrile:water extraction of the quinazoline liver sample were further extracted with methanol water. This procedure did not extract significant residue, and was not applied to the phenyl label sample. The combined extracts accounted for 42–61% of the TRR. While fenazaquin was not detected in either sample of liver, 2-hydroxy-fenazaquin acid was the predominant residue accounting for 14–15% of the TRR (0.05–0.11 mg eq/kg). Considering the solvents did not extract sufficient residues, the P-label and Q-label PES samples were extracted with acetone and ethyl acetate. However, these solvents were also ineffective in releasing significant amounts of the bound residue. Mild acid and base hydrolyses carried out on portions of both liver PES samples also resulted in poor solubilization (< 5% of TRR). The use of 1N sodium hydroxide at 100 °C for 2 hours released 0.08 and 0.12 mg eq/kg respectively, from the quinazoline and phenyl label liver PES samples. Strong refluxing acid (6N HCl at 100 °C for 17hr) released 0.21 mg eq/kg (Q-label) and 0.10 mg eq/kg (P-label). These acid hydrolysates were neutralized and analysed by HPLC. 4-Hydroxyquinazoline was the only metabolite identified in the hydrolyzed Q-label sample (9% of the TRR; 0.04 mg eq/kg). In the acid hydrolysate from the P-label sample, only Metabolite G and other unidentified components were detected, yet none exceeded 6% of the TRR.

The kidney samples were extracted in a similar manner to liver samples, with 88-89% of the TRRs being extracted. No fenazaquin was detected in either sample. The most abundant identified metabolites were 2-hydroxy-fenazaquin acid (29% of the TRR; 0.023 mg eq/kg) and Metabolite G (P-label only; 9% of the TRR [0.008 mg eq/kg]). Total characterized residues accounted for 54% of the TRR (≤ 0.05 mg eq/kg).

The phenyl label flank muscle sample was extracted twice with acetonitrile:water (1:1) and once with acetonitrile, resulting in 84% of the TRRs being extracted. Fenazaquin was not detected in

flank muscle. The predominant metabolite observed was 2-hydroxy-fenazaquin acid (20% of the TRR; 0.005 mg eq/kg). Total characterized residues accounted for 64% of the TRR (≤ 0.02 mg eq/kg).

Greater than 92% of the TRRs in both composite fat samples were extracted, following two extractions with acetone:hexane (1:4 v/v) and a third extraction with 100% acetone. The residue present in the extracted fat (dissolved in hexane) that could not be back-extracted into acetonitrile for analysis accounted for 9–12% of the TRR. The primary component of the residue in both composite fat samples was fenazaquin (0.073 to 0.085 mg eq/kg; > 75% of the TRR). A trace (0.001 mg eq/kg) of 2-hydroxy-fenazaquin was detected in both fat samples, and a trace of 2-hydroxy-fenazaquin acid was detected in the phenyl label sample.

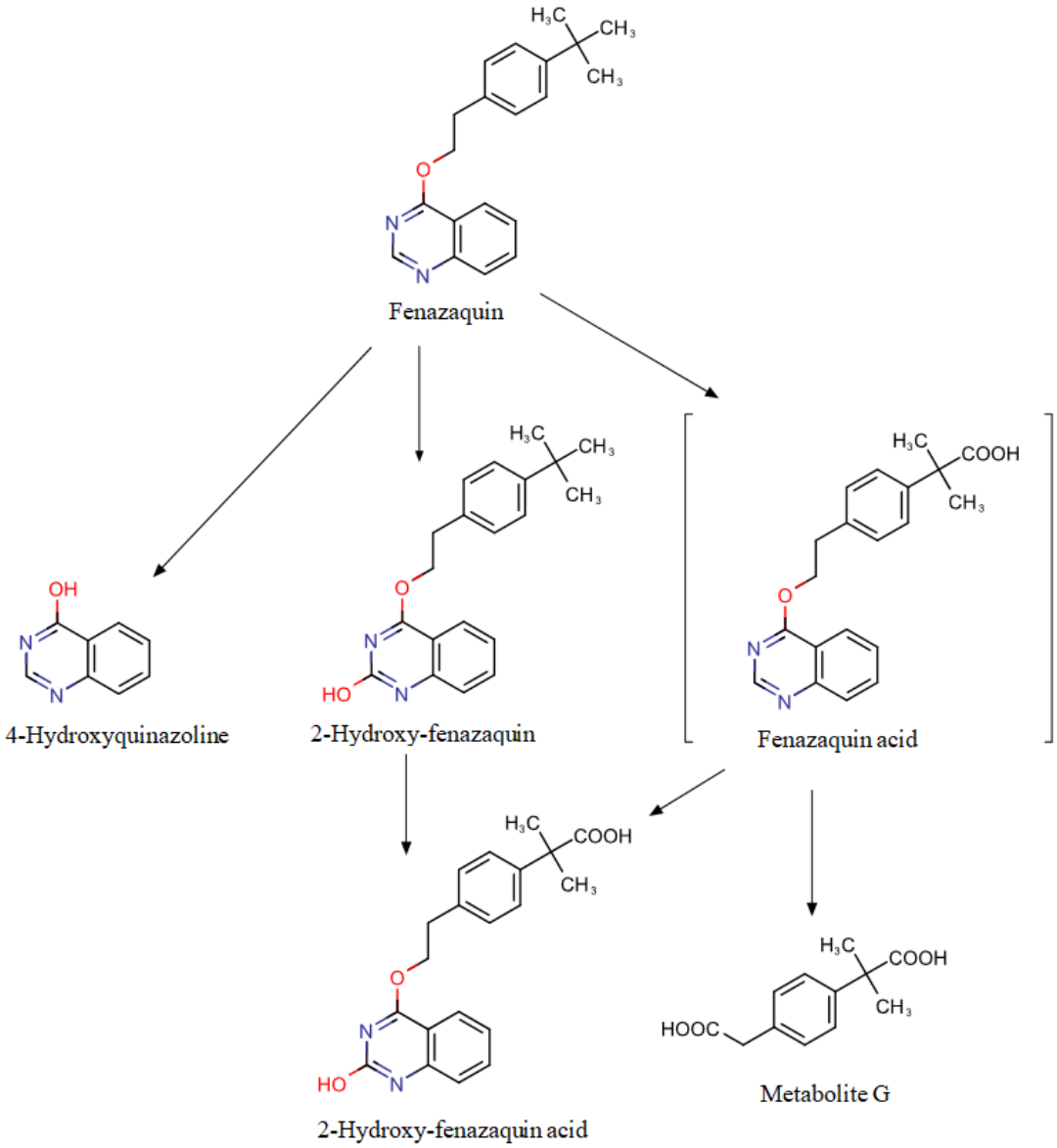


Figure 2 Proposed Metabolic Pathway of Fenazaquin in Goats

Laying Hen

Twenty laying hens (*Gallus gallus* domesticus), weighing on average 1.4 kg, were dosed orally once daily for seven consecutive days with [^{14}C-quinazoline]fenazaquin (specific activity: 0.144 mCi/mg)

at a dose level of 12.3 mg/kg feed, or [^{14}C-phenyl]fenazaquin (specific activity: 0.167 mCi/mg) at a dose level of 12.4 mg/kg feed (Quistad *et al.,* 2009, 1866W). The test substances were administered using cellulose-filled gelatin capsules. Eggs were collected twice daily, in the morning before and in the afternoon after administration, while excreta were collected once daily. The animals were sacrificed approximately 21–23 hours after the last dose and the liver, breast and thigh muscle, omental fat, subcutaneous fat and gastrointestinal tracts (with contents) were collected and pooled by treatment group. The total radioactive residues (TRRs) were determined by liquid scintillation counting (LSC) for liquid samples and by combustion/LSC or tissue solubilisation/LSC for solid samples.

Approximately 89% of the administered dose (AD) was recovered, most of which (88% of the AD) was excreta-related. Total radioactive residues (TRR) in eggs accounted for 0.1–0.3% of the AD. The tissue burden was low (< 0.1% of the AD) with highest concentrations found in fat (0.06% of the AD), followed by liver (0.04% of the AD) and muscle (0.04% of the AD).

Table 21 Balance of radioactivity in hens following oral administration of [^{14}C]fenazaquin for 4 days

	P-Label		Q-Label	
Sample	%AD	mg eq/kg	%AD	mg eq/kg
Eggs	0.04		0.31	
Omental Fat	0.0	0.181	0.0	0.162
Subcutaneous fat	0.0	0.170	0.0	0.170
Thigh muscle	0.0	0.020	0.0	0.068
Breast muscle	0.0	0.005	0.0	0.058
Liver	0.0	0.057	0.0	0.096
GI Tract	0.3		0.4	
Excreta	89.5		87.6	
Total Recovery	89.8		88.3	

Table 22 Average [a] TRRs in eggs following oral administration of [^{14}C]fenazaquin for 7 days

Day	P-Label		Q-Label	
	%AD	mg eq/kg	%AD	mg eq/kg
1	ND	ND	ND	ND
2	0.0	0.004	0.01	0.015
3	0.0	0.007	0.03	0.086
4	0.0	0.013	0.04	0.102
5	0.01	0.025	0.05	0.116
6	0.01	0.028	0.06	0.132
7	0.01	0.030	0.06	0.128
8	0.01	0.025	0.06	0.143
Total	0.04		0.31	

[a] Average of the morning and evening samples

Eggs, muscle and liver samples were extracted twice with acetonitrile:water (l:l, v:v) and once with acetonitrile. The extracts were combined, concentrated, partitioned with hexane and analysed by both HPLC and TLC. The quinazoline radiolabel PES was treated with 0.1 M KOH, (1 hr at room temperature), followed by 24% KOH overnight at room temperature. The KOH extracts were combined, acidified, and then partitioned with ethyl acetate.Isolation of metabolites was performed by fractionation using HPLC. The fractions were analysed using LC-MS, NMR or 2-D TLC. The nature of the residues in extracts was elucidated using LC-MS, ^{1}H-NMR or 2-D TLC or by co-chromatography against authentic reference standards.

Fat samples were extracted with acetone:hexane (1:4, v:v) and 100% acetone. All three extracts were combined, concentrated by rotoevaporation redissolved in hexane and partitioned with acetonitrile prior to analysis by HPLC and TLC. The hexane phases were loaded onto preconditioned columns and then rinsed with 100% hexane followed by 5% ethyl acetate in hexane. The ethyl acetate fractions were concentrated to dryness, redissolved in acetonitrile, and then analysed by HPLC and TLC.

Table 23 Characterization and identification of radioactivity in eggs and fat

Component	Day 7 Eggs				Omental Fat				Subcutaneous Fat			
	P-Label [0.023 mg eq /kg]		Q-Label [0.142 mg eq/kg]		P-Label [0.175 mg eq/kg]		Q-label [0.155 mg eq/kg]		P-Label [0.179 mg eq/kg]		Q-Label [0.161 mg eq/kg]	
	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
Extracted	73.9	0.017	90.8	0.129	100	0.175	99.4	0.154	100.0	0.179	98.1	0.158
Aqueous soluble	39.1	0.009	85.9	0.122	64.6	0.113	72.3	0.112	70.9	0.127	79.5	0.128
Fenazaquin	*13.0*	*0.003*	*2.1*	*0.003*	*64.0*	*0.112*	*68.4*	*0.106*	*70.9*	*0.127*	*64.6*	*0.104*
Fenazaquin acid	*13.0*	*0.003*	*1.4*	*0.002*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*	*N/D*
Benzoyleneurea	*N/D*	*N/D*	*82.4*	*0.117*	*N/D*	*N/D*	*3.2*	*0.005*	*N/D*	*N/D*	*14.9*	*0.024*
Unknowns	*13.1*	*0.003*	*N/D*	*N/D*	*0.6*	*0.001*	*0.7*	*0.001*	*N/D*	*N/D*	*N/D*	*N/D*
Hexane soluble	34.8	0.008	4.9	0.007	35.4	0.062	27.1	0.042	29.1	0.052	18.6	0.030
Fenazaquin	*N/D*	*N/D*	*N/D*	*N/D*	*26.2*	*0.046*	*26.5*	*0.041*	*17.9*	*0.032*	*18.6*	*0.030*
Unknown	*N/D*	*N/D*	*N/D*	*N/D*	*9.1*	*0.016*	*0.6*	*0.001*	*11.2*	*0.020*	*N/D*	*N/D*
Unextracted	26.1	0.006	9.2	0.013	0	N/A	0.6	N/A	0	N/A	1.9	0.003
0.1 M KOH Hydrolysate	N/A	N/A	2.8	0.004	N/A	N/A	N/A	N/A	N/A	N/A	N/A	NA
24% KOH Hydrolysate	N/A	N/A	6.3	0.009	N/A	N/A	N/A	N/A	N/A	N/A	N/A	NA
Total Extracted [a]	N/A	N/A	100	0.142	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total identified [b]	*26.0*	*0.006*	*85.9*	*0.122*	*89.1*	*0.156*	*98.1*	*0.152*	*88.8*	*0.159*	*98.1*	*0.158*
Total Characterized [c]	*13.1*	*0.003*	*14.1*	*0.020*	*9.1*	*0.016*	*1.3*	*0.002*	*11.2*	*0.020*	--	--
Accountability	100		100		100		100		100		100	

Note: TRRs reported reflect those determined based on extraction and not combustion

N/A- Not applicable; N/D- Not detected

[a] Total extracted = Solvent extracted + 0.1 M KOH Hydrolysate + 24% KOH Hydrolysate

[b] Total identified = fenazaquin + fenazaquin acid + Benzoyleneurea

[c] Total Characterized = Unknowns + hexane soluble + 0.1 M KOH Hydrolysate + 24% KOH Hydrolysate

Table 24 Characterization and identification of radioactivity in muscle and liver

Component	Thigh Muscle				Breast Muscle				Liver			
	P-Label [0.016 mg eq /kg]		Q-Label [0.060 mg eq/kg]		P-Label [0.005 mg eq/kg]		Q-label [0.054 mg eq/kg]		P-Label [0.043 mg eq/kg]		Q-Label [0.161 mg eq/kg]	
	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg
Extracted	75.0	0.012	71.7	0.043	80.0	0.004	75.9	0.041	39.5	0.017	79.3	0.069
Fenazaquin	*68.8*	*0.011*	*5.0*	*0.003*	*20.0*	*0.001*	*1.9*	*0.001*	*N/D*	*N/D*	*N/D*	*N/D*
Fenazaquin acid	*N/D*	*N/D*	*N/D*	*N/D*	*20.0*	*0.001*	*N/D*	*N/D*	*7.0*	*0.003*	*4.6*	*0.004*
Benzoyleneurea	*N/D*	*N/D*	*63.3*	*0.038*	*N/D*	*N/D*	*70.4*	*0.038*	*ND*	*ND*	*52.9*	*0.046*
Unknowns	*6.2*	*0.001*	*3.4*	*0.002*	*20.0*	*0.001*	*3.8*	*0.002*	*32.5*	*0.014*	*13.7*	*0.012*
Unextracted	N/A	N/A	23.3	0.014	N/A	N/A	16.7	0.009	46.5	0.020	18.4	0.016
0.1 M KOH Hydrolysate	N/A	N/A	8.3	0.005	N/A	N/A	5.6	0.003	4.7	0.002	3.4	0.003
24% KOH Hydrolysate	N/A	N/A	15.0	0.009	N/A	N/A	11.1	0.006	41.9	0.018	14.9	0.013
Total Extracted [a]	75.0	0.012	95.0	0.057	80.0	0.004	92.6	0.050	86.0	0.037	97.7	0.085
Total identified [b]	*68.8*	*0.011*	*68.3*	*0.041*	*40.0*	*0.002*	*72.3*	*0.039*	*7.0*	*0.003*	*67.8*	*0.059*
Total Characterized [c]	*6.2*	*0.001*	*26.7*	*0.016*	*20.0*	*0.001*	*20.5*	*0.011*	*79.0*	*0.034*	*32.0*	*0.028*
Total Unextracted	25.0	0.004	5.0	0.003	20.0	0.001	7.4	0.004	14.0	0.006	2.3	0.002
Accountability	100		100		100		100		100		100	

Note: TRRs reported reflect those determined based on extraction and not combustion

N/A - Not applicable; N/D - Not detected

[a] Total extracted = Solvent extracted + 0.1 M KOH Hydrolysate + 24% KOH Hydrolysate

[b] Total identified = Fenazaquin + fenazaquin acid + benzoyleneurea

[c] Total characterized = Unknowns + 0.1 M KOH Hydrolysate + 24% KOH Hydrolysate

Acetonitrile:water extracted greater than 74% of the TRRs in P- and Q-label eggs. The major residues in P-label eggs were fenazaquin (13% TRR; 0.003 mg eq/kg) and fenazaquin acid (13% TRR; 0.003 mg eq/kg). None of the individual unknowns accounted for more than 4% TRR (0.001 mg eq/kg). The PES contained 26% TRR (0.006 mg eq/kg). Fenazaquin and fenazaquin acid were also present in Q-label eggs (2% TRR; 0.003 mg eq/kg and 1% TRR; 0.002 mg eq/kg, respectively). Benzoyleneurea, the major residue in Q-label eggs, accounted for 82% TRR (0.12 mg eq/kg). Treatment of the Q-label PES with 0.1 M KOH in methanol/water (1:1) followed by 24% KOH released an additional 9% TRR (0.01 mg eq/kg). When acidified and partitioned with ethyl acetate, most of the residue remained in the aqueous phase with a limited amount in the ethyl acetate phase. Neither the aqueous or ethyl acetate phases were analysed as the radioactivity was too low. All the radioactivity in PES was released following hydrolysis.

All of the P- and Q-radiolabel in omental and subcutaneous fats were readily extracted with acetone and hexane. The predominant residue in omental and subcutaneous fats was fenazaquin (83–95% TRR; 0.13–0.16 mg eq/kg). While no other residue was identified in P-label fats, benzoyleneurea was detected in the Q-label fats (3–15% TRR, 0.005–0.02 mg eq/kg). The PES contained ≤ 0.003 mg eq/kg (< 2% TRR).

In the P-label thigh and breast muscles, 75–80% of the TRR were extracted. Fenazaquin (20–69% TRR; 0.01–0.011 mg eq/kg) and fenazaquin acid (20% TRR; 0.001 mg eq/kg) were the only residues detected. In the Q-label thigh and breast muscles, benzoyleneurea was the major residue found (63–70% TRR; 0.04 mg eq/kg) while fenazaquin accounted for ≤ 5% TRR (≤ 0.003 mg eq/kg). Treatment of the Q-label PES with 0.1 M KOH in methanol/water (1:1) followed by 24% KOH released an additional up to 23% TRR (0.01 mg eq/kg). When acidified and partitioned with ethyl acetate, most of the residue remained in the aqueous phase with a minimal amount being partitioning into the ethyl acetate. The PES contained ≤ 7% TRR (≤ 0.004 mg eq/kg).

The combined acetonitrile/water and acetonitrile extracts represented 40–79% of the TRR in liver. No fenazaquin was detected in either P- or Q-label liver. Moreover, in the P-label liver, fenazaquin acid was the only residue identified (7% TRR; 0.003 mg eq/kg), while in the Q-label liver, benzoyleneurea was the predominant residue accounting for 53% TRR (0.05 mg eq/kg). Hydrolysis of the PES with 0.1 M KOH in methanol/water (1:1) followed by 24% KOH released an additional 18–46% TRR (0.02 mg eq/kg), of which 6–19% TRR (< 0.01 mg eq/kg) partitioned into the organic phase. The solids after hydrolysis accounted for ≤ 14% TRR (≤ 0.006 mg eq/kg).

According to the metabolic pathway of fenazaquin in the laying hen, oxidation of the tert-butyl group gives fenazaquin acid. It appears that the methylene group adjacent to the oxygen of the ether is oxidized to release the 4-hydroxyquinazoline that is further oxidized to benzoyleneurea.

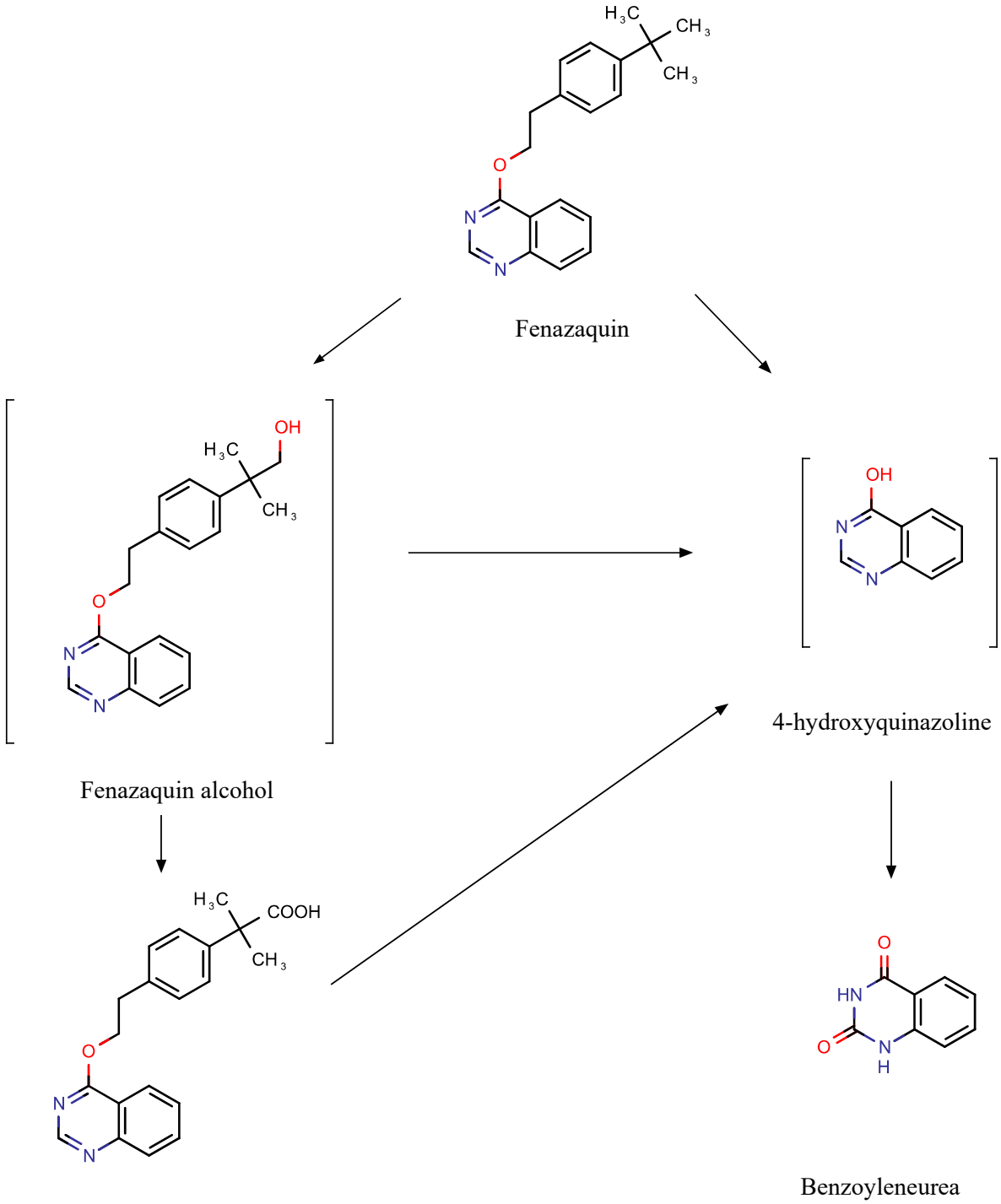


Fig Fenazaquin acid Pathway of Fenazaquin in Laying Hens

The Meeting concluded that, in all species investigated (goats, hens and rats), the total administered radioactivity was predominantly eliminated in excreta. The metabolic profiles differed quantitatively between the species, yet qualitatively there are no major differences with the exception that the metabolism in goats was more extensive. The routes and products of metabolism were similar across all animals, resulting from oxidation of the tert-butyl group giving rise to the hydroxyl-fenazaquin (goats only), fenazaquin alcohol (postulated), fenazaquin acid and its hydroxylated form. The methylene group adjacent to the oxygen of the ether is also oxidized to release the 4-hydroxyquinazoline that is further oxidized to benzoyleneurea (laying hens only).

ENVIRONMENTAL FATE IN SOIL

The FAO Manual on the Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed (2016) explains the data requirements for studies of environmental fate. The focus should be on those aspects that are most relevant to MRL setting. Aerobic degradation in soil is relevant, as well as the normal requirements for hydrolysis, photolysis.

While the Meeting received information on soil aerobic metabolism, hydrolysis and photolysis properties of fenazaquin, the Meeting did not receive studies on the behaviour of [^{14}C]fenazaquin in confined rotational crops since the crops currently being considered are permanent or semi-permanent and are not typically rotated.

Route of Degradation in Soil

Aerobic degradation in soil

A number of studies have investigated the aerobic degradation of [^{14}C]fenazaquin in soil.

The degradation of ^{14}C-fenazaquin, uniformly labelled in the tert-butylphenyl ring was studied in four soils in the laboratory under aerobic conditions (Osborne *et al*., 1992, 722-002). The soils were characterised as a loamy sand, a silty sand, a sandy clay loam and a clayish silt.

Parameter	Soil Characteristics			
Particle size	Loamy sand	Silty sand	Sandy clay loam	Clayish silt
Sand (%)	47	7	92	86
Silt (%)	27	79	13	11
Clay (%)	26	14	5	3
Organic carbon (%)	4.3	1.2	2.3	0.6
pH	7.4	7.0	6.3	6.5
CEC (m Eq/100g)	25.0	11.0	9.7	4.0

The soils were incubated in the dark at 20 ºC. Fenazaquin was applied to each soil subsample at a rate of 0.27 mg/kg. Volatiles were collected in trapping solutions. In parallel to the samples of microbially active soil, sterilised samples were also prepared. Samples of soil were removed for analysis after 0, 1, 3, 7 and 14 days after application (DAA)and 1, 3 and 6 months. The sterilised soil was sampled after 3 and 6 months.

The soils were extracted by refluxing with acetonitrile and shaking with acetonitrile/0.1N sodium hydroxide. In addition the one- and three-month samples were extracted by shaking with methanolic sodium hydroxide or methanol. Radioactivity in the extracts was determined by liquid scintillation counting. Unextracted radioactivity was measured by combustion of the extracted soils. All extracts which contained greater than 10% of the applied radioactivity (AR) were analysed by thin layer chromatography. The extracts were co-chromatographed in parallel to unlabelled reference compounds.

The degradation of fenazaquin started slowly. The predominant degradation product in all soils was 2-oxy-fenazaquin which reached a maximum of 9.1% of the AR in the clayish silt at the end of the study. A similar trend was observed in the loamy sand where it reached a maximum of 5% by six months. In the sandy clay loam it reached its maximum of 5% after one month and declined thereafter. In the silty sand it peaked (2.1%) at three months while fenazaquin acid reached its

maximum concentration (2.1%) at 14 DAA in the loamy sand. A similar trend was observed in the sandy clay loam and clayish silt. In the silty sand, the maximum (1.4%) was observed in the three-month sample. In addition to these two metabolites, Metabolite G was detected in the loamy sand at 1.1% at three months and six months. This metabolite was not detected in the other soils.

Metabolite H was detected at 0.8% of the AR, at three and six month time points in the loamy sand. In the silty sand, it reached its maximum of 2.0% at six months. It was not detected in the other two soils. An unknown metabolite was also detected at 1.2%, each in the loamy sand at clayish silt at three months, and in the silty sand at 3.1% at the end of the study. For all soils the mineralisation to carbon dioxide was high. The value reached by six months ranged from 30% in the silty sand to 38% in the loamy sand. The residual, unextracted residue increased to between 14% and 27% after six months. The half-lives (DT_{50}) determined using single first-order (SFO) ranged from 48.1 days in the sandy clay loam, to 57.6 days in the clayish silt, to 88.1 days in the loamy sand and finally to 114.1 days in the silty sand. The results of the sterilised control samples showed that the degradation of fenazaquin was mainly microbiological.

Table 25 Distribution of the applied radioactivity (% AR) in the various soil types

	Time after application							
	0 day	1 day	3 day	7 day	14 day	1 month	3 month	6 month
	Loamy sand							
ACN extracts	88.5	91.8	903	86.1	85.7	77.9	51.6	31.9
Fenazaquin	88.3	91.8	90.3	86.1	81.7	72.7	42.9	25.0
2-Oxy-fenazaquin	N/A	N/A	N/A	N/A	1.9	2.3	3.8	5.0
Fenazaquin acid	N/A	N/A	N/A	N/A	2.1	2.1	1.8	N/A
Metabolite G	N/A	N/A	N/A	N/A	N/A	0.8	1.1	1.1
Metabolite H	N/A	N/A	N/A	N/A	N/A	N/A	0.8	0.8
Unknowns	N/A	N/A	N/A	N/A	N/A	N/A	1.2	N/A
0.1 N NaOH/ACN	13.9	14.8	15.5	15.2	15.7	14.7	11.4	6.5
Fenazaquin	13.9	14.8	15.5	15.2	14.4	11.8	5.2	1.1
Metabolites (sum)	N/A	N/A	N/A	N/A	1.3	2.9	6.2	5.4
MeOH or methanolic NaOH	N/A	N/A	N/A	N/A	N/A	1.1	1.9	N/A
Volatiles	--	0.1	0.3	1.1	2.4	7.1	21.5	37.7
Unextracted	1.7	1.7	1.9	3.1	4.1	6.3	10.9	19.3
Total Recovered	104.1	108.4	108.0	105.5	107.9	107.1	97.3	95.4
	Silty Sand							
ACN extracts	88.2	81.7	85.6	91.7	74.0	77.2	54.9	40.1
Fenazaquin	88.2	81.7	85.6	91.7	71.4	72.0	48.8	33.7
2-Oxy-fenazaquin	N/A	N/A	N/A	N/A	0.9	1.6	2.1	0.7
Fenazaquin acid	N/A	N/A	N/A	N/A	0.9	1.3	1.4	0.3
Metabolite G	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Metabolite H	N/A	N/A	N/A	N/A	N/A	1.1	1.5	2.0
Unknowns	N/A	N/A	N/A	N/A	0.8	1.2	1.2	3.1
0.1 N NaOH/ACN	11.1	11.4	10.0	9.0	11.4	12.7	9.2	8.5
Fenazaquin	11.1	11.4	10.0	9.0	10.9	11.3	7.2	2.3
Metabolites (sum)	N/A	N/A	N/A	N/A	0.8	1.4	2.0	6.2
Volatiles	--	<0.1	0.2	1.1	2.7	6.2	19.6	30.2
Unextracted	2.1	1.4	1.4	2.0	2.8	4.9	10.8	13.9
Total Recovered	101.4	94.6	97.2	103.8	91.2	101.0	94.5	92.7
	Sandy Clay Loam							
ACN extracts	89.8	90.2	94.8	87.5	80.0	37.2	33.3	21.1
Fenazaquin	89.8	90.2	93.0	83.9	74.7	60.6	27.4	16.0
2-Oxy-fenazaquin	N/A	N/A	1.8	2.3	3.5	5.0	4.7	3.6
Fenazaquin acid	N/A	N/A	N/A	1.3	1.8	1.3	1.2	0.9
Metabolite G	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Metabolite H	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Unknowns	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
0.1 N NaOH/ACN	12.6	14.9	13.1	15.0	15.9	13.7	8.9	5.0
Fenazaquin	12.6	14.9	13.1	12.2	10.7	8.8	1.7	0.7
Metabolites (sum)	NA	NA	NA	2.8	5.2	4.9	7.2	4.3
MeOH or methanolic NaOH	N/A	N/A	N/A	N/A	N/A	1.8	2.5	N/A

	Time after application							
	0 day	1 day	3 day	7 day	14 day	1 month	3 month	6 month
Volatiles	--	0.1	0.3	1.1	3.8	10.5	30.5	37.3
Unextracted	3.8	2.2	5.4	4.6	7.7	7.5	19.9	26.8
Total Recovered	106.2	107.4	113.6	108.2	107.4	100.7	95.1	90.2
	Clayish silt							
ACN extracts	93.5	94.9	91.3	87.8	84.5	70.4	40.7	27.2
Fenazaquin	93.5	94.9	90.9	82.9	78.1	61.9	30.8	17.2
2-Oxy-fenazaquin	N/A	N/A	0.9	3.5	4.5	5.5	7.2	9.1
Fenazaquin acid	N/A	N/A	N/A	1.4	1.9	1.8	1.6	N/A
Metabolite G	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Metabolite H	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Unknowns	N/A	N/A	N/A	N/A	N/A	N/A	1.2	1.1
0.1 N NaOH/ACN	10.5	13.2	11.1	16.9	15.7	13.5	10.3	7.3
Fenazaquin	10.5	13.2	11.1	14.9	11.2	6.6	5.2	3.0
Metabolites (sum)	N/A	N/A	N/A	2.0	4.5	6.9	5.1	N/A
MeOH or methanolic NaOH	N/A	N/A	N/A	N/A	N/A	1.3	1.9	N/A
Volatiles	NA	0.1	0.3	1.0	2.5	7.3	23.6	33.3
Unextracted	3.6	1.4	4.2	3.8	6.5	12.3	18.7	22.8
Total Recovered	107.6	109.6	106.9	109.5	109.2	104.8	95.2	90.6

N/A- Not analysed

Table 26 Summary of DT_{50} for fenazaquin under aerobic soil conditions

Soil	DT_{50} (days)	DT_{90} (days)
Sandy clay loam	34.2	307.3
Clayish silt	57.6	300.8
Loamy sand	88.1	292.6
Silty sand	114.1	--

-- could not be calculated

The metabolism and rate of degradation of ^{14}C-fenazaquin was also investigated in a sandy loam soil (Berard, 1992, 722-001). The soil contained 66% sand, 21% silt, 13% clay, and 1.5% organic matter. The soil had a pH of 7.7, a cation exchange capacity of 8.8 meq/100 g and a moisture content of 75%. A mixture of ^{14}C-quinazoline labelled, ^{14}C-phenyl labelled and unlabelled fenazaquin (specific activity: 5.0 µCi/mg) was applied at 0.56 g ai/ha. Soil samples were incubated under aerobic conditions in the dark at 22–23 °C. Volatiles were collected in trapping solutions. Samples were taken immediately after application and at 3, 7, 14, 28, 56, 84, 112, 252, 336 and 365 days after application (DAA). In addition, soil samples, maintained in an open system, were treated with the same mixture at an exaggerated application rate (56 g ai/ha) for the structural determination of metabolites. Samples from the open system were taken after 168, 288 and 365 days after application.

Soil samples treated at the lower application rate were extracted with acetonitrile under reflux conditions for one hour. Extracted soil samples, collected after day 84, were subsequently extracted further with a solution of acetonitrile:1 N NaOH (1:1, v:v) for two hours at ambient temperature. After filtration, the extracts were adjusted to pH 7 and partitioned with equal volumes of dichloromethane. The aqueous phase was partitioned with ethyl acetate before and after adjusting the solution to pH 2. Soil samples from day 365 were subjected to organic matter fractionation to determine the amount of radioactivity bound to fulvic acid, humic acid and the humin fraction. The soil samples treated at the exaggerated rate were extracted in a similar manner to soil samples treated at the lower application rate, however, the extracts from these soil samples were fractionated by silica gel column chromatography using gradient elution. Isolated metabolites from the soil treated at the higher application rate were used to characterize/identify the metabolites in the extracts of soil treated at the lower application rate.

Table 27 Recovery and extraction of radioactivity from sandy loam soil treated with ^{14}C fenazaquin at a nominal level of 0.5 mg ai/kg and incubated at 22 °C under aerobic conditions (results expressed as percentage of applied radioactivity)

Incubation time [days]	Extracted	Unextracted	$^{14}CO_2$	Organic Volatiles	Total
0	99.5	0.5	nd	nd	100
3	96.8	2.2	0.02	0.00	99.02
7	96.1	5.4	0.22	0.01	101.73
14	92.2	9.0	0.73	0.02	101.95
28	80.2	16.6	1.79	0.05	98.64
56	68.0	24.6	4.72	0.09	97.41
84	81.1	8.3	7.57	0.10	97.07
112	76.7	9.6	10.28	0.12	96.70
168	67.0	11.1	14.48	0.15	92.73
252	60.0	12.4	18.45	0.16	91.01
336	51.5	14.8	20.42	0.16	86.88
365	50.5	15.4	20.93	0.17	87.00

The total amount of extracted residues declined from 99.5% of applied radioactivity (AR) immediately after application to 50.5% at day 365, with a corresponding increase in the unextracted residues from 0.5% AR to a maximum of 24.6% AR after 56 days of incubation. Afterwards, the soil samples were subjected to additional extractions and therefore, the amount unextracted decreased to 8.3% AR at day 84. Thereafter, the unextracted increased up to 15.4% AR at day 365.

Volatilization was not a significant dissipation pathway for fenazaquin or its metabolites, which is demonstrated by the low amount of radioactivity (< 0.2% AR) recovered in the charcoal filter. During incubation, the mineralisation to CO_2 continuously increased until the end of incubation and reached maximum values of 20.9% AR.

Table 28 Distribution of fenazaquin in soil extracts treated with ^{14}C-fenazaquin at a nominal level of 0.5 mg ai/kg and incubated at 22 °C under aerobic conditions (results expressed as a percentage of applied radioactivity)

Incubation time [days]	Fenazaquin
0	98.6
3	94.1
7	88.9
14	83.7
28	66.9
56	50.6
84	42.6
112	34.1
168	24.8
252	17.7
336	12.6
365	13.0

No single compound exceeded 10% of applied radioactivity at the lower application rate, however, at the higher application rate, the major metabolite was 2-oxy-fenazaquin, reaching a maximum level of 8.1% at day 168. The metabolism of fenazaquin appeared to proceed via hydrolysis of the ether linkage between the quinazoline ring system and the tertiary butyl-phenyl portions of the parent molecule, hydroxylation of the quinazoline ring system, and oxidation of the tertiary butyl group and phenethyl alcohol formed following hydrolysis of fenazaquin.

The amount of applied fenazaquin decreased steadily from 98.6% (day 0) to 12.6% of applied radioactivity (day 336), resulting in a calculated DT_{50} of 51 days, determined using SFO.

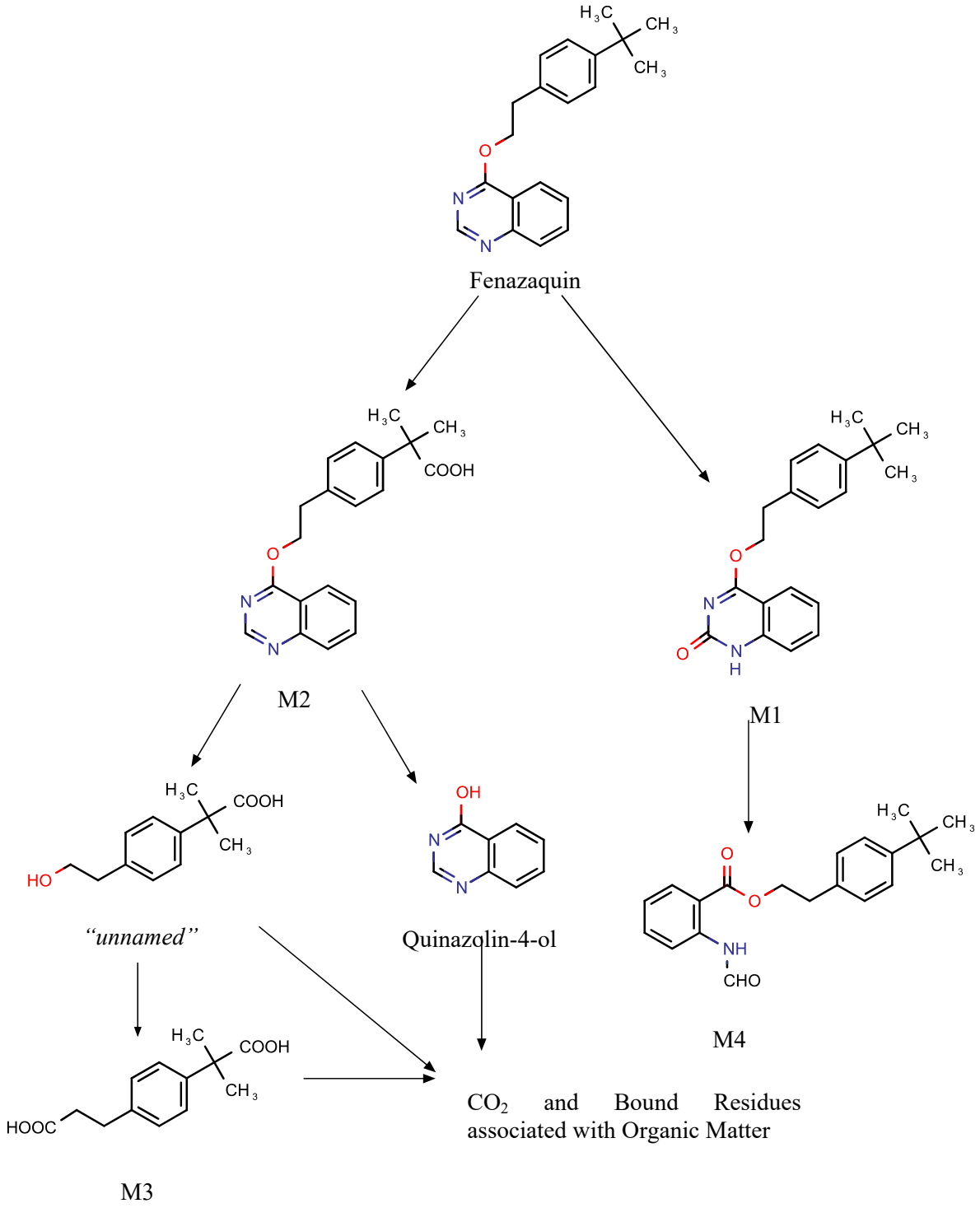


Figure 4 Proposed degradation pathway of ^{14}C-Fenazaquin in soil under aerobic conditions

Unnamed degradation products were identified by NMR- and MS-spectroscopy in soil treated at an exaggerated application rate (50 mg ai/kg). The same metabolite pattern was found as that in the soil extracts treated at "normal" rate (Osborne *et al*., 1992, 722-002).

Field Dissipation

The dissipation of ^{14}C-Fenazaquin in soil under field conditions was investigated at one US test site (Berard, 1993, 723-001). ^{14}C-quinazoline or ^{14}C-phenyl labelled fenazaquin, formulated as emulsifiable concentrate formulations, were applied to bare soil in June 1988 (plot 1) and May 1989 (plot 2), at an application rate of 0.224 kg ai/ha. The soil contained 6% sand, 47.6% silt, 22.8% clay and 2.2% organic matter. The soil had a pH of 6.7 and a cation exchange capacity of 10.2 mEq/100g. Soil cores were taken to a depth of 7.6 cm immediately after treatment, to 15.2 cm after 7 days, to 22.9 cm after 56 days and to 30.5 cm at day 168. After sampling, soil cores were separated into 7.6 cm segments. Samples were taken immediately after application and after 7, 14, 28, 56, 83 or 84, 112 and 168 (one plot only) days. Daily maximum and minimum temperatures, rainfall and supplemental irrigation were measured. During 1988, maximum temperatures over the study period of 112 days were frequently greater than 32 °C and rainfall was limited to 185 mm. Irrigation was used to supply additional water. In 1989, the climate was generally cooler and the rainfall over the first 112 days of the study period reached approximately 508 mm and 750 mm in total within 168 days after application.

Aliquots of soil samples were combusted and the total radioactivity was determined by LSC. Soil segments containing more than 92% of radioactivity in the entire soil core were extracted with methanol for two hours and with acetonitrile under reflux conditions or only with acetonitrile under reflux conditions for 1.5 hours. The extracts were concentrated and analysed by TLC. In addition, soil samples from day 112 (0 - 7.6 cm segments) were submitted to organic matter fractionation to determine the amount of radioactivity bound to fulvic acid, humic acid and the humin fraction.

Table 29 Distribution of radioactivity in soil in a field dissipation study in the USA (results expressed in percentage radioactivity of total soil core)

Soil Layers	Days after application							
	0	7	14	28	56	83/84[a]	112	168
Plot 1 (date of application: May 1988)								
0–7.6 cm	100	89.9	75.8	97.8	92.0	97.6	98.1	Ns
7.6–15.2 cm	ns	10.1	24.2	2.2	2.1	1.2	0.7	Ns
15.2–22.9 cm	ns	ns	ns	ns	5.9	1.2	1.2	Ns
Plot 2 (date of application: May 1989)								
0–7.6 cm	100	99.1	97.7	96.3	96.8	94.3	92.1	90.1
7.6–15.2 cm	ns	0.9	2.3	3.7	1.6	2.7	4.2	6.0
15.2–22.9 cm	ns	ns	ns	ns	1.6	3.0	3.7	2.9
22.9–30.5 cm	ns	ns	ns	ns	ns	ns	ns	1.0

ns no samples

[a] from plot 1 and 2 samples were taken at day 84 and 83, respectively

Fenazaquin and its degradation products were mainly restricted to the upper soil layer (0–7.6 cm). More than 90% radioactivity of the entire soil core remained in the upper soil layer throughout the study, except in plot 1 at days 7 and 14. Since plot 1 received only 5 mm precipitation during the first 14 days after application, the presence of radioactivity in lower soil layer 7.6–15.2 cm at days 7 and 14 was apparently caused by the movement of surface soil into cracks formed in the dry plot.

The radioactivity decreased in the upper soil layer (0–7.6 cm) after application of ^{14}C-fenazaquin. In plot 1, the radioactivity expressed as percent of day 0 sample was found to be 100% and 107.3% at days 0 and 7, respectively, and decreased afterwards to 57.8% at day 14 and to 31.7% at day 112. In plot 2, the radioactivity expressed as percent of day 0 sample decreased steadily from 100% at day 0 to 54.3% at day 28. At day 56 the radioactivity was 67.6% yet decreased afterwards to 24.7% at day 168.

Table 30 Residue level in soil in a field dissipation study in the USA (results expressed in % of radioactivity of day 0 sample)

Soil Layers	Days after application							
	0	7	14	28	56	83/84[a]	112	168
Plot 1 (date of application: May 1988)								
0–7.6 cm	100.0	107.3	57.8	61.6	52.5	56.9	31.7	Ns
Total core	100.0	119.7	84.4	67.5	60.8	62.3	33.0	Ns
Plot 2 (date of application: May 1989)								
0–7.6 cm	100.0	83.2	90.7	54.3	67.6	38.2	32.1	24.7
Total core	100.0	90.1	92.4	52.4	67.7	36.4	35.5	26.5

ns no samples

[a] from plot 1 and 2 samples were taken at day 84 and 83, respectively

Table 31 Dissipation of ^{14}C-fenazaquin in the upper soil layer (0–7.6 cm)

	Days after application						
	0	7	14	28	56	83/84[a]	112
Plot 1 (date of application: May 1988)							
% of radioactivity present as fenazaquin in total soil core	92.9	75.4	65.4	57.1	28.3	20.4	11.2
% of fenazaquin of day 0 sample	92.9	90.3	35.2	38.5	17.2	12.7	3.7
Plot 2 (date of application: May 1989)							
% of radioactivity present as fenazaquin in total soil core	95.6	75.3	58.1	33.1	22.0	14.7	8.8
% of fenazaquin of day 0 sample	95.6	67.8	53.7	17.3	14.9	5.4	3.1

[a] from plot 1 and 2 samples were taken at day 84 and 83, respectively

Despite the different climatic conditions, the dissipation of fenazaquin and its related compounds and the rate of fenazaquin degradation were similar at both plots. The amount of applied fenazaquin decreased in the upper soil layer (0–7.6 cm) in both plots. In plot 1, the amount of fenazaquin decreased from 92.9% of radioactivity present in the entire soil core to 11.2% at day 112. Related to the radioactivity detected in day 0 sample, 3.7% were identified as fenazaquin at day 112. In plot 2, the amount of fenazaquin decreased from 95.6% of radioactivity present in the entire soil core to 8.8% at day 112. Related to the radioactivity detected in day 0 sample, 3.1% were identified as fenazaquin at day 112.

The DT_{50} values for fenazaquin ranged from 34 to 36 days and the corresponding DT_{90} values from 113 to 126 days in the two plots applying SFO kinetics.

The dissipation of fenazaquin in soil under field conditions was investigated at 5 sites located in geographically and climatically different areas of Germany (Gambie *et al*., 1993, 723-003 and Butcher *et al*., 1993, 723-005). Four replicate subplots were set up at each test site (3 treated and 1 untreated control plot).

Parameter	Soil Characteristics				
Particle size	Loamy silt	Loamy sand	Loamy silt	Silty loam	Silt
Sand (%)	3	68	4	20	7
Silt (%)	83	21	80	60	78
Clay (%)	14	11	18	20	8
Organic carbon (%)	0.97	2.63	1.31	1.97	1.02
pH	5.8	6.4	5.9	7.0	5.0

A suspension concentrate of fenazaquin was applied to bare soil at an application rate of 0.15 kg ai/ha. Soil cores were collected to a depth of 20 cm one day before, immediately after application, 11 to 14 days after application and 1, 3, 5, 7, 10 and 12 months after application. Soil cores were separated into three sections (0 to 5 cm, 5 to 10 cm and 10 to 20 cm).

Soil cores were extracted with acetonitrile/water under reflux conditions. The extracts were concentrated and after addition of a sodium bicarbonate solution, fenazaquin was partitioned into hexane. The hexane solution was dried and the residues were diluted in dichloromethane. This solution was cleaned-up and fenazaquin was eluted with an ethyl acetate/dichloromethane solution, after which the eluate was evaporated to dryness and the residues were diluted in hexane. This solution was also cleaned-up and fenazaquin was eluted with acetone/hexane. The eluate was evaporated to dryness and the residues were diluted in trimethylpentane, prior to analysis using GC-MS.

Table 32 Levels of fenazaquin in soil in field dissipation studies conducted in Germany (results expressed in mg ai/kg)

Herford-Eickum									
Days after application	-1	0	14	30	90	153	216	325	363
Layer 0–5 cm	< LOD	0.186	0.082	0.111	0.022	0.013	0.013	N/A	N/A
Layer 5–10 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	N/A	N/A
Adelshausen									
Days after application	-1	0	14	30	90	153	215	325	363
Layer 0–5 cm	< LOD	0.253	0.082	0.082	0.010	0.007	0.006	N/A	N/A
Layer 5–10 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	N/A	N/A
Lauter									
Days after application	-1	0	14	30	92	153	226	325	363
0–5 cm	< LOD	0.118	0.127	0.040	0.006	< LOD	< LOD	< LOD	< LOD
0–10 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Landsberg									
Days after application	-1	0	11	30	92	153	227	304	365
0–5 cm	< LOD	0.141	0.172	0.069	0.042	0.020	0.019	0.009	0.007
0–10 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Grebin									
Days after application	-1	0	14	29	92	153	221	330	360
Layer 0–5 cm	< LOD	0.080	0.078	0.040	0.008	0.006	0.006	< LOD	< LOD
Layer 0–10 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

LOD limit of detection (0.005 mg/kg)

N/A - Not analysed

Fenazaquin was only detected in the upper soil layer (0–5 cm). The residues in the soils from all sites, below a soil depth of 5 cm, were below the limit of detection (0.005 mg ai/kg).

The amount of applied fenazaquin declined relatively rapidly in the upper soil layer (0–5 cm) at all sites, most notably in the Lauter soil where the residues of fenazaquin reached non-quantifiable levels shortly after day 92.

The DT_{50} values of fenazaquin were calculated to range from 26–66 days and the corresponding DT_{90} values ranged from 85–223 days, applying 1st-order reaction kinetics.

The dissipation of fenazaquin in soil under field conditions was also investigated at 2 sites located in a typical orchard area of Italy (Long *et al*., 1996, 723-006). Four replicate plots were set up at each test site (three treated and one untreated control plot). A suspension concentrate of fenazaquin was applied to bare soil at an application rate of approximately 0.20 kg ai/ha. Soil cores were taken from the treated and untreated plots to a depth of 25 cm one day before application, immediately after application, at 13 to 14 days, 1, 3, 5, 7 and 12 months after application. Soil cores were separated into

two sections (0 to 10 cm and 10 to 20 cm). The lowest section of the soil core (20 to 25 cm) was discarded.

Soil samples were extracted with acetonitrile/water (9:1, v:v) under reflux conditions. The extracts were concentrated and after addition of a sodium bicarbonate solution fenazaquin was partitioned into hexane. The hexane solution was dried and the residues were diluted in dichloromethane. This solution was cleaned-up using after which fenazaquin was eluted with ethyl acetate/dichloromethane (1:9, v:v) solution. The extract was further purified using a solid phase cartridge. Fenazaquin was eluted with acetone/hexane (99:1, v:v) and the eluate was evaporated to dryness and the residues were diluted in trimethylpentane, prior to analysis by GC-MS.

Table 33 Levels of fenazaquin in soil in a field dissipation study in Italy (results expressed in mg ai/kg)

Grugno								
Days after application	pre-treatment	0	14	29	92	153	203	369
0–10 cm	< LOD	0.065	0.055	0.039	0.015	0.006	< 0.005	< 0.005
10–20 cm	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Fognamo								
Days after application	pre-treatment	0	13	28	91	152	201	368
0–10 cm	< LOD	0.155	0.105	0.023	0.019	0.007	< 0.005	0.006
10–20 cm	< LOD	< 0.005	0.011	< 0.005	< 0.005	< LOD	< LOD	< LOD

LOD - limit of detection (0.001 mg/kg)

Detection of fenazaquin was restricted to the upper soil layer (0–10 cm). Only at the Fognamo site were very low levels of fenazaquin residues detected in the lower soil layer (10–20 cm) accounting for 0.011 mg ai/kg at day 13 and < 0.005 mg ai/kg at days 0, 28 and 91, respectively.

Residues of fenazaquin decreased relatively quickly in the upper soil layer (0–10 cm) at both sites. In the Grugno soil, fenazaquin residues were 0.065 mg ai/kg immediately after application and decreased steadily to < 0.005 mg ai/kg at day 203. In the Fognamo soil, fenazaquin accounted for 0.155 mg ai/kg immediately after application and decreased to < 0.005 mg ai/kg at day 201.

Based on the residue data from the top 10 cm soil layer the DT_{50} values for fenazaquin were calculated to be 71 and 75 days for the Grugno and Fognamo site assuming SFO and linear regression, respectively. The corresponding DT_{90} values were 235 days and 250 days based on the same methods of calculation as those of the DT_{50}.

Photolysis - Soil

The photodegradation of ^{14}C-fenazaquin was investigated under the conditions of a laboratory (Saunders, 1991, 724-001). The soil classification was a sandy loam soil with 63, 23 and 14% sand, silt and clay, respectively. The soil organic matter, pH, CEC and moisture at field capacity were 1.7, 7.0, 5.3 mEq/100g, and 12.9%, respectively.

Soil samples were treated separately with 40 µg of ^{14}C-quinazoline labelled fenazaquin or ^{14}C-phenyl labelled fenazaquin dissolved in acetone to obtain a nominal concentration of 40 mg ai/kg. Provisions were made to trap neutral organic volatiles and $^{14}CO_2$ by installing a tube containing activated charcoal. Immediately after dosing, the treated soils were exposed to natural summer sunlight for up to 30 days. The temperature of the samples was maintained constant at 25 ± 1 °C using a water bath. Control soil samples were incubated at 25 °C in the dark. Duplicate irradiated soil samples were collected at day 3, 8, 16, 22, 28 and 30. Control samples were taken at days 4, 22 and 30.

Soil samples were extracted by sonication with water followed by acetonitrile. The mixture was heated at 52 °C for 2 hours and sonicated for one minute. After filtration the radioactivity in the extract was quantified by LSC. Extracts from the soil samples treated with the ^{14}C-quinazoline label were concentrated, re-dissolved in acetonitrile and analysed by TLC by co-chromatography with

authentic reference substances. Extracts from the ^{14}C-phenyl label were analysed by HPLC, directly after centrifugation, by co-chromatography with authentic reference standards. The identity of the degradation products characterised by TLC and HPLC was confirmed by GC-MS. Extracted soil samples were combusted to determine levels of non-extracted residues. The Mallcosorb filter was combusted to determine the level of trapped radioactivity. The charcoal filter was extracted twice with dichloromethane and the radioactivity present was determined by LSC.

Additionally, the extracted soil samples irradiated for 30 days were extracted further with a solution of acetonitrile/1 N NaOH (1:1, v:v) for two hours at ambient temperature, prio to filtration. The filtrates were partitioned with dichloromethane and the remaining aqueous phase was partitioned twice with ethyl acetate after adjusting the solution to pH 2. Radioactivity in the extracts and aqueous phases was quantified LSC. The ethyl acetate extracts were analysed by TLC.

Table 34 Distribution of radioactivity in irradiated and dark control samples treated with ^{14}C-quinazoline labelled fenazaquin (results expressed in % of applied radioactivity)

Time after application [days]	Soil extracted	Non-extracted residues	Charcoal Filter [a]	Mallcosorb Filter [b]	Total ^{14}C
Irradiated samples					
0	100.3	0.3	0.0	0.0	100.5
3	98.0	1.5	0.0	0.0	99.5
8	95.3	3.5	0.0	0.2	99.0
16	96.5	4.6	0.0	0.5	100.1
22	93.7	5.7	0.0	0.7	99.2
30	90.2	7.6	0.0	1.3	101.6
Dark control sample					
4	101.3	0.3	0.0	0.0	100.8
22	100.3	0.5	0.0	0.0	100.3
30	99.8	0.6	0.0	0.0	98.4

[a] to trap organic volatiles,

[b] to trap CO_2

Table 35 Distribution of radioactivity in irradiated and dark control samples treated with ^{14}C-phenyl labelled fenazaquin (results expressed in % of applied radioactivity)

Time after application [days]	Soil extracted	Non-extracted residues	Charcoal Filter [a]	Mallcosorb Filter [b]	Total ^{14}C
Irradiated samples					
0	98.1	0.3	0.0	0.0	98.4
3	94.5	2.3	0.4	0.1	97.3
8	88.4	4.4	2.3	0.5	95.6
16	86.9	5.4	2.0	1.4	95.5
22	84.4	3.2	4.2	2.7	94.4
30	76.9	7.4	6.8	4.0	95.1
Dark control sample					
4	98.6	0.4	0.0	0.0	100.0
22	99.7	0.3	0.0	0.0	100.0
30	99.0	0.4	0.0	0.0	99.5

[a] to trap organic volatiles,

[b] to trap CO_2

In irradiated and dark control samples treated with ^{14}C-quinazoline labelled fenazaquin, the recovery of radioactivity ranged from 98% to 102% of applied radioactivity (AR). In irradiated samples treated with ^{14}C-quinazoline label, extracted residues decreased slightly from 100% to 90% of AR, whereas in the dark control samples the amount of the extracted radioactivity remained relatively unchanged (101 to 99.8% of AR). The unextracted residues increased during the irradiation period from 0.3% at day 0 to 7.6% of AR at day 30. Conversely, in the dark control samples the

amount of unextracted did not exceed 1% of AR during incubation. In the charcoal filter no radioactivity was detected while in the Mallcosorb filter the trapped radioactivity was 1.3% of AR after 30 days irradiation.

In irradiated and dark control samples treated with ^{14}C-phenyl label, the recovery of radioactivity ranged from 94% to 100% of applied radioactivity. The extracted residues decreased steadily from 98% to 77% of AR during irradiation. In the dark control samples the amount of the extracted residues remained constant in a range of 98.6 to 99.0% of AR during incubation. The unextracted residues increased during irradiation period from 0.3% at day 0 to 7.4% of applied radioactivity at day 30. In the dark control samples the amount of unextracted residues did not exceed 0.5% of AR during incubation. During irradiation, the trapped radioactivity increased continuously reaching levels of 6.8% and 4.0% of AR in the charcoal and Mallcosorb filter, respectively. No radioactivity was found in the filters from the dark control samples.

The amount of ^{14}C-quinazoline labelled fenazaquin decreased from 92.8% to 42.3% of applied radioactivity during 30 days irradiation. In the dark control samples the amount of fenazaquin decreased slightly from 95.4% at day 4 to 87.1% of applied radioactivity at day 30.

The amount of ^{14}C-phenyl labelled fenazaquin decreased from 90.7% of applied radioactivity immediately after application to 34.7% at day 30 in irradiated samples. In the dark control samples the amount of fenazaquin decreased from 91.2% at day 4 to 68.1% of applied radioactivity at day 30.

Table 36 Characterisation of radioactivity by TLC in soil extracts from irradiated and dark control samples treated with ^{14}C-quinazoline labelled fenazaquin (results expressed as percentage of applied radioactivity)

Time after application [days]	TLC Zone							
	1	2	3	4-Hydroxy-quinazoline	5	6	Fenazaquin	8
Irradiated samples								
0	0.2	0.1	0.2	0.4	0.6	0.1	92.8	0.1
3	0.3	0.2	0.6	11.0	0.4	1.0	78.9	0.1
8	0.6	0.4	0.8	21.4	0.4	1.6	61.6	0.2
16	1.0	0.6	1.6	27.0	0.5	1.6	55.7	0.2
22	1.2	0.7	1.8	32.0	0.4	1.9	50.4	0.2
30	1.7	1.0	2.7	36.7	0.6	2.0	42.3	0.3
Dark control samples								
4	0.2	0.0	0.1	1.1	0.1	0.1	95.4	0.1
22	0.2	0.1	0.3	4.0	0.1	0.1	91.9	0.2
30	0.2	0.1	0.6	6.9	0.2	0.2	87.1	0.2

Table 37 Characterisation of radioactivity by HPLC in soil extracts from irradiated and dark control samples treated with ^{14}C-phenyl labelled fenazaquin (results expressed as percentage of applied radioactivity)

Time after application [days]	4-Tert-butylphenylacetic acid	TBPE	Unknown	Fenazaquin
Irradiated samples				
0	0.0	1.4	0.2	90.7
3	1.6	6.6	2.1	77.7
8	2.7	13.5	2.7	57.1
16	4.8	15.5	3.0	51.7
22	5.7	16.8	3.4	45.9
30	7.3	17.9	3.6	34.7
Dark control samples				
4	0.0	2.4	0.2	91.2
22	0.1	6.1	0.2	88.2
30	0.1	8.4	0.2	68.1

In samples treated with ^{14}C-quinazoline labelled fenazaquin up to 6 minor degradation products were detected, none of which exceeded 3% of AR in the irradiated and dark control samples. One major degradation product was 4-hydroxyquinazoline. During the study, the amount of 4-hydroxyquinazoline increased continuously from 0.4 to 37% of AR and from 1.1% to 6.9% in the irradiated and dark control samples, respectively.

In samples treated with ^{14}C-phenyl labelled fenazaquin two degradation products were identified as 4-tert-butyphenylacetic acid and TBPE. The amount of 4-tert-butylphenylacetic acid increased continuously during irradiation to 7.3% of AR and did not exceed 0.1% in dark control samples. The amount of the major degradation product TBPE increased continuously from 1.4 to 18% of AR (irradiated samples) and from 2.4% to 8.4% (dark control samples) immediately after application to day 30, respectively. One unidentified degradation product peaked at 3.6% of AR at day 30 in the irradiated samples and did not exceed 0.2% in the dark control samples. The organic volatile degradation product trapped in the charcoal filter was identified as 4-tert-butylstyrene accounting for a maximum level of 6.1% of AR. No other volatiles were detected in significant amounts. The half-life of fenazaquin on soil surface was calculated to be 15 days in summer sunlight.

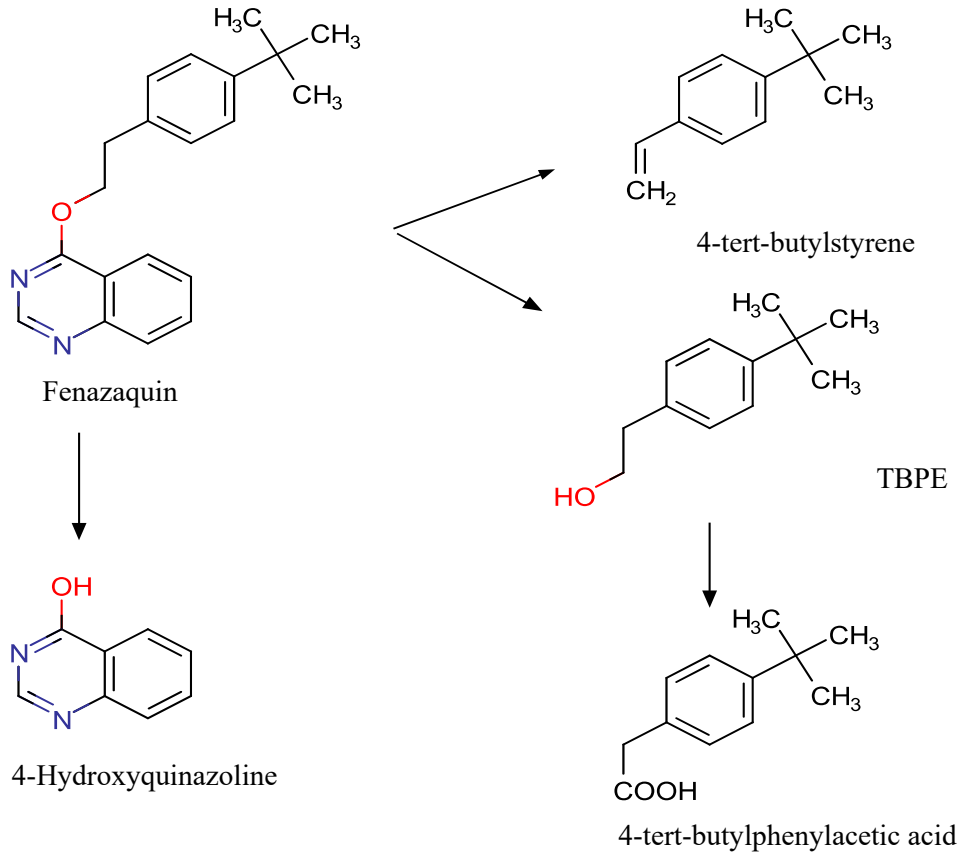


Figure 5 The proposed photolytic degradation pathway of ^{14}C-fenazaquin on soil surface

Photolysis – Aqueous Solution

The photolytic stability of ^{14}C-fenazaquin was studied in a laboratory using distilled water (Saunders, 1991, 712-001). Stock solutions of ^{14}C-quinazoline and ^{14}C-phenyl labelled fenazaquin in acetonitrile were prepared at a concentration of 25 mg ai/L and applied to distilled water, resulting in a nominal concentration of 0.1 mg ai/L. Test vessels containing the test solutions were exposed to summer sunlight for up to 32 days at 25 ± 1 °C. The control samples were maintained under the same conditions but in the dark. Duplicate exposed samples of each radiolabel were collected at 0, 5, 15, 7,

28 and 32 days after application. Duplicate control samples were taken at day 32 after application. Before incubation and during the study, the pH values of the distilled water ranged from pH 7.5 to 7.7.

Samples of distilled water were extracted with dichloromethane/ethyl acetate (1:1, v:v) and the radioactivity was quantified by LSC. Afterwards the extracts and extracted aqueous solutions were analysed by TLC. ^{14}C-phenyl treated samples which were taken at days 28 and 32 were analysed by HPLC.

The mass balance in the light exposed test samples treated with ^{14}C-quinazoline labelled fenazaquin ranged from 93.9 to 101.8%. In the dark control sample taken after 32 days total recovery was 101.6% of applied radioactivity. The mass balance in the light exposed test samples treated with ^{14}C-phenyl labelled fenazaquin ranged from 97.2 to 99.9% after 0, 5 and 15 days of incubation.

Table 38 Characterisation of radioactivity in distilled water after photolytic degradation of ^{14}C-Fenazaquin (results expressed as % of applied radioactivity)

Time after application [days]	Q-Label		P-Label		
	4-Hydroxyquinazoline	Fenazaquin	TBPE	4-tert-butylstyrene	Fenazaquin
Irradiated samples					
0	0.5	98.1	0.4	N/A	97.0
5	6.2	85.8	2.0	N/A	85.8
15	18.7	70.0	7.2	N/A	60.7
28	28.5	49.4	17.0	9.2	32.8
32	32.4	40.2	18.6	7.5	28.3
Dark control samples					
32	9.0	87.7	11.2	0.2	80.6

N/A - Not analysed

In light exposed samples ^{14}C-quinazoline labelled fenazaquin decreased from 98.1% to 40.2% of applied radioactivity. In the dark control solution the amount of fenazaquin was 87.7% at day 32. The amount of ^{14}C-phenyl labelled fenazaquin decreased from 97.0% to 28.3% of applied radioactivity in the light exposed samples. In the dark control solution the amount of fenazaquin was 80.6% at day 32.

Three degradation products of fenazaquin were detected in the distilled water of both light exposed and dark control samples and identified as 4-hydroxyquinazoline, TBPE and 4-tert-butylstyrene. In light exposed samples treated with the ^{14}C-quinazoline label, the amount of 4-hydroxyquinazoline increased continuously reaching a maximum level of 32.4% of applied radioactivity after 32 days of exposure. In dark control samples, 4-hydroxyquinazoline reached 9.0% of applied radioactivity after 32 days. In light exposed samples treated with the ^{14}C-phenyl label, the amount of TBPE and 4-tert-butylstyrene increased during exposure time and peaked at 18.6% and 9.2% of applied radioactivity at days 32 and 28, respectively. In dark control samples TBPE and 4-tert-butylstyrene reached 11.2% and 0.2% of applied radioactivity after 32 days, respectively.

Fenazaquin degraded rapidly in distilled water under natural sunlight. The DT_{50} value of fenazaquin was calculated to be 15 days.

The persistence of fenazaquin was also studied in three different soils, namely, Gangetic alluvial (pH 6.9), laterite (pH 5.3), and terai (pH 5.1) soil and in water at three different pH values (4.0, 7.0, and 9.2) under laboratory-simulated conditions (Bhattacharyya *et al*., 2010). Samples were analysed up to 60 days at regular intervals. Fenazaquin was applied at 5 and 10 µg/g to each soil and at 0.2 and 0.4 µg/mL to each water sample. Dissipation of fenazaquin in soil and water followed first-order kinetics irrespective of any treatments. The half-life of fenazaquin was found in the range of 47–64 days in soils and 3.49–37.6 days in water irrespective of dose. The persistence of fenazaquin in soil significantly increased in the order of Gangetic alluvial soil (pH 6.9) > laterite soil (pH 5.3) > terai soil (pH 5.1), whereas in water the trend of persistence was pH 9.2 ≥ pH 7.0 > pH 4.0. The dissipation of fenazaquin in soil and water was found to be dependent on pH irrespective of doses.

The photodecomposition of fenazaquin was studied in aqueous methanolic and 2-propanolic solution under UV light (30 hours) and sunlight (70 hours) separately (Bhattacharyya *et al*., 2003). The photolytic half-lives in aqueous methanolic solution were found to be 17.1 hours (UV) and 38.1 hours (sunlight), whereas these were 12.9 hours (UV) and 29.2 hours (sunlight) for aqueous 2-propanolic solution. All followed first-order reaction kinetics. Six photoproducts were obtained: TBPE, 4-hydroxyquinazoline, 4-tert-butylstyrene, 2,4-dihydroxyquinazoline, 4-tert-butylphenyl acetic acid, and 2-methyl-2-[4-(2-hydroxyethyl)phenyl]propanoic acid. These degradation products were formed as a result of the cleavage of the ether bridge linking the quinazoline and phenyl ring systems of the molecule, oxidation of the tert-butyl substituent, and oxidation of the heterocyclic portion of the quinazoline ring. These findings are also supported by the results of the plant metabolism studies which demonstrated that photolysis is a major degradation pathway.

In summary, the dissipation half-lives (SFO, DT_{50}) estimated for fenazaquin in field studies ranged from 48–114 days, indicating that fenazaquin is moderately persistent in soil under field conditions. Fenazaquin also exhibited moderate to high persistence in the soil laboratory incubations under aerobic conditions in the dark, forming the minor metabolite 2-oxy-fenazaquin. Mineralisation of the phenyl ring and phenyl-quinazoline ring radiolabels to carbon dioxide was an important pathway. Based on the results of the field dissipation studies, fenazaquin is considered immobile in soil. Under the conditions of a laboratory soil photolysis study, degradation of fenazaquin was enhanced compared to that which occurred in the dark with the major (>10% AR) metabolites 4-hydroxyquinazoline and TBPE being formed. Fenazaquin degraded rapidly in distilled water under natural sunlight in the laboratory. Three degradation products were detected and identified as 4-hydroxyquinazoline, TBPE and 4-tert-butylstyrene. The degradation products 4-hydroxyquinazoline and TBPE were only formed under artificial and sterile conditions of the photolysis and hydrolysis study.

METHODS OF RESIDUE ANALYSIS

Analytical methods

The Meeting received descriptions and validation data for analytical methods for residues of fenazaquin in plant and animal matrices, which were determined to be suitable.

Residues in food of plant origin

Residue analytical method R A4167 (Perny, 2006)

Briefly, fenazaquin is extracted from crops by macerating and heating (60 °C) with acetonitrile:water (90:10, v:v). After cooling, clean-up is by partitioning into hexane, followed by solid phase chromatography using a Florisil cartridge and eluting fenazaquin with dichloromethane:ethyl acetate (90:10, v:v). Additional purification is achieved using an aminopropyl solid phase cartridge, with hexane/acetone (98:2, v:v) as the elution solvent. The final eluate is evaporated and the residuum reconstituted in isooctane containing 1,4-dibromonaphthalene as an internal standard. Quantification of the residue is achieved by GC-MS using selected monitoring (*mz* 117.2→145.2). The validated LOQ was 0.01 mg/kg for all crops.

The linearity of the detector response covered a working range of 7.5–300 ng/mL of fenazaquin for matrix-matched solutions (r^2>0.990).

Table 39 Recovery data obtained during validation of R A4167

Matrix	Fenazaquin Fortification Levels (mg/kg)	Average Recovery (%)	Range of Recoveries (%)	% RSD	Number of Recoveries
Grapes	0.01	94.6	86.6-100.4	7.6	3
	1.0	79.4	78.3-81.1	1.9	3
Apples	0.01	80.6	72.5-85.2	8.7	3
	1.0	84.1	81.3-87.3	3.6	3
Oranges whole	0.01	77.3	72.7-79.5	3.7	5

Matrix	Fenazaquin Fortification Levels (mg/kg)	Average Recovery (%)	Range of Recoveries (%)	% RSD	Number of Recoveries
fruit	1.0	77.5	73.8-80.1	3.3	5
Oranges flesh	0.01	83.0	74.9-86.9	6.0	5
	1.0	83.9	80.0-87.2	3.4	5
Oranges peel	0.01	81.6	72.9-89.2	8.0	5
	1.0	87.1	80.6-93.6	6.0	5
Peaches	0.01	75.7	74.2-78.0	2.7	3
	1.0	91.6	87.4-97.9	6.1	3
Melon whole fruit	0.01	95.6	91.4-99.4	3.2	5
	1.0	79.3	76.5-86.8	5.4	5
Melon flesh	0.01	95.6	87.1-100.6	6.3	5
	1.0	77.1	70.6-89.7	10.1	5
Melon peel	0.01	86.4	76.8-104.3	13.1	5
	1.0	80.6	72.3-89.2	7.6	5
Zucchini	0.01	103.9	101.8-105.5	1.8	3
	1.0	94.6	91.8-97.5	3.0	3
Strawberries	0.01	88.6	80.9-93.1	7.6	3
	1.0	97.9	93.3-100.6	4.1	3
Tomato	0.01	104.7	99.1-108.9	4.8	3
	1.0	94.1	80.4-108.7	15.1	3
Sweet Pepper	0.01	85.5	82.2-87.2	3.3	3
	1.0	82.8	80.6-85.8	3.3	3

Ricerca residue analytical method from report 024119-1 and report 028973-1 (Cassidy, 2009, Oden, 2012)

Samples (20 g) were extracted with acetonitrile and the extract was partitioned with water and methylene chloride. The methylene chloride layer was evaporated and the residue was reconstituted in hexane. The hexane was placed on a preconditioned SPE cartridge. Fenazaquin was eluted with 5 mLs hexane:ethyl acetate (40:60, v:v).The eluate was evaporated and reconstituted in acetonitrile. Determination and quantitation of fenazaquin residue were conducted using LC-MS/MS for the detection of fenazaquin by both primary and secondary transition ions; quantification (m/z 307.0→161.2) and confirmation (m/z 307.2→147.2). The LOQ was 0.01 ppm for all crops investigated.

The linearity of the detector response covered a working range of 0.2 to 20 ng/mLfor all crops ($r^2 > 0.99$).

Table 40 Recovery data obtained during validation of method from report 028973-1 method

Matrix	Fenazaquin Fortification Levels (mg/kg)	Average Recovery (%)	Range of Recoveries (%)	% RSD	Number of Recoveries
Quantification (m/z 307.0→161.2)					
Corn Grain	0.01	101	91-106	6	5
	0.1	111	105-114	4	5
Tomatoes	0.01	101	97-111	6	5
	0.1	109	103-115	4	5
Almonds	0.01	78	75-80	2	5
	0.1	81	77-96	3	5
Lemons	0.01	103	96-108	4	5
	0.1	105	102-111	3	5
Leafy Mint	0.01	88	85-93	3	5
	0.1	95	89-103	7	5
Confirmation (m/z 307.2→147.2)					
Corn Grain	0.01	107	89-119	11	5
	0.1	110	105-113	3	5
Tomatoes	0.01	90	77-96	8	5
	0.1	108	100-113	5	5
Almonds	0.01	81	80-84	2	5

Matrix	Fenazaquin Fortification Levels (mg/kg)	Average Recovery (%)	Range of Recoveries (%)	% RSD	Number of Recoveries
	0.1	80	77-85	3	5
Lemons	0.01	104	85-136	20	5
	0.1	107	103-114	4	5
Leafy Mint	0.01	80	76-83	3	5
	0.1	96	91-104	6	5

Residues in food of animal origin

Fenazaquin - Validation of an analytical method for the determination of fenazaquin in animal products from report 272-023 (Weisner et al., 2012)

Briefly, to all animal matrices except milk, water was added to adjust the water volume present during extraction. Afterwards, acetonitrile was added. The samples were allowed to stand and then shaken vigorously by hand. A QuEChERS salt mixture (4 g magnesium sulphate,

Each sample had 1 g sodiuin chloride, 1 g trisodiuin citrate and 0.5 g disodiuin hydrogen citrate) added prior to vigorously being shaken by hand. After shaking the samples were centrifuged. For all matrices except fat, after centrifugation, an aliquot of the upper acetonitrile phase was diluted with ultra pure water and acetonitrile:water (1:1, v:v) prior to final determination by LC-MSIMS. For fat, after centrifugation, an aliquot of the upper acetonitrile phase was put into a deep-freezer for about 4 hours to freeze out coextracted lipids, then centrifuged for a few minutes, and allowed to stand to achieve room teinperature. An aliquot of of the supernatant was then transferred into an autosainpler vial, and diluted with ultra pure water for the final determination by LC-MS/MS for the detection of fenazaquin by both primary and secondary transition ions; quantification (m/z 307.0→57) and confirmation (m/z 307.0→161). The LOQ was 0.01 ppm for all animal matrices investigated.

The linearity of the LC-MS/MS detector response was confirrned for fenazaquin, for both mass

transitions, covering the working range of 0.25 ng/mL to 25 ng/mL(r^2 >0.995). The method was successfully validated by an independent laboratory.

Table 41 Recovery data obtained during validation of analytical method for the determination of fenazaquin in animal products, report 272-023

Matrix/ Ion transition	Fenazaquin Fortification Levels (mg/kg)	Average Recovery (%)	Range of Recoveries (%)	% RSD	Number of Recoveries
Meat	0.01	84	81-88	3.7	5
m/z 307.0→57	0.10	90	87-93	2.7	5
Meat	0.01	84	82-85	1.4	5
m/z 307.0→161	0.10	89	85-92	3.8	5
Liver	0.01	81	7-84	3.8	5
m/z 307.0→57	0.10	80	74-83	4.7	5
Liver	0.01	80	76-82	3.2	5
m/z 307.0→161	0.10	77	72-81	4.3	5
Milk	0.01	86	76-91	7.1	5
m/z 307.0→57	0.10	90	85-92	3.2	5
Milk	0.01	87	79-95	7.9	5
m/z 307.0→161	0.10	94	87-100	5.1	5
Eggs	0.01	83	80-88	3.8	5
m/z 307.0→57	0.10	87	86-89	1.3	5
Eggs	0.01	81	78-84	3.0	5
m/z 307.0→161	0.10	85	84-87	1.3	5
Fat	0.01	76	70-81	6.4	5
m/z 307.0→57	0.10	77	70-89	9.7	5
Fat	0.01	73	66-79	7.7	5
m/z 307.0→161	0.10	75	70-87	9.2	5

Applicability of multi-residue methods

A liquid chromatography–tandem quadrupole mass spectrometry (LC-MS/MS) multi-residue method for the simultaneous target analysis of a wide range of pesticides and metabolites in fruit, vegetables and cereals has been developed (Hiemstra *et al.*, 2007). Gradient elution has been used in conjunction with positive mode electrospray ionization tandem mass spectrometry to detect up to 171 pesticides and/or metabolites in different crop matrices using a single chromatographic run. Pesticide residues were extracted/partitioned from the samples with acetone/dichloromethane/light petroleum. The analytical performance was demonstrated by the analysis of extracts from lettuce, orange, apple, cabbage, grapes and wheat flour, spiked at three concentration levels ranging from 0.01 to 0.10 mg/kg for each pesticide and/or metabolite. In general, recoveries ranging from 70 to 110%, with relative standard deviations better than 15%, were obtained. The recovery and repeatability data are in good accordance with EU guidelines for pesticide residue analysis. The limit of quantification for all targeted pesticides and metabolites tested was 0.01 mg/kg. The selectivity and robustness of the LC–MS/MS method was demonstrated by a 1-year comparison of its analytical results with those obtained from our validated GC and LC multi-residue methods applied to more than 3500 routine samples. The validated LC-MS/MS method has been implemented in our analytical scheme since 2004, replacing four of the conventional detection methods, i.e. GC-flame-photometric detection (acephate, methamidophos, etc.), GC-nitrogen-phosphorus detection, LC-UV detection (carbendazim, thiabendazole, imazalil and prochloraz) and LC-fluorescence detection (N-methylcarbamate pesticides). During a 3-year period, the LC-MS/MS method has been applied to the analyses of more than 12 000 samples

Additional methods have also been reported in the scientific literature for the analysis of fenazquin in various fruits, vegetables and spices, including multi-residue screens (Walorczyk, 2014, Zang et al., 2011, Wang et al., 2009, Lukasz et al., 2013, Sung Woo Lee et al. 2011, Bienvenida et al. 2010, Lehotay et al. 2005).

Testing of fenazaquin through the US Food and Drug Adminstration's Multiresidue Methods in PAM Vol. 1 indicated that fenazaquin was adequately recovered from whole oranges and orange oil.

Stability of residues in stored analytical samples

The freezer storage stability of fenazaquin in homogenised plant samples fortified with fenazaquin and/or metabolites was studied.

The stability of fenazaquin and its metabolites in animal commodities was not provided to the Meeting.

Stability of residues in plant products

Cassidy (2010/2011, 023643-1/2) studied the stability of fenazaquin and fenazaquin dimer in tomatoes, corn (stover, forage and grain), mint and almond nutmeat. Samples were fortified with each analyte at a concentration of 0.1 mg/kg and stored frozen at -25 °C to -10 °C with brief excursions above this temperature due to power failures. Samples were taken for analysis at intervals up to 25 months. Samples were analysed using the validated analytical method from Report 024119-1. All procedural recoveries were within the acceptable range of 70–120% at each storage interval.

In all commodities, residues of fenazaquin seemed to dissipate within the storage periods of 6.6–12.2 months, yet there was no evidence of residue decline at later storage intervals of 17.6 and 25.9 months. Conversely, residues of the fenazaquin dimer declined by ~ 30% in all commodities within the tested storage intervals, however, in the case of almond nutmeats, the % remaining after 25.9 months of storage seemed to increase. The recoveries of fenazaquin and the fenazaquin dimer after frozen storage at -25 °C to -10 °C are summarized in Table 42.

Table 42 Stability of fenazaquin and fenazaquin dimer residues in various commodities during frozen storage

Storage Period (months)	Procedural Recoveries (%)	Stored Sample Residues (mg/kg)	Mean % Remaining	Procedural Recoveries (%)	Stored Sample Residues (mg/kg)	Mean % Remaining
	Fenazaquin			Fenazaquin Dimer		
Tomatoes						
0	--	0.118, 0.106, 0.116, 0.115	100	--	0.112, 0.106, 0.108, 0.111	100
1.5	98, 100	0.081, 0.091, 0.088	76	88, 93	0.076, 0.084, 0.084	74
3.5	100, 95	0.072, 0.083, 0.084	70	107, 103	0.063, 0.068, 0.067	61
6.6	95, 88	0.078, 0.080, 0.080	70	92, 77	0.084, 0.079, 0.083	75
9.4	83, 82	0.065, 0.068, 0.065	58	70, 70	0.068, 0.069, 0.074	64
12.2	88, 91	0.057, 0.057, 0.055	49	98, 100	0.070, 0.070, 0.074	65
17.6	84, 83	0.072, 0.086, 0.069	66	81, 76	0.075, 0.080, 0.081	72
25.9	69, 74	0.072, 0.069, 0.071	62	Not analysed		
Field Corn Stover						
0	--	0.095, 0.098, 0.095, 0.094	100	--	0.100, 0.103, 0.101, 0.096	100
3.5	70, 74	0.117, 0.075, 0.084	99	81, 97	0.137, 0.095, 0.108	113
6.6	78, 82	0.080, 0.097, 0.103	100	71, 76	0.104, 0.124, 0.133	120
9.4	119, 117	0.094, 0.088, 0.095	99	104, 111	0.117, 0.115, 0.130	121
12.2	70, 72	0.082, 0.076, 0.081	86	72, 84	0.114, 0.102, 0.117	111
17.6	75, 73	0.119, 0.099, 0.174	141	118, 120	0.136, 0.114, 0.121	124
25.9	93, 94	0.062, 0.072, 0.068	72	Not analysed		
Field Corn Forage						
0	-	0.096, 0.101, 0.106, 0.101	100	-	0.098, 0.098, 0.107, 0.098	100
3.0	87, 86	0.077, 0.074, 0.076	75	118, 120	0.097, 0.085, 0.086	89
6.1	79, 81	0.080, 0.074, 0.070	74	82, 83	0.063, 0.075, 0.084	74
8.9	69, 73	0.064, 0.067, 0.064	64	70, 67	0.081, 0.068, 0.076	75
11.8	69, 72	0.057, 0.058, 0.058	57	85, 80	0.066, 0.066, 0.064	65
17.2	89, 95	0.057, 0.092, 0.084	77	72, 79	0.090, 0.086, 0.085	87
25.5	73, 88	0.088, 0.165[a], 0.125	125	Not analysed		
Field Corn Grain						
0	-	0.109, 0.113, 0.107, 0.105	100	-	0.108, 0.126, 0.104, 0.104	100
1.5	104, 105	0.088, 0.084, 0.085	80	98, 96	0.087, 0.086, 0.084	78
3.5	112, 109	0.080, 0.080, 0.081	74	118, 118	0.084, 0.088, 0.085	78
6.6	0[b], 0[b]	0.064, 0.064, 0.073	62	0[b], 0[b]	0.072, 0.072, 0.078	67
9.4	72, 75	0.060, 0.064,	58	70, 75	0.061, 0.068,	62

Storage Period (months)	Procedural Recoveries (%)	Stored Sample Residues (mg/kg)	Mean % Remaining	Procedural Recoveries (%)	Stored Sample Residues (mg/kg)	Mean % Remaining
	Fenazaquin			Fenazaquin Dimer		
		0.064			0.077	
12.2	71, 76	0.052, 0.051, 0.054	48	75, 78	0.058, 0.061, 0.057	53
17.6	79, 88	0.064, 0.073, 0.067	63	111, 85	0.067, 0.078, 0.068	64
25.2	106, 97	0.116, 0.102, 0.102	99	Not analysed		
Mint						
0	-	0.143, 0.107, 0.120, 0.108	100	-	0.106, 0.094, 0.092, 0.085	100
3.5	100, 82	0.068, 0.076, 0.069	59	110, 85	0.046, 0.053, 0.050	53
6.6	73, 74	0.064, 0.062, 0.063	52	73, 62	0.048, 0.054, 0.050	54
9.4	80, 72	0.074, 0.071, 0.076	61	79, 66	0.059, 0.055, 0.049	58
12.2	61, 86	0.067, 0.071, 0.069	58	63, 80	0.042, 0.045, 0.046	47
17.6	107, 115	0.115, 0.118, 0.119	98	103, 91	0.081, 0.052, 0.052	66
25.2	112, 110	0.108, 0.106, 0.106	89	Not analysed		
Almond Nutmeats						
0	-	0.088, 0b, 0.092, 0.090	100	-	0.082, 0[b], 0.077, 0.081	100
3.5	99, 105	0.060, 0.056, 0.056	64	112, 119	0.071, 0.060, 0.060	79
6.6	82, 84	0.047, 0.048, 0.048	53	89, 74	0.062, 0.052, 0.058	72
9.4	68, 70	0.037, 0.038, 0.042	43	64, 66	0.037, 0.042, 0.048	53
12.2	74, 74	0.028, 0.036, 0.032	36	70, 74	0.046, 0.042, 0.033	50
17.6	81, 100	0.084, 0.110, 0.099	108	78, 107	0.092, 0.123, 0.106	134
25.2	72, 82	0.076, 0.079, 0.083	88	Not analysed		

[a] Sample was fortified at 0.2 ppm.

[b] Sample was not fortified.

Introduction to use patterns

Fenazaquin is a quinazoline-derived insecticide/miticide used to control mites and whiteflies, currently registered in the USA as an emulsifiable concentrate (EC) or a soluble concentrate (SC) for use on almonds, cherries and hops. In the USA, tank-mixing of the products with non-ionic surfactants is permitted. Fenazaquin is also registered in the Philippines as an emulsifiable concentrate (EC) or a soluble concentrate (SC) for use on pineapples. The formulated end use products are applied as foliar sprays using ground equipment.

Table 43 Selected registered uses of fenazaquin

Crop	Country	Form	Rate (kg ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
Cherries	USA	192 g ai/L, EC	0.336-0.504	936	1	N/A	3
Pineapple	Philippines	200 g ai/L, SC	0.50-1.0	1871	2	7-14	14-28

Crop	Country	Form	Rate (kg ai/ha)	Water (L/ha)	No.	Interval (days)	PHI (days)
		100 g ai/L, EC	0.50-1.0	1871	2	7-14	14
Almonds	USA	192 g ai/L, EC	0.336-0.504	468	1	N/A	7
Hops	USA	192 g ai/L, SC	0.336-0.504	936	1	N/A	7

N/A - Not applicable

RESULTS OF SUPERVISED RESIDUE TRIALS ON CROPS

The Meeting received information on supervised field trials for fenazaquin on the following crops or crop groups:

Crop	Table No.
Stone fruits	52
Pineapple	53
Tree nuts	54
Hops	55

Trials were generally well documented with laboratory and field reports. Laboratory reports included method validation with procedural recoveries from spiking at levels similar to those occurring in samples from the supervised trials. Dates of analyses or duration of residue sample storage were also provided. Although trials included control plots, no control data are recorded in the tables. Unless stated otherwise, residue data are recorded unadjusted for recovery. Residues and application rates have generally been rounded to two significant figures or, for residues near the LOQ, to one significant figure. Residue values from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels. Those results included in the evaluation are underlined. Conditions of the supervised residue trials were generally well reported in detailed field reports. Trial designs used non-replicated plots. Field reports provided data on the sprayers used, plot size, field sample size and sampling date.

Where duplicate field samples from an un-replicated plot were taken at each sampling time and were analysed separately, the mean of the two analytical results was taken as the best estimate of the residues in the plot. Similarly where samples were collected from replicate plots the mean result is reported.

Stone Fruit (Sweet and Tart Cherries, Peaches, Plums)

A total of twenty-one supervised trials were conducted on stone fruit (sweet and tart cherries [6], peaches [9], and plums [6]) during the 2008–2009 growing seasons in the USA (Carringer 2010, TCI-08-215). The treated plots received one foliar application, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a rate of 500 g ai/ha. The spray volumes ranged from 496–655 L/ha for concentrate spray mixes and 1132–1946 L/ha for dilute spray mixes. A non-ionic surfactant was included in the spray mixes. At all test sites, samples were harvested 3 days after application (DAA). Additional decline samples were collected from one sweet cherry, one peach and one plum trial at 0, 7 and 12–14 days after application. Samples of sweet and tart cherries weighed at least 1kg each. Samples of peach and plum samples were comprised of at least 24 fruit and weighed at least 2 kg.

Residues of fenazaquin and the fenazaquin dimer were quantified using the validated LC-MS/MS Ricerca method 024119-1. The LOQ of the method was 0.01 mg/kg per analyte. As residues of the dimer were non quantifiable (< 0.01 mg/kg) in all stone fruit samples, these were not reported in Table 46.

The storage intervals from collection to extraction ranged from 25 to 127 days.

Table 44 Fenazaquin residues in sweet and tart cherries, peaches and plums from supervised trials in the USA

Location Year Trial ID (variety)	Application					DAA	Commodity	Fenazaquin Residues [average], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.			
USA GAP - Cherries	192 g ai/L EC	36-54	936	336-504	1	3		
Conklin, MI, 2008 TCI-08-215-01 (Sam)	200 g/L SC	28.7	1740	500	1	3	Sweet Cherry	0.488, 0.487 [0.488]
Conklin, MI, 2008 TCI-08-215-02 (Montmorency)	200 g/L SC	82.2	608	500	1	3	Tart Cherry	0.965, 0.863 [0.914]
Marengo, IL, 2009 TCI-08-215-03 (North Star)	200 g/L SC	79.7	627	500	1	3	Tart Cherry	0.277, 0.233 [0.255]
Plainview, CA, 2009 TCI-08-215-04 (Tulare)	200 g/L SC	27.3	1833	500	1	0	Sweet Cherry	0.459* {0.020, 0.459}, 0.679* {0.601, 0.757} [0.569]
						3		0.371* {0.361, 0.381}, 0.577* {0.616, 0.538} [0.474]
						7		0.301, 0.300 [0.301]
						14		0.091, 0.149 [0.120]
Royal City, WA, 2008 TCI-08-215-05 (Bing)	200 g/L SC	76.3	655	500	1	3	Sweet Cherry	0.658, 0.451 [0.555]
Weiser, ID, 2009 TCI-08-215-06 (Montmorency)	200 g/L SC	44.2	1132	500	1	3	Tart Cherry	0.712, 0.959 [0.836]
Alton, NY, 2008 TCI-08-215-07 (Glohaven)	200 g/L SC	89.1	561	500	1	3	Peach	0.268, 0.241 [0.255]
Monetta, SC, 2008 TCI-08-215-08 (Contender)	200 g/L SC	38.5	1300	500	1	3	Peach	0.349, 0.521 [0.435]
Chula, GA, 2008 TCI-08-215-09 (Hawthorne)	200 g/L SC	97.3	514	500	1	3	Peach	0.511, 0.315 [0.413]
Montezuma, GA, 2008 TCI-08-215-10 (Flame Prince)	200 g/L SC	25.7	1946	500	1	3	Peach	0.232, 0.246 [0.239]
Conklin, MI, 2008 TCI-08-215-11 (Bellaire)	200 g/L SC	82.2	608	500	1	3	Peach	0.378, 0.378 [0.378]
D'Hanis, TX, 2008,	200 g/L	33.4	1497	520	1	3	Peach	1.20, 0.570 [0.885]

Location Year Trial ID (variety)	Application					DAA	Commodity	Fenazaquin Residues [average], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.			
TCI-08-215-12 (La Feliciana)	SC							
Porterville, CA, 2008 TCI-08-215-13 (Fay Alberta)	200 g/L SC	81.8	599	490	1	0	Peach	0.288, 0.325 [0.307]
						3		0.290, 0.115 [0.203]
						7		0.091, 0.114 [0.103]
						12		0.075, 0.085 [0.080]
Exeter, CA, 2008 TCI-08-215-14 (Klamt)	200 g/L SC	28.9	1730	500	1	3	Peach	0.856, 0.448 [0.652]
Strathmore, CA, 2008 TCI-08-215-15 (Ceres Carson)	200 g/L SC	81.5	589	480	1	3	Peach	0.221, 0.199 [0.220]
Conklin, MI, 2008 TCI-08-215-16 (Stanley)	200 g/L SC	86.2	580	500	1	3	Plum	0.183, 0.174 [0.179]
Poplar, CA, 2008 TCI-08-215-17 (French Plum)	200 g/L SC	26.2	1908	500	1	3	Plum	0.253, 0.216 [0.235]
Ducor, CA, 2008 TCI-08-215-18 (Black Cat)	200 g/L SC	78.6	636	500	1	0	Plum	0.015, 0.012 [0.014]
						3		< 0.01, < 0.01 [< 0.01]
						7		< 0.01, < 0.01 [< 0.01]
						14		< 0.01, < 0.01 [< 0.01]
Exeter, CA, 2008 TCI-08-215-19 (Flavour Fall)	200 g/L SC	36.6	1366	500	1	3	Plum	0.112, 0.090 [0.101]
Dinuba, CA, 2008 TCI-08-215-20 (Fryer's)	200 g/L SC	105.0	496	520	1	3	Plum	0.021, < 0.01 [0.016]
Monmouth, OR, 2008 TCI-08-215-21 (Moyer)	200 g/L SC	34.1	1525	520	1	3	Plum	0.194, 0.170 [0.182]

*Samples were reanalysed to confirm results. The values in braces are the original and reanalysed values. Except for the 0.0198 mg/kg value for Trial -04, the original values were confirmed. The mean of the two values (original and reanalysis) was used for statistical purposes except for Trial -04 where 0.459 mg/kg is considered the more reliable value based on its duplicate sample and other residue values for this trial.

Note: Cherry trials TCI-08-215-01/ TCI-08-215-02 were deemed dependant based on timing of application being within one week.

Pineapple

A total of eight supervised trials were conducted on pineapples during the 2013 growing season in Hawaii (1), in Costa Rica (4) and the Philippines (3) (Carringer 2014, TCI-13-352). The treated plots received two foliar applications, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a rate of 1 kg ai/ha/application at 35 and 21 days before harvest. The spray volumes ranged from 2345–2616 L/ha. A locally available surfactant at the recommended concentration was added to spray mixes at all applications. At all test sites, samples were harvested 20–21 days after the last application (DALA). Additional decline samples were collected from one of the trials at 8, 14, 28, 35 days after application. Each RAC sample consisted of 12 fruits (without crowns), which were subsampled such that each primary sample generally comprised two opposing quarters.

Residues of fenazaquin in pineapple RAC, pulp, peel, and processed commodities were quantitated using the validated analytical method No. R A4167. The LOQ was 0.01 mg/kg. Residues of the fenazaquin dimer were not analysed.

The pineapple RAC samples were stored frozen for 49–140 days between sample collection and extraction for analysis.

Table 45 Fenazaquin residues in pineapple from supervised trials in the USA, Costa Rica and the Philippines

Location Year Trial ID (variety)	Application							DALA	Commodity	Fenazaquin Residues [average], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.	RTI (days)	Max/ year (g ai/ha)			
Philippines	200 g ai/L, SC	27-54	1871	500-1000	2	7-14	2000	14-28		
	100 g ai/L, EC	27-54	1871	500-1000	2	7-14	2000	14		
General Santos City, South Cotabato, PH, 2013 TCI-13-352-01 (MG3)	200 g/L SC	42.8 43.0	2475 2396	1060 1030	2	13	2090	20	Whole Fruit	0.030, 0.025 [0.028]
General Santos City, South Cotabato, PH, 2013 TCI-13-352-02 (F200)	200 g/L SC	43.0 42.7	2390 2387	1030 1020	2	14	2050	20	Whole Fruit	0.032, 0.036 [0.034]
General Santos City, South Cotabato, PH, 2013 TCI-13-352-03 (MG3)	200 g/L SC	42.0 42.6	2616 2604	1100 1110	2	13	2210	20	Whole Fruit	0.078, 0.071 [0.074]
									Peel	0.150, 0.205 [0.178]
									Pulp	< 0.01, < 0.01 [< 0.01]
Nuevo Arenal, Alajuela, Costa Rica, 2013 TCI-13-352-04 (Dole-11)	200 g/L SC	42.3 42.3	2363 2362	1000 1000	2	14	2000	21	Whole Fruit	0.023, 0.018 [0.020]
Herediana, Limón, Costa Rica, 2013 TCI-13-352-05 (MD-2)	200 g/L SC	42.6 42.5	2345 2353	1000 1000	2	14	2000	21	Whole Fruit Peel	0.037, 0.033 [0.035] 0.114, 0.127 [0.121]
									Pulp	< 0.01, < 0.01 [< 0.01]
La Rita, Limón, Costa Rica, 2013 TCI-13-352-06 (Dole-11)	200 g/L SC	42.5 42.3	2354 2366	1000 1000	2	14	2000	21	Whole Fruit	0.015, 0.013 [0.014]
									Peel	0.079, 0.101 [0.090]
									Pulp	< 0.01, < 0.01 [< 0.01]
Puerto Viejo, Heredia, Costa Rica, 2013 TCI-13-352-07 (MD-2)	200 g/L SC	42.4 42.6	2359 2349	1000 1000	2	14	2000	7	Whole Fruit	0.122, 0.154 [0.138]
								14		0.049, 0.121 [0.085]
								21		0.027, 0.033 [0.030]

Location Year Trial ID (variety)	Application							DALA	Commodity	Fenazaquin Residues [average], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.	RTI (days)	Max/ year (g ai/ha)			
								28		0.020, 0.011 [0.016]
								35		< 0.01, < 0.01 [< 0.01]
Kunia, HI, USA, 2013 TCI-13-352-08 (Sweet Gold)	200 g/L SC	42.6 42.6	2348 2348	1000 1000	2	14	2000	21	Whole Fruit	0.124, 0.117 [0.121]
									Peel	0.254, 0.376 [0.315]
									Pulp	< 0.01, < 0.01 [< 0.01]

Note: Trials TCI-13-352-01/ TCI-13-352-02 and TCI-13-352-05/ TCI-13-352-06 were deemed dependant based on timing of application being within 30 days.

Tree Nuts (Pecans and Almonds)

A total of ten supervised trials were conducted in the USA on pecans [5] and almonds [5] during the 2008 growing season (Carringer 2010, TCI-08-219). The treated plots received one foliar application, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a rate of 500 g ai/ha. The spray volumes ranged from 561–655 L/ha for concentrate spray mixes and 963–1600 L/ha for dilute spray mixes. A non-ionic surfactant was included in the spray mixes. At all test sites, samples were harvested 6–7 days after the last application. Additional decline samples were collected from one pecan and one almond trial at 0–1, 14 and 21 days after application. The almond whole nuts were shelled on the day of collection and whole pecan samples were shelled 0–1 days after collection. Samples of pecan and almond nutmeats each weighed 1kg. Residues of fenazaquin and the fenazaquin dimer were quantified using the validated LC-MS/MS Ricerca method 024119-1. The LOQ of the method was 0.01 mg/kg per analyte. As residues of the dimer were non quantifiable (< 0.01 mg/kg) in all tree nutmeat samples, these were not reported in Table 54.

The pecan and almond nutmeat samples were stored frozen for 68–86 days and 58–78 days, respectively.

Table 46 Fenazaquin residues in pecans and almonds (nutmeat) from supervised trials in the USA

Location Year Trial ID (variety)	Application					DAA	Commodity	Fenazaquin Residues [averages], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.			
USA GAP - Almonds	192 g ai/L, EC	72-108	468	336-504	1	7		
Girard, GA, 2008 TCI-08-219-01 (Desirables)	200 g/L SC	54.0	963	520	1	6	Pecan	< 0.01, < 0.01 [< 0.01]
Montezuma, GA, 2008 TCI-08-219-02 (Money Makers)	200 g/L SC	31.2	1600	500	1	7	Pecan	< 0.01, < 0.01 [< 0.01]
Alexandria, LA, 2008 TCI-08-219-03 (Creek)	200 g/L SC	49.0	1001	490	1	0	Pecan	0.017, 0.019 [0.018]
						7		< 0.01, < 0.01 [< 0.01]
						14		< 0.01, < 0.01 [< 0.01]
						21		< 0.01, < 0.01 [< 0.01]

Location Year Trial ID (variety)	Application					DAA	Commodity	Fenazaquin Residues [averages], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.			
Pearsall, TX, 2008 TCI-08-219-04 (Wichita)	200 g/L SC	87.3	561	490	1	7	Pecan	< 0.01, < 0.01 [< 0.01]
Anton, TX, 2008 TCI-08-219-05 (Western Schley)	200 g/L SC	76.0	645	490	1	7	Pecan	0.013, 0.015 [0.014]
Terra Bella, CA, 2008 TCI-08-219-06 (Non-Pareil)	200 g/L SC	87.6	571	500	1	1	Almond	0.047, 0.023 [0.035]
						7		< 0.01, 0.012 [0.011]
						14		< 0.01, < 0.01 [< 0.01]
						21		0.016, < 0.01 [0.013]
Dinuba, CA, 2008 TCI-08-219-07 (Carmel)	200 g/L SC	42.6	1151	490	1	7	Almond	< 0.01, < 0.01 [< 0.01]
Wasco, CA, 2008 TCI-08-219-08 (Price)	200 g/L SC	76.3	655	500	1	7	Almond	< 0.01, < 0.01 [< 0.01]
Wasco, CA, 2008 TCI-08-219-10 (Non-Pareil)	200 g/L SC	90.0	589	530	1	7	Almond	< 0.01, < 0.01 [< 0.01]
Strathmore, CA, 2008 TCI-08-219-09 (Non-Pareil)	200 g/L SC	47.1	1104	520	1	7	Almond	< 0.01, < 0.01 [< 0.01]

Note: Almond trials TCI-08-219-08/ TCI-08-219-10 were deemed independent as the timing of the applications was greater than 30 days and the variety was different.

Hops

Three supervised trials were conducted on hops during the 2008 growing season in the US (Wyatt 2010, TCI-08-218). An additional four trials were conducted on hops during the 2014 growing season in the USA (Carringer, S.J., 2015, TCI-14-403). The treated plots received one foliar application, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a nominal rate of 500 g ai/ha, 7 days after application (DAA). The spray volumes ranged from 935–2049 L/ha. A locally available non-ionic surfactant was added to spray mixes at all applications. In 2014, additional treated samples were collected from two sites at 0, 14 and 21 DAA.

Treated samples were comprised of cones from a minimum of twelve separate areas, avoiding the edges or ends of the treated plot. Green hop cones were hand-picked. The duplicate treated samples were collected by making two separate passes through the treated plot. The green hop cones were dried using a commercial oven. The drying temperature was 46 °C to 66 °C for 4 to 36 hours with moisture contents ranging from 8 to 10%. When the dried cones reached the targeted moisture, they were placed directly into pre-labeled residue bags and placed in the freezer storage. The dried hop cone samples weighed at least 0.5 kg.

Residues of fenazaquin and the fenazaquin dimer in hops, dried cones, were quantitated using the validated LC-MS/MS Ricerca analytical method 024119-1, with minor modifications. The LOQ was 0.01 mg/kg/analyte. Samples collected from the 2014 trials were not analysed for residues of the dimer.

The dried cone samples were stored frozen for 439–445 days (2008) and 6–50 days (2014) between sample collection and extraction for analysis.

Table 47 Fenazaquin residues in hops from supervised trials in the USA

Location Year Trial ID (variety)	Application					DAA (days)	Commodity	Fenazaquin Residues [averages], mg/kg	Fenazaquin Dimer Residues [averages], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.				
USA GAP	192 g ai/L, SC	36-54	936	336-504	1	7			
Moxee, WA. 2008 TCI-08-218-01 Columbus	200 g/L SC	35.0	1412	500	1	7	Hops, dried cones	0.973, 1.27 [1.12]	0.722, 0.708 [0.715]
Wilder, ID, 2008 TCI-08-218-02 Zeus	200 g/L SC	36.0	1403	500	1	7	Hops, dried cones	1.09, 0.359 [0.724]	0.337, 0.101 [0.219]
Independence, OR, 2008 TCI-08-218-03 Galena	200 g/L SC	26.0	2049	530	1	7	Hops, dried cones	0.816, 1.21 [1.01]	0.316, 0.490 [0.403]
Ephrata, WA 2014 TCI-14-403-01 Cascade	200 g/L SC	27.0	1871	506	1	0	Hops, dried cones	35.2, 28.3 [31.8]	N/A
						7		11.2, 10.3 [10.8]	
						14		7.54, 5.21 [6.38]	
						21		3.99, 5.48 [4.74]	
Ephrata, WA 2014 TCI-14-403-02 Columbus	200 g/L SC	54.0	935	504	1	0	Hops, dried cones	20.1, 23.4 [21.8]	N/A
						7		9.21, 8.71 [8.96]	
						14		4.20, 3.83 [4.02]	
						21		1.98, 2.50 [2.24]	
Gervais, OR 2014 TCI-14-403-03 Willamette	200 g/L SC	26.0	1945	508	1	7	Hops, dried cones	8.78, 10.9 [9.84]	N/A
Mt. Angel, OR 2014 TCI-14-403-04 Super Galena	200 g/L SC	48.0	1038	500	1	7	Hops, dried cones	11.3, 12.0 [11.6]	N/A

N/A: Not analysed

Note: Trials TCI-14-403-01/ TCI-14-403-02 were deemed independent based on timing of application being greater than 30 days.

PRIMARY FEED COMMODITIES OF PLANT ORIGIN

Almond hulls

A total of five supervised trials were conducted in the USA on almonds during the 2008 growing season (Carringer 2010, TCI-08-219). The treated plots received one foliar application, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a nominal rate of 500 g ai/ha. The spray volumes ranged from 571–655 L/ha for concentrate spray mixes and 1104–1151 L/ha for dilute spray mixes. A non-ionic surfactant was included in the spray mixes. At all test sites, samples were harvested 7 days after the last application. Additional decline samples were collected from one almond trial at 0–1, 14 and 21 days after application. The almond whole nuts were shelled on the day of collection. Samples of almond hulls weighed 1kg.

Residues of fenazaquin and the fenazaquin dimer were quantified using the validated LC-MS/MS Ricerca method 024119-1. The LOQ of the method was 0.01 mg/kg per analyte.

The almond hull samples were stored frozen for 160–350 days.

Table 48 Residues of fenazaquin and fenazaquin dimer in almond hulls from supervised trials in the USA

Location Year Trial ID (variety)	Application					DALA	Fenazaquin Residues [average], mg/kg	Fenazaquin Dimer Residues [average], mg/kg
	Form	Rate (g ai/hL)	Spray Volume (L/ha)	Rate (g ai/ha)	No.			
USA GAP - Almonds	192 g ai/L, EC	72-108	468	336-504	1	7		
Terra Bella, CA, 2008 TCI-08-219-06 (Non-Pareil)	200 g/L SC	87.6	571	500	1	1	1.80, 1.91 [1.86]	< 0.01, < 0.01 [< 0.01]
						7	1.01, 1.17 [1.09]	0.036, 0.035 [0.036]
						14	1.23, 1.52 [1.38]	0.057, 0.076 [0.067]
						21	1.33, 1.22 [1.28]	0.053, 0.052 [0.053]
Dinuba, CA, 2008 TCI-08-219-07 (Carmel)	200 g/L SC	42.6	1151	490	1	7	1.67, 1.27 [1.47]	0.165, 0.143 [0.154]
Wasco, CA, 2008 TCI-08-219-08 (Price)	200 g/L SC	76.3	655	500	1	7	0.312, 0.461 [0.387]	0.020, < 0.01 [0.015]
Wasco, CA, 2008 TCI-08-219-10 (Non-Pareil)	200 g/L SC	90.0	589	530	1	7	0.217, 0.315 [0.268]	0.033, 0.036 [0.035]
Strathmore, CA, 2008 TCI-08-219-09 (Non-Pareil)	200 g/L SC	47.1	1104	520	1	7	1.28, 1.12 [1.20]	0.015, 0.024 [0.020]

Note: Almond trials TCI-08-219-08/ TCI-08-219-10 were deemed independent as the timing of the applications was greater than 30 days.

Fate of residues during processing

High temperature hydrolysis

The hydrolytic stability of [^{14}C]-fenazaquin uniformly labelled in the phenyl portion of the quinazoline ring (specific activity: 3.091 MBq/mg) was investigated in aqueous buffer solutions at three pH values and temperatures to simulate processing practice (Diehl 2003, 849767). The study was performed at pH 4, 5 and 6 at temperatures of 90 °C, 100 °C and 120 °C, respectively, for 20 or 60 minutes. The range of hydrolytic conditions used represents the processes of pasteurisation, baking/brewing/boiling and sterilisation.

Duplicate samples (except time 0) were set-up for each pH value. Buffer solutions containing the radiolabelled fenazaquin were incubated in glass vessels placed in an oil bath or an autoclave depending on the temperature. The test item was tested at concentrations of about 0.1 mg/L buffer solution. At time 0 and after 20 or 60 minutes incubation, the samples were taken, measured for total radioactivity by means of LSC and analysed for the nature of degradates by means of HPLC. Samples were also analysed by TLC to confirm the HPLC results. The temperatures were maintained constant throughout incubation and no significant variation of the pH values was observed in the buffered solutions.

Table 49 Degradation of fenazaquin under various hydrolysis conditions

Analyte	% Applied Radioactivity					
	pH 4 (90 °C)		pH 5 (100 °C)		pH 6 (120 °C)	
	0 min	20 min	0 min	60 min	0 min	20 min
Parent	99.4	35.0	99.5	61.6	101.0	79.4
4-Hydroxyquinazoline	N/D	61.0	N/D	35.5	N/D	17.7
Unknown	N/D	N/D	N/D	1.6	N/D	N/D

N/D – Not Detected

[^{14}C]-Fenazaquin was shown to be hydrolytically unstable for all hydrolytic conditions tested in this study: at pH 4 and 90 °C simulating pasteurisation, at pH 5 and 100 °C simulating baking/brewing/boiling and at pH 6 and 120 °C simulating the process of sterilisation. Up to two hydrolysis products were formed, one of which was characterised as 4-hydroxyquinazoline.

4-Hydroxyquinazoline, the major hydrolysis product at all pH values, accounted for 61.0% (pH 4, 90 °C, 20 minutes), 35.5% (pH 5, 100 °C, 60 minutes) and 17.7% (pH 6, 120 °C, 20 minutes) of the applied radioactivity at the end of incubation. The second hydrolysis product (M2), which was only detected at pH 4 and 5, showed a more polar character than the parent and accounted for 3.1% (pH 4, 90 °C, 20 minutes) and 1.6% of the AR (pH 5, 100 °C, 60 minutes).

Processing

Plums

A treated plot received one foliar application, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a rate of 2.52 kg ai/ha. Plums were harvested 3 days after application (DAA) and washed using a ratio of 2 kg of cold water to each 1 kg of fruit for 5 minutes. The washed fruit were placed in each of three drying trays. The balance of the washed fruit was discarded. The drying trays were placed in a preheated air dryer at a set temperature of 68–79 °C. The positions of the drying trays were rotated periodically to ensure even drying. The fruit was removed when the appropriate average moisture contents were achieved. The prunes were allowed to cool for approximately 20 minutes. The cooled prunes were packaged, labelled, and placed in frozen storage.

Residues of fenazaquin and thh fenazaquin dimer were quantified using the LC-MS/MS Ricerca method 024119-1. The LOQ was 0.01 ppm/analyte. As residues of the fenazaquin dimer were non quantifiable (<LOQ) in the plums and prunes, these were not reported herein. The storage intervals, from collection to extraction was 33 days.

Table 50 Fenazaquin Prune Processing Study

Trial Identification (City, State/Year)	Total Application Rate (kg ai/ha)	DAA (days)	Processed Commodity	Fenazaquin Residues (ppm)	Processing Factor
Poplar, CA, 2008 TCI-08-215-17	2.50	3	Plum fruit	0.707	-
				0.689	
				Mean = 0.698	
			Prunes	3.62	4.8
				3.12	
				Mean = 3.37	

Pineapple

The treated plots received two foliar applications, of a suspension concentrate formulation containing 200 g/L of fenazaquin, at a rate of 5 kg ai/ha/application at 35 and 21 days before harvest. The spray volumes ranged from 2345–2616 L/ha. A locally available surfactant at the recommended concentration was added to spray mixes at all applications. Samples were harvested 20–21 days after the last application (DALA). The whole fruit samples were processed to generate pulp (fruit without peel, cores included), peel, juice (pasteurized), canned fruit, and process residue (wet bran). For

production of fresh juice, whole pineapples (without crowns) were chopped in a bowl chopper to a uniform consistency. The chopped material was pressed with a hydraulic press to separate fresh juice from the pulp (wet bran). After pressing, the moisture content of the wet bran was determined with an electronic moisture analyser. Fractions of wet bran were collected and placed into frozen storage.

Fresh juice was screened with a 100 mesh sieve. To produce pasteurized pineapple juice, the fresh juice was heated to 93.3–99.9 °C by pumping through a steam heated heat exchanger. After passing through the heat exchanger, the juice entered a chilled heat exchanger and was cooled. Pasteurized juice was collected in sterilized containers and placed into frozen storage.

For production of pulp (fresh fruit without peel, cores included) and peel, pineapples were cored. Prior to coring, approximately 1/2 inch was sliced off the top and bottom of each whole pineapple. Pineapples were then cored using a pineapple corer. During coring, the following pieces were produced from a single fruit: cylinder, core, and peel with eradicator meat (in 3 pieces). After coring, cylinders and cores were sliced horizontally and vertically to produce 1/4 sections, which were further sliced into 1/8 sections. Pulp (fruit without peel, cores included) samples were collected and placed in frozen storage. Top and bottom sections and peel were trimmed to remove eradicator meat which was later discarded. After removing the meat, representative peel fractions were collected and placed in frozen storage. Cylinder pieces remaining after collection of pulp samples were sliced into chunks, representative of all whole pineapple cored. Pineapple chunks were placed into sterilized cans which were filled to approximately 90% capacity with fresh juice and sealed. Cans were placed into boiling water and heated to 94.4–100 °C. Temperature range was maintained for 12–18 minutes under atmospheric conditions. Canned pineapple was removed and cooled in a cold water bath for 30 minutes. Canned fruit were placed into frozen storage.

The RAC and processed commodity samples were analysed using the validated LC-MS/MS method R A4167.The Limit of Quantitation (LOQ) for fenazaquin was 0.01 ppm for all pineapple RAC, peel, pulp, and processed commodities samples.

The pineapple pulp and peel samples were stored frozen for 80–148 days and 82–144 days, respectively. The juice, canned fruit, and process residue (wet bran) were stored 124–134 days, 138–148 days, and 125–135 days, respectively.

Table 51 Fenazaquin pineapple processing study

Trial Identification (City, State/Year)	Total Application Rate (kg ai/ha)	DAA	Processed Commodity	Fenazaquin Residues (ppm)	Processing Factor
Kunia, HI, 2013 TCI-13-352-08	10	21	Pineapple Whole Fruit (RAC)	0.696	-
				0.612	
				Mean = 0.654	
			Pulp (fruit without peel, cores included)	0.014	<1
				0.031	
				Mean = 0.022	
			Peel	1.58	2.05
				1.10	
				Mean = 1.34	
			Juice (pasteurized)	0.083	<1
				0.091	
				Mean = 0.087	
			Canned Fruit	0.067	<1
				0.068	
				Mean = 0.067	
			Process Residue (wet bran)	2.21	3.52
				2.38	
				Mean = 2.30	
Waialua, HI, 2013 TCI-13-352-09	10	21	Pineapple Whole Fruit (RAC)	0.848	NA
				0.896	
				Mean = 0.872	
			Pulp (fruit without peel,	<LOQ	<1
				<LOQ	

Trial Identification (City, State/Year)	Total Application Rate (kg ai/ha)	DAA	Processed Commodity	Fenazaquin Residues (ppm)	Processing Factor
			cores included)	Mean <LOQ	
			Peel	2.07	2.34
				2.00	
				Mean = 2.04	
			Juice (pasteurized)	0.104	<1
				0.122	
				Mean = 0.113	
			Canned Fruit	0.043	<1
				0.051	
				Mean = 0.047	
			Process Residue (wet bran)	1.89	2.21
				1.97	
				Mean = 1.93	

RESIDUES IN ANIMAL COMMODITIES

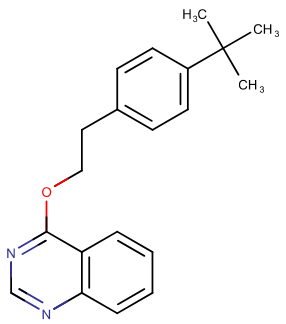
Livestock feeding studies were not made available to the Meeting.

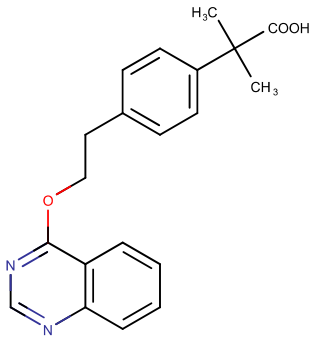
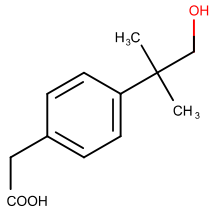
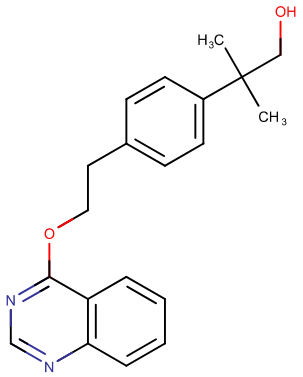
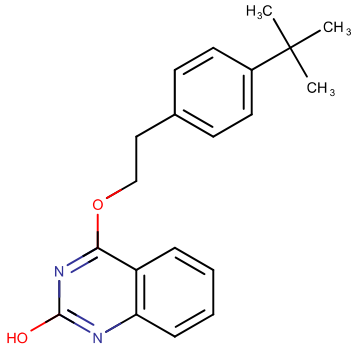
APPRAISAL

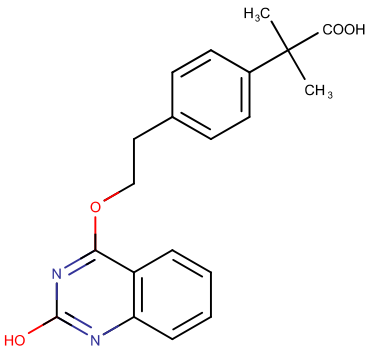
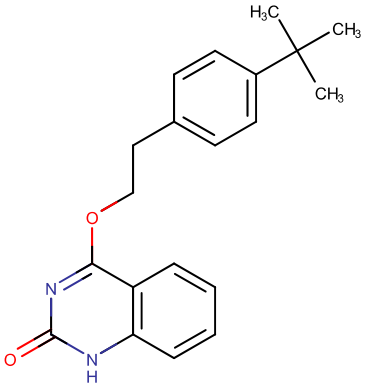
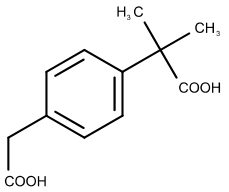
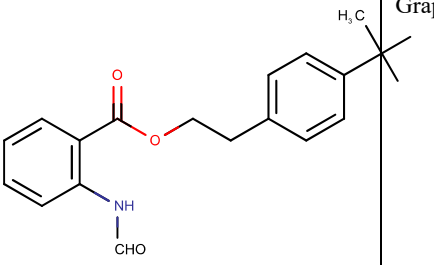
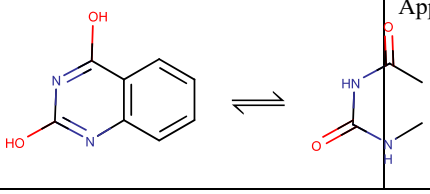
Fenazaquin is a quinazoline insecticide/acaricide which exhibits contact and ovicidal activity against a broad spectrum of mites in grapes, pome fruit, citrus, peaches, cucurbits, tomatoes, cotton and ornamentals. At the 48th Session of the CCPR (2016), it was scheduled for evaluation as a new compound by the 2017 JMPR.

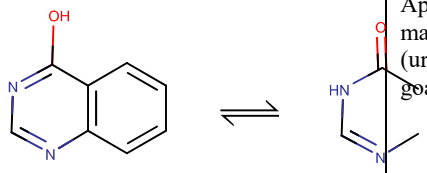
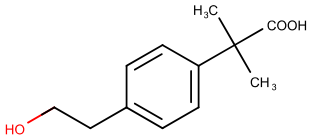
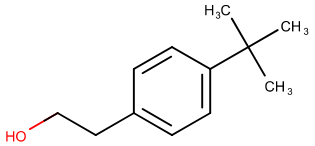
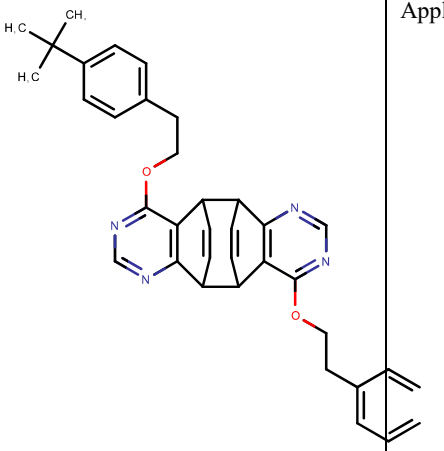
The Meeting received information on the metabolism of fenazaquin in apples, oranges, grapes and corn, lactating goats and laying hens, methods of residue analysis, freezer storage stability, GAP information, supervised residue trials on stone fruits, pineapples, tree nuts (almonds and pecans), hops and processing studies.

In this document, the common names, chemical structures and chemical names of the metabolites were as follows:

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
Fenazaquin	4-[2-(4-tert-Butyl-phenyl)-ethoxy]-quinazoline		Apples, oranges, grapes, maize, rat, goats, hens, soil

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
Fenazaquin acid	2-methyl-2-{4-[2-(quinazolin-4-yloxy)ethyl]phenyl}propanoic acid		Maize, grapes, rat (faeces, F-2), hen, soil
Metabolite B	2-[4-(1-hydroxy-2-methylpropan-2-yl)phenyl]acetic acid		Rat (urine, AN-1)
Metabolite C	2-Methyl-2-{4-[2-(quinazolin-4-yloxy)-ethyl]-phenyl}-propan-1-ol		Apples, grapes, rat (faeces, F-1)
2-Hydroxy-fenazaquin	4-[2-(4-tert-butylphenyl)ethoxy]quinazolin-2-ol/ Exists in tautomeric equilibrium with 2-oxy-fenazaquin		Maize, goats, soil

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
2-Hydroxy-fenazaquin acid	2-(4-{2-[(2-hydroxyquinazolin-4-yl)oxy]ethyl}phenyl)-2-methylpropanoic acid		Apples, oranges, goats, rats (faeces, F-3)
2-Oxy-fenazaquin	4-[2-(4-tert-butylphenyl)ethoxy]-1,2-Dihydroquinazolin-2-one Exists in tautomeric equilibrium with 2-hydroxy-fenazaquin		Maize, soil
Metabolite G	2-[4-(carboxymethyl)phenyl]-2-methylpropanoic acid		Goats, soil
Metabolite H	2-(4-tert-butylphenyl)ethyl 2-formamidobenzoate		Grapes, soil
Dihydroxyquinazoline	Equilibrium exists between the two tautomeric forms: quinazoline-2,4-diol and 1,2,3,4-tetrahydroquinazoline-2,4-dione		Apples, grapes

List of metabolites and degradates of fenazaquin			
Common name	Chemical name	Chemical structure	Occurrence in
4-Hydroxyquinazoline	Equilibrium exists between the two tautomeric forms: quinazoline-4-ol and 3,4-dihydroquinazolin-4-one		Apples, grapes, maize, rats (urine, 4-OH), goats, soil
Metabolite K	2-[4-(2-hydroxyethyl)phenyl]-2-methylpropanoic acid		Grapes
Tertiarybutylphenylethanol (TBPE)	2-(4-tert-butylphenyl)ethan-1-ol		Apples, grapes, maize, soil
Fenazaquin Dimer	7,15-bis[2-(4-tert-butylphenyl)ethoxy]-4,6,12,14-tetraazapentacyclo[8.6.2.2^{2,9}.0^{3,8}.0^{11,16}]icosa-3(8),4,6,11(16),12,14,17,19-octaene		Apples

Plant metabolism

Apples

Study 1

Four semi-dwarf *Golden Delicious* apple trees, grown outdoors, were treated with a single foliar application of ^{14}C-fenazaquin labelled in the phenyl ring of the tert-butyl phenyl portion of the molecule (P-Label) and with ^{14}C-fenazaquin uniformly labelled in the phenyl portion of the quinazoline ring (Q-Label). Two trees (one per radiolabel) were sprayed at a rate of 0.45 kg ai/ha in late June when the apples were 2–3 cm in diameter (early season application), while the remaining two trees were sprayed at the same rate approximately 4–5 weeks prior to harvest when the apples were 6–7 cm in diameter (late season application).

In order to study the effect of photolysis on the decline of the fenazaquin residues, six apples on the tree receiving the late season application of the P-label were covered shortly after application with bags made of a white muslin cloth.

Apples from trees receiving the early season application were sampled at 0, 4, 7, 14, 29, 57, and 92 days after treatment (DAT), while apples from trees receiving the late season application were sampled at 0, 7, 14, 28, and 42 DAT. The covered apples from the photolysis study were sampled at 7 and 14 DAT.

The ^{14}C levels in the peel, pulp, and whole fruit of apples from the late season application (1.92–2.47 mg eq/kg, 0.05–0.06 mg eq/kg, 0.37–0.49 mg eq/kg, respectively) were up to 3 times greater than the levels in the corresponding fractions of apples receiving the early season application (0.65–0.80 mg eq/kg, 0.03 mg eq/kg, 0.14–0.16 mg eq/kg, respectively). The radioactivity in peel was consistently higher than that in pulp, demonstrating limited penetration of the radioactivity.

Once harvested, fruits were rinsed with various organic solvents, The levels of fenazaquin in the surface washes declined relatively rapidly from 85–90% of the TRR on day 0 to 2–6% of the TRR on day 92. The decline of residues in the washes appeared to be associated with a corresponding increase in peel and pulp residues. In apples that were covered from the sun, the decline in fenazaquin residues was insignificant as was the accumulation of residues in pulp and peel. Photolysis appears to be a major route of degradation of fenazaquin.

Characterization of the residues in peel of washed mature fruit showed 30–53% of the TRR to be extracted using acetonitrile:water (75:25, v:v) with 38–57% of the TRRs remaining unextracted. Analysis of the peel extracts, showed fenazaquin to be the major component (up to 20% of the total peel residue at harvest). No metabolites with the intact ether bridge were present at measurable levels in the peel. All of the metabolites found were confirmed as being cleavage products of fenazaquin. Since cleavage of the fenazaquin ether bridge is known to occur as a result of photolysis, it was assumed that most of these metabolites were photoproducts. A total of six or more cleavage products were observed in the peel extracts, with 4-hydroxyquinazoline and TBPE tentatively identified as the two major cleavage constituents (2–5% of the TRR). All of the other observed cleavage metabolites accounted for < 3% of the TRR. Therefore, the structure of these could not be elucidated further. The unextracted residues in peel appeared to be cleavage products which had been incorporated into natural products.

Characterization of the residues in apple pulp showed 14–40% of the TRR to be extracted using acetonitrile:water (75:25, v:v) with 5–13% of the TRR being unextracted. Analysis of the extracted residues showed no presence of fenazaquin in the pulp. All of the observed metabolites were confirmed as being cleavage products, most of which were different from those observed in the peel. No individual metabolite in the pulp represented more than 3% of the whole fruit residue. As with the peel metabolites, none of the pulp metabolites were present at levels high enough to facilitate isolation and identification.

Study 2

Fenazaquin, labelled in the phenyl ring (P-Label) or in the quinazoline ring (Q-Label), and formulated as a suspension concentrate, was applied to *Golden Delicious* apple trees, maintained outdoor, at total seasonal application rates of 3.3 g ai/hL or 13.3 g ai/hL. The first application was made to apple trees when fruits had reached an average size of 2 cm in diameter.

Fruits were harvested on the day of application, 7, 14, 28 days after treatment (DAT) and at maturity (105 DAT). Five weeks later, a separate cluster of apple trees were treated at the low and high application rates. Apples were harvested immediately after treatment (0-DAT) and at maturity (70 DAT). In a separate experiment, a single application of P-labelled fenazaquin was made to one tree at the low rate. Following treatment, the fruit were covered with aluminum foil-lined plastic plant pots, the open end being covered with mesh to exclude light but allow air exchange.

In all scenarios, the total radioactivity in fruits declined relatively rapidly over the duration of the study.

Following the early season application, the TRRs removed by surface washing were greater than 50% of the TRR at all sampling intervals except at 105 DAT where surface washing removed 20–40% of the TRR from fruit treated at the low rate. Similarly, at the 70-DAT interval following the

higher application rate, 26–35% of the TRR were removed by surface washing. After the surface washes, most of the radioactivity remaining in the fruit was found in the peel for both the low and high rates, following early and late season applications, demonstrating limited penetration.

Fenazaquin accounted for greater than 92% of the TRR in whole fruits collected 0-DAT and declined rapidly thereafter following low and high rates of application made early season and late season. Starting on day 7, the fenazaquin dimer was a major metabolite accounting for 7–33% of the TRR, following early and late season applications at the low and high rates. The Metabolites C/TBPE, which could not be separated either by HPLC or TLC, accounted for up to 2.3% of the TRR for both radiolabels at the low and high rates following early and late season applications. As Metabolites C and TBPE were present predominantly in the P-labeled samples, the Meeting concluded that it likely represents TBPE. Several other unknown metabolites were characterized, none of which exceeded 5% of the TRR.

For the mature 14 DAT covered fruits, surface residues were comprised solely of fenazaquin, and peel extracts were fenazaquin and Metabolites C/TBPE. The absence of the dimer on these samples tends to confirm that it is a product of photochemistry.

Oranges

^{14}C-Phenyl-fenazaquin (P-label) and ^{14}C-quinazoline-fenazaquin (Q-label) were formulated as emulsifiable concentrates (EC) and were separately applied at 0.45 kg ai/ha to orange trees, variety *Valencia*, grown outdoors. Applications were made approximately 6 months prior to fruit maturity (early season application, when fruit were 3 cm in diameter) and approximately 2 months prior to fruit maturity (late season application when fruit were 6 cm in diameter).

Fruits were collected at 0, 28, 112 and 191 days after treatment (DAT) following the early season application and at 0, 19 and 63 DAT following the late season application.

In an effort to examine the effect of photolysis on the nature of the residues, some of the fruit from the late season application were covered with muslin cloth shortly after the application of fenazaquin. Samples of covered fruit were collected at 9, 19 and 63 DAT.

Following the early season application, the TRRs in whole fruit averaged 2.3 mg eq/kg at 0 DAT and declined rapidly to an average of 0.77 mg eq/kg by 28 DAT, and then declined more slowly to an average of 0.34 mg eq/kg by fruit maturity (191 DAT). Following the late-season application, TRRs in whole fruit were 0.53 mg eq/kg at 0 DAT and remained relatively unchanged (P label), or increased slightly (Q label), until maturity, at 63 DAT (0.45–0.90 mg eq/kg). Regardless of the application timing or sampling interval, the two radiolabels yielded similar TRRs. There was no apparent decline in TRRs in the covered fruit from the late season application.

The distribution of the TRRs between peel and pulp fractions was similar for mature fruits for both ^{14}C-labels. For the early and late season applications, the peel accounted for 86–99% of the TRR in mature fruits, and the pulp accounted for less than 14% of the TRR. These findings confirmed that there was limited penetration from the peel into the pulp.

Analyses of surfaces washes and solvent extracts of the mature fruit (191 DAT) from the early season application demonstrated that parent, fenazaquin, was the major residue in the fruit (39–52% of the TRR; 0.13–0.19 mg eq/kg). Minor amounts of 2-hydroxy-fenazaquin (5–8% of the TRR; 0.02–0.03 mg eq/kg) were also identified with the remaining radioactivity comprised largely of unknowns, each present at < 5% TRR.

For the late season application, TLC analyses of solvent washes and fruit extracts of mature fruit (63 DAT), identified parent as the primary residue (55–66% of the TRR; 0.30–0.50 mg eq/kg), along with minor amounts of 2-hydroxy-fenazaquin (≤ 1% of the TRR; ≤0.008 mg eq/kg).

For the mature covered fruits (late season application; 63 DAT), the concentrations of the parent compound were higher (81–84% TRR; 0.15–0.46 mg eq/kg) than in the uncovered fruit. Levels of 2-hydroxy-fenazaquin in the mature covered fruit were comparable to those in the uncovered mature fruits (≤1% of the TRR; 0.001–0.006 mg eq/kg). The limited degradation of the parent

molecule that occurred in/on covered fruits suggests that photolysis of surface residues plays an important role in the degradation of fenazaquin residues on the surface of these fruits.

Grapes

Field grown grapes, variety *Cabernet Sauvignon,* were treated with single applications of an EC formulation of [^{14}C]fenazaquin labelled in the phenyl ring of the molecule (P-label) or in the quinazoline portion of the molecule (Q-label) at 15 g ai/hL. An early season application was made to grape bunches approximately two to three weeks after the end of flowering (BBCH 68). A late season application was made to separate grape bunches approximately 7 weeks later (nine to ten weeks after the end of flowering). To facilitate the characterisation of any fenazaquin metabolites, applications at 150 g ai/hL were also made to 10 grape bunches spread over three to five vines at the late application time point with both P- and Q-labelled fenazaquin.

Grape bunches from the early season application were sampled at 0, 49 and 76 days after treatment (DAT) while grape bunches from the late season application were sampled at 0 and 28 DAT.

To determine the potential for fenazaquin and its metabolites to translocate from the site of application, individual branches on two separate vines were treated with ^{14}C-fenazaquin at the time of the early season application.

Following early season application, the levels of radioactivity, removed following the surface washes decreased from 78–81% of the TRR of day 0 to 29–34% of the TRR on day 76. Conversely, the amount of radioactivity extracted using acetonitrile:water (9:1, v/v) increased with increased duration from 16–18% of the TRR on day 0 to 39–45% of the TRR on day 76 as did the radioactivity in unextracted residues, from 3.2–5.0% of the TRR on day 0 to 22–32% of the TRR on day 76. No distinct differences between P- and Q-labelled fenazaquin were observed.

Characterisation of the residues in the surface washes of samples collected following the early season application showed the major component was fenazaquin, ranging from 24–29% of the TRR (0.22–0.31 mg eq/kg). Analysis of the extracts showed the presence of three metabolites derived from the intact fenazaquin molecule: fenazaquin acid, Metabolite C and Metabolite H. The remaining metabolites were products formed as a result of the cleavage of the ether bridge linking the quinazoline and the ethylphenylbutyl portions of the molecule. These minor metabolites, identified as dihydroxyquinazoline, 4-hydroxyquinazoline, Metabolite K and TBPE, each accounted for 1–6% of the TRR (0.004–0.06 mg eq/kg). A total of 8 unknown metabolites were characterized, two of which contained both the P- and Q-labels, four contained only the P-label and two included the Q-label only. In total, unknowns accounted for 6–9% of the TRR (0.06–0.09 mg eq/kg).

Following the late season application (28 DAT), the radioactivity recovered in the surface washes ranged from 61–71% of the TRR, corresponding to lower radioactivity in the extracts (21–27% of the TRR) and unextracted residues (8–12% of the TRR). Fenazaquin was the predominant residue in the P- and Q-labelled surface washes (46–64% of the TRR; 0.96–1.19 mg eq/kg). Five metabolites were also identified: Metabolite C, Metabolite H, 4-hydroxyquinazoline, dihydroxyquinazoline, and TBPE) in amounts of 0.4 to 3% of the TRR (0.01 to 0.07 mg eq/kg).

In the extracts, the parent fenazaquin, TBPE, dihydroxyquinazoline and 4-hydroxyquinazoline were detected, each accounting for < 6% of the TRR (< 0.16 mg eq/kg).

The translocation experiment showed that, following application of fenazaquin to branches, no radioactivity was found in grape bunches despite the measurable TRRs in the sprayed branches (10 mg eq/kg), thus confirming that translocation of fenazaquin and/or any degradation products beyond the site of application did not occur.

Treatment of grape bunches at the higher application rate (150 g ai/hL) were included in the experiment to assist in the identification of metabolites. The distribution of radioactivity in the higher rate samples was similar to that observed with the late season application samples with surface washes removing up to 60% of the TRR. The radioactivity in the washed fruit samples accounted for 40–44%

of the TRR. Analysis of the surface washes showed the presence of the parent compound, fenazaquin which represented 38% of the TRR and a number of unknown components, each accounting for less than 4% of the TRR. Analysis of the extracts showed the presence of more components than in the washes but fenazaquin was still the predominant analyte, accounting for an average of 14% of the TRR.

An additional experiment was conducted to assess whether the radioactivity was mainly associated with the grape skin. Grapes were peeled and the peel and pulp analysed separately. The results showed that 68% of the TRR was associated with the grape skin confirming that the predominant route of fenazaquin degradation is due to photolytic breakdown as the majority of the radioactivity had not yet reached the pulp tissue where metabolic processes are more likely to occur.

Maize

Applications of [^{14}C]Fenazaquin labelled in the phenyl ring (P-label) and in the quinazoline portion (Q-label) as aqueous suspensions were made at an average rate of 0.55 kg ai/ha by foliar application to maize (*Zea mays*) plants at the soft dough stage of development. The plants were grown in above ground wooden boxes located outdoors. Maize grain (corn-on-the-cob) and stover were harvested 20 DAT.

Acetonitrile extraction of the Q-label maize grain released 46% of the TRR, with fenazaquin identified as the major residue (23% of the TRR, 0.003 mg eq/kg). Fenazaquin dimer was also found at 7.7% of the TRR (0.001 mg eq/kg).

Extraction of the stover with acetonitrile:water followed by tetrahydrofuran released a minimum of 94% of the TRR . The major components identified in these combined extracts were fenazaquin (30–49% of the TRR; 2.0–3.0 mg eq/kg) and fenazaquin dimer (20–54% TRR; 1.2–3.6 mg eq/kg)). Minor components identified included 2-hydroxy-fenazaquin (0.5–1.0% of the TRR; 0.03–0.07 mg eq/kg)), TBPE (2% of the TRR; 0.12 mg eq/kg), fenazaquin acid (0.5% of the TRR; 0.03 mg eq/kg) and 4-hydroxyquinazoline (7% of the TRR; 0.42 mg eq/kg).

Fenazaquin did not readily translocate, as evidenced by the low levels of radioactive residue in the grain as compared to the higher levels in stover. The major route of transformation was conversion to the fenazaquin dimer. The presence of the minor identified metabolites, 4-hydroxyquinazoline and TBPE, suggested cleavage of the ether linkage. The intact fenazaquin appeared to have also been oxidized on the quinazoline ring to yield an alcohol or oxidized on the tert-butyl group to yield a carboxylic acid.

In summary, the metabolism of fenazaquin in fruits and cereals is well understood. The majority of the radioactive residues were located on the surface of the crops with limited penetration from the peel to the pulp. Furthermore, there was no evidence of translocation from the site of application to the untreated parts of the plant. While photolysis was the major route of degradation, the metabolic pathways of fenazaquin include: 1) formation of the dimer; 2) cleavage of the ether linkage to form 4-hydroxyquinazoline and TBPE; 3) oxidation of the quinazoline ring to yield 2-hydroxy-fenazaquin or oxidation on the tert-butyl group to yield fenazaquin acid followed by subsequent hydroxylation of these various metabolites. The metabolites, fenazaquin acid and 2-hydroxy-fenazaquin acid were identified as major metabolites in rats while 4-hydroxyquinazoline was a minor metabolite.

Animal metabolism

Lactating goats

The metabolism of fenazaquin was investigated in two lactating goats (*Capra hircus,* Alpine breed), weighing on average 42 kg, dosed orally once daily for 5 consecutive days, by a balling gun, with [^{14}C-quinazoline]fenazaquin (Q-label) or [^{14}C-phenyl]fenazaquin (P-label) at a dose level of 34 mg/day equivalent to 14 ppm feed. At sacrifice (within 22 hours after the final dose) samples of liver, kidney, muscle (loin and leg), fat (omental and perirenal), blood, bile and GI tract were collected.

The major route of elimination of the radioactivity was via the feces which accounted for 64–91% of the total administered dose (AD), while urine accounted for approximately 5% of the AD and milk accounted for 0.1% of the AD. The tissue burden was low (<1% of the AD). The overall recovery of the administered dose averaged 94% for both radiolabels.

The total radioactive residues (TRR) were highest in liver (0.41–0.79 mg eq/kg) followed by fat (0.09–0.12 mg eq/kg for composite fat and 0.07–0.15 mg eq/kg for P-label renal, omental and subcutaneous fat), kidney (0.04–0.09 mg eq/kg) and muscle (0.005 mg eq/kg for Q-label composite muscle and 0.007–0.03 mg eq/kg for P-label flank and loin muscle). The tissues from goats administered the [^{14}C-phenyl]fenazaquin had consistently higher concentrations of radioactive residues.

Greater than 88% of the TRR in milk was extracted following sequential extraction with acetonitrile and acetonitrile:water (1:1, v:v). Fenazaquin was detected as a predominant component of the residue in both P-label and Q-label milk samples (15–47% of the TRR; 0.004–0.02 mg eq/kg). 4-Hydroxyquinazoline (23% of the TRR; 0.006 mg eq.kg) was found in the Q-label milk sample only. The phenyl label sample was separated into milk fat and skim milk before analysis. The fenazaquin in this sample was found almost entirely in the milk fat portion of the whole milk sample.

Liver samples from both goats were extracted twice with acetonitrile:water (1:1), and once with acetonitrile. The solids remaining after the acetonitrile:water extraction of the Q-label liver sample were further extracted with methanol:water. The combined extracts accounted for 42–61% of the TRR. While fenazaquin was not detected in either sample of liver, 2-hydroxy-fenazaquin acid was the predominant residue representing 14–15% of the TRR (0.05–0.11 mg eq/kg). 4-Hydroxyquinazoline was the only metabolite identified in the hydrolyzed quinazoline PES sample (9% of the TRR; 0.04 mg eq/kg), while in the acid hydrolysate from the P-label sample, only Metabolite G and other unidentified components were detected, yet none exceeded 6% of the TRR.

The kidney samples were extracted in a similar manner to liver samples, with 88–89% of the TRR being extracted. No fenazaquin was detected. The identified metabolites were 2-hydroxy-fenazaquin acid (25–29% of the TRR; 0.01–0.02 mg eq/kg), Metabolite G (9% of the TRR; 0.01 mg eq/kg: P-label only) and 4-hydroxyquinazoline (6% of the TRR; 0.002 mg eq/kg: Q-label only). Unidentified residues accounted for 54% of the TRR (≤ 0.05 mg eq/kg). These accounted for extracted components observed on HPLC that did not match available reference standards (no more than 4 components; none of which exceeded 25% of the TRR [≤ 0.023 mg eq/kg]), unknowns (no more than 6 accounting for a total of 14% of the TRR [0.013 mg eq/kg]) and the remaining methanol:water extract which was not characterized or identified.

The P-label flank muscle sample was extracted twice with acetonitrile:water (1:1) and once with acetonitrile, resulting in 84% of the TRRs being extracted. Fenazaquin was not detected in flank muscle. The predominant metabolite observed was 2-hydroxy-fenazaquin acid (20% of the TRR; 0.005 mg eq/kg). Unidentified residues accounted for 64% of the TRR (≤ 0.02 mg eq/kg). Similar to kidney, these accounted for extracted components observed on HPLC that did not match available reference standards (no more than 3 components none exceeding 36% of the TRR [≤ 0.009 mg eq/kg]), unknowns (no more than 6 accounting for a total of 12% of the TRR [0.003 mg eq/kg]) and the remaining methanol:water extract which was not characterized or identified.

Greater than 92% of the TRR in P- and Q-label composite fat samples were extracted, following two extractions with acetone:hexane (1:4, v:v) and a third extraction with 100% acetone. The primary component of the residue was fenazaquin (77–83% of the TRR; 0.07–0.08 mg eq/kg). A trace of 2-hydroxy-fenazaquin was detected (1% of the TRR; 0.001 mg eq/kg) in both fat samples, and a trace of 2-hydroxy-fenazaquin acid (< 1% of the TRR; < 0.001 mg eq/kg) was detected in the phenyl label sample.

Laying hens

Twenty laying hens (*Gallus gallus domesticus*), weighing on average 1.4 kg, were dosed orally once daily for seven consecutive days with [^{14}C-quinazoline]fenazaquin at a dose level of 12.3 ppm feed, or [^{14}C-phenyl]fenazaquin at a dose level of 12.4 ppm feed. The test substances were administered using cellulose-filled gelatin capsules. The animals were sacrificed approximately 21–23 hours after the last dose and the liver, breast and thigh muscle, omental fat, subcutaneous fat and gastrointestinal tracts (with contents) were collected and pooled by treatment group.

Approximately 90% of the administered dose (AD) was recovered, most of which (an average of 89% of the AD) was excreta-related. Total radioactive residues (TRR) in eggs accounted for 0.04–0.3% of the AD. The tissue burden was low (< 0.1% of the AD). Highest concentrations of radioactivity were found in fat (0.16–0.18 mg eq/kg) followed by liver (0.06–0.1 mg eq/kg) and muscle (0.005–0.07 mg eq/kg).

Acetonitrile:water extracted 74–91% of the TRR in P- and Q-label eggs. The major residues in P-label eggs were fenazaquin (13% of the TRR; 0.003 mg eq/kg) and fenazaquin acid (13% of the TRR; 0.003 mg eq/kg). None of the individual unknowns accounted for more than 4% of the TRR. While 26% of the TRR (0.006 mg eq/kg) remained in the P-label PES, there was insufficient radioactivity for any further work-up. Fenazaquin and fenazaquin acid were also present in Q-label eggs (2% of the TRR [0.003 mg eq/kg] and 1% of the TRR [0.002 mg eq/kg], respectively), however, benzoyleneurea was the major residue in eggs, accounting for 82% of the TRR (0.12 mg eq/kg).

All of the radioactivity in the omental and subcutaneous fats was readily extracted with acetone and hexane (> 98% of the TRR). The predominant residue was fenazaquin (83–95% of the TRR; 0.134–0.158 mg eq/kg). While no other residue was identified in P-label fats, benzoyleneurea was detected in the Q-label fats (3–15% of the TRR; 0.005–0.02 mg eq/kg). The PES contained < 2% of the TRR.

In the P-label thigh and breast muscles, 75–80% of the TRR were extracted using acetonitrile:water. Fenazaquin (20–69% of the TRR; 0.001–0.01 mg eq/kg) and fenazaquin acid (20% of the TRR; 0.001 mg eq/kg) were the only residues detected. In the Q-label thigh and breast muscles, 72–76% of the TRR was extracted, with benzoyleneurea identified as the major residue (63–70% TRR; 0.04 mg eq/kg) and fenazaquin present at lower concentrations (≤ 5% of the TRR; < 0.003 mg eq/kg).

Extraction with acetonitrile:water and acetonitrile released 40–79% of the TRR in liver. No fenazaquin was detected. Moreover, in the P-label liver, fenazaquin acid was the only residue identified (7% of the TRR; 0.003 mg eq/kg), while in the Q-label liver, benzoyleneurea was the predominant metabolite accounting for 53% of the TRR (0.05 mg eq/kg).

The Meeting concluded that, in all species investigated (goats, hens and rats), the total administered radioactivity was predominantly eliminated in excreta. The metabolic profiles differed quantitatively between the species, yet qualitatively there are no major differences with the exception that the metabolism in goats was more extensive. The routes and products of metabolism were similar across all animals, resulting from oxidation of the tert-butyl group giving rise to the hydroxyl-fenazaquin (goats only), fenazaquin alcohol (postulated), fenazaquin acid and its hydroxylated form. The methylene group adjacent to the oxygen of the ether is also oxidized to release the 4-hydroxyquinazoline that is further oxidized to benzoyleneurea (laying hens only).

Environmental fate

The FAO Manual (FAO, 2016) explained the data requirements for studies of environmental fate. The focus should be on those aspects that are most relevant to MRL setting. For fenazaquin, supervised residue trials were received for foliar spray applications on permanent and semi-permanent crops. Therefore, according to the FAO Manual, only studies on rotational crops (confined, field), aerobic degradation, hydrolysis and soil photolysis are required.

Confined and field rotational crops

Confined/field rotational crop studies were not provided to the Meeting as cherries, pineapples, almonds and hops were considered permanent/semi-permanent crops.

In the future, should maximum residue levels be requested for non-permanent crops, the Meeting recommends that confined and field rotational crop studies should be provided.

Aerobic degradation in soil

The degradation of ^{14}C-fenazaquin, uniformly labelled in the tert-butylphenyl ring (P-label) was studied in four soil types in the laboratory under aerobic conditions. The soils were incubated in the dark at 20 °C. Fenazaquin was applied to each soil subsample at a rate of 0.27 mg ai/kg.

The predominant degradation product in all soils was 2-oxy-fenazaquin which reached a maximum of 9% of the applied radioactivity (AR). Fenazaquin acid reached its maximum concentration (2% of the AR) at 14 DAA. In addition to these two minor metabolites, Metabolites G, H and additional unknowns were also detected at < 3% of the AR by the end of the study.

The half-lives (DT_{50}) determined using single first-order (SFO) kinetics ranged from 48 days to 114 days depending on the soil type. The results of the sterilised control samples showed that the degradation of fenazaquin was mainly microbiological.

The rate of degradation of ^{14}C-fenazaquin was also investigated in a laboratory where ^{14}C-quinazoline labelled (Q-label), ^{14}C-phenyl labelled (P-label) and unlabelled fenazaquin were individually applied to a sandy loam soil at 0.56 g ai/ha. In addition, soil samples maintained in an open system, were treated with the same mixture at an exaggerated application rate (56 g ai/ha) for the structural determination of metabolites. Less than 0.2% of the AR was lost by day 365 as a result of volatilization, indicating that it was not a significant dissipation pathway for fenazaquin or its metabolites. During incubation, the mineralisation to CO_2 continuously increased until the end of incubation (≤ 21% of the AR).

No single degradate exceeded 10% of the AR at the lower application rate, however, at the higher application rate, the major metabolite was 2-oxy-fenazaquin, reaching a maximum level of 8% at day 168. The amount of applied fenazaquin decreased steadily from 99% (day 0) to 13% of the AR (day 336), resulting in a calculated DT_{50} of 51 days, determined using SFO kinetics.

In summary, the degradation of fenazaquin in soil appeared to proceed via hydrolysis of the ether linkage between the quinazoline ring system and the tertiary butyl-phenyl portions of the parent molecule, hydroxylation of the quinazoline ring system, and oxidation of the tertiary butyl group and phenylethyl alcohol, formed following hydrolysis of fenazaquin.

Field dissipation

The dissipation of ^{14}C-fenazaquin in soil under field conditions was investigated at one USA test site. ^{14}C-Quinazoline or ^{14}C-phenyl labelled fenazaquin, formulated as emulsifiable concentrate formulations, were applied to bare soil at an application rate of 0.224 kg ai/ha.

The amount of applied fenazaquin decreased from 93–96% of the AR to 9–11% of the AR by day 112, resulting in DT_{50} values of 34 to 36 days, applying single first order kinetics.

The dissipation of fenazaquin in soil under field conditions was also investigated in Germany and Italy. A suspension concentrate of fenazaquin was applied to bare soil at application rates of 0.15 and 0.20 kg ai/ha, respectively. The amount of applied fenazaquin declined relatively rapidly where the residues of fenazaquin reached non-quantifiable levels (< 0.005 mg/kg) 3 months after application.

The DT_{50} values of fenazaquin were calculated to range from 26–75 days applying 1st-order reaction kinetics.

The Meeting concluded that fenazaquin is moderately persistent in soil.

Photolysis - Soil

The photodegradation of ^{14}C-fenazaquin was investigated under laboratory conditions using a sandy loam soil. Soil samples were treated separately with 40 µg of ^{14}C-quinazoline labelled fenazaquin (Q-label) or ^{14}C-phenyl labelled fenazaquin (P-label). Immediately after dosing, the treated soils were exposed to simulated summer sunlight for up to 30 days.

Fenazaquin decreased from 91–93% to 35–42% of the AR during 30 days of irradiation. In the dark control samples the amount of fenazaquin decreased slightly from 91–95% at day 4 to 68–87% of the AR at day 30. In the Q-labelled study, up to 6 minor degradation products were detected, none of which exceeded 3% of the AR in the irradiated and dark control samples. One major degradation product was 4-hydroxyquinazoline (irradiated samples: 0.4 to 37% of the AR; dark control samples: 1% to 7% of the AR). In the P-labelled study, two degradation products were identified, 4-tert-butylphenylacetic acid and TBPE. The amount of 4-tert-butylphenylacetic acid increased continuously during irradiation to 7.3% of the AR and did not exceed 0.1% in dark control samples. The amount of TBPE increased continuously from 1.4 to 18% of the AR (irradiated samples) and from 2% to 8% of the AR (dark control samples) immediately after application to day 30, respectively. The metabolite 4-tert-butylstyrene was also detected and accounted for a maximum level of 6% of the AR. No other volatiles were detected in significant amounts.

The half-life of fenazaquin on the soil surface was calculated to be 15 days in summer sunlight.

The Meeting concluded that the photolysis of fenazaquin in soil, under sunlight conditions, was an important degradation pathway. This is further supported by the photolysis experiments of the plant metabolism studies which demonstrated that the degradation of fenazaquin was enhanced in the presence of sunlight.

Photolysis – Aqueous Solution

The photolytic stability of ^{14}C-fenazaquin was studied in a laboratory using distilled water. Stock solutions of ^{14}C-quinazoline and ^{14}C-phenyl labelled fenazaquin in acetonitrile were prepared at a concentration of 2.5 g ai/hL and applied to distilled water, resulting in a nominal concentration of 0.01 g ai/hL. Test vessels containing the test solutions were exposed to simulated summer sunlight for up to 32 days at 25 ± 1 °C.

Three degradation products of fenazaquin were detected in the distilled water of both light exposed and dark control samples and identified as 4-hydroxyquinazoline, TBPE and 4-tert-butylstyrene. In light exposed samples treated with the ^{14}C-quinazoline label, the amount of 4-hydroxyquinazoline increased continuously reaching a maximum level of 32% of the AR after 32 days of exposure. In dark control samples, 4-hydroxyquinazoline reached 9% of the AR after 32 days. In light exposed samples treated with the ^{14}C-phenyl label, the amount of TBPE and 4-tert-butylstyrene increased during exposure time and peaked at 19% and 9% of the AR at days 32 and 28, respectively. In dark control samples, TBPE and 4-tert-butylstyrene reached 11% and 0.2% of the AR after 32 days, respectively.

The DT_{50} value of fenazaquin was calculated to be 15 days.

Similar to the soil photolysis study, fenazaquin degraded rapidly in the presence of sunlight.

In summary, the Meeting concluded that fenazaquin is moderately persistent in soil under field conditions (DT_{50s} ranging from 26–114 days). Under laboratory conditions, the photolytic degradation of fenazaquin in soil was enhanced under sunlight as was its degradation in distilled water.

Methods of analysis

Methods have been reported in the scientific literature for the analysis of fenazaquin in food, including multi-residue methods. These methods do not involve a hydrolysis step, therefore, the measured residue is reported as fenazaquin, *per se*.

The Meeting received the description and validation data for various analytical methods capable of quantifying fenazaquin in plant commodities. All residue analytical methods rely on GC-MS or LC-MS/MS. The typical LOQ achieved for the plant commodities is 0.01 mg/kg. Methods were successfully validated by independent laboratories, demonstrating good reproducibility.

The Meeting also received the description and validation data for an LC-MS/MS analytical method capable of quantifying fenazaquin in animal commodities (muscle, fat, liver, milk and eggs). The LOQ achieved for all animal commodities is 0.01 mg/kg. The method was successfully validated by an independent laboratory.

Stability of pesticide residues in stored analytical samples

The Meeting received storage stability studies on fenazaquin and the fenazaquin dimer in tomatoes, corn (stover, forage and grain), mint and almond nutmeat. Individual samples were fortified with both analytes at a concentration of 0.1 mg/kg/analyte and stored frozen at -25 °C to -10 °C. Samples were taken for analysis at intervals up to 26 months.

Storage stability studies showed that fenazaquin was stable for 7 months in tomatoes (high water), 26 months in maize stover, 6 months in maize forage (high water) and 3.5 months in maize grain (high starch). The fenazaquin dimer was observed to be stable for 1.5 months in tomatoes, 18 months in maize stover, 17 months in maize forage, 3.5 months in maize grain and 7 months in almond nutmeats. The Meeting could not determine the demonstrated storage stability interval for fenazaquin and its dimer in mint or the stability of fenazaquin in almond nutmeats as > 30% dissipation was observed within the first 3 months of storage in these commodities.

Since livestock feeding studies were not provided to the Meeting, neither was a freezer storage stability study for fenazaquin and metabolite residues in animal commodities.

In the future, should maximum residue levels be requested for crops, from which may be derived feed items, that are likely to contribute significantly to the livestock diet, the Meeting recommends that freezer storage stability studies for animal commodities should be provided, if samples of animal matrices in the livestock feeding studies are stored for greater than 30 days.

Definition of the residue

The nature of the fenazaquin residues was investigated in apples, oranges, grapes and maize following foliar application.

The predominant residue in edible parts of the mature food crops was the parent, fenazaquin, accounting for 10–66% of the TRR. The fenazaquin dimer was also observed in mature apples (8–32% of the TRR) and maize grain (8% of the TRR). Several minor metabolites were observed, none of which exceeded 10% of the TRR. Under simulated processing conditions, quinazoline ring-labelled fenazaquin was degraded to 4-hydroxyquinazoline, the major hydrolysis product formed under pasteurisation conditions (pH 4, 90 °C, 20 minutes), accounting for 61% of the applied radioactivity (approximately 2-fold higher than that of the parent).

Confined and field rotational crop studies were not required as the uses being considered by this Meeting are semi-permanent/permanent crops.

Fenazaquin was the only analyte present as a major compound in all tested matrices. Suitable analytical methods are available to analyse the parent compound.

The Meeting considered that fenazaquin was a suitable marker for enforcement of MRLs for fruits and cereal crops.

From the dietary risk perspective, the Meeting considered the potential exposure and toxicity of the fenazaquin dimer and 4-hydroxyquinazoline. Levels of these metabolites in apples, grapes and maize grain, sampled 7–28 DAT, 28 DAT and 20 DAT, respectively, in the metabolism studies, were considered as these encompassed the range of PHIs of the critical GAPs (1–14 days) provided to the Meeting. In the orange metabolism study, fruits were collected 63 and 191 DAT, therefore, this study was not considered further, as the residues of these metabolites would likely overestimate those

expected at shorter PHIs. The percentage of the fenazaquin dimer in comparison to the parent in whole apples (7–14 DAT) ranged from 13–57%, and was 27% in maize grain (20 DAT). No dimer was observed in grapes. The metabolite 4-hydroxyquinazoline accounted for 0.5–9% and 4% of the fenazaquin concentrations in whole apples (7–28 DAT) and grapes (28 DAT), respectively. 4-Hydroxyquinazoline was not observed in maize.

While the fenazaquin dimer, a photoproduct, was not observed in rats, the metabolite is unlikely to be readily absorbed due to its high molecular weight and lipophilic properties and is thus not likely to be more toxic than the parent compound. The 4-hydroxyquinazoline metabolite was observed in rats and based on the metabolite-specific toxicity studies, it was determined to be no more toxic than the parent.

In light of the residue profile, measurable residues of the predominant metabolites, fenazaquin dimer and 4-hydroxyquinazoline, are not expected in crops harvested at short PHIs. Considering that these compounds are not more toxic than parent fenazaquin, the Meeting concluded that fenazaquin is the only residue pertinent for assessing dietary risk.

The nature of the fenazaquin residues was investigated in livestock following oral administration of the test substance.

In the lactating goat metabolism study, fenazaquin and 4-hydroxyquinazoline were the predominant residues in milk (15–47% of the TRR and 23% of the TRR, respectively) while fenazaquin was the major residue in fat (77–83% of the TRR). The parent was not detected in liver, kidney or muscle. In these tissues, 2-hydroxy-fenazaquin acid was observed as the major metabolite accounting for 14–29% of the TRR.

In the laying hen metabolism study, fenazaquin was a major residue (13–71% of the TRR) in eggs and tissues (except liver) while fenazaquin acid was the predominant metabolite in eggs and muscle (13–20% of the TRR). In Q-label eggs, fat, muscle and liver, the cleavage product, benzoyleneurea, was the major component (15–82% of the TRR).

Fenazaquin is the predominant residue in milk and fat of lactating goats and is detectable in eggs and all poultry tissues. The parent compound was not detected in kidney, liver and muscle of lactating goats. In these tissues, the 2-hydroxy-fenazaquin acid was identified as the principal metabolite. A valid enforcement analytical method is available for fenazaquin; however, a method for the 2-hydroxy-fenazaquin acid was not provided.

The Meeting concluded that fenazaquin and 2-hydroxy-fenazaquin acid are suitable markers for enforcement of MRLs for livestock commodities.

In lactating goats, 2-hydroxy fenazaquin acid may contribute to the consumer exposure as it accounts for the majority of the TRRs in liver (up to 15% of the TRR [0.11 mg eq/kg]), kidney (up to 29% of the TRR [up to 0.02 mg eq/kg]) and muscle (20% of the TRR [0.005 mg eq/kg]), where no parent is present. In milk, 2-hydroxy-fenazaquin acid was up to 25% of the parent residues while in fat, it accounted for less than 2% of the parent residues. The metabolite 4-hydroxyquinazoline is predominantly found in milk accounting for 1.5-fold the fenazaquin residues. In tissues, 4-hydroxyquinazoline was either not detected or detected at lower levels than those of the metabolite 2-hydroxy-fenazaquin acid. The Meeting concluded that the 2-hydroxy-fenazaquin acid and 4-hydroxyquinazoline metabolites are not likely to be more toxic than the parent fenazaquin.

For laying hens, the Meeting considered the potential exposure and toxicity of the metabolites fenazaquin acid and benzoyleneurea, which were significant residues in poultry matrices. Fenazaquin acid occurred at levels similar to parent fenazaquin in eggs, and muscle. Fenazaquin acid was a minor residue in liver, where no fenazaquin was detected. The Meeting determined that fenazaquin acid is a predominant metabolite in rats and no more toxic than the parent compound. The benzoyleneurea was the major residue in eggs (concentration *ca*. 40-fold greater than parent), muscle (*ca*. 13-fold greater than parent), and liver (*ca*. 50% TRR, 0.05 mg eq/kg; no fenazaquin observed). As the benzoyleneurea is structurally similar to the 4-hydroxyquinazoline, it is not expected to be more toxic than the parent fenazaquin. The Meeting noted that, at this time, there are no fenazaquin-treated

poultry feed items; therefore, exposure to fenazaquin-related residues from poultry commodities is not expected.

In summary, based on the above, the Meeting recommended the following residue definition for compliance with MRLs and for dietary risk assessment:

Definition of the residue for plant commodities for enforcement of MRLs and for dietary risk assessment: *fenazaquin*

Definition of the residue for animal commodities for enforcement of MRLs and for dietary risk assessment: *sum of fenazaquin and the metabolites 2-(4-{2-[(2-hydroxyquinazolin-4-yl)oxy]ethyl}phenyl)-2-methylpropanoic acid (2-hydroxy-fenazaquin acid) and quinazoline-4-ol and 3,4-dihydroquinazolin-4-one (4-hydroxyquinazoline), expressed as fenazaquin equivalents.*

The Log Kow of fenazaquin is greater than 5, indicating its propensity to sequester into fat. In lactating goats, the ratio of residues (sum of fenazaquin, 2-hydroxy-fenazaquin acid and 4-hydroxyquinazoline) in fat to muscle was greater than 10-fold. Therefore, the Meeting considered the residue fat soluble.

Results of supervised residue trials on crops

Cherries

In the USA, the critical GAP for fenazaquin on sweet and tart cherries is a single foliar spray application at 504 g ai/ha and a PHI of 3 days.

A total of five independent trials were conducted in the USA on sweet and tart cherries during the 2008 and 2009 growing seasons. All five trials were conducted in accordance with the critical GAP. Residues in samples harvested at the PHI of 3 days were: 0.26, 0.47, 0.56, 0.84, 0.91 mg/kg with the highest analytical result reported at 0.965 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg, an HR of 0.965 mg/kg and an STMR of 0.56 mg/kg for fenazaquin on the subgroup of cherries.

While supervised residue trials were provided for peaches and plums, in the absence of a critical GAP for these crops, maximum residue levels could not be estimated.

Pineapples

In the Philippines, the GAP for fenazaquin on pineapples is two foliar spray applications of 1.0 kg ai/ha and a minimum PHI of 14 days.

Only one trial in Costa Rica during the 2013 growing season was conducted in accordance with the Philippines GAP, where the average residue was reported to be 0.085mg/kg

The Meeting could not estimate a maximum residue level in the absence of a sufficient number of trials conducted in accordance with the minimum PHI of 14 days prescribed on the Philippine label.

Almonds

In the USA, the critical GAP for fenazaquin on almonds is a single foliar spray application at 504 g ai/ha and a PHI of 7 days.

A total of five independent trials were conducted in the USA on almonds during the 2008 growing season. All five trials were conducted in accordance with the critical GAP in the USA. Almonds were harvested at the 7-day PHI and residues in almond nutmeats were: < 0.01 (4) and 0.01 mg/kg.

As the supervised field trial residues were not supported by the demonstrated storage stability data for almond nutmeats, the Meeting could not estimate a maximum residue level.

Hops

In the USA, the critical GAP for fenazaquin on hops is a single foliar spray application at 504 g ai/ha and a PHI of 7 days.

A total of seven independent trials were conducted in the USA on hops during the 2008 growing season. All seven trials were conducted in accordance with the critical GAP in the USA. Green hop cones were harvested at the 7-day PHI and dried for 4 to 36 hours using a commercial oven maintained at 46 ºC to 66 ºC. Residues in dried hop cones were: 0.72, 1.0, 1.1, 9.0, 9.8, 11 and 12 mg/kg.

No information on the metabolism of fenazaquin in/on leafy vegetables was provided to the Meeting. However, according to the metabolism study in maize, fenazaquin is the predominant residue observed in stover at a preharvest interval (20-DAT) that is almost 3-fold longer than the GAP PHI, demonstrating limited metabolism of the parent compound. Therefore, the Meeting estimated a maximum residue level of 30 mg/kg and an STMR of 9.0 mg/kg for fenazaquin on hops.

Animal feed items

Almond hulls

In the USA, the critical GAP for fenazaquin on almonds is a single foliar spray application at 504 g ai/ha and a PHI of 7 days.

As the supervised field trial residues were not supported by the demonstrated storage stability data for almond hulls, the Meeting could not estimate a maximum residue level.

Fate of residues during processing

High temperature hydrolysis

In the high temperature hydrolysis study, quinazoline ring-labelled fenazaquin was degraded to a significant extent to 4-hydroxyquinazoline under simulated pasteurisation conditions (pH 4, 90 °C, 20 minutes), accounting for 61% of the applied radioactivity at the end of incubation which represented almost two fold the levels detected for the parent, fenazaquin. For processes simulating baking/brewing/boiling (pH 5, 100 °C, 60 minutes) and sterilisation (pH 6, 120 °C and 60 minutes), 4-hydroxyquinazoline represented 22–60% of the parent concentrations, at the end of incubation. The fate of the phenyl ring moiety under processing conditions has not been provided to the Meeting.

Processing

The Meeting received information on the fate of fenazaquin residues during the processing of plums and pineapples. No recommendations could be made for any of the processed commodities as there is no GAP for plums and there were an insufficient number of trials conducted in accordance with the critical GAP for pineapple.

Animal commodities maximum residue level estimation

The information provided to the Meeting on almond hulls, the only potential livestock feed item, could not be considered in the calculation of the dietary burden. Therefore, maximum residue levels for animal commodities were not estimated.

RECOMMENDATIONS

On the basis of the data from supervised trials, the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits and for IESTI and IEDI assessments.

Definition of the residue for plant commodities for enforcement of MRLs and for dietary risk assessment: *fenazaquin*

Definition of the residue for animal commodities for enforcement of MRLs and for dietary risk assessment: *sum of fenazaquin and the metabolites 2-(4-{2-[(2-hydroxyquinazolin-4-yl)oxy]ethyl}phenyl)-2-methylpropanoic acid (2-hydroxy-fenazaquin acid) and quinazoline-4-ol and 3,4-dihydroquinazolin-4-one (4-hydroxyquinazoline), expressed as fenazaquin equivalents.*

CCN	Commodity Name	Recommended maximum residue level, mg/kg	STMR, mg/kg	HR, mg/kg
DH 1100	Hops, dry	30	9	-
FS 0013	Subgroup of cherries	2	0.56	0.965

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The International Estimated Dietary Intakes (IEDIs) of fenazaquin were calculated for the 17 GEMS/Food cluster diets using STMRs estimated by the current Meeting (Annex 3 to the 2017 Report). The ADI is 0–0.05 mg/kg bw and the calculated IEDIs were 0–0.2% of the maximum ADI. The Meeting concluded that the long-term dietary exposure to residues of fenazaquin resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term dietary exposure

The ARfD for fenazaquin is 0.1 mg/kg bw. The International Estimate of Short Term Intake (IESTI) for fenazaquin was calculated for the food commodities for which STMRs or HRs were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2017 JMPR Report. The IESTI represented 0–10% of the ARfD for the general population including children. The Meeting concluded that the short-term dietary exposure to residues of fenazaquin, from the uses that have been considered by the present Meeting, is unlikely to present a public health concern.

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