Ethiprole (304)

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EXPLANATION

Residue and analytical aspects of ethiprole were considered for the first time by the 2018 JMPR.

Ethiprole is a broad spectrum non-systemic insecticide of the phenylpyrazole class for control of plant hoppers, thrips, aphids, weevils, flies and maggots, grasshoppers, psyllids, leaf minders and some species of whitefly. Ethiprole acts by interfering with the passage of chloride ions through the γ-aminobutyric acid GABA regulated chloride channel, thereby disrupting central nervous system activity and causing death.

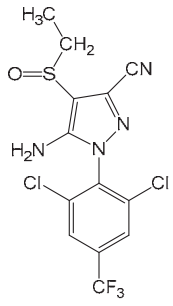
It was scheduled for evaluation as a new compound at the Forty-ninth Session of the CCPR (2017).

The manufacturer supplied information on identity, metabolism and environmental fate, methods of residue analysis, freezer storage stability, registered use patterns, supervised residue trials, fate of residues in processing and farm animal feeding studies.

SPECIFICATIONS

Specifications for ethiprole have not been developed by FAO.

IDENTITY

ISO common name:	Ethiprole
IUPAC name:	5-Amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-ethylsulfinylpyrazole-3-carbonitrile
Chemical Abstract name:	1H-pyrazole-3-carbonitrile, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)- (9CI)
CAS No.:	181587-01-9
CIPAC No.:	Not allocated
Manufacturer's experimental name:	RPA 107382
Molecular Formula:	$C_{13}H_9Cl_2F_3N_4OS$
Structural Formula:	 Ethiprole
Molecular Weight:	397.2 g/mol

PHYSICAL AND CHEMICAL PROPERTIES

Pure Active Ingredient (except where noted as technical grade)

Property	Results	Test material purity	Reference
Ethiprole			
Melting point	No melting point observed before the decomposition point. Decomposition point 164.5 °C.	99.6% w/w	Bascou 2000b, M-191984-01-2

Property	Results	Test material purity	Reference
	No melting point observed before the decomposition point. Decomposition point 165.1 °C.	97.2% w/w (technical grade)	
Boiling point	No boiling point observed before the decomposition point.	99.6% w/w	
	No boiling point observed before the decomposition point.	97.2% w/w (technical grade)	
Relative Density	Active substance, pure: 1.54 g/mL at 20 °C	99.6% w/w	
	Active substance, technical grade: 1.56 g/mL at 20 °C	97.2% w/w (technical grade)	
Vapour pressure	9.1×10^{-8} Pa at 25 °C.	99.6% w/w	Bascou 2001a, M-191486-01-2
Henry's law constant	(Calculation) K = 2.15×10^{-6} Pa m^3 mol^{-1} at 20 °C	Not given	Bascou 2002, M-214281-01-2
Description of the physical state and colour, purity of the ai. and of technical grade	White crystalline powder	99.6% w/w	Bascou 2000b, M-191984-01-2
	Pale brown crystalline powder	97.2% w/w (technical grade)	
	Light yellow powder	95.8% w/w (technical grade)	Ziemer and Eyrich 2013, M-458874-01-1
Solubility of purified active substance in water	9.2 mg/L at 20 °C	99.6% w/w	Bascou 2001c, M-202032-01-2
Solubility in organic solvents	[g/L at 20 °C] acetone 90.7 ethyl acetate 24.0 methanol 47.2 acetonitrile 24.5 dichloromethane 19.9 n-octanol 2.4 n-heptane 0.004 toluene 1.0	97.2% w/w (technical grade)	
n-Octanol/ water partition coefficient	log P_{ow} 2.9 at 20 °C	purity 99.6% w/w	Bascou 2000a, M-191980-01-2
Hydrolysis rate at pH 4, 7 and 9 under sterile and dark conditions	DT_{50} (days) pH 4 No hydrolysis after 31 days pH 5 No hydrolysis after 31 days pH 7 No hydrolysis after 31 days pH 9 121 days by extrapolation Ethiprole is stable at pH 4, 5 and 7 and was slowly hydrolysed at pH 9. At 25°C: The only significant hydrolysis product detected at pH 9 was the ethiprole-amide metabolite (RPA 112916)	[^{14}C] RPA 107382, radiochemical purity 97.9%	Shepler 1998, M-191939-01-2
Direct phototransformation in sterile water using artificial light	DT_{50} = 6.46 hours under a xenon lamp corresponding to 1.3 days of summer sunlight in Florida. The major photodegradation metabolite products of ethiprole were: RPA 157925 (RT 25.6 mins) AE 0813783 (RT 10.3 mins) AE 0813782 (RT 9.3 mins)	[^{14}C] RPA 107382, radiochemical purity 97.2%	Corgier and Turier 2002, M-192004-02-1
Quantum yield of direct transformation	A mean quantum yield of Φ = 0.00646 was calculated.	[phenyl-U-^{14}C] RPA 107382, radiochemical purity 100%	Mamouni 2001, M-199902-01-1
Dissociation in water of purified active substance	Not relevant since the substance is not ionisable in water. However, the potentiometric and the spectrophotometric methods have been applied without success. The estimated pKa has been calculated using modelling software and was found to be: $pKa_{(calculated)}$ = -3.9	99.6% w/w	Bascou 2001b, M-191482-01-2

Property	Results	Test material purity	Reference
pH	4.4 at approximately 22 °C (conc. of 1% (w/v) suspension in water containing 2% v/v acetonitrile)	97.2% w/w (technical grade)	

Formulations

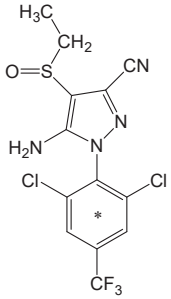
Various ethiprole formulations are available in the following products.

Active substance and content	Formulation type
100 g/L ethiprole	SC
200 g/L ethiprole	SC
100 g/L ethiprole 100 g/L imidacloprid	SC
400 g/kg ethiprole 400 g/kg imidacloprid	WG
20 g/kg ethiprole 8 g/kg imidacloprid	GR
20 g/kg ethiprole 40 g/kg metominostrobin	GR
7.5 g/kg chlorantraniliprole 40 g/kg ethiprole 20 g/kg imidacloprid	GR
2.5 g/kg ethiprole 4 g/kg silafluofen 5 g/kg tricyclazole	DP
30.6 g/L ethiprole 71.4 g/L silafluofen	SE
2.5 g/kg ethiprole 4 g/kg silafluofen	DP
100 g/kg ethiprole 200 g/kg silafluofen	WP
30 g/kg ethiprole 70 g/kg silafluofen 80 g/kg tricyclazole	SC
115 g/L ethiprole 287.5 g/L thiacloprid	SC
5 g/kg ethiprole	DP
350 g/L ethiprole	FS
15 g/kg ethiprole	GR
103 g/L ethiprole	SC
20 g/kg ethiprole	GR

METABOLISM AND ENVIRONMENTAL FATE

General

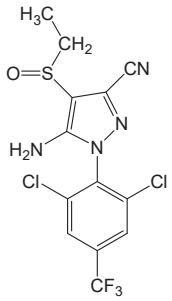
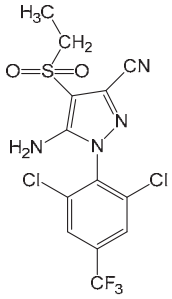
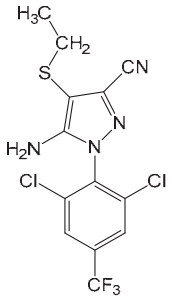
The studies for plant metabolism (rice, sweet pepper and cotton), animal metabolism (poultry, goat and rats) and confined rotational crops, as well as the environment (soil and water) were conducted with the test material shown below, with the label position indicated in the following structural formula:

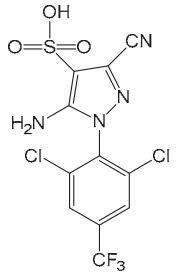
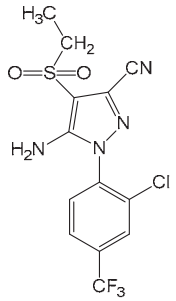
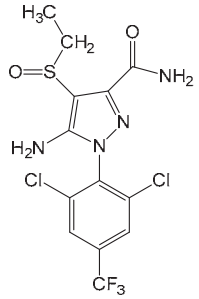
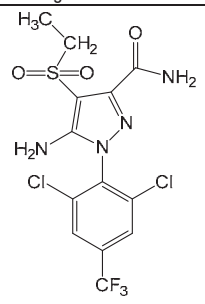


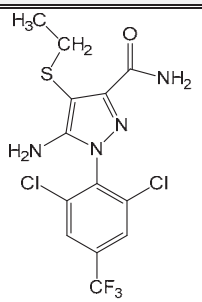
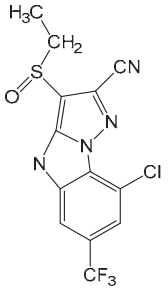
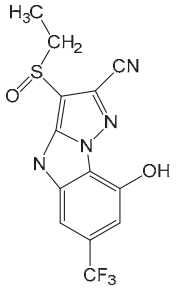
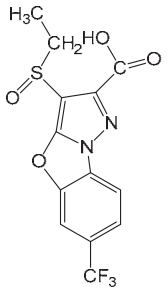
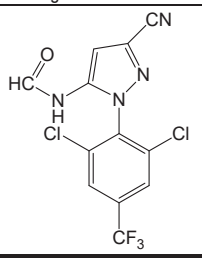
[phenyl-UL-^{14}C] ethiprole
(RPA 107382)

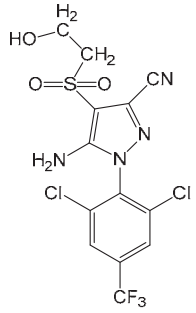
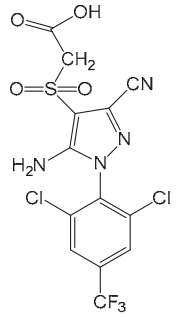
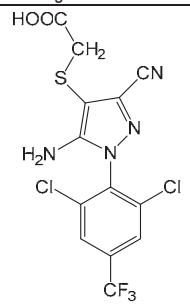
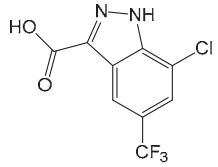
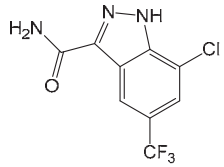
Table 1 summarises the names, codes, and structures of the parent and principal metabolites found in plant, livestock, rat, rotational crop and environmental studies.

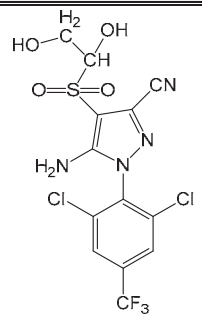
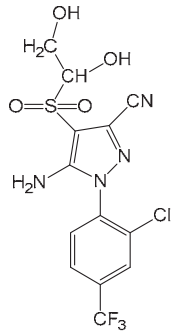
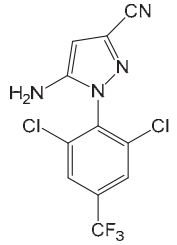
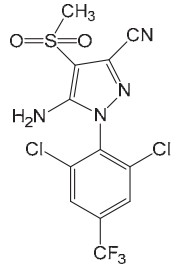
Table 1 Ethiprole and metabolites/degradates found in metabolism and environmental fate studies

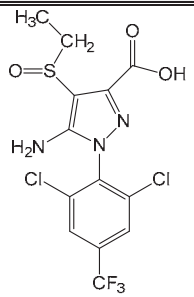
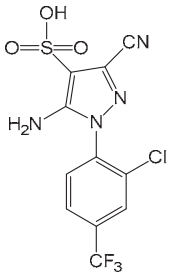
Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
Active substance Ethiprole RPA 107382	 $C_{13}H_9Cl_2F_3N_4OS$ 397.2 g/mol CAS-No.: 181587-01-9	5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-ethylsulfinylpyrazole-3-carbonitrile (IUPAC)	All matrices
Ethiprole–sulfone RPA 097973	 $C_{13}H_9Cl_2F_3N_4O_2S$ 413.2 g/mol CAS-No.: 120068-68-0	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfonylpyrazole-3-carbonitrile	Rat, goat, hen Rice (soil & foliar application), Sweet pepper, cotton Rotational crops: radish, lettuce, wheat Soil, water-sediment
Ethiprole–sulfide RPA 107566	 $C_{13}H_9Cl_2F_3N_4S$	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylthiopyrazole-3-carbonitrile	Rat (faeces), goat, hen Sweet pepper Soil, water-sediment

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
	381.2 g/mol		
Ethiprole–sulfonic acid RPA 104615	 $C_{11}H_5Cl_2F_3N_4O_3S$ 401.2 g/mol	5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-4-sulfonic acid	Rat, goat, hen Rice, sweet pepper, cotton Rotational crops: radish, lettuce, wheat Soil
Ethiprole–des-chloro-sulfone RPA 115369	 $C_{13}H_{10}ClF_3N_4O_2S$ 378.8 g/mol	5-amino-1-[2-chloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfonyl)-1H-pyrazole-3-carbonitrile	Rice, cotton
Ethiprole-amide RPA 112916	 $C_{13}H_{11}Cl_2F_3N_4O_2S$ 415.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1H-pyrazole-3-carboxamide	Rat, goat, hen Rice, sweet pepper, Rotational crops: radish, lettuce, wheat Soil-Water
Ethiprole–sulfone-amide RPA 112917	 $C_{13}H_{11}Cl_2F_3N_4O_3S$ 431.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfonyl)-1H-pyrazole-3-carboxamide	Rice, sweet pepper Rotational crops: radish, lettuce, wheat Soil

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
Ethiprole–sulfide-amide RPA 112915	 $C_{13}H_{11}Cl_2F_3N_4OS$ 399.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfanyl)-1H-pyrazole-3-carboxamide	Rotational crops: radish, lettuce, wheat
Ethiprole-benzimidazole RPA 157925	 $C_{13}H_8ClF_3N_4OS$ 360.7 g/mol	8-chloro-3-(ethylsulfinyl)-6-(trifluoromethyl)-4H-pyrazolo[1,5-a]benzimidazole-2-carbonitrile	Water (aqueous photolysis in sterile and natural water)
Ethiprole-des-chloro-hydroxy-benzimidazole AE 0764815	 $C_{13}H_9F_3N_4O_2S$ 342.3 g/mol	3-(ethylsulfinyl)-8-hydroxy-6-(trifluoromethyl)-4H-pyrazolo[1,5-a]benzimidazole-2-carbonitrile	Water (aqueous photolysis in natural water)
Ethiprole-des-chloro-carboxy-benzoxazole AE 0966829	 $C_{13}H_9F_3N_2O_4S$ 346.3 g/mol	3-(ethylsulfinyl)-6-(trifluoromethyl)pyrazolo[5,1-b][1,3]benzoxazole-2-carboxylic acid	Water (aqueous photolysis in sterile water)
Ethiprole-formamide RPA 103343		N-{3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazol-5-yl}formamide	Cotton, rice

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
	$C_{12}H_5Cl_2F_3N_4O$ 349.1 g/mol		
Ethiprole–sulfone -hydroxide RPA 114345	 $C_{13}H_9Cl_2F_3N_4O_3S$ 429.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(2-hydroxyethyl)sulfonyl]-1H-pyrazole-3-carbonitrile	Rat, goat, hen
Ethiprole–sulfone-carboxy RPA 112705	 $C_{13}H_7Cl_2F_3N_4O_4S$ 443.2 g/mol	({5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazol-4-yl}sulfonyl)acetic acid	Rat, hen (egg white)
Ethiprole–sulfide -carboxy RPA 112716	 $C_{13}H_7Cl_2F_3N_4O_2S$ 411.2 g/mol	({5-amino-3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-1H-pyrazol-4-yl}sulfanyl)acetic acid	Rat, hen
Ethiprole–benzpyrazole-carboxy AE 0813782	 $C_9H_4ClF_3N_2O_2$ 264.6 g/mol	7-chloro-5-(trifluoromethyl)-1H-indazole-3-carboxylic acid	Water (aqueous photolysis in sterile water)
Ethiprole – benzpyrazole-carboxamide AE 0813783	 $C_9H_5ClF_3N_3O$ 263.6 g/mol	7-chloro-5-(trifluoromethyl)-1H-indazole-3-carboxamide	Water (aqueous photolysis in sterile water)

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
Ethiprole dihydroxy-sulfone Dihydroxy- RPA 097973	 $C_{13}H_9Cl_2F_3N_4O_4S$ 445.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1,2-dihydroxyethyl)sulfonyl]-1H-pyrazole-3-carbonitrile	Hen (egg white)
Ethiprole monochloro-dihydroxy-sulfone	 $C_{13}H_{10}ClF_3N_4O_4S$ 410.8 g/mol	5-amino-1-[2-chloro-4-(trifluoromethyl)phenyl]-4-[(1,2-dihydroxyethyl)sulfonyl]-1H-pyrazole-3-carbonitrile	Cotton
MB 45897 RPA 097920	 $C_{11}H_5Cl_2F_3N_4$ 321.1 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile	Rat, goat, hen Sweet pepper Rotational crops: radish, lettuce, wheat
Ethiprole methyl sulfone RPA 094569	 $C_{12}H_7Cl_2F_3N_4O_2S$ 399.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-methylsulfonyl-1H-pyrazole-3-carbonitrile	Goat, hen

Abbreviation or Code	Chemical Structure	IUPAC Name	Found in
Ethiprole acid	 $C_{13}H_{10}Cl_2F_3N_3O_3S$ 416.2 g/mol	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1H-pyrazole-3-carboxylic acid	Rice
Ethiprole monochloro sulfonic acid Ethiprole-deschloro-sulfonic acid Monochloro-RPA 104615	 $C_{11}H_6ClF_3N_4O_3S$ 366.7 g/mol	5-amino-1-[2-chloro-4-(trifluoromethyl)phenyl]-3-methyl-1H-pyrazole-4-sulfonic acid	Cotton (Gin trash)

Plant metabolism

The metabolism of ethiprole (RPA 107382) has been investigated in rice following foliar and soil application, and in sweet pepper and cotton following foliar application. A confined rotational crop study has also been conducted on lettuce, radish, wheat and sorghum grown after four plant back intervals.

Rice – foliar application

A study was carried out to investigate the metabolism of phenyl ring labelled ^{14}C-ethiprole in rice (*Oryza sativa*) following foliar application of ethiprole at a total seasonal rate of 0.67 kg ai/ha (Guyton 2000, M-191923-01-2). ^{14}C-ethiprole was dissolved in acetonitrile and mixed with a wetting agent. The solution was applied twice to greenhouse grown rice. The first application, representing two thirds of the total seasonal rate (to enhance metabolite levels) was conducted 25 days prior to crop maturity. The second application, representing one third of the total season rate, was conducted 11 days later at 14 days prior to harvest. Separate rice plants were treated at 5× the anticipated use rate (seasonal rate of 3.35 kg ai/ha) so that samples with higher residues would be available to help in metabolite identification if necessary. Irrigation was conducted at the appropriate growth stage with watering three to four times per week in order to maintain a water level at least 10 cm above the soil.

Forage and panicle (green seedhead) samples were collected after each application and also immediately before the second application. At harvest, 14 days after the second application, straw and grain samples were collected. Some of the rice grain samples were passed through a commercial dehuller and separated into kernels and hulls.

All plant samples were analysed in order to determine the total radioactive residues (TRR) by LSC or combustion/LSC. At harvest, 14 days after the last application, the TRR amounted to 6.27 mg eq/kg in rice straw and 2.12 mg eq/kg in grain (Table 2). The residues in grain were concentrated in the hulls (3.95 mg eq/kg) whereas the kernels contained very low TRR levels (0.15 mg eq/kg).

Table 2 Total radioactive residues in rice matrices from pre-harvest and harvest samples

Crop Fraction	Total Radioactive Residue (mg eq/kg)							
	Post 1st Application		Pre 2nd Application		Post 2nd Application		Harvest	
	1×	5×	1×	5×	1×	5×	1×	5×
Forage	0.67	3.33	0.45	4.85	1.09	4.89	NA	NA
Panicle	1.01	6.67	0.60	4.52	0.88	5.21	NA	NA
Straw	NA	NA	NA	NA	NA	NA	6.27	31.52
Grain	NA	NA	NA	NA	NA	NA	2.12	11.11

Crop Fraction	Total Radioactive Residue (mg eq/kg)							
	Post 1st Application		Pre 2nd Application		Post 2nd Application		Harvest	
	1×	5×	1×	5×	1×	5×	1×	5×
Kernel	NA	NA	NA	NA	NA	NA	0.15	2.28
Hull	NA	NA	NA	NA	NA	NA	3.95	33.54

NA = not analysed / no sample taken

Harvest samples were extracted with acetonitrile/water (80:20, v/v) giving extraction efficiencies ranging from 94 -100% of the TRR in rice kernels, grain, straw and hulls for the 1**×** samples (Table 3). Additional portions of weakly bound residues could be released by treatment with a detergent (1% Triton X100) accounting for 14% of TRR from straw and 3.8% of TRR from grain and by using acid digestion to release cellulose and acid sensitive material (5.1% of TRR from straw and 2.4% of TRR from grain).

Table 3 Extraction accountability of rice matrices from 1× and 5× harvest samples

Fraction	Rice straw		Rice grain		Rice kernel		Rice hull	
	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
1× application rate								
TRR	100.0	6.27	100.0	2.12	100.0	0.15	100.0	3.95
ACN/H_2O soluble	98.6	6.18	100.0	2.12	94.5	0.14	100.2	3.96
Triton soluble	14.2	0.89	3.8	0.08	-	-	-	-
Acid digestion	5.1	0.32	2.4	0.05	-	-	-	-
Final cake (solid residues)	3.4	0.21	2.4	0.05	6.7	0.01	7.3	0.29
Total	121.2	7.60	108.5	2.30	101.2	0.15	107.6	4.25
5× application rate								
TRR	100.0	31.52	100.0	11.11	100.0	2.28	100.0	33.54
ACN/H_2O soluble	113.4	35.75	85.4	9.49	98.4	2.24	85.4	28.65
Final cake (solid residues)	21.7	6.85	5.6	0.62	5.3	0.12	4.1	1.38
Total	135.2	42.60	91.0	10.11	103.7	2.36	89.5	30.03

Extracts were analysed by ^{14}C-HPLC and MS to identify metabolites. Samples treated at the 1× rate contained sufficient radioactivity for residue identification so the 5× rate samples did not need to be analysed. The residue components extracted with acetonitrile/water and Triton consisted predominantly of the parent ethiprole which was the major component seen in all samples, ranging from 67% TRR (0.10 mg eq/kg) in kernels to 75% TRR (4.70 mg eq/kg) in straw (Table 4). The second most abundant metabolite was ethiprole-sulfone (RPA 097973), which was detected in all samples from 20–35% TRR (0.03 mg eq/kg in kernels – 2.17 mg eq/kg in straw). Low levels of ethiprole-sulfonic acid RPA 104615 (≤2.6% TRR, ≤0.16 mg eq/kg) and ethiprole-amide RPA 112916 (≤0.8% TRR, ≤0.05 mg eq/kg) were found in grain and straw. These were not detected at any significant level in kernel or hulls.

Table 4 Identification of organosoluble residues* in harvest rice matrices treated with [^{14}C]-ethiprole at the 1× rate

Residue component	Rice straw		Rice grain		Rice kernel		Rice hull	
	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
TRR	100.0	6.27	100.0	2.12	100.0	0.15	100.0	3.95
Ethiprole	75.0	4.70	72.6	1.54	66.7	0.10	74.1	2.92
Ethiprole-sulfone RPA 097973	34.6	2.17	25.5	0.54	20.0	0.03	23.8	0.94
Ethiprole-amide RPA 112916	0.8	0.05	0.5	0.01	-	-	-	-
Ethiprole-sulfonic acid RPA 104615	2.6	0.16	1.9	0.04	-	<0.01	-	-
Total identified	112.9	7.08	100.5	2.13	86.7	0.13	97.9	3.86
Unknowns	None		ACN/H_2O - Four, none > 0.05 mg eq/kg (2% TRR), Triton - Two, none > 0.01 mg eq/kg		Two, none > 0.01 mg eq/kg		Two, none > 0.06 mg eq/kg (2% TRR)	

*Sum of metabolite levels in ACN/H_2O and Triton extracts

The metabolism of ethiprole in/on rice after foliar application proceeds predominantly through oxidation to the parent sulfone RPA 097973.

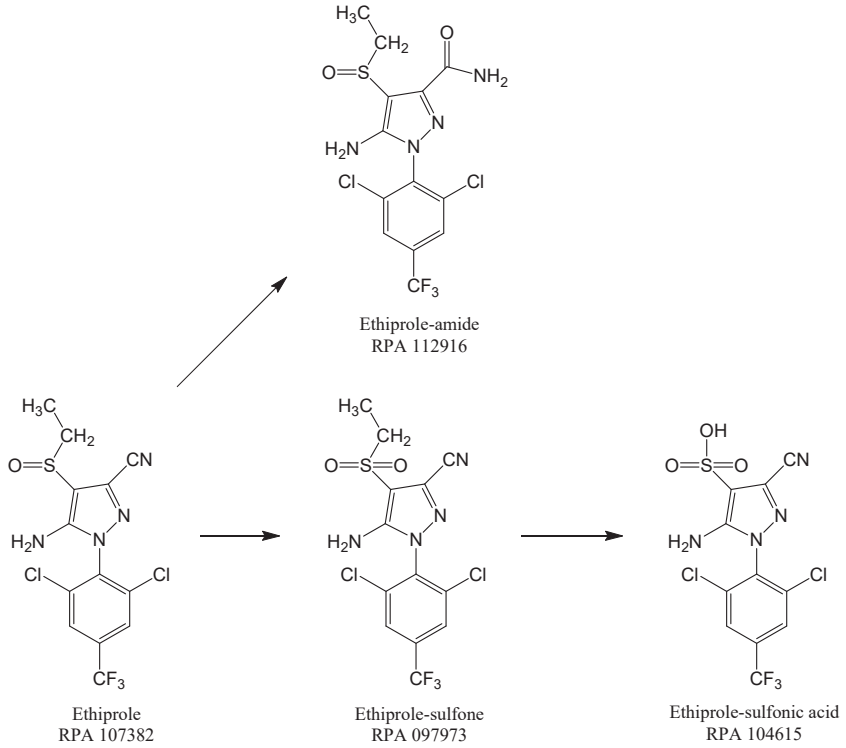


Figure 1 Proposed metabolic pathway for ethiprole in rice after foliar application

Rice – soil application

A study was carried out to investigate the metabolism of phenyl ring labelled ^{14}C-ethiprole in rice (*Oryza sativa*) following soil application of ethiprole, in order to simulate application of a granule formulation to water in paddy rice (Preu 2008, M-231707-01-2). The rice was grown under paddy conditions in a climatic chamber. Phenyl -UL-^{14}C ethiprole was dissolved in acetonitrile, diluted with water and poured onto the soil using a watering can. Two applications of approximately 0.6 kg ai/ha were made. The first application was made at full flowering (BBCH 65), while the second application was made 8 days later, when the plants had reached a growth stage between milk stage and ripening (BBCH 69 - 89).

Harvest was conducted at maturity 30 days after the last application, 116 days after transplantation. First, the panicles bearing the rice grain of all plants were cut off from the plants using scissors. The panicles were then dried at ambient temperature for four days. After drying, the panicles were separated into unhulled rice grains and rachis using forceps. The rachis portion was added to the straw samples. The unhulled rice was husked in an automatic laboratory rice husker and the obtained fractions were sorted manually to produce hulled rice grain and husks (chaff). An aliquot of the hulled rice grain was subjected to polishing in a coffee mill in which the outer skin of the grains was removed. The sample was subsequently sieved to separate polished rice grain and bran. After the panicles had been cut off, the rest of the plants were cut with scissors about 3-4 cm above the soil surface level to obtain straw.

All plant samples were analysed in order to determine the TRR by LSC or combustion/LSC. Most of the recovered radioactivity was found in rice straw (23.97 mg eq/kg) with lower levels in rice husk (5.69 mg eq/kg), and even lower levels in hulled rice grain (0.280 mg eq/kg). In hulled rice grain higher levels of radioactivity were found in the bran fraction (1.381 mg eq/kg) as compared to polished rice grain (0.116 mg eq/kg).

The samples of rice straw, rice husks (chaff) and hulled rice grain were extracted with acetonitrile/ water (4:1, v/v, 3×) followed by acetonitrile. This procedure extracted more than 93% of the radioactivity from hulled rice grain and 62 and 87% from

rice husks and rice straw respectively. After evaporation of the organic solvent the combined extracts were partitioned with dichloromethane. The majority of the TRR (59–92% TRR) was extracted in the dichloromethane phase. A microwave-assisted extraction applied to rice straw and husks yielded an additional 9 and 34% of the TRR respectively. Non-extracted residues were low for all RACs, ranging from 3-7% TRR. No extractions were performed on polished rice and bran (TRRs = 0.116 and 1.381 mg eq/kg). Total extraction accountability for rice straw, husks and hulled rice grain is shown in Table 5.

Table 5 Extraction accountability of rice matrices

Fraction	Rice straw		Rice husks		Hulled rice grain	
	% TRR	mg eq/ kg	% TRR	mg eq/ kg	% TRR	mg eq/ kg
Total Radioactive Residue (TRR)	100.0	23.97	100.0	5.69	100.0	0.280
ACN/H_2O	86.7	20.79	62.1	3.54	93.1	0.261
Dichloromethane-phase	78.6	18.84	58.5	3.33	91.9	0.257
Aqueous phase	8.1	1.95	3.6	0.21	1.2	0.003
Microwave-extract [a]	9.3	2.23	34.4	1.96	-	-
Solids (non-extracted residue)[b]	4.0	0.95	3.5	0.20	6.9	0.019

[a] Acetonitrile/water extraction was enhanced with microwave treatment for straw and husk samples only.

[b] Residue remaining after microwave extraction

Organic and aqueous extracts were analysed by ^{14}C-HPLC with co-eluting radiolabelled and non-labelled reference standards to determine the composition of the radioactive residues. The metabolites isolated from straw were identified by spectroscopy using LC-MS/MS and ^{1}H-NMR. The composition of the ethiprole residues in rice straw, husks and hulled grain is presented in Table 6.

Table 6 Identification of residues in rice matrices treated with [^{14}C]-ethiprole

Residue component	Rice straw		Rice husks		Hulled rice grain	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole	42.2	10.12	62.0	3.53	62.3	0.175
Ethiprole-sulfone RPA 097973	23.4	5.60	19.9	1.13	18.1	0.051
Ethiprole-amide RPA 112916	11.2	2.68	7.7	0.44	8.3	0.023
Ethiprole acid	4.9	1.17	1.7	0.10	-	-
Ethiprole-sulfone amide RPA 112917	1.9	0.45	1.9	0.11	1.8	0.005
Ethiprole-monochloro sulfone RPA 115369	0.8	0.18	0.3	0.02	-	-
Total identified	84.3	20.20	93.4	5.32	90.5	0.254
Unknowns	Twelve, ≤ 0.59 mg eq/kg (≤ 2.4% TRR)		Five, ≤ 0.11 mg eq/kg (≤ 2.0% TRR)		One, 0.004 mg eq/kg (1.4% TRR)	
Total characterised	11.8	2.82	3.1	0.18	1.4	0.004
Aqueous phase (no HPLC)	-	-	-	-	1.2	0.003
Solids (non-extracted residue)	4.0	0.95	3.5	0.20	6.9	0.019
TRR	100.0	23.97	100.0	5.69	100.0	0.280

Parent ethiprole was the major component seen in all samples, ranging from 42% TRR (10.12 mg eq/kg) in straw to 62% TRR in husks and hulled rice grain. The most abundant metabolite was the parent sulfone (RPA 097973), which was detected in all samples between 18–23% TRR (0.051–5.60 mg eq/kg), the highest residue being observed in straw. Levels of the parent amide (RPA 112916) were greatest in straw (11% TRR, 2.68 mg eq/kg), but were <10%TRR (≤0.44 mg eq/kg) in husks and hulled grain. Low levels of ethiprole acid (<5% TRR, ≤1.17 mg eq/kg) were found in straw and husks, but not in hulled grain. Ethiprole-sulfone-amide (RPA 112917) was also detected in all samples, but at low levels (≤1.9% TRR, ≤0.45 mg eq/kg).

The metabolism of ethiprole in/on rice from soil application proceeds predominantly through oxidation to sulfone compounds, hydrolysis of the nitrile moiety to amides and carboxylic acid groups and loss of chlorine to the monochloro compound. The metabolic routes observed for rice straw, husks and hulled rice grain are very similar. The proposed metabolic pathway for ethiprole in rice after application of a graule formulation to water is shown in Figure 2.

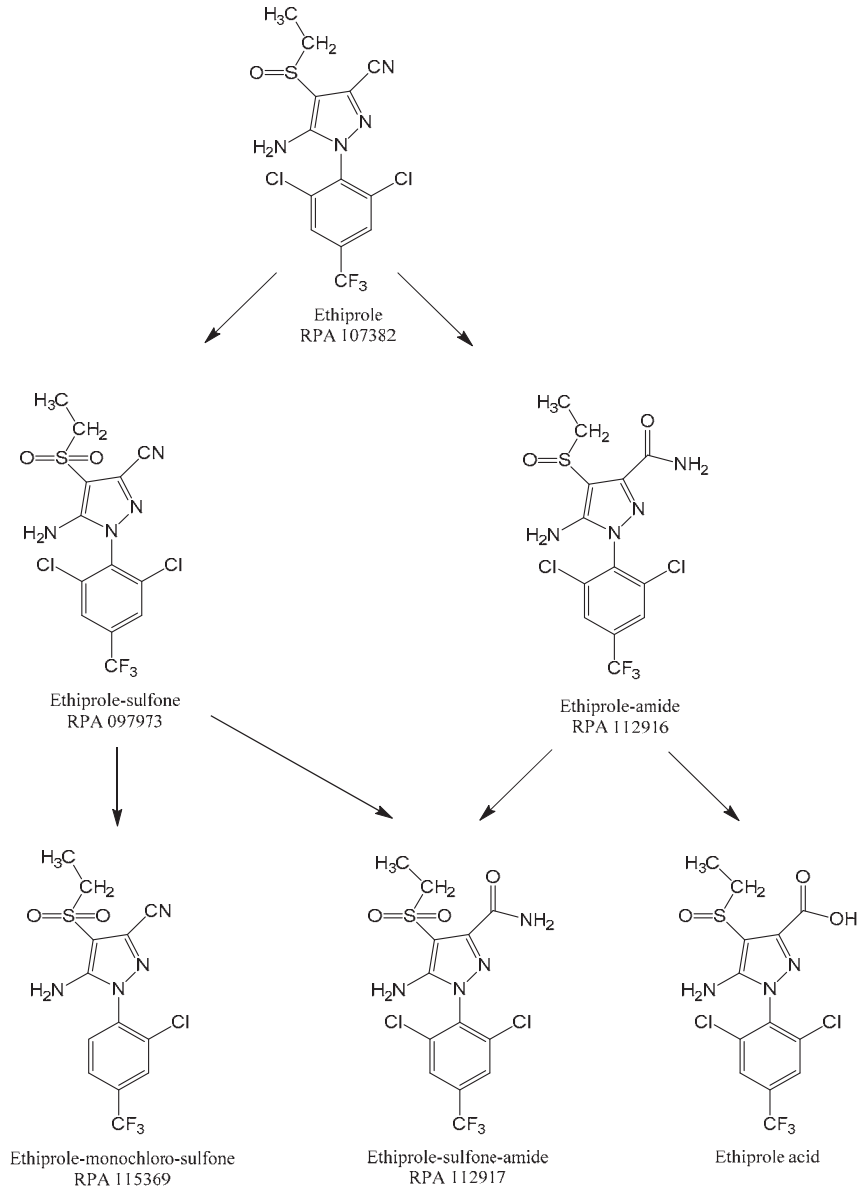


Figure 2 Proposed metabolic pathway for ethiprole in rice after soil application

Sweet pepper

The metabolism of uniformly phenyl ring labelled ^{14}C-ethiprole was investigated in sweet pepper (*Capsicum annum*) following foliar application at a total seasonal rate of 0.67 kg ai/ha (Quarmby and Jesudason 1999, M-191915-02-2). ^{14}C-ethiprole was dissolved in acetonitrile and mixed with a wetting agent. The solution was applied twice to greenhouse grown sweet pepper plants. The first application, representing two-thirds of the total seasonal rate, was made 26 days prior to crop maturity. The second application, representing one-third of the total season rate, was made 12 days later, 14 days prior to harvest. Separate sweet pepper plants were treated at 5× the anticipated use rate (seasonal rate of 3.35 kg ai/ha) so that samples with higher residues would be available to help in metabolite identification if necessary.

Plant samples treated at the 1× rate were collected 2-4 hours after the first application (foliage only), prior to the second application (fruit and foliage), 2-4 hours after the second application (fruit and foliage), and at final harvest 14 days after the second application (fruit and foliage). Samples treated at the 5× rate were collected only at final harvest. Red (mature) and green (immature) peppers were processed separately. In addition, fruit samples collected at final harvest for both treatment rates were

separated into fruits that were present on the plant during both applications and fruits that were present only during the last application. All plant samples were analysed to determine the total radioactive residue (TRR) by combustion/LSC.

Total radioactive residues (TRR) in the various crop fractions as determined by combustion/LSC are presented in Table 7. Radioactivity was significantly higher in/on foliage (36-184 mg eq/kg for the 1× samples) than in/on fruits (0.3–0.7 mg eq/kg for the 1× samples).

Table 7 Total radioactive residues in pepper matrices from pre-harvest and harvest samples

Crop Fraction	Total Radioactive Residue (mg eq/kg)				
	Post 1st Application	Pre 2nd Application	Post 2nd Application*	Harvest	
	1×	1×	1×	1×	5×
Foliage	183.69	36.04	117.74	44.57	163.20
Green Fruit	NA	0.450[a]	0.591[b]	0.676[c] 0.505[b]	0.400[d] 1.134[e]
Red Fruit	NA	0.312[a]	0.549[b]	0.450[b]	1.708[e]

Post 1st or 2nd application: 2-4 hours post application

Pre 2nd application: 1 day prior to application

*Fruit were not separated into those that had received both sprays and those that had received only the second spray, therefore some fruit may not have been treated at 0.67 kg ai/ha. This separation was conducted on fruits sampled at harvest.

NA = not analysed / no sample taken

[a] Total 0.45 kg ai/ha applied to fruit

[b] Total 0.67 kg ai/ha applied to fruit

[c] Total 0.22 kg ai/ha applied to fruit (new fruit formed after the first treatment)

[d] Total 1.12 kg ai/ha applied to fruit (new fruit formed after the first treatment)

[e] Total 3.35 kg ai/ha applied to fruit

Fruit samples were extracted with acetonitrile/water (80:20, v/v) followed as necessary by aqueous Triton to release bound residues. The acetonitrile/water extraction recovered 85-101% of TRR while only 1–5% of TRR were recovered during the Triton extraction (Table 8). The unextractable residues in the solids accounted for up to 6% TRR, with total recoveries ranging from 94–105% TRR. The acetonitrile/water (80:20, v/v) solvent mixture extracted 88–100% of TRR from the foliage samples treated at the 1× rate (77% of TRR from the foliage samples treated at the 5× rate) and no Triton extraction was considered necessary.

Acid extraction was used on samples following the Triton extraction when less than 90% of the sample TRR was extracted after both the initial organo-aqueous extraction and the Triton extraction.

Table 8 Extraction accountability of green and red pepper fruit

Sample matrix	TRR mg/kg	ACN/water (4:1) extraction		Triton X-100 extraction		Total extractable		Non- extractable		Total recovered	
		% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
1× application rate											
Red fruit, pre-2nd app 0.45 kg ai/ha	0.312	93.9	0.293	-	-	94.1	0.293	1.3	0.004	95.2	0.297
Red fruit, post-2nd app 0.67 kg ai/ha	0.549	96.1	0.527	-	-	96.1	0.527	1.1	0.006	97.2	0.533
Green fruit post-2nd app 0.67 kg ai/ha	0.591	96.1	0.568	-	-	96.0	0.568	2.4	0.014	98.5	0.582
Red fruit, final harvest (2 sprays) 0.67 kg ai/ha	0.450	94.0	0.423	1.1	0.005	95.1	0.428	3.8	0.017	98.9	0.445
Green fruit, final harvest (1 spray) 0.22 kg ai/ha	0.676	85.2	0.576	4.7*	0.032*	90.1	0.609	3.9	0.026	93.8	0.634
Green fruit, final harvest (2 sprays) 0.67 kg ai/ha	0.505	93.3	0.471	1.2	0.006	94.5	0.477	5.7	0.029	100.2	0.506
5× application rate											
Red fruit, final harvest (2 sprays) 3.35 kg ai/ha	1.708	96.0	1.639	0.5	0.009	96.5	1.648	0.9	0.016	97.4	1.664
Green fruit, final harvest (1 spray) 1.12 kg ai/ha	0.400	93.8	0.375	0	ND	93.8	0.375	5.5	0.022	99.3	0.397
Green fruit, final harvest (2 sprays) 3.35 kg ai/ha	1.134	101.3	1.149	0.6	0.007	101.9	1.156	3.0	0.034	104.9	1.190

* Included 2% with 1.5 N acid digestion and 2% with acetonitrile/water extract.

The foliage and fruit samples treated at the 1× rate contained sufficient levels of radioactivity for residue identification. The acetonitrile/water extracts from these samples were analysed by ^{14}C-HPLC and by MS.

In the foliage from the 1× rate application, ethiprole accounts for the majority of the TRR at each time point (Table 9). Extensive metabolism was not observed, despite the reduction in total residue levels between applications and between the second application and harvest. The reduction was thought to be mainly the result of growth dilution, rather than residue dissipation.

Table 9 Identification of organosoluble* residues in pepper plant foliage treated with [^{14}C]-ethiprole at the 1× rate

Residue component	Post 1st Application		Pre 2nd Application		Post 2nd Application		Harvest	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100.0	183.69	100.0	36.04	100.0	117.74	100.0	44.57
Ethiprole	93.0	170.91	85.4	30.77	98.7	116.23	83.3	37.13
Ethiprole-amide RPA 112916	-	-	1.7	0.620	-	-	2.5	1.109
Ethiprole-sulfonic acid RPA 104615	-	-	0.8	0.283	0.8	0.985	2.9	1.308
RPA 097920	-	-	0.5	0.171	-	-	1.5	0.670

Residue component	Post 1st Application		Pre 2nd Application		Post 2nd Application		Harvest	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfone-amide RPA 112917	-	-	-	-	-	-	0.9	0.420
Total identified	93.0	170.91	88.4	31.84	99.6	117.21	91.2	40.63

*Sum of metabolite levels in ACN/H_20 and Triton extracts

More extensive metabolism was observed in fruits. In green (immature) fruits, the amide RPA 112916 and the sulfone RPA 097973 were both present at levels >10% TRR while in red (mature) fruits at harvest the sulfone RPA 097973 accounted for 16% TRR with the amide RPA 112916 representing < 10% TRR. The total identified radioactivity was in the range of 55–92% TRR in green fruit and 84–96% TRR in red fruit. The total radioactivity characterised and identified by HPLC was in the range of 83–96% TRR in green fruit and 94–96% TRR in red fruit.

Table 10 Identification of organosoluble* residues in green pepper fruit treated with [^{14}C]-ethiprole at the 1× rate

Residue component	Post 2nd Application		Harvest [a]		Harvest [b]	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100.0	0.591	100.0	0.505	100.0	0.676
Ethiprole	79.4	0.469	43.2	0.218	22.2	0.150
Ethiprole-sulfone RPA 097973	8.5	0.050	12.7	0.064	9.3	0.063
Ethiprole-sulfide RPA 107566	-	-	-	-	1.2	0.008
Ethiprole-amide RPA 112916	4.4	0.026	14.7	0.074	18.1	0.122
Ethiprole-sulfone-amide RPA 112917	-	-	1.0	0.005	4.1	0.028
Total identified	92.2	0.545	71.5	0.361	54.9	0.371
Unknowns on HPLC	1 compound accounting for 3.7% TRR		6 compounds, each accounting for 0.6-7.1% TRR		7 compounds, each accounting for 1.3-6.4% TRR	
Total identified/ characterised	95.9	0.567	93.3	0.471	83.3	0.563

*Sum of metabolite levels in ACN/H_20 and Triton extracts

[a] Total 0.67 kg ai/ha applied to fruit (2 sprays)

[b] Total 0.225 kg ai/ha applied to fruit (1 spray, new fruit formed after the first treatment)

Table 11 Identification of organosoluble* residues in red pepper fruit treated with [^{14}C]-ethiprole at the 1× rate

Residue component	Pre 2nd Application		Post 2nd Application		Harvest [a]	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100.0	0.312	100.0	0.549	100.0	0.450
Ethiprole	85.3	0.266	91.6	0.503	59.8	0.269
Ethiprole-sulfone RPA 097973	9.0	0.028	4.4	0.024	16.4	0.074
Ethiprole-sulfonic acid RPA 104615	-	-	-	-	2.7	0.012
Ethiprole-amide RPA 112916	-	-	-	-	5.3	0.024
Total identified	94.2	0.294	96.0	0.527	84.2	0.379
Unknowns on HPLC	None		None		5 compounds, each accounting for < 2.5% TRR	
Total identified/ characterised	94.2	0.294	96.0	0.527	93.6	0.421

* Sum of metabolite levels in ACN/H_20 and Triton extracts

[a] Total 0.67 kg ai/ha applied to fruit (2 sprays)

The identified residues in green and red pepper fruit from the 5× rate application are shown in the following table. Ethiprole and the sulfone were the dominant residues.

Table 12 Identification of organosoluble* residues in green and red pepper fruit treated with [^{14}C]-ethiprole at the 5× rate

Residue component	Green fruit – Harvest (1 spray)[b]		Green fruit – Harvest (2 sprays)[a]		Red fruit – Harvest (2 sprays)[a]	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100.0	0.400	100.0	1.134	100.0	1.708
Ethiprole	48.8	0.195	83.3	0.945	89.8	1.534
Ethiprole-sulfone RPA 097973	5.8	0.023	9.1	0.103	5.3	0.090
Ethiprole-sulfide RPA 107566	-	-	-	-	0.8	0.014
Ethiprole-sulfonic acid RPA 104615	-	-	1.0	0.011	-	-
Ethiprole-amide RPA 112916	9.8	0.039	3.8	0.043	-	-
Ethiprole-sulfone-amide RPA 112917	1.8	0.007	-	-	-	-
Total identified	66.0	0.264	97.2	1.102	95.9	1.638
Unknowns on HPLC	9 compounds, each accounting for < 9.0% TRR		3 compounds, each accounting for < 1.6% TRR		-	
Total identified/ characterised	92.8	0.371	101.3	1.149	95.9	1.638

*Sum of metabolite levels in ACN/$H_2$0 and Triton extracts

[a] Total 3.35 kg ai/ha applied to fruit (2 sprays)

[b] 1.1 kg ai/ha applied to fruit

5× foliage, TRR 163.20 mg eq/kg: Ethiprole 120.58 mg eq/kg, 73.9% of TRR; RPA 112916 0.577 mg eq/kg, 0.4% of TRR; RPA 104615 2.339 mg eq/kg, 1.4% of TRR; M&B45897 1.510 mg eq/kg, 0.9% of TRR; Identified 125.01 mg eq/kg, 76.6% TRR; Non-extracted 3.847 mg eq/kg, 2.4% TRR. Total recovered 128.85 mg eq/kg, 79.0% TRR.

The main metabolic reactions of ethiprole in sweet pepper were oxidation of the sulfoxide group to give the sulfone RPA 097973 and hydrolysis of the nitrile moiety to form the amide RPA 112916. Minor pathways resulted in the sulfone amide RPA 112917 that could be formed either by hydrolysis of the nitrile group of RPA 097973 or by oxidation of the sulfoxide group of RPA 112916. Reduction of the sulfoxide group of ethiprole resulted in the sulfide metabolite RPA 107566. Oxidation of RPA 097973 leads to the sulfonic acid RPA 104615 and subsequent degradation to MB 45897 (RPA 097920).

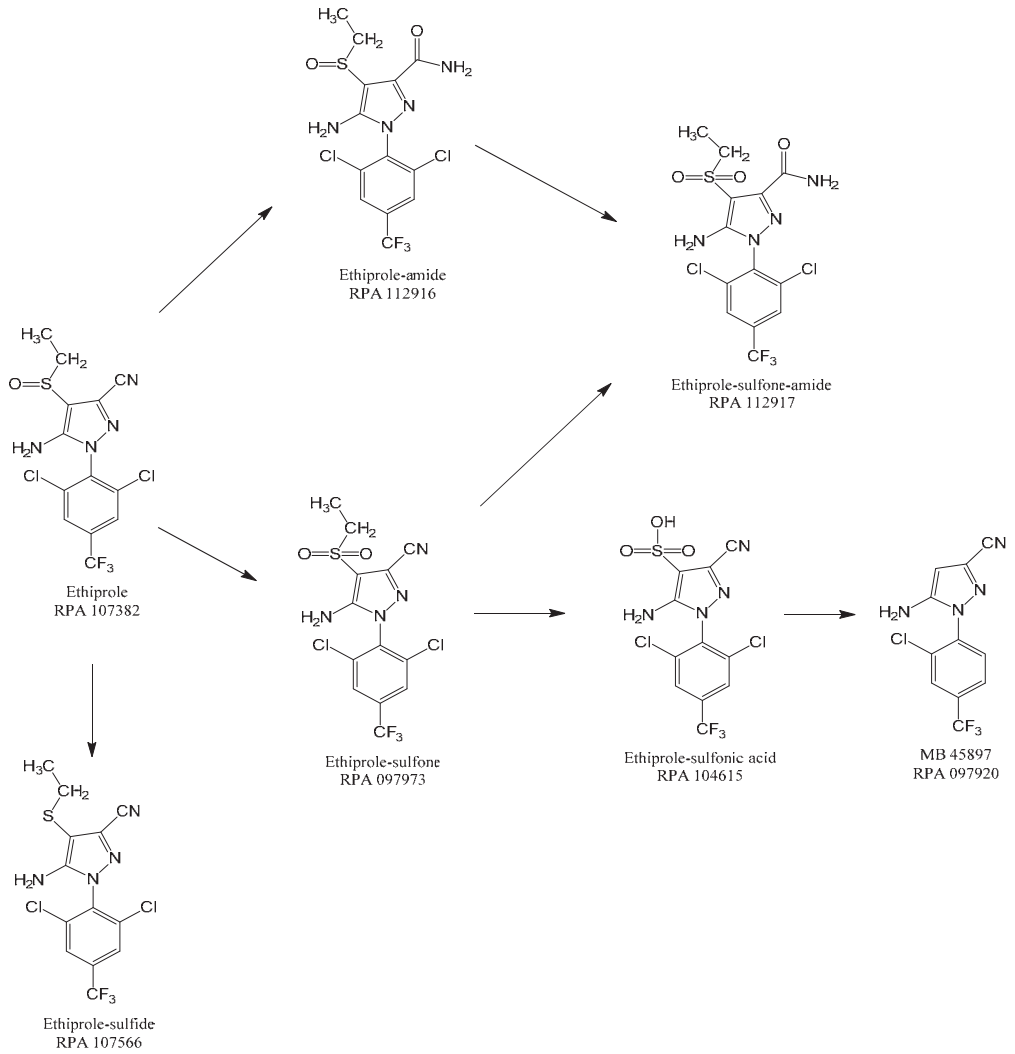


Figure 3 Proposed metabolic pathway for ethiprole in sweet pepper

Cotton

The metabolism of uniformly phenyl ring labelled ^{14}C-ethiprole was investigated in cotton (*Gossypium hirsutum*) following two foliar applications at a total seasonal rate of 0.67 kg ai/ha (Guyton and Jesudason 2000, M-191927-02-2). ^{14}C-Ethiprole was dissolved in acetonitrile and mixed with a wetting agent and the resultant solution applied twice to field grown cotton. The first application, representing two-thirds of the total seasonal rate, was made 61 days prior to harvest. The second application, representing one-third of the total season rate, was made 48 days prior to harvest. Further cotton plants were treated at 10× the anticipated use rate (seasonal rate of 6.7 kg ai/ha) so that samples with higher residues would be available to help in metabolite identification if necessary.

Plant samples were collected just before the second application (foliage, old and new growth), after the second application (foliage) and at harvest 48 days after the second application (bolls and gin trash). The cotton bolls were ginned to yield lint and seed. All plant samples were analysed to determine the TRR by combustion/LSC.

Radioactive residues (TRR) in the various crop fractions as determined by combustion/LSC are presented in Table 13. ^{14}C-Residues were detected in new growth foliage samples collected just before the second application (13 days after the first application), which indicates translocation of residues in the plant. At harvest, the gin trash samples showed much higher levels of total residues than the seed samples. The residues in the samples treated at the 10× rate were approximately an order of magnitude higher than the residues in the samples treated at the 1× rate.

Table 13 Total radioactive residues in cotton matrices from pre-harvest and harvest samples

Crop Fraction	Total Radioactive Residue (mg eq/kg)							
	Post 1st Application		Pre 2nd Application		Post 2nd Application		Harvest	
	1×	10×	1×	10×	1×	10×	1×	10×
Foliage	55.25	347.90	-	-	46.69	483.60	-	-
New foliage	-	-	4.96	136.04	-	-	-	-
Old foliage	-	-	15.91	256.35	-	-	-	-
Gin trash	-	-	-	-	-	-	4.55	60.10
Cottonseed	-	-	-	-	-	-	0.07	0.57
Lint	-	-	-	-	-	-	0.12	2.49

Selected samples of foliage, gin trash and cotton seed were extracted for metabolite identification and quantification (Table 14). Extraction was performed with acetonitrile/water (80/20, v/v) followed by aqueous Triton to release loosely bound residues, followed by other digestion techniques (Soxhlet extraction with ACN/H_2O, harsh acid, lignin or caustic digest). Analysis of extracts was by HPLC with confirmation by mass spectrometry.

Table 14 Extraction accountability of cotton matrices from the 1× and 10× [^{14}C]-ethiprole treatments

Fraction	Foliage Pre 2nd Application (1×)		Gin trash Harvest (1×)		Cottonseed Harvest (1×)		Cottonseed Harvest (10×)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR	100.0	15.91	100.0	4.55	100.0	0.07	100.0	0.57
ACN/H_2O soluble	84.6	13.46	76.1	3.46	41.4	0.029	54.0	0.308
Triton Soluble	4.8	0.76	3.6	0.16	5.1	0.004	6.7	0.038
Soxhlet (ACN/H_2O)	-	-	-	-	ND	ND	2.3	0.013
Acid Digestion	-	-	5.7	0.26	11.4	0.008	13.4	0.077
Lignin Digestion	-	-	4.4	0.20	-	-	-	-
Caustic Digestion	-	-	-	-	5.7	0.004	5.3	0.030
Final cake	14.7	2.35	8.8	0.40	11.4	0.008	10.5	0.060
Total	104.1	16.57	98.5	4.48	75.7	0.053	92.3	0.526

ND = None Detected

An overview of the residue components identified or characterised in the acetonitrile/water (80:20, v/v) extracts is provided in Table 15.

Table 15 Identification of residues in cotton matrices from treatments with [^{14}C]-ethiprole at the 1× and 10× rate

Residue component	Foliage Pre 2nd Application (1×)		Gin trash Harvest (1×)		Cottonseed Harvest (1×)		Cottonseed Harvest (10×)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
TRR [a]	100.0	15.91	100.0	4.55	100.0	0.07	100.0	0.57
Ethiprole	21.0	3.34	16.3	0.74	1.4	0.001	7.0	0.040
Ethiprole-sulfone RPA 097973	14.8	2.36	26.4	1.20	2.9	0.002	2.1	0.012
Ethiprole-sulfonic acid RPA 104615	9.1	1.44	7.9	0.36	-	-	2.4	0.014
Ethiprole formamide RPA 103343	8.2	1.31	0.4	0.02	1.4	0.001	-	-
Ethiprole-deschloro-sulfone RPA 115369			9.5	0.43			-	-
Mono-chloro RPA 104615	-	-	2.4	0.11	-	-	-	-
Total identified	53.1	8.45	62.9	2.86	5.7	0.004	11.5	0.066
Polar unknown	-	-	-	-	10.0	0.007	7.9	0.045
Polar unknown	-	-	-	-	7.1	0.005	12.6	0.072

Residue component	Foliage Pre 2nd Application (1×)		Gin trash Harvest (1×)		Cottonseed Harvest (1×)		Cottonseed Harvest (10×)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Other unknowns on HPLC	33 compounds totalling 31.5% TRR, 5.01 mg eq/kg		8 polar compounds totalling 6.2% TRR, 0.28 mg eq/kg + 8 non-polar cpds totalling 6.4% TRR, 0.29 mg eq/kg		16 polar compounds totalling 8.6% TRR, 0.006 mg eq/kg + 20 non-polar cpds totalling 4.3% TRR, 0.003 mg eq/kg		10 polar compounds totalling 11.2% TRR, 0.064 mg eq/kg + 7 non-polar cpds totalling 7.2% TRR, 0.041 mg eq/kg	
Total identified or characterised [b]	84.6	13.46	76.1	3.46	41.4	0.029	50.5	0.288

[a] In the sample before extraction.

[b] In the acetonitrile/water (80:20) extract.

Overall, the data indicate extensive metabolism of ethiprole in cotton. Parent ethiprole and the sulfone RPA 097973 were the main residue components in foliage and gin trash, comprising 16–21% TRR for ethiprole and 15–26% TRR for RPA 097973. In cottonseed, the matrix most relevant to dietary risk assessment, parent ethiprole and the sulfone RPA 097973 were identified at very low levels (≤7% TRR, ≤0.04 mg eq/kg). Minor levels of ethiprole deschoro-sulfone (RPA 115369) and ethiprole-formamide (RPA 103343) were observed in the 1× seed samples but not confirmed in the 10× samples. Sulfonic acid RPA 104615 was only observed in the cottonseed 10× samples at a low level (2.4% TRR, 0.014 mg eq/kg) and at 7.9% TRR in gin trash (0.36 mg eq/kg) and 9.1% TRR (1.44 mg eq/kg) in foliage. Two unknowns near or just above 10% TRR were observed in both 1× and 10× samples. HPLC qualitative analysis confirmed both unknowns to be more polar than the highly polar sulfonic acid metabolite RPA 104615, and evidence that they were not conjugated metabolites was provided by showing the unknowns were not converted to non-polar compounds by mild acid hydrolysis.

In cotton treated with a foliar spray of [phenyl-^{14}C]-ethiprole, it was shown that ethiprole is extensively metabolised in cotton. The major metabolic pathway is oxidation of parent to sulfone RPA 097973, which can be further metabolised to sulfonic acid and mono-chloroaryl derivatives. A proposed metabolic pathway is shown in Figure 4.

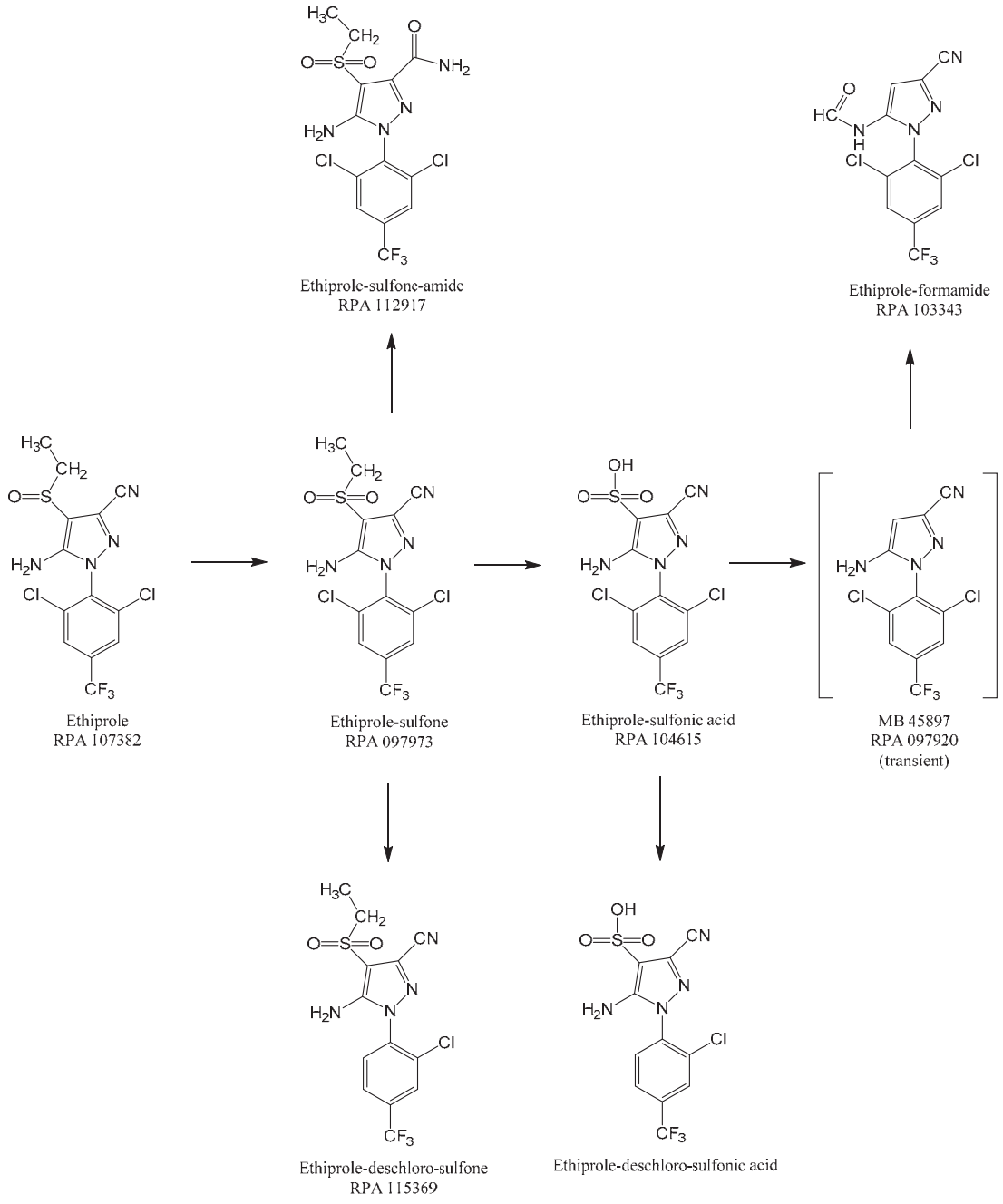


Figure 4 Proposed metabolic pathway for ethiprole in cotton

Supplementary analyses on the metabolic fate in pepper foliage, rice straw and cotton gin trash

A supplemental study was conducted to evaluate qualitatively for the presence of traces of minor metabolites with a sensitive confirmatory technique, LC-MS/MS, in samples generated in the metabolism studies (Quarmby 2009, M-214263-01-3). Pepper foliage and rice straw from foliar application and cotton gin trash from the metabolism studies were used for this study. Attention was focused on evaluating for residues of ethiprole-deschloro-sulfone (RPA 115369) in pepper foliage and rice straw. Extracts were also analysed for ethiprole and ethiprole sulfone (RPA 097973). In addition, cotton gin trash from the cotton metabolism study was analysed to confirm the storage stability of RPA 115369 in that matrix, and for residues of ethiprole, RPA 097973 and ethiprole-amide (RPA 112916).

Due to long freezer storage times of approximately two to three years, each of the processed plant matrices was combusted to determine total radioactive residues (TRR). Table 16 compares the TRR values found in this study with those TRR values determined in the original studies. Extraction of each of the plant matrices was conducted using acetonitrile/water. The extraction efficiencies are given in the following table and are compared to those of the initial studies. This comparison indicates that the extractability remains very good even after a long storage duration. Total recovery ranged from 98% to 102% of TRR in all matrices.

Table 16 TRR comparison and extractability comparison in plant matrices

Matrix	TRR (mg eq/kg)		%TRR extracted		Solid unextracted residue in supplemental study (% TRR)
	Initial study	Supplemental study	Initial study	Supplemental study	
Pepper foliage Final harvest	44.57	46.65	91.2	95.3	4.4
Rice Straw Harvest	6.27	4.75	98.6	83.7	13.9
Cotton gin trash Harvest	4.55	4.75	76.1	74.2	28.2

Extracts were analysed by HPLC in order to determine storage stability. Both the rice straw and cotton gin trash showed chromatographic profiles similar to those from the original metabolism studies, indicating good storage stability. The pepper foliage extracts showed that over the approximate two year storage period, a small percentage (14%) of ethiprole was converted to the sulfone metabolite RPA 097973 not detected previously.

Overall, this data supports the fact that the major metabolites are stable over the duration of storage and the results are consistent with those obtained in the original studies.

Extracts were submitted for mass spectral analysis using LC-MS/MS. The analyses of the pepper foliage and rice straw extracts confirmed that ethiprole and RPA 097973 were present in the samples. The ethiprole-deschloro-sulfone metabolite (RPA 115369) was not detected in the rice straw. In the pepper foliage extracts, the amount of RPA 115369 detected was similar in both the control and treated samples, indicating that the compound did not arise from ethiprole metabolism. The analysis of the cotton gin trash samples confirmed the presence of ethiprole, the sulfone RPA 097973 and RPA 115369, with the amide RPA 112916 being present as a minor metabolite.

Summary of plant metabolism

Plant metabolism studies have been conducted with [phenyl-UL-^{14}C]-ethiprole that was applied to rice (foliar and paddy application), sweet pepper (foliar) and cotton (foliar) at rates covering the anticipated maximum total seasonal application rates. The test substance was labelled in the phenyl moiety.

The metabolism of ethiprole is comparable in all crops investigated and is consistent with what would be expected based upon major metabolic processes defined in the rat. Most of the radioactivity was recovered in the organosoluble extraction, with the majority of this being identified as parent ethiprole and ethiprole-sulfone (RPA 097973). The sulfone compound is common to all crop metabolism studies and was found in rice straw and grain, pepper green and red fruit and cotton gin trash at levels >10% TRR. Ethiprole-amide (RPA 112916) was observed in the rice and pepper metabolism studies (not cotton), but was observed at <10% TRR in all but green (immature) pepper fruit (up to 18% TRR) and rice straw from soil application (11% TRR). Ethiprole sulfonic acid (RPA 104615) is common to all crop metabolism studies except the rice soil application study, but was observed at <5% TRR in all but cotton foliage and gin trash (8–9% TRR).

Rice

After foliar application, ethiprole and the sulfone accounted for greater than 98% of the TRR in rice grain and greater than 97% of the normalised TRR in rice straw. In the study mimicking paddy application, ethiprole and ethiprole-sulfone accounted for greater than 80% of the TRR in rice grain and greater than 65% of TRR in rice straw. Ethiprole-amide accounted for 11% of TRR in straw.

Sweet pepper

In sweet pepper, ethiprole and the sulfone accounted for approximately 83% of the TRR in foliage, 56% of TRR in immature green fruit and 76% of the TRR in mature red fruit at harvest. Ethiprole-amide was found at >10% of TRR in the immature green pepper fruit but in mature fruit the level was only 5% of TRR.

Cotton

In cotton seed, the very low TRR (0.07 mg eq/kg) made metabolite identification difficult, but ethiprole and the sulfone comprised the majority of the identified residues. In cotton gin trash, ethiprole and the sulfone comprised about 43% of the TRR, or about 68% of the identified residues. In cotton gin trash two other minor metabolites were found at levels greater than 5% of the TRR, identified as ethiprole-deschloro-sulfone (RPA 115369) and ethiprole-sulfonic acid (RPA 104615). RPA 104615 was also found as a very minor metabolite in the other crops.

The metabolic pathway of ethiprole in plants proceeds *via* oxidation to the sulfone RPA 097973 and hydrolysis to the amide RPA 112916. A metabolic pathway for ethiprole in plants is shown in Figure 5.

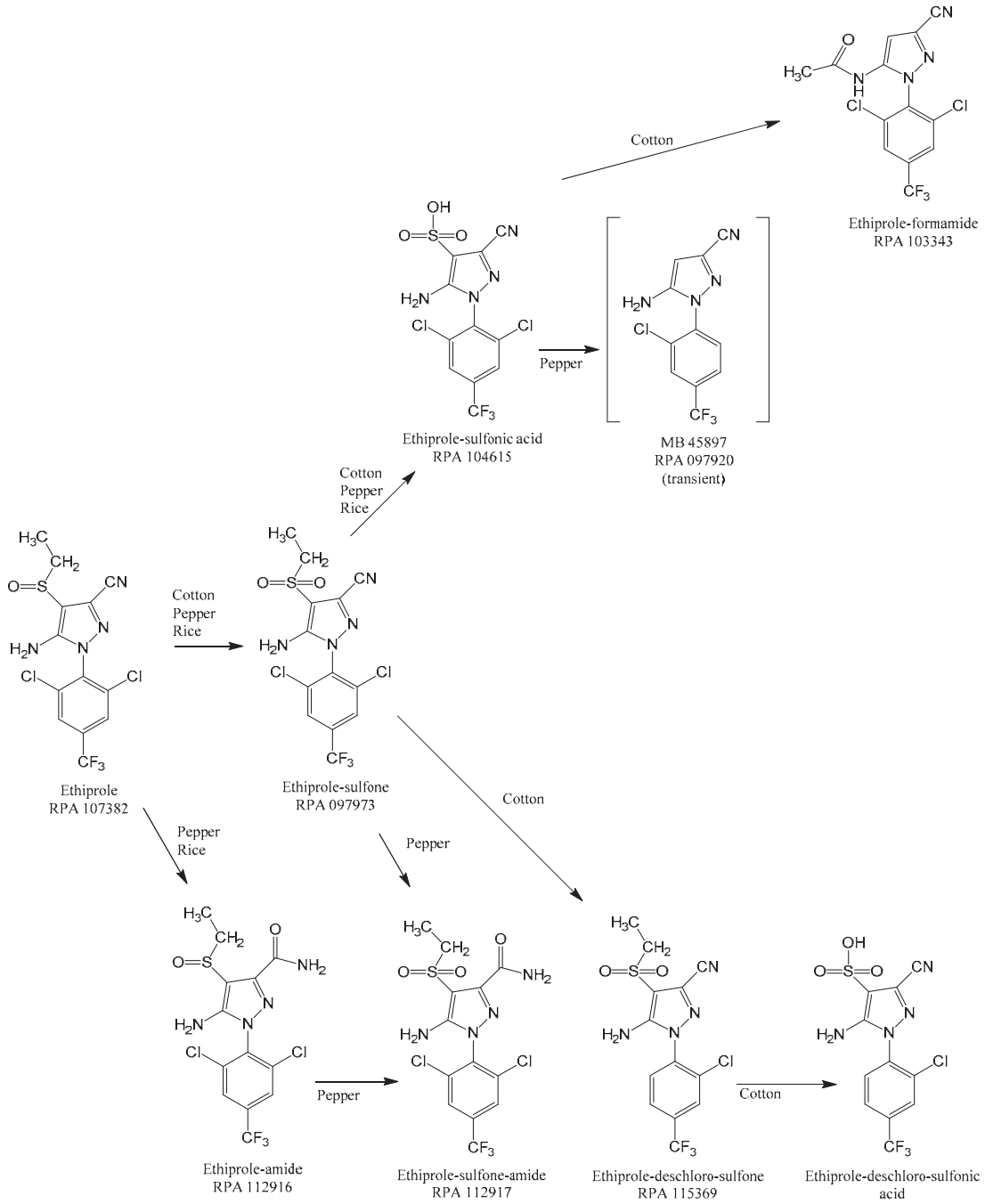


Figure 5 The metabolic pathway of ethiprole in plants (rice, sweet pepper and cotton)

Confined rotational crop study

The consideration of succeeding crops is not required, as rice and coffee are permanent or semi-permanent crops.

A confined rotational crop study was conducted to investigate the metabolism of [phenyl-UL-^{14}C]ethiprole outdoors in succeeding crops following uniform broadcast application of the test substance to sandy loam soil at a total seasonal rate of 0.74 kg ai/ha (Mislankar 2002, M-240827-01-1). Lettuce, radish, wheat and sorghum were sown at the plant back intervals (PBIs) of 30, 90, 150 and 365 days after application. Radish tops, radish roots, lettuce, sorghum straw, sorghum grain, wheat straw, and wheat grain were harvested at maturity. Sorghum and wheat forage were collected approximately at half maturity.

All samples were frozen, ground and homogenised in the presence of dry ice. The homogenised samples were radioassayed to determine the total radioactive residue (TRR) and extracted conventionally with acetonitrile/water (4:1, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) if the TRR was >0.01 mg eq/kg. The extracts were filtered, concentrated to near dryness, reconstituted in acetonitrile/water (1:1, v/v) and analysed by radio-HPLC (LOQ *ca.* 0.001 mg eq/kg). Extracted solids still containing residues > 0.01 mg eq/kg were hydrolysed with 1N HCl in methanol and 1N NaOH in water. Non-extracted residues in straw were digested additionally with cellulase and dioxane/2N HCl (9:1, v/v) for release of radioactivity attached to cellulose and lignin. The final solids with non-extracted residues were combusted and radioassayed. LC-MS/MS analysis was used for final identification of the metabolites.

TRRs in these crops were determined by combustion and radioassaying of homogenised samples. The results are shown in Table 17. TRRs ranged between 0.053 and 0.763 mg eq/kg at the earliest rotation and between 0.013 and 0.199 mg eq/kg at the latest rotation. The highest TRR levels were detected in dry straw, the lowest levels in wheat and sorghum grain. In general, the total radioactive residues observed in the crops declined with increasing plant back interval (PBI, exception radish). The TRRs in edible commodities were low, with the highest being 0.294 mg eq/kg in lettuce.

Table 17 Total radioactive residues (TRR) in lettuce, radish and wheat/sorghum sown between 30 and 365 day after application of [^{14}C]-ethiprole to bare soil at a use rate on 0.74 kg ai/ha

Plant back interval, PBI [days]	30	90	150	365
Plant commodities	Total radioactive residue, TRR [mg eq/kg]			
Lettuce	0.294	0.085	0.075	0.032
Radish leaf	0.227	0.079	0.159	0.026
Radish root	0.098	0.043	0.055	0.023
Wheat forage	0.304	0.207	0.058 [a]	0.036
Wheat fodder/straw	0.763	0.655	0.298 [a]	0.199
Wheat grain	0.053	0.041	0.027 [a]	0.013

[a] Sorghum forage/straw/grain

An overview of the extraction of radioactive residues from the rotational crop material is given in Tables 18-23.

The radioactive residues were extracted conventionally with acetonitrile/water (80:20, v/v, 3×) and acetonitrile/water/acetic acid (60:40:1, v/v/v, 3×). The conventional extraction efficiency was very good for lettuce and radish leaves accounting for approximately 77–104% of TRR and sufficient for wheat/sorghum forage and straw accounting for 65–88% of TRR at all PBIs. A high portion of TRR could also be extracted from wheat grain collected at the 30 and 90 days PBIs amounting to 83 and 86% of TRR. However, the extraction efficiency of the conventional extraction was low for radish roots at all PBIs and for wheat/sorghum grain at the 150 and 365 days PBIs amounting to 52–69% of TRR.

Exhaustive extraction procedures (acid and alkaline digestion and cellulose digestion and lignin extraction for wheat/sorghum straw) were performed additionally when the efficiency of conventional extraction was below 90% of TRR. Acid and alkaline hydrolysis steps released an additional 3–9% of TRR from lettuce and radish leaves, wheat forage of the PBIs of 30 and 90 days, and from wheat grain of the 30 days PBI. An additional 5–14% of TRR could be released from wheat and sorghum straw by acid and alkaline hydrolysis and an extra portion of 11–14% of TRR by cellulose and lignin digestion.

Finally, the non-extracted residues amounted to 1–17% of TRR in lettuce, radish leaves and roots and wheat/sorghum forage and straw at all PBIs. The non-extracted residues in wheat/sorghum grain accounted to 16–27% of TRR. However, this non-extracted portion corresponded only to 0.003–0.008 mg eq/kg.

The total accountability comprising conventional and exhaustive extraction, as well as combustion/radioassaying of non-extracted residues ranged from 80% of TRR (0.018 mg eq/kg) for 365 days PBI radish root to 111% of TRR (0.177 mg eq/kg) for 150 days PBI radish leaf.

The identification of the organosoluble residue was performed by radio-HPLC with reference substances co-chromatographed. The identity was confirmed by LC-MS/MS. The resulting residue pattern of the rotated crops lettuce, radish, wheat and sorghum are shown also in Tables 18–23.

Table 18 Extraction efficiency and radioactive residues in lettuce

PBI [days]	30		90		150		365	
Lettuce	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR	0.294	100	0.085	100	0.075	100	0.032	100
Organosoluble extraction [a]	0.283	96	0.089	104	0.062	82	0.028	88
Ethiprole-sulfonic acid RPA 104615	0.007	2	0.001	1	-	-	-	-
Ethiprole-amide RPA 112916	0.029	10	0.005	7	0.003	4	0.003	9
Ethiprole-sulfone-amide RPA 112917	0.031	11	0.017	21	0.004	5	0.004	12
Ethiprole-sulfide-amide RPA 112915	0.015	5	0.003	4	0.004	6	-	-
Ethiprole	0.043	15	0.003	4	0.004	6	-	-
RPA 097920 M45897	0.008	3	0.002	2	0.003	4	-	-
Ethipole-sulfone RPA 097973	0.134	46	0.038	45	0.043	57	0.012	36
Unknown	0.005	2	0.001	1	-	-	0.001	3
Polar metabolites [b]	0.009	3	0.01	12	0.008	10	0.003	10
Total identified/ Characterised	0.281	95	0.081	96	0.068	91	0.022	69
Exhaustive extraction [c]	-	-	-	-	0.004	5	-	-
Non extracted residue	0.018	6	0.004	5	0.003	4	0.003	9
Total accountability	0.301	102	0.093	109	0.069	91	0.031	97

[a] Comprising acetonitrile/water (80:20, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) extraction.

[b] Polar fraction analysed under various HPLC conditions, contains *ca.* 13 components

[c] Comprising 1N HCl and 1N NaOH extraction.

Table 19 Extraction efficiency and radioactive residues in radish leaves

PBI [days]	30		90		150		365	
Radish leaves	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR	0.227	100	0.079	100	0.159	100	0.026	100
Organosoluble extraction [a]	0.209	92	0.072	91	0.159	100	0.020	77
Ethiprole-sulfonic acid RPA 104615	0.023	10	0.017	22	0.023	15	0.005	19
Ethiprole-amide RPA 112916	0.017	8	0.005	6	0.014	9	0.002	8
Ethiprole-sulfone-amide RPA 112917	0.009	4	0.007	9	0.013	8	0.004	15
Ethiprole-sulfide-amide RPA 112915	0.015	7	0.003	4	-	-	-	-
Ethiprole	0.020	9	0.001	2	0.008	5	-	-
RPA 097920 MB45897	0.010	4	0.003	3	0.01	6	-	-
Ethiprole-sulfone RPA 097973	0.071	31	0.019	24	0.048	30	0.004	14
Unknowns	1 unknown (0.008 mg eq/kg, 4%		1 unknown (0.002 mg eq/kg, 3%		-	-	-	-

PBI [days]	30		90		150		365	
Radish leaves	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
	TRR)		TRR)					
Polar metabolites [b]	0.030	13	0.012	15	0.036	22	0.004	16
Total identified/ Characterised	0.203	89	0.069	88	0.152	96	0.019	72
Exhaustive extraction [c]	-	-	-	-	-	-	0.002	6
Non extracted residue	0.022	9	0.009	11	0.018	11	0.001	4
Total accountability	0.230	101	0.081	102	0.177	111	0.024	87

[a] Comprising acetonitrile/ water (80:20, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) extraction.

[b] Polar fraction analysed under various HPLC conditions, contains up to 13 components

[c] Comprising 1N HCl and 1N NaOH extraction.

Table 20 Extraction efficiency and radioactive residues in radish root

PBI [days]	30		90		150		365	
Radish root	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR	0.098	100	0.043	100	0.055	100	0.023	100
Organosoluble extraction [a]	0.064	66	0.030	69	0.036	66	0.012	52
Ethiprole-sulfonic acid RPA 104615	0.011	11	0.013	29	0.006	10	0.002	8
Ethiprole-amide RPA 112916	-	-	-	-	0.002	3	-	-
Ethiprole-sulfone-amide RPA 112917	-	-	0.002	4	0.001	3	0.002	4
Ethiprole	0.018	19	-	-	0.003	6	-	-
RPA 097920 MB45897	-	-	0.009	22	0.003	5	-	-
Ethipole-sulfone RPA 097973	0.036	36	-	-	0.015	27	0.007	28
Polar metabolites [b]	-	-	0.005	11	0.002	4	-	-
Total identified/ Characterised	0.065	66	0.029	66	0.031	57	0.011	42
Exhaustive extraction [c]	0.015	16	0.006	12	0.007	12	0.002	11
Non extracted residue	0.014	15	0.005	11	0.006	10	0.004 [d]	17
Total accountability	0.093	97	0.041	92	0.047	86	0.018	80

[a] Comprising acetonitrile/ water (80:20, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) extraction.

[b] Polar fraction analysed under various HPLC conditions, contains up to 13 components

[c] Comprising 1N HCl and 1N NaOH extraction.

[d] Combustion of filter paper plus unextracted residue

Table 21 Extraction efficiency and radioactive residues in wheat/sorghum forage

PBI [days]	30		90		150 [d]		365	
Wheat/sorghum forage	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR	0.304	100	0.207	100	0.058	100	0.036	100
Organosoluble extraction [a]	0.198	65	0.176	84	0.051	88	0.030	82
Ethiprole-sulfonic acid RPA 104615	0.006	2	0.016	8	0.008	15	-	-
Ethiprole-amide RPA 112916	0.005	2	0.014	7	-	-	0.002	5
Ethiprole-sulfone-amide RPA 112917	0.010	3	0.026	13	-	-	0.007	19
Ethiprole	0.015	5	-	-	-	-	-	-

PBI [days]	30		90		150 [d]		365	
Wheat/sorghum forage	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
RPA 097920 MB45897	0.024	8	0.016	8	-	-	-	-
Ethipole-sulfone RPA 097973	0.103	34	0.081	39	0.026	46	0.016	46
Unknown	-	-	-	-	0.004	6	-	-
Polar metabolites [b]	0.047	16	0.035	17	0.011	19	0.004	12
Total identified/ Characterised	0.211	69	0.187	91	0.05	85	0.029	82
Exhaustive extraction [c]	0.026	9	0.014	7	-	-	-	-
Non-extracted residue	0.024	8	0.011	7	0.004	8	0.001	3
Total accountability	0.248	82	0.201	98	0.055	96	0.031	85

[a] Comprising acetonitrile/ water (80:20, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) extraction.

[b] Polar metabolites analysed under various HPLC conditions, contains up to 13 components

[c] Comprising 1N HCl and 1N NaOH extraction.

[d] Sorghum forage at PBI 150 days, wheat forage at 30, 90 and 365 days

Table 22 Extraction efficiency and radioactive residues in wheat/sorghum straw

PBI [days]	30		90		150 [d]		365	
Wheat/sorghum straw	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR	0.763	100	0.655	100	0.298	100	0.199	100
Organosoluble extraction [a]	0.627	82	0.574	88	0.196	66	0.17	69
Ethiprole-sulfonic acid RPA 104615	0.115	15	0.109	17	0.015	5	0.017	8
Ethiprole-amide RPA 112916	0.022	3	0.025	4	-	-	-	-
Ethiprole-sulfone-amide RPA 112917	0.058	3	0.065	10	0.016	5	0.022	11
Ethiprole-sulfide-amide RPA 112915	0.011	1	-	-	-	-	-	-
Ethiprole	-	-	-	-	-	-	-	-
RPA 097920 M45897	0.039	4	0.015	2	-	-	0.004	2
Ethipole-sulfone RPA 097973	0.174	23	0.120	18	0.082	28	0.107	54
Unknowns [b]	Sum 0.141 mg eq/kg, 19% TRR		Sum 0.106 mg eq/kg, 16% TRR)		Sum 0.078 mg eq/kg, 27% TRR)		Sum 0.017 mg eq/kg, 9% TRR)	
Unknowns	1 unknown (0.015 mg eq/kg, 2% TRR)		2 unknowns (max. 0.012 mg eq/kg, 2% TRR)		-	-	1 unknown (0.004 mg eq/kg, 2% TRR)	
Total identified/ Characterised	0.575	70	0.462	71	0.194	65	0.171	86
Exhaustive extraction [c]	0.056	7	0.046	7	0.044	14	0.0012	5
Cellulose/Lignin extraction [e]	-	-	-	-	0.02/ 0.012	7/ 4	0.014/ 0.014	7/ 7
Non-extracted residue	0.046	6	0.052	8	0.003	1	0.010	5
Total accountability	0.729	95	0.672	103	0.275	92	0.185	93

[a] Comprising acetonitrile/ water (80:20, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) extraction.

[b] These fractions were analysed under various HPLC conditions and contain *ca.* 13 components

[c] Comprising 1N HCl and 1N NaOH extraction of straw sampled after a PBI of 150 and 365 days

[d] Sorghum straw at PBI 150 days, wheat straw at 30, 90 and 365 days

[e] By incubation with cellulase followed by dioxan/2N HCl (9/1)

Table 23 Extraction efficiency and radioactive residues in wheat/sorghum grain

PBI [days]	30		90		150 [d]		365	
Wheat/sorghum grain	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR	0.053	100	0.041	100	0.027	100	0.013	100
Organosoluble extraction [a]	0.044	83	0.035	86	0.018	65	0.008	61
Ethiprole-sulfonic acid RPA 104615	0.012	22	0.016	40	0.002	8	-	-
Ethiprole-amide RPA 112916	-	-	0.001	2	-	-	-	-
Ethiprole-sulfide-amide RPA 112915	-	-	-	-	0.001	4	-	-
Ethiprole	-	-	-	-	-	-	-	-
Ethipole-sulfone RPA 097973	0.002	4	0.002	9	0.004	12	-	-
Unknowns [b]	Sum 0.030 mg eq/kg, 57% TRR		Sum 0.013 mg eq/kg, 34% TRR)		Sum 0.010 mg eq/kg, 39% TRR)		0.008 mg eq/kg, 62% TRR)	
Total identified/ Characterised	0.042	82	0.033	80	0.017	62	0.008	62
Exhaustive extraction [c]	0.001	3	-	-	-	-	-	-
Non-extracted residue	0.008	16	0.008	20	0.007	27	0.003	23
Total accountability	0.053	102	0.043	106	0.025	92	0.011	84

[a] Comprising acetonitrile/ water (80:20, v/v) and acetonitrile/water/acetic acid (60:40:1, v/v/v) extraction.

[b] These fractions were analysed under various HPLC conditions, contain multiple components

[c] Comprising 1N HCl and 1N NaOH extraction.

[d] Sorghum grain at PBI 150 days, wheat grain at 30, 90 and 365 days

Parent ethiprole was intensively metabolised as it could be detected only at low levels in lettuce, radish and wheat forage of the first rotation (PBI 30 days) amounting to 5–19% of TRR (0.015–0.043 mg/kg). At later rotations its residue level was <0.01 mg/kg. Ethiprole was not at all detectable in wheat/sorghum straw and grain, and not detected in wheat/sorghum forage at the PBIs 90, 150 and 365 days. However, the sulfone metabolite RPA 097973 was the main residue component in almost all crop commodities and all PBIs amounting to 36–57% of TRR in lettuce, 14–31% of TRR in radish leaves, 27–36% of TRR in radish roots (except PBI 90 days), 34–46% of TRR in wheat/sorghum forage, and 18–54% of TRR in wheat/sorghum straw, but only to 4–12% of TRR in wheat/sorghum grain (except 365 days). The main residue component in wheat and sorghum grain was the sulfonic acid metabolite RPA 104615 accounting for 8–40% of TRR at the PBIs 30, 90 and 120 days. At the last PBI of 365 days the total residues were too low (TRR = 0.013 mg eq/kg) for metabolite identification.

In lettuce, the main residue component was the sulfone (RPA 097973) which accounted for 0.012 (PBI 365 days) to 0.134 mg eq/kg (PBI 30 days). A further major metabolite was the sulfone amide RPA 112917 accounting for 5–21% of TRR (0.004–0.031 mg eq/kg). Minor metabolites (≤10% of TRR) were the amide RPA 112916, the sulfide amide RPA 112915, RPA 097920 and the sulfonic acid RPA 104615. In addition, up to 14 minor unknowns were detected (Table 18).

In radish leaves, the main residue component was the sulfone which accounted for 0.071 (31% of TRR) and 0.048 (30% of TRR) mg eq/kg at the PBIs 30 and 150 days and declined to 0.004 mg eq/kg at PBI 365 day. Another major metabolite was the sulfonic acid RPA 104615 amounting to 0.023 mg eq/kg each at PBI 30 days (10% of TRR) and PBI 150 days (15% of TRR). It amounted to 0.017 mg eq/kg (22% of TRR) at PBI 90 days and to 0.005 mg eq/kg (19% of TRR) at PBI 365 days. Minor metabolites were ethiprole-amide RPA 112916, ethiprole-sulfide-amide RPA 112915, ethiprole-sulfone-amide RPA 112917 and RPA 097920. In addition, at least 15 minor unknown metabolites were observed (Table 19).

In radish root, the parent sulfone was also a major metabolite at the PBIs 30, 150 and 365 days (0.007–0.036 mg eq/kg, 27–36% of TRR), but it was not detected at PBI 90 days. A further major metabolite was the sulfonic acid RPA 104615 accounting for 0.0011 mg eq/kg (11% of TRR) and 0.013 mg eq/kg (29% of TRR) in the first and second rotation, respectively. Minor metabolites were occasionally detected as the parent amide RPA 112916, sulfone-amide RPA 112917, RPA 097920 and approximately 13 unknown polar residue components (Table 20).

In wheat/sorghum forage, the main residue component, the sulfone RPA 097973, was found at 0.103 mg eq/kg (34% of TRR) at a PBI of 30 days. This declined to 0.081 mg eq/kg (39% of TRR) at a PBI of 90 days, to 0.026 mg eq/kg (46% of TRR) at a PBI of 150 days and finally to 0.016 mg eq/kg (46% of TRR) at a PBI of 365 days. Some metabolites showed a maximum residue level only at the second rotation (PBI 90 days) followed by a decrease at longer PBIs. These peak levels were 0.016 mg eq/kg (8% of TRR) for the sulfonic acid RPA 104615, 0.014 mg eq/kg (7% of TRR) for the parent-amide RPA 112916, and 0.026 mg eq/kg (13%

of TRR) for the sulfone-amide RPA 112917. The metabolite RPA 097920 peaked at the first PBI of 30 days amounting to 0.024 mg eq/kg (8% of TRR). In addition, approximately 14 polar unknown metabolites were detected amounting in total to 0.004–0.047 mg eq/kg (12–19% of TRR) (Table 21).

In wheat/sorghum straw, the sulfone RPA 097973 was again the major residue component accounting for 0.082 mg eq/kg at a PBI of 150 days to 0.174 mg eq/kg at a PBI of 30 days (18–54% of TRR). Another major metabolite was identified as the sulfonic acid RPA 104615 accounting for 0.017–0.115 mg eq/kg (5–17% of TRR). Some minor metabolites were detected at ≤11% of TRR. These metabolites were the amide RPA 112916, the sulfone amide RPA 112917, the sulfide amide RPA 112915 and RPA 097920. Approximately 13 unknown polar metabolites were found accounting for 11–27% of the TRR. In addition, acid and alkaline hydrolysis after conventional extraction released an extra portion of 5–14% of TRR and cellulose/lignin digestion an additional 11–14% of TRR (Table 22).

In wheat/sorghum grain, the main residue component was the sulfonic acid RPA 104615 amounting to 0.012 mg eq/kg (22% of TRR) at a PBI of 30 days, 0.016 mg eq/kg (40% of TRR) at a PBI of 90 days and 0.002 mg eq/kg (8% of TRR) at a PBI of 150 days. No residue component could be identified at the last PBI of 365 days. Minor metabolites were detected as the sulfone RPA 097973, the amide RPA 112916 and the sulfide amide RPA 112915, all of them amounting to <0.01 mg eq/kg. In addition, two medium polar unknown metabolites accounted for 10–19% of TRR, but did not exceed 0.01 mg eq/kg. Finally, a lot of very minor polar unknowns were detected accounting in total for 0.002–0.013 mg eq/kg (6–62% of TRR) (Table 23).

The samples were extracted and analysed within four months of storage so no storage stability is required. Nevertheless, a nine-month freezer storage stability of a lettuce sample was demonstrated by repeated extraction of the same sample and concentration and radio-HPLC analysis of the extracts. The extraction efficiency was the same at both extraction events and the two HPLC profiles of the extracts were found to be similar.

Ethiprole was extensively metabolised in confined rotational crops. The main parallel metabolic reactions involved were oxidation of the sulfoxide group to form the sulfone metabolite RPA 097973, and hydrolysis of the nitrile moiety to the amide metabolite RPA 112916. The sulfone metabolite RPA 097973 could further be oxidized to the sulfonic acid RPA 104615. Elimination of the sulfonic acid substituent resulted in RPA 097920. The amide RPA 112916 was also oxidized to sulfone amide metabolite RPA 112917 or reduced to the sulfide metabolite RPA 112915. The sulfone metabolite was the main residue component in almost all samples of the four rotations. However, the sulfonic acid metabolite was the main residue component in grain, at PBIs of 30 and 90 days.

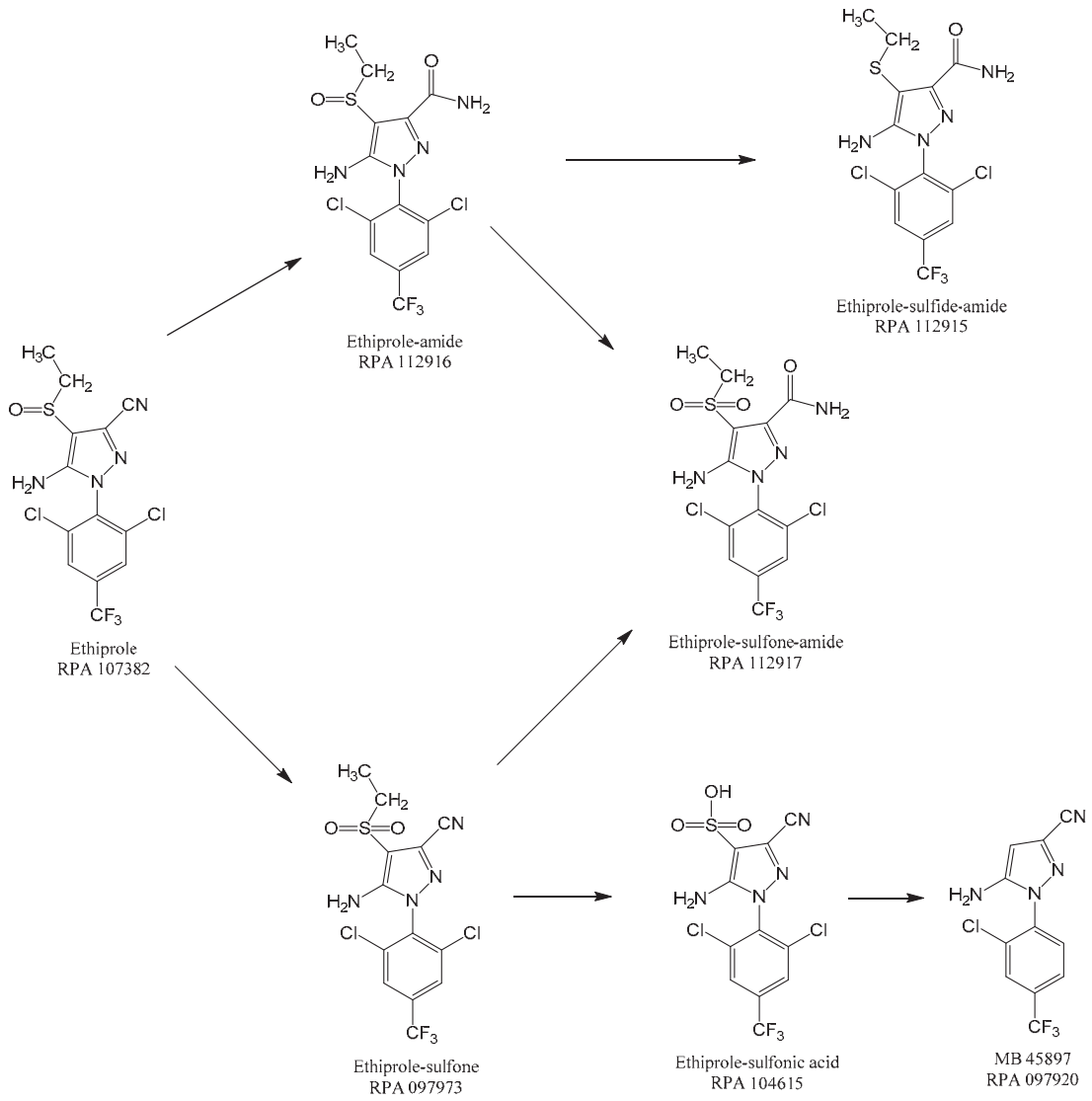


Figure 6 Proposed metabolic pathway for ethiprole in rotational crops

Animal metabolism

Metabolism in rat

Evaluation of the metabolism studies in rodents was carried out by the WHO Core Assessment Group.

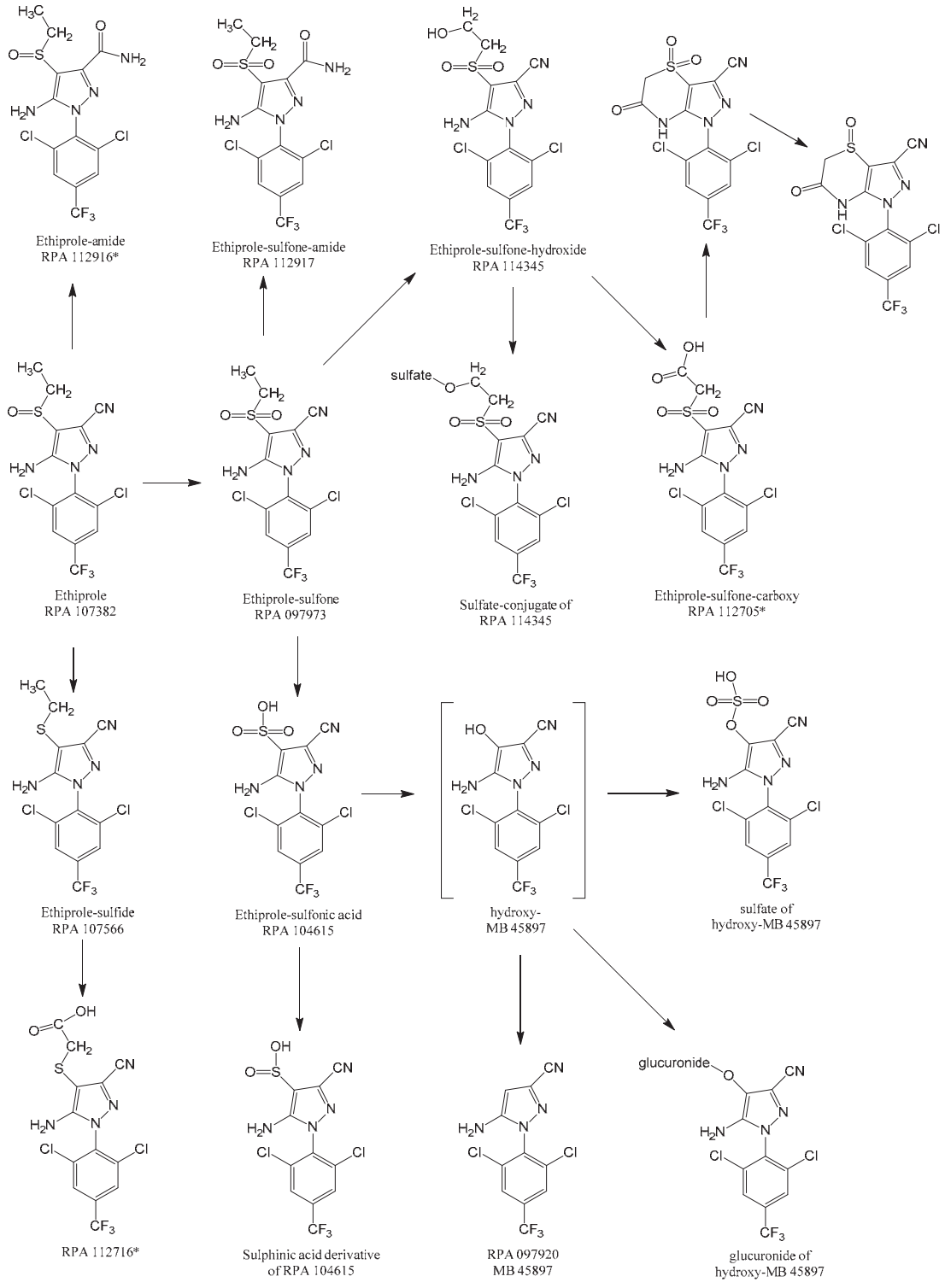
Rats

Analysis of the excreted radioactivity in rats indicated that orally administered [^{14}C]-Ethiprole was extensively metabolised. The pattern of metabolites in urine was similar in male and female rats following administration of the low dose. The major components were the polar glucuronide conjugate of hydroxy-MB 45897, the sulfonic acid, as well as the less polar (non-conjugated) MB 45897 and in female urine ethiprole-sulfone-carboxy. The excreted radioactive components could be identified to a proportion of approximately 65 or 80% of the administered radioactivity at the low or high dose level.

Three primary parallel metabolic pathways could be derived from the metabolites observed followed by further reactions including conjugate formation: 1) Hydrolysis of the nitrile group of ethiprole to form ethiprole-amide; 2) Reduction of the sulfoxide

group of ethiprole to form ethiprole-sulfide. A subsequent alkyl oxidation results in ethiprole-sulfide-carboxy, and 3) Oxidation of the sulfoxide group of ethiprole to form the major metabolite, ethiprole-sulfone, followed by further metabolic reactions:

- The sulfone can be alkyl hydroxylated to generate ethiprole-sulfone-hydroxide, which is subsequently metabolised by alkyl oxidation to form ethiprole-sulfone-carboxy and by conjugation with sulfuric acid. Further metabolic steps can follow.
- An oxidative desalkylation of the sulfone forms ethiprole-sulfonic acid. Replacement of the sulfuric acid group by a hydroxy group generates the intermediate hydroxy-MB 45897 that subsequently can be stabilised as a sulphate or glucuronide conjugate.
- The nitrile group of ethiprole-sulfone can be further hydrolysed to the amide resulting in ethiprole-sulfone-amide.



* Tentatively identified by co-chromatography with a reference standard
[] Postulated intermediate

Figure 7 Proposed metabolic pathway for ethiprole in rats

Lactating goats

A study on the metabolism of ethiprole in lactating goats was conducted with the test compound ^{14}C-labelled in the phenyl position (McCorquodale *et al.,* 1999, M-192557-01-3). Two lactating goats were orally dosed twice daily for 7 consecutive days at approximately 8.30 and 16.00 hours. Goats 1 and 2 received doses at nominal levels of 10 ppm and 1 ppm of daily food consumption, respectively. The actual daily dose rates were 14.2 ppm feed (high dose) and 1.2 ppm feed (low dose). A third goat, goat 3, received an empty gelatin capsule. The goats were of a locally used breed and weighed 61, 65 and 66 kg.

The goats were milked in the morning prior to administration of the first dose, and twice daily throughout the study period. Urine and faeces were collected during the day prior to the first dose and at 24 hour intervals thereafter. Each animal was sacrificed approximately 23 hours after the last dose and selected tissues collected. All biological samples were assayed for total radioactivity by liquid scintillation counting, either directly or following sample combustion.

The total recovery of radioactivity was 86.5% for the high dose and 87.3% for the low dose. The majority of the radioactivity was excreted with the faeces, accounting for 68.6 and 62.2% of the total dose for the high (10 ppm) and low (1 ppm) dose respectively, while excretion *via* urine accounted for 8.5 and 15.1% for the high and low dose levels respectively.

A summary of the distribution, excretion and recovery of administered radioactivity is given in the following table:

Table 24 Total radioactive residues following administration of [phenyl-^{14}C]-ethiprole to lactating goats at 1 and 10 ppm in the diet

Matrix	Total Radioactive Residues (TRR)	
	10 ppm dose	1 ppm dose
	% of administered dose	% of administered dose
Urine	8.45	15.1
Faeces	68.6	62.2
Cage wash	0.25	0.99
Kidneys	0.03	0.03
Liver	0.47	0.58
Milk	0.42	0.34
GI tract and contents	8.31	8.06
Total Recovery	86.5	87.3

The radioactivity levels and concentrations measured in the milk ranged from 0.013 mg eq/kg at 8 hours after the first dose to 0.070 mg eq/kg at 152 hours after the first administration for the 10 ppm dose level, and 0.002 mg eq/kg at 8 hours after the first dose to 0.009 mg eq/kg at 152 (and 168) hours for the 1 ppm dose level. Residues had declined for both dose levels at the time of sacrifice (175 hours post first dose), to 0.039 and 0.007 mg eq/kg, indicating that plateau levels had been reached. The total recovery of radioactivity in milk at 175 hours post first dose, accounted for only 0.42 and 0.34% of the administered doses (10 and 1 ppm levels respectively).

Table 25 Mean total radioactive residues in milk over time following administration of [phenyl-^{14}C]-ethiprole to lactating goats at 1 and 10 ppm in the diet

Time after first administration [h]	10 ppm feed/day (high dose)	1 ppm feed/day (low dose)
	[mg eq/kg]	
8	0.013	0.002
24	0.017	0.003
32	0.029	0.003
48	0.027	0.004
56	0.040	0.005
72	0.040	0.005
80	0.051	0.006
96	0.045	0.006
104	0.053	0.007
120	0.053	0.007
128	0.033	0.006
144	0.055	0.008
152	0.070	0.009
168	0.059	0.009
175	0.039	0.007
Time to reach the residue plateau	152 hours post 1st dose	152–168 hours post 1st dose

The highest TRR values in tissues for the 10 ppm dosed cow were found in liver (0.685 mg eq/kg, 0.47% of the administered dose), renal fat (0.659 mg eq/kg), omental fat (0.612 mg eq/kg), and kidney (0.206 mg eq/kg, 0.03% of the administered dose). For the low dose the highest TRR values in tissues were also found in liver (0.094 mg eq/kg, 0.58% of the administered dose), renal fat (0.094 mg eq/kg), omental fat (0.081 mg eq/kg) and kidney (0.033 mg eq/kg, 0.03% of the administered dose).

Table 26 Total radioactive residues in tissues following administration of [phenyl-^{14}C]-ethiprole to lactating goats at 10 and 1 ppm in the diet

Sampling Time	Total Radioactive Residues (TRR)	
	10 ppm feed	1 ppm feed
	mg eq/kg	mg eq/kg
Fat-omental	0.612	0.081
Fat-renal	0.659	0.094
Kidneys	0.206	0.033
Liver	0.685	0.094
Muscle	0.086	0.010
Whole blood	0.087	0.000
Plasma	0.107	0.014

For analysis of parent compound and metabolites, faeces, milk, liver, renal fat, omental fat, muscle and kidney were extracted with methanol (3×), radioactivity levels in extracts quantified by LSC and extracts concentrated and re-dissolved for HPLC analysis. Radioactivity remaining in the post-extracted solids was quantified by combustion analysis and LSC.

Extraction of residues with methanol at ambient temperature ranged from 90.1% of the TRR (liver) to 99.5% TRR (milk) for the high dose. For the low dose the extraction efficiency ranged from 88.0% (liver) to 99.3% (milk).

The residue pattern in liver, kidney, muscle, fat (23 hours after the last dose, 175 hours after the first dose) and milk (72 and 168 hours samples) is shown in Tables 27-30.

Analysis of extracts from liver, kidney, muscle, renal and omental fat and milk of the high dose group showed ethiprole-sulfone RPA 097973 to be the major residue component, representing 32–77% of the TRR in high dose samples. Parent ethiprole was identified in kidney, muscle, renal and omental fat and milk and the metabolite ethiprole methyl sulfone (RPA 094569) was identified in liver. A major metabolite in liver and kidney co-chromatographed with ethiprole-sulfonic acid (RPA 104615), but was not confirmed by LC-MS. Further MS analysis indicated that this peak co-chromatographed with the N-glucuronide of RPA 107566. None of the unidentified minor metabolites in organs and tissues represented more than 6.8% of the TRR.

The metabolite pattern in the low dose group was similar to the high dose group with slight variations. Besides the main residue component, ethiprole-sulfone (35–79% of TRR), the parent substance was only detected in muscle (9.7% of TRR), in fat (8.8–10% of TRR) and in milk (18–22% of TRR). In muscle, ethiprole-sulfone-hydroxide (RPA 114345) and ethiprole methyl sulfone (RPA 094569) were additionally detected at a low level (≤3.6% of TRR). In liver, ethiprole sulfone-amide RPA 112917 (1.6% TRR) and ethiprole sulfone-hydroxide RPA 114345 (1.4% TRR) were observed instead of ethiprole methyl sulfone RPA 094569 and ethiprole sulfide RPA 107566. In kidney, RPA 0979720 (MB 45897, 3.8% TRR) was found.

Table 27 Residue components in organs of a goat 23 hours after the last of 14 twice daily doses of ^{14}C-Ethiprole for 7 consecutive days at a high dose level of 10 ppm feed

Compound	Liver		Kidney		Muscle	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfonic acid RPA 104615/ N-glucuronide of RPA 107566	13.1	0.090	20.0	0.041	-	-
Ethiprole-amide RPA 112916	5.7	0.039	-	-	-	-
Ethiprole (parent)	-	-	4.1	0.008	17.0	0.015
Ethiprole methyl sulfone RPA 094569	2.0	0.013	-	-	-	-
Ethiprole-sulfone RPA 097973	55.4	0.379	32.2	0.066	66.3	0.057
Ethiprole-sulfide RPA 107566	4.7	0.032	4.4	0.009	5.1	0.004
Total identified	80.8	0.553	60.7	0.124	88.4	0.076
Characterised (number of characterised)	5.9 (1)	0.041	10.5 (2)	0.022	0 (0)	0 (0)
Total extractable	90.1	0.617	93.8	0.193	95.0	0.082
Non extractable	9.9	0.068	6.2	0.013	5.0	0.004

Table 28 Residue components in fat and milk of a goat 23 hours after the last of 14 twice daily doses of ^{14}C-Ethiprole for 7 consecutive days at a high dose level of 10 ppm feed

Compound	Renal fat		Omental fat		Milk (72h)		Milk (168 h)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfone-hydroxide RPA 114345	-	-	-	-	0.5	<0.001	-	-
Ethiprole (parent)	17.3	0.114	15.2	0.093	28.7	0.010	20.5	0.013
Ethiprole-sulfone RPA 097973	77.2	0.509	77.0	0.471	51.6	0.019	60.5	0.037
Ethiprole-sulfide RPA 107566	4.1	0.027	4.2	0.025	4.9	0.002	5.0	0.003
Total identified	98.6	0.650	96.4	0.589	85.7	0.031	86.0	0.053
Characterised (number of characterised)	0 (0)	0 (0)	0 (0)	0 (0)	0.8 (1)	<0.001 (1)	0 (0)	0 (0)
Total extractable	99.0	0.652	99.2	0.607	99.6	0.036	99.5	0.061
Non extractable	1.0	0.007	0.8	0.005	0.4	<0.001	0.5	<0.001

Table 29 Residue components in organs of a goat 23 hours after the last of 14 twice daily doses of ^{14}C-Ethiprole for 7 consecutive days at a low dose level of 1 ppm feed

Compound	Liver		Kidney		Muscle	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfonic acid RPA 104615/ N-glucuronide of RPA 107566	18.2	0.017	26.1	0.009	-	-
Ethiprole-amide RPA 112916	5.2	0.005	-	-	-	-
Ethiprole-sulfone-amide RPA 112917	1.6	0.001	-	-	-	-
Ethiprole-sulfone-hydroxide RPA 114345	1.4	0.001	-	-	2.4	0.000
RPA 097920	-	-	3.8	0.001	-	-

Compound	Liver		Kidney		Muscle	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
= MB 45897						
Ethiprole (parent)	-	-	-	-	9.7	0.001
Ethiprole methyl sulfone RPA 094569	-	-	-	-	3.6	0.000
Ethiprole-sulfone RPA 097973	35.3	0.033	42.4	0.014	59.1	0.006
Ethiprole-sulfide RPA 107566	-	-	-	-	2.0	0.000
Total identified	61.7	0.057	72.3	0.024	76.8	0.007
Characterised (number of characterised)	5.9 (2)	0.005	8.9 (1)	0.003	0 (0)	0 (0)
Total extractable	88.0	0.083	93.4	0.031	95.0	0.010
Non extractable	12.0	0.011	6.6	0.002	5.0	<0.001

Table 30 Residue components in fat and milk of a goat 23 hours after the last of 14 twice daily doses of ^{14}C-Ethiprole for 7 consecutive days at a low dose level of 1 ppm feed

Compound	Renal fat		Omental fat		Milk (72h)		Milk (168 h)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole (parent)	8.8	0.008	10.4	0.008	21.7	0.001	17.7	0.002
Ethiprole methyl sulfone RPA 094569	2.1	0.002	3.0	0.002	-	-	-	-
Ethiprole-sulfone RPA 097973	76.1	0.072	78.8	0.064	66.0	0.003	68.5	0.006
Ethiprole-sulfide RPA 107566	2.4	0.002	2.3	0.002	3.9	0.000	2.0	0.000
Total identified	89.4	0.084	94.5	0.076	91.6	0.004	88.2	0.008
Characterised (number of characterised)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total extractable	99.0	0.093	98.1	0.079	99.0	0.005	99.3	0.009
Non extractable	1.0	0.001	1.9	0.002	1.0	<0.001	0.7	<0.001

Characterised = unknown components characterised by retention time or enzymatic treatment

Total identified = components identified by co-chromatography with an authentic reference standard and by LC-MS or by co-chromatography solely

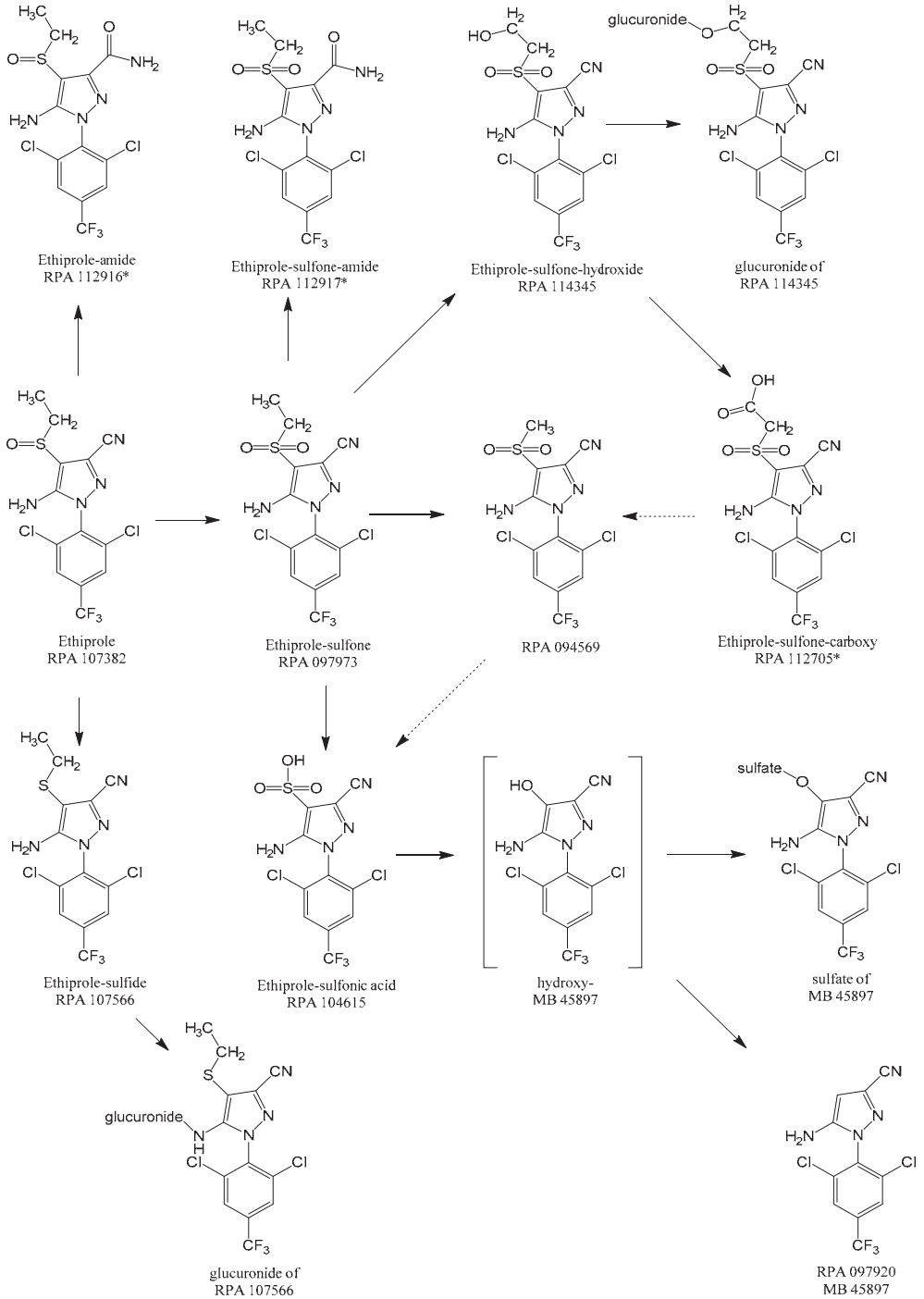
Total extractable = sum of all components in combined extracts before concentration

Non extractable = components in the post-extracted solids

[^{14}C]-Ethiprole was metabolised in the lactating goat *via* three primary routes: 1) oxidation (hydrolysis) of the nitrile group to form the minor amide metabolite RPA 112916; 2) reduction of the sulfoxide group of ethiprole to the sulfide metabolite RPA 107566 observed in milk and all organs and tissues and which was further metabolised by conjugation of the primary amine to N-glucuronide-RPA 107566; and 3) oxidation of the sulfoxide group to form the major sulfone metabolite RPA 097973 detected in all samples as the main residue component.

The sulfone metabolite was further metabolised *via* four different pathways: 1) hydroxylation of the ethyl group linked to the sulfone substituent to form ethiprole-sulfone-hydroxide RPA 114345, which was subsequently conjugated with glucuronic acid or oxidized to the transient carboxylic metabolite RPA 112705 that can be decarboxylated to RPA 094569; 2) oxidative dealkylation of RPA 097973 to produce the sulfonic acid metabolite RPA 104615, which was further metabolised by substitution of the sulfate function by a hydroxyl group to produce the transient hydroxy-MB 45897 (hydroxy-RPA 097920). This aglycon was conjugated with sulfuric acid. The metabolite MB 45897 (without hydroxy substituent) was also identified in kidney and likely formed from hydroxy-MB 45897 by reduction of the hydroxy group; 3) oxidation (hydrolysis) of the nitrile function of RPA 097973 to form the minor metabolite ethiprole-sulfone-amide RPA 112917, which was tentatively identified by co-chromatography during HPLC analysis and 4) demethylation of RPA 097973 to form the very minor methyl sulfoxide RPA 094569, although it could also be formed by decarboxylation of metabolite RPA 112705.

The proposed metabolic pathway for ethiprole in goats is shown in Figure 8.



* Tentatively identified by co-chromatography with a reference standard
[] Postulated intermediate

Figure 8 Proposed metabolic pathway for ethiprole in lactating goats

Laying hens

A study on the metabolism of ethiprole in laying hens was conducted with the test compound ^{14}C-labelled in the phenyl position (McCorquodale and Anderson 1999, M-192553-02-2). Two dose groups of five hens each (locally used breed, 7-8 months, 1.38-2.00 kg body weight at the time of initial dosing) were dosed orally once daily in the morning for 14 consecutive days, at a nominal dose rate of either 1 or 10 ppm of daily food consumption. The actual dose rates were 0.84–1.17 ppm feed (low dose) and 11.46–15.25 ppm feed (high dose). Based on the body weight of the birds these doses corresponded to 0.09-0.13 mg/kg bw/day (low dose) and 0.93-1.35 mg/kg bw/day (high dose).

Eggs and excreta were collected on a daily basis. The animals were sacrificed 23 hours after the last administration. Total radioactive residues (TRR) were determined daily in the pooled eggs and pooled excreta and after sacrifice in the dissected tissues and organs (muscle, fat, liver, skin, egg white and egg yolk and eggs from oviduct).

The TRR in each sample was determined by liquid scintillation counting (LSC), either directly or following solubilisation or combustion. The total recovery of radioactivity was 94.4% for the high dose group and 91.3% for the low dose group. The majority of the administered dose was eliminated in the excreta, accounting for 90.9% and 87.8% of the total dose for the high and low dose hen groups respectively.

Table 31 Total radioactive residues in eggs, tissues and excreta following administration of [phenyl-^{14}C]-ethiprole to laying hens at 1 and 10 ppm in the diet

Matrix	Total Radioactive Residues (TRR)	
	10 ppm dose	1 ppm dose
	% of administered dose	% of administered dose
Excreta	89.2	85.2
Cage wash	1.73	2.56
Total excreted	90.9	87.8
Egg yolks	1.92	1.88
Egg whites	0.39	0.30
Total in eggs	2.31	2.18
Organs/tissues/blood	1.20	1.36
Total Recovery	94.4	91.3

Low levels of radioactivity were detected in eggs in both dose groups with the residues in egg white reaching a steady state approximately 4 days after the first administration and in egg yolk 10 days after the first administration, with a slight increase in yolk residues until the end of the collection period (day 14). The residue plateau in egg white and egg yolk accounted for approximately 0.222 and 3.681 mg eq/kg at the high dose and approximately 0.015 and 0.297 mg eq/kg at the low dose.

The highest mean TRR levels detected in egg white and egg yolk in the high dose group were 0.288 mg eq/kg and 3.790 mg eq/kg, respectively, and for the low dose group 0.019 mg eq/kg and 0.317 mg eq/kg, respectively. A total of 2.3% and 2.2% of the dose was recovered in the eggs at high and low dose hens respectively.

Table 32 Mean total radioactive residues in eggs over time following administration of [phenyl-^{14}C]-ethiprole to laying hens at 10 and 1 ppm in the diet

Time after first administration [days]	10 ppm feed/day (high dose)		1 ppm feed day (low dose)	
	egg white	egg yolk	egg white	egg yolk
	[mg eq/kg]			
1	0.081	0.056 [a]	0.006 [a]	0.003 [a]
2	0.170	0.342	0.012	0.023
3	0.214	0.617	0.018	0.064
4	0.288	1.263	0.019	0.109
5	0.239	1.970	0.015	0.151
6	0.220	2.645	0.016	0.210
7	0.228	3.120	0.016	0.262
8	0.161	3.513	0.015	0.289
9	0.241	3.390	0.011	0.279
10	0.211	3.637	0.014	0.295
11	0.218	3.567	0.014	0.274
12	0.241	3.652	0.014	0.296
13	0.207	3.790	0.016	0.304
14	0.183	3.759	0.015	0.317
Time to reach the residue	4	10	4	10

Time after first administration [days]	10 ppm feed/day (high dose)		1 ppm feed day (low dose)	
	egg white	egg yolk	egg white	egg yolk
	[mg eq/kg]			
plateau (days)				
Residue plateau (mg eq/kg, arithmetic mean)	0.222 ± 0.033 (days 4–14)	3.681 ± 0.092 (day 10–14)	0.015 ± 0.002 (days 4–14)	0.297 ± 0.016 (days 10–14)

[a] Mean includes one result calculated from data less than 30 dpm above background

Highest tissue residues were observed in abdominal fat, liver and combined skin and fat (0.896–1.383 mg eq/kg at the high dose and 0.088–0.131 mg eq/kg at the low dose).

Table 33 Total radioactive residues in tissues following administration of [phenyl-^{14}C]-ethiprole to laying hens at 10 and 1 ppm in the diet

Sampling Time	Total Radioactive Residues (TRR)	
	10 ppm feed	1 ppm feed
	mg eq/kg	mg eq/kg
Abdominal fat	1.383	0.131
Breast muscle	0.128	0.012
Thigh muscle	0.203	0.026
Liver	1.290	0.119
Skin plus fat	0.896	0.088
Partially formed eggs	3.553	0.303

Pooled samples of excreta, liver, abdominal fat, skin and fat, breast muscle, thigh muscle, egg white (day 5 and 13) and egg yolk (day 5 and 13) from high and low dose groups were extracted using methanol (3×). Radioactivity levels in extracts quantified by LSC and radioactivity remaining in the post-extracted solids (PES) was quantified by combustion analysis and LSC. The solvent extracts were combined, concentrated under nitrogen and re-dissolved in methanol for HPLC analysis and isolation of metabolites. The PES samples were further extracted with ethyl acetate.

Extraction of residues with methanol at ambient temperature ranged from 87.7% of the TRR (breast muscle) to 99.5% TRR (abdominal fat) for the high dose. For the low dose the extraction efficiency ranged from 81.7% (liver) to 99.2% (abdominal fat).

The residue pattern in tissues and eggs is shown in Tables 34 to 39.

LC-MS analysis of extracts from tissues and organs showed ethiprole-sulfone RPA 097973 to be the major residue component, representing 54–91% of the TRR in high dose samples and 35–93% of TRR in the low dose samples. Parent ethiprole was only identified at a very low level (1.5–2.5% of TRR) in muscle of the low dose hens.

A major metabolite in liver co-chromatographed with the reference standard ethiprole sulfonic acid RPA 104615. This was thought to be the same peak identified in goat liver as being comprised of two metabolites, the ethiprole sulfonic acid RPA 104615 (co-chromatography) and a N-glucuronide of ethiprole-sulfide RPA 107566.

LC-MS analysis of sample extracts from liver, breast muscle, thigh muscle and abdominal fat and skin and fat confirmed a number of minor metabolites, i.e. ethiprole methyl sulfone (RPA 094569) in all tissues (0.9–3.2% of TRR); ethiprole-sulfone-hydroxide (RPA 114345 in all tissues, 0.5–6.7% of TRR), as well as RPA 097920 (MB 45897) in all tissues (0.8–3.1% of TRR), except breast muscle. None of the unidentified minor metabolites in liver, muscle, skin and fat represented more than 4.8% of TRR.

Egg yolk samples contained predominantly the sulfone metabolite RPA 097973 (49–72% TRR at days 5 and 13, low and high dose). Other compounds observed were parent ethiprole (2.6–7.9% TRR) and the minor metabolites ethiprole sulfonic acid (RPA 104615), ethiprole-sulfone-hydroxide RPA (114345), ethiprole methyl sulfone (RPA 094569), RPA 0979720 and the proposed sulfate conjugate of hydroxy-RPA 097920, all of them accounting for <10% of TRR.

In egg white, the main residue component was dihydroxy-RPA 097973 (38-53% TRR on days 5 and 13 and low and high dose, respectively), followed by ethiprole-sulfone (RPA 097973, 12% TRR, high dose) and ethiprole-amide RPA 112916 (19% TRR, low dose).

Table 34 Residue components in liver and muscle of hens 23 hours after the last of 14 daily doses of ^{14}C-Ethiprole at a high dose of 10 ppm feed (mean of 5 birds)

Compound	Liver	Breast muscle	Thigh muscle

	% of TRR	mg eq/kg	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Ethiprole-sulfonic acid RPA 104615/ N-glucuronide of RPA 107566	11.0	0.142	-	-	-	-
Ethiprole-amide RPA 112916	-	-	2.7	0.003	-	-
Ethiprole-sulfone-hydroxide RPA 114345	4.8	0.062	6.7	0.009	4.6	0.009
RPA 097920 = M&B 45897	2.1	0.027	-	-	1.0	0.002
Ethiprole (parent)	-	-	-	-	-	-
Ethiprole methyl sulfone RPA 094569	1.6	0.021	3.2	0.004	2.6	0.005
Ethiprole-sulfone RPA 097973	53.9	0.696	60.4	0.077	77.2	0.157
Total identified	73.5	0.948	73.0	0.093	85.4	0.173
Characterised (number of characterised)	13.6 (5)	0.176 (5)	7.2 (3)	0.010 (3)	2.7 (1)	0.006
Total extracted	88.5	1.14	87.7	0.112	91.1	0.185
Non extracted	11.5	0.148	12.3	0.016	8.9	0.018

Table 35 Residue components in skin and fat of hens 23 hours after the last of 14 daily doses of ^{14}C-Ethiprole at a high dose of 10 ppm feed (mean of 5 birds)

Compound	Abdominal fat		Skin and fat	
	% of TRR	mg eq/kg	% of TRR	mg eq/kg
Ethiprole-sulfone-hydroxide RPA 114345	0.5	0.006	1.2	0.011
RPA 097920 = M&B 45897	1.8	0.024	3.1	0.027
Ethiprole (parent)	-	-	-	-
Ethiprole methyl sulfone RPA 094569	1.9	0.026	2.2	0.019
Ethiprole-sulfone RPA 097973	91.0	1.25	78.0	0.699
Ethiprole-sulfide RPA 107566	0.63	0.009	0.9	0.008
Total identified	95.3	1.315	85.3	0.764
Characterised (number of characterised)	1.5 (2)	0.021	5.4 (3)	0.049
Total extracted	99.5	1.376	95.8	0.858
Non extracted	0.5	0.007	4.2	0.038

Table 36 Residue components in eggs of hens collected after 5 and 13 daily doses of ^{14}C-Ethiprole at a high dose of 10 ppm feed (mean of 5 birds)

Compound	Day 5 egg white		Day 13 egg white		Day 5 egg yolk		Day 13 egg yolk	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfonic acid RPA 104615/ N-glucuronide of RPA 107566	0.9	0.002	-	-	1.8	0.036	-	-
Sulfate of RPA 097920-OH	2.0	0.005	2.4	0.005	3.8	0.074	3.5	0.131
Ethiprole sulfone-amide RPA 112917	7.7	0.018	7.9	0.016	-	-	-	-
Ethiprole-sulfone-hydroxide RPA 114345	-	-	-	-	5.1	0.101	5.5	0.207
RPA 097920 = MB 45897	1.6	0.004	1.7	0.003	1.2	0.024	1.1	0.041
Ethiprole (parent)	-	-	-	-	3.6	0.071	2.6	0.097
Ethiprole methyl sulfone	-	-	-	-	1.8	0.035	1.7	0.066

Compound	Day 5 egg white		Day 13 egg white		Day 5 egg yolk		Day 13 egg yolk	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
RPA 094569								
Ethiprole-sulfone RPA 097973	12.2	0.029	11.5	0.024	72.2	1.422	70.9	2.687
Dihydroxy of RPA 097973	50.5	0.121	52.8	0.109	-	-	-	-
Total identified	74.8	0.179	76.3	0.157	89.5	1.763	85.2	3.229
Characterised (number of characterised)	13.9 (3)	0.034	15.9 (3)	0.033	2.75 (1)	0.054	5.3 (1)	0.201
Total extracted	98.1	0.234	96.5	0.200	92.7	1.826	91.0	3.449
Non extracted	1.9	0.005	3.5	0.007	7.3	0.144	9.0	0.341

Table 37 Residue components in liver and muscle of hens 23 hours after the last of 14 daily doses of ^{14}C-Ethiprole at a low dose of 1 ppm feed (mean of 5 birds)

Compound	Liver		Breast muscle		Thigh muscle	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfonic acid RPA 104615/ N-glucuronide of RPA 107566	11.7	0.014	-	-	-	-
Ethiprole-amide RPA 112916	-	-	0.3	<0.001	-	-
Ethiprole-sulfone-hydroxide RPA 114345	1.4	0.002	1.8	<0.001	3.3	0.001
RPA 097920 = MB 45897	0.8	0.001	-	-	-	-
Ethiprole (parent)	-	-	1.5	<0.001	2.5	<0.001
Ethiprole methyl sulfone RPA 094569	0.9	0.001	1.8	<0.001	1.9	<0.001
Ethiprole-sulfone RPA 097973	34.9	0.042	58.4	0.007	73.2	0.019
Total identified	49.7	0.060	63.8	0.007	80.9	0.022
Characterised (number of characterised)	10.9 (5)	0.012	4.4 (5)	<0.001	6.4 (4)	0.002
Total extracted	81.7	0.097	82.1	0.010	91.7	0.024
Non extracted	18.3	0.022	17.9	0.002	8.3	0.002

Table 38 Residue components in skin and fat of hens 23 hours after the last of 14 daily doses of ^{14}C-Ethiprole at a low dose of 1 ppm feed (mean of 5 birds)

Compound	Abdominal fat		Skin and fat	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfone RPA 097973	92.5	0.121	88.4	0.078
Total identified	92.5	0.121	88.4	0.078
Characterised (number of characterised)	-	-	-	-
Total extractable	99.2	0.130	96.6	0.085
Non extracted	0.8	0.001	3.4	0.003

Table 39 Residue components in eggs of hens collected after 5 and 13 daily doses of ^{14}C-Ethiprole at a low dose of 1 ppm feed (mean of 5 birds)

Compound	Day 5 egg white		Day 13 egg white		Day 5 egg yolk		Day 13 egg yolk	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Ethiprole-sulfonic acid RPA 104615/ N-glucuronide of RPA 107566	-	-	-	-	1.1	0.002	-	-
Ethiprole-sulfone-carboxy RPA 112705	5.6	0.001	-	-	-	-	-	-

Compound	Day 5 egg white		Day 13 egg white		Day 5 egg yolk		Day 13 egg yolk	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Sulfate of RPA 097920	4.8	0.001	7.3	0.001	2.7	0.004	4.2	0.013
Ethiprole-amide RPA 112916	-	-	18.9	0.003	-	-	-	-
Ethiprole-sulfone-hydroxide RPA 114345	-	-	-	-	4.3	0.006	6.4	0.020
Ethiprole, (parent)	-	-	-	-	4.3	0.006	7.9	0.024
Ethiprole methyl sulfone RPA 094569	-	-	-	-	0.9	0.001	-	-
Ethiprole-sulfone RPA 097973	-	-	1.0	<0.001	68.8	0.104	48.7	0.148
Dihydroxy of RPA 097973	38.4	0.006	47.2	0.009	-	-	-	-
Total identified	48.8	0.008	74.4	0.013	82.1	0.123	67.2	0.205
Characterised (number of characterised)	33.3 (4)	0.006	11.1 (4)	0.004	5.0 (1)	0.007	8.1 (4)	0.025
Total extracted	95.6	0.014	98.2	0.016	89.4	0.135	86.9	0.264
Non extracted	4.4	<0.001	1.8	<0.001	10.6	0.016	13.1	0.040

The main metabolic reactions of ethiprole in the laying hen are: 1) oxidation of the sulfoxide group of ethiprole to the sulfone to form RPA 097973 detected in all samples as a major residue component; 2) oxidation (hydrolysis) of the nitrile group of the parent ethiprole to the amide metabolite RPA 112916; and 3) reduction of the sulfoxide group of ethiprole to a sulfide function with formation of RPA 107566, which was conjugated at the primary amine with glucuronic acid to produce N-glucuronide of RPA 107566 or which was alkyl oxidised to form ethiprole sulphide carboxy (RPA 112716) which was only detected in the excreta.

The major metabolite ethiprole-sulfone, was further metabolised *via* four different possible routes: 1) alkyl hydroxylation to form ethiprole-sulfone-hydroxide (RPA 114345), which was further hydroxylated to dihydroxy-RPA 097973 or further oxidised to RPA 112705 (only in egg white). RPA 112705 is suggested to be decarboxylated to form RPA 094569; 2) oxidative dealkylation to form ethiprole-sulfonic acid RPA 104615, which was further metabolised to the intermediate hydroxy-MB 45897 (hydroxy-RPA 097920) by substitution of the sulfate function with a hydroxy group. The latter one was dehydroxylated to RPA 097920 (MB 45897) or conjugated with sulfuric acid to form the corresponding sulfate conjugate; 3) hydroxylation of the nitrile group to an amide function to form ethiprole-sulfone-amide RPA 112917; and 4) demethylation of RPA 097973 to form ethiprole methyl sulfone RPA 094569 (can also be formed *via* decarboxylation of RPA 112705. RPA 094569 is supposed to be metabolised to sulfate metabolite RPA 104615 (minor pathway).

The pattern of observed residues in hen liver, meat, fat and egg yolk revealed the sulfone metabolite RPA 097973 as the main residue component. In egg white, the dihydroxy-RPA 097973 metabolite proved to be the main residue component. The proposed metabolic pathway is shown in Figure 9.

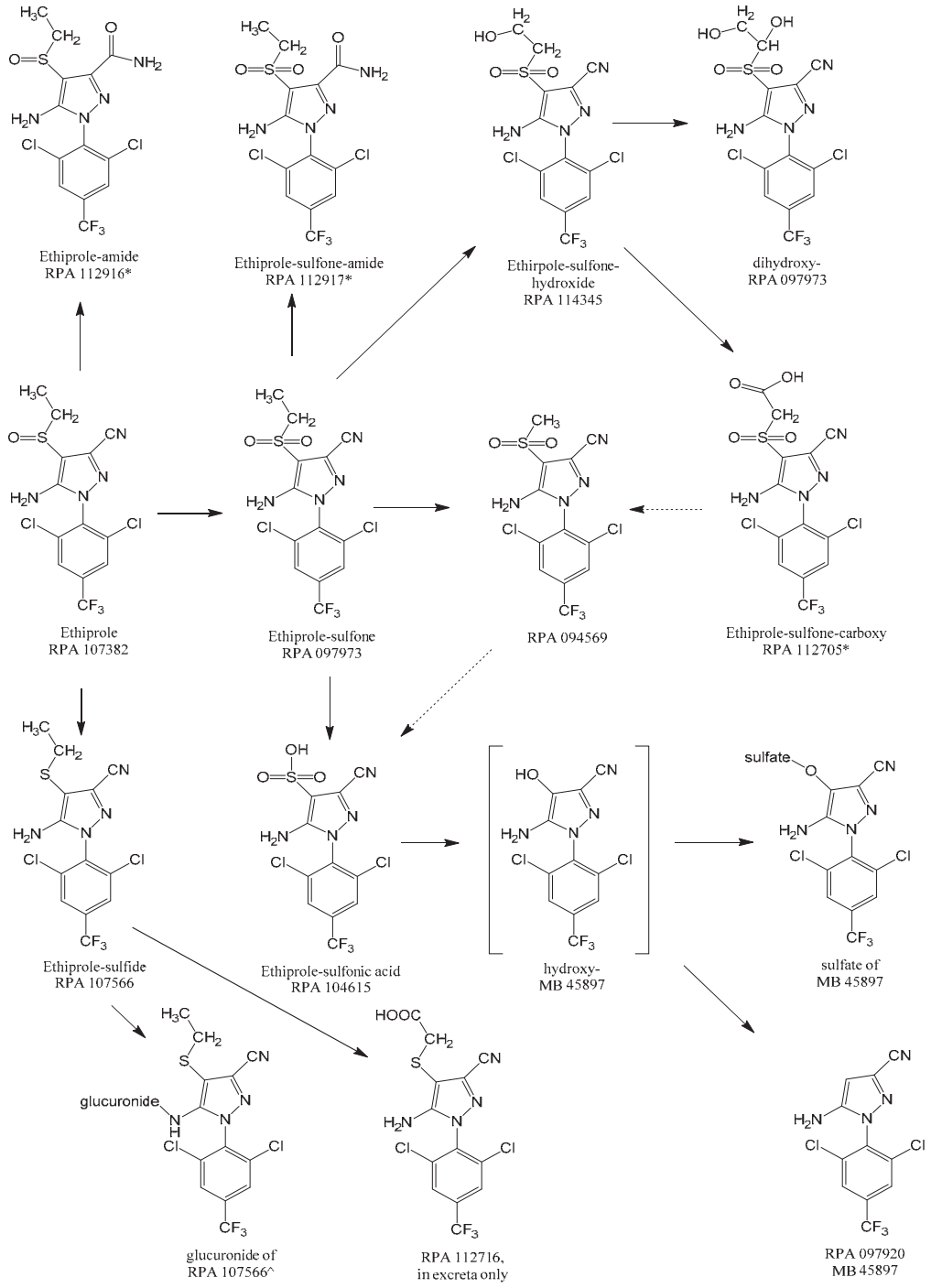


Figure 9 Proposed metabolic pathway for ethiprole in poultry

Summary of animal metabolism

The Meeting received animal metabolism studies with ethiprole in rats, laying hens and lactating goats.

Analysis of the excreted radioactivity in rats indicated that orally administered [^{14}C]-Ethiprole was extensively metabolised. Levels of metabolites were low. However, in the proposed rat metabolism pathway, there were three identified breakdown pathways, each starting with either ethiprole-sulfone (RPA 097973), ethiprole-amide (RPA 112916), or ethiprole-sulfide (RPA 107566). The major components were the polar glucuronide conjugate of hydroxy-MB 45897, the sulfonic acid, as well as the less polar (non-conjugated) MB 45897 and in female urine ethiprole-sulfone-carboxy. The excreted radioactive components could be identified to a proportion of approximately 65 or 80% of the administered radioactivity at the low or high dose level.

In both laying hen and lactating goat the majority of the administered dose is rapidly excreted. The metabolism of ethiprole in poultry and ruminants shows a comparable metabolite profile. Ethiprole was extensively metabolised in both the goat and the hen, which proceeds *via* oxidation, reduction, and hydrolysis followed by additional metabolism pathways such as conjugation.

Ethiprole was extensively metabolised in the goat. Ethiprole-sulfone was the major residue component, representing 32–79% of the TRR in liver, kidney, muscle, fat and milk. Parent ethiprole was identified in kidney (4% TRR in the high dose goat only), muscle (10–17% TRR), renal (9–17% TRR) and omental fat (10–15% TRR) and milk (18–29% TRR). A major metabolite in liver and kidney co-chromatographed with ethiprole-sulfonic acid (RPA 104615), but was not confirmed by LC-MS; further MS analysis indicated that this peak co-chromatographed with the N-glucuronide of ethiprole-sulfide (RPA 107566) (total 13–18% TRR in liver, 20–26% TRR in kidney).

Metabolism of ethiprole in poultry showed a comparable metabolite profile. Ethiprole-sulfone was the major residue component in tissues, representing 35–93% of TRR in liver, breast and thigh muscle, abdominal fat and skin and fat. Parent ethiprole was only identified at a very low level (2–3% of TRR) in muscle of the low dose hens. A major metabolite in liver (11–12% TRR) co-chromatographed with the reference standard ethiprole-sulfonic acid. This was thought to be the same peak identified in goat liver as being comprised of the two metabolites, ethiprole-sulfonic acid (co-chromatography) and a N-glucuronide of ethiprole-sulfide.

Egg yolk samples contained predominantly the sulfone metabolite (49–72% TRR) while parent ethiprole was present at 3–8% TRR. In egg white, the main residue component was dihydroxy-RPA 097973 (38–53% TRR on days 5 and 13 and low and high dose, respectively). This compound was not present in egg yolks or in any tissues. Ethiprole-sulfone (12% TRR, high dose) and ethiprole-amide RPA 112916 (19% TRR, low dose) were also present.

Environmental fate

The Meeting received information on soil photolysis, the route and rate of aerobic metabolism (degradation) of ethiprole and ethiprole-sulfone, field dissipation, confined rotational crops, hydrolysis, phototransformation in sterile and natural water, phototransformation of ethiprole-sulfone and ethiprole-sulfide in sterile water, degradation in aerobic and anaerobic water sediment systems and fate in paddy soil under field conditions. Only those studies relevant to the current evaluation are reported here.

Phototransformation in sterile water

The aqueous phototransformation of [U-^{14}C-phenyl]-ethiprole was studied at an initial concentration of 3.0 mg/L in pH 5 buffer at 25 ± 1 °C (Corgier and Turier 2002, M-192004-02-1). Acetonitrile as a co-solvent was at a concentration of 1%. Light was provided by a Xenon lamp with wavelengths <290 nm filtered out. Duplicate samples were removed after 0, 3, 5, 8, 12.3 and 16 hours of irradiation. Dark control samples were also incubated for 16 hours. For each individual sample, the radioactivity balance ranged from 94–101% of the initial applied radioactivity. Samples were analysed by HPLC.

In the irradiated samples, ^{14}C-ethiprole showed a decrease from 100% applied radioactivity (AR) at time zero to approximately 19% after 16 hours. No degradation of ethiprole was observed in the dark controls. Ethiprole is quickly photodegraded in an aqueous medium. Its half-life (DT_{50}) = 6.46 hours for irradiation under the Xenon lamp, calculated by applying a simple first-order kinetic model, corresponds to 1.3 days of summer sunlight in Florida, so sunlight was considered to be an important route of ethiprole degradation in an aqueous environment.

The metabolic pathway resulting from photolytic degradation in sterile aqueous buffer is shown in Figure 10. Except for RPA 157925, all metabolites shown below were only tentatively identified with LC-MS.

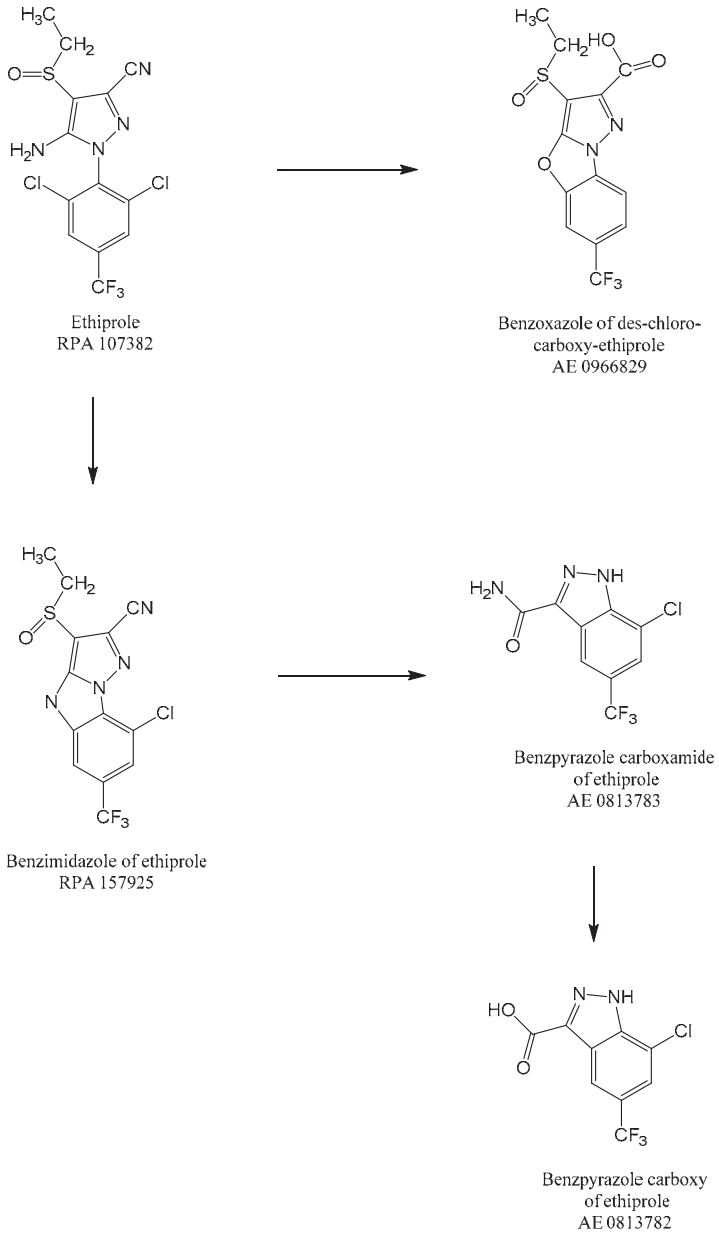


Figure 10 Proposed metabolic pathway for the photolysis of ethiprole in sterile aqueous buffer solution

Phototransformation in natural water

The phototransformation of [^{14}C-phenyl]-ethiprole was studied in natural (pond) water collected from a pond system (Ormalingen BL in Switzerland) at a depth of 10–20 cm below the surface (van der Gaauw 2002, M-210934-02-1). Simulated sunlight from a xenon arc lamp, with filters to remove wavelengths below 290 nm and having an intensity of 52.0 W/m^2 within the 300–400 nm range of the spectrum, was used to irradiate a 4.35 mg/L solution of [^{14}C]-ethiprole in pond water maintained at 25.0 ± 0.2°C. Irradiation was for a continuous period of up to 4 days with a corresponding control sample maintained under the same conditions but in the dark. Samples were taken for analysis at a range of time intervals up to 4 days (irradiated) and 2 days (dark control) with radiochemical quantification by LSC and chromatographic analysis by HPLC. The mean recoveries during the study were 96.9% ± 2.2% of the AR and 97.6% ± 1.7% for the irradiated and dark control samples respectively.

The concentration of the test item declined rapidly in irradiated pond water, with ethiprole repesenting 75 and 35% AR after 2 and 8 hours of irradiation respectively. At the end of irradiation (day 4) it accounted for 2.2% AR. Ethiprole was rapidly

degraded to form up to 21 photodegradation products. The two main products were identified by LC/MS, as well as by HPLC and TLC, as the benzimidazole of ethiprole (RPA 157925) and the benzimidazole of des-chloro-hydroxy-ethiprole (AE 0764815) respectively. Already present after 2 hours of irradiation, RPA 157925 and AE 0764815 rapidly increased to reach maximum levels of 33% and 47% AR after 8 hours and 1 day of irradiation, respectively. These major photolysis products were then rapidly degraded to several highly polar molecules (<12% AR), that were not well resolved by HPLC.

Mineralisation of ethiprole and its degradation products was high in the irradiated samples, with radioactive carbon dioxide accounting for a maximum of 15% AR at the end of irradiation (day 4). No degradation of ^{14}C-ethiprole was observed in the dark control samples.

The experimental photolytic DT_{50} of ^{14}C-ethiprole was calculated to be 0.2 days (1.3 spring sunlight days in Tokyo, Japan). The experimental photolytic DT_{50} of the major metabolites was 0.4 days (RPA 157925) and 0.9 days (AE 0764815).

It was concluded that direct photodegradation in aqueous solution is expected to contribute to the elimination of ethiprole in the aquatic environment, particularly in shallow surface water where significant levels of UV light can penetrate. A proposed photolytic pathway in natural water is shown in Figure 11.

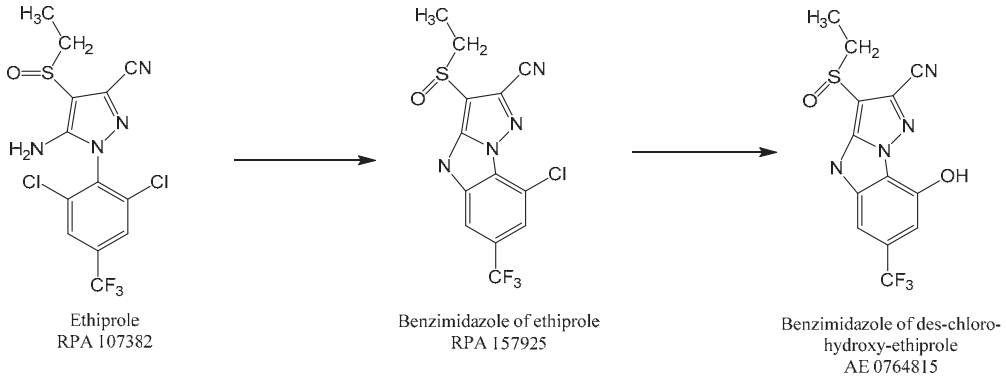
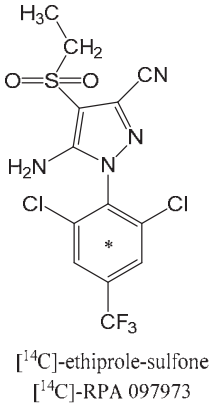


Figure 11 Proposed metabolic pathway for the photolysis of ethiprole in sterile natural water

Phototransformation of [^{14}C]-RPA 097973 (ethiprole-sulfone) in sterile water

The aqueous phototransformation of [^{14}C]-RPA 097973 (ethiprole-sulfone) was studied at an initial concentration of 3.75 mg/L by dissolving the test item in acetonitrile and diluting with sterile pH 5 buffer at 25 ± 1 °C (Keirs 2001a, M-199198-01-1). Light was provided by a xenon lamp with wavelengths <290 nm and > 800 nm filtered out. Duplicate samples were removed after 0, 6, 12, 24, 48 and 72 hours of irradiation. Dark control samples were also incubated for and sampled over 72 hours.



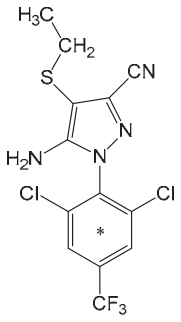
Overall recoveries for the irradiated samples were in the range of 95–103% (mean = 100%) and radioactivity in the incubates accounted for 76–102% (mean = 97%). Overall recoveries for the dark control samples were in the range of 99–101% (mean = 100%) and radioactivity recovered in the incubates accounted for 97–100% (mean = 99%).

HPLC analysis demonstrated that [^{14}C]-RPA 097973 was rapidly photodegraded over the irradiation period. At 0 hours, RPA 097973 quantitatively accounted for the radioactivity present. As the incubation progressed, levels of parent sulfone material declined with only low levels of parent evident in the study termination samples (3%). Levels of an unknown component and polar material increased as the irradiation period progressed and accounted for approximately 54 and 42% of the applied radioactivity in study termination samples. Another unknown component was detected at intervals throughout the irradiation period and appeared to be an intermediate in the photodegradation process. Only parent material was observed in the dark control samples.

[^{14}C]-RPA 097973 was shown to be photolytically unstable in buffered aqueous solution (pH 5). The rate of photodegradation of [^{14}C]-RPA 097973 in buffered aqueous solution was calculated from the HPLC data using linear regression assuming first order kinetics. The half-life (DT_{50}) and the corresponding DT_{90} value were 14.9 hours and 49.4 hours respectively.

Phototransformation of [^{14}C]-RPA 107566 (ethiprole-sulfide) in sterile water

The aqueous phototransformation of [^{14}C]-RPA 107566 (ethiprole-sulfide) was studied at an initial concentration of 0.75 mg/L by dissolving the test item in acetonitrile and diluting with sterile pH 5 buffer at 25±1 °C (Keirs 2001b, M-199196-01-1). Light was provided by a xenon lamp with wavelengths <290 nm and > 800 nm filtered out. Duplicate samples were removed after 0, 1.5, 3, 6, 12 and 24 hours of irradiation. Dark control samples were also incubated for and sampled over 24 hours.



[^{14}C]-ethiprole-sulfide
[^{14}C]-RPA 107566

Overall recoveries for the irradiated samples were in the range of 99–104% (mean = 102%) while radioactivity in the incubates accounted for 97–103% (mean = 101%). Overall recoveries for the dark control samples were in the range of 103–105% (mean = 104%) while radioactivity recovered in the incubates accounted for 102–104% (mean = 103%).

HPLC analysis demonstrated that [^{14}C]-RPA 107566 was rapidly photodegraded over the irradiation period. At 0 hours in the main study, RPA 107566 quantitatively accounted for the radioactivity present. As the irradiation period progressed, levels of parent material declined with approximately 17% of the radioactivity characterised as parent at 12 hours. No parent was evident in the 24 hour study termination samples. Levels of three unknown components and polar material increased as the irradiation period progressed and accounted for 18, 29, 9 and 41% of the applied radioactivity respectively at 24h. Another unknown component was detected at intervals throughout the irradiation period and appeared to be an intermediate in the photodegradation process. Only parent material was observed in the dark control samples.

[^{14}C]-RPA 107566 was shown to be photolytically unstable in buffered aqueous solution (pH 5). The rate of photodegradation of [^{14}C]-RPA 107566 in was calculated from the HPLC data using linear regression assuming first order kinetics. The half-life (DT_{50}) and the corresponding DT_{90} value were 4.6 hours and 15.1 hours respectively.

Degradation in water-sediment systems (aerobic conditions)

The degradation of ethiprole was studied under aerobic conditions in two different UK (Ongar and Manningtree, Essex; Oddy and Doble 1999, M-192511-02-1) and one USA (Clayton, North Carolina; Jesudason and Mackie 2002, M-192578-02-1) water-sediment systems.

In general, ethiprole was degraded by one major pathway, reduction of the sulfoxide group to the sulfide, RPA 107566, with up to 80% of the applied at study end in the Ongar water-sediment system at day 100. Oxidation of the sulfoxide group to the corresponding sulfone RPA 097973 also occurred in maximum quantities of 15% of the applied at day 100, also in the Ongar water-sediment system. Under aerobic aquatic conditions, ethiprole is rapidly transferred from the water to the sediment where it is reduced *via* the sulphoxide group to one major metabolite RPA 107566 which is primarily present in the sediment together with minor amounts of RPA 097973. No significant amounts of $^{14}CO_2$ or other volatiles were observed in any test system.

Oddy and Doble 1999, M-192511-02-1 (2 UK water-sediment systems, 20 °C, 100 days)

The degradation of [^{14}C]-ethiprole, applied at a rate equivalent to 0.648 kg ai/ha, was studied in two different water-sediment systems over a period of 100 days at 20 ± 2 °C in the dark. The incubation was performed in glass flasks containing sediment to associated water in an average ratio of 1:7.5 (Manningtree) and 1:6 (Ongar). The water-sediment systems were incubated for approximately 4 weeks to enable acclimatisation, prior to [^{14}C]-ethiprole application to the surface of the water. The incubation flasks were attached to a system where moist air was passed into the water layer in each flask at a constant rate and the effluent air passed through an ethylene glycol trap to capture liberated volatiles and through two potassium hydroxide traps to trap any evolved CO_2.

Good radiochemical balances were obtained for both water-sediment systems with an overall mean recovery of 104% (range 98–108%) for the Manningtree system and 106% (range 101–111%) for the Ongar system. No significant volatiles products were formed (<0.2% of applied). Unextracted residues remained below 8% of the applied radioactivity.

In both water-sediment systems ethiprole steadily transferred from the water phase to the sediment phase. The radioactivity recovered from the water phase declined from slightly greater than 100% at time zero to approximately 12–15% of the applied radioactivity at day 100. At the same time the radioactivity recovered from the sediment increased from zero at time zero to 84 and 90% in the two sediments at the end of the study.

In both water-sediment systems, on transfer to the sediment, ethiprole was readily reduced to the major metabolite ethiprole-sulfide RPA 107566 (mean values of 77% and 72% of applied radioactivity at 100 days in each system, total and sediment, respectively). The oxidised metabolite ethiprole-sulfone (RPA 097973) was gradually transferred from the water phase to the sediment (mean values 9% and 11% of applied radioactivity in the systems at 100 days). Less than 3% of ethiprole was found in the sediment at 100 days. Other minor metabolites were found but never reached more than 2% of applied radioactivity. In the water, ethiprole was rapidly degraded to less than 4% of applied radioactivity by 100 days in both systems; six minor metabolites were seen but none of these exceeded 10% of the applied radioactivity during the study except for ethiprole-sulfide which reached a mean value of 11% in the Ongar system at 14 days but had declined to 3% by 100 days.

Jesudason and Mackie 2002a, M-192578-02-1 (1 USA water-sediment system, 20 °C, 12 months)

The degradation of [^{14}C]-ethiprole, applied as a single application to the surface water in a flask at a rate equivalent to 0.52 kg ai/ha, was studied over a period of 12 months at 20 ± 1 °C in the dark. The incubation was performed in glass flasks containing sediment to water in an average ratio of 1:4 (w/w). The water-sediment systems were acclimatised for 20 days. Moistened air was supplied under positive pressure on the surface of the water in each unit. The effluent air passed through ethylene glycol and 2-ethoxyethanol: monoethanolamine (2:1 v/v) to trap organic volatiles and liberated CO_2 respectively. Duplicate samples were analysed at intervals of 0, 3, 7 and 14 days and 1, 2, 3, 6, 9 and 12 months after application.

The average material balance of ^{14}C-radioactivity ranged from 91–107% of the applied dose with an overall mean recovery of 95%. Volatiles accounted for less than 1% of applied radioactivity at all sampling intervals. The radioactivity extracted from the sediment increased throughout the study as the radioactive content of the sediment increased. The unextractable residues as determined by combustion after the solvent extractions were at or below 5% of applied radioactivity at all sampling intervals.

Ethiprole-sulfide (RPA 107566) was the most substantial metabolite in both water and sediment throughout the study. The concentration of RPA 107566 in the total system increased from 14% of the initial application at day 3 to 80% of the applied dose at 1 month and then slowly declined to 41% at 12 months. Ethiprole-sulfone (RPA 097973) in the total system was initially detected from 2 to 12 months and the levels detected ranged from 1 to 9% of the applied dose.

The dissipation of ethiprole in water was rapid. At 14 days after application ethiprole represented less than 10% of the applied radioactivity in water and <4% in the sediment. Throughout the test, ethiprole was detected in both the water and sediment phases. At 12 months, approximately 2% was detected in water and 7% was detected in sediment. The rate of dissipation of ethiprole in water and the rate of degradation of ethiprole in the sediment and total system was calculated using the data obtained from the first 14 days of the test.

The half-life DT_{50} and DT_{90} values for ethiprole in the water and the water-sediment for the two UK systems and one USA system were calculated and are shown in Table 40.

Table 40 Best fit DT_{50} and DT_{90} values for the dissipation from water and degradation in total water-sediment systems (aerobic) of ethiprole at 20 °C

Program	Model	Applied to degradation in	DT_{50} [days]	DT_{90} [days]	Reference
Manningtree, Essex [loam, pH 6.0 ($CaCl_2$), OC 5.6%]	Power rate (KIM program)	Water	12.0	68.1	Oddy and Doble 1999, M-192511-02-1
		Water + sediment	16.1	86.1	
	First-order kinetics	Water	21.1	70.1	
		Water + sediment	23.7	78.7	

Ongar, Essex, UK [clay loam, pH 7.2 ($CaCl_2$), OC 4.4%]	Power rate (KIM program)	Water	13.5	57.4	
		Water + sediment	15.0	67.5	
	First-order kinetics	Water	18.5	61.6	
		Water + sediment	20.3	67.6	
Clayton, North Carolina, USA [loamy sand, pH 5.7, OC 1.8%]	First-order kinetics	Water	4	14	Jesudason and Mackie 2002, M-192578-02-1
		Sediment	11	38	
		Water + sediment	5	16	

It is concluded that ethiprole is not likely to persist in an aerobic aquatic environment. The degradation is mainly through oxidation of the parent to the sulfone and reduction of the parent to the sulfide. The metabolic pathway for ethiprole in a water-sediment system under aerobic conditions in depicted below.

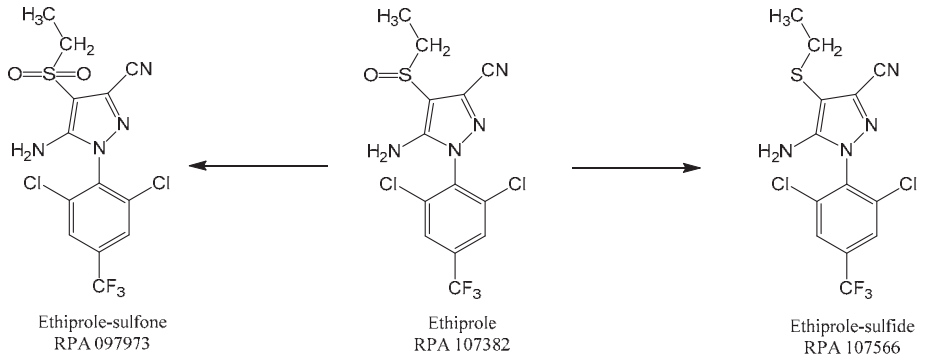


Figure 12 Proposed metabolic pathway for ethiprole in a water-sediment system under aerobic conditions

Degradation in water-sediment systems (anaerobic conditions)

Degradation of ethiprole was studied in water-sediment systems under anaerobic conditions (Jesudason and Mackie 1999, M-192563-01-1 and Zheng 2011, M-436141-01-1). In general ethiprole was degraded to only one major product, the sulfide of ethiprole (RPA 107566), which accounted for up to 86% of the applied dose at day 14, before declining to 56% of applied radioactivity at study end.

Jesudason and Mackie 2002b, M-192563-01-1 (1 USA water-sediment system, 20 °C, 12 months)

The degradation of [^{14}C]-ethiprole, applied as a single application to the surface water in a flask at a rate equivalent to 0.52 kg ai/ha, was studied over a period of 12 months at 20 ± 2.5 °C. The incubation was performed in glass flasks containing sediment to water in an average ratio of 1:4 (w/w). The flasks were maintained in the dark under anaerobic conditions in a growth chamber. The water-sediment systems were acclimatised for 20 days. Moistened nitrogen was supplied under positive pressure into the surface of the water in each unit. The effluent nitrogen passed through ethylene glycol and 2-ethoxyethanol: monoethanolamine (2:1, v/v) to trap organic volatiles and liberated CO_2 respectively. Duplicate samples were analysed at intervals of 0, 3, 7 and 14 days and 1, 2, 3, 6, 9 and 12 months after application.

The average material balance of ^{14}C-radioactivity ranged from 90–106% of the applied dose with an overall mean recovery of 96%. Volatiles accounted for less than 1% of applied radioactivity at all sampling intervals. There was a gradual transfer of the radioactivity from the water to the sediment. The average radioactivity in the water decreased from 85% at 0 time to 4% at 12 months while the sediment radioactivity increased from 21% to 93% during the same period. The radioactivity extracted from the sediment increased throughout the study. The unextractable residues as recovered by combustion after the solvent extractions were ≤ 4% of applied radioactivity at all sampling intervals.

Ethiprole-sulfide (RPA 107566) was the only significant metabolite in both water and sediment throughout the study. The concentration of RPA 107566 in the total system increased from 48% of the initial application at day 3 to 86% of the applied dose at 14 days and then slowly declined to 56% at 12 months.

The dissipation of ethiprole in water was very rapid. At 7 days after application ethiprole represented about 11% of the applied radioactivity in water and <1% in the sediment. Less than 1% of parent was detected in the one and six month water samples. In the sediment after one month ≤3% of parent was seen at each sampling except at 9 months (13%).

The rate of dissipation of ethiprole in water and the rate of degradation of ethiprole in the sediment and total system was calculated using the data obtained from the first 7 days of the test.

Zheng 2011, M-436141-01-1 (1 China water-sediment system, 25 °C, 9 days)

A dissipation study was carried out in a Chinese water-sediment system under anaerobic conditions. The water sediment system (water: sediment = 3:1 v/v) was pre-cultivated at 25±1°C in darkness for 14 days before being treated to achieve a final concentration of 10.0 mg/kg ethiprole in water phase. Nitrogen was introduced to maintain anaerobic conditions during the study period. Sampling intervals for duplicate samples for sediment were 0, 0.25, 0.5, 1, 2, 3, 5, 7 and 9 days after application.

The concentration of ethiprole in the water phase decreased with time and the degradation percentage at day 9 was 99.5%. In the sediment phase, the concentration of ethiprole gradually increased within the first 3 days after the incubation and then started to decrease and the degradation percentage at 9 days was 98%. The degradation percentage for the whole system was 99%.

The half-life DT_{50} and DT_{90} values for ethiprole in the water, sediment and the water-sediment for the USA and China systems were calculated and are shown in Table 41.

Table 41 Best fit DT_{50} and DT_{90} values for the dissipation from water and degradation in total water-sediment systems (anaerobic) of ethiprole at 20 °C

Program	Model	Applied to degradation in	DT_{50} [days]	DT_{90} [days]	Reference
Clayton, North Carolina, USA [loamy sand, pH 5.7, OC 1.8%]	First-order kinetics	Water	2	8	Jesudason and Mackie 1999, M-192563-01-1
		Sediment	2	7	
		Water + sediment	2	8	
Shangzhuang, Beijing, China [loamy sand, pH 7.9 (water), OC 2.3%]	First-order kinetics	Water	1.5	-	Zheng 2011, M-436141-01-1
		Sediment	1.7	-	
		Water + sediment	1.6	-	

It was concluded that ethiprole is unlikely to persist in an anaerobic aquatic environment. The metabolic pathway for ethiprole in a water-sediment environment under anaerobic conditions is depicted below.

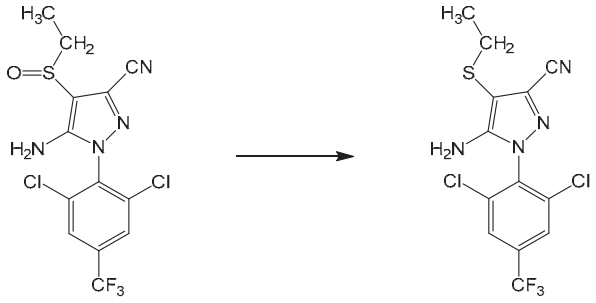


Figure 13 Proposed metabolic pathway for ethiprole in a water/ sediment system under anaerobic conditions

Fate in paddy soil under field conditions

A number of field (Garside *et al.*, 2009, M-356426-02-1; Garside *et al.*, 2009, M-356427-02-1) and semi-field (Garside and Odanaka 2009, M-356423-02-1; Garside *et al.*, 2009, M-356470-02-1) studies were conducted in Japan to address the possibility that ethiprole and its metabolites could be persistent under field conditions and hence have the potential to accumulate in rice paddy fields, following repeated application of ethiprole over successive growing seasons.

The dissipation of ethiprole and its metabolites ethiprole-sulfone (RPA 097973) and ethiprole-sulfide (RPA 107566), were monitored in all studies, while ethiprole-benzimidazole (RPA 157925), AE0764815, ethiprole-amide (RPA 112916) and ethiprole-sulfone-amide (RPA 112917) were monitored in some of the studies. The dissipation behaviour in sediment was assessed in field studies conducted in rice paddy fields in Japan with a foliar application of an ethiprole flowable formulation or a submerged application of an ethiprole granule formulation. Additionally, the dissipation in water was determined in semi-field studies in Japan

following foliar application or submerged application. The target compounds were selected due to their occurrence in laboratory studies with ethiprole.

Trials conducted at two rice paddy field plots in different locations in Japan by the Japanese Plant Protection Research Institute (JPPA Research and JPPA Kochi)

The dissipation in paddy soil, of ethiprole and potential metabolites following *foliar* application to rice was investigated under field conditions (Garside *et al.,* 2009, M-356426-02-1). The target compounds for analysis were ethiprole, RPA 097973, RPA 107566, RPA 112916, RPA 112917 and RPA 157925. Ethiprole was applied to transplanted rice plants in two different field plots in different locations in Japan with different underlying paddy soils. Two applications were made of a diluted spray solution of a suspension concentrate (10% w/w flowable concentrate) containing 110 g/L ethiprole, at 200g ai /ha, at a 7-day retreatment interval.

Paddy soil samples were collected from each system before application 1 and at 0, 7, 14, 30, 60, 150, 210 and 270 days after treatment 2 and analysed for the target compounds by HPLC with UV-detection. The limit of quantification for all compounds was 0.01 mg/kg. Ethiprole residues dissipated very rapidly following application with a half-life of ethiprole estimated to be approximately 4 days in both test systems. The maximum concentration of ethiprole was 0.12 mg/kg (JPPA research) and 0.10 mg/kg (JPPA Kochi) on the day of second treatment. The half-life for the total ethiprole residues was estimated to be 54 days in the JPPA research system and 5.4 days in the JPPA Kochi test. The maximum concentration of ethiprole residues (sum of ethiprole, RPA 097973 and RPA 107566) was 0.33 mg/kg (0.35 mg/kg including RPA157925) at JPPA Research and 0.17 mg/kg at JPPA Kochi, the residues declined to 0.09 mg/kg (26%) and 0.03 mg/kg (18%) at the final sampling point. The metabolites were detected at low concentrations.

Table 42 Summary of ethiprole residues in paddy soil following foliar application

	Concentration (mg eq/kg)						
Time after 2nd application	Ethiprole	RPA 097973	RPA 107566	RPA 157925	RPA 112916	RPA 112917	Total residues (ethiprole eq.)
JPPA Research							
0	0.12	0.01	0.2	0.02	<0.01	<0.01	0.35
7	0.02	0.01	0.16	0.02	<0.01	<0.01	0.22
14	0.02	0.02	0.19	0.03	<0.01	<0.01	0.27
30	0.02	0.01	0.2	0.01	<0.01	<0.01	0.25
60	0.01	0.02	0.11	0.01	<0.01	<0.01	0.15
90	0.03	0.03	0.02	0.01, <0.01	<0.01	<0.01	0.08
150	0.04	0.06	0.02	0.01	0.01, <0.01	<0.01	0.13
216	0.01	0.03	0.01	<0.01	<0.01	<0.01	0.05
270	0.03	0.05	0.01	<0.01	<0.01	<0.01	0.09
JPPA Kochi							
0	0.10	0.01	0.06	<0.01	<0.01	<0.01	0.17
7	0.01	0.01	0.04	0.01	<0.01	<0.01	0.07
14	<0.01	<0.01	0.05	<0.01	<0.01	<0.01	0.05
30	<0.01	<0.01	0.04	<0.01	<0.01	<0.01	0.04
59	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	0.02
91	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	0.02
150	0.01	<0.01	0.02	<0.01	<0.01	<0.01	0.03
210	0.01, <0.01	0.01, <0.01	0.01	<0.01	<0.01	<0.01	0.01
270	<0.01	0.01	0.01	<0.01	<0.01	<0.01	0.02

The dissipation in paddy soil of ethiprole and potential metabolites following *submerged* application of a 1.5% w/w granular formulation to rice was investigated under field conditions (Garside *et al.,* 2009, M-356427-02-1). The target compounds for analysis were ethiprole, RPA097973 and RPA107566. Ethiprole was applied in two rice paddy field plots (JPPA Research and JPPA Kochi).

Ethiprole 1.5% GR, formulated as a granule 1.5% w/w product containing 15g ethiprole / kg, was applied once as a submerged application (water depth approximately 10 cm) 6 to 10 days before heading of the rice plants. A mixture of an equal volume of dry paddy soil and the test item was prepared and applied manually. The application rate was 600 g ai/ha at both sites.

Paddy soil samples (to 10 cm depth) were collected from each system shortly before treatment and at 0, 7, 14, 30, 90 and 181 days after treatment. Additional samples were collected from JPPA Research at 60 and 120 days and at JPPA Kochi at 58, 121 and 240 days after treatment. Soil samples were analysed for the target compounds by HPLC with LC/MS detection. The limit of quantification for all compounds was 0.01 mg/kg for ethiprole and RPA 090973 and 0.009 mg/kg for RPA 107566.

Ethiprole dissipated rapidly following application with a half-life of ethiprole estimated to be approximately 1.8 days at the JPPA Research site and at the JPPA Kochi site. The maximum concentration of ethiprole was 1.86 mg/kg at JPPA Research and 0.48 mg/kg at JPPA Kochi, both on the day of application. The residues of ethiprole declined to 0.07 mg/kg after 181 days at JPPA Research and 0.04 mg/kg after 240 days at JPPA Kochi, representing approximately 4% and 8% of the initial residues. The half-life of total ethiprole residues was estimated as 2.8 days at the JPPA Research site and 11 days in the JPPA Kochi site. The maximum concentration of total ethiprole residues was 2.37 mg/kg at JPPA Research and 0.70 mg/kg at JPPA Kochi both on the day of application. The total residues declined rapidly to 0.18 mg/kg at the end of the study at JPPA Research (181 day) and 0.15 mg/kg at JPPA Kochi (240 days). The metabolites were detected at low concentrations.

Table 43 Summary of ethiprole residues in paddy soil following submerged application

Time after application	Concentration (mg eq/kg)			
	Ethiprole	RPA097973	RPA107566	Total residues (ethiprole eq.)
JPPA research				
0	1.86	0.03	0.48	2.37
7	0.12	0.03	0.28	0.43
14	0.05	0.03	0.26	0.34
30	0.02	0.02	0.16	0.20
60	0.05	0.03	0.14	0.22
90	0.07	0.05	0.06	0.18
120	0.08	0.06	0.07	0.21
181	0.07	0.06	0.05	0.18
JPPA Kochi				
0	0.48	<0.01	0.21	0.70
7	0.03	<0.01	0.42	0.46
14	<0.01	<0.01	0.27	0.29
30	<0.01	<0.01	0.20	0.22
58	0.06	0.02	0.12	0.20
90	0.06	0.03	0.05	0.14
121	0.04	0.04	0.04	0.12
181	0.07	0.06	0.05	0.18
240	0.04	0.08	0.03	0.15

Trials conducted in semi-field plots at the Institute of Environmental Toxicology, Ibaragi, Japan

The dissipation in water of ethiprole and potential metabolites following *foliar* application to rice was investigated under semi-field conditions (Garside and Odanaka 2009, M-356423-02-1). The target compounds for analysis were ethiprole, RPA 097973 (ethiprole-sulfone), RPA 107566 (ethiprole-sulfide), RPA 157925 (ethiprole-benzimidazole) and AE0764815.

Ethiprole was applied as a single application of a diluted spray application of a suspension concentrate (10% w/w flowable of ethiprole), in one application to transplanted rice plants in two semi-field plots with different paddy soil types and textures. The application rate of the formulation was equivalent to 200g ethiprole / ha.

Water samples were collected from each system at 0, 1, 3, 7 and 14 days after treatment and analysed for the target compounds by HPLC with UV-detection. The limit of quantification for all compounds was 0.001 mg/L. Ethiprole dissipated very rapidly following application with a half-life of ethiprole estimated to be approximately 1.9 days in both test systems. The maximum concentration of ethiprole was 0.220 mg/L in plot 1 and 0.285 mg/L in plot 2 immediately after application. This corresponds to 55 and 71% of the theoretical maximum application rate (20 mg/m^2). The concentration of ethiprole declined to 0.001 mg/L after 14 days in plot 1 and 0.002 mg/L in plot 2. Total ethiprole residues declined from 0.234 mg/L at day 0 to 0.001 mg/L at day 14 in plot 1 and from 0.308 mg/L at day 0 to 0.004 mg/L at day 14 in plot 2.

The parent ethiprole was the main residue in water at all sampling intervals. The metabolites were detected at low concentrations and dissipated rapidly with estimated 1-5 day half-lives for the individual metabolites and 2 days for the total metabolite residues.

Table 44 Summary of ethiprole residues in paddy water following foliar application

Time after application	Concentration (mg/L)					
	Ethiprole	AE0764815	RPA097973	RPA107566	RPA157925	Total residues (ethiprole eq.)
PLOT 1						

Time after application	Concentration (mg/L)					
	Ethiprole	AE0764815	RPA097973	RPA107566	RPA157925	Total residues (ethiprole eq.)
0	0.22	<0.001	0.006	0.004	0.004	0.234
1	0.182	<0.001	0.006	0.002	0.007	0.198
3	0.028	<0.001	0.003	<0.001	0.004	0.035
7	0.004	<0.001	0.001	<0.001	<0.001	0.005
14	0.001	<0.001	<0.001	<0.001	<0.001	0.001
PLOT 2						
0	0.285	<0.001	0.007	0.005	0.010	0.308
1	0.213	0.001	0.007	0.003	0.022	0.248
3	0.056	0.002	0.005	0.001	0.019	0.085
7	0.010	<0.001	0.003	<0.001	0.005	0.018
14	0.002	<0.001	0.001	<0.001	0.001	0.004

The dissipation in water of ethiprole and potential metabolites following *submerged* application to rice was investigated under semi-field conditions (Garside *et al.,* 2009, M-356470-02-1). The target compounds for analysis were ethiprole, RPA 097973 (ethiprole-sulfone), RPA 107566 (ethiprole-sulfide), RPA 157925 (ethiprole-benzimidazole) and AE0764815.

Ethiprole 1.5% GR, formulated as a granule 1.5% w/w product, was applied to transplanted rice plants in two semi-field plots simulating rice paddy fields with different paddy soil types and textures. It was applied in one submerged application, with an application rate of 4g product/ m^2, equivalent to an application rate of 600 g ethiprole/ha.

Water samples were collected from each system before treatment and at 0, 1, 3, 7 and 14 days after treatment and analysed for the target compounds by HPLC with UV-detection. The limit of quantification for all compounds was 0.001mg/L. Ethiprole dissipated very rapidly following application with a half-life estimated to be approximately 2 to 4 days. The maximum concentration of ethiprole was 0.481 mg/L and 0.290 mg/L in plot 1 and 2 respectively, one day after application, equivalent to approximately 40 and 24% of the theoretical applied amount (60 mg/m^2). The concentration of ethiprole declined rapidly to 0.004 mg/L and 0.005 mg/L after 14 days respectively in the two plots. The majority of the residues found in the water were due to parent ethiprole. The metabolites were usually detected at low concentrations and dissipated rapidly with estimated half-lives for each metabolite and the total residues of 2 to 5 days. The total residues of ethiprole declined from a maximum of 0.561 mg/L at day 1 to 0.006 mg/L at day 14 in plot 1. In plot 2 the concentration declined from 0.345 mg/L at day 1 to 0.010 mg/L at day 14.

These semi-field studies in Japan with simulated rice paddy fields (1m × 1m) demonstrated the very rapid dissipation of ethiprole and its metabolites RPA 097973, RPA 107566 and RPA 157925 in the paddy water with half-lives of approximately 1 - 5 days for each of the substances.

Table 45 Summary of ethiprole residues in paddy water following submerged application

Time after application	Concentration (mg/L)					
	Ethiprole	AE0764815	RPA097973	RPA107566	RPA157925	Total residues (ethiprole eq.)
PLOT 1						
0	0.298	0.002	0.005	0.003	0.031	0.342
1	0.481	0.003	0.015	0.005	0.052	0.561
3	0.172	0.003	0.014	0.005	0.056	0.256
7	0.024	<0.001	0.007	0.001	0.014	0.047
14	0.004	<0.001	0.001	<0.001	0.001	0.006
PLOT 2						
0	0.204	0.002	0.004	0.003	0.026	0.242
1	0.290	0.002	0.009	0.004	0.036	0.345
3	0.194	0.002	0.014	0.006	0.049	0.270
7	0.036	<0.001	0.008	0.004	0.015	0.064
14	0.005	<0.001	0.002	0.001	0.002	0.010

Summary

From the results of the four Japanese studies it can be concluded that neither ethiprole nor its metabolites will persist in paddy soil following a foliar or a submerged application. The degradation half-lives of ethiprole and the metabolites following either foliar

or submerged applications sections are summarised in Table 46. The DT_{50} obtained was not dependent on the application technique.

Table 46 Half-lives of parent ethiprole, the total residues of ethiprole and metabolites in paddy water and paddy soil following either foliar or submerged application

	DT_{50} in water (days) (semi-field study)				DT_{50} in paddy soil (days) (field study)			
Site	Plot 1		Plot 2		JPPA Research		JPPA Kochi	
Application method	Foliar[c]	Sub-merged[d]	Foliar[c]	Sub-merged[d]	Foliar[a]	Sub-merged[b]	Foliar[a]	Sub-merged[b]
Ethiprole	1.9	2.5	1.9	4.2	4.2	1.8	3.9	1.8
Ethiprole-sulfide RPA107566	*ca* 1	*ca* 2	*ca* 2	*ca* 5	63	30	45	30
Ethiprole-sulfone RPA097973	*ca* 2	ca 4	*ca* 5	*ca* 5	*	*	*	*
Ethiprole-benzimidazole RPA157925	*ca* 3	*ca* 2	*ca* 5	*ca* 2	*	-	26	-
AE0764815	*	*ca* 2	*	*ca* 2	-	-	-	-
Ethiprole-amide RPA112916	-	-	-	-	*	-	*	-
Ethiprole-sulfone-amide RPA112917	-	-	-	-	*	-	*	-
Total residues[#]	*ca* 2	*ca* 2	*ca* 2	*ca* 3	54	2.8	5.4	11

= ethiprole + measured metabolites

* = values could not be calculated due to low formation

[a] Garside *et al.*, 2009, M-356426-02-1

[b] Garside *et al.*, 2009, M-356427-02-1

[c] Garside and Odanaka 2009, M-356423-02-1

[d] Garside *et al.*, 2009, M-356470-02-1

Terrestrial rice field studies in Japan indicated that ethiprole and the major metabolites RPA ethiprole-sulfide (107566) and ethiprole-sulfone (RPA 097973), potentially formed under the conditions of paddy rice growing, are not persistent. The degradation half life of RPA107566 in paddy soil ranged from 30 to 63 days under rice paddy field conditions. In the paddy water, the dissipation half lives of ethiprole and its metabolites RPA 097973 and RPA 107566 did not exceed 5 days. As a consequence, it was concluded that potential accumulation of ethiprole and its metabolites RPA 107566 and RPA 097973 in paddy water and soil following repeated application of ethiprole in successive seasons can be excluded.

RESIDUE ANALYSIS

Analytical methods

Details of analytical methods including validation data were supplied for the determination of ethiprole and key metabolites in plant and animal matrices, soil and water and are considered satisfactory. A summary of all analytical methods for plants and animals is given in Table 47.

Table 47 Summary of analytical methods developed for plant and animal matrices

Method No.	Analyte	Detection system	Substrate	LOQ	Reference
Plant Matrices – Monitoring and Enforcement					
01128	Ethiprole RPA 097973 RPA 112916	HPLC-MS/MS	Orange fruit Tomato Dry bean (seeds) Wheat grain Tea (black or green tea) Avocado	0.002 mg/kg	Schwarz 2008, M-311022-01-2
ILV of 01128	Ethiprole RPA 097973	HPLC-MS/MS	Tea (black or green tea)	0.02 mg/kg (tea leaves, green)	Rzepka 2008, M-312599-01-2

Method No.	Analyte	Detection system	Substrate	LOQ	Reference
			Tomato Rice grain	0.002 mg/kg (other matrices)	
ILV of 01128	Ethiprole RPA 097973	HPLC-MS/MS	Coffee beans	0.002 mg/kg	Winter and Geisler 2017, M-589070-01-1
Plant Matrices – Data Generation					
01053 (previously AR 243-00)	Ethiprole RPA 097973 RPA 115369	HPLC-MS/MS	Potato Apple Tomato Lettuce	0.002 mg/kg	Cavezza *et al.*, 2000, M-192000-01-2
R016922	Ethiprole RPA 097973 RPA 107566 RPA 115369 RPA 112916 RPA 103343	HPLC-MS/MS	Orange (fruit, dry pulp, juice, oil) Cotton (seed, gin trash, meal, hulls, oil) Rice (straw, grain)	0.001 mg/kg	Zheng and Arjmand 1999, M-192650-01-2
01128	Ethiprole (primary transition) RPA 097973 RPA 112916	HPLC-MS/MS	Coffee (grain) Soya bean (grain) Wheat (grain) Sugar cane (stalk) Citrus (fruit) Beans (grain)	0.01 mg/kg	Santiago 2012, M-455162-01-2
Animal Matrices – Monitoring and Enforcement					
01YQ18289	Ethiprole + RPA 097973 (common moiety)	GC/ECD	Bovine liver, fat, meat, milk Poultry egg, muscles	0.01 mg/kg	Howell 2001, M-240553-01-2
ILV of 01YQ18289	Ethiprole + RPA 097973 (common moiety)	GC/ECD	Bovine, fat Poultry egg	0.2 mg/kg 0.02 mg/kg	Gould 2009, M-327549-01-1
Animal Matrices – Data Generation					
Study 019-034	Ethiprole RPA 097973 RPA 094569 RPA 115369	LC-MS/MS	Milk Eggs Liver Kidney Muscle Fat	0.001 mg/kg	Zheng *et al.*, 1999, M-192648-01-1
01431 MR-14/113	Ethiprole RPA 097973 RPA 104615 RPA 107566	HPLC-MS/MS	Cow muscle Cow fat Cow liver Cow kidney Milk Eggs	0.01 mg/kg (tissues and eggs) 0.005 mg/kg (milk)	Glaubitz and Keppels 2015, M-543785-01-1

Plant commodities

Method 01128 (enforcement): Method 01128 for the determination of ethiprole and its metabolites ethiprole-sulfone (RPA 097973) and and ethiprole-amide (RPA 112916) in plant matrices by means of high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) was reported by Schwarz in 2008 (M-311022-01-2). This was the data generation method used in the coffee field trials.

Ethiprole and its metabolites RPA 097973 and RPA 112916 are extracted from plant material with acetonitrile/water (9:1, v/v). After blending and centrifugation and dilution with acetonitrile/water (1/9, v/v) the final solution is analysed by HPLC-MS/MS. The residues are determined by LC-MS/MS using the characteristic *m/z* 395/330 (parent), *m/z* 411/375 (RPA 097973) and *m/z* 415/398 (RPA 112916) MRM transitions for quantification and *m/z* 395/250 (parent), *m/z* 411/282 (RPA 097973) and *m/z* 415/255 (RPA 112916) for confirmation with external calibrations with matrix-matched standards.

The accuracy of the method was assessed on the basis of the determined recovery rates. The materials tested included orange (fruit), tomato (fruit), dry bean (seeds), wheat (grain), tea (leaves) and avocado (fruit). Samples were fortified with parent ethiprole, RPA 097973 and RPA 112916 at concentrations of 0.002 and 0.02 mg/kg. Mean recoveries per fortification level for parent ethiprole, RPA 097973 and RPA 112916 for all matrices were in a range of 78-110%, using the primary conditions, with acceptable RSD values.

Confirmatory procedures for parent ethiprole, RPA 097973 and RPA 112916 using the same chromatographic system, but using different MRM transitions gave mean recoveries ranging from 76-109% for all matrices, with acceptable RSD values.

The limit of quantitation (LOQ) for ethiprole, RPA 097973 and RPA 112916, defined as the lowest validated fortification level, was 0.002 mg/kg in all matrices tested. All metabolite levels are expressed in parent equivalents.

Method linearity was validated over the range 0.025 to 5.0 or 10.0 ng/mL (matrix-matched calibration solutions) for all three analytes. Correlation coefficients (r) were ≥ 0.994 for each analyte and both MS/MS transitions.

Table 48 Recoveries for method 01128: ethiprole in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 395 → 330			Confirmatory Transition 395 → 250		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Orange (fruit)	Ethiprole	5	0.002	82-95	89	5.7	89-96	92	3.1
		5	0.02	85-90	88	2.2	87-94	90	4.0
Tomato (fruit)	Ethiprole	5	0.002	101-105	104	1.6	97-110	103	4.5
		5	0.02	95-98	96	1.4	90-98	94	3.4
Dry bean (seeds)	Ethiprole	5	0.002	106-111	108	1.8	104-113	109	3.6
		5	0.02	101-110	107	3.7	99-110	106	4.0
Wheat (grain)	Ethiprole	5	0.002	107-115	110	2.7	102-118	107	6.3
		5	0.02	108-110	109	0.8	106-111	109	1.6
Tea (leaves)	Ethiprole	5	0.002	91-118	107	9.3	100-120	109	6.8
		5	0.02	88-110	98	8.8	90-110	99	7.8
Avocado (fruit)	Ethiprole	5	0.002	99-106	103	3.2	95-109	101	6.1
		5	0.02	90-98	94	3.7	91-99	94	3.2

Table 49 Recoveries for method 01128: ethiprole-sulfone (RPA 097973) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 411 → 375			Confirmatory Transition 411 → 282		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Orange (fruit)	RPA 097973	5	0.002	77-87	81	5.0	74-85	79	5.4
		5	0.02	92-96	94	1.7	88-92	91	1.6
Tomato (fruit)	RPA 097973	5	0.002	91-99	95	3.2	86-93	91	3.2
		5	0.02	98-109	103	3.9	98-109	104	4.3
Dry bean (seeds)	RPA 097973	5	0.002	102-110	108	3.2	108-110	109	0.8
		5	0.02	108-112	110	1.4	105-111	108	2.4
Wheat (grain)	RPA 097973	5	0.002	99-105	102	2.6	93-100	97	2.7
		5	0.02	104-109	108	2.1	103-109	106	2.4
Tea (leaves)	RPA 097973	5	0.002	90-104	97	7.1	84-110	96	11.1
		5	0.02	91-109	100	8.1	88-109	99	9.2
Avocado (fruit)	RPA 097973	5	0.002	90-97	94	3.3	91-100	96	4.1
		5	0.02	92-101	98	3.4	89-102	97	4.8

Table 50 Recoveries for method 01128: ethiprole-amide (RPA 112916) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 415 → 398			Confirmatory Transition 415 → 255		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Orange	RPA 112916	5	0.002	75-82	78	3.6	73-80	76	3.3

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 415 → 398			Confirmatory Transition 415 → 255		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
(fruit)		5	0.02	81-83	82	0.8	81-84	83	1.3
Tomato	RPA 112916	5	0.002	91-99	96	4.1	78-104	94	11.7
(fruit)		5	0.02	88-91	90	1.4	85-90	88	2.1
Dry bean	RPA 112916	5	0.002	98-102	101	1.4	90-105	97	7.3
(seeds)		5	0.02	97-106	101	3.5	97-105	100	3.0
Wheat	RPA 112916	5	0.002	101-108	105	2.8	101-105	103	1.6
(grain)		5	0.02	105-108	107	0.9	108-109	109	0.4
Tea	RPA 112916	5	0.002	88-94	92	2.7	87-97	92	4.4
(leaves)		5	0.02	92-96	94	2.1	92-96	93	1.5
Avocado	RPA 112916	5	0.002	86-95	90	3.9	88-100	95	4.9
(fruit)		5	0.02	88-96	91	3.3	83-93	89	4.0

An independent laboratory validation (ILV) was conducted for method 01128 (Rzepka 2008, M-312599-01-2). As the metabolite ethiprole-amide was not observed in significant amounts in the field residue trials this ILV study focussed on the determination of ethiprole parent compound and ethiprole-sulfone (RPA 097973). Samples of green tea leaf, tomato fruit and rice grain were fortified with ethiprole parent compound and ethiprole-sulfone at the nominal fortification levels of 0.02 and 0.20 mg/kg (tea leaves) and 0.002 and 0.02 mg/kg (tomato and rice).

Analysis of samples was performed according to method 01128 (Schwarz 2009, M-311022-01-2). Two MRM transitions were measured for parent ethiprole and RPA 097973, one for quantification and the second for confirmation. For all three matrices, for both fortification levels, and for both MRM transitions monitored, the mean recoveries were between 76% and 110%, with relative standard deviations of < 20%. A summary of the independent laboratory validation results is given in Tables 51 and 52.

The limit of quantitation (LOQ) for ethiprole and RPA 097973 was 0.002 mg/kg for tomato and rice and 0.02 mg/kg for tea expressed in parent equivalents.

The linearity of detector response was confirmed by injecting seven external standard solutions in the range of 0.033–20.0 ng/mL of ethiprole and its metabolite RPA 097973 (expressed as parent equivalent) in solvent and green tea (leaf) extract and by injecting six external standard solutions in the range of 0.033–10.0 ng/mL of ethiprole and its metabolite RPA 097973 (expressed as parent equivalent) in tomato (fruit) and rice (grain) extract.

Table 51 Recoveries for method 01128: ethiprole in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 395 → 330			Confirmatory Transition 395 → 250		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Green tea leaves	Ethiprole	5	0.02	103-107	105	1.4	101-107	104	2.3
		5	0.20	102-114	110	4.6	106-115	110	3.6
Tomato fruit	Ethiprole	5	0.002	103-112	107	3.1	90-125	107	13
		5	0.02	77-81	78	2.2	73-81	77	4.4
Rice grain	Ethiprole	5	0.002	78-111	91	14	73-91	81	10
		5	0.02	80-87	84	3.3	83-86	84	1.5

Table 52 Recoveries for method 01128: ethiprole-sulfone (RPA 097973) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 411 → 375			Confirmatory Transition 411 → 282		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Green tea leaves	RPA 097973	5	0.02	107-111	108	1.6	105-107	106	0.8
		5	0.20	106-114	110	2.9	102-121	110	6.5
Tomato fruit	RPA 097973	5	0.002	88-112	99	8.8	93-111	102	7.4
		5	0.02	73-82	76	4.5	76-78	77	0.9
Rice grain	RPA 097973	5	0.002	106-113	109	3.2	107-115	110	2.7
		5	0.02	86-89	87	1.5	85-89	86	2.0

Another independent laboratory validation was conducted for method 01128 (Winter and Geisler 2017, M-589070-01-1). Samples of coffee bean were fortified with ethiprole parent, ethiprole-sulfone and ethiprole-amide at the nominal fortification levels of 0.002 and 0.02 mg/kg.

Analysis of samples was performed according to method 01128. Two MRM transitions were measured for parent ethiprole, ethiprole-sulfone (RPA 097973) and RPA 112916, one for quantification and the second for confirmation. For both fortification levels, and for both MRM transitions monitored, the mean recoveries were between 87% and 97%, with relative standard deviations of < 20%. A summary of the independent laboratory validation results is given in Tables 53 - 55.

The limit of quantitation (LOQ) for ethiprole, RPA 097973 and RPA 112916, was 0.002 mg/kg in coffee beans (green) expressed in parent equivalents.

The linearity of detector response for ethiprole and its two metabolites RPA 097973 and RPA 112916 was established using matrix matched standard solutions in the range of 0.03–1.5 ng/mL (calculated as parent equivalent). The coefficients of determination (R^2) were always > 0.98.

Table 53 Recoveries for method 01128: ethiprole in coffee beans

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 395 → 330			Confirmatory Transition 395 → 250		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Coffee bean	Ethiprole	5	0.002	86-108	94	11	77-99	87	12
		5	0.02	84-99	93	8.1	82-97	91	8.3

Table 54 Recoveries for method 01128: ethiprole-sulfone (RPA 097973) in coffee beans

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 411 → 375			Confirmatory Transition 411 → 282		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Coffee bean	RPA 097973	5	0.002	87-95	90	3.6	91-96	94	2.4
		5	0.02	94-98	97	1,7	94-98	96	1.7

Table 55 Recoveries for method 01128: ethiprole-amide (RPA 112916) in coffee beans

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Primary Method: Transition 415 → 398			Confirmatory Transition 415 → 255		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Coffee bean	RPA 112916	5	0.002	91-103	96	5.1	87-106	97	8.2
		5	0.02	94-102	97	3.5	93-103	97	4.2

Method 01053 – previously called AR 243-00 (data collection):

A residue analytical method, 01053, which was previously called AR 243-00, was developed as a data collection method for the determination of ethiprole and its metabolites ethiprole-sulfone (RPA 097973) and ethiprole-des-chloro-sulfone (RPA 115369) in/on plant materials using LC-MS/MS (Cavezza *et al.,* 2000, M-192000-01-2).

The residues of ethiprole and its metabolites are extracted from each matrix twice by homogenisation with acetonitrile/water (9:1, v/v). After filtration the extracts are combined and analysed by LC-MS/MS using a C-18 column for chromatographic separation. Quantitation of ethiprole and metabolites is by LC-MS/MS using external standards. As this is a data collection method, only one MRM transition was required.

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples were fortified with parent ethiprole, RPA 097973 and RPA 115369 at concentrations of 0.002, 0.01 and 0.5 mg/kg in all matrices (potato, lettuce, apple

and tomato). Metabolite levels were expressed in parent equivalents. Mean recoveries per fortification level and overall mean recoveries and RSD values for ethiprole and metabolites were acceptable.

The limit of quantitation (LOQ) for parent ethiprole, RPA 097973 and RPA 115369, was 0.002 mg/kg in all matrices tested.

The linearity of detector response for ethiprole and its two metabolites RPA 097973 and RPA 115369 was established in the range of 0.04–10 µg/L, using six different concentration levels. Correlation coefficients (r) were > 0.999 for each analyte.

Table 56 Recoveries for method 10503: ethiprole in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 330		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Potato	Ethiprole	2	0.002	103, 120	112	-
		2	0.01	105, 117	111	-
		2	0.5	98, 102	100	-
Lettuce	Ethiprole	2	0.002	105, 111	108	-
		2	0.01	84, 90	87	-
		2	0.5	91, 101	96	-
Apple	Ethiprole	2	0.002	70, 72	71	-
		2	0.01	91, 98	95	-
		2	0.5	86, 88	87	-
Tomato	Ethiprole	2	0.002	101, 102	102	-
		2	0.01	86, 88	87	-
		2	0.5	93, 95	94	-

Table 57 Recoveries for method 01053: ethiprole-sulfone (RPA 097973) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 411 → 375		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Potato	RPA 097973	2	0.002	108, 112	110	-
		2	0.01	88, 98	93	-
		2	0.5	96, 100	98	-
Lettuce	RPA 097973	2	0.002	105, 108	107	-
		2	0.01	88, 92	90	-
		2	0.5	95, 99	97	-
Apple	RPA 097973	2	0.002	100, 109	105	-
		2	0.01	97, 112	105	-
		2	0.5	89, 91	90	-
Tomato	RPA 097973	2	0.002	99, 108	104	-
		2	0.01	94, 98	96	-
		2	0.5	90, 94	92	-

Table 58 Recoveries for method 01053: ethiprole-des-chloro-sulfone (RPA 115369) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 377 → 341		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Potato	RPA 115369	2	0.002	102, 110	106	-
		2	0.01	98, 108	103	-
		2	0.5	97, 98	98	-
Lettuce	RPA 115369	2	0.002	97, 103	100	-
		2	0.01	87, 87	87	-
		2	0.5	95, 102	99	-
Apple	RPA 115369	2	0.002	100, 101	101	-
		2	0.01	91, 99	95	-
		2	0.5	88, 88	88	-
Tomato	RPA 115369	2	0.002	107, 111	109	-
		2	0.01	100, 107	104	-
		2	0.5	92, 93	93	-

Method R016922 (data collection):

A validated data collection method (R016922) has been reported for the determination of ethiprole and its metabolites ethiprole-sulfone (RPA 097973), ethiprole-sulfide (RPA 107566), ethiprole-deschloro-sulfone (RPA 115369), ethiprole-amide (RPA 112916) and ethiprole-formamide (RPA 103343) in/on plant materials using HPLC-MS/MS (Zheng and Arjmand 1999, M-192650-01-2).

The residues of ethiprole and its metabolites are extracted from each matrix, except oil, by shaking with an acetonitrile/water solution in the presence of celite and graphitised carbon, followed by filtration. The oil sample is first dissolved in hexane, then extracted into the acetonitrile/ water solution. The extract is partially purified by liquid-liquid partitioning with hexane. After removal of acetonitrile, the residues are partitioned into dichloromethane. After removal of the dichloromethane, the residues are cleaned by solid-phase extraction. Compounds of interest are eluted with 10% methanol/acetonitrile. Quantitation of ethiprole and metabolites is by LC-MS/MS in multiple ion reaction monitoring (MRM) mode. As this is a data collection method, only one MRM transition is monitored for in each matrix tested.

The accuracy of the method was assessed on the basis of the determined recovery rates in different matrices. Samples of orange fruit, orange dried pulp, orange juice, orange oil, cotton seed, cotton gin trash, cotton meal, cotton hulls, cotton seed oil, rice grain and rice straw were fortified with ethiprole, RPA 103343, RPA 097973, RPA 107566 and RPA 112916 (not rice grain) at various concentrations (RPA 115369 orange oil, cotton gin trash and rice straw only). Metabolites were expressed in parent equivalents.

The limit of quantitation (LOQ) for parent ethiprole, RPA 103343, RPA 097973, RPA 107566, RPA 115369 and RPA 112916 was 0.001 mg/kg in all matrices tested except RPA 112916 for cotton gin trash (0.02 mg/kg).

The linearity of detector response for ethiprole and its metabolites was established, using at least four different concentration levels. Correlation coefficients (r) were > 0.99 for each analyte.

Table 59 Recoveries for method R016922: ethiprole in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 330		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Orange fruit	Ethiprole	2	0.001	81, 93	87	-
		2	0.005	86, 87	87	-
		2	0.05	93, 102	98	-
Orange dried pulp	Ethiprole	2	0.001	73, 76	75	-
		2	0.005	81, 87	84	-
		2	0.05	88, 93	91	-
Orange juice	Ethiprole	2	0.001	101, 90	96	-
		2	0.005	101, 107	104	-
		2	0.05	111, 109	110	-

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 330		
				Range [%]	Mean	RSD [%]
Orange oil	Ethiprole	4	0.001	98-122	105	11
		2	0.005	108, 110	109	-
		4	0.05	99-107	104	3.3
Cotton seed	Ethiprole	2	0.001	84, 88	86	-
		2	0.005	91, 92	91	-
		2	0.05	92, 93	92	-
Cotton gin trash	Ethiprole	4	0.001	78-104	89	14
		2	0.005	88, 91	90	-
		4	0.05	85-93	90	3.8
Cotton meal	Ethiprole	2	0.001	91, 92	91	-
		2	0.005	98, 103	100	-
		2	0.05	93, 110	102	-
Cotton hulls	Ethiprole	2	0.001	77, 84	81	-
		2	0.005	83, 83	83	-
		2	0.05	101, 110	106	-
Cottonseed oil	Ethiprole	2	0.001	87, 90	89	-
		2	0.005	82, 100	91	-
		2	0.05	91, 97	94	-
Rice grain	Ethiprole	2	0.001	95, 107	101	-
		2	0.005	103, 105	104	-
		2	0.05	91, 96	93	-
Rice straw	Ethiprole	4	0.001	87-108	95	9.5
		2	0.005	86, 87	86	-
		4	0.05	89-93	91	2.0

Table 60 Recoveries for method R016922: ethiprole-formamide (RPA 103343) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 347 → 283		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Orange fruit	RPA 103343	2	0.001	87, 104	95	-
		2	0.005	83, 93	88	-
		2	0.05	97, 104	101	-
Orange dried pulp	RPA 103343	2	0.001	82, 83	82	-
		2	0.005	88, 91	89	-
		2	0.05	90, 96	93	-
Orange juice	RPA 103343	2	0.001	89, 94	92	-
		2	0.005	97, 111	104	-
		2	0.05	108, 110	109	-
Orange oil	RPA 103343	2	0.001	104, 107	106	-
		2	0.005	110, 113	112	-
		2	0.05	112, 113	113	-
Cotton seed	RPA 103343	2	0.001	85, 87	86	-
		2	0.005	80, 92	86	-
		2	0.05	88, 88	88	-
Cotton gin trash	RPA 103343	2	0.001	99, 108	103	-
		2	0.005	93, 95	94	-
		2	0.05	87, 91	89	-
Cotton meal	RPA 103343	2	0.001	94, 101	98	-
		2	0.005	98, 102	100	-
		2	0.05	91, 102	96	-
Cotton hulls	RPA 103343	2	0.001	85, 90	87	-
		2	0.005	83, 85	84	-
		2	0.05	93, 98	95	-
Cottonseed oil	RPA 103343	2	0.001	96, 103	99	-
		2	0.005	82, 89	85	-
		2	0.05	84, 86	85	-

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 347 → 283		
				Range [%]	Mean	RSD [%]
Rice grain	RPA 103343	2	0.001	93, 106	99	-
		2	0.005	93, 96	94	-
		2	0.05	88, 96	92	-
Rice straw	RPA 103343	2	0.001	90, 97	94	-
		2	0.005	88, 90	89	-
		2	0.05	89, 90	89	-

Table 61 Recoveries for method R016922: ethiprole-sulfone (RPA 097973) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 411 → 375		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Orange fruit	RPA 097973	2	0.001	80, 100	90	-
		2	0.005	83, 86	85	-
		2	0.05	97, 106	102	-
Orange dried pulp	RPA 097973	2	0.001	82, 83	82	-
		2	0.005	88, 91	90	-
		2	0.05	89, 91	90	-
Orange juice	RPA 097973	2	0.001	87, 95	91	-
		2	0.005	99, 100	99	-
		2	0.05	108, 109	109	-
Orange oil	RPA 097973	4	0.001	119-128	123	3.4
		2	0.005	106, 106	106	-
		4	0.05	94-109	102	6.1
Cotton seed	RPA 097973	2	0.001	86, 86	86	-
		2	0.005	88, 87	88	-
		2	0.05	89, 92	90	-
Cotton gin trash	RPA 097973	4	0.001	88-97	92	4.6
		2	0.005	90, 93	91	-
		4	0.05	85-94	88	4.8
Cotton meal	RPA 097973	2	0.001	98, 101	99	-
		2	0.005	98, 100	99	-
		2	0.05	94, 104	99	-
Cotton hulls	RPA 097973	2	0.001	87, 94	90	-
		2	0.005	89, 94	91	-
		2	0.05	92, 98	95	-
Cottonseed oil	RPA 097973	2	0.001	94, 98	96	-
		2	0.005	86, 98	92	-
		2	0.05	91, 93	92	-
Rice grain	RPA 097973	2	0.001	85, 99	92	-
		2	0.005	85, 93	89	-
		2	0.05	86, 91	89	-
Rice straw	RPA 097973	4	0.001	82-95	87	6.6
		2	0.005	81, 83	82	-
		4	0.05	84-91	87	3.9

Table 62 Recoveries for method R016922: ethiprole-sulfide (RPA 107566) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 379 → 314		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Orange fruit	RPA 107566	2	0.001	75, 93	84	-
		2	0.005	83, 87	85	-
		2	0.05	92, 103	98	-

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 379 → 314		
				Range [%]	Mean	RSD [%]
Orange dried pulp	RPA 107566	2	0.001	75, 75	75	-
		2	0.005	84, 84	84	-
		2	0.05	85, 90	87	-
Orange juice	RPA 107566	2	0.001	86, 92	89	-
		2	0.005	92, 98	95	-
		2	0.05	103, 104	104	-
Orange oil	RPA 107566	4	0.001	80-119	99	20
		2	0.005	108, 110	109	-
		4	0.05	65-102	83	23
Cotton seed	RPA 107566	2	0.001	74, 82	78	-
		2	0.005	87, 91	89	-
		2	0.05	89, 91	90	-
Cotton gin trash	RPA 107566	4	0.001	67-74	70	4.6
		2	0.005	75, 79	77	-
		4	0.05	78-85	81	3.8
Cotton meal	RPA 107566	2	0.001	78,81	79	-
		2	0.005	88, 92	90	-
		2	0.05	89, 103	96	-
Cotton hulls	RPA 107566	2	0.001	67, 73	70	-
		2	0.005	80, 82	81	-
		2	0.05	87, 87	87	-
Cottonseed oil	RPA 107566	2	0.001	73, 79	76	-
		2	0.005	73, 88	80	-
		2	0.05	89, 95	92	-
Rice grain	RPA 107566	2	0.001	75, 77	76	-
		2	0.005	84, 90	87	-
		2	0.05	86, 88	87	-
Rice straw	RPA 107566	4	0.001	63-77	68	9.7
		2	0.005	75, 80	77	-
		4	0.05	83-88	85	3.0

Table 63 Recoveries for method R016922: ethiprole-amide (RPA 112916) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 413 → 304		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Orange fruit	RPA 112916	3	0.001	70-82	75	8.3
		1	0.02	115	115	-
		1	0.05	89	89	-
		1	0.1	105	105	-
		2	0.2	78, 90	84	-
		1	1	117	117	-
Orange dried pulp	RPA 112916	1	0.001	85	85	-
		1	0.200	90	90	-
		1	1	102	102	-
Orange juice	RPA 112916	1	0.001	61	61	-
		1	0.200	76	76	-
		1	1	84	84	-
Orange oil	RPA 112916	1	0.001	87	87	-
		1	0.005	125	125	-
Cotton seed	RPA 112916	3	0.001	86-107	93	13
		1	0.020	91	91	-
		4	0.05	63-88	82	15
		1	1	87	87	-

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 413 → 304		
				Range [%]	Mean	RSD [%]
Cotton gin trash	RPA 112916	5	0.02	75-107	92	16
		1	0.05	100	100	-
		2	0.5	74, 85	80	-
		1	2	85	85	-
Cotton meal	RPA 112916	1	0.001	67	67	-
		1	0.02	71	71	-
		1	1	71	71	-
Cotton hulls	RPA 112916	1	0.001	89	89	-
		1	0.02	84	84	-
		1	1	87	87	-
Cottonseed oil	RPA 112916	1	0.001	71	71	-
		1	0.02	74	74	-
		1	1	81	81	-

Table 64 Recoveries for method R016922: ethiprole-deschloro-sulfone (RPA 115369) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 377 → 341		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Orange oil	RPA 115369	2	0.001	106, 109	108	-
		2	0.05	98, 101	100	-
Cotton gin trash	RPA 115369	2	0.001	85, 87	86	-
		2	0.05	84, 87	86	-
Rice straw	RPA 115369	2	0.001	90, 94	92	-
		2	0.05	92, 97	95	-

Method 01128 (data collection):

Method 01128 is the enforcement method described above. A study was conducted to validate method 01128 for the determination of residues of 01128 and its metabolites ethiprole-sulfone (RPA 097973) and ethiprole-amide (RPA 112916) in soya beans (grains), wheat (grains), coffee (grains), sugar cane (culms), citrus (fruit) and beans (grains) (Santiago 2013, M-455162-01-2).

Samples of soya bean, wheat, tomato and rice grain were fortified with ethiprole parent compound, RPA 097973 and RPA 112916 at nominal fortification levels of 0.01 and 1.0 mg/kg (at least five samples at each level).

Analysis of samples was performed according to method 01128 (Schwarz 2009, M-311022-01-2). Two MRM transitions were measured for parent ethiprole, RPA 097973 and RPA 112916, one for quantification and the second for confirmation, for all matrices except for ethiprole in coffee grains, where only the primary transition was measured. For all six matrices, for both fortification levels, and for both MRM transitions monitored, the mean recoveries were between 72% and 112%, with relative standard deviations of ≤ 13%. Control samples used for fortification had residues of less than 30% of the LOQ, confirming the selectivity of the method. A summary of the independent laboratory validation results is given in Tables 65-67.

Table 65 Recoveries for method 01128: ethiprole in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 250			Transition 395 → 330		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Soya beans (grains)	Ethiprole	6	0.010	100-110	106	3.5	93-108	101	6.3
		5	1.0	89-104	96	6.6	88-105	97	6.6
Wheat (grains)	Ethiprole	5	0.010	93-105	97	5.2	83-107	100	9.9
		5	1.0	99-106	103	3.0	96-109	102	4.7
Coffee (grains)	Ethiprole	5	0.010	78-101	92	10.9	-	-	-
		6	1.0	80-114	96	12.0	-	-	-
Sugar cane (culms)	Ethiprole	5	0.010	65-76	72	6.7	68-88	81	10.2
		5	1.0	94-103	99	3.6	93-103	99	4.1

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 250			Transition 395 → 330		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
Citrus (fruit)	Ethiprole	5	0.010	93-101	98	3.2	90-109	99	6.9
		5	1.0	94-111	101	7.6	91-113	101	9.7
Beans (grains)	Ethiprole	5	0.010	88-111	103	8.7	90-115	103	8.6
		5	1.0	95-112	103	7.6	98-115	104	7.1

Table 66 Recoveries for method 01128: ethiprole-sulfone (RPA 097973) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 411 → 375			Transition 411 → 282		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Soya beans (grains)	RPA 097973	7	0.010	97-120	112	6.9	108-116	112	3.2
		5	1.0	81-100	91	9.2	82-101	90	9.1
Wheat (grains)	RPA 097973	5	0.010	94-103	98	3.9	96-104	98	3.3
		5	1.0	98-106	102	2.8	98-106	102	2.8
Coffee (grains)	RPA 097973	7	0.010	100-124	112	7.4	98-121	108	7.4
		7	1.0	77-111	97	12.8	75-110	96	13.0
Sugar cane (culms)	RPA 097973	5	0.010	71-84	77	7.8	67-87	77	11.5
		5	1.0	97-101	98	1.7	96-101	98	2.6
Citrus (fruit)	RPA 097973	6	0.010	97-104	100	3.1	96-108	100	4.9
		5	1.0	81-105	94	10.9	81-104	94	10.8
Beans (grains)	RPA 097973	6	0.010	104-117	108	4.4	100-117	107	5.7
		5	1.0	87-113	101	12.1	85-113	99	13.0

Table 67 Recoveries for method 01128: ethiprole-amide (RPA 112916) in plants

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 415 → 398			Transition 415 → 255		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Soya beans (grains)	RPA 112916	5	0.010	94-108	103	5.5	96-99	98	1.6
		5	1.0	92-104	97	4.7	88-101	94	5.4
Wheat (grains)	RPA 112916	5	0.010	92-107	99	6.5	78-97	88	8.3
		5	1.0	97-107	100	4.2	96-107	99	4.8
Coffee (grains)	RPA 112916	5	0.010	86-102	96	6.5	84-99	93	8.1
		5	1.0	78-110	92	12.8	79-110	93	13.0
Sugar cane (culms)	RPA 112916	5	0.010	71-81	77	5.2	72-89	81	8.9
		5	1.0	91-95	93	1.6	90-96	93	2.7
Citrus (fruit)	RPA 112916	5	0.010	90-103	97	5.3	86-102	98	6.9
		5	1.0	97-103	100	2.4	98-103	101	2.1
Beans (grains)	RPA 112916	5	0.010	95-102	99	3.1	91-99	94	3.9
		5	1.0	96-101	99	2.5	96-100	98	1.8

Multi-residue method DFG S19:

A study was undertaken to determine whether the German multi-residue enforcement method DFG S19 is applicable to the determination of residues of ethiprole and its metabolites ethiprole-sulfone (RPA 097973) and ethiprole-amide (RPA 112916) in plant matrices (Class 2008, M-308635-01-1). The original version of the method DFG S19 involves measurement of sample extracts by gas chromatography (*e.g.* GC/ECD, GC/NPD or GC/MS). The study showed that parent ethiprole and its metabolite RPA 112916 are not amenable to gas chromatographic determination since they undergo thermal degradation on the column and/or in the injector. Therefore, the multi-residue method DFG S19 is not suitable for the enforcement of ethiprole-derived residues in plant (or animal) commodities.

Animal commodities

Method 01YQ18289 (enforcement method)

Method 01YQ18289 was developed for the determination of the residues of ethiprole parent, and its metabolite ethiprole-sulfone (RPA 097973) in/on animal matrices (Howell 2001, M-240553-01-2).

The residues are extracted twice with acetonitrile/water (4:1, v/v). After filtration, an aliquot of the extract was evaporated to remove the acetonitrile, then submitted for HR-P cleanup. The column eluate was vacuum-evaporated to dryness and subjected to oxidation, coverting parent ethiprole to the sulfone RPA 097973. The oxidised solution was diluted with water and partitioned with dichloromethane and the latter evaporated to dryness. The residue was dissolved in acetonitrile and quantitated by GC/ED. A confirmatory technique for ethiprole utilises a different GC column.

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples of bovine milk, poultry eggs, poultry muscle, bovine fat and bovine liver were fortified with parent or RPA 097973 at concentrations of 0.01 and 0.10 mg/kg, expressed in parent equivalents. Mean recoveries per fortification level for the primary method for both analytes and all matrices were in a range of 85-115%. Using the confirmatory transition for ethiprole, mean values per fortification level were 79-115% in all matrices except for eggs, at 0.01 mg/kg (123%) and liver at 0.01 mg/kg (69%). In both cases of higher recovery, the RSD was low (11 and 6.1%), so that these higher values were considered to be acceptable. The results are summarised below in Tables 68 and 69.

Table 68 Recoveries for method 01YQ18289: ethiprole in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Range [%]	Mean	RSD [%]	Confirmatory Column		
							Range [%]	Mean	RSD [%]
Bovine milk	Ethiprole	5	0.01	76-124	94	20.5	102-119	109	8.33
		5	0.1	74-105	92	12.3	80-104	95	10.4
Poultry eggs	Ethiprole	5	0.01	97-118	108	8.64	105-138	123	10.8
		5	0.1	78-102	92	10.5	103-115	109	5.11
Poultry muscle	Ethiprole	5	0.01	81-115	100	13.9	103-127	115	8.59
		5	0.1	92-108	102	6.28	96-110	106	5.28
Bovine fat	Ethiprole	5	0.01	102-124	114	8.02	86-93	89	3.03
		5	0.1	96-114	102	7.06	85-93	88	3.65
Bovine liver	Ethiprole	5	0.01	80-94	85	7.16	65-76	69	6.07
		5	0.1	79-102	89	11.2	68-91	79	11.4

Table 69 Recoveries for method 01YQ18289: ethiprole-sulfone (RPA 097973) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Range [%]	Mean	RSD [%]
Bovine milk	RPA 097973	5	0.01	100-128	115	9.18
		5	0.1	88-112	103	9.56
Poultry eggs	RPA 097973	5	0.01	72-105	92	14.1
		5	0.1	87-100	94	5.97
Poultry muscle	RPA 097973	5	0.01	77-115	94	16.0
		5	0.1	90-106	98	6.75
Bovine fat	RPA 097973	5	0.01	106-123	114	6.99
		5	0.1	98-110	105	4.70
Bovine liver	RPA 097973	5	0.01	81-100	88	8.48
		5	0.1	69-99	88	13.1

An independent laboratory validation was performed for method 01YQ18289 (Gould 2009, M-327549-01-1). Samples of bovine fat were fortified with ethiprole parent compound and sulfone RPA 097973 at the nominal fortification levels of 0.10, 0.20 and 1.0 mg/kg, *i.e.* the LOQ and 2× and 10× LOQ, while samples of chicken eggs were fortified with ethiprole parent compound and sulfone RPA at 0.01, 0.02 and 0.10 mg/kg. Crude extracts were oxidised to convert ethiprole to ethiprole sulfone and results are reported as ethiprole sulfone residues.

Analysis of samples was performed according to method 01YQ18289 (Howell 2001, M-240553-01-2). Ethyl acetate was used instead of acetonitrile as the reconstitution solvent, since it is more amenable for GC analysis. The use of the DB 5 column for confirmatory analysis was not investigated during the independent laboratory validation.

For both matrices the mean recoveries were between 85% and 114% (one recovery of 148% for bovine fat fortified at 2 mg/kg was considered to be an outlier), with relative standard deviations of < 15%.

Table 70 Recoveries for method 01YQ18289: ethiprole + RPA 097973 in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Range [%]	Mean	RSD [%]
Bovine fat	Ethiprole + RPA 097973	5	0.2	87-93	90	2.6
		5	0.4	78-107	95	13
		5	2	110-118, 148*	114	3.1
Chicken eggs	Ethiprole + RPA 097973	5	0.02	83-97	88	7.1
		5	0.04	73-95	85	9.2
		5	0.2	99-116	106	6.5

*Considered to be an outlier and not considered in calculations

Method 019-034 (data collection)

A residue analytical method, 019-034, was developed as a data collection method for the determination of the residues of ethiprole (parent compound), and its metabolites ethiprole-sulfone (RPA 097973), ethiprole-methyl sulfone (RPA 094569) and ethiprole-des-chloro-sulfone (RPA 115369) in/on animal matrices (Zheng *et al.,* 1999, M-192648-01-1).

The residues are extracted from animal matrices by homogenising with methanol followed by filtration. The extract is partially purified by liquid-liquid partitioning with hexane. After removal of methanol, the residues are partitioned into dichloromethane for all the matrices except cow milk. After solvent exchange back to methanol, the residues are cleaned by solid-phase extraction. Compounds of interest are eluted with 70% methanol/acetone. The final solution is analysed by HPLC-MS/MS with a multiple ion reaction monitoring (MRM) mode or by LC-MS with a single-ion monitoring mode. As this is a data collection method, only one MRM transition was required.

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples of chicken fat, muscle, liver and egg and cow milk, muscle, fat, liver and kidney, were fortified with parent ethiprole, RPA 097973, RPA 094569 and RPA 115369 at concentrations of 0.001, 0.005 and 0.05 mg/kg (2 tests for each). Mean recoveries (73-116% per fortification level) and RSD values determined for analyte/ matrix combinations (≤ 18.1%) for parent and all metabolites were acceptable.

The correlation between the injected amount of substance and the detector response was linear in the range from 0.25 to 5 ng/mL, using at least 5 different concentration levels, for all compounds. The correlation coefficients were > 0.99 in all cases.

The limit of quantitation (LOQ) for parent ethiprole, RPA 097973, RPA 094569 and RPA 115369, defined as the lowest validated fortification level, was 0.001 mg/kg in all matrices tested.

Table 71 Recoveries for method 019-034: ethiprole in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 330		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Chicken fat	Ethiprole	2	0.001	65, 101	83	-
		2	0.005	93, 94	94	-
		2	0.05	81, 101	91	-
Chicken muscle	Ethiprole	2	0.001	87, 106	97	-
		2	0.005	91, 95	93	-
		2	0.05	84, 98	91	-
Chicken liver	Ethiprole	2	0.001	101, 109	105	-
		2	0.005	110, 111	111	-
		2	0.05	101, 106	104	-
Chicken egg	Ethiprole	2	0.001	74, 76	75	-
		2	0.005	91, 93	92	-
		2	0.05	97, 110	104	-
Cow milk	Ethiprole	2	0.001	71, 88	80	-
		2	0.005	80, 97	89	-
		2	0.05	85, 90	88	-
Cow muscle	Ethiprole	2	0.001	80, 92	86	-
		2	0.005	81, 81	81	-
		2	0.05	78, 89	84	-

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 395 → 330		
				Range [%]	Mean	RSD [%]
Cow fat	Ethiprole	2	0.001	86, 102	94	-
		2	0.005	84, 87	86	-
		2	0.05	89, 90	90	-
Cow liver	Ethiprole	2	0.001	84, 96	90	-
		2	0.005	87, 101	94	-
		2	0.05	85, 92	89	-
Cow kidney	Ethiprole	2	0.001	110, 122	116	-
		2	0.005	88, 92	90	-
		2	0.05	90, 101	96	-

Table 72 Recoveries for method 019-034: ethiprole-sulfone (RPA 097973) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 411 → 375		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Chicken fat	RPA 097973	2	0.001	81, 109	95	-
		2	0.005	98, 106	102	-
		2	0.05	87, 109	98	-
Chicken muscle	RPA 097973	2	0.001	88, 90	89	-
		2	0.005	93, 99	96	-
		2	0.05	97, 99	98	-
Chicken liver	RPA 097973	2	0.001	100, 105	103	-
		2	0.005	110, 115	113	-
		2	0.05	105, 113	109	-
Chicken egg	RPA 097973	2	0.001	76, 88	82	-
		2	0.005	92, 93	93	-
		2	0.05	98, 104	101	-
Cow milk	RPA 097973	2	0.001	95, 105	100	-
		2	0.005	96, 97	97	-
		2	0.05	97, 97	97	-
Cow muscle	RPA 097973	2	0.001	87, 93	90	-
		2	0.005	79, 82	81	-
		2	0.05	84, 92	88	-
Cow fat	RPA 097973	2	0.001	101, 105	103	-
		2	0.005	89, 102	96	-
		2	0.05	92, 102	97	-
Cow liver	RPA 097973	2	0.001	79, 90	85	-
		2	0.005	86, 90	88	-
		2	0.05	85, 90	88	-
Cow kidney	RPA 097973	2	0.001	94, 96	95	-
		2	0.005	90, 91	91	-
		2	0.05	93, 100	97	-

Table 73 Recoveries for method 019-034: ethiprole-methyl sulfone (RPA 094569) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 397 → 361		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Chicken fat	RPA 094569	2	0.001	79, 99	89	-
		2	0.005	92, 100	96	-
		2	0.05	84, 102	93	-
Chicken muscle	RPA 094569	2	0.001	93, 99	96	-
		2	0.005	93, 99	96	-
		2	0.05	97, 100	99	-

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 397 → 361		
				Range [%]	Mean	RSD [%]
Chicken liver	RPA 094569	2	0.001	99, 99	99	-
		2	0.005	106, 111	109	-
		2	0.05	104, 110	107	-
Chicken egg	RPA 094569	2	0.001	70, 76	73	-
		2	0.005	93, 99	96	-
		2	0.05	107, 112	110	-
Cow milk	RPA 094569	2	0.001	76, 87	82	-
		2	0.005	73, 89	81	-
		2	0.05	85, 89	87	-
Cow muscle	RPA 094569	2	0.001	88, 89	89	-
		2	0.005	69, 86	78	-
		2	0.05	77, 88	83	-
Cow fat	RPA 094569	2	0.001	93, 102	98	-
		2	0.005	90, 98	94	-
		2	0.05	95, 101	98	-
Cow liver	RPA 094569	2	0.001	73, 86	80	-
		2	0.005	83, 83	83	-
		2	0.05	91, 93	92	-
Cow kidney	RPA 094569	2	0.001	91, 105	98	-
		2	0.005	87, 88	88	-
		2	0.05	92, 98	95	-

Table 74 Recoveries for method 019-034: ethiprole-des-chloro-sulfone (RPA 115369) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 377 → 341		
				Range [%]	Mean	RSD [%]
				HPLC-MS/MS		
Chicken fat	RPA 115369	2	0.001	80, 103	92	-
		2	0.005	92, 97	95	-
		2	0.05	85, 99	92	-
Chicken muscle	RPA 115369	2	0.001	92, 99	96	-
		2	0.005	90, 94	92	-
		2	0.05	91, 95	93	-
Chicken liver	RPA 115369	2	0.001	103, 108	106	-
		2	0.005	111, 118	115	-
		2	0.05	105, 111	108	-
Chicken egg	RPA 115369	2	0.001	74, 81	78	-
		2	0.005	85, 90	88	-
		2	0.05	96, 104	100	-
Cow milk	RPA 115369	2	0.001	79, 86	83	-
		2	0.005	76, 79	78	-
		2	0.05	83, 89	86	-
Cow muscle	RPA 115369	2	0.001	91, 92	92	-
		2	0.005	75, 81	78	-
		2	0.05	84, 89	87	-
Cow fat	RPA 115369	2	0.001	98, 105	102	-
		2	0.005	94, 99	97	-
		2	0.05	94, 104	99	-
Cow liver	RPA 115369	2	0.001	81, 92	87	-
		2	0.005	83, 87	85	-
		2	0.05	88, 92	90	-
Cow kidney	RPA 115369	4	0.001	96, 96	96	-
		2	0.005	85, 94	90	-
		4	0.05	90, 100	95	-

Method 01431 (data collection)

A residue analytical method, 01431, was developed as a data collection method for the determination of the residues of ethiprole (parent compound), and its metabolites ethiprole-sulfone (RPA 097973), ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) in/on animal tissues, milk and eggs (Glaubitz and Kuppels 2015, M-543785-01-1).

The residues are extracted from animal matrices by shaking with methanol (3×) followed by filtration. After addition of internal standards the combined extracts were made up to 100 mL with an aqueous solution of 0.1 mL/L formic acid. The extracts were further diluted by a factor of 5 (tissues and eggs) or 2.5 (milk) and then analysed by HPLC-MS/MS. The use of HPLC-MS/MS with two independent mass transitions for quantitation and confirmatory analysis (qualification) allow a high degree of specificity.

The accuracy of the method was assessed on the basis of the determined recovery rates. Samples of hen eggs and cow milk, muscle, fat, liver and kidney were fortified with parent ethiprole, RPA 097973, RPA 104615 and RPA 107566 at concentrations of the LOQ (0.005 mg/kg for milk and 0.01 mg/kg for the other matrices) and 10× LOQ (5 recovery tests for each). Metabolite levels were expressed in parent equivalents. Mean recoveries and RSD values per fortification level for parent and metabolites were generally acceptable.

The correlation between the injected amount of substance and the detector response was linear in the range from 0.025 to 2.50 µg/L for ethiprole and 0.025 to 5 µg/L for the metabolites RPA 097973, RPA 104615 and RPA 107566. The correlation coefficients were > 0.99 in all cases.

The limit of quantitation (LOQ) for parent ethiprole, RPA 097973, RPA 104615 and RPA 107566, defined as the lowest validated fortification level, was 0.01 mg/kg in eggs, muscle, fat, liver and kidney and 0.005 mg/kg in milk.

Table 75 Recoveries for method 01431: Ethiprole in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 397 → 255			Confirmatory Transition 397 → 351		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Hen egg	Ethiprole	5	0.01	93-102	99	3.7	99-107	102	3.1
		5	0.10	102-110	106	3.6	104-111	108	3.0
Cow milk	Ethiprole	5	0.005	92-105	97	5.2	101-125	108	8.8
		5	0.05	100-112	105	4.5	97-113	106	5.5
Cow muscle	Ethiprole	5	0.01	95-107	101	4.6	101-107	104	2.3
		5	0.10	103-109	105	2.5	104-113	109	3.9
Cow fat	Ethiprole	5	0.01	92-100	96	3.3	92-104	98	5.0
		5	0.10	103-109	107	2.2	97-112	107	5.6
Cow liver	Ethiprole	5	0.01	98-102	100	1.5	94-103	101	3.9
		5	0.10	100-105	102	1.8	100-104	102	2.0
Cow kidney	Ethiprole	5	0.01	96-101	99	2.1	99-104	101	2.1
		5	0.10	98-105	102	2.9	100-105	102	1.8

Table 76 Recoveries for method 01431: ethiprole-sulfone (RPA 097973) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 413 → 213			Confirmatory Transition 413 → 324		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Hen egg	RPA 097973	5	0.01	98-103	100	2.0	101-111	104	3.9
		5	0.10	102-106	104	1.7	105-110	107	1.7
Cow milk	RPA 097973	5	0.005	82-100	94	7.7	91-105	96	6.2
		5	0.05	94-104	99	3.7	91-104	97	5.7
Cow muscle	RPA 097973	5	0.01	87-100	94	5.2	99-120	105	8.4
		5	0.10	102-110	106	2.7	106-111	108	2.2
Cow fat	RPA 097973	5	0.01	97-103	99	2.5	90-107	99	6.4
		5	0.10	99-106	102	2.6	93-101	97	3.1
Cow liver	RPA 097973	5	0.01	87-112	98	9.5	96-114	104	6.8
		5	0.10	100-112	106	4.1	101-110	104	3.4
Cow kidney	RPA 097973	5	0.01	97-107	100	4.3	90-103	98	5.4
		5	0.10	98-104	102	2.4	69-117	98	18.1

Table 77 Recoveries for method 01431: ethiprole-sulfonic acid (RPA 104615) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 399 → 283			Confirmatory Transition 399 → 183		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Hen egg	RPA 104615	5	0.01	93-109	102	6.8	103-115	110	4.0
		5	0.10	100-109	103	3.6	110-133	118	8.3
Cow milk	RPA 104615	5	0.005	79-96	90	7.5	83-117	102	15.5
		5	0.05	90-98	95	3.4	89-107	97	6.9
Cow muscle	RPA 104615	5	0.01	95-102	97	2.9	79-139	101	22.9
		5	0.10	100-103	101	1.3	96-116	103	7.9
Cow fat	RPA 104615	5	0.01	88-95	91	3.0	81-123	97	16.4
		5	0.10	91-103	97	4.5	90-100	95	4.6
Cow liver	RPA 104615	5	0.01	90-107	98	7.5	76-133	98	24.4
		5	0.10	81-106	98	10.1	97-145	111	17.8
Cow kidney	RPA 104615	5	0.01	96-107	100	4.2	92-141	109	18.2
		5	0.10	96-116	102	8.0	88-114	102	10.8

Table 78 Recoveries for method 01431: ethiprole-sulfide (RPA 107566) in animal matrices

Matrix	Analyte	No. of tests	Spiking level [mg/kg]	Transition 381 → 265			Confirmatory Transition 381 → 92		
				Range [%]	Mean	RSD [%]	Range [%]	Mean	RSD [%]
				HPLC-MS/MS			HPLC-MS/MS		
Hen egg	RPA 107566	5	0.01	98-116	106	6.7	98-116	108	7.0
		5	0.10	100-110	106	3.8	90-108	99	6.4
Cow milk	RPA 107566	5	0.005	88-100	93	5.3	88-113	96	10.7
		5	0.05	91-104	98	5.2	93-106	99	5.0
Cow muscle	RPA 107566	5	0.01	94-112	102	6.6	90-115	103	8.8
		5	0.10	103-112	106	3.5	95-107	102	4.6
Cow fat	RPA 107566	5	0.01	92-110	102	6.9	82-107	94	9.5
		5	0.10	96-106	102	4.6	93-108	101	5.5
Cow liver	RPA 107566	5	0.01	89-103	97	5.5	93-121	106	9.5
		5	0.10	103-111	105	3.2	97-102	99	2.3
Cow kidney	RPA 107566	5	0.01	82-104	95	8.6	91-109	100	6.8
		5	0.10	100-102	101	0.8	93-103	99	3.8

The extraction efficiency of the residue method 01431 for the determination of the relevant residues of ethiprole in animal matrices, consisting of the parent compound and its metabolites ethiprole-sulfone (RPA 097973), ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566), was investigated (Glaubitz 2016, M-570514-01-1).

Cream, muscle, fat, liver and kidney samples from the ethiprole cow feeding study and eggs from the hen feeding study were extracted as in method 01431, as well as according to the procedure described in the ethiprole goat metabolism study. Samples were chosen because they were found to contain resdues of ethiprole and its metabolites above the limit of quantitation (LOQ = 0.005 mg/kg in cream and 0.01 mg/kg in tissues and eggs).

In method 01431 the samples are extracted with three portions of methanol using an overhead shaker while in the goat metabolism study the samples were extracted with three portions of methanol using an ultra turrax.

The extracts obtained using both procedures were analysed by HPLC-MS/MS following addition of appropriate isotopically labelled internal standards, using the same approach as for method 01431. Each matrix was analysed at least four times using each extraction procedure. Regardless of the method the analyte ethiprole-sulfone was found in cream, egg, muscle, fat, liver and kidney at levels above the LOQ (0.005 mg/kg in cream and 0.01 mg/kg in tissues and egg) so the extraction efficiency can be estimated based on the analysis of all relevant matrices. Parent ethiprole was found at levels above the LOQ in cream, egg and fat only so the extraction efficiency was estimated on the basis of analyses of only these three matrices. Similarly, residues of ethiprole-sulfide ≥ LOQ were only observed in cream, so the extraction efficiency was only evaluated based on this matrix. Ethiprole-sulfonic acid residues ≥ LOQ were not found in any matrix, so the extraction efficiency of method 01431 could not be determined for this analyte.

Satisfactory extraction efficiences (in the range of 90-111%) were obtained for ethiprole, ethiprole-sulfone and ethiprole-sulfide in animal commodities using the method 01431 (Table 79).

Table 79 Extraction efficiencies for ethiprole, ethiprole-sulfone (RPA 097973) and ethiprole-sulfide (RPA 107566) using method 01431

Analyte	Matrix	Extraction efficiency (%) Method 01431
Ethiprole	Cream	101.8
	Egg	93.3
	Fat	101.4
Ethiprole-sulfone (RPA 097973)	Cream	110.8
	Egg	89.8
	Muscle	104.0
	Fat	100.1
	Liver	102.3
	Kidney	101.7
Ethiprole-sulfide (RPA 107566)	Cream	106.8

Stability of pesticide residues in stored analytical samples

Plant matrices

To determine the freezer storage stability of the residues of ethiprole, ethiprole-sulfone (RPA 097973) and ethiprole-amide (RPA 112916) in plant materials, individual 5g control samples of homogenised sugarcane stalks (high water content), soya bean seed (high oil content commodity), dry bean seed (high protein content commodity), wheat grain (high starch content commodity) and citrus fruit (acidic fruit) were fortified individually (one analyte spiked to each sample) resulting in a fortification level of 0.10 mg/kg of ethiprole and its metabolites ethiprole-sulfone and ethiprole-amide (both expressed as parent equivalents) (Sarti 2016, M-551442-01-1). Except for the day-0 analysis, samples were stored in amber glass bottles in a freezer at an average temperature of -18 ± 2 °C until analysis.

For day-0 analysis, the series for each matrix and analyte consisted of one control sample, 5 fortified samples, one freshly fortified sample at 0.01 mg/kg (LOQ) and one freshly fortified sample at 0.10 mg/kg (10×LOQ).

Fortified control samples were taken out of storage for analysis after about 1, 3, 6, 12-13, 18 and 24 months of storage to determine recoveries from fortified samples. At each of these intervals, unfortified samples were taken out of storage and freshly fortified with ethiprole, RPA 097973 or RPA 112916 in order to determine concurrent recoveries. The analytical series for each matrix and analyte consisted of one stored control sample, 3 stored fortified samples and 2 freshly fortified samples at 0.10 mg/kg.

Analyses of ethiprole, RPA 097973 and RPA 112916 were carried out using method 01128. The storage stability data for ethiprole and its metabolites are summarised below in Table 80.

Table 80 Stability of ethiprole-derived residues in plant commodities following storage at -18 ± 2 °C

Commodity	Level (mg/kg)	Storage interval (days)	Ethiprole			RPA 097973 (Ethiprole-sulfone)			RPA 112916 (Ethiprole-amide)		
			Concurrent recovery (mean, %)	Recovery		Concurrent recovery (mean, %)	Recovery		Concurrent recovery (mean, %)	Recovery	
				Mean % Nominal spiking level	% Day 0		Mean % Nominal spiking level	% Day 0		Mean % Nominal spiking level	% Day 0
Sugarcane (stalk)	0.10	0	85	83	100	85	84	100	94	88	100
		30	101	92	111	95	88	105	98	91	103
		90	91	75	90	91	83	99	84	75	85
		181	97	84	101	88	83	99	100	87	99
		400	92	85	102	94	90	107	101	74	84
		547	95	84	101	96	90	107	98	75	85

Commodity	Level (mg/kg)	Storage interval (days)	Ethiprole			RPA 097973 (Ethiprole-sulfone)			RPA 112916 (Ethiprole-amide)		
			Concurrent recovery (mean, %)	Recovery		Concurrent recovery (mean, %)	Recovery		Concurrent recovery (mean, %)	Recovery	
				Mean % Nominal spiking level	% Day 0		Mean % Nominal spiking level	% Day 0		Mean % Nominal spiking level	% Day 0
		756	93	83	100	93	88	105	95	74	84
Soya bean (seed)	0.10	0	100	102	100	93	96	100	100	96	100
		29	103	103	101	112	101	105	102	99	103
		91	95	97	95	102	101	105	102	91	95
		182	112	111	109	101	105	109	98	97	101
		358	111	96	94	117	97	101	103	94	98
		542	85	95	93	108	101	105	97	90	94
		738	103	100	98	108	98	102	98	89	93
Dry bean (seed)	0.10	0	90	85	100	88	92	100	90	90	100
		32	87	98	115	86	97	105	85	83	92
		92	98	99	116	96	96	104	97	96	107
		181	94	92	108	97	91	99	99	96	107
		371	91	92	108	95	100	109	105	94	104
		558	97	96	113	103	98	107	112	93	103
		728	105	90	106	107	105	114	102	95	106
Wheat grain	0.10	0	91	84	100	95	90	100	93	92	100
		31	97	102	121	96	97	108	97	95	103
		90	91	95	113	89	100	111	95	96	104
		186	103	96	114	100	97	108	101	92	100
		367	92	107	127	91	100	111	99	89	97
		552	88	101	120	88	109	121	98	89	97
		724	96	97	115	103	104	116	104	82	89
Citrus (fruit)	0.10	0	90	90	100	100	88	100	91	90	100
		31	101	102	113	99	94	107	100	96	107
		91	93	89	99	94	88	100	97	93	103
		176	96	91	101	99	97	110	103	94	104
		373	96	97	108	95	97	110	94	89	99
		526	94	99	110	94	97	110	94	88	98
		730	93	93	103	92	92	105	92	82	91

The concurrent recoveries for individual matrices and storage intervals were in the range of 85-112% for ethiprole, 85-117% for RPA 097973 and 84-112% for RPA 112916. The corresponding average recoveries from stored fortified samples were in the range of 75-111% for ethiprole, 83-109% for RPA 097973 and 74-99% for RPA 112916. Overall the data demonstrate that the residues of ethiprole, RPA 097973 and RPA 112916 are stable in all tested plant matrices for at least 24 months when stored at -18 ± 2 °C.

Cotton processed commodities

As part of a cotton processing study, the frozen storage stability of ethiprole, and its metabolites RPA 097973 (ethiprole-sulfone), RPA 112916 (ethiprole-amide) and RPA 115369 (monochloroaryl analogue of ethiprole-sulfone) was investigated in cottonseed, cotton gin trash, cottonseed meal, cotton hulls and refined oil (Mackie 2001, M-238783-01-2). Control samples of the various cotton commodities were fortified at 0.03 mg/kg with parent and the metabolites and placed in frozen storage at ≤ -10 °C. The storage stability was determined by analysing samples immediately after fortification and after 1, 3, 6, 9 and 12 months of storage. At each storage interval, one control sample, two stored fortified samples and one freshly fortified sample were analysed for each compound. The analyses were conducted by LC-MS/MS according to method R016922 that was specifically developed for the determination of ethiprole-derived residues in processed commodities. The LOQ was established at 0.001 mg/kg for each analyte in each matrix.

An overview of the storage stability results is presented in Table 81. The average recoveries determined in the fortified samples stored for 12 months ranged between 62% and 104%. The low average recovery of 62% was calculated for parent ethiprole in meal. It is due to a low recovery of 38% in one of the two replicate samples. The other meal sample analysed at the same storage interval gave an acceptable recovery of 85% for ethiprole.

It was concluded that the residues of ethiprole, RPA 097973, RPA 112916 and RPA 115369 are stable under frozen storage (≤ -10 °C) for at least 12 months in cotton seed, gin trash, hulls, meal and oil.

Table 81 Stability of ethiprole-derived residues in cotton commodities following storage at ≤ -10 °C

Commodity	Storage Interval (months)	Recovered residues (%)							
		Ethiprole		RPA 097973 (Ethiprole-sulfone)		RPA 112916 (Ethiprole-amide)		RPA 115369 (Monochloroaryl analogue of ethiprole-sulfone)	
		Stored	Con-current	Stored	Con-current	Stored	Con-current	Stored	Con-current
Cotton seed	0	83	95	91	98	79	95	85	106
	1	96	151	95	99	74	88	98	110
	3	87	97	79	91	67	70	78	91
	6	71	88	74	91	72	83	69	96
	9	86	106	76	100	58	97	81	105
	12	101	101	79	97	80	85	89	98
Cotton gin trash	0	77	77	99	94	56	68	95	99
	1	121	122	94	106	84	85	104	110
	3	79	88	64	86	72	78	68	86
	6	83	81	67	74	71	81	80	79
	9	110	96	89	87	98	91	99	97
	12	99	99	87	98	80	89	92	103
Cotton hulls	0	88	82	101	82	78	62	86	81
	1	91	89	102	96	85	73	94	95
	3	86	89	82	77	85	73	82	85
	6	74	80	90	95	81	77	81	89
	9	84	101	75	104	87	102	90	118
	12	76	79	84	87	74	93	87	79
Cottonseed meal	0	105	108	109	112	82	81	107	109
	1	101	100	95	95	84	75	95	89
	3	83	80	79	86	68	72	80	84
	6	95	83	95	98	81	88	94	94
	9	117	107	113	73	105	125	115	120
	12	62	71	83	80	78	87	82	94
Cotton oil	0	115	100	104	113	73	84	115	110
	1	106	91	95	98	82	74	103	99
	3	111	70	81	114	80	67	110	92
	6	105	92	86	112	90	94	103	104
	9	101	89	87	135	91	98	126	119
	12	104	114	91	94	92	80	104	101

Orange processed commodities

As part of an orange processing study, the storage stability of ethiprole, and its metabolites RPA 097973 (ethiprole-sulfone), RPA 112916 (ethiprole-amide) and RPA 115369 (ethiprole-deschloro-sulfone) was investigated in orange fruit, orange juice, orange dry pulp and orange oil (Gough 2002, M-238818-02-2). Control samples of the various orange commodities were fortified at 0.05 mg/kg with parent and the metabolites and placed in frozen storage at ≤ -10 °C. The storage stability was determined by analysing samples immediately after fortification and after 1, 3, 6, 9, 12-14 and 16 months of storage. At each storage interval, one control sample, two stored fortified samples and two freshly fortified samples were analysed for each compound. The analyses were conducted by LC-MS/MS according to method R016922. The LOQ was established at 0.001 mg/kg for each analyte in each matrix.

The results of the study are shown in the following table. The average recoveries determined in the stored fortified samples after 16 months of storage ranged between 93-122%. It was concluded that residues of ethiprole, RPA 097973, RPA 112916 and RPA 115369 are stable under frozen storage (≤ -10 °C) for at least 16 months in orange fruit, juice, dry pulp and oil.

Table 82 Stability of ethiprole-derived residues in orange following storage at ≤ -10 °C

Commodity	Storage Interval (months)	Recovered residues (%)*							
		Ethiprole		RPA 097973 (Ethiprole-sulfone)		RPA 112916 (Ethiprole-amide)		RPA 115369 (ethiprole-deschloro- sulfone)	
		Stored	Con-current	Stored	Con-current	Stored	Con-current	Stored	Con-current
Orange fruit	0	87	75	94	82	86	73	90	83
	1	96	100	95	103	97	89	92	104
	3	91	102	85	103	80	88	89	107
	6	73	81	77	85	73	74	75	84
	9	99	105	99	103	91	93	99	106
	12	81	96	81	105	82	90	96	105
	16	110	97	103	91	108	106	106	94
Orange juice	0	92	91	92	95	86	89	97	97
	1	85	91	91	94	87	86	94	96
	3	89	97	94	97	87	85	89	94
	6	80	82	75	87	74	91	85	84
	9	102	107	106	106	80	112	100	106
	12	96	112	93	105	87	110	93	103
	16	108	109	107	99	107	105	109	109
Orange dry pulp	0	99	102	97	96	84	84	97	96
	1	83	101	93	101	79	77	85	101
	3	99	107	99	107	81	84	82	86
	6	78	77	83	80	77	73	77	81
	9	102	114	109	106	90	97	94	101
	12	89	107	88	112	81	92	93	79
	16	118	111	93	84	109	103	114	101
Orange oil	0	124	113	85	81	77	78	93	81
	1	116	107	95	95	67	78	113	107
	3	135	100	104	119	85	80	105	94
	6	86	73	101	75	85	76	87	77
	9	122	92	110	105	113	107	114	107
	12	116	113	101	106	107	83	104	98
	16	118	116	104	104	107	98	122	120

*Stored and concurrent recoveries were the mean of two samples

Rice and tea

The freezer storage stability of ethiprole and its metabolite RPA 097973 (ethiprole-sulfone) was investigated in rice grain and tea leaves (Miller 2010, M-368556-01-1). Control samples of rice grain and tea leaves were fortified separately with 0.10 mg/kg of parent ethiprole and RPA 097973 and placed in frozen storage at an average temperature of -23 °C. The storage stability was determined by analysing samples immediately after fortification and after 1, 3, 6, 9 and 12 months of storage. At each storage interval, one control sample, two stored fortified samples and one or two freshly fortified samples were analysed for each compound.

The analyses were conducted by LC-MS/MS according to method 01053. The LOQ was established at 0.002 mg/kg for each analyte in each matrix.

An overview of the storage stability results is presented in the following table. Based on the average recoveries determined in the stored fortified samples no degradation of ethiprole and RPA 097973 was observed during frozen storage. The data show an apparent increase of the ethiprole and RPA 097973 levels but this evolution is paralleled by the increase of the corresponding concurrent recoveries. At the end of the 12 month storage period the average recoveries determined in the stored fortified samples ranged between 107% and 132%. After correction for concurrent recoveries from freshly fortified samples, these recoveries ranged between 101% and 108%. It was concluded that the residues of ethiprole and RPA 097973 are stable under frozen storage (≤ -23 °C) for at least 12 months in rice grain and tea leaves.

Table 83 Stability of ethiprole and RPA 097973 residues in rice grain and tea leaves following storage at -23 °C

Commodity	Level (mg/kg)	Storage interval (days)	Ethiprole				RPA 097973 (Ethiprole-sulfone)			
			Fresh recovery (mean, %)	Recovery			Fresh recovery (mean, %)	Recovery		
				% Nominal spiking level	% Day 0	Corrected % stability		% Nominal spiking level	% Day 0	Corrected % stability
Rice grain	0.10	0	91	91	-	-	98	97	-	-
		30	97	98	108	101	101	103	106	102
		90	93	97	107	103	99	101	103	102
		181	113	145	159	129	97	108	110	110
		272	102	107	118	105	105	111	114	106
		365	111	119	131	108	106	107	110	101
Tea leaves	0.10	0	81	81	-	-	84	84	-	-
		30	97	81	101	84	80	83	99	103
		90	75	85	106	114	81	76	90	93
		181	99	108	134	109	94	98	117	104
		272	107	128	159	120	127	131	155	102
		365	126	132	163	104	115	123	146	107

Corrected stability: (% recovery of stored samples/ average % concurrent recovery) × 100

Animal matrices

To determine the stability of ethiprole and its metabolites (RPA 097973, RPA 104615 and RPA 107566) in sample extracts, the 10× LOQ sample extracts from the initial validation analyses for method 01431 were re-analysed after a storage period of at least 12 days and then again after at least 26 days at 5 °C ± 3 °C (Glaubitz and Kuppels 2015, M-543785-01-1). Mean recoveries after storage for all four analytes ranged from 94-107%. In hen eggs, cow muscle, cow liver and cow kidney all analytes were found to be stable for at least 29 days, while in cow milk and cow fat, all analytes were found to be stable for at least 26 days.

USE PATTERN

Information on registered uses made available to this Meeting is shown in Table 84.

Table 84 Registered uses of ethiprole on cereal grains (rice) and coffee

Crop	Country	Formulation		Application				PHI [days]
		g ai/L or [g ai/kg]	Type	Method	Timing [Interval – days]	Rate [g ai/ ha] (g ai/100L)	Season Max. [g ai/ ha/year] or (no. per crop)	
Cereal Grains								
Rice	Brazil	200	SC	Foliar	-	50 (25)	[50] (1)	75
Rice	India	[400]	WG	Foliar	-	50	[150] (3)	15
Rice	Indonesia	100	SC	Foliar	-	125	[a]	20
Rice	Japan	0.5%	DP	Foliar	-	200	(2)	14
Rice	Japan	2%	GR	Submerged spraying	-	600	(2, 1 up to transplanting)	14
Rice	Japan	10%	SC	Foliar	-	200	(2, 1 up to	14

Crop	Country	Formulation		Application				PHI [days]
		g ai/L or [g ai/kg]	Type	Method	Timing [Interval – days]	Rate [g ai/ ha] (g ai/100L)	Season Max. [g ai/ ha/year] or (no. per crop)	
				Aerial spray (ULV)	-	100	transplanting)	
Rice	Thailand	100	SC	Foliar	-	94 (25)	[b]	14
Coffee								
Coffee	Brazil	200	SC	Foliar	30	500	[1000] (2)	60
Coffee	El Salvador	100	SC	Foliar	45	200	[400] (2)	90
Coffee	Guatemala	100	SC	Foliar	45	200	[400] (2)	90
Coffee	Honduras	100	SC	Foliar	45	200	[400] (2)	90

[a] Maximum number of applications are not given on the label. The label says to follow the local recommendation.

[b] Maximum number of applications are not given on the label.

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on supervised trials for the uses of ethiprole on cereals (rice), and coffee.

Trials were well documented with laboratory and field reports. The former included method validation including recoveries with spiking at residue levels similar to those occurring in samples from the supervised trials. Dates of analyses or duration of sample storage were also provided. Samples were collected and stored frozen immediately or soon after sampling. Although trials included control plots, no control data are recorded in the Tables because, unless noted, residues in control samples did not exceed the LOQ. Residues are unadjusted for recoveries.

Residues from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels and dietary risk assessment and are underlined. If a higher residue level was observed at a longer PHI than the GAP, the higher value has been used in MRL setting and dietary risk assessment. Where residues were not detected or less than the LOQ they are shown as below the LOQ (*i.e.* <0.002 mg/kg) and the LOQ value was utilised for maximum residue level estimation and dietary intake assessment.

For replicate samples (from the same plot), the mean value was used for maximum residue level estimation and dietary intake assessment. Parent, ethiprole-sulfone and ethiprole-amide residues were added as unrounded values. The results of these supervised trials are shown in the following tables:

Group	Commodity	Country/ Countries	Table No.
GC Cereal Grains	Rice	China and Thailand	85
	Rice	India	86
SB Seeds for Beverages and Sweets	Coffee Beans	Brazil	87
	Coffee Beans	Colombia, Costa Rica, Mexico	88

Cereal Grains

Supervised trials were carried out on rice (9 trials –Table 85) in Thailand (7 trials) and China (2) during the 2007 or 2008 growing seasons in order to determine residues after foliar applications of ethiprole (Balluff 2008, M-308810-02-2). Four foliar applications (three in trial TH07W006R due to early ripening) were made at approximately 14 day retreatment intervals using Ethiprole 100 SC. All foliar applications were made using ground-based equipment with no adjuvant. Trial TH07W001R also contains a processing study, in which applications were made at double rate, which is discussed in the relevant section of this evaluation.

Samples were taken at 0 (plant and panicle) and 14-16 (grain) days after the last application. Plant samples were cut approximately 15 cm above the ground while panicles were cut directly below the panicle allowing the flag leaf. Grain samples were obtained by threshing manually or using a combiner (CN07W001R, CN07W002R). Samples were deep-frozen before analysis.

Residues of ethiprole and ethiprole sulfone in rice were determined using LC-MS/MS Method 01053. Acceptable concurrent recovery data were obtained for both analytes (ethiprole in rice grain and polished rice: spike 0.002 mg/kg, n = 5, range of recoveries 69-110%, mean recovery 90%, RSD 17%; spike 0.02 mg/kg, n = 3, range of recoveries 88-116%, mean recovery 99%, RSD 15%; spike 2 mg/kg, n = 1, 79%; overall result, mean recovery 92%, RSD = 16%), (ethiprole in rice plant, panicle, hulls and bran: spike 0.002 mg/kg, n = 2, 99 and 107%, mean recovery 103%; spike 0.02 mg/kg, n = 2, 79 and 103%, mean recovery 91%; spike

0.2 mg/kg, n = 1, 103%; overall result, mean recovery 98%, RSD = 11%; also spike 10 mg/kg, n = 3, range of recoveries 71-81%, mean recovery 75%, RSD = 7%); (ethiprole sulfone in rice grain and polished rice: spike 0.002 mg/kg, n = 5, range of recoveries 91-118%, mean recovery 108%, RSD 10%; spike 0.02 mg/kg, n = 3, range of recoveries 91-116%, mean recovery 102%, RSD 13%; spike 2 mg/kg, n = 1, 86%; overall result, mean recovery 103%, RSD = 12%), (ethiprole sulfone in rice plant, panicle, hulls and bran: spike 0.002 mg/kg, n = 2, 114 and 118%, mean recovery 116%; spike 0.02 mg/kg, n = 2, 86 and 91%, mean recovery 89%; spike 0.2 mg/kg, n = 1, 79%; overall result, mean recovery 98%, RSD = 18%; also spike 10 mg/kg, n = 3, range of recoveries 63-69%, mean recovery 66%, RSD = 5%).

Table 85 Residues in rice from the foliar application of ethiprole to rice in China and Thailand

Trial No., Location Year Variety	Application				Sample	DALA	Residues as ethiprole equivalents (mg/kg)		
	No. (RTI, days)	Growth Stage	Rate (g ai/ha)	Volume (L/ha)			Parent	RPA 097973 (Ethiprole sulfone)	Sum of parent + RPA 097973
GAP, Thailand, Rice			94			14			
CN07W001R, Juncheng Village, Zhejiang Province, China, 2007 (Chunjiang 683)	4 (14)	65 n.a. 77 87	106 108 108 110	689 699 700 712	Plant	0	13	2.0	15
					Panicle	0	17	1.3	18
					Grain	14	1.3	0.42	
							1.2	0.35	
							1.3 (mean)	0.39 (mean)	1.6
CN07W002R, Paitou, Zhejiang Province, China, 2007 (Jishou 09)	4 (14)	73 74 77 87	110 107 104 107	713 696 677 697	Plant	0	12	1.8	14
					Panicle	0	13	1.2	14
					Grain	14	1.3	0.34	
							1.3	0.37	
							1.3 (mean)	0.36 (mean)	1.7
TH07W001R, Pathumthani Province, Thailand, 2008 (Suphan Buri 1)	4 (14)	37 47 69 77	100 98 103 103	250 246 258 258	Plant	0	2.8	0.21	3.0
					Panicle	0	5.8	0.19	6.0
					Grain	16	0.33	0.22	
							0.29	0.21	
							0.31 (mean)	0.22 (mean)	0.53
TH07W002R, Prachinburi Province, Thailand, 2008 (Suphan Buri 1)	4 (14, 14, 13)	37 53 69 85	96 101 95 92	240 252 238 230	Plant	0	4.9	0.60	5.5
					Panicle	0	5.4	0.53	5.9
					Grain	14	0.41	0.26	
							0.43	0.27	
							0.42 (mean)	0.27 (mean)	0.69
TH07W003R, Phitsanulok Province, Thailand, 2008 (RD29)	4 (14)	39 47 61 69	102 101 102 99	254 252 256 248	Plant	0	2.6	0.65	3.3
					Panicle	0	6.0	0.32	6.3
					Grain	14	0.38	0.33	
							0.43	0.38	
							0.41 (mean)	0.36 (mean)	0.76
TH07W004R, Chachoengsao Province, Thailand, 2008 (Phitsanulok 2)	4 (14, 14, 13)	32 36 53 77	101 91 101 98	252 228 252 246	Plant	0	4.2	0.16	4.4
					Panicle	0	5.7	0.11	5.8
					Grain	14	0.17	0.13	
							0.17	0.12	
							0.17 (mean)	0.13 (mean)	0.30
TH07W005R, Lopburi Province, Thailand, 2008 (Pathum Thani No. 1)	4 (14, 14, 13)	37 61 75 77-83	96 96 92 101	240 240 230 252	Plant	0	5.3	0.29	5.6
					Panicle	0	6.8	0.17	7.0
					Grain	15	0.10	0.06	
							0.12	0.08	
							0.11 (mean)	0.07 (mean)	0.18
TH07W006R, Ratchaburi Province, Thailand, 2008 (Chai Nat 1)	3 (14)	39 65 n.a.	108 93 99	269 232 248	Panicle	0	0.39	0.86	1.3
					Grain	14	0.41	0.36	
							0.44	0.38	
							0.43 (mean)	0.37 (mean)	0.80
TH07W007R,	4	28	99	248	Plant	0	2.9	0.27	3.2

Trial No., Location Year Variety	Application				Sample	DALA	Residues as ethiprole equivalents (mg/kg)		
	No. (RTI, days)	Growth Stage	Rate (g ai/ha)	Volume (L/ha)			Parent	RPA 097973 (Ethiprole sulfone)	Sum of parent + RPA 097973
Suphanburi Province, Thailand, 2008 (Not reported)	(14, 15, 12)	43 69 85	102 109 98	254 272 244	Panicle	0	5.5	0.19	5.7
					Grain	14	0.21	0.16	
							0.18	0.13	
							0.20 (mean)	0.15 (mean)	0.34

LOQ is 0.002 mg/kg for each of parent ethiprole and the metabolite RPA 097973 (Ethiprole sulfone) (parent equivs.)

Supervised trials were carried out on rice (3 trials –Table 86) in India during the 2008 growing season in order to determine residues after four foliar applications of ethiprole (Manjunatha 2008, M-312556-01-2). Foliar applications of Ethiprole SC 100 (0.2% solution) were made using hand operated knapsack sprayer. The first spray was at booting stage then at 14-day intervals.

For 0-day sampling, the shoot samples were collected immediately after the last spray (i.e within 2 hours). For sampling at harvest, the crop was harvested as per the PHI (14 days after the last spray). Rice grain samples were separated from straw after the samples were sun-dried for 2-3 days followed by shade drying for 1-2 days as per local practice. All samples when brought from the field trial locations were stored in a deep-freezer until taken for analysis.

Residues of ethiprole and ethiprole sulfone in rice were determined using LC-MS/MS Method 01053. Acceptable recovery data were obtained for both analytes (ethiprole in rice grain: spike 0.002 mg/kg, n = 3, range of recoveries 93-116%, mean recovery 107%, RSD 12%; spike 0.02 mg/kg, n = 3, range of recoveries 99-110%, mean recovery 106%, RSD 6%), (ethiprole in rice shoot: spike 0.002 mg/kg, n = 3, range of recoveries 77 - 88%, mean recovery 82%, RSD 5%; spike 0.02 mg/kg, n = 3, range of recoveries 97 - 106%, mean recovery 101%, RSD 5%), (ethiprole sulfone in rice grain: spike 0.002 mg/kg, n = 3, range of recoveries 89-105%, mean recovery 96%, RSD 8%; spike 0.02 mg/kg, n = 3, range of recoveries 97-108%, mean recovery 101%, RSD 6%), (ethiprole sulfone in rice shoot: spike 0.002 mg/kg, n = 3, range of recoveries 109-113%, mean recovery 111%, RSD 2%; spike 0.02 mg/kg, n = 3, range of recoveries 107 - 114%, mean recovery 111%, RSD 3%).

Table 86 Residues in rice from the foliar application of ethiprole to rice in India

Trial No., Location Year Variety	Application		Sample	DALA	Residues as ethiprole equivalents (mg/kg)		
	No. (RTI, days)	Rate (g ai/ha)			Parent	RPA 097973 (Ethiprole sulfone)	Sum of parent + RPA 097973
GAP, Thailand, Rice		94		14			
G5076 – Trial E1, Mandya, Karnataka, India, 2008 (Jaya)	4 (14)	100	Shoot	0	0.974	0.262	
					0.971	0.262	
					0.97 (mean)	0.26 (mean)	1.2
			Grain	14	0.118	0.100	
					0.122	0.092	
					0.12 (mean)	0.096 (mean)	0.22
G5076 – Trial E2, Shimoga District, Karnataka, India, 2008 (Jyothi)	4 (14)	100	Shoot	0	0.845	0.287	
					0.860	0.290	
					0.85 (mean)	0.29 (mean)	1.1
			Grain	14	0.142	0.111	
					0.136	0.103	
					0.14 (mean)	0.11 (mean)	0.25
G5076 – Trial E3, Mangalore, Karnataka, India, 2008 (Jaya)	4 (14)	100	Shoot	0	0.894	0.032	
					0.965	0.033	
					0.93 (mean)	0.033 (mean)	0.96
			Grain	14	0.137	0.102	
					0.150	0.110	
					0.14 (mean)	0.11 (mean)	0.25

LOQ is 0.002 mg/kg for each of parent ethiprole and the metabolite RPA 097973 (parent equivs.)

Seeds for beverages and sweets

Supervised trials were carried out on coffee (5 trials –Table 87) in Brazil during the 2014/2015 growing season in order to determine residues after two foliar applications of ethiprole (Sarti 2015, M-543220-01-3). Foliar applications of Curbix 200 SC were made with ground equipment with no adjuvant.

Sampling of coffee fruits (cherries) was carried out at 40, 50, 60, 70 and 80 days after the last application (DALA) in the 3 decline trials (except for trial I15-001-03 for which sampling at 80 DALA was not possible), while sampling in the two harvest trials was at 60 DALA. After sampling the cherries were spread out separately to dry in the sun. After the drying process the fruits were hulled in a machine to take off the husks and parchment to generate the RAC commodity of coffee green beans.

Residues of ethiprole, ethiprole sulfone and ethiprole amide in coffee beans were determined using LC-MS/MS Method 01128. Acceptable concurrent recovery data were obtained for all analytes (ethiprole: spike 0.002 mg/kg, n = 5, range of recoveries 85-99%, mean recovery 92%, RSD 5%; spike 1.0 mg/kg, n = 5, range of recoveries 92-100%, mean recovery 96%, RSD 4%; overall result, mean recovery 94%, RSD = 5%), (ethiprole sulfone: spike 0.002 mg/kg, n = 5, range of recoveries 93-99%, mean recovery 97%, RSD 3%; spike 1.0 mg/kg, n = 5, range of recoveries 99-110%, mean recovery 103%, RSD 5%; overall result, mean recovery 100%, RSD = 5%), (ethiprole amide: spike 0.002 mg/kg, n = 5, range of recoveries 93-105%, mean recovery 99%, RSD 5%; spike 1.0 mg/kg, n = 5, range of recoveries 97-100%, mean recovery 99%, RSD 1%; overall result, mean recovery 99%, RSD = 4%).

Table 87 Residues in green coffee beans from the foliar application of ethiprole to coffee in Brazil

Trial No., Location Year Variety	Application				Sample	DALA	Residues as ethiprole equivalents (mg/kg)			
	No. (RTI, days)	Growth Stage	Rate (g ai/ha)	Volume (L/ha)			Parent	RPA 097973 (Ethiprole sulfone)	RPA 112916 (Ethiprole amide)	Sum of parent + RPA 097973 + RPA 112916
GAP, Brazil, Coffee	2		500			60				
I15-001-01, Rio Claro, Sao Paulo, Brazil, 2015 (Catuai)	2 (29)	79 81	507 504	508 504	Bean, Green	40	0.021	0.008	0.003	
							0.019	0.006	0.002	
							0.020 (mean)	0.007 (mean)	0.003 (mean)	0.030
						50	0.048	0.017	0.005	
							0.037	0.013	0.005	
							0.043 (mean)	0.015 (mean)	0.005 (mean)	0.063
						60	0.014	0.006	0.002	
							0.018	0.007	0.002	
							0.016 (mean)	0.007 (mean)	0.002 (mean)	0.025
						70	0.008	0.003	<0.002	
							0.007	0.003	<0.002	
							0.008 (mean)	0.003 (mean)	<0.002 (mean)	0.013
						80	0.010	0.004	<0.002	
							0.012	0.004	<0.002	
							0.011 (mean)	0.004 (mean)	<0.002 (mean)	0.017
I15-001-02, Pocos de Caldas, Minas Gerais, Brazil, 2015 (Catuai)	2 (30)	75 81	509 501	511 504	Bean, Green	40	0.043	0.012	0.003	
							0.036	0.011	0.002	
							0.040 (mean)	0.012 (mean)	0.003 (mean)	0.054
						50	0.056	0.014	0.003	
							0.057	0.015	0.003	
							0.057 (mean)	0.015 (mean)	0.003 (mean)	0.074
						60	0.005	0.003	<0.002	
							0.013	0.006	<0.002	
							0.009 (mean)	0.005 (mean)	<0.002 (mean)	0.016
						70	0.009	0.005	<0.002	
							0.007	0.004	<0.002	
							0.008 (mean)	0.005 (mean)	<0.002 (mean)	0.015
						80	0.015	0.008	0.002	

Trial No., Location Year Variety	Application No. (RTI, days)	Growth Stage	Rate (g ai/ha)	Volume (L/ha)	Sample	DALA	Residues as ethiprole equivalents (mg/kg) Parent	RPA 097973 (Ethiprole sulfone)	RPA 112916 (Ethiprole amide)	Sum of parent + RPA 097973 + RPA 112916
GAP, Brazil, Coffee	2		500			60				
							0.013	0.007	<0.002	
							0.014 (mean)	0.008 (mean)	0.002 (mean)	0.024
I15-001-03, Andradas, Minas Gerais, Brazil, 2015 (Mundo Novo)	2 (30)	75 81	535 508	538 511	Bean, Green	40	0.007	0.004	<0.002	
							0.008	0.004	<0.002	
							0.008 (mean)	0.004 (mean)	<0.002 (mean)	0.014
						50	0.016	0.009	0.002	
							0.021	0.011	0.002	
							0.019 (mean)	0.010 (mean)	0.002 (mean)	0.031
						60	0.014	0.007	<0.002	
							0.022	0.011	<0.002	
							0.018 (mean)	0.009 (mean)	<0.002 (mean)	0.029
						70	0.010	0.005	<0.002	
							0.011	0.005	<0.002	
							0.011 (mean)	0.005 (mean)	<0.002 (mean)	0.018
I15-001-04, Leme, Sao Paulo, Brazil, 2015 (Mundo Novo)	2 (29)	77 79	504 509	503 508	Bean, Green	60	0.045	0.013	0.003	
							0.042	0.014	0.003	
							0.044 (mean)	0.014 (mean)	0.003 (mean)	0.060
I15-001-06, Campinas, Sao Paulo, Brazil, 2015 (Catuai Vermelho)	2 (29)	81 85	521 504	521 503	Bean, Green	60	0.021	0.005	<0.002	
							0.023	0.005	<0.002	
							0.022 (mean)	0.005 (mean)	<0.002 (mean)	0.029

LOQ is 0.002 mg/kg for each of parent ethiprole and the metabolites RPA 097973 and RPA 112916 (parent equivs.) in green coffee beans.

Supervised trials were carried out on coffee (5 trials –Table 88) in Colombia (2 trials), Costa Rica (2) and Mexico (1) during the 2015 or 2016 growing season in order to determine residues after two foliar applications of ethiprole (Harbin 2017, M-581972-03-1). Foliar applications of CURBIX (Ethiprole 200 SC) at a target 30-day application interval were made with ground equipment, with no adjuvant. The first application was made at a target 90-days prior to normal commercial harvest of coffee. The first applications were made between growth stage BBCH 75 (development of fruit 50%) and BBCH 79 (nearly all fruits have reached final size).

At each sampling interval, duplicate composite samples (two separate runs through the plot) of coffee cherries were harvested from the treated plot when the cherries were at or prior to commercial maturity. Coffee cherries were collected at PHIs of 38–40, 48–50, 58–60, 68–70 and 78–80 days following the second application. The only exception to this was for trial EH005-15DA, where due to the lack of cherries, only a single sample was harvested from the treated plot at the target 50-day PHI. Single composite samples of coffee cherries were harvested from the control plot of each trial on the same day the target 60-day PHI samples were harvested from the treated plots. Coffee cherries were processed using wet processing methods typical of commercial coffee production in Central America, followed by air drying and parchment removal to generate the RAC commodity of coffee green beans.

Residues ethiprole, ethiprole sulfone and ethiprole amide in coffee beans were determined using LC-MS/MS Method 01128. Acceptable concurrent recovery data were obtained for all analytes (ethiprole: spike 0.002 mg/kg, n = 9, range of recoveries 99–120%, mean recovery 109%, RSD 7%; spike 0.10 mg/kg, n = 5, range of recoveries 85–133%, mean recovery 106%, RSD 17%), (ethiprole sulfone: spike 0.002 mg/kg, n = 9, range of recoveries 81–119%, mean recovery 102%, RSD 12%; spike 0.10 mg/kg, n = 5,

range of recoveries 83–116%, mean recovery 93%, RSD 15%), (ethiprole amide: spike 0.002 mg/kg, n = 9, range of recoveries 85–116%, mean recovery 104%, RSD 9%; spike 0.10 mg/kg, n = 5, range of recoveries 75–112%, mean recovery 92%, RSD 18%).

Table 88 Residues in green coffee beans from the foliar application of ethiprole to coffee in Colombia, Costa Rica and Mexico

Trial No., Location, Year (Variety)	Application				Sample	DALA	Residues as ethiprole equivalents (mg/kg)			
	No. (RTI, days)	Growth Stage (BBCH)	Rate (g ai/ha)	Volume (L/ha)			Parent	RPA 097973 (Ethiprole sulfone)	RPA 112916 (Ethiprole amide)	Sum of parent + RPA 097973 + RPA 112916
GAP, Brazil, Coffee	2		500			60				
EH001-15DA, Chinchina, Risaralda, Colombia 2015 (Supremo)	2 (29)	79 79	502 496	325 323	Bean, Green	40	0.007	0.003	0.003	
							0.008	0.004	0.003	
							0.007 (mean)	0.003 (mean)	0.003 (mean)	0.014
						49	0.008	0.006	0.005	
							0.007	0.005	0.003	
							0.008 (mean)	0.005 (mean)	0.004 (mean)	0.017
						60	0.008	0.007	0.004	
							0.008	0.007	0.004	
							0.008 (mean)	0.007 (mean)	0.004 (mean)	0.019
						70	0.007	0.009	0.005	
							0.006	0.008	0.004	
							0.007 (mean)	0.009 (mean)	0.005 (mean)	0.020
						80	0.006	0.011	0.005	
							0.005	0.008	0.004	
							0.005 (mean)	0.009 (mean)	0.004 (mean)	0.019
EH002-15DA, Pereira, Risaralda, Colombia 2015 (Castillo)	2 (29)	77 79	509 502	379 387	Bean, Green	40	0.004	<0.002	<0.002	
							0.003	<0.002	<0.002	
							0.003 (mean)	<0.002 (mean)	<0.002 (mean)	0.007
						48	0.003	0.003	0.002	
							0.005	0.005	0.003	
							0.004 (mean)	0.004 (mean)	0.003 (mean)	0.011
						60	0.004	0.006	0.003	
							0.003	0.005	0.004	
							0.004 (mean)	0.005 (mean)	0.003 (mean)	0.013
						69	0.003	0.007	0.005	
							0.004	0.006	0.005	
							0.004 (mean)	0.007 (mean)	0.005 (mean)	0.015
						80	0.003	0.006	0.004	
							0.003	0.007	0.005	
							0.003 (mean)	0.007 (mean)	0.004 (mean)	0.014
EH004-15DA, San Pedro de Poas, Alejuela, Costa Rica 2015 (Catuai Red)	2 (30)	75 79	498 498	300 306	Bean, Green	40	0.004	0.002	<0.002	
							0.005	0.002	<0.002	
							0.005 (mean)	0.002 (mean)	<0.002 (mean)	0.009
						50	0.003	<0.002	<0.002	
							0.005	0.003	<0.002	
							0.004 (mean)	0.003 (mean)	<0.002 (mean)	0.008
						59	0.006	0.003	<0.002	
							0.005	0.002	<0.002	
							0.005	0.003 (mean)	<0.002	0.010

Trial No., Location, Year (Variety)	Application				Sample	DALA	Residues as ethiprole equivalents (mg/kg)			
	No. (RTI, days)	Growth Stage (BBCH)	Rate (g ai/ha)	Volume (L/ha)			Parent	RPA 097973 (Ethiprole sulfone)	RPA 112916 (Ethiprole amide)	Sum of parent + RPA 097973 + RPA 112916
							(mean)		(mean)	
						68	0.005	0.003	<0.002	
							0.005	0.003	<0.002	
							0.005 (mean)	0.003 (mean)	<0.002 (mean)	0.009
						78	0.005	0.003	<0.002	
							0.005	0.003	<0.002	
							0.005 (mean)	0.003 (mean)	<0.002 (mean)	0.011
EH005-15DA, Coatepec, Veracruz, Mwxico, 2015 (Mundo Novo Hybrid)	2 (28)	76 77	511 505	338 362	Bean, Green	40	0.005	0.003	0.003	
							0.006	0.005	0.003	
							0.006 (mean)	0.004 (mean)	0.003 (mean)	0.013
						49	0.009	0.008	0.004	0.020
						59	0.010	0.016	0.005	
							0.012	0.020	0.006	
							0.011 (mean)	0.018 (mean)	0.005 (mean)	0.035
						69	0.013	0.025	0.006	
							0.011	0.023	0.007	
							0.013 (mean)	0.025 (mean)	0.007 (mean)	0.043
						80	0.005	0.009	0.005	
							0.005	0.012	0.006	
							0.005 (mean)	0.010 (mean)	0.006 (mean)	0.021
EH006-15DA, San Juan Norte, Alejuela, Costa Rica, 2016 (Villa Sarchi)	2 (25)	75 77	508 500	393 398	Bean, Green	38	0.008	0.004	<0.002	
							0.006	0.003	<0.002	
							0.007 (mean)	0.004 (mean)	<0.002 (mean)	0.013
						48	0.004	0.003	<0.002	
							0.007	0.003	<0.002	
							0.006 (mean)	0.003 (mean)	<0.002 (mean)	0.010
						58	0.005	0.006	<0.002	
							0.007	0.007	0.003	
							0.006 (mean)	0.006 (mean)	0.002 (mean)	0.015
						69	0.004	0.005	<0.002	
							0.005	0.006	<0.002	
							0.005 (mean)	0.005 (mean)	<0.002 (mean)	0.012
						78	0.005	0.004	<0.002	
							0.005	0.005	<0.002	
							0.005 (mean)	0.004 (mean)	<0.002 (mean)	0.011

LOQ is 0.002 mg/kg for each of parent ethiprole and the metabolites RPA 097973 and RPA 112916 (parent equivs.) in green coffee beans.

Due to climatic conditions, the coffee cherries from trial EH005-15DA were harvested, per PHI, at immaturity (BBCH 80 to 85) and the green beans (RAC) incurred breakage during processing. Therefore the weights obtained for the RAC samples were less than requested in the protocol.

FATE OF RESIDUES IN STORAGE AND PROCESSING

Residues after processing

The fate of ethiprole residues during processing of raw agricultural commodities was investigated in cereals (rice) and coffee.

As a measure for the transfer of residues into processed products, a transfer factor (TF) was used, which is defined as:

$$TF = \frac{\text{Residue in processed products (mg/kg)}}{\text{Residue in raw agricultural commodity (mg/kg)}}$$

The high temperature hydrolysis of residues of ethiprole and its major plant metabolite ethiprole-sulfone (RPA 097973) was studied under varying conditions (Spiegel 2009, M-348589-01-1). An acetonitrile solution of [phenyl-UL-^{14}C]-ethiprole or [phenyl-UL-^{14}C]- ethiprole-sulfone was added to aqueous buffer solutions and subjected to hydrolysis at pH 4, 5, 6 or 7 at high temperature, at concentrations of 0.96, 0.84, 0.98 and 0.88 mg/L respectively for ethiprole and concentrations of 0.90, 0.80, 0.92 and 0.93 mg/L respectively for ethiprole-sulfone. The conditions were selected to simulate hydrolysis under processing conditions and included:

- The effect of pasteurisation (pH 4 at 90 °C for 20 minutes)
- The effect of baking, brewing and boiling (pH 5 at 100 °C for 60 minutes)
- The effect of sterilisation (pH 6 and autoclaving at 120 °C for 20 minutes)
- The effect of tea preparation/ steaming of rice (pH 7 at 100 °C for 40 minutes).

For ethiprole the pH ranged from 3.96 (before incubation) - 4.05 (after incubation) for the pH 4 samples, 5.01 (before incubation) - 5.05 (after incubation) for the pH 5 samples, 6.08 (before incubation) - 6.09 (after incubation) for the pH 6 samples and from 7.01 (before incubation) – 7.02 (after incubation) for the pH 7 samples. For ethiprole-sulfone the pH ranged from 3.97 (before incubation) - 4.03 (after incubation) for the pH 4 samples, 5.02 (before incubation) - 5.05 (after incubation) for the pH 5 samples, 6.08 (before incubation) - 6.09 (after incubation) for the pH 6 samples and from 7.01 (before incubation) – 7.02 (after incubation) for the pH 7 samples.

No major loss of radioactivity material occurred; pH 4 at 90 °C experiment – 104.1% of total applied radioactivity remained after the test for ethiprole and 110.2% for ethiprole-sulfone, pH 5 at 100 °C experiment – 120.7% of total applied radioactivity remained after the test for ethiprole and 125.8% for ethiprole-sulfone, pH 6 at 120 °C experiment – 99.2% of total applied radioactivity remained after the test for ethiprole and 105.3% for ethiprole-sulfone; and pH 7 at 100 °C experiment – 114.7% of total applied radioactivity remained after the test for ethiprole and 106.4% for ethirpole-sulfone.

No hydrolysis products of [phenyl-UL-^{14}C]-ethiprole or [phenyl-UL-^{14}C]-ethiprole-sulfone were detected under the following conditions of processing:

- pH 4/ 90 °C (20 minutes)
- pH 5/ 100 °C (60 minutes).
- Ethiprole-amide was detected as a minor degradation product of ethiprole under the following conditions of processing:
- pH 6/ 120 °C (20 minutes); ethiprole-amide approximately 6% of radioactivity
- pH 7/ 100 °C (40 minutes); ethiprole-amide approximately 5% of radioactivity.
- Ethiprole-sulfone-amide was detected as a minor degradation product of ethiprole-sulfone under the following conditions of processing:
- pH 6/ 120 °C (20 minutes); ethiprole-sulfone-amide approximately 4% of radioactivity
- pH 7/ 100 °C (40 minutes); ethiprole-sulfone-amide approximately 3% of radioactivity.

In summary, the data show that ethiprole and ethiprole-sulfone were stable under the conditions of pasteurisation (pH 4, 90 °C, 20 minutes), and baking, boiling and brewing (pH 5, 100 °C, 60 minutes) whereas minor degradation was observed under the conditions of sterilisation (pH 6, 120 °C, 20 minutes) and infusing tea/ cooking of rice (pH 7, 100 °C, 40 minutes). The observed degradation products were identical to well-known plant metabolites.

Rice

The effect of processing (laboratory scale) on residues of ethiprole in rice (Table 89) was investigated in a trial carried out in Thailand during the 2007 growing season in order to determine the residues of ethiprole in husked rice and then after processing, in brown rice, polished rice, hulls and bran (Balluff 2008, M-308810-02-2). Four foliar applications were made using Ethiprole 100 SC. All foliar applications were made using ground-based equipment with no adjuvant.

Rice grain for processing were collected and was first sun-dried according to local practice until a moisture content of 14% was achieved. Four days after harvest the whole sample of rice grain was passed through a cleaning machine to separate out dirt and other seeds, generating (cleaned) rice grain. The rice was then passed through the dehusking machine to separate husk from the grains, generating from this process husk and husked rice (brown rice). In the final process the brown rice was treated with a polishing machine (1 min for 100 g of rice grain) to obtain polished rice and bran.

Samples of cleaned rice grain, husked rice, polished rice, husks and bran were analysed for residues of ethiprole and its metabolite ethiprole-sulfone (RPA 097973) by LC-MS/MS according to the method 01053. This analytical method has been validated with an LOQ of 0.002 mg/kg. Samples were analysed within 96 days (approximately 3 months) from sampling.

Table 89 Residues in rice processed fractions from the foliar application of ethiprole to rice in Thailand (Balluff 2008, M-308810-02-2)

Trial No., Location, Year (Variety)	Sample	DAT	Residues as parent (mg/kg)			Processing Factor (PF)	
			Parent	Ethiprole-sulfone RPA 097973	Parent + RPA 097973	Parent	Parent + RPA 097973
GAP, Thailand, Rice 94g ai/ha		14					
TH07W001R, Pathumthani Province, Thailand, 2007 (Suphan Buri 1) 4 applications Growth Stages 37, 47, 69, 77 203, 200, 211, 195 g ai/ha 254, 250, 264, 244 L/ha	Rice grain (cleaned)*	16	0.44	0.38	0.82	-	-
	Husked (brown) rice**	16	0.16	0.10	0.26	0.36	0.32
	Polished rice	16	0.047	0.024	0.07	0.11	0.09
	Hulls	16	0.67	0.51	1.18	1.5	1.4
	Bran	16	0.50	0.46	0.96	1.1	1.2

LOQ is 0.002 mg/kg for each of parent ethiprole and the metabolite RPA 097973 (Ethiprole sulfone) (parent equivs.)

The RAC is dried rice, but dried rice was not analysed in the study. It was thought that the cleaning step to generate cleaned rice would not be expected to impact the residue level significantly, therefore for the purposes of estimating the processing factors cleaned rice was taken as the RAC.

*The cleaned rice was called husked rice in the processing study. This has been changed in the table above.

**The husked rice was called brown rice in the processing study.

It was concluded that these results indicate that total residues according to the residue definition for risk assessment (sum of ethiprole and ethiprole-sulfone RPA 097973 expressed as ethiprole) are concentrated in the outer parts of the grain: hulls (PF = 1.4) and bran (PF = 1.2). Significantly lower total residues were present in the inner parts of the processed grain: brown grain (PF = 0.32) and polished grain (PF = 0.09).

For MRL setting the following processing factors were obtained: PF = 0.11 (polished rice), 1.5 (rice hulls) and 1.1 (rice bran).

Coffee

The effect of processing (laboratory scale) on residues of ethiprole in coffee (Table 90) was investigated in two trials carried out in Mexico (EH012-13PA) and Colombia (EH013-13PA) during the 2014 growing season in order to determine the residues of ethiprole in green coffee beans and then after mild and normal processing, in roasted coffee beans and instant coffee (Lemke and Woodard 2016, M-553056-02-1). Three broadcast spray applications were made to the foliage using Ethiprole 200 SC (100 g ethiprole/L + 100 g imidacloprid/L). All foliar applications were made using ground-based equipment with no adjuvant. Imidacloprid residues were also determined in the processing study, but the results are not discussed any further.

Duplicate, composite samples of treated coffee cherries were harvested from the treated plots at a target pre-harvest interval (PHIs) of 60 days with sampling corresponding to growth stages ranging from BBCH 88 (fruit fully ripe and ready for picking)

to BBCH 89 (advanced ripening). Composite samples of coffee cherries were also harvested from the control plots on the same day as the 60-day PHI samples were collected from the treated plots.

In the Mexican trial (EH012-13PA, "dry" method sampling) the coffee cherries were sun-dried for several weeks with periodic turning to ensure uniform drying, before removing the outer part (husk and parchment) to obtain the green coffee bean. In the Colombian trial (EH013-13PA, "wet" method sampling), the green coffee beans were obtained by removal of husk from the harvested cherries, fermentation of the bean and remaining pulp, removal of pulp, air-drying for several days with frequent turning and removal of parchment to obtain green coffee beans. Due to the duration of the drying steps, the overall time required to obtain the green coffee beans was 21 days in the Mexican trial and 14 days in the Colombian trial.

Samples of the green coffee beans were sent to two different processing facilities; GLP Technologies (Navasota, Texas, USA) and University of Idaho Food Technology Center (Caldwell, Idaho, USA). At each processing facility the samples of coffee green beans were processed into roasted coffee beans and instant coffee but according to slightly different procedures.

The overall processing scheme at the two facilities was similar. All the treated RAC samples had already reached the appropriate moisture content (10-13%), so no drying step was necessary except the control sample for trial EH-012-13PA-A101. The green coffee bean samples were first cleaned by aspiration and/or screening to remove light impurities, loose hulls and foreign material. After cleaning, the green coffee beans were roasted and then allowed to cool at ambient temperature. The roasted, cooled beans were cleaned either by aspiration or by screening (University of Idaho) to remove chaff loosened during the roasting process. Aliquots of roasted, cleaned beans were taken for residue analysis. The roasted beans were ground to an average particle size of 20 mesh (0.84 mm) and the ground coffee was brewed with boiling water/ steam. The brewed extract was cooled and filtered (GLP Technologies) or centrifuged (University of Idaho). The extract was first concentrated in a laboratory vacuum evaporator and the concentrated extract was then freeze-dried. The freeze-dried coffee was milled to a uniform particle size (optional, only performed at University of Idaho). Aliquots of freeze dried coffee (= instant coffee) were taken for residue analysis.

The main differences between the procedures applied at the two processing facilities pertain to:

1. The roasting temperature: At GLP Technologies the green coffee beans were roasted under so-called mild conditions (188–204 °C for 2–15 minutes) while at University of Idaho roasting involved higher, 'normal', temperatures (215–225 °C for approximately 6 minutes). This temperature difference is likely to significantly impact the levels of ethiprole-derived residues since parent ethiprole is known to decompose from 165 °C onwards; and
2. The brewing process and the subsequent concentration step: At GLP Technologies the ground coffee was brewed with 10- to 16- fold amount of water (on a weight basis). At University of Idaho 1.2- to 1.6- fold amount of water was used. As a result, the GLP Technologies coffee extract was more diluted and required extensive concentration in the laboratory vacuum evaporator, compared to that from the University of Idaho.

Residues of ethiprole and metabolites in coffee RAC and processed commodities were quantitated by LC-MS/MS according to the Method 01128. This analytical method has been validated with an LOQ of 0.002 mg/kg in green coffee beans and 0.005 mg/kg in roasted and instant coffee for parent, ethiprole-sulfone and ethiprole-amide (parent equivalents). Samples were stored frozen (-10 °C) and analysed within approximately 10 months from sampling.

Acceptable concurrent recovery data were obtained for all analytes (ethiprole in green coffee beans: spike 0.002 mg/kg, n = 7, range of recoveries 102–112%, mean recovery 110%, RSD 3%; spike 0.20 mg/kg, n = 3, range of recoveries 108–115%, mean recovery 112%, RSD 3%), (ethiprole in instant coffee: spike 0.005 mg/kg, n = 3, range of recoveries 102–116%, mean recovery 108%, RSD 7%; spike 0.60 mg/kg, n = 3, range of recoveries 108–112%, mean recovery 109%, RSD 2%), (ethiprole in roasted coffee: spike 0.005 mg/kg, n = 3, range of recoveries 107–110%, mean recovery 109%, RSD 1%; spike 0.60 mg/kg, n = 3, range of recoveries 105–112%, mean recovery 109%, RSD 3%), (ethiprole-sulfone in green coffee beans: spike 0.002 mg/kg, n = 7, range of recoveries 89–115%, mean recovery 102%, RSD 10%; spike 0.20 mg/kg, n = 3, range of recoveries 105–113%, mean recovery 109%, RSD 4%), (ethiprole-sulfone in instant coffee: spike 0.005 mg/kg, n = 3, range of recoveries 95–111%, mean recovery 101%, RSD 8%; spike 0.60 mg/kg, n = 3, range of recoveries 104–109%, mean recovery 107%, RSD 3%), (ethiprole-sulfone in roasted coffee: spike 0.005 mg/kg, n = 3, range of recoveries 99–108%, mean recovery 105%, RSD 5%; spike 0.60 mg/kg, n = 3, range of recoveries 92–103%, mean recovery 99%, RSD 6%), (ethiprole-amide in green coffee beans: spike 0.002 mg/kg, n = 7, range of recoveries 101–120%, mean recovery 114%, RSD 7%; spike 0.20 mg/kg, n = 3, range of recoveries 116–117%, mean recovery 116%, RSD 1%), (ethiprole-amide in instant coffee: spike 0.005 mg/kg, n = 3, range of recoveries 80–96%, mean recovery 89%, RSD 9%; spike 0.60 mg/kg, n = 3, range of recoveries 106–108%, mean recovery 107%, RSD 1%), (ethiprole-amide in roasted coffee: spike 0.005 mg/kg, n = 3, range of recoveries 82–96%, mean recovery 91%, RSD 9%; spike 0.60 mg/kg, n = 3, range of recoveries 112–113%, mean recovery 113%, RSD 1%).

Table 90 Residues in coffee processed fractions from the foliar application of ethiprole to coffee in Mexico and Colombia (Lemke and Woodard 2016, M-553056-02-1)

Trial No., Location, Location, Year, (Variety)	Sample	PHI	Residues as parent (mg/kg)				Processing Factor	
			Parent	Ethiprole-sulfone RPA 097973	Ethiprole-amide RPA 112916	Parent + RPA 097973 + RPA 112916	Parent	Parent + RPA 097973 + RPA 112916
GAP, Brazil, Coffee 2×500 g ai/ha		60						
EH012-13PA, Texcoco, Mexico, 2014 (Costa Rica 95) 3 applications Growth Stages 75, 77 and 81 1.28, 1.26 and 1.26 kg ai/ha 358, 380 and 374 L/ha 28 and 30 day RTI	Green bean (RAC)-GLP Dry sampling method	60	0.0379 0.0379 0.0232 0.033 (mean)	0.0439 0.0439 0.0229 0.0369 (mean)	0.0146 0.0158 0.0086 0.013 (mean)	0.083	-	-
	Roasted coffee -GLP	60	0.0729 0.0861 0.0769 0.079 (mean)	0.0526 0.0660 0.0570 0.059 (mean)	0.0320 0.0327 0.0326 0.032 (mean)	0.17	2.4	2.0
	Instant coffee -GLP	60	0.0527 0.0573 0.0650 0.058 (mean)	0.0571 0.0678 0.0725 0.066 (mean)	0.0336 0.0328 0.0379 0.035 (mean)	0.16	1.8	1.9
	Green bean (RAC)-UoI Dry sampling method	60	0.0357 0.0365 0.0370 0.036 (mean)	0.0415 0.0420 0.0412 0.042 (mean)	0.0139 0.0149 0.0140 0.014 (mean)	0.092	-	-
	Roasted coffee -UoI	60	<0.005 <0.005 <0.005 <0.005 (mean)	0.0282 0.0287 0.0295 0.029 (mean)	0.0072 0.0065 0.0065 0.0067 (mean)	0.041	<0.14	0.45
	Instant coffee -UoI	60	<0.005 <0.005 <0.005 <0.005 (mean)	<0.005 <0.005 <0.005 <0.005 (mean)	0.0066 0.0072 0.0077 0.0072 (mean)	0.017	<0.14	0.18
EH013-13PA, Pereira, Colombia, 2014 (Arabica) 3 applications Growth Stages 75, 79 and 85 1.23, 1.25 and 1.25 kg ai/ha	Green bean (RAC)-GLP Wet method sampling	60	0.0149 0.0131 0.0137 0.014 (mean)	0.0189 0.0168 0.0191 0.018 (mean)	0.0113 0.0091 0.0103 0.010 (mean)	0.042	-	-
	Roasted coffee -GLP	60	0.0232 0.0191 0.0217 0.021 (mean)	0.0267 0.0251 0.0245 0.025 (mean)	0.0210 0.0192 0.0176 0.019 (mean)	0.066	1.5	1.6
	Instant coffee -GLP	60	0.0244 0.0230 0.0219 0.023 (mean)	0.0387 0.0378 0.0352 0.037 (mean)	0.0263 0.0241 0.0230 0.024 (mean)	0.085	1.6	2.0
	Green bean (RAC)-UoI Wet method sampling	60	0.0120 0.0137 0.0157 0.014 (mean)	0.0159 0.0199 0.0201 0.019 (mean)	0.0087 0.0115 0.0106 0.010 (mean)	0.043	-	-
	Roasted coffee -UoI	60	<0.005 <0.005 <0.005 <0.005	0.0170 0.0153 0.0172 0.017 (mean)	0.0064 0.0074 0.0061 0.0066	0.028	<0.36	0.65

Trial No., Location, Location, Year, (Variety)	Sample	PHI	Residues as parent (mg/kg)				Processing Factor	
			Parent	Ethiprole-sulfone RPA 097973	Ethiprole-amide RPA 112916	Parent + RPA 097973 + RPA 112916	Parent	Parent + RPA 097973 + RPA 112916
			(mean)		(mean)			
314, 311 and 321 L/ha 28 and 25 day RTI	Instant coffee -UoI	60	<0.005 <0.005 <0.005 <0.005 (mean)	<0.005 <0.005 <0.005 <0.005 (mean)	<0.005 <0.005 <0.005 <0.005 (mean)	<0.015	<0.36	<0.35

GLP = Processing at GLP Technologies (mild processing); UoI = Processing at University of Idaho (normal processing).

LOQ is 0.002 mg/kg for each of parent ethiprole and the metabolites RPA 097973 and RPA 112916 (parent equivs.) in green coffee beans

LOQ is 0.005 mg/kg for each of parent ethiprole and the metabolites RPA 097973 and RPA 112916 (parent equivs.) in roasted coffee and instant coffee

Where the residues are < LOQ, the processing factor cannot be calculated accurately, but the LOQ value has been used.

Dry sampling method – coffee cherries collected, air dried for no more than 21 days and then the hulls and parchment were removed

Wet sampling method – coffee cherries collected and outer hulls removed on the day of harvest, air dried for no more than 14 days and then parchment was removed

It was concluded that these results indicate that total residues according to the residue definition for risk assessment (sum of ethiprole, ethiprole-sulfone RPA 097973 and ethiprole-amide RPA 112916 expressed as ethiprole) are concentrated in the roasted and instant coffee.

A significant difference is observed when comparing the residue levels following mild and 'normal' processing. Upon mild roasting an average 1.95-fold concentration of parent ethiprole residues was observed in roasted beans and an average 1.7-fold concentration in instant coffee. Upon normal roasting the residues of parent ethiprole were found to degrade almost completely while the residues of ethiprole-sulfone degraded to a lesser extent. Due to the low residues it was not possible to reliably estimate the processing and conversion factors. The mild roasting therefore represents the worst case and these values are used to set processing and conversion factors.

The mean processing factor (enforcement) for roast coffee was 1.95 and for instant coffee was 1.7. The mean processing factor (risk assessment) for roast coffee was 1.80 and for instant coffee was 1.95.

The results of the processing factors are summarised in Table 91 below:

Table 91 Summary of processing factors for ethiprole residues

Raw Agricultural Commodity (RAC)	Processed Commodity	Calculated Processing factors (Parent)	Best Estimate Processing Factor (Parent + ethiprole-sulfone)
Rice grain*	Husked (brown) rice	0.36	0.32
	Polished rice	0.11	0.09
	Hulls	1.5	1.4
	Bran	1.1	1.2
Coffee beans	Roasted coffee (mild processing)	1.5, 2.4 (mean 1.95)	1.6, 2.0 (mean 1.80)
	Instant coffee (mild processing)	1.6, 1.8 (mean 1.7)	1.9, 2.0 (mean 1.95)

*Cleaned rice was taken as the RAC.

RESIDUES IN ANIMAL COMMODITIES

Dairy cattle transfer study (preliminary)

A preliminary study was conducted in the USA to determine the level of ethiprole residues in milk and tissues of dairy cows following the oral administration of ethiprole at a nominal rate corresponding to 12 ppm in the feed (Tew 2001, M-240497-01-4).

One lactating cow was dosed each morning after milking for 28 days. A second cow served as a control. The cows were milked twice daily. Milk samples from days 17, 21, 24 and 28 were collected for analysis. At the end of the 28-day dosing period, the cows were euthanised and samples of muscle, liver, kidney and fat were collected for analysis. Also, milk fat was separated from the 28-day milk collection for analysis.

Tissue and milk samples were analysed for ethiprole and its metabolites ethiprole-sulfone (RPA 097973) and ethiprole-methyl sulfone (RPA 094569) according to LC-MS/MS method 019-034. The limit of quantification was 0.001 mg/kg for each analyte (in parent equivalents) in all matrices.

Acceptable concurrent recovery data were obtained for all analytes: (ethiprole in whole milk: spike 0.001 mg/kg, n = 1, recovery 92%; spike 0.050 mg/kg, n = 1, recovery 111%), (ethiprole in cow milk fat: spike 0.001 mg/kg, n = 1, recovery 119%; spike 0.050 mg/kg, n = 1, recovery 106%), (ethiprole in kidney: spike 0.001 mg/kg, n = 1, recovery 76%; spike 0.500 mg/kg, n = 1, recovery 125%), (ethiprole in fat: spike 0.001 mg/kg, n = 1, recovery 102%; spike 0.500 mg/kg, n = 1, recovery 98%), (ethiprole in liver: spike 0.001 mg/kg, n = 1, recovery 62%; spike 0.500 mg/kg, n = 1, recovery 105%), (ethiprole in muscle: spike 0.001 mg/kg, n = 1, recovery 92%; spike 0.500 mg/kg, n = 1, recovery 93%); (ethiprole-sulfone in whole milk: spike 0.001 mg/kg, n = 1, recovery 119%; spike 0.050 mg/kg, n = 1, recovery 112%), (ethiprole-sulfone in cow milk fat: spike 0.001 mg/kg, n = 1, recovery 127%; spike 0.050 mg/kg, n = 1, recovery 110%), (ethiprole-sulfone in kidney: spike 0.001 mg/kg, n = 1, recovery 71%; spike 0.500 mg/kg, n = 1, recovery 135%), (ethiprole-sulfone in fat: spike 0.001 mg/kg, n = 1, recovery 102%; spike 0.500 mg/kg, n = 1, recovery 104%), (ethiprole-sulfone in liver: spike 0.001 mg/kg, n = 1, recovery 73%; spike 0.500 mg/kg, n = 1, recovery 127%), (ethiprole-sulfone in muscle: spike 0.001 mg/kg, n = 1, recovery 96%; spike 0.500 mg/kg, n = 1, recovery 101%); (ethiprole-methyl sulfone in whole milk: spike 0.001 mg/kg, n = 1, recovery 112%; spike 0.050 mg/kg, n = 1, recovery 114%), (ethiprole-methyl sulfone in cow milk fat: spike 0.001 mg/kg, n = 1, recovery 88%; spike 0.050 mg/kg, n = 1, recovery 118%), (ethiprole-methyl sulfone in kidney: spike 0.001 mg/kg, n = 1, recovery 76%; spike 0.500 mg/kg, n = 1, recovery 136%), (ethiprole-methyl sulfone in fat: spike 0.001 mg/kg, n = 1, recovery 101%; spike 0.500 mg/kg, n = 1, recovery 97%), (ethiprole-methyl sulfone in liver: spike 0.001 mg/kg, n = 1, recovery 60%; spike 0.500 mg/kg, n = 1, recovery 121%), (ethiprole-methyl sulfone in muscle: spike 0.001 mg/kg, n = 1, recovery 88%; spike 0.500 mg/kg, n = 1, recovery 95%);

Residues of ethiprole and metabolites in milk and cattle tissue are reported in the table below:

Table 92 Ethiprole and its metabolites (RPA 097973 and RPA 094569) in milk and cattle tissues

Commodity	Residue (mg/kg) expressed as parent equivalents			
	Ethiprole	Ethiprole-sulfone (RPA 097973)	Ethiprole-methyl sulfone (RPA 094569)	Parent + Ethiprole-sulfone
Milk				
17 days	0.004	0.112	0.001	0.116
21 days	0.002	0.091	0.001	0.093
24 days	0.005	0.062	0.001	0.067
28 days	0.005	0.070	0.001	0.075
Milk Fat	0.038	1.605	0.012	1.643
Kidney	0.009	0.341	0.004	0.350
Fat	0.050	1.836	0.014	1.886
Liver	0.020	1.596	0.021	1.616
Muscle	0.005	0.179	0.002	0.184

LOQ is 0.001 mg/kg for each of parent ethiprole and the metabolites RPA 097973 and RPA 094569 in milk, milk fat, muscle, liver, kidney and fat

The milk samples collected daily from day 17 to day 28 (last day of dosing) were analysed to determine the level of residue and verify that residues had reached a plateau level. The highest total residue (sum of ethiprole and RPA 097973 expressed as ethiprole equivalents) occurred at day 17 (0.116 mg eq/kg) and a plateau of 0.080 mg eq/kg was reached for the remainder of the dosing period. Residues at the plateau and throughout the study consisted almost entirely of the metabolite RPA 097973. Residues of parent ethiprole (RPA 107382) were ≤0.005 mg eq/kg and residues of RPA 094569 were ≤0.001 mg eq/kg, which is consistent with the goat metabolism study. A portion of the day 28 milk was centrifuged to separate the milk fat. The residues of all three components were found to concentrate in milk fat. The concentration factors with respect to whole milk were 7.6 for ethiprole, 23 for RPA 097973 and 12 for RPA 094569.

In tissues, RPA 097973 was the major component of the residue, followed by parent ethiprole and then RPA 0945569. The residues were higher in fat and liver with a total residue (sum of ethiprole and RPA 097973 expressed as ethiprole equivalents) of 1.89 and 1.62 mg eq/kg, respectively. The total residues in muscle were <0.2 mg eq/kg.

The transfer factors derived from the feeding study (ratio of residue level in milk and tissues over the residue level in the feed) are summarised in the following table.

Table 93 Transfer factors for ethiprole-derived residues in milk and cow tissues

Feeding level	12 ppm dry feed	
Commodity	Total ethiprole (mg/kg)	Transfer factor
Milk	0.116	0.010
Milk Fat	1.643	0.137
Kidney	0.350	0.029
Fat	1.886	0.157
Liver	1.616	0.135
Muscle	0.184	0.015

Dairy cattle transfer study

A study was conducted to determine residues of ethiprole and its metabolites ethiprole-sulfone (RPA 097973), ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) in milk and tissues of dairy cows orally dosed with ethiprole for 28 days (Glaubitz and Rehagen 2016, M-564842-01-1).

A group of 15 Holstein Frisian dairy cows (426–611 kg at the beginning of the second week of the acclimatisation phase and approximately 2.3–3.8 years of age) were dosed with ethiprole by gelatin capsule daily for 28 consecutive days. Three cows were allocated to a control group (0×) or 4 treated groups (1×, 3×, 10× and 10×E). The target dose rates of ethiprole were 0.005 mg/kg bw/day test item for the 1× dose group, 0.015 mg/kg bw/day for the dose group 3× and 0.05 mg/kg bw/day for the dose groups 10× and 10×E. The exact amounts of test item to be administered daily to each cow were calculated based on the body weights measured at the beginning of the second week of the acclimatisation phase.

The cows were fed with a combination of cob mix and feed concentrate for dairy cattle, which were supplemeted with minerals. The amount of feed consumed was monitored daily. Based on actual feed intake, the dose rates simulated residue concentrations in feed dry matter (expressed as ethiprole) of 0.14 ppm (1× group), 0.41 ppm (3× group), 1.31 ppm (10× group) and 1.38 ppm (10×E group).

Milk samples of the cows of the 0, 1, 3 and 10× groups were taken twice before the first dosing, at least every third day during the first three weeks of dosage and twice during the last week of dosage. The milk samples for the 10× group at day 30 were separated into cream and skim milk (whey). The animals were sacrificed on the day after the final application, less than 24 hours after the final dose. Liver, muscle, kidney and fat (perirenal, subcutaneous and mesenteric) were taken for analysis.

The three dairy cows of the 10×E group were dosed at the rate of 0.05 mg/kg bw/day for 28 consecutive days simultaneously with the animals from dose group 10×. Thereafter dosing was stopped and the animals were kept alive for a further 5–15 days in order to investigate the depuration of ethiprole, ethiprole-sulfone (RPA 097973), ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) in milk and tissues after the end of application. During the depuration phase milk was collected periodically for analysis. Depending on the animal the depuration phase extended for 5, 8 or 15 days. Thereafter, each animal was sacrificed and liver, muscle, kidney and fat (perirenal, subcutaneous and mesenteric) were taken for analysis.

Tissue and milk samples were analysed for ethiprole and the three metabolites by method 01431. All analyses were conducted within less than 30 days of sampling and the samples that were not analysed within 24 hours of sampling were stored deep frozen until analysed. All extracts of milk and tissues were analysed within 20 days of extraction. This period is covered by the storage stability experiment conducted during the development of method 01431, which demonstrated the stability of extracts for at least 26 days in all matrices relevant for this study.

Acceptable concurrent recovery data were obtained for all analytes: (ethiprole in milk: spike 0.005–0.05 mg/kg, n = 97, range of recoveries 87–121%, mean recovery 107%, RSD 5.5%), (ethiprole in cream: spike 0.005–1.0 mg/kg, n = 13, range of recoveries 102–119%, mean recovery 110%, RSD 4.9%), (ethiprole in whey: spike 0.005–0.05 mg/kg, n = 5, range of recoveries 95–109%, mean recovery 102%, RSD 5.2%), (ethiprole in muscle: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 99–115%, mean recovery 108%, RSD 4.7%), (ethiprole in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 104–117%, mean recovery 111%, RSD 4.0%), (ethiprole in perirenal fat: spike 0.01–1.0 mg/kg, n = 12, range of recoveries 102–117%, mean recovery 111%, RSD 4.4%), (ethiprole in mesenteric fat: spike 0.01–1.0 mg/kg, n = 11, range of recoveries 82–120%, mean recovery 110%, RSD 10.1%), (ethiprole in liver: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 102–116%, mean recovery 108%, RSD 4.0%), (ethiprole in kidney: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 102–114%, mean recovery 108%, RSD 4.4%);

(ethiprole-sulfone in milk: spike 0.005–0.05 mg/kg, n = 97, range of recoveries 66-128%, mean recovery 106%, RSD 7.8%), (ethiprole-sulfone in cream: spike 0.005–1.0 mg/kg, n = 13, range of recoveries 101–119%, mean recovery 110%, RSD 5.6%), (ethiprole-sulfone in whey: spike 0.005–0.05 mg/kg, n = 5, range of recoveries 102–115%, mean recovery 105%, RSD 5.3%), (ethiprole-sulfone in muscle: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 99–120%, mean recovery 109%, RSD 5.3%),

(ethiprole-sulfone in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 103–120%, mean recovery 111%, RSD 5.1%), (ethiprole-sulfone in perirenal fat: spike 0.01–1.0 mg/kg, n = 12, range of recoveries 103–127%, mean recovery 112%, RSD 6.0%), (ethiprole-sulfone in mesenteric fat: spike 0.01–0.5 mg/kg, n = 10, range of recoveries 92–137%, mean recovery 112%, RSD 10.4%), (ethiprole-sulfone in liver: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 102–116%, mean recovery 109%, RSD 4.4%), (ethiprole-sulfone in kidney: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 102–117%, mean recovery 109%, RSD 4.8%);

(ethiprole-sulfonic acid in milk: spike 0.005–0.05 mg/kg, n = 98, range of recoveries 75–119%, mean recovery 104%, RSD 7.8%), (ethiprole-sulfonic acid in cream: spike 0.005–1.0 mg/kg, n = 13, range of recoveries 101–120%, mean recovery 109%, RSD 6.1%), (ethiprole-sulfonic acid in whey: spike 0.005–0.05 mg/kg, n = 6, range of recoveries 95–107%, mean recovery 102%, RSD 4.5%), (ethiprole-sulfonic acid in muscle: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 98–116%, mean recovery 106%, RSD 5.5%), (ethiprole-sulfonic acid in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 104–116%, mean recovery 109%, RSD 3.8%), (ethiprole-sulfonic acid in perirenal fat: spike 0.01–1.0 mg/kg, n = 12, range of recoveries 106–120%, mean recovery 112%, RSD 3.9%), (ethiprole-sulfonic acid in mesenteric fat: spike 0.01–0.5 mg/kg, n = 10, range of recoveries 85–120%, mean recovery 108%, RSD 8.6%), (ethiprole-sulfonic acid in liver: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 97–109%, mean recovery 103%, RSD 3.7%), (ethiprole-sulfonic acid in kidney: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 101–114%, mean recovery 107%, RSD 3.7%);

(ethiprole-amide in milk: spike 0.005–0.05 mg/kg, n = 99, range of recoveries 70–145%, mean recovery 106%, RSD 11.5%), (ethiprole-amide in cream: spike 0.005–1.0 mg/kg, n = 12, range of recoveries 83–116%, mean recovery 103%, RSD 7.5%), (ethiprole-amide in whey: spike 0.005–0.05 mg/kg, n = 6, range of recoveries 99–120%, mean recovery 105%, RSD 7.1%), (ethiprole-amide in muscle: spike 0.01–0.10 mg/kg, n = 8, range of recoveries 98–115%, mean recovery 107%, RSD 5.6%), (ethiprole-amide in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 106–120%, mean recovery 112%, RSD 4.6%), (ethiprole-amide in perirenal fat: spike 0.01–1.0 mg/kg, n = 12, range of recoveries 97–119%, mean recovery 111%, RSD 5.9%), (ethiprole-amide in mesenteric fat: spike 0.01–0.5 mg/kg, n = 9, range of recoveries 92–115%, mean recovery 108%, RSD 7.2%), (ethiprole-amide in liver: spike 0.01–1.0 mg/kg, n = 9, range of recoveries 103–118%, mean recovery 108%, RSD 4.2%), (ethiprole-amide in kidney: spike 0.01–0.10 mg/kg, n = 8, range of recoveries 91–118%, mean recovery 108%, RSD 8.3%).

Residues in milk and tissues are displayed in Table 94.

No residues of ethiprole or the three metabolites were observed in any of the milk or tissues control samples.

The residues of ethiprole and its metabolites ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) were less than the LOQ of 0.005 mg eq/kg in all milk samples in all dose groups.

Residues of ethiprole-sulfone above the LOQ of 0.005 mg eq/kg were found in the milk samples of the doses groups 3×, 10× and 10×E. The highest residue level of the sulfone compond in milk was 0.0373 mg eq/kg which was found in a sample of the 10×E group. The milk samples collected on the overall study day 30 of dosing from the 0× and the 10× group animals were separated by centrifugation into skim milk (whey) and cream. The cream samples were found to contain up to 0.015 mg eq/kg of ethiprole, 0.41 mg eq/kg of RPA 097973 and 0.007 mg eq/kg of RPA 107566 while the residues of RPA 104615 were less than the LOQ of 0.005 mg eq/kg. The skim milk (whey) samples showed no residues of ethiprole or its metabolites above the LOQ of 0.005 mg eq/kg.

No residues of ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) at or above the LOQ of 0.01 mg eq/kg were found in any of the tissue samples of any dose group. The residues of ethiprole were found to be < 0.01 mg/kg in the tissue samples of the 0×-, 1×- and 3×-groups. Residues of ethiprole above the LOQ were only found in fat (10× group). The fat samples of the depuration group (10×E) did not contain residues of ethiprole >0.01 mg/kg.

Residues of ethiprole-sulfone (RPA 097973) above the LOQ were found in muscle only in the samples of the 10× group with a highest level of 0.036 mg eq/kg. From the muscle samples of the depuration group (10×E), only the 5 day depuration animal contained residues of RPA 097973 above the LOQ (0.01 mg eq/kg).

Residues of RPA 097973 in fat were found at levels >0.01 mg eq/kg (LOQ) in the dose groups 1×, 3×, 10× and 10×E. The highest residue at the 10× dose level was 0.45 mg eq/kg. After a depuration phase of 15 days the residues of RPA 097973 in fat had decreased to <0.01 mg eq/kg.

Residues of RPA 097973 in liver were found at levels >0.01 mg eq/kg (LOQ) in the dose groups 1×, 3×, 10× and 10×E. The highest residue at the 10× dose level was 0.24 mg eq/kg. After a depuration phase of 15 days the residues of RPA 097973 in liver had decreased to <0.01 mg eq/kg.

Residues of RPA 097973 in kidney were found at levels >0.01 mg eq/kg (LOQ) in the dose groups 3×, 10× and 10×E. The highest residue at the 10× dose level was 0.079 mg eq/kg. After a depuration phase of 15 days the residues of RPA 097973 in kidney had decreased to <0.01 mg eq/kg.

Overall the residues of ethiprole-sulfone (RPA 097973) in milk, fat, liver and kidney were found to increase fairly linearly with the dose level of ethiprole.

The results of the study are summarised in the following table.

Table 94 Residues of ethiprole and metabolites in milk and tissues

Animal Commodity	Dose Level of Ethiprole (ppm)	Ethiprole (mg/kg)		Ethiprole-sulfone (RPA 097973) (mg/kg) [a]		Ethiprole + Ethiprole-sulfone [a](mg/kg)		Ethiprole-sulfonic acid (RPA 104615) (mg/kg) [a]		Ethiprole-sulfide (RPA 107566) (mg/kg) [a]	
		Mean	Highest	Mean	Highest	Mean	Highest[b]	Mean	Highest	Mean	Highest
Subcutaneous Fat	0.14 (1×)	<0.01	<0.01	0.0166	0.0196	0.0266	0.0296	<0.01	<0.01	<0.01	<0.01
	0.41 (3×)	<0.01	<0.01	0.0463	0.0575	0.0563	0.0675	<0.01	<0.01	<0.01	<0.01
	1.31 (10×)	<0.01	<0.01	0.153	0.178	0.163	0.188	<0.01	<0.01	<0.01	<0.01
	1.38 (10×E) (5 days depuration)		<0.01		0.1269				<0.01		<0.01
	1.38 (10×E) (8 days depuration)		<0.01		0.0497				<0.01		<0.01
	1.38 (10×E) (15 days depuration)		<0.01		<0.01				<0.01		<0.01
Mesenteric Fat	0.14 (1×)	<0.01	<0.01	0.0319	0.0335	0.0419	0.0435	<0.01	<0.01	<0.01	<0.01
	0.41 (3×)	<0.01	<0.01	0.0701	0.0746	0.0801	0.0846	<0.01	<0.01	<0.01	<0.01
	1.31 (10×)	0.0119	0.013	0.291	0.419	0.303	0.432	<0.01	<0.01	<0.01	<0.01
	1.38 (10×E) (5 days depuration)		<0.01		0.1392				<0.01		<0.01
	1.38 (10×E) (8 days depuration)		<0.01		0.0589				<0.01		<0.01
	1.38 (10×E) (15 days depuration)		<0.01		<0.01				<0.01		<0.01
Perirenal Fat	0.14 (1×)	<0.01	<0.01	0.0279	0.0317	0.0379	0.0417	<0.01	<0.01	<0.01	<0.01
	0.41 (3×)	<0.01	<0.01	0.0752	0.0807	0.0852	0.0907	<0.01	<0.01	<0.01	<0.01
	1.31 (10×)	0.0114	0.0121	0.320	0.447	0.331	0.459	<0.01	<0.01	<0.01	<0.01
	1.38 (10×E) (5 days depuration)		<0.01		0.1345				<0.01		<0.01
	1.38 (10×E) (8 days depuration)		<0.01		0.0507				<0.01		<0.01
	1.38 (10×E) (15 days depuration)		<0.01		<0.01				<0.01		<0.01
Muscle	0.14 (1×)	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	0.41 (3×)	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	1.31 (10×)	<0.01	<0.01	0.031	0.036	0.041	0.046	<0.01	<0.01	<0.01	<0.01
	1.38 (10×E) (5 days depuration)		<0.01		0.0130				<0.01		<0.01
	1.38 (10×E) (8 days depuration)		<0.01		<0.01				<0.01		<0.01
	1.38 (10×E) (15 days depuration)		<0.01		<0.01				<0.01		<0.01
Liver	0.14 (1×)	<0.01	<0.01	0.0271	0.0288	0.0371	0.0388	<0.01	<0.01	<0.01	<0.01
	0.41 (3×)	<0.01	<0.01	0.0601	0.0626	0.0701	0.0726	<0.01	<0.01	<0.01	<0.01
	1.31 (10×)	<0.01	<0.01	0.218	0.242	0.228	0.252	<0.01	<0.01	<0.01	<0.01
	1.38 (10×E) (5 days depuration)		<0.01		0.1007				<0.01		<0.01
	1.38 (10×E) (8 days depuration)		<0.01		0.0481				<0.01		<0.01
	1.38 (10×E) (15 days depuration)		<0.01		<0.01				<0.01		<0.01
Kidney	0.14 (1×)	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	0.41 (3×)	<0.01	<0.01	0.0174	0.0182	0.0274	0.0282	<0.01	<0.01	<0.01	<0.01
	1.31 (10×)	<0.01	<0.01	0.070	0.079	0.080	0.089	<0.01	<0.01	<0.01	<0.01
	1.38 (10×E) (5 days depuration)		<0.01		0.0329				<0.01		<0.01
	1.38 (10×E) (8 days depuration)		<0.01		0.0113				<0.01		<0.01
	1.38 (10×E)		<0.01		<0.01				<0.01		<0.01

Animal Commodity	Dose Level of Ethiprole (ppm)		Ethiprole (mg/kg)		Ethiprole-sulfone (RPA 097973) (mg/kg) [a]		Ethiprole + Ethiprole-sulfone [a](mg/kg)		Ethiprole-sulfonic acid (RPA 104615) (mg/kg) [a]		Ethiprole-sulfide (RPA 107566) (mg/kg) [a]	
			Mean	Highest	Mean	Highest	Mean	Highest[b]	Mean	Highest	Mean	Highest
	(15 days depuration)											
Milk	0.14 (1×)	Day 1	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 3	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 4	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 7	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 9	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 11	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 14	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 16	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 18	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 21	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 23	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 29	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
	0.41 (3×)	Day 2	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 3	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 4	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 7	<0.005	<0.005	0.0063	0.0066	0.0113	0.0116	<0.005	<0.005	<0.005	<0.005
		Day 9	<0.005	<0.005	0.0066	0.0069	0.0116	0.0119	<0.005	<0.005	<0.005	<0.005
		Day 11	<0.005	<0.005	0.0066	0.0071	0.0116	0.0121	<0.005	<0.005	<0.005	<0.005
		Day 14	<0.005	<0.005	0.0070	0.0077	0.0120	0.0127	<0.005	<0.005	<0.005	<0.005
		Day 16	<0.005	<0.005	0.0065	0.0073	0.0115	0.0123	<0.005	<0.005	<0.005	<0.005
		Day 18	<0.005	<0.005	0.0077	0.0079	0.0127	0.0129	<0.005	<0.005	<0.005	<0.005
		Day 21	<0.005	<0.005	0.0064	0.0072	0.0114	0.0122	<0.005	<0.005	<0.005	<0.005
		Day 23	<0.005	<0.005	0.0069	0.0073	0.0119	0.0123	<0.005	<0.005	<0.005	<0.005
		Day 30	<0.005	<0.005	0.0066	0.0076	0.0116	0.0126	<0.005	<0.005	<0.005	<0.005
	1.31 (10×)	Day 3	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 4	<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005
		Day 7	<0.005	<0.005	0.0161	0.0187	0.0211	0.0237	<0.005	<0.005	<0.005	<0.005
		Day 9	<0.005	<0.005	0.0210	0.0231	0.0260	0.0281	<0.005	<0.005	<0.005	<0.005
		Day 11	<0.005	<0.005	0.0228	0.0267	0.0278	0.0317	<0.005	<0.005	<0.005	<0.005
		Day 14	<0.005	<0.005	0.0240	0.0306	0.0290	0.0356	<0.005	<0.005	<0.005	<0.005
		Day 16	<0.005	<0.005	0.0249	0.0271	0.0299	0.0321	<0.005	<0.005	<0.005	<0.005
		Day 18	<0.005	<0.005	0.0237	0.0278	0.0287	0.0328	<0.005	<0.005	<0.005	<0.005
		Day 21	<0.005	<0.005	0.0241	0.0258	0.0291	0.0308	<0.005	<0.005	<0.005	<0.005
		Day 23	<0.005	<0.005	0.0258	0.0295	0.0308	0.0345	<0.005	<0.005	<0.005	<0.005
		Day 30	<0.005	<0.005	0.0245	0.0260	0.0295	0.0310	<0.005	<0.005	<0.005	<0.005
		Day 31	<0.005	<0.005	0.0277	0.0356	0.0327	0.0406	<0.005	<0.005	<0.005	<0.005
	1.38 (10×E)	Day 31	<0.005	<0.005	0.0338	0.0373	0.0388	0.0423	<0.005	<0.005	<0.005	<0.005
		Day 35	<0.005	<0.005	0.0139	0.0153	0.0189	0.0203	<0.005	<0.005	<0.005	<0.005
		Day 38	<0.005	<0.005	0.0056	0.0059	0.0106	0.0109	<0.005	<0.005	<0.005	<0.005
		Day 45	-	<0.005	-	<0.005	-	<0.01	-	<0.005	-	<0.005
Cream (Day 30)	1.38 (10×)		0.012	0.0149	0.349	0.413	0.361	0.4279	<0.005	<0.005	0.006	0.007
Whey (Day 30)	1.38 (10×)		<0.005	<0.005	<0.005	<0.005	<0.01	<0.01	<0.005	<0.005	<0.005	<0.005

LOQ is 0.005 mg/kg for each of parent ethiprole and the metabolites RPA 097973, RPA 104615 and RPA 107566 (parent equivs.) in milk, cream and whey.

LOQ is 0.01 mg/kg for each of parent ethiprole and the metabolites RPA 097973, RPA 104615 and RPA 107566 (parent equivs.) in tissues (muscle, fat, liver and kidney).

[a] Calculated as parent equivalents.

[b] The highest total residues is from one animal, not from the addition of highest ethiprole + ethiprole-sulfone from more than one animal.

During the whole study no residues of ethiprole-sulfonic acid (RPA 104615) above the respective LOQ of 0.005 mg eq/kg or 0.01 mg eq/kg were found in any milk, cream, skimmed milk or tissue sample while residues of ethiprole-sulfide (RPA 107566) above the LOQ were only found in cream (maximum of 0.007 mg eq/kg). The only samples which were found to contain residues of

ethiprole above the LOQ were cream and fat samples of the 10× and 10×E groups. The highest residue of ethiprole was 0.015 mg/kg in cream and 0.013 mg/kg in fat.

Residues of the metabolite ethiprole-sulfone (RPA 097973) were present above the LOQ in milk and cream of the dose groups 3×, 10× and 10×E. The highest residues were 0.037 mg eq/kg in milk (observed for the 10×E group) and 0.41 mg eq/kg in cream (10× group). The sulfone was present above the LOQ in muscle of the 10× group (highest residue 0.036 mg eq/kg), in kidney of the 3×, 10× and 10×E dose groups (highest residue 0.079 mg eq/kg), in liver of the 1×, 3×, 10× and 10×E dose groups (highest residue 0.24 mg eq/kg), and fat of the 1×, 3×, 10× and 10×E dose groups (highest residue 0.45 mg eq/kg).

Laying hen transfer study (preliminary)

A preliminary study was conducted in the USA to determine the level of ethiprole residues in the eggs and tissues of hens following the oral administration of ethiprole at a nominal rate corresponding to 8 ppm in the feed (Tew 2001, M-240498-01-2).

Five hens were dosed each mornng after egg collection for 28 days. A second group of five hens served as control. Eggs were collected twice each day. Egg samples from days 17, 21, 24 and 28 were collected for analysis. At the end of the 28-day dosing period, the hens were euthanised and samples of muscle, liver and fat were collected for analysis.

Tissue and eggs samples were analysed for ethiprole and its metabolites ethiprole-sulfone (RPA 097973) and ethiprole-methyl sulfone (RPA 094569) according to LC-MS/MS method 019-034. The limit of quantification was 0.001 mg/kg for each analyte (in parent equivalents) in all matrices.

Concurrent recovery data were obtained for all analytes: (ethiprole in eggs: spike 0.001 mg/kg, n = 1, recovery 102%; spike 0.500 mg/kg, n = 1, recovery 110%), (ethiprole in fat: spike 0.001 mg/kg, n = 1, recovery 809%; spike 0.500 mg/kg, n = 1, recovery 85%), (ethiprole in liver: spike 0.001 mg/kg, n = 1, recovery 11%; spike 0.500 mg/kg, n = 1, recovery 98%), (ethiprole in muscle: spike 0.001 mg/kg, n = 1, recovery 23%; spike 0.500 mg/kg, n = 1, recovery 130%);

(ethiprole-sulfone in eggs: spike 0.001 mg/kg, n = 1, recovery 122%; spike 0.500 mg/kg, n = 1, recovery 114%), (ethiprole-sulfone in fat: spike 0.001 mg/kg, n = 1, recovery 169%; spike 0.500 mg/kg, n = 1, recovery 104%), (ethiprole-sulfone in liver: spike 0.001 mg/kg, n = 1, recovery 98%; spike 0.500 mg/kg, n = 1, recovery 111%), (ethiprole-sulfone in muscle: spike 0.001 mg/kg, n = 1, recovery 114%; spike 0.500 mg/kg, n = 1, recovery 122%);

(ethiprole-methyl sulfone in eggs: spike 0.001 mg/kg, n = 1, recovery 94%; spike 0.500 mg/kg, n = 1, recovery 111%), (ethiprole-sulfone in fat: spike 0.001 mg/kg, n = 1, recovery 112%; spike 0.500 mg/kg, n = 1, recovery 100%), (ethiprole-sulfone in liver: spike 0.001 mg/kg, n = 1, recovery 86%; spike 0.500 mg/kg, n = 1, recovery 108%), (ethiprole-sulfone in muscle: spike 0.001 mg/kg, n = 1, recovery 82%; spike 0.500 mg/kg, n = 1, recovery 125%).

In tissue samples, four recovery values for spikes at the LOQ were outside the normally acceptable range. These recoveries were 11%, 23% and 809% for parent in liver, muscle and fat respectively and 169% for ethiprole-sulfone in fat. These were assumed to be due to apparent contamination in the control samples.

Residues of ethiprole and metabolites in eggs and hen tissues are reported in the table below:

Table 95 Ethiprole and its metabolites (RPA 097973 and RPA 094569) in poultry eggs and tissues

Commodity	Residue (mg/kg) expressed as parent equivalents			
	Ethiprole	Ethiprole-sulfone (RPA 097973)	Ethiprole-methyl sulfone (RPA 094569)	Parent + Ethiprole-sulfone
Eggs				
17 days	0.009	0.296	0.009	0.305
21 days	0.048	0.277	0.008	0.325
24 days	0.009	0.307	0.010	0.316
28 days	0.011	0.315	0.020	0.326
Fat	0.004	0.689	0.020	0.693
Liver	0.011	0.347	0.011	0.358
Muscle	<0.001	0.038	0.002	0.039

LOQ is 0.001 mg/kg for each of parent ethiprole and the metabolites RPA 097973 and RPA 094569 in eggs, muscle, liver and fat

The egg samples collected daily from day 17 to day 28 (last day of dosing) were analysed to determine the level of residue and verify that residues had reached a plateau level. The total residue (sum of ethiprole and RPA 097973, expressed as ethiprole equivalents) in eggs was consistent at 0.30–0.33 mg eq/kg for days 17 to 28. Thus, the total residues of ethiprole in eggs reached a plateau at or before 17 days after the first dose. The residues at the plateau and throughout the study consisted almost entirely of the ethiprole-sulfone. This is consistent with results from the hen metabolism study. With one exception (day 21), the residues

of parent ethiprole and RPA 094569 were ≤ 0.01 mg eq/kg. On day 21, the residues of ethiprole were 0.048 mg eq/kg, while the residues of RPA 097973 were correspondingly lower than on the other sampling days.

In tissues, the residues were highest in fat with a total residue of 0.69 mg eq/kg followed by liver with 0.36 mg eq/kg. The total residues in muscle were much lower than in any other matrix at 0.04 mg eq/kg. These results are also consistent with the hen metabolism study.

The transfer factors derived from the feeding study (ratio of residue level in eggs and tissues over the residue level in the feed) are summarised in the following table.

Table 96 Transfer factors for ethiprole-derived residues in eggs and poultry tissues

Feeding level	8 ppm dry feed	
Commodity	Total ethiprole (mg/kg)	Transfer factor
Egg	0.326	0.041
Fat	0.693	0.087
Liver	0.358	0.045
Muscle	0.039	0.005

Laying hen transfer study

A study was conducted to determine residues of ethiprole and its metabolites ethiprole-sulfone (RPA 097973), ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) in eggs and tissues of laying hens orally dosed with ethiprole for 28 days (Glaubitz 2017, M-586814-02-1).

Forty-two hens of a common commercial strain (Gallus gallus domesticus, egg laying young adults, 1.50–2.07 kg at the beginning of the third week of the acclimatisation) were dosed with ethiprole by gelatin capsule daily for 28 consecutive days. The animals were allocated to a control group (0×, 6 hens) and 4 treated groups (1×, 6×, 30× and 30×E, 9 hens in each). The target dose rates of ethiprole were 0.005 mg/kg bw/day test item for the 1× dose group, 0.030 mg/kg bw/day for the dose group 6× and 0.15 mg/kg bw/day for the dose groups 30× and 30×E.

The hens were fed with non-supplemented commercial laying hen meal and the amount of feed consumed was monitored daily. Based on actual feed intake, the dose rates simulated residue concentrations in feed dry matter (expressed as ethiprole) of 0.084 ppm (1× group), 0.50 ppm (6× group), 2.51 ppm (30× group) and 2.46 ppm (30×E group).

Egg samples of the hens of the 0, 1, 6 and 30× groups were taken once before the first dose administration, at least every third day during the first three weeks of dosage and twice during the last week of dosage. The animals were sacrificed on the day after the 28th and final test item administration, approximately 24 hours after the final dose. Liver, muscle and fat with adhering skin were taken for analysis.

The nine laying hens of the 30×E group were dosed at the rate of 0.05 mg/kg bw/day for 28 consecutive days simultaneously with the animals from dose group 30×. Thereafter dosing was stopped and the animals were kept alive for further 5–15 days in order to investigate the depuration of ethiprole, ethiprole-sulfone (RPA 097973), ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) in eggs and tissues after the end of test item administration. During the depuration phase eggs were collected periodically for analysis. Depending on the animal the depuration phase extended for 5, 8 or 15 days. Thereafter, each animal was sacrificed and liver, muscle and fat with adhering skin were taken for analysis.

Tissue and egg samples were analysed for ethiprole and the three metabolites by method 01431. All analyses were conducted within less than 30 days of sampling and the samples that were not analysed within 24 hours of sampling were stored deep frozen until analysed. All extracts of eggs and tissues were analysed within 20 days of extraction. This period is covered by the storage stability experiment conducted during the development of method 01431, which demonstrated the stability of extracts for at least 26 days in all matrices relevant for this study.

Acceptable concurrent recovery data were obtained for all analytes (ethiprole in egg: spike 0.01–1.0 mg/kg, n = 78, range of recoveries 81–116%, mean recovery 97%, RSD 6.0%), (ethiprole in yolk: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 90–103%, mean recovery 99%, RSD 5.2%), (ethiprole in egg white: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 94–109%, mean recovery 101%, RSD 5.2%), (ethiprole in muscle: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 96–110%, mean recovery 102%, RSD 5.1%), (ethiprole in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 93–111%, mean recovery 102%, RSD 6.6%), (ethiprole in liver: spike 0.01–1.0 mg/kg, n = 7, range of recoveries 99–107%, mean recovery 102%, RSD 2.7%);

(ethiprole-sulfone in egg: spike 0.01–1.0 mg/kg, n = 78, range of recoveries 82–117%, mean recovery 97%, RSD 6.5%), (ethiprole-sulfone in yolk: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 91–111%, mean recovery 101%, RSD 6.9%), (ethiprole-sulfone in egg white: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 92–108%, mean recovery 101%, RSD 6.0%), (ethiprole-

sulfone in muscle: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 95–109%, mean recovery 100%, RSD 5.4%), (ethiprole-sulfone in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 91–114%, mean recovery 101%, RSD 8.5%), (ethiprole-sulfone in liver: spike 0.01–1.0 mg/kg, n = 7, range of recoveries 96–111%, mean recovery 102%, RSD 5.2%);

(ethiprole-sulfonic acid in egg: spike 0.01–1.0 mg/kg, n = 78, range of recoveries 82–111%, mean recovery 96%, RSD 6.1%), (ethiprole-sulfonic acid in yolk: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 91–104%, mean recovery 99%, RSD 4.6%), (ethiprole-sulfonic acid in egg white: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 95–110%, mean recovery 105%, RSD 5.1%), (ethiprole-sulfonic acid in muscle: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 89–104%, mean recovery 97%, RSD 5.6%), (ethiprole-sulfonic acid in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 93–108%, mean recovery 101%, RSD 6.4%), (ethiprole-sulfonic acid in liver: spike 0.01–1.0 mg/kg, n = 7, range of recoveries 93–108%, mean recovery 100%, RSD 6.2%);

(ethiprole-amide in egg: spike 0.01–1.0 mg/kg, n = 78, range of recoveries 77–127%, mean recovery 98%, RSD 8.4%), (ethiprole-amide in yolk: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 100–110%, mean recovery 105%, RSD 4.3%), (ethiprole-amide in egg white: spike 0.01–1.0 mg/kg, n = 6, range of recoveries 97–115%, mean recovery 106%, RSD 5.7%), (ethiprole-amide in muscle: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 83–108%, mean recovery 100%, RSD 8.9%), (ethiprole-amide in subcutaneous fat: spike 0.01–1.0 mg/kg, n = 8, range of recoveries 87–115%, mean recovery 100%, RSD 10.1%), (ethiprole-amide in liver: spike 0.01–1.0 mg/kg, n = 7, range of recoveries 103–116%, mean recovery 109%, RSD 4.1%).

Residues in eggs and tissues are displayed in Table 97.

No residues of ethiprole or the three metabolites were observed in any of the eggs or tissues control samples.

Residues of the metabolites ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) were less than the LOQ of 0.01 mg eq/kg in all egg samples in all dose groups. Residues of ethiprole at measurable levels were found in some egg samples of the dose 30× (at the LOQ of 0.01 mg/kg). Residues of RPA 097973 above the LOQ of 0.01 mg eq/kg were found in the egg samples of the doses groups 6×, 30× and 30×E. These residues increased during the first days of dosing and reached a plateau about 11 to 14 days after the beginning of dosing. The highest residue of RPA 097973 in eggs was 0.178 mg eq/kg and was found in a sample of the 30× group on day 23. After a depuration phase of 15 days the residues of RPA 097973 in eggs had decreased to <0.01 mg eq/kg.

The egg samples collected on the overall study day 30 from the 30× group animals were separated into yolk and egg white. The yolk samples were found to contain up to 0.016 mg/kg of ethiprole and 0.44 mg eq/kg of RPA 097973 while the residues of RPA 104615 and RPA 107566 were <0.01 mg eq/kg. The egg white samples were found to contain only residues of RPA 097973 up to 0.032 mg eq/kg while the residues of ethiprole, RPA 104615 and RPA 107566 were <0.01 mg eq/kg.

Residues of ethiprole, ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) were <LOQ in all the tissue samples of all dose groups. The residues of RPA 097973 were found to be <0.01 mg eq/kg in all tissue samples of the 1×-group and in the muscle samples of the 6× group.

Residues of RPA 097973 in muscle were found at levels above the LOQ of 0.01 mg eq/kg in the samples of the 30× group only, with a highest level of 0.02 mg eq/ kg. The muscle samples of the depuration group (30×E) did not contain residues of RPA 097973 above the LOQ.

Residues of RPA 097973 in fat were found at levels above the LOQ of 0.01 mg eq/kg in the dose groups 6× and 30×. The highest residue at the 30× dose level was 0.168 mg eq/kg. After a depuration phase of 5 days the residues of RPA 097973 in fat had decreased to < 0.01 mg eq/kg.

Residues of RPA 097973 in liver were found at levels above the LOQ of 0.01 mg eq/kg in the dose groups 6× and 30×. The highest residue at the 30× dose level was 0.128 mg eq/kg. After a depuration phase of 5 days the residues of RPA 097973 in liver had decreased to < 0.01 mg eq/kg.

Overall the residues of RPA 097973 in egg, muscle, fat and liver were found to increase fairly linearly with the dose level of ethiprole.

The results of the study are summarised in the following table.

Table 97 Residues of ethiprole and metabolites in eggs and tissues

Animal Commodity	Dose Level of Ethiprole (ppm)	Ethiprole (mg/kg)		Ethiprole-sulfone (RPA 097973) (mg/kg) [a]		Ethiprole + Ethiprole-sulfone (mg/kg) [a]		Ethiprole-sulfonic acid (RPA 104615) (mg/kg) [a]		Ethiprole-sulfide (RPA 107566) (mg/kg) [a]	
		Mean	Highest	Mean	Highest	Mean	Highest[b]	Mean	Highest	Mean	Highest
Muscle	0.084 (1×)	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	0.50 (6×)	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01

Animal Commodity	Dose Level of Ethiprole (ppm)		Ethiprole (mg/kg)		Ethiprole-sulfone (RPA 097973) (mg/kg) [a]		Ethiprole + Ethiprole-sulfone (mg/kg) [a]		Ethiprole-sulfonic acid (RPA 104615) (mg/kg) [a]		Ethiprole-sulfide (RPA 107566) (mg/kg) [a]	
			Mean	Highest	Mean	Highest	Mean	Highest[b]	Mean	Highest	Mean	Highest
	2.51 (30×)		<0.01	<0.01	0.0167	0.0200	0.0267	0.0300	<0.01	<0.01	<0.01	<0.01
	2.46 (30×E) (5 days depuration)			<0.01		<0.01				<0.01		<0.01
	2.46 (30×E) (8 days depuration)			<0.01		<0.01				<0.01		<0.01
	2.46 (30×E) (15 days depuration)			<0.01		<0.01				<0.01		<0.01
Fat	0.084 (1×)		<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	0.50 (6×)		<0.01	<0.01	0.0347	0.0380	0.0447	0.048	<0.01	<0.01	<0.01	<0.01
	2.51 (30×)		<0.01	<0.01	0.1483	0.1680	0.153	0.178	<0.01	<0.01	<0.01	<0.01
	2.46 (30×E) (5 days depuration)			<0.01		<0.01				<0.01		<0.01
	2.46 (30×E) (8 days depuration)			<0.01		<0.01				<0.01		<0.01
	2.46 (30×E) (15 days depuration)			<0.01		<0.01				<0.01		<0.01
Liver	0.084 (1×)		<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	0.50 (6×)		<0.01	<0.01	0.0260	0.0290	0.0360	0.0390	<0.01	<0.01	<0.01	<0.01
	2.51 (30×)		<0.01	<0.01	0.113	0.1280	0.123	0.138	<0.01	<0.01	<0.01	<0.01
	2.46 (30×E) (5 days depuration)			<0.01		<0.01				<0.01		<0.01
	2.46 (30×E) (8 days depuration)			<0.01		<0.01				<0.01		<0.01
	2.46 (30×E) (15 days depuration)			<0.01		<0.01				<0.01		<0.01
Eggs	0.084 (1×)	Day 1	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 3	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 4	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 7	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 9	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 11	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 14	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 16	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 18	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 21	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 23	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 29	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
	0.50 (6×)	Day 2	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 3	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 4	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 7	<0.01	<0.01	0.0177	0.0190	0.0277	0.0290	<0.01	<0.01	<0.01	<0.01
		Day 9	<0.01	<0.01	0.0257	0.0290	0.0357	0.0390	<0.01	<0.01	<0.01	<0.01
		Day 11	<0.01	<0.01	0.0303	0.0320	0.0403	0.0420	<0.01	<0.01	<0.01	<0.01
		Day 14	<0.01	<0.01	0.0300	0.0300	0.0400	0.0400	<0.01	<0.01	<0.01	<0.01
		Day 16	<0.01	<0.01	0.0317	0.0360	0.0417	0.0460	<0.01	<0.01	<0.01	<0.01
		Day 18	<0.01	<0.01	0.0270	0.0320	0.0370	0.0420	<0.01	<0.01	<0.01	<0.01
		Day 21	<0.01	<0.01	0.0317	0.0360	0.0417	0.0460	<0.01	<0.01	<0.01	<0.01
		Day 23	<0.01	<0.01	0.0320	0.0330	0.0420	0.0430	<0.01	<0.01	<0.01	<0.01
		Day 30	<0.01	<0.01	0.0340	0.0370	0.0440	0.0470	<0.01	<0.01	<0.01	<0.01
	2.51 (30×)	Day 3	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 4	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.01	<0.01	<0.01	<0.01
		Day 7	<0.01	<0.01	0.0607	0.0700	0.0707	0.0800	<0.01	<0.01	<0.01	<0.01
		Day 9	<0.01	<0.01	0.1090	0.1210	0.1190	0.1310	<0.01	<0.01	<0.01	<0.01
		Day 11	<0.01	0.010	0.1340	0.1490	0.1440	0.1590	<0.01	<0.01	<0.01	<0.01
		Day 14	<0.01	0.010	0.1503	0.1690	0.1603	0.1790	<0.01	<0.01	<0.01	<0.01
		Day 16	<0.01	0.010	0.1477	0.1680	0.1577	0.1780	<0.01	<0.01	<0.01	<0.01
		Day 18	<0.01	0.010	0.1493	0.1780	0.1593	0.1880	<0.01	<0.01	<0.01	<0.01

Animal Commodity	Dose Level of Ethiprole (ppm)		Ethiprole (mg/kg)		Ethiprole-sulfone (RPA 097973) (mg/kg) [a]		Ethiprole + Ethiprole-sulfone (mg/kg) [a]		Ethiprole-sulfonic acid (RPA 104615) (mg/kg) [a]		Ethiprole-sulfide (RPA 107566) (mg/kg) [a]	
			Mean	Highest	Mean	Highest	Mean	Highest[b]	Mean	Highest	Mean	Highest
		Day 21	<0.01	<0.01	0.1533	0.1730	0.1633	0.1830	<0.01	<0.01	<0.01	<0.01
		Day 23	<0.01	0.010	0.1617	0.1780	0.1717	0.1880	<0.01	<0.01	<0.01	<0.01
		Day 31	<0.01	<0.01	0.1470	0.1550	0.1570	0.1650	<0.01	<0.01	<0.01	<0.01
	2.46 (30×E)	Day 21	<0.01	<0.01	0.1443	0.1580	0.1543	0.1680	<0.01	<0.01	<0.01	<0.01
		Day 23	<0.01	<0.01	0.1427	0.1470	0.1527	0.1570	<0.01	<0.01	<0.01	<0.01
		Day 31 Depuration	<0.01	<0.01	0.1360	0.1470	0.1460	0.1570	<0.01	<0.01	<0.01	<0.01
		Day 35 Depuration	<0.01	<0.01	0.0727	0.0810	0.0827	0.0910	<0.01	<0.01	<0.01	<0.01
		Day 38 Depuration	<0.01	<0.01	0.0180	0.0200	0.0280	0.0300	<0.01	<0.01	<0.01	<0.01
		Day 45 Depuration	-	<0.01	-	<0.01	-	<0.02	-	<0.01	-	<0.01
Yolk (Day 30)	2.51 (30×)		0.0123	0.0160	0.3367	0.4440	0.3490	0.4600	<0.01	<0.01	<0.01	<0.01
Egg white (Day 30)	2.51 (30×)		<0.01	<0.01	0.0290	0.0320	0.0390	0.0420	<0.01	<0.01	<0.01	<0.01

LOQ is 0.01 mg/kg for each of parent ethiprole and the metabolites RPA 097973, RPA 104615 and RPA 107566 (parent equivalents.) in eggs and tissues.

[a] Calculated as parent equivalents.

[b] The highest total residues is from one animal, not from the addition of highest ethiprole + ethiprole-sulfone from more than one animal

During the whole study no residues of ethiprole-sulfonic acid (RPA 104615) and ethiprole-sulfide (RPA 107566) at or above above the respective LOQ of 0.01 mg eq/kg were found in any egg, yolk, egg white or tissue sample. The only samples which were found to contain residues of ethiprole at or above the LOQ were egg and yolk samples of the 30× group. The highest residues of ethiprole were 0.010 mg/kg in egg and 0.016 mg/kg in yolk.

Residues of the metabolite ethiprole-sulfone (RPA 097973) were present above the LOQ in egg, yolk and egg white of the dose groups 6×, 30× and 30×E. The highest residues (observed for the 30E group) were 0.178 mg eq/kg in egg, 0.44 mg eq/kg in yolk and 0.032 mg eq/kg in egg white. The sulfone was present at or above the LOQ in muscle of the 30× group (highest residue 0.02 mg eq/kg), in liver of the 6× and 30× dose groups (highest residue 0.128 mg eq/kg) and fat of the 6× and 30× dose groups (highest residue 0.168 mg eq/kg).

Residues of ethiprole and its metabolites RPA 104615 and RPA 107566 were below the LOQ of 0.01 mg eq/kg in all egg and tissue samples of the depuration group (30×E).

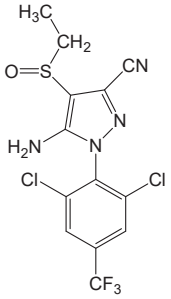
APPRAISAL

Ethiprole is an insecticide belonging to the chemical class of phenylpyrazoles. Ethiprole acts by interfering with the passage of chloride ions through the γ-aminobutyric acid GABA regulated chloride channel, thereby disrupting an insects central nervous system activity and causing death.

It was scheduled for evaluation as a new compound by the 2018 JMPR at the Forty-ninth Session of the CCPR (2017).

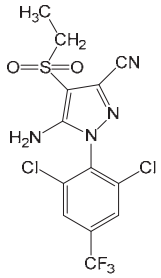
The manufacturer supplied information on identity, metabolism and environmental fate, methods of residue analysis, freezer storage stability, registered use patterns, supervised residue trials, fate of residues in processing and farm animal feeding studies.

The IUPAC name is 5-Amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-ethylsulfinylpyrazole-3-carbonitrile.

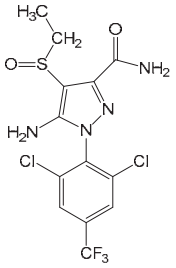


Ethiprole

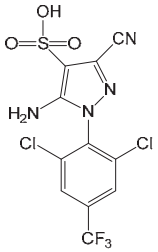
The structures of the key metabolites discussed are shown below:



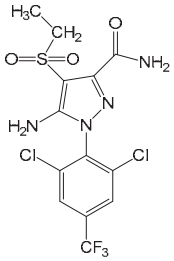
ethiprole-sulfone
(RPA 097973)



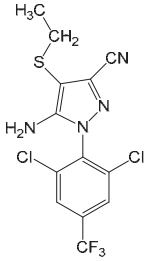
ethiprole-amide
(RPA 122916)



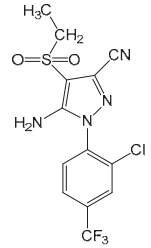
ethiprole-sulfonic acid
(RPA 104615)



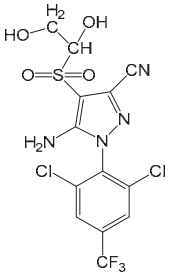
ethiprole-sulfone-amide
(RPA 112917)

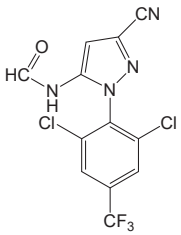


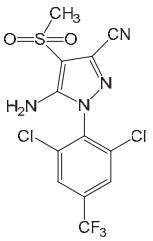
ethiprole-sulfide
(RPA 107566)



ethiprole-deschloro-sulfone
(RPA 115369)



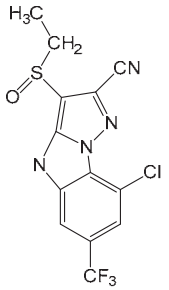




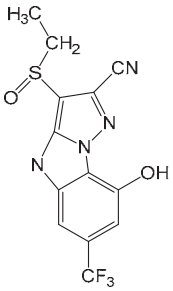
ethiprole-dihydroxy-sulfone
(dihydroxy-RPA 097973)

ethiprole-formamide
(RPA 103343)

ethiprole-methyl-sulfone
(RPA 094569)



RPA 157925



AE 0764815

Physical and chemical properties of ethiprole

Ethiprole is not volatile. It generally has a higher solubility in organic solvents in comparison to water. The n-octanol water partition coefficient log Pow is 2.9 at 20 °C, suggesting that the parent has the potential to partition into fat. Ethiprole was shown to be hydrolytically stable at pH 4, 5 and 7, but slowly degrades at pH 9, with ethiprole-amide the only detected hydrolysis product. Ethiprole is photolytically unstable in aqueous media.

Plant metabolism

Ethiprole metabolism in primary crops was investigated following either foliar applications (rice, sweet pepper and cotton), or by soil application (rice) using 14C-ethiprole labelled on the phenyl moiety.

Two different methods of application to rice were described. In one, the metabolism of 14C-ethiprole was investigated following foliar application of ethiprole to greenhouse grown rice at a total seasonal rate of 670 g ai/ha. Separate rice plants were treated at 5× that rate. The first application was made 25 days prior to crop maturity, with the second application 11 days later, 14 days prior to harvest. In another study, soil application of ethiprole was performed, in order to simulate application of a granule formulation to water in paddy rice. 14C-ethiprole was applied twice by soil drench applications to paddy rice at 600g ai/ha at BBCH 65 (= full flowering) and BBCH 69–89 (= between milk stage and ripening). Harvest was 30 days after the last application.

TRRs from foliar application were 6.3 mg eq/kg for rice straw, 2.1 mg eq/kg for rice grain, 0.15 mg eq/kg for brown rice and 4.0 mg eq/kg for rice hulls, TRRs from soil application were 24.0 mg eq/kg for rice straw, 5.7 mg eq/kg for rice husks and 0.28 mg eq/kg for hulled rice grain.

Acetonitrile and water extraction of rice, resulted in extraction efficiencies of 87–113% for rice straw, 85–100% for paddy rice grain and hulled (brown) rice grain and 62–100% for rice hulls/husks.

The main compound was parent in all rice matrices [67–75% of the TRR for foliar application (0.10–4.7 mg eq/kg) and 42–62% of the TRR for soil application (0.18–10.1 mg eq/kg)]. The major metabolite was ethiprole-sulfone which was found at significant levels in all matrices [20–35% TRR for foliar application (0.03–2.2 mg eq/kg) and 18–23% TRR for soil application (0.051–5.6 mg eq/kg)]. Ethiprole-amide was observed at 11% TRR in rice straw from soil application, while it was observed in husks and brown rice grain at 8% TRR. It was present in rice matrices after foliar application at < 1% TRR. No other metabolites were present at > 5% TRR in any rice matrices.

The metabolism of 14C-ethiprole in greenhouse sweet peppers was investigated following foliar application at a total seasonal rate of 670 g ai/ha. The two applications (450 and 220 g ai/ha) were made 26 and 14 days prior to harvest. In addition separate fruit were treated at 5× this seasonal rate. Plant samples treated at the 1× rate were collected 2–4 hours after the first application (foliage only), prior to the second application (fruit and foliage), 2–4 hours after the second application (fruit and foliage), and at final harvest (fruit and foliage). Samples treated at the 5× rate were collected only at final harvest.

TRRs in foliage were 184 mg eq/kg after the first application, 36.0 mg eq/kg before the second application, 118 mg eq/kg after the second application, and 44.6 mg eq/kg at harvest for the 1× application. TRRs were 0.45–0.68 mg eq/kg in green pepper fruit and 0.31–0.55 mg eq/kg in red peppers for the 1× application.

Acetonitrile and water extraction resulted in extraction efficiencies of 77–100% for pepper foliage and 85–101% for green and red pepper fruit.

Ethiprole accounted for the majority of the TRR in the foliage (83–99% TRR, 30.8–171 mg eq/kg) at each time point (after the first application, before and after the second application and at harvest) for the 1× application. More extensive metabolism was observed in sweet peppers, with ethiprole present at 22–92% TRR. In green (immature) peppers, ethiprole-amide and ethiprole-sulfone were both present at levels >10% TRR. Ethiprole-amide and ethiprole-sulfone were observed at up to 15% TRR and 13% TRR, respectively at harvest after two applications. Ethiprole-amide was also observed at 18% TRR after application of 1 spray at 220 g ai/ha to new fruit formed after the first treatment. In red (mature) fruits, ethiprole-sulfone accounted for up to 16% TRR with ethiprole-amide observed at 5% TRR. No other metabolites were present at >4% TRR in either foliage or fruit.

The metabolism of 14C-ethiprole in field grown cotton was investigated following two foliar applications at a total seasonal rate of 670 g a.i./ha. The first application, representing two-thirds of the seasonal rate, was made 61 days prior to harvest and the second application was made 48 days prior to harvest. Further cotton plants were treated at 10× that seasonal use rate (i.e. two applications for a total of 6.7 kg a.i./ha). Plant samples were collected just before the second application [foliage [TRR = 55 (1×)–348 (10×) mg eq/kg)], old [TRR = 15.9 (1×)–256 (10×) mg eq/kg] and new growth [TRR = 5.0 (1×)–136 (10×) mg eq/kg], after the second application [foliage, TRR = 46.7 (1×)–484 (10×) mg eq/kg] and at harvest 48 days after the second application [bolls and gin trash (TRR = 4.6 (1×)–60 (10×) mg eq/kg)]. The cotton bolls were ginned to yield lint [TRR = 0.12 (1×)–2.5 (10×) mg eq/kg] and seed [TRR = 0.07 (1×)–0.57 (10×) mg eq/kg].

Acetonitrile and water extraction resulted in extraction efficiencies of 85% for cotton foliage, 76% for cotton gin trash and 41–54% for cotton seed. Triton X-100 was added to release loosely bound residues followed by harsher techniques.

In cottonseed, ethiprole and the sulfone were identified at very low levels in the 1× and 10× samples (1–7% TRR, ≤0.04 mg eq/kg). Minor levels of ethiprole-deschloro-sulfone and ethiprole-formamide (1% TRR, 0.001 mg eq/kg) were observed in the 1× seed samples but not in the 10× samples. Ethiprole-sulfonic acid was only observed in the cottonseed 10× samples at a low level (< 3% TRR, 0.014 mg eq/kg)

Parent ethiprole (16–21% TRR, 0.74–3.3 mg eq/kg) and ethiprole-sulfone (15–26% TRR, 1.2–2.4 mg eq/kg) were the main residue components in foliage and gin trash. Minor levels of ethiprole-sulfonic acid, ethiprole-deschloro-sulfone and ethiprole-formamide were observed at < 10% TRR total in foliage and gin trash. A large number of unidentified polar and non-polar compounds were characterised in all matrices.

As photolysis studies in water indicate that significant photodegradation may occur, the Meeting discussed whether the metabolism studies conducted in a greenhouse (two rice studies and one pepper study) were representative for uses in the field. In all three studies, artificial light was supplied to mimic sunlight. In particular, the light spectrum used in the rice study carried out in a climatic chamber to simulate application of a granule formulation to water in paddy rice, was similar to that used in the photolysis studies. The Meeting therefore decided that the supplied metabolism studies were adequate to demonstrate metabolism under field and glasshouse conditions.

Summary of plant metabolism

The metabolism of ethiprole is comparable in all crops investigated. Most of the radioactivity was recovered in the organosoluble extraction, with the majority of this being identified as parent and ethiprole-sulfone. The metabolic pathway of ethiprole in plants proceeds *via* oxidation to the sulfone and hydrolysis to ethiprole-amide. Significant plant metabolites, including ethiprole-sulfone and ethiprole-amide were also observed in the rat metabolism studies.

Environmental fate

The Meeting received information on soil photolysis, the route and rate of aerobic metabolism (degradation) of ethiprole and ethiprole-sulfone, field dissipation, hydrolysis, phototransformation in sterile and natural water, phototransformation of ethiprole-sulfone and ethiprole-sulfide in sterile water, degradation in aerobic and anaerobic water sediment systems and fate in paddy soil under field conditions. Only those studies relevant to the current evaluation are reported here.

Confined Rotational Crops

A study was undertaken to investigate the metabolism of ethiprole in the representative crops lettuce, radish, wheat and sorghum from four consecutive rotations using [^{14}C]-ethiprole sprayed onto the soil at a total seasonal rate of 740 g ai/ha. Lettuce and radish were each sown at 30, 90, 150 and 365 days after the soil application, wheat at 30, 90 and 365 days and sorghum at 150 days after the soil application. Radish tops (TRRs = 0.026–0.23 mg eq/kg), radish roots (TRRs = 0.023–0.098 mg eq/kg), lettuce (TRRs = 0.032–0.29 mg eq/kg), sorghum straw (TRR = 0.30 mg eq/kg), sorghum grain (TRR = 0.027 mg eq/kg), wheat straw (TRRs = 0.20–0.76 mg eq/kg), and wheat grain (TRRs = 0.013–0.053 mg eq/kg) were harvested at maturity. Sorghum (0.058 mg eq/kg) and wheat forage (0.036–0.30 mg eq/kg) were collected approximately at half-maturity.

Acetonitrile and water extraction or acetonitrile/water/acetic acid extraction resulted in extraction efficiencies of 82–104% for lettuce, 77–100% for radish leaves, 52–69% for radish root, 65–88% for wheat/sorghum forage, 66–88% for wheat/sorghum straw and 61–86% in wheat/sorghum grain.

Parent ethiprole was extensively metabolised as it could be detected only at low levels in lettuce, radish and wheat forage of the first rotation (PBI 30 days) amounting to 5–19% TRR (0.015–0.043 mg/kg). At later PBIs, its residue level was < 0.01 mg/kg.

Ethiprole-sulfone was the main residue component in almost all crop commodities and all PBIs amounting to 36–57% TRR in lettuce (0.012–0.13 mg eq/kg), 14–31% TRR in radish leaves (0.004–0.071 mg eq/kg) , 27–36% TRR in radish roots (0.007–0.036 mg eq/kg, except PBI 90 days), 34–46% of TRR in wheat/sorghum forage (0.016–0.10 mg eq/kg) and 18–54% of TRR in wheat/sorghum straw (0.082–0.17 mg eq/kg) but only 4–12% of TRR in wheat/sorghum grain (≤ 0.004 mg eq/kg, not observed at PBI 365 days).

The main residue component in wheat and sorghum grain was ethiprole-sulfonic acid accounting for 8–40% of TRR at PBIs 30, 90 and 150 days (up to 0.016 mg eq/kg). It was also a significant metabolite in radish leaves (10–22% TRR, 0.005–0.023 mg eq/kg), radish roots (8–29% TRR, 0.002–0.013 mg eq/kg), wheat/sorghum forage (2–15% TRR, up to 0.016 mg eq/kg) and wheat/sorghum straw (5–17% TRR, 0.015–0.12 mg eq/kg). Ethiprole-sulfone amide was present in lettuce at 5–21% TRR (0.004–0.031 mg eq/kg), radish leaves at 4–15% TRR (up to 0.013 mg eq/kg), radish roots at 3–4% TRR (up to 0.002 mg eq/kg), wheat forage at 3–19% TRR (up to 0.026 mg eq/kg) and wheat/sorghum straw at 3–11% TRR (0.016–0.065 mg eq/kg).

Hydrolysis

Ethiprole was shown to be hydrolytically stable at pH 4, 5 and 7 over 31 days at 25 °C in the dark. Ethiprole degrades slowly at pH 9, with ethiprole-amide the only detected hydrolysis product. The DT_{50} is 121 days by extrapolation.

Phototransformation in sterile water

An aqueous phototransformation study showed ethiprole is quickly photodegraded in an aqueous medium. Its half-life (DT_{50}) = 6.46 hours for irradiation under a Xenon lamp. Except for the benzimidazole of ethiprole (RPA 157925), all metabolites in the sterile water system were only tentatively identified.

Phototransformation in natural water

The aqueous phototransformation of ethiprole was studied in natural (pond) water collected from a pond system. The experimental photolytic DT_{50} of ^{14}C-ethiprole was calculated to be 0.2 days. Up to 21 photodegradation products were formed. The major photolysis products, RPA 157925 and AE 0764815, rapidly degraded after reaching maxima after 8 hours and 1 day respectively.

Phototransformation of ethiprole-sulfone and ethiprole-sulfide in natural water

The photochemical breakdown of the ethiprole metabolites ^{14}C-ethiprole-sulfone and ^{14}C-ethiprole-sulfide was investigated in buffered aqueous solution (pH 5) during irradiation with artificial sunlight. The DT_{50} values for ^{14}C-ethiprole-sulfone and ^{14}C-ethiprole-sulfide were approximately 15 hours and 5 hours respectively.

Degradation in water-sediment systems (aerobic and anaerobic conditions)

The degradation of ethiprole was studied under aerobic conditions in water-sediment systems in the UK (two) and the USA (one). Under aerobic aquatic conditions, ethiprole is rapidly transferred from the water to the sediment where it is reduced *via* the sulfoxide group to one major metabolite ethiprole-sulfide, which is primarily present in the sediment together with minor amounts of ethiprole-sulfone. DT_{50} values for ethiprole were 4–14 days in water and 5–16 days in the total system. It was concluded that ethiprole is not likely to persist in an aerobic aquatic environment.

The degradation of ethiprole was also studied in water-sediment systems under anaerobic conditions. Ethiprole was degraded to only one major product, ethiprole-sulfide. In water and the total system, ethiprole had a DT_{50} value of 2 days. It was concluded that ethiprole is unlikely to persist in an anaerobic aquatic environment.

Fate in paddy field under field conditions

Four terrestrial rice field studies conducted in Japan indicated that ethiprole and the major metabolites ethiprole-sulfone and ethiprole-sulfide, potentially formed under the conditions of paddy rice growing, are not persistent. The degradation half-life of ethiprole and ethiprole-sulfide in paddy soil ranged from 2–4 and 30–63 days respectively under rice paddy field conditions. In the paddy water, the dissipation half-lives of ethiprole and its metabolites ethiprole-sulfone and ethiprole-sulfide did not exceed 5 days. Ethiprole benzimidazole and AE 0764815, were seen to dissipate quickly with DT_{50} values of 2–5 days in water and 26 days (ethiprole benzimidazole) in paddy soil. Potential accumulation of ethiprole and its major metabolites ethiprole-sulfone and ethiprole-sulfide in paddy water and soil, following repeated application of ethiprole in successive seasons, can be excluded.

Based on the findings of the confined rotational study, the terrestrial rice field studies, and the other environmental fate studies, the Meeting concluded that the uptake of quantifiable residues of ethiprole and its associated metabolites in secondary crops is unlikely.

Animal metabolism

The Meeting received animal metabolism studies with ethiprole in hens and goats. Evaluation of the metabolism studies in rats was carried out by the WHO Core Assessment Group.

Goats

A study on the metabolism of [phenyl-^{14}C]ethiprole was conducted with two lactating goats orally dosed twice daily for 7 consecutive days at 14.2 ppm feed (high dose) or 1.2 ppm feed (low dose) of daily feed consumption. The goats were milked in the morning immediately prior to each administration, and then twice daily throughout the study period. Urine and faeces were collected during the day prior to the first dose and at 24 hour intervals thereafter. Each animal was sacrificed approximately 23 hours after the last dose and selected tissues collected.

The total recovery of radioactivity was 87% for both doses. The majority of the radioactivity was excreted with the faeces, accounting for 62–69% of the total dose, while excretion via urine accounted for 8–15%.

The radioactivity levels and concentrations measured in the milk ranged from 0.013 mg eq/kg at 8 hours after the first dose to the plateau level of 0.070 mg eq/kg at 152 hours for the high-dose level, and 0.002 mg eq/kg at 8 hours after the first dose to the plateau level of 0.009 mg eq/kg at 152 (and 168) hours for the low-dose level. The total recovery of radioactivity in milk at 175 hours post first dose, accounted for < 1% of the administered doses.

The highest TRR values in tissues were found in liver, renal fat and omental fat (0.612–0.685 mg eq/kg at the high dose and 0.081–0.094 mg eq/kg at the low dose). TRRs in muscle and kidney were 0.086–0.21 mg eq/kg for the high dose and 0.010–0.033 mg eq/kg for the low dose.

Extraction of residues with methanol at ambient temperature ranged from 88% of the TRR (liver) to 99% TRR (milk).

Ethiprole was extensively metabolised in the goat. Analysis of extracts from liver, kidney, muscle, renal and omental fat and milk showed ethiprole-sulfone to be the major residue component, representing 32–79% of TRR. Parent ethiprole was identified in kidney (4% TRR in high dose only), muscle (10–17% TRR), renal (9–17% TRR) and omental fat (10–15% TRR) and milk (18–29% TRR). A major metabolite in liver and kidney was thought to be ethiprole-sulfonic acid which co-chromatographed with the N-glucuronide of ethiprole-sulfide (total 13–26% TRR, 0.041–0.090 mg eq/kg high dose and 0.009–0.017 mg eq/kg low dose). All other identified metabolites in milk and tissues were present at <6% TRR.

Laying hens

A study on the metabolism of ethiprole in laying hens was conducted with the test compound ^{14}C-labelled in the phenyl position.

Two dose groups of five laying hens each were dosed orally once daily for 14 consecutive days at nominal levels of 10 ppm and 1 ppm of daily food consumption. The animals were sacrificed 23 hours after the last administration.

The total recovery of radioactivity was 94% for the high-dose group and 91% for the low-dose group. The majority of the administered dose was eliminated in the excreta, accounting for 91% and 88% of the total dose for the high and low-dose hen groups respectively. Low levels of radioactivity were detected in eggs in both dose groups, with the residues in egg white reaching a plateau level approximately 4 days after the first administration and in egg yolk 10 days after the first administration. The residue plateau in egg white and egg yolk accounted for approximately 0.22 and 3.7 mg eq/kg at the high dose and approximately 0.015 and 0.30 mg eq/kg at the low dose. A total of 2.2–2.3% of the dose was recovered in the eggs.

Highest tissue residues were observed in abdominal fat, liver and combined skin and fat (0.90–1.4 mg eq/kg at the high dose and 0.088 – 0.131 mg eq/kg at the low dose).

Extraction of residues with methanol ranged from 82% (liver and breast muscle) to 99% (abdominal fat).

Ethiprole-sulfone was the major residue component in tissues, representing 35–93% of TRR. Parent ethiprole was only identified at a very low level (2–3% of TRR) in muscle of the low-dose hens. As observed in the goat, ethiprole-sulfonic acid, which co-chromatographed with a N-glucuronide of ethiprole-sulfide, was observed in the liver at a total 11–12% TRR (0.014–0.14 mg eq/kg). All other identified metabolites in tissues were observed at < 7% TRR.

Egg yolk samples contained predominantly the sulfone metabolite (49–72% TRR). Parent ethiprole was present at 3–8% TRR as well as a number of minor metabolites, all of them accounting for < 6% of TRR. In egg whites, the main residue component was ethiprole-dihydroxy-sulfone (38–53% TRR, 0.006–0.009 mg eq/kg at the low-dose and 0.11–0.12 mg eq/kg at the high dose), followed by ethiprole-sulfone (12% TRR, high dose) and ethiprole-amide (19% TRR, low dose). All other identified metabolites in egg whites were observed at < 8% TRR.

Summary of animal metabolism

In rats, laying hens and lactating goats the majority of the administered dose is rapidly excreted. Ethiprole was extensively metabolised in each, and proceeds *via* oxidation, reduction, and hydrolysis followed by additional metabolism pathways such as conjugation. Most of the major metabolites identified in goat and hen metabolism studies were also observed in the rat.

Methods of analysis

The Meeting received information on LC-MS/MS analytical methods suitable for the determination of residues of parent, ethiprole-sulfone, ethiprole-deschloro-sulfone, ethiprole-amide, ethiprole-sulfide and ethiprole-formamide in plant matrices. LOQs for plant commodities are generally 0.001–0.002 mg/kg (up to 0.02 mg/kg). No extraction efficiency study was submitted for plant matrices, but the solvent system used for extraction is acetonitrile/water, as was used in the metabolism studies, and is acceptable.

LC-MS/MS and GC/ECD analytical methods are available for the determination of residues of parent, ethiprole-sulfone, ethiprole-methyl sulfone, ethiprole-deschloro-sulfone, ethiprole-sulfonic acid and ethiprole-sulfide in animal matrices. LOQs for animal commodities range from 0.001–0.2 mg/kg. Methods involve extraction with methanol or acetonitrile/water. One method involved an oxidation step, converting the parent to sulfone. Satisfactory extraction efficiencies were obtained for ethiprole, ethiprole-sulfone and ethiprole-sulfide in animal commodities using Method 01431, which was used in the feeding studies.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the freezer storage stability of ethiprole, ethiprole-sulfone and ethiprole-amide in plant commodities. The residues are stable for at least 24 months in sugarcane stalks (high water), citrus fruit (high acid), soya bean seeds (high oil content), dry bean seed (high protein) and wheat grain (high starch content) matrices, when stored frozen at approximately -18 °C.

Additional storage stability data showed that residues of ethiprole and ethiprole-sulfone are stable frozen for at least 12 months in rice grain and tea leaves (-23 °C), while ethiprole, ethiprole-sulfone, ethiprole-amide and ethiprole-deschloro-sulfone are stable under frozen storage for at least 12 months in cotton seed, gin trash, hulls, meal and oil and at least 16 months in orange fruit, juice, dry pulp and oil (≤-10 °C). The storage periods in the storage stability studies cover the sample storage intervals in the residue trials.

All samples in the ethiprole dairy cow and laying hen feeding studies were analysed within thirty days of collection. Therefore there was no necessity for freezer storage stability data.

Definition of the residue

Plant commodities

Following application of ethiprole to crops, the parent compound and the sulfone were the major residues. Parent ethiprole was the major identified residue found in all rice commodities from foliar and soil application (42–75% TRR), in peppers (22–92% TRR), in cottonseed from 10× application (7% TRR) and in cotton foliage (21% TRR). Ethiprole-sulfone was found in rice grain and straw (18–35% TRR), peppers (4–16% TRR), and gin trash (26% TRR), and was the major residue detected in all matrices of all rotations in the confined rotation study, with the exception of radish leaves at 365 days, radish root at 90 days and wheat/sorghum grain.

With the exception of ethiprole-amide in green pepper fruit (15–18% TRR, 0.074–0.12 mg eq/kg) and in rice straw from soil application (11% TRR, 2.68 mg eq/kg), other metabolites were observed at less than 10% TRR or less than 0.01 mg eq/kg in primary crop metabolism studies.

Residues of ethiprole were consistently greater than the sulfone and much greater than the amide across all foods for human consumption in the metabolism studies. Similarly, parent was usually the dominant residue in the crop trials.

A suitable analytical method to determine parent compound in plant matrices is available.

The Meeting therefore considered that a residue definition of "*Ethiprole*" is appropriate for plant commodities for compliance with MRLs (enforcement).

In deciding if additional compounds should be included in the residue definition for risk assessment, the Meeting considered the likely occurrence of the compounds and the toxicological properties of those compounds.

Ethiprole-sulfone is found in plant metabolism and secondary crops studies and crop field trials at levels that are significant relative to the parent compound in most samples and is considered to have no greater toxicity than the parent ethiprole. The Meeting therefore considered that ethiprole-sulfone should be included for risk assessment.

Ethiprole-amide was observed in the pepper and rice plant metabolism studies. The amide was observed in the coffee field trials and in significant levels compared to parent, and has no greater toxicity than the parent. The Meeting decided that ethiprole-amide contributes significantly to the total exposure of ethiprole through the diet.

The Meeting therefore considered that a residue definition of "*Sum of ethiprole, ethiprole-amide and ethiprole-sulfone expressed as parent equivalents*" is appropriate for plant commodities for dietary risk assessment.

Animal commodities

Ethiprole was extensively metabolised in goat and poultry. Ethiprole-sulfone was observed as a major residue across all goat matrices (52–69% of TRR in milk, 59–66% of TRR in muscle, 76–79% of TRR in fat, 35–55% of TRR in liver, and 32–42% of TRR in kidney) and was observed in hen liver (35–54% TRR), hen muscle (58–77% TRR), hen fat (91–93% TRR), hen skin and fat (78–88% TRR), egg yolks (49–72% TRR) and egg whites (up to 12% TRR). Parent ethiprole was identified in goat muscle (10–17% TRR), fat (9–17% TRR), milk (18–29% TRR) and kidney (up to 4% TRR), and at levels <10% of TRR in hen muscle and eggs.

In the dairy cattle feeding study, residues of ethiprole above the LOQ were found in fat samples of the highest dose group while ethiprole-sulfone was present in muscle of the highest group, milk and kidney samples of the two highest dose groups, and in liver and fat samples from all dose groups. Residues of parent and ethiprole-sulfone were observed in cream samples. Ethiprole was found at 0.01 mg/kg in egg samples from the highest dose group of the laying hen feeding study, while ethiprole-sulfone was present above the LOQ in muscle of hens fed from the highest dose group and fat, liver and eggs of hens from the two highest dose groups.

A suitable analytical method to determine parent compound and ethiprole-sulfone in animal matrices is available.

The Meeting decided that a residue definition of the "*Sum of ethiprole and ethiprole-sulfone, expressed as ethiprole*", is a suitable marker for compliance in livestock commodities.

In addition to the residues for compliance, dietary exposure from consumption of livestock commodities may occur for ethiprole-sulfonic acid, the N-glucuronide of ethiprole-sulfide and ethiprole-dihydroxy-sulfone.

A fraction observed in both liver (13–18% of the TRR) and kidney (20–26% of the TRR) of goats, and in the liver (11–12% of the TRR) of hens, was thought to be due to ethiprole-sulfonic acid which co-chromatographed with the N-glucuronide of ethiprole-sulfide. The proportion of the two compounds was not determined in each matrix.

Since ethiprole-sulfonic acid was not observed in the lactating dairy cattle and laying hen feeding studies, it will not be included in the risk assessment definition for animal commodities.

The N-glucuronide of ethiprole-sulfide was detected in considerable amounts in liver and kidney. As no specific data were available on the toxicity of the metabolite the TTC approach was applied[2]. The estimated exposure based on potential animal commodities (0.3 µg/kg bw) was below the applicable threshold of toxicological concern. The Meeting concluded that dietary exposure to the N-glucuronide of ethiprole-sulfide from the uses considered by the current Meeting is unlikely to present a public health concern.

In egg white, the main residue component was ethiprole-dihydroxy-sulfone (38–53% TRR). The latter compound was not detected in any other matrix. Although not a rat metabolite, it was considered to be of no greater toxicity than the parent due to structural similarity. As the residue concentration observed in egg white, in comparison with the whole egg, are insignificant, the Meeting decided that the overall contribution to the dietary burden is negligible, so it will not be included in the definition for risk assessment.

No additional metabolites were observed in animal matrices at greater than 10% TRR.

It is therefore considered that a residue definition of "*Sum of ethiprole and ethiprole-sulfone, expressed as parent equivalents*" is appropriate for animal commodities for dietary risk assessment.

The log K_{ow} of ethiprole (log K_{ow} 2.9) suggests that parent ethiprole has the potential to partition into fat. The ratio of ethiprole + ethiprole-sulfone residues in muscle/fat and milk/cream in the dairy cattle feeding study and muscle/fat in the laying hen feeding study, and muscle/fat in the goat and hen metabolism studies, support the conclusion that the components of the compliance definition are fat-soluble.

Definition of the residue for compliance with the MRL for plant commodities: *ethiprole.*

Definition of the residue for dietary risk assessment for plant commodities: sum of ethiprole, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1H-pyrazole-3-carboxamide (ethiprole-amide) and 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfonylpyrazole-3-carbonitrile (ethiprole-sulfone), expressed as parent equivalents.

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[2] See Toxicology section for further details

Definition of the residue for compliance with the MRL and for dietary risk assessment for animal commodities: sum of ethiprole and 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfonylpyrazole-3-carbonitrile (ethiprole-sulfone), expressed as parent equivalents.

The Meeting agreed that the residue be designated as fat-soluble.

Results of supervised residue trials on crops

Supervised trials were available for the use of ethiprole on rice and coffee.

Product labels were available from Brazil, India, Indonesia, Japan and Thailand for rice and Brazil, El Salvador, Guatemala and Honduras for coffee.

For dietary risk assessment the residues are expressed as the sum of ethiprole, ethiprole-amide and ethiprole-sulfone expressed as ethiprole (referred to as "total").

Rice

The Japanese GAPs were the critical GAPs for rice. None of the submitted trial data matched the Japanese GAPs for rice, so these will not be referred to further.

GAP for ethiprole in Thailand is for foliar applications (number not indicated on the registered label) at 94 g ai/ ha with a 14-day PHI.

Twelve trials were conducted in China, India and Thailand approximating this GAP [four applications (three in one trial) were made at 91–110g ai/ha, with samples harvested 14 to 16 days after treatment]. Although the number of spray applications made in Thailand could be higher than four, it is considered that any applications made earlier in the crop cycle, i.e. further away from harvest, would not significantly increase the residues, noting the short half-life of ethiprole and ethiprole-sulfone in soil and water.

Although rice samples were not analysed for ethiprole-amide, one of the components of the residue definition, the Meeting considered this to be acceptable, as the results of the foliar rice metabolism study showed that significant residues of the amide, in comparison with parent and ethiprole-sulfone, are not expected.

Residues of ethiprole in rice grains from 12 supervised trials conducted in China, India and Thailand in ranked order were (n = 12): 0.11, 0.12, 0.14 (2), 0.17, 0.20, 0.31, 0.41, 0.42, 0.43 and 1.3 (2) mg/kg.

Total residues in ranked order were (n = 12): 0.18, 0.22, 0.25 (2), 0.30, 0.34, 0.53, 0.69, 0.76, 0.80, 1.6 and 1.7 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg and a STMR of 0.44 mg/kg for ethiprole in rice grain (paddy rice).

Coffee

GAP for ethiprole in coffee in Brazil is 2 foliar applications at 500 g ai/ ha, with a 60-day PHI.

Ten residue trials were conducted according to established local practices in Brazil, Colombia, Costa Rica and Mexico according to the Brazilian GAP, giving residues of ethiprole in green coffee beans in ranked order of (n = 10): 0.004, 0.005, 0.006, 0.008, 0.013, 0.014, 0.016, 0.018, 0.022 and 0.044 mg/kg.

Total residues were in ranked order (n = 10): 0.011, 0.015 (2), 0.020, 0.024, 0.025, 0.029 (2), 0.043 and 0.060 mg/kg.

The Meeting estimated a maximum residue level of 0.07 mg/kg, and a STMR of 0.0245 mg/kg for ethiprole in coffee beans.

Fate of residues during processing

The Meeting received data which showed that ethiprole and ethiprole-sulfone were not degraded during the simulation of pasteurisation (pH 4, 90 °C, 20 minutes), and baking, boiling and brewing (pH 5, 100 °C, 60 minutes). Minor degradation was observed under the conditions of sterilisation (pH 6, 120 °C, 20 minutes) and infusing tea/cooking of rice (pH 7, 100 °C, 40 minutes). Ethiprole-amide (5–6%) and ethiprole-sulfone-amide (3–4%) were detected as minor degradation products of ethiprole and ethiprole-sulfone respectively, under those conditions. These residues are either addressed by the residue definition (ethiprole-amide) or considered to be minor (ethiprole-sulfone-amide).

The Meeting also received processing studies for rice and coffee. The table below summarises maximum residue levels calculated on the determined processing factors for parent and STMR-Ps calculated on the determined processing factors for parent ethiprole, ethiprole-amide and ethiprole-sulfone.

Processing Factors from the Processing of Raw Agricultural Commodities (RACs) with Field-Incurred Residues from Foliar Treatment with Ethiprole

RAC	Processed Commodity	Best Estimate Processing Factor (parent)	RAC Maximum residue level	Processed Commodity Maximum residue level	Best Estimate Processing Factor (parent + ethiprole-sulfone + ethiprole-amide)[b]	RAC STMR	Processed Commodity STMR-P
Rice [a]	Husked (brown) rice	0.36	3	1.5	0.32	0.44	0.14
	Polished rice	0.11		0.4	0.09		0.040
	Hulls	-		-	1.4		0.62
	Bran	-		-	1.2		0.53
Coffee	Roasted coffee	1.95	0.07	0.2	1.8	0.0245	0.044
	Instant coffee	-		-	1.95		0.048

[a] Cleaned paddy rice taken as the RAC. As previous experience of the JMPR shows this does not have a significant effect on observed residues, this is considered to be acceptable.

[b] The amide metabolite was not measured in rice as it was not found in significant amounts in the corresponding metabolism study.

Residues in animal commodities

Estimated maximum and mean dietary burdens of farm animals

Dietary burdens were calculated for beef cattle, dairy cattle, broilers and laying poultry. The dietary burdens, estimated using the OECD diets listed in Appendix IX of the 2016 edition of the FAO manual, are presented in Annex 6 and summarised below.

Potential cattle feed items include rice bran, rice grain and rice hulls. Potential poultry feed items include rice grain. The dietary burden was calculated from total residues of ethiprole, ethiprole-amide and ethiprole-sulfone.

Summary of livestock dietary burden for ethiprole (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Beef cattle	0.188	0.188	-	-	0.441[a]	0.441[c]	0.118	0.118
Dairy cattle	0.188	0.188	0.118	0.118	0.346[b]	0.346[d]	0.059	0.059
Poultry Broiler	0.159	0.159	0.059	0.059	0.368	0.368	0.029	0.029
Poultry Layer	0.159	0.159	0.029	0.029	0.368[e]	0.368[f]	0.118	0.118

[a] Highest maximum beef or dairy cattle dietary burden suitable for HR and maximum residue level estimates for mammalian tissues

[b] Highest maximum dairy cattle dietary burden suitable for HR and maximum residue level estimates for mammalian milk

[c] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian tissues

[d] Highest mean dairy cattle dietary burden suitable for STMR estimates for mammalian milk

[e] Highest maximum poultry dietary burden suitable for HR and maximum residue level estimates for poultry tissues and eggs

[f] Highest maximum poultry dietary burden suitable for STMR estimates for poultry tissues and eggs

Farm animal feeding studies

The Meeting received a lactating dairy cow feeding study which provided information on residues of ethiprole arising in tissues and milk when dairy cows were dosed for 28 days, at feeding levels equivalent to 0, 0.14, 0.41, 1.31 and 1.38 (depuration group) ppm ethiprole in the diet. Residues of parent, ethiprole-sulfone, ethiprole-sulfonic acid and ethiprole-sulfide were determined.

Total ethiprole (parent + ethiprole-sulfone) residues in milk from the 1.31 ppm feed group reached plateau levels within approximately 14 days of consecutive dosing and declined rapidly, from 0.042 mg/kg to < 0.01 mg/kg, at 15 days after cessation of dosing. The residue in milk was ethiprole-sulfone only, but residues of parent + ethiprole-sulfone did concentrate in cream.

Residues of ethiprole-sulfone were observed in fat and liver samples at every feeding level and in kidney and muscle samples at higher feeding levels and it was the dominant residue. Parent was only observed in fat samples at the highest feeding level. Residues of parent + ethiprole-sulfone in all tissues were <LOQ within 15 days of cessation of dosing.

The Meeting also received information on residues arising in tissues and eggs when laying hens were dosed with ethiprole for 28 days, at feeding levels equivalent to 0, 0.084, 0.50, 2.51 and 2.46 (depuration group) ppm in the diet. Residues of parent, ethiprole-sulfone, ethiprole-sulfonic acid and ethiprole-sulfide were determined.

Total ethiprole residues in eggs from the 2.51 ppm feed group reached plateau levels within approximately 14 days of consecutive dosing and declined rapidly, from approximately 0.16 mg/kg to < 0.02 mg/kg, at 15 days after cessation of dosing. Ethiprole-sulfone was again the dominant residue (residues up to 0.178 mg eq/kg) with residues of parent (maximum 0.01 mg/kg) observed in eggs at the highest feeding level only. Total residues in egg yolk were approximately 10× the residues in egg white.

Residues of parent were <LOQ in all tissue samples of all dose groups. Ethiprole-sulfone was observed at the highest feeding level in muscle and in the two highest feeding levels in fat and liver (residues up to 0.168 mg eq/kg). Residues of ethiprole-sulfone in all tissues were <LOQ within 5 days of cessation of dosing.

No quantifiable residues of ethiprole-sulfonic acid were observed in any milk, cream or skimmed milk or tissue (subcutaneous, mesenteric and perirenal fat, muscle, liver and kidney) samples at any feeding level in the dairy cattle transfer study or in any eggs, muscle, fat and liver samples at any feeding level in the laying hen transfer study.

No quantifiable residues of ethiprole-sulfide were observed in milk or tissues in the dairy cattle study or in any eggs or tissues samples in the laying hen transfer study. Residues were observed in cream (0.007 mg eq/kg) after feeding at the 1.38 ppm feeding level.

Animal commodity maximum residue levels

Cattle- STMR, HR and maximum residue levels

For highest residue level estimation, the high residues in the cattle tissues were calculated by interpolating the maximum dietary burden for beef cattle (0.441 ppm) between the relevant feeding levels (0.41 and 1.31 ppm) in the dairy cow feeding study and using the highest tissue concentrations from individual animals within those feeding groups. For highest residue level estimation, the high residues in the cattle milk were calculated by interpolating the maximum dietary burden for dairy cattle (0.346 ppm) between the relevant feeding levels (0.14 and 0.41 ppm) in the dairy cow feeding study and using the highest mean milk concentrations from those feeding groups.

The STMR values for the tissues were calculated by interpolating the mean dietary burden for beef cattle (0.441 ppm) with the 0.41 and 1.31 ppm feeding levels from the dairy cow feeding study and using the mean tissue concentrations from those feeding groups. The STMR values for the milk were calculated by interpolating the mean dietary burden for dairy cattle (0.346 ppm) with the 0.14 and 0.41 ppm feeding levels from the dairy cow feeding study and using the mean milk concentrations from those feeding groups.

Ethiprole Feeding Study	Feed Level (ppm) for milk residues	Total residues (mg eq/kg) in milk	Feed Level (ppm) for tissue residues	Total residues (mg eq/kg)			
				Muscle	Liver	Kidney	Fat [a]
HR Determination (beef or dairy cattle)							
Feeding Study	0.14 0.41	< 0.01 0.0129	0.41 1.31	< 0.02 0.046	0.0726 0.252	0.0282 0.089	0.0907 0.459
Dietary burden and estimate of highest residue	0.346	0.012	0.441	0.021	0.079	0.030	0.10
STMR Determination (beef or dairy cattle)							
Feeding Study	0.14 0.41	< 0.01 0.0112	0.41 1.31	< 0.02 0.041	0.0701 0.228	0.0274 0.080	0.0852 0.331
Dietary burden and estimate of STMR	0.346	0.011	0.441	0.021	0.076	0.029	0.094

[a] Mesenteric fat

The Meeting estimated the following STMR values: milk 0.011 mg/kg; muscle 0.021 mg/kg; edible offal (based on liver) 0.076 mg/kg, kidney 0.029 mg/kg and fat 0.094 mg/kg.

The Meeting estimated the following HR values: muscle 0.021 mg/kg; edible offal (based on liver) 0.079 mg/kg, kidney 0.030 mg/kg and fat 0.10 mg/kg.

The Meeting estimated the following maximum residue levels: milk 0.015 mg/kg; meat (mammalian except marine mammals) 0.15 mg/kg (fat), edible offal (based on liver) 0.1 mg/kg and mammalian fats (except milk fats) 0.15 mg/kg.

In the Day 30 sample the mean residues observed in cream were 12.2 times the residues in milk. It is therefore calculated that the estimated STMR in cream will be 0.13 mg/kg. It is assumed that cream is 40% fat, therefore the Meeting estimated a maximum residue level for milk fats of 0.5 mg/kg.

Poultry- STMR, HR and maximum residue levels

For highest residue level estimation, the high residues in the hen tissues and eggs were calculated by interpolating the maximum dietary burden (0.368 ppm) with the 0.084 and 0.50 ppm feeding levels in the laying hen feeding study and using the highest tissue concentrations from individual animals within that feeding group and using the highest mean egg concentration from those feeding groups.

The STMR values for the tissues and eggs were calculated by interpolating the mean dietary burden (0.368 ppm) with the 0.084 and 0.50 ppm feeding levels from the poultry feeding study and using the mean tissue and egg concentrations from those feeding groups.

Ethiprole Feeding Study	Feed Level (ppm) for egg residues	Total residues (mg eq/kg) in egg	Feed Level (ppm) for tissue residues	Total residues (mg eq/kg)		
				Muscle	Liver	Fat
HR Determination (poultry broiler or layer)						
Feeding Study	0.084 0.50	< 0.02 0.0470	0.084 0.50	< 0.02 < 0.02	< 0.02 0.0390	< 0.02 0.048
Dietary burden and estimate of highest residue	0.368	0.038	0.368	< 0.02	0.033	0.039
STMR Determination (poultry broiler or layer)						
Feeding Study	0.084 0.50	< 0.02 0.0342	0.084 0.50	< 0.02 < 0.02	< 0.02 0.0360	< 0.02 0.0447
Dietary burden and estimate of STMR	0.368	0.030	0.368	< 0.02	0.031	0.037

The Meeting estimated the following STMR values: egg 0.030 mg/kg; muscle 0.02 mg/kg; edible offal (based on liver) 0.031 mg/kg and fat 0.037 mg/kg.

The Meeting estimated the following HR values: egg 0.038 mg/kg; muscle 0.02 mg/kg; edible offal (based on liver) 0.033 mg/kg and fat 0.039 mg/kg.

The Meeting estimated the following maximum residue levels: eggs 0.05 mg/kg; poultry fats 0.05 mg/kg; poultry meat 0.05 mg/kg and poultry edible offal (based on liver) 0.05 mg/kg.

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 IEDI and IESTI assessment.

The Meeting recommended the following residue definitions for ethiprole:

Definition of the residue for compliance with the MRL for plant commodities: *ethiprole.*

Definition of the residue for dietary risk assessment for plant commodities: sum of ethiprole, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-(ethylsulfinyl)-1H-pyrazole-3-carboxamide (ethiprole-amide) and 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfonylpyrazole-3-carbonitrile (ethiprole-sulfone), expressed as parent equivalents.

Definition of the residue for compliance with the MRL and for dietary risk assessment for animal commodities: sum of ethiprole and 5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-ethylsulfonylpyrazole-3-carbonitrile (ethiprole-sulfone), expressed as parent equivalents.

The Meeting considers the residue to be fat-soluble.

Commodity		Recommended maximum residue level, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
CCN	Name			
SB 0716	Coffee beans	0.07	0.0245	
SM 0716	Coffee beans, roasted	0.2	0.044	
MO 0105	Edible offal (mammalian)	0.1	Kidney: 0.029 Liver: 0.076	Kidney: 0.030 Liver: 0.079
PE 0112	Eggs	0.05	0.030	0.038
MF 0100	Mammalian fats (except milk fats)	0.15	Fat: 0.094	Fat: 0.10
MM 0095	Meat (from mammals other than marine mammals)	0.15 (fat)	Muscle: 0.021 Fat: 0.094	Muscle: 0.021 Fat: 0.10

Commodity CCN	Name	Recommended maximum residue level, mg/kg	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
FM 0183	Milk fats	0.5	0.33	
ML 0106	Milks	0.015	0.011	
PM 0110	Poultry meat	0.05 (fat)	Muscle: 0.02 Fat: 0.037	Muscle: 0.02 Fat: 0.039
PO 0111	Poultry, edible offal of	0.05	Liver 0.031	Liver 0.033
PF 0111	Poultry fats	0.05	Fat 0.037	Fat 0.039
GC 0659	Rice	3	0.44	
CM 0649	Rice, husked	1.5	0.14	
GC 1205	Rice, polished	0.4	0.040	

Dietary exposure and feed burden only

Commodity CCN	Name	STMR or STMR-P, mg/kg	HR or HR-P, mg/kg
	Coffee beans, instant	0.048	
CM 1206	Rice bran, unprocessed	0.53	
CM 1207	Rice hulls	0.62	

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for ethiprole is 0–0.005 mg/kg bw. The International Estimated Daily Intakes (IEDIs) for ethiprole were estimated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2018 JMPR Report. The IEDIs ranged from 1–6% of the maximum ADI.

The Meeting concluded that long-term dietary exposure to residues of ethiprole from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The ARfD for ethiprole is 0.005 mg/kg bw. The International Estimate of Short Term Intakes (IESTIs) for ethiprole were calculated for the food commodities and their processed commodities for which HRs/HR-Ps or STMRs/STMR-Ps were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2018 JMPR Report. The IESTIs varied from 0–80% of the ARfD for children and 0–40% for the general population.

The Meeting concluded that acute dietary exposure to residues of ethiprole from uses considered by the present Meeting is unlikely to present a public health concern

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