FENITROTHION (37)

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EXPLANATION

Fenitrothion was evaluated under the CCPR Periodic Review Programme by the 2003 JMPR, which recommended an MRL of 10 mg/kg for cereals and listed as desirable additional information on metabolism in cereals (including rice) after pre-harvest treatment, a validated analytical method for the determination of fenitrothion in animal commodities, freezer storage stability of residues in animal commodities, farm animal transfer studies and a processing study on rice.

Data have now been submitted to address these questions, together with result of supervised trials on apples, pears, soya beans, beans and peas.

The 2004 CCPR agreed to retain for four years the existing Codex MRLs for meat (0.05* mg/kg (fat)), milks (0.002* mg/kg), unprocessed rice bran (20 mg/kg), polished rice (1 mg/kg), processed wheat bran (2 mg/kg), wheat flour (2 mg/kg) and wheat wholemeal (5 mg/kg), which had been recommended for withdrawal by the 2003 JMPR.

IDENTITY

The Meeting was informed of the following additional synonyms and trade names: accothion, agrothion, Bay 41831, Cytel, Folithion, MEP, Novathion, Nuvanol, and S-5660.

Reference compounds used in various study reports are listed below.

Abbreviation	Synonyms	Adapted and chemical name(s)	Found as or in
-	FNT	fenitrothion (parent)	quail, hens, soil
AA-FNT	-	acetylaminofenitrothion IUPAC: *O,O*-dimethyl *O*-4-acetylamino-*m*-tolyl phosphorothioate *O,O*-dimethyl *O*-(3-methyl-4-acetylaminophenyl) phosphorothioate *O,O*-dimethyl *O*-(3-methyl-4-acetamidophenyl) phosphorothioate *O,O*-dimethyl *O*-4-acetamido-*m*-tolyl phosphorothioate	metabolite in goat; metabolite in water/sediment
AM-FNT	-	aminofenitrothion IUPAC: *O,O*-dimethyl *O*-4-amino-*m*-tolyl phosphorothioate *O,O*-dimethyl *O*-(3-methyl-4-aminophenyl) phosphorothioate	metabolite in goat; metabolite in water/sediment; metabolite in rice
DM-FNO	DM-SMO	desmethylfenitrooxon IUPAC: methyl hydrogen 4-nitro-*m*-tolyl phosphate *O*-methyl *O*-hydrogen *O*-(3-methyl-4-nitrophenyl) phosphate	photolyis product; metabolite in quail and hens; metabolite in soil; rice storage
DM-FNT	DM-SMT	desmethylfenitrothion IUPAC: *O*-methyl *O*-hydrogen *O*-4-nitro-*m*-tolyl phosphorothioate *O*-methyl *O*-hydrogen *O*-(3-methyl-4-nitrophenyl) phosphorothioate	hydrolysis and photolysis product; metabolite in quail and hens; metabolite in grapes; metabolite in soil; rice storage; metabolite in rice
DMPTA	-	*O-O*-dimethyl *O*-hydrogen phosphorothioate dimethylphosphorothioic acid	wheat storage; metabolite in rice

Abbreviation	Synonyms	Adapted and chemical name(s)	Found as or in
FNO	SMO	fenitrooxon IUPAC: dimethyl 4-nitro-*m*-tolyl phosphate *O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphate	photolysis product; metabolite in quail; metabolite in soil; rice storage; metabolite in rice
NMC	4 NMC; MNP	4-nitro-*m*-cresol IUPAC: 3-methyl-4-nitrophenol *p*-nitrocresol	impurity; hydrolysis and photolysis product, metabolite in quail and hens; metabolite in grapes, rice and tomatoes; metabolite in soil and water/sediment; rice storage
NMC-B-glc	-	NMC-ß-glucoside IUPAC: 1-*O*-β-D-(glucopyranosyl)-3-methyl-4-nitrophenol 3-methyl-4-nitrophenol-beta-glucoside	metabolite in grapes, rice and tomatoes
SM-FNT	SCH_3-SMT; S-methyl-SMT	fenitrothion *S*-isomer IUPAC: *O*-methyl *S*-methyl *O*-4-nitro-*m*-tolyl phosphorothioate *O*-methyl *S*-methyl *O*-(3-methyl-4-nitrophenyl) phosphorothiolate *O*,*S*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothiolate *O*,*S*-dimethyl *O*-4-nitro-*m*-tolyl phosphorothiolate	photolysis product; rice storage; metabolite in rice

Additional abbreviations	Meaning
ACN	acetonitrile
DCM	dichloromethane; methylene chloride
2D-TLC	two dimensional thin layer chromatography
DAT	days after treatment
DALT	days after last treatment
GC-ECD	gas chromatography with electron capture detection
GC-FPD	gas chromatography with flame photometric detection
GC-FTD	gas chromatography with flame thermoionic detection = GC-AFID (alkali flame ionisation detection)
GC-NPD	gas chromatography with nitrogen-phosphorus detection
HPLC-UV	high performance liquid chromatography with ultraviolet detection
MBq	mega Becquerel
meq	milliequivalents
om	organic matter
PTD	phosphorus thermionic detector
PES	post-extraction solids
TRR	total recovered (radioactive) residue

METABOLISM AND ENVIRONMENTAL FATE

Animal metabolism

No additional data were reported.

Plant metabolism

The 2003 JMPR evaluated the fate of fenitrothion in grapes and tomatoes after spray applications, and also in stored rice. Two additional studies on the fate of fenitrothion after pre-harvest spray applications to rice under simulated paddy growing conditions were reported.

Rice. In a trial in Japan in 1964 plants were transplanted to a prepared paddy field in a vinyl tent (Miyamoto and Sato, 1965), and sprayed with fenitrothion formulations (see Table 1). At each

interval 3 Hatsushimo rice plants were cut down at the base, and the leaf sheath and leaf blade separated. At normal harvest mature grain was harvested from all varieties, and separated into bran and polished rice.

Table 1. Application of fenitrothion to rice plants.

Test material	Variety	Dose (kg ai/ha)	Application	DAT
50% EC containing [^{32}P]fenitrothion (>99% radiochemical purity)	Hatsushimo	1x 0.375	25 Aug 1964; 18 days after transplanting	plants 0-10 grain 46
50% EC; non-radioactive	Kin-nampu	1x 0.357	28 Aug 1964	62
5% granule; non-radioactive	Ginga	1x 1.50	3 Aug 1964	38
2% dust; non-radioactive	Aichi-asahi	1x 0.800	23 Aug 1964	66

Leaf samples were washed with acetone. Sample homogenates were suspended in distilled water/perchloric acid, extracted with chloroform and the chloroform extract purified and analysed by TLC using a single solvent system (Miyamoto *et al.*, 1965). The aqueous layers from radiolabelled leaves were lyophilised and analysed by paper chromatography using 5 solvent systems (Miyamoto *et al.*, 1963). The aqueous layers from radiolabelled grain were analysed by ion exchange chromatography using solvent gradients from 0.01 M HCl to concentrated HCl/water/MeOH=1:1:6 (Miyamoto *et al.*, 1963). Compounds were identified by co-chromatography with reference standards of the parent, FNO, DM-FNO, DMPTA, DM-FNT, phosphorothionic acid, phosphoric acid, dimethylphosphoric acid, monomethylphosphorothioic acid and monomethylphosphoric acid. In addition the parent compound and the sodium salt of NMC were quantified in the chloroform extract of unlabelled rice grain by TLC separation followed by isolation, re-chromatography on TLC, isolation again, dissolution in 1 M NaOH in 50% EtOH for 3 h at 85°C and colorimetric analysis (Miyamoto *et al.*, 1965).

The decrease of residues of [^{32}P]fenitrothion in immature rice plants is shown in Table 2. Fenitrothion adhered to the leaf blade about 20 times more than to the leaf sheath and decrease of the attached fenitrothion proceeded at nearly the same rate in both parts (data not shown).

Compounds identified in chloroform and aqueous extracts from immature plants are shown in Table 2. Total radioactive residues were not reported. In the chloroform extracts of leaf sheaths and blades, the parent and FNO were identified, and in the aqueous extracts radioactivity increased in both. One week after application radioactivity levels in the aqueous extracts were 80% of the TRR in leaf blades (data not shown). DMPTA, phosphorothionic acid and phosphoric acid were considered to be the main degradation products in blades. DM-FNT was also detected for a maximum of 2 days after application. In the sheaths, DMPTA, phosphorothionic acid and phosphoric acid were detected (data not shown). DM-FNO, dimethylphosphoric acid, monomethylphosphorothioic and monomethyl-phosphoric acid were not detected in sheaths or blades. In both leaf blade and leaf sheath an appreciable amount of radioactivity was unextractable but exact values in mg/kg or as a percentage of the TRR were not reported.

Table 2. Decrease of residues of [^{32}P]fenitrothion in immature rice plants.

DAT	Immature rice plants			Leaf sheath; chloroform extract		Leaf blades; Chloroform extract		Leaf blades; aqueous layer, mg/kg eq		
	TRR mg/kg eq	Acetone surface wash, % of TRR	'penetrated' radioactivity, % of TRR	parent mg/kg	FNO mg/kg eq	parent mg/kg	FNO mg/kg eq	DM-FNT	DMPTA	Phosphoro-thionic and/or phosphoric acid
0	12	100	0.0							
1	7.0	19	81	0.19	0.03	4.5	0.83	0.45	1.2	3.5
2	5.8	13	87	0.06	0.02	1.5	0.86	0.02	1.8	5.5
4	5.5	9.8	90	0.02	<0.01	0.33	0.27	-	1.8	7.1
7	5.0	6.4	94	0.01	0.01	0.23	0.15	-	1.5	6.8
10	4.8	6.9	93	0.01	<0.01	0.16	0.08			

Compounds identified in chloroform and aqueous extracts from mature rice grain are shown in Table 3. FNO, DM-FNT, DM-FNO, dimethylphosphoric acid, monomethylphosphorothioic acid and monomethylphosphoric acid were not detected. In addition grains contained some unidentified radioactive phosphorus (0.33 mg/kg eq). The authors presumed this came from normal constituents of tissues containing the newly incorporated ^{32}P.

Table 3. Compounds identified in grain from rice treated with ^{32}P-labelled fenitrothion.

		Rice bran	Polished rice
Parent	mg/kg	0.01	0.0003
Phosphoric acid	mg/kg eq	2.6	0.18
Phosphorothionic acid	mg/kg eq	1.0	0.06
DMPTA	mg/kg eq	0.2	0.03

Parent and free NMC in rice bran and polished rice from unlabelled grains are shown in Table 4 with concentrations corrected for controls.

Table 4. Concentrations of the parent and NMC in rice bran and polished rice after the application of unlabelled fenitrothion.

Variety	Rice bran		Polished rice	
	Parent mg/kg	NMC mg/kg[1]	Parent mg/kg	NMC mg/kg[1]
Kin-nampu	<0.1	0.13	<0.1	<0.1
Ginga	<0.1	<0.1	<0.1	<0.1
Aichi-sahi	<0.1	0.36	<0.1	<0.1

[1] as NMC

In a second trial in 2003-2004 Nihonbare rice was grown in a glasshouse to simulate rice paddy growing conditions (Herczog, 2004). The seedlings were planted in pots filled with a loam soil (pH 7.7, 3.6% om; CEC 15.5 meq/100 g; 15% clay) and the pots were flooded with 3-5 cm water throughout. Rice was treated with four spray applications of an EC formulation containing phenyl-[^{14}C]fenitrothion with a radiochemical purity of 98.8% (formulation) and a specific activity of 13.8 MBq/mg (undiluted) at a nominal dose rate of 0.75 kg ai/ha per application (actually 0.754-0.798 kg ai/ha) at 81, 28, 21 and 14 days before harvest at maturity (the first application was 2 months after planting). For twelve days before harvest the plants were unwatered, and they were harvested by cutting the stems above the soil surface. Plants, and soil and root samples were collected and the plants were separated into unhulled whole grain and straw (leaf/stem). The unhulled whole grain was further processed to give brown rice and chaff (hulls). Brown rice was further processed to give 90% (w/w) polished rice and 11% (w/w) bran.

Unhulled whole grain and straw were washed in ACN before grinding. Root balls were washed with water to remove soil. The total radioactivity was determined by combustion, extraction and LSC. Samples were extracted with ACN/water (90+10) and filtered. The filtrates were analysed by LSC, and the remaining solids further extracted using ACN/water (60+40) and ACN/water/HCl (60+40+1.7)). The pooled extracts were concentrated by evaporation and analysed by LSC and radio-HPLC. The results are shown in Table 5.

Table 5. Distribution of radioactivity in rice fractions following rice plant treatment with [^{14}C]fenitrothion.

Sample	TRR combustion + surface wash	TRR extracts + PES	ACN, surface wash	ACN-water	ACN-water-HCl	Total extracted	PES	Total
	mg/kg eq		% of TRR					
Unhulled (whole) grain	2.5	2.3	5.5	56	11	73	27	100
Brown rice	0.59	0.61	-	82	6.4	89	11	100
Polished rice	0.10	0.11	-	72	13	85	15	100
Bran	3.5	3.8	-	85	3.5	89	11	100
Chaff (hulls)	9.1	10	7.1	49	9.3	66	34	100
Straw	8.4	7.9	9.6	70	3.4	83	17	100
Roots	0.11	-	-	-	-	-	-	-
Soil	0.01	-	-	-	-	-	-	-

PES: post-extracted solids
% of TRR values based on total recovered radioactive residues in extracts + PES, because these values were in good agreement with the TRR values based on combustion (93%-111% recovery)

The main residues were fractionated by HPLC, and identified by radio-HPLC-UV and 2-D TLC with co-chromatography. Reference standards were the parent, AA-FNT, AM-FNT, DM-FNO, DM-FNT, FNO, SM-FNT, NMC, and NMC-B-glc. Unresolved regions were isolated, treated with β-glucosidase (pH 5.0, 37°C, 16 h) and 6 M HCl (80°C, 24 h) and re-analysed by HPLC and TLC. The distribution of radioactivity in rice fractions is shown in Table 6.

Table 6. Distribution of metabolites in extracts of rice treated with [^{14}C]fenitrothion.

	Unhulled whole grain	Brown	Polished	Bran	Chaff (hulls)	Straw
TRR (mg/kg eq)	2.3	0.61	0.11	3.8	10	7.9
	% of TRR					
Fenitrothion	13	4.5	2.8	2.8	14	9.9
SM-FNT	1.1	-	-	-	1.3	-
FNO	6.3	1.5	-	1.1	8.4	3.4
NMC (free)	8.3	10	16	7.0	11	8.2
NMC conjugates	35	68	65	73	22	42
Unknowns [1]	1.3	4.8	-	3.1	2.2	6.1
Polar region	2.4	0.2	-	0.1	1.7	2.1
Minor unknowns [2]	5.6	0.1	1.1	1.4	5.4	12
Total extracted	73	89	85	89	66	83

[1] 1-4 minor unknown conjugates characterized as free metabolites after enzyme and acid hydrolysis
[2] 1-14 minor unknowns, each containing <2% of TRR (<0.03-0.16 mg/kg eq)

About 65-89% of the TRR was extractable. Fenitrothion was detectable in all rice fractions (0.003-0.3 mg/kg). NMC (3-methyl-4-nitrophenol), the main metabolite in unhulled whole grain, brown rice, polished rice, chaff, bran and straw (about 0.09-3.9 mg/kg fenitrothion equivalents), was present in a mixture of free and conjugated forms. The conjugates were hydrolysed to the free form using enzymatic (β-glucosidase) and acid hydrolyses. Residues of fenitrothion were 0.30 mg/kg in unhulled whole grain, 0.027 mg/kg in brown and 0.003 mg/kg in polished rice, equivalent to 8.9% transference for brown rice and 1.0% for polished rice.

Post-extraction solids (PES) were treated by sequential acid/base hydrolysis (1M HCl at 40°C, 6M HCl at 80°C and 1N NaOH at 40°C, each 16 h). Results are shown in Table 7. Most of the PES were released by acid/base hydrolysis. NMC was detected in all hydrolysates except polished rice. Minor amounts (<2.5% of the TRR) of AM-FNT, FNO, SM-FNT and fenitrothion were also released.

The acid hydrolysates of all PES contained a polar fraction (1-5% of the TRR), which did not correspond to known metabolites but was shown to have a similar retention time to glucose. These polar products can be formed as a result of metabolism of fenitrothion to small molecules and/or CO_2, which are incorporated into cellulose or starch.

HPLC profiles of extracts were shown to be stable in stored samples for 116-133 days (maximum periods tested).

The main metabolic pathways of fenitrothion in rice plants involved cleavage of the P-O aryl linkage to form NMC. The primary metabolite was further metabolised to more polar products by conjugation with plant components. The metabolic pathways in rice are shown in Figure 1.

Table 7. Distribution of metabolites in acid/base hydrolysates of post-extracted solids (PES).

	Unhulled whole rice grain	Brown rice	Polished rice	Bran	Chaff (hulls)	Straw
TRR (mg/kg eq)	2.3	0.61	0.11	3.8	10	7.9
	% of TRR					
PES (total)	27	11	15	11	34	17
Fenitrothion	0.2	-	-	-	0.1	-
SM-FNT	-	1.3	1.7	-	1.6	-
FNO	0.3	-	-	-	0.2	-
NMC	6.3	0.6	-	1.4	10	1.6
AM-FNT	2.2	0.8	-	1.0	0.9	1.2
Unknowns [1]	3.6	2.4	7.9	1.9	12	3.4
Polar region	5.2	0.1	1.1	3.0	2.6	2.2
Diffuse ^{14}C	3.0	4.0	2.1	2.3	1.6	0.9
Total hydrolysed	21	9.1	13	9.6	30	9.1
Remaining solids	6.1	2.1	2.1	1.4	4.6	8.1

[1] 1-7 unknowns, each containing <2% of TRR (0.005-0.043 mg/kg eq), except in chaff (1 unknown 7.4% of TRR) and polished rice (4.1% of TRR, 1 M HCl hydrolysate not analysed).

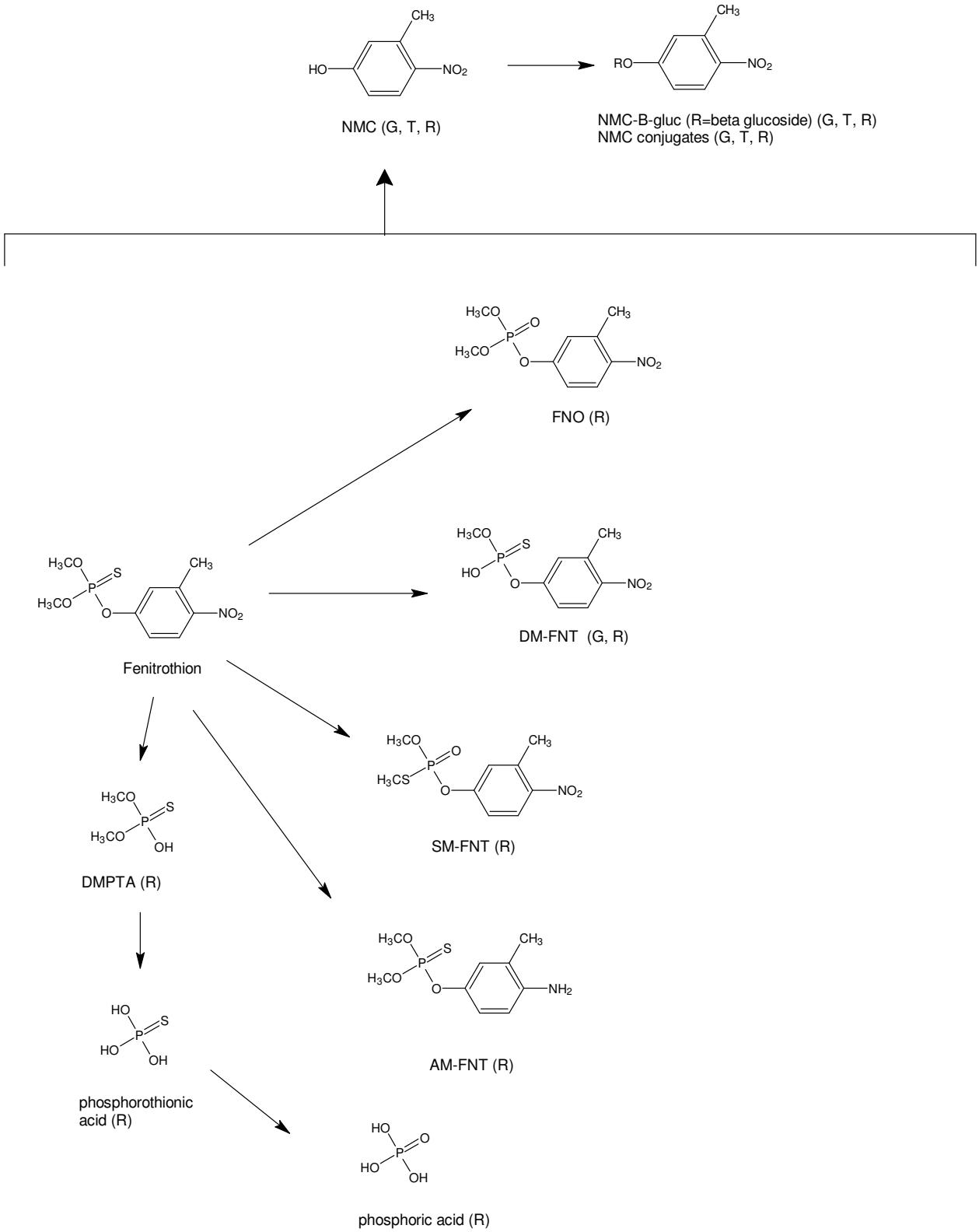


Figure 1. Proposed degradation pathway of fenitrothion in plants (G= grapes, T = tomato, R = rice) after pre-harvest spray treatment with fenitrothion

Sample	LOQ mg/kg[1]	Spike mg/kg	Recovery, mean	No.	Control mg/kg	Ref.
Poultry liver	0.05	0.05	64 60-68	2	<0.3LOQ (1)	idem
Poultry fat	0.05	0.05	82 82-82	2	<0.3LOQ (1)	idem

[1] LOQ reported: this is not necessarily the validated LOQ

Analytical methods used in study reports

The Meeting received information on analytical methods for the determination of fenitrothion in foodstuffs of plant, animal or environmental origin as used in various additionally submitted reports of residue trials, storage stability of analytical samples, processing and feeding studies. There were no code names for the analytical methods.

Table 9 shows the validation results for the methods used in the supervised field trials on apples and pears. In trials HR-21-0004 and HR-41-0006 the residue was extracted with water/MeOH/ACN (2+1+4) and in trials JA-001 to JA-0010 with acetone.

Table 9. Validation results for the determination of fenitrothion in pome fruits.

Sample	GC detec -tor	LOQ reported mg/kg	Spike mg/kg	Recovery mean range	RSD, %	No.	Control (mg/kg)	Linearity	Ref.
Apple whole fruit	FTD	0.001	0.01 0.05 1.0	100 94-106 98 96-102 103 98-108	6.0 3.3 4.9	3 3 3	<0.001 (12)	0.1-0.8 mg/kg; linear by graph	HR-21-0004
Apple whole fruit	FTD	0.001	0.05	96 -	-	1	<0.001 (3)	not verified	HR-41-0006
Pear whole fruit	FTD	0.001	0.01	100 -	-	1	<0.001 (1)	not verified	HR-41-0006
Apple pulp	FPD	0.01	0.1 1	98 98-99 94 92-94	0.7 0.8	6 6	<0.01 (4)	4 point curve range 0.02-0.6 mg/l standard in acetone r>0.999	JA-001
Apple pulp	FPD	0.01	0.1 1	97 94-98 93 92-94	1.6 1.4	4 4	<0.01 (4)	4 point curve range 0.02-0.6 mg/l standard in acetone r>0.999	JA-002
Apple pulp	NPD	0.01	0.1 1	92 86-96 89 88-91	4.9 1.8	4 4	<0.01 (4)	5 point curve range 0.02-0.4 mg/l standard in acetone r>0.999	JA-003
Apple pulp	FPD	0.01	0.1 1	95 90-98 92 91-92	3.8 0.4	4 4	<0.01 (4)	4 point curve range 0.05-1 mg/l standard in acetone linear by graph	JA-004
Apple (pulp plus peel)	FPD	0.01	0.1	95 94-95	-	2	<0.01 (4)	4 point curve range 0.05-1 mg/l standard in acetone linear by graph	JA-005
Apple pulp	NPD	0.01	0.5	98 -	-	1	<0.01 (4)	4 point curve range 0.05-2 mg/l standard in acetone r>0.999999	JA-006
Apple pulp	FPD	0.01	0.1 1.0	93 92-94 94 91-97	1.0 3.3	4 4	<0.01 (4)	4 point curve range 0.05-1 mg/l standard in acetone linear by graph	JA-007

Sample	GC detec -tor	LOQ reported mg/kg	Spike mg/kg	Recovery mean range	RSD, %	No.	Control (mg/kg)	Linearity	Ref.
Apple pulp	NPD	0.01	0.5	94 92-96	-	2	<0.01 (2)	4 point curve range 0.1-3 mg/l standard in acetone r>0.999999	JA-008
Apple pulp	FPD	0.01	0.4	88 86-91	2.4	4	<0.01 (2)	5 point curve range 0.05-1 mg/l standard in acetone linear by graph	JA-009
Apple (pulp plus peels)	FPD	0.01	0.4	91 90-95	2.6	4	<0.01 (2)	4 point curve range 0.05-1 mg/l standard in acetone linear by graph	JA-010

Table10 shows the validation results for the methods used in the supervised field trials on green and dry beans and dry peas. In trial JB-006 green soya beans were extracted with MeOH/ACN and dry soya beans with ACN, as were the dry kidney beans in trial JB-012. In all other trials the residue was extracted with acetone. In trials JB-008/JB-009 control samples were contaminated with fenitrothion (<0.01-0.029 mg/kg): this was confirmed by a second laboratory.

Table 10. Validation results for the determination of fenitrothion in dry peas, green and dry beans, and soya beans.

Sample	GC detec -tor	LOQ reported mg/kg	Spike mg/kg	Recovery mean range	RSD, %	No.	Control (mg/kg)	Linearity	Ref.
Dry soya bean seeds	NPD	0.01	1	89 88-89	-	2	<0.01 (4)	5 point curve range 0.05-2.0 mg/l standard in acetone linear by graph	JB-002
Green soya beans (seeds plus pods)	NPD	0.01	0.5	88 86-89	-	2	<0.01 (4)	5 point curve range 0.1-3.0 mg/l standard in acetone linear by graph	JB-003
Dry soya bean seeds	FPD	0.01	0.4	92 89-93	2.2	4	<0.01 (4)	6 point curve range 0.02-1.0 mg/l standard in hexane linear by graph	JB-004
Green soya beans (seeds plus pods)	FPD	0.01	0.4	92 88-97	4.1	4	<0.01 (4)	6 point curve range 0.02-1.0 mg/l standard in hexane linear by graph	JB-005
Green soya bean seeds	FTD	0.001	0.02	98 98-98	-	2	<0.001 (2)	5 point curve; range 0.5-6 pg; standard in acetone linear by graph	JB-006
Dry soya bean seeds	FTD	0.001	0.02	98 98-98	-	2	<0.001 (2)	5 point curve; internal standard; ratio range 0.2-2.0 linear by graph	JB-006
Dry soya bean seeds	FPD	0.005	0.1	102 98-106	-	2	<0.005 (4)	4 point curve range 0.25-1.0 mg/l standard in acetone linear by graph	JB-007
Dry soya bean seeds	FPD	0.01	0.25 0.50	101 - 103 -	- -	1 1	<0.01-0.02 (4)	7 point curve range 0.1-1.0 mg/l standard in acetone	JB-008

								linear by graph	
Dry soya bean seeds	FPD	0.005	0.2	83 76-88	6.1	4	0.013-0.029 (4)	6 point curve range 0.05-1.2 mg/l standard in hexane linear by graph	JB-009
Dry soya bean seeds	FTD	0.005	0.1	80 80-80	-	2	<0.005 (4)	5 point curve range 0.02-0.4 mg/l standard in acetone linear by graph	JB-010
Dry soya bean seeds	FPD	0.004	0.011 0.056 0.11	83 83-83 100 98-102 94 93-95	- - -	2 2 2	<0.004 (4)	4 point curve range 0.01-0.56 mg/l standard in acetone linear by graph	JB-011
Dry bean seeds (kidney bean; String beans)	NPD	0.01	0.5	96 95-98	-	2	<0.01 (4)	5 point curve range 0.05-2.0 mg/l standard in acetone linear by graph	JB-012
Dry bean seeds (kidney bean; String beans)	FPD	0.01	0.4	86 84-88	2.1	4	<0.01 (4)	6 point curve range 0.02-1.0 mg/l standard in hexane linear by graph	JB-013
Dry adzuki bean seeds	FPD	0.005	0.1	85 83-86	-	2	<0.005 (4)	4 point curve range 0.05-1.0 mg/l standard in acetone linear by graph	JB-014
Dry adzuki bean seeds	FTD	0.002	0.01 0.05 0.2	99 98-100 98 96-99 99 98-101	- - -	2 2 2	<0.002 (4)	4 point curve range 0.01-0.2 mg/l standard in acetone linear by graph	JB-015
Dry peas	FPD	0.01	0.4	99 98-100	-	2	<0.01 (4)	5 point curve range 0.01-0.4 mg/l standard in toluene linear by graph	JB-016
Dry peas	FPD	0.01	0.4	90 86-92	2.8	4	<0.01 (4)	6 point curve range 0.025-0.5 mg/l standard in hexane linear by graph	JB-017
Green broad bean seeds	FTD	0.001	0.1	88 86-90	-	2	<0.001 (4)	6 point curve range 0.05-0.5 mg/l standard in acetone linear by graph	JB-018
Green broad bean seeds	FPD	0.01	0.1 1	96 95-96 97 97-97	- -	2 2	<0.01 (4)	4 point curve range 0.05-1.0 mg/l standard in acetone linear by graph	JB-019

Analytical methods in processing study 1 on rice were based on extraction with benzene (for unpolished rice, polished rice, bran) or hexane/EtOH (10+2, for cooked rice), clean-up, and determination by GC-FTD. The proposed LOQ was 0.002 mg/kg. Validation data were not available.

Analytical methods in processing study 2 on barley and rice were based on extraction with EtOH (without clean-up) and determination by GC-FPD. Only a summary of validation results was provided. The average recovery from cooked rice was 99% at 0.25-2.0 mg/kg, and recoveries from barley, oats, sorghum and unhusked rice at 1-8 mg/kg and from polished and milled rice, and malted barley at 0.25-2.0 mg/kg were similar (±10%). The LOQ was assumed to be 0.1 mg/kg.

The analytical method (ALM/OP/1, updated version 02.98 JP) used for processing study 3 on rice involved extraction with MeOH, clean-up, and determination by GC-FPD. Recovery, precision and linearity were not reported. The proposed LOQ was 0.05 mg/kg. Apparent residues in control samples were <LOQ, except in pollard which showed 0.08 mg/kg fenitrothion, so the LOQ for pollard

Environmental fate in soil

No additional data were reported.

Environmental fate in water/sediment systems

No additional data submitted.

METHODS OF RESIDUE ANALYSIS

Analytical methods for enforcement and monitoring

The 2003 JMPR evaluated enforcement and monitoring methods for plant commodities. Two additional analytical methods were reported to the Meeting for use on animal commodities (RRC 78-32 and RRC 78-32A).

Method RRC 78-32 and RRC 78-32A (1978). This is for animal commodities. Method RRC 78-32 is used for the determination of parent fenitrothion in milk, cream and cattle tissues (Katague, 1978a), and Method RRC 78-32A is a modification for eggs and poultry tissues (Katague, 1978b). Although validation results are also available for FNO, AM-FNT and NMC, they are not included here because these compounds are not included in the proposed definition of the residue.

In method RRC-78-32 (Katague, 1978a) cows' milk or cream (100 g) is blended with acetone and filter aid (hyflo-supercel), and tissues (50 g) are macerated with ACN/MeOH/water and filter aid. Mixtures are filtered, saturated NaCl solution is added, the mixture extracted with DCM and drained through anhydrous sodium sulfate. An aliquot of the extract (equivalent to 10-20 g sample) is evaporated to near dryness, dissolved in hexane and partitioned into ACN. The ACN phase is evaporated to near dryness, dissolved in ethyl acetate/hexane (1+1) and purified on a silica acid column using ethyl acetate/hexane (1+1) as final eluant. The eluate is then evaporated. Fenitrothion is determined by GC-FPD (Pyrex packed column, 10% OV-101 on 80/100 Gas Chrom Q, 185°C, phosphorus mode). The results are shown in Table 8.

Potentially interfering organophosphorus pesticides and their metabolites (41 compounds) were examined (Katague, 1978a). With the OV-101 column used in the method, paraoxon, fenthion oxygen analogue, and fenchlorphos (ronnel) interfered with the fenitrothion peak.

In Method RRC 78-32A extraction and quantification are essentially the same as for cattle tissues, except that rinsing volumes and sample weights are modified, and column temperatures different. The results are shown in Table 8.

Table 8. Validation results for the determination of fenitrothion using method RRC 78-32. Linearity was not determined.

Sample	LOQ mg/kg[1]	Spike mg/kg	Recovery, mean		No.	Control mg/kg	Ref.
Cattle milk	0.01	0.01	99	97-100	2	<0.3LOQ (1)	Katague, 1978a
Cattle cream	0.01	0.01	88	79-97	2	<0.3LOQ (1)	idem
Cattle muscle	0.05	0.05	98	-	1	<0.3LOQ (1)	idem
Cattle liver	0.05	0.05	88	-	1	<0.3LOQ (1)	idem
Cattle kidney	0.05	0.05	94	-	1	<0.3LOQ (1)	idem
Cattle fat	0.05	0.05	96	-	1	<0.3LOQ (1)	idem
Whole eggs	0.05	0.05	101	98-104	2	<0.3LOQ (1)	Katague, 1978b
Poultry muscle	0.05	0.05	66	62-70	2	<0.3LOQ (1)	idem

has to be increased to a minimum of 0.08 x 10/3 = 0.3 mg/kg, indicating that this method is unsuitable for pollard.

The methods used for pasture in a feeding study were based on extraction with MeOH, clean-up, and determination by HPLC-UV. Validation data were not available.

The analytical method used for animal fat in a feeding study involved melting the fat, extraction by the assisted distillation technique (Heath and Black, 1987), clean-up, and determination by GC with a phosphorus-specific detector. Individual recoveries were not reported. The average recovery was 75%, precision 10% (at 0.5 mg/kg). Linearity was not verified. The proposed LOQ was 0.02 mg/kg. Because control samples contained residues, the valid LOQ should be increased to a minimum of 0.064 x 10/3 = 0.2 mg/kg, indicating that the method is not suitable for fat.

In the method for meat and liver in a feeding study extraction with ethyl acetate was followed by clean-up and determination by GC with a phosphorus-specific detector. Individual recoveries were not reported. The average recovery was 68% for meat and liver (at 0.2 mg/kg) and precision and linearity were not verified. The proposed LOQ was 0.02 mg/kg and control samples were <LOQ (4 determinations per sample).

Soil was analysed in a feeding study by extraction with acetone without clean-up and determination by GC with a phosphorus-specific detector (not further specified). Individual recoveries were not reported. Average recoveries were 90%, precision 10%, but linearity was not verified and control samples were not analysed. The reported LOQ was 0.01 mg/kg.

Stability of pesticide residues in stored analytical samples

The 2003 JMPR evaluated the storage stability of residues in dry crops with starch and protein (wheat, barley and rice grain, and rice straw). Additional data on residues in crops with a high water content (apples, green soya beans and green broad beans), as well as dry crops with starch and protein (soya bean seeds, kidney beans, and peas) have now been provided.

Apples. In supervised residue trials on apples, homogenised samples with added fenitrothion were stored at -20°C and analysed using several methods. The results show that residues of fenitrothion in apple commodities are stable for at least 192 days at -20°C (Table 11). The longest storage period in the apple trials was 189 days.

Table 11. Stability of fenitrothion in apples stored at -20°C.

Sample	Ref	Method	Storage (days)	Spike (mg/kg)	Recovery (uncorrected) Mean	range (n=2), %	Concurrent recovery, %
Pulp	JA-008	GC-NPD	25	0.5	97	94-100	92-96
Pulp	JA-006	GC-NPD	36	0.5	86	84-88	98
Pulp	JA-008	GC-NPD	46	0.5	100	97-102	92-96
Pulp	JA-003	GC-NPD	52	1.0	92	92-92	86-96
Pulp plus peel	JA-010	GC-FPD	54	1.0	89	88-91	90-95
Pulp	JA-007	GC-FPD	56	1.0	90	89-92	91-97
Pulp	JA-006	GC-NPD	59	0.5	92	92-92	98
Pulp	JA-007	GC-FPD	80	1.0	93	92-93	91-97
Pulp plus peel	JA-005	GC-FPD	80	1.0	93	92-93	94-95
Pulp plus peel	JA-010	GC-FPD	92	1.0	83	83-83	90-95
Pulp	JA-009	GC-FPD	100	1.0	88	88-89	86-91
Pulp	JA-003	GC-NPD	109	1.0	83	82-84	86-96
Pulp	JA-002	GC-FPD	112	1.0	92	92-92	92-98
Pulp	JA-001	GC-FPD	116	1.0	85	83-87	92-99
Pulp	JA-009	GC-FPD	119	1.0	90	90-90	86-91

Sample	Ref	Method	Storage (days)	Spike (mg/kg)	Recovery (uncorrected) Mean range (n=2), %	Concurrent recovery, %
Pulp	JA-004	GC-FPD	124	1.0	92 92-92	90-98
Pulp	JA-002	GC-FPD	128	1.0	86 86-87	92-98
Pulp	JA-001	GC-FPD	172	1.0	84 84-85	92-99
Pulp	JA-001	GC-FPD	176	1.0	84 83-85	92-99
Pulp	JA-004	GC-FPD	192	1.0	87 87-88	90-98

Legumes. In supervised trials homogenised samples with added fenitrothion were stored at -20°C and analysed using several methods.

The results show that fenitrothion residues are stable for at least 155 days at -20°C (Table 12), covering the frozen storage periods in the trials.

Table 12. Storage stability for fenitrothion in green legumes stored at -20°C.

Sample	Ref	Method	Storage (days)	Spike (mg/kg)	Recovery (uncorrected), % mean range (n=2)	Recovery (corrected), % mean range (n=2)	Concurrent recovery, %
Broad beans (seeds)	JB-019	GC-FPD	13	1.0	95 94-95		95-97
Soya beans (seeds + pods)	JB-003	GC-NPD	19	0.5	ns	95 94-97	86-89
Broad beans (seeds)	JB-019	GC-FPD	20	1.0	95 94-96		95-97
Soya beans (seeds + pods)	JB-003	GC-NPD	35	0.5	ns	95 94-96	86-89
Soya beans (seeds + pods)	JB-005	GC-FPD	142	1.0	89 87-92	-	88-97
Soya beans (seeds + pods)	JB-005	GC-FPD	155	1.0	88 86-90	-	88-97

ns: not stated

Pulses. In supervised residue trials on beans and peas homogenised samples with added fenitrothion stored at -20°C were analysed using several methods. The results are summarized in Table 13.

Fenitrothion residues in dry crops with starch and protein are stable for at least 149 days in cereals (2003 evaluation) and 98 days in pulses when stored at -20°C. Stability at higher temperatures was not determined.

Table 13. Storage stability for fenitrothion in dry pulses stored at -20°C.

Sample	Ref	Method	Storage (days)	Spike (mg/kg)	Recovery (uncorrected), % mean range (n=2)	Recovery (corrected), % mean range (n=2)	Concurrent recovery, %
Pea seeds	JB-017	GC-FPD	2	1.0	93 91-95	-	87-92
Kidney bean seeds (string beans)	JB-013	GC-FPD	28	1.0	83 82-83	-	84-88
Kidney bean seeds (string beans)	JB-012	GC-NPD	30	0.5	ns	100 99-100	95-98
Pea seeds	JB-016	GC-FPD	45	1.0	82 81-83	-	98-100
Pea seeds	JB-016	GC-FPD	45	1.0	86 85-88	-	98-100
Soya bean seeds	JB-002	GC-NPD	55	0.5	ns	94 93-94	86-89
Soya bean seeds	JB-004	GC-FPD	70	1.0	92 91-92	-	88-97
Soya bean seeds	JB-002	GC-NPD	81	0.5	ns	93 92-94	86-89
Kidneybean seeds	JB-013	GC-FPD	90	1.0	86 86-87	-	84-88
Soya bean seeds	JB-004	GC-FPD	96	1.0	92 91-94	-	88-97
Kidney bean seeds	JB-012	GC-NPD	98	0.5	ns	100 99-102	95-98

ns: not stated

USE PATTERN

Fenitrothion is a broad spectrum contact organophosphorus pesticide for use on a wide variety of crops against chewing, sucking and boring insects. It is useful for controlling weevils, bugs, stem borers, worms, nematodes, chafers, grass grubs, grasshoppers and locusts in orchards, vineyards and various field and forage crops and also for flour beetles, moths, weevils and grain borers in stored grain and seeds.

Fenitrothion is registered for use in Argentina, Australia, Brazil, Japan, Russia, South Korea and Vietnam for control of insect pests on citrus fruit; pome fruit (apples, pears, quinces); stone fruit (plums, peaches, cherries, Japanese apricots); berries and small fruit (strawberries, mulberries, grapes); miscellaneous tropical and sub-tropical fruit (olives, Japanese persimmons, avocado, guava, kaki, mango, pineapple, figs); bulb vegetables (onions; garlic); brassica vegetables (cauliflower, broccoli, head cabbages); fruiting vegetables (tomatoes, sweet pepper, egg plant, cucumbers, summer squash, melons, oriental pickling melons, watermelons, pumpkins); leafy vegetables (lettuce, spinach, wild chicory, kale); legume vegetables (garden peas, green beans, broad beans, green soya beans); pulses (peas, beans, soya beans, azuki beans, kidney beans) both in the field and as stored seeds; root and tuber vegetables (carrots, beetroot, sugar beet, potatoes, yams, burdock, konjac); stalk and stem vegetables (leeks, udo); cereals (maize, rice, barley, oats, rye, wheat, millet, sorghum), both in the field and as stored grain; oilseeds (cotton, peanuts, hemp); cashew nuts; cocoa; sugar cane; tea; coffee; medicinal crops (nalta jute (leaves and shoots), *Murraya paniculata* jack (roots)); forage crops (pasture, lucerne) and pastures for seed production. Fenitrothion is not authorized for use in Germany or The Netherlands.

Because only registered uses on cereals were supported by the results of supervised residue trials, only these uses were listed by the 2003 JMPR. As the present Meeting received additional trials data on pome fruits, legumes and pulses, these uses are listed below. For all uses, original labels were available, together with an English translation.

Table 14. Registered uses of fenitrothion for pre-harvest applications to pome fruits.

Crop	Country	Form	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	No. (interval)	
Apple	Australia [1]	EC 1000	foliar spray	0.27-0.55	ns	ns	14
Apple	Argentina	EC 1000	foliar spray	ns	0.080	ns	14
Apple	Brazil	EC 500	foliar spray	0.525-1.3	0.075-0.10	ns (10-15 d)	14
Apple	Japan	EC 500	foliar spray	1.75-3.5[2]	0.025-0.050	3	30
Apple	Russia	EC 500	foliar spray	0.8-2.0	ns	ns	20
Apple	South Korea	EC 500	foliar spray[3]	ns	0.050	max 4	15
Pear	Argentina	EC 1000	foliar spray	ns	0.060	ns	14
Pear	Brazil	EC 500	foliar spray	0.525-0.975	0.075	ns (10-15d)	14
Pear	Russia	EC 500	foliar spray	0.8-2.0	ns	ns	20
Pear	Japan	EC 500	foliar spray; pears with bag	1.75-3.5[2]	0.025-0.050	6	14
Pear	Japan	EC 500	foliar spray	1.75-3.5[2]	0.025-0.050	6	21
Quince	Brazil	EC 500	foliar spray	0.525-0.975	0.075	ns (10-15d)	14

ns: not stated
[1] GAP information provided by national government
[2] application volume 7000 l/ha not stated on label, but provided by manufacturer.
[3] application 20 days before blooming and after end of flowering, and early part of August.

Table 15. Registered uses of fenitrothion for pre-harvest applications to legumes and pulses.

Crop	Country	Form	Application				PHI, days
			Method	Rate kg ai/ha	Spray conc, kg ai/hl	No.	
Azuki beans	Japan	EC 500	foliar spray	0.75-1.5[1]	0.025-0.050	4	21
Beans	Argentina	EC 1000	foliar spray	ns	0.040	ns	14
Beans	Brazil	EC 500	foliar spray by tractor	0.50-0.75	0.17-0.75	ns (10-15d)	14
Beans	Brazil	EC 500	foliar spray by aeroplane	0.50-0.75	1.25-2.5	ns (10-15d)	14
Kidney beans	Japan	EC 500	foliar spray	0.75-1.5[1]	0.025-0.050	4	21
Peas (dry/green)	Japan	EC 500	foliar spray	0.75-1.5[1]	0.025-0.050	4	21
Soya beans (dry/green)	Australia [2]	EC 1000	foliar spray	0.27-0.55	ns	3	14 (WHP=14d)
	Australia [2]	EC 1000	foliar spray by aeroplane	0.27-0.55	ns	3	14 (WHP=14d)
Soya beans	Argentina	EC 1000	foliar spray	0.4-0.5	0.40-0.71	ns	14
	Argentina	EC 1000	foliar spray by aeroplane	0.4-0.5	min 2.0-3.3	ns	14
Soya beans	Brazil	EC 500	foliar spray by tractor	0.5-1.0	0.17-1.0	ns (10-15d)	7
	Brazil	EC 500	foliar spray by aeroplane	0.5-1.0	1.25-3.3	ns (10-15d)	7
Soya beans (dry/green)	Japan	EC 500	foliar spray	0.75-1.5[1]	0.025-0.050	4	21
Soya beans (dry)	Japan	EC 500	foliar spray by unmanned helicopter	0.50	6.25	4	21
Soya beans (dry)	Japan	EC 500	foliar spray	0.75	2.5	4	21

ns: not stated
WHP: graze or feed withholding period
[1] application volume 3000 l/ha not stated on label, but provided by manufacturer.
[2] GAP information provided by national government

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The 2003 JMPR evaluated residue trials on cereal grains. The present Meeting received additional information on supervised residue trials for:

Fruits	Table 16	Apple (whole fruit)
	Table 17	Apple without pedicel, stylar scar and cores
	Table 18	Apple without pedicel, stylar scar, cores and hulls
	Table 19	Pear
Vegetables	Table 20	Green broad beans
	Table 21	Green soya beans
Pulses	Table 22	Dry soya beans
	Table 23	Dry beans
	Table 24	Dry peas

Residue levels and application rates were reported as fenitrothion (parent). Unquantifiable residues are shown as below the reported LOQ (e.g. <0.01 mg/kg). Residues, application rates and spray concentrations have been rounded to two figures, and the results are unadjusted for percentage recoveries or residue values in control samples unless otherwise stated. Residues from the trials conducted according to maximum GAP have been used for the estimation of maximum residue levels. These results are double-underlined.

Concurrent method recoveries were reported to be within 70%-110% limits in all trials and most control samples were below the LOQ. Trials where residues in control samples exceeded the LOQ are indicated; actual LOQs were increased accordingly.

Dates of analyses or durations of residue sample storage were also provided, except for the Canadian pome fruit trials. All trials in which samples were stored at -20°C were within the maximum storage stability periods tested. In some of the trials on legumes and pulses, samples were stored at different temperatures (-15° - +10°C). Storage stability data for samples stored at these temperatures are desirable.

For some Japanese trials, duplicate samples were analysed by two different laboratories.. The results shown are the mean of all four when the results were similar and are the mean of duplicate results from one laboratory when results were around the LOQ and the LOQ differed between the laboratories or when results from a later time were significantly lower.

Pome fruit. Nineteen trials on apples and two trials on pears were conducted in Canada in 1972-1973 (Tables 16 and 19). The storage period was not stated. The results are corrected for recovery; uncorrected results are not available.

In 24 trials on apples in Japan in 1989-1995 (Tables 17 and 18) analytical samples excluded (hulls), pedicel, stylar scars and cores. In the trials included in Table 17 the hulls were not removed but in Table 18 they were.

Table 16. Fenitrothion residues in whole apples after pre-harvest spray treatments in the field in Canada.

Location, year, report no.	Variety	Form	No.	Inter-val (days)	kg ai /hl	Last treatment	DALT	Residues (mg/kg)
Thornbury, Ontario, 1972, HR-21-004	-	WP 400	1	na	0.144	28 Sept 21 Sept 14 Sept 5 Sept[1]	0 7 14 23	0.13 (2); mean 0.13 0.048; 0.057; mean 0.052 0.016; 0.017; mean 0.016 0.007; 0.008; mean 0.008
Thornbury, Ontario, 1972, HR-21-004	-	WP 400	6	10-14-11-10-56	0.144	14 Sept	3 7 14	0.038; 0.046; mean 0.042 0.012; 0.015; mean 0.014 0.002 (2); mean 0.002
Thornbury, Ontario, 1972, HR-21-004	-	WP 400	4	10-14-11	0.096	10 Jul	80	0.006; 0.009; mean 0.008
Thornbury, Ontario, 1972, HR-21-004	-	WP 400	6	10-14-11-10-56/63/67	0.096	25 Sept 21 Sept 14 Sept[1]	3 7 14	0.12; 0.17; mean 0.15 0.053; 0.062; mean 0.058 0.018; 0.020; mean 0.019
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	1	na	0.192	8 June	117	0.005; 0.007; mean 0.006
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	2	95	0.192	11 Sept	22	0.022 (2); mean 0.022
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	3	95-7	0.192	18 Sept	15	0.12; 0.16; mean 0.14
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	4	95-7-8	0.192	26 Sept	7	0.23 (2); mean 0.23
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	5	95-7-8-4	0.192	30 Sept	3	0.30; 0.31; mean 0.31
West Bank, British Columbia,	-	WP 400	1	na	0.096	8 June	117	0.005; 0.009; mean 0.007

Location, year, report no.	Variety	Form	No.	Inter-val (days)	kg ai /hl	Last treatment	DALT	Residues (mg/kg)
1972, HR-21-004								
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	2	95	0.096	11 Sept	22	0.019; 0.020; mean 0.020
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	3	95-7	0.096	18 Sept	15	0.062; 0.075; mean 0.068
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	4	95-7-8	0.096	26 Sept	7	0.21 (2); mean 0.21
West Bank, British Columbia, 1972, HR-21-004	-	WP 400	5	95-7-8-4	0.096	30 Sept	3	0.037; 0.049; mean 0.043
Chateauguau Centre, Quebec, 1972, HR-21-004	-	WP 400	2	25	0.192	30 June	45	0.011 (2); mean 0.011
Chateauguau Centre, Quebec, 1972, HR-21-004	-	WP 400	4	25-13-13	0.192	26 July	19	0.018; 0.019; mean 0.018
Millgrove, Ontario, 1973, HR-41-006	Golden Delicious	WP 400	10	11-21-10-10-14-9-8-8-11	0.072	14 Aug	7	0.05; 0.10; 0.15; mean 0.10
D'Abbotsford, Quebec, 1973, HR-41-006	-	WP 400	7	9-10-18-12-16-6	0.072	9 Aug	8	0.19; 0.23; 0.26; mean 0.23
D'Abbotsford, Quebec, 1973, HR-41-006	-	WP 400	6	9-10-18-28-6	0.072	9 Aug	7	0.16; 0.18; 0.31; mean 0.22

DALT: days after last treatment
na: not applicable
[1] Reverse decay trial , different last treatment day but same harvest day

HR-21-0004. (French, 1972). Non-GLP. Weather conditions, soil, plot size and application equipment not stated. Field samples 20 pieces stored at -20°C (period not stated). Analytical method GC-FTD, method HR-21-0004/HR-41-0006. Results not corrected for sample interferences (<0.001 mg/kg, n=8) but were for concurrent method recoveries (94%-108%). Uncorrected results not given. Results from replicate field samples, average value taken.

HR-41-0006. (Van Doornik, 1974). Non-GLP. Wet spring, hot dry summer. Soil not stated (Millgrove), loam (D'Abbotsford). Hose and gun power sprayer. Sample sizes not stated. Samples stored at -20°C (period not stated). Analytical method GC-FTD, method HR-21-0004/HR-41-0006. Results not corrected for sample interferences (<0.001 mg/kg, n=3) or for concurrent method recovery (96%). Results from three replicate field samples, average value taken.

Table 17. Fenitrothion residues in apples (excluding pedicel, stylar scar and cores but including hulls) after pre-harvest spray treatments in the field in Japan.

Location, year, report no.	Variety	Form	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	DALT	Residues (mg/kg)
Gunma, 1989, JA-010	Fuji	EC 500	3	7-10	0.05	-	-	30 Oct	14 21	1.5[1] 0.52[1]
Gunma, 1989, JA-010	Fuji	EC 500	3	7-11	0.05	-	-	13 Oct	31 45	0.41[1] 0.25[1]
Nagano, 1989, JA-010	Fuji	EC 500	3	8-6	0.05	4000	2.0	21 Sept	14 21	0.22[1] 0.18[1]
Nagano, 1989, JA-010	Fuji	EC 500	3	7-7	0.05	4000	2.0	5 Sept	30 45	0.10[1] 0.01[1]
Iwate, 1992, JA-005	Senshu	EC 500	3	7-7	0.05	3000	1.5	7 Sept	21 30	0.14[1] 0.11[1]
Iwate, 1990, JA-008/JA-009	Fuji	EC 500	3	7-7	0.05	4000	2.0	24 Sept	14 21 30	0.30[2] 0.15[2] 0.12[2]
Iwate, 1990,	Fuji	EC 500	5	7-7-7-7	0.05	4000	2.0	24 Sept	14	0.31[2]

Location, year, report no.	Variety	Form	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	DALT	Residues (mg/kg)
JA-008/JA-009									21 30	0.25[2] 0.16[2]
Toyama, 1990, JA-008/JA-009	Fuji	EC 500	2	6	0.05	4000	2.0	15 Oct	14 21 30	0.28[2] 0.14[2] 0.11[2]
Toyama, 1990, JA-008/JA-009	Fuji	EC 500	5	7-8-7-6	0.05	4000	2.0	15 Oct	14 21 30	0.51[2] 0.32[2] 0.11[2]
Hokkaido, 1993, JA-004	Tsugaru	EC 500	3	7-7	0.05	4000	2.0	31 Aug	30 45	0.02[1] 0.01[1]
Niigata, 1993, JA-004	Tsugaru	EC 500	3	7-7	0.05	3000	1.5	20 Jul	30 45	≤0.01[1] <0.01[1]
Iwate, 1994, JA-003	Hokuto	EC 500	3	7-7	0.05	4000	2.0	27 Sept	29	0.10[1]
Ibaraki, 1994, JA-003	Tsugaru	EC 500	3	7-7	0.05	4000	2.0	2 Aug	30	0.01[1]
Nagano, 1995, JA-001	Sensyu	EC 500	3	10-10	0.05	4000	2.0	21 Aug	30	0.01[1]
Ishikawa, 1995, JA-001	Hatsuaki	EC 500	3	16-11	0.05	3000	1.5	14 Aug	31	0.01[1]
Gifu, 1995, JA-001	Fuji	EC 500	3	15-14	0.05	3000	1.5	17 Oct	30	0.02[1]
Fukushima, 1995, JA-002	Fuji	EC 500	3	7-7	0.05	3000	1.5	16 Oct	30	0.08[1]
Gunma, 1995, JA-002	Fuji	EC 500	3	7-7	0.05	3000	1.5	2 Oct	30	0.04[1]

[1] Mean of duplicate analyses.
[2] Mean of four analytical portions (2 different methods, each with duplicate analyses)

JA-010. (Kuroda and Higuchi, 1989). Non-GLP. Normal weather conditions. Rainfall occurred within 24 h of first 2 treatment dates for trial with DALT 31 and 45 at Gunma. Soil humic volcanic ash deposit (Gunma), clay loam (Nagano). Plot size: 2 trees (Gunma), 3 trees (Nagano). Power spraying equipment (Gunma), small-scale power equipment (Nagano). Field samples 10 pieces (Gunma), 2 kg (Nagano). Samples stored at -20°C for 43-104 days. Analytical method GC-FPD, method JA-010. Results not corrected for sample interferences (<0.01 mg/kg, n=4), or for concurrent method recoveries (90%-95%).

JA-005. (Hirota, 1994). Non-GLP. Normal weather conditions. Soil: diluvial humus volcanic ash. Plot size: 3 trees. Engine sprayer. Field samples 2 kg. Samples stored frozen (temperature not stated) for 59-68 days. Analytical method GC-FPD, method JA-005. Results not corrected for sample interferences (<0.01 mg/kg, n=4), or for concurrent method recoveries (94%-95%).

JA-008/JA-009. (Matano and Kobayashi, 1991a; Kuroda and Higuchi, 1991a). Non-GLP. Normal weather conditions. Soil: clay (Iwate), sandy loam (Toyama). Plot size: 1 tree (Iwate), 3 trees (Toyama). Shoulder spraying equipment (Iwate), power spraying equipment (Toyama). Field samples 4-5 kg. Samples stored at -20°C for 9-45 days. Analytical method GC-NPD, method JA-008. Samples re-analysed by second laboratory after 86-135 days using method GC-FPD, method JA-009. In the Toyama control sample 0.05-0.06 mg/kg fenitrothion was found by both methods, confirmed by GC-MS, and it was concluded that the tree was treated with a fenitrothion rather than a blank solution. Because results from the second analysis (JA-009) were identical to the first (JA-008), mean results for all four analytical portions are shown in the Table. Results not corrected for sample interferences (<0.01 mg/kg, n=2, each method), or for concurrent method recoveries (92%-96%).

JA-004. (Hirota and Kikuchi, 1994). Non-GLP. Normal weather conditions. Soil: brown acid forest (Hokkaido), sandy loam (Niigata). Plot size: 1 tree (Niigata), 3 trees (Hokkaido). Automatic sprayer (Niigata), backpack automatic spraying equipment (Hokkaido). Field samples 2 kg (Hokkaido), 10 pieces (Niigata). Samples stored at -20°C for 132-189 days. Analytical method GC-FPD, method JA-004. Results not corrected for sample interferences (<0.01 mg/kg, n=4), or for concurrent method recoveries (90%-98%).

JA-003. (Suzuki, 1995). Non-GLP. Normal weather conditions. Soil: humic volcanic ash (Iwate), not stated (Ibaraki). Plot size: one tree (Iwate), not stated (Ibaraki). Equipment not stated (Iwate), backpack power spraying equipment (Ibaraki). Field samples 2 kg (Iwate), 50 pieces (Ibaraki). Samples stored at -20°C for 51-106 days. Analytical method GC-NPD, method JA-003. Results not corrected for sample interferences (<0.01 mg/kg, n=4), or for concurrent method recoveries (86%-96%).

JA-001/JA-002. (Suzuki, 1996a,b). Non-GLP. Normal weather conditions. Soil: sandy loam (Nagano), clay loam (Ishikawa, Fukushima), loam (Gunma), fine grained yellow earth (Gifu). Plot size: one tree (Nagano, Fukushima), four trees (Ishikawa, Gifu, Gunma). Backpack power atomizer (Nagano), power spraying equipment (Ishikawa, Fukushima), backpack power spraying equipment (Gifu, Gunma). Field samples 2-4 kg. Samples stored at -20°C for 112-180 days. Analytical method GC-FPD, method JA-001/JA-002. Results not corrected for sample interferences (<0.01 mg/kg, n=4), or for concurrent method recoveries (92%-99%).

Table 18. Fenitrothion residues in apples (excluding hulls, pedicel, stylar scars and cores) after pre-harvest spray treatments (WP 400) in the field in Japan in 1992.

Location, year, report no.	Variety	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	DALT	Residues (mg/kg)
Iwate, JA-006/JA-007	Senshu	2	7	0.05	3000	1.5	14 Sept	14 21	0.06[1] 0.08[1]
Iwate, JA-006/JA-007	Senshu	3	7-7	0.05	3000	1.5	14 Sept	14 21	0.09[1] 0.07[1]
Iwate, JA-006/JA-007	Senshu	4	7-7-7	0.05	3000	1.5	14 Sept	14 21	0.11[1] 0.07[1]
Nagano, JA-006/JA-007	Fuji	2	7	0.05	3000	1.5	7 Oct	14 21	0.21[1] 0.16[1]
Nagano, JA-006/JA-007	Fuji	3	6-7	0.05	3000	1.5	7 Oct	14 21	0.32[1] 0.19[1]
Nagano, JA-006/JA-007	Fuji	4	8-6-7	0.05	3000	1.5	7 Oct	14 21	0.42[1] 0.37[1]

[1] Mean of four analytical portions (2 different methods, each with duplicate analyses)

JA-006/JA-007. (Matano and Kobayashi, 1992; Hirota and Umemoto, 1993). Non-GLP. Normal weather conditions. Soil: diluvial humus volcanic ash (Iwate), volcanic ash, black soil (Nagano). 1 tree (Nagano), 3 trees (Iwate). Engine sprayers. Field samples 2 kg stored at -20°C for 27-52 days. Analytical method GC-NPD, method JA-006. Samples re-analysed by another laboratory after 50-79 days storage using method GC-FPD, method JA-007. Because results from JA-007 were identical to those from JA-006, mean results for all four analyses are summarized in the Table. Results not corrected for sample interferences (<0.01 mg/kg, n=4, each method), or for concurrent method recoveries (91%-98%).

Table 19. Fenitrothion residues in pear (whole fruits) after pre-harvest spray treatments in the field in St Catherines, Canada, in 1973.

Report no	Variety	Form	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	DALT	Residues (mg/kg)
HR-41-006	Bartlett & Bosc	WP 400	1	na	-	-	1.12	7 Sept 31Aug 24 Aug 17 Aug[1]	0 7 14 21	1.4 0.48 0.36 0.12
HR-41-006	Bartlett & Bosc	WP 400	5	10-39-31-28	-	-	0.67	24 Aug	7 14	0.77; 0.80; 0.88; mean 0.82 0.02; 0.25; 0.48; mean 0.25

na : not applicable

[1] Reverse decay trial, different last treatment day but same harvest day

HR-41-0006. (Van Doornik, 1974). Non-GLP. Wet spring and hot dry summer. Soil: not stated. Hose and gun power sprayer. Sample sizes not stated. Samples stored at -20°C (period not stated). Analytical method GC-FTD, method HR-21-0004/HR-41-0006. Results not corrected for sample interferences (<0.001 mg/kg, n=1) or for concurrent method recoveries (100%). Results for 5 treatments from three replicate field samples, average value taken.

Legumes. Three trials on green broad beans (seeds only) and seven trials on green soya beans (beans with pods) were conducted in 1971-1995 in Japan (Tables 20 and 21). In trial JB-006 samples were stored at a temperature for which no storage stability data are available (3-4 days at +5°C).

Table 20. Fenitrothion residues in green broad bean seeds after pre-harvest spray treatments in the field in Japan in 1995.

Location, report no.	Variety	Form	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	PHI (days)	Residues (mg/kg)
Ehime, JB-018/JB-019	Ryosai Issun	EC 500	3	7-7; 6-7; 8-6	0.05	3000	1.5	19 May; 15 May; 8 May[1]	3 7 14	0.008[2] 0.010[2] 0.006[2]
Kagawa, JB-	Shimizu	EC	3	8-6	0.05	3000	1.5	8 May	3	0.035[2]

Location, report no.	Variety	Form	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	PHI (days)	Residues (mg/kg)
018/JB-019	Issun	500							7	0.018[2]
Kagawa, JB-018/JB-019	Shimizu Issun	EC 500	3	7-8	0.05	3000	1.5	2 May	13	0.007[2]

[1] Reverse decay trial, different treatment days but same harvest day.
[2] Mean of four analytical portions (2 different analytical methods, each duplicate analyses)
JB-018/JB-019. (Ishimaru, 1996; Hirota and Nakanishi, 1995). Non-GLP. Normal weather conditions. Soil: loam (Ehime), clay loam (Kagawa). Plot size 30-66 m^2. Power sprayer. Field samples 2-4 kg. Samples were stored at -20°C for 0-42 days. Analytical method GC-FTD, method JB-018. Samples re-analysed in same period by another laboratory, using GC-FPD method JB-019. Because results from the second analysis were identical to first, mean results for all four analyses are summarized in the Table. Results not corrected for sample interferences (<0.001 mg/kg, n=4, for JB-018 or <0.01 mg/kg, n=4, for JB-019) or for concurrent method recoveries (86%-97%).

Table 21. Fenitrothion residues in young immature green soya beans after pre-harvest spray treatments (EC 500) in the field in Japan.

Location, year, report no.	Variety	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	Sample	DALT	Residues (mg/kg)
Tokyo, 1971, JB-006	Furisode	2	10	0.07	1000	0.7	14 Sept	green seeds	3 13	0.028[1] 0.002[1]
Tokyo, 1971, JB-006	Furisode	3	10-13	0.07	1000	0.7	27 Sept	green seeds	3 11	0.018 0.002[1]
Hokkaido, 1971, JB-006	Toyosuzu	2	10	0.07	1000	0.7	3 Sept	green seeds	7 14	0.019[1] 0.010[1]
Hokkaido, 1971, JB-006	Toyosuzu	3	11-10	0.07	1000	0.7	3 Sept	green seeds	7 14	0.049[1] 0.006[1]
Yamagata, 1990, JB-003/JB-005	Tachiyutaka	4	7-7-7	0.05	2500	1.25	31 Aug	green soya bean in pod	21 30	0.12[1] 0.09[1]
Ishikawa, 1990, JB-003/JB-005	Enrei	4	7-7-7	0.05	2500	1.25	17 Aug	green soya bean in pod	21	0.18[1]
Ishikawa, 1990, JB-003/JB-005	Enrei	4	7-7-7	0.05	2500	1.25	8 Aug	green soya bean in pod	30	<0.01[1]

[1] Results mean of duplicate analyses.
JB-006. (Takimoto, 1972). Non-GLP. Normal weather conditions. Soil: Kanto loam (Tokyo), sandy loam (Hokkaido). Plot size 40-100 m^2. Shoulder sprayer. Field samples 1 kg stored at 5°C for 3-4 days. Analytical method GC-FTD, method JB-006. Results not corrected for sample interferences (<0.001 mg/kg, n=2), or for concurrent method recoveries (98% at 0.02 mg/kg).
JB-003/JB-005. (Goto *et al.*, 1991b, Kuroda and Higuchi, 1991c). Non-GLP. Normal weather conditions. Soil: humid kokuboku soil with humus at surface (Yamagata), clay loam (Ishikawa). Plot size 22-36 m^2. Backpack sprayer (Yamagata), small power sprayer (Ishikawa). Field samples 2-4 kg stored at -20°C for 3-39 days. Analytical method GC-NPD, method JB-003. Results not corrected for sample interferences (<0.01 mg/kg, n=4), or for concurrent method recoveries (86%-89% at 0.5 mg/kg). Samples re-analysed after 127-165 days by another laboratory, using GC-FPD method JB-005. Because results from JB-005 were slightly lower than results from JB-003, only results from first analysis are summarized in this Table.

Pulses. Ninetreen trials on soya beans (Table 22), four trials on beans (Table 23) and two trials on peas (Table 24) were carried out in 1971-1990 in Japan. Soya beans were treated by broadcast dust, ground spray or spray from a helicopter, beans were ground sprayed, and pea trials were conducted indoors. In trials JB-006, JB-007, JB-008/JB-009 and JB-014/JB-015 samples were stored at temperatures for which no storage stability data were available (66 days at -15°C for JB-008/JB-009, 140 days at -10°C for JB-006, 147 days at +5°C for JB-007 and 34 days at +10°C for JB-014/JB-015). In trials JB-008/JB-009 control samples were contaminated with fenitrothion (max. 0.029 mg/kg).

Table 22. Fenitrothion residues in dry harvested soya bean seeds after pre-harvest ground or aerial spray treatments in Japan.

Location, year report no.	Variety	Method	Form	No.	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treat-ment	DALT	Residues (mg/kg)
Tokyo, 1971, JB-006	Furisode	Ground spray	EC 500	2	10	0.07	1000	0.70	14 Sept	56	0.002[1]
Tokyo, 1971, JB-006	Furisode	Ground spray	EC 500	3	10-13	0.07	1000	0.70	27 Sept	43	0.002[1]
Hokkaido, 1971, JB-006	Toyosuzu	Ground spray	EC 500	2	10	0.07	1000	0.70	3 Sept	55	0.004[1]
Hokkaido, 1971, JB-006	Toyosuzu	Ground spray	EC 500	3	11-10	0.07	1000	0.70	3 Sept	55	0.001[1]
Mito-shi, Ibaraki, 1980, JB-007	Kakushin no 1	Spray by helicopter	EC 500	3	10-10	2.5	30	0.75	8 Sept	45	<0.005[1]
Mito-shi, Ibaraki, 1980, JB-007	Kakushin no 1	Ground spray	EC 500	3	10-10	0.05	1800	0.90	8 Sept	45	<0.005[1]
Hitachiota-shi, Ibaraki, 1980, JB-007	Kakushin no 1	Spray by helicopter	EC 500	3	10-10	2.5	30	0.75	8 Sept	45	<0.005[1]
Hitachiota-shi, Ibaraki, 1980, JB-007	Kakushin no 1	Ground spray	EC 500	3	10-10	0.05	1800	0.90	8 Sept	45	<0.005[1]
Daiyu-Mura, 1981, JB-008/JB-009	Shiro-Sennari	1x ground spray; 2x spray by helicopter	EC 500 (mix)	3	47-12	1x 0.05; 2x 9.4	1x 1200; 2x 8	1x 0.60; 2x 0.75	7 Sept	38	0.013[1]
Daiyu-Mura, 1981, JB-008/JB-009	Shiro-Sennari	Ground spray	EC 500 (mix)	3	47-12	0.05	1x 1200; 2x 1500	1x 0.60; 2x 0.75	7 Sept	38	0.022[1]
Yokote City, Akita, 1981, JB-008/JB-009	Shiro-Sennari	Spray by helicopter	EC 500 (mix)	2	12	9.4	8	0.75	7 Sept	38	0.026[1]
Yokote City, Akita, 1981, JB-008/JB-009	Shiro-Sennari	Ground spray	EC 500 (mix)	3	13-12	0.05	1500	0.75	7 Sept	38	0.12[1]
Nagano, 1981, JB-011/JB-010	Enrei	Broadcast	DP 30	4	7-7-7	na	na	1.2	28 Sept	11	<0.004[1]
Nagano, 1981, JB-011/JB-010	Enrei	Broadcast	DP 30	4	7-7-7	na	na	1.2	21 Sept	18	0.004[1]
Tottori, 1981, JB-011/JB-010	Tamahomare	Broadcast	DP 30	4	7-8-7	na	na	1.2	13 Oct	13	0.004[1]
Tottori, 1981, JB-011/JB-010	Tamahomare	Broadcast	DP 30	4	7-7-8	na	na	1.2	6 Oct	20	0.004[1]
Yamagata, 1990, JB-002/JB-004	Tachiyutaka	Ground spray	EC 500	4	7-8-6	0.05	2500	1.25	14 Sept	21 31	≤0.01[1] <0.01[1]
Ishikawa, 1990, JB-002/JB-004	Enrei	Ground spray	EC 500	4	7-7-7	0.05	2500	1.25	17 Sept	21	≤0.01[2]
Ishikawa, 1990, JB-002/JB-004	Enrei	Ground spray	EC 500	4	7-7-7	0.05	2500	1.25	8 Sept	30	<0.01[2]

na: not applicable;
[1.] Results mean of duplicate analyses;
[2.] Results mean of four analyses (2 different analytical methods, each duplicate analyses)
JB-006. (Takimoto, 1972). Non-GLP. Normal weather conditions. Soil: loam (Tokyo), sandy loam (Hokkaido). Plot size 40-100 m^2. Shoulder sprayer. Field samples 1-2 kg. Harvested samples naturally seasoned outdoors for 10 days (Tokyo), at the farm for 4 days (Hokkaido). Soya bean seeds removed from pods using flails. Samples stored at -10°C for 135-140 days.

Analytical method GC-FPD, method JB-006. Results not corrected for sample interferences (<0.001 mg/kg, n=2), or for concurrent method recoveries (98%).

JB-007. (Goto, 1981). Non-GLP. Normal weather conditions. Soil: alluvial. Plot size 2-4 ha (helicopter), 30-50 m^2 (ground spray). Powered ground sprayer or spray by helicopter. Field samples 4 kg. Harvested samples left to dry for unstated period, stored cool (+5°C) for 140-147 days. Analytical method GC-FPD, method JB-007. Results not corrected for sample interferences (<0.005 mg/kg, n=4), or for concurrent method recoveries (98%-106%).

JB-008/JB-009. (Yamaya, 1982; Kuroda and Araya, 1981). Non-GLP. Normal weather conditions. First treatment with fenitrothion only; 2nd and 3rd treatment formulations 40% thiophanate-methyl and 50% fenitrothion. Soil: peat-muck. Plot size 5 ha (helicopter), 30 m^2 (ground spray). Equipment and sample sizes not stated. Harvested samples left to dry for unstated period, stored for 46-48 days at -15°C. Analytical method GC-FPD, method JB-009. Samples re-analysed by another laboratory after 56-66 days storage. Analytical method GC-FPD, method JB-008. Because results from JB-008 lower than from JB-009, only results from the latter are summarized in the Table. Results not corrected for sample interferences (0.013-0.029 mg/kg, n=8), or for concurrent method recoveries (101%-103%). **Results from both laboratories confirmed control samples contained residues, indicating contamination. Results for this trial are considered unreliable.**

JB-010/JB-011. (Kato, 1982; Shimada, 1981) Non-GLP. Normal weather conditions. Soil: clay loam. Plot size 45-133 m^2. Manual dust sprayer (Nagano), machine dust sprayer (Tottori). Field samples 2-4 kg. Harvested samples from Nagano dried in greenhouse for 21 days and shells removed. Drying procedure for Tottori not stated. Samples stored at -20°C for 38-50 days. Analytical method GC-FPD, method JB-011. Results not corrected for sample interferences (<0.004, n=4), or for concurrent method recoveries (83-102%). Samples re-analysed after 110-114 days storage by another laboratory, using GC-FTD method JB-010. Because the LOQ (0.005 mg/kg) for the second analysis was higher, only results from the first are summarized in this Table.

JB-002/JB-004. (Goto *et al.*, 1991a, Kuroda and Higuchi, 1991b). Non-GLP. Normal weather conditions. Soil: humid kokuboku with humus at surface (Yamagata), clay loam (Ishikawa). Plot size 22-36 m^2. Backpack sprayer (Yamagata), small power sprayer (Ishikawa). Field samples 1.5-4 kg. Harvested samples were dried on shelves in a greenhouse for 16-42 days and then threshed. Samples stored at -20°C for 70-101 days. Analytical method GC-NPD, method JB-002/JB-003. Samples were re-analysed after 89-115 days storage by another laboratory, using GC-FPD method JB-002/JB-004. Because results from second analysis were identical to those from the first, mean results for all four analyses are summarized in the Table. Results were not corrected for sample interferences (<0.01 mg/kg, n=8) or for concurrent method recoveries (88%-93%).

Table 23. Fenitrothion residues in dry harvested bean seeds after four pre-harvest spray treatments in the field in Japan.

Location, year report no.	Commodity; variety	Form	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	DALT	Residues (mg/kg)
Yubari-gun, Hokkaido, 1984, JB-014/JB-015	Adzuki beans; Erimo Shozu	EC 500	7-7-7	0.05	2500	1.25	11 Sept	14 21	0.048[1] 0.068[1]
Hokkaido, 1984, JB-014/JB-015	Adzuki beans; Takara Adzuki	EC 500	10-10-10	0.05	2500	1.25	30 Aug	14 21	0.075[1] 0.061[1]
Ishikawa, 1990, JB-012/JB-013	Kidney beans; Shin-Edogawa	EC 500	7-7-7	0.05	2500	1.25	9 Oct	21 30	0.01[2] <0.01[2]
Okinawa, 1990-1991, JB-012/JB-013	Kidney beans (string beans); FS-Edogawa	EC 500	7-7-7	0.05	2500	1.25	28 Dec	21 30	0.02[2] 0.02[2]

[1] Mean of duplicate analyses;

[2.] Mean of four analyses (2 different analytical methods, each duplicate analyses)

JB-014/JB-015. (Goto, 1985; Nakajima and Saito, 1985). Non-GLP. Normal weather conditions. Soil: clay loam (Yubari-gun), brown volcanic soil (Hokkaido). Plot size 86-108 m^2. Portable power sprayer. Field samples 2 kg. From Ybari-gun DALT 14 samples air-dried for 4 days and shelled, DALT 21 samples shelled at harvest. Hokkaido samples dried for 14 days in mesh room. Thereafter samples stored cool (+10°C) for 22-34 days. Analytical method GC-FPD, method JB-014. Results not corrected for sample interferences (<0.005 mg/kg, n=4), or for concurrent method recoveries (83%-86%). Thereafter samples stored at -20°C for a further 54-65 days and re-analysed by another laboratory using GC-FTD method JB-015. Because results from the second analysis (JB-015) were significantly lower than results from the first analysis (JB-014), only the results from the first analysis are summarized in this Table.

JB-012/JB-013. (Matano and Kobayashi, 1991b; Kuroda and Higuchi, 1991d). Non-GLP. Normal weather conditions. Soil: clay loam (Ishikawa), mineral heavy, alkali (Okinawa). Plot size 49 m^2 (Ishikawa), 450 m^2 (Okinawa). Backpack fully-automatic sprayer (Ishikawa), power sprayer (Okinawa). Field samples 0.65-1.9 kg. Kidney beans shelled in the field - no extra drying. String kidney beans dried in glass room for 3-4 days, shelled manually and dried for an unstated period. Thereafter samples stored at -20°C for 22-121 days. Analytical method GC-NPD, method JB-012. Samples re-analysed after 28-127 days storage by another laboratory, using GC-FPD method JB-013. Because results from JB-013 were identical to those from JB-012, mean results for all four analyses are summarized in the Table. Results not corrected for sample interferences (<0.01 mg/kg, n=8) or for concurrent method recoveries (84%-98%).

Table 24. Fenitrothion residues in dry harvested pea seeds after four pre-harvest indoor spray treatments in Japan.

Location, year report no.	Commodity; variety	Form	Interval (days)	kg ai /hl	Water (l/ha)	kg ai /ha	Last treatment	PHI (days)	Residues[1] (mg/kg)
Chiba, 1991, JB-016/JB-017	Field pea; Mizasa	EC 500	7-7-7	0.05	2000	1.00	30 Apr	21 30	<0.01 <0.01
Nagano, 1991, JB-016/JB-017	Pea; Nimura Akahana No. 2	EC 500	7-7-7	0.05	2500	1.25	16 Apr	21 30	0.04 <0.01

[1] Mean of four analyses (2 different analytical methods, each duplicate analyses)

JB-016/JB-017. (Komatsu and Yabusaki, 1991; Kuroda and Higuchi, 1991e). Non-GLP. Normal weather conditions. Soil: tertiary viscous CL (Chiba), diluvium, alkali (Okinawa). Plot size 10-30 m^2. Manual sprayer (Chiba), small power sprayer (Nagano). Field samples 1.0-3.4 kg. Peas sun-dried in greenhouse for 8-22 days and shelled. Peas from Nagano steamed during drying because of high moisture content of shells. All samples stored at -20°C for 7-41 days. Analytical method GC-FPD, method JB-016. Samples re-analysed in the same period by another laboratory, using GC-FPD method JB-017. Because results from JB-017 were identical to those from JB-016, mean results for all four analyses are summarized in the Table. Results not corrected for sample interferences (<0.01 mg/kg, n=8) or for concurrent method recoveries (87%-100%).

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

No additional data reported.

In processing

The 2003 JMPR evaluated processing studies on rice and wheat. Three studies on the processing of cereal grains were reported to the present Meeting. A fourth study (Booth, 1979) was not evaluated because it was a review on wheat and rice processing and contained no new material, and was of limited value because only processing from white wheat flour to bread and cooking of husked/polished rice was included and processing from raw agricultural product (RAC) to end product was not described.

Processing of rice grain, study 1. Treated unpolished rice was separated into polished rice and bran with a rice-cleaning machine (Ito *et al.*, 1976). The mass ratio grain:bran was 87:13. Polished grain (3 kg) was washed with 300 ml distilled water and boiled as follows.

(1) In an electric cooking apparatus for 15 min (180 g grain in 200 ml distilled water) and kept warm for a further 15 min.
(2) Autoclaved at 110°C for 10 min at 1.5 atm pressure (10 g grain in 10 ml distilled water).
(3) Autoclaved at 120°C for 10 min. at 2.1 atm pressure (10 g grain in 10 ml distilled water).

Samples were analysed by GC-FTD, method 1 (Ito *et al.*, 1976; Sato *et al.*, 1968). The distribution of residues is shown in Table 25 and calculated processing factors in Table 26.

Note. Details of rice treatments and storage, and concurrent method recoveries were not reported, no method validation was available nor were storage conditions for analytical samples stated.

Table 25. Distribution of fenitrothion residues in processed rice fractions.

Months after treatment	Amount applied (mg/kg)	Unpolished rice (mg/kg)	Bran (mg/kg)	Polished rice (mg/kg)	Washed polished rice (mg/kg)	Washing water (mg/kg[1])	Cooking conditions	Cooked washed polished rice (mg/kg)
0	15	9.4	65	1.0	-	0.60	1	0.26
12	2	0.61	4.0	0.09	0.03	0.05	1 2 3	0.02 0.01 0.01
12	6	1.7	12	0.25	0.08	0.16	1 2 3	0.05 0.03 0.01
12	15	4.4	32	0.55	0.18	0.37	1 2 3	0.12 0.07 0.04

[1] Equivalent in polished grains before washing

Table 26. Processing factors for fenitrothion residues in processed rice fractions.[1]

Months after treatment	Polished rice	Bran	Washed polished rice	Cooked washed polished rice condition 1	Cooked washed polished rice condition 2	Cooked washed polished rice condition 3
0	0.11	6.9	-	0.028	-	-
12	0.15	6.6	0.049	0.033	0.016	0.016
12	0.15	7.0	0.048	0.030	0.018	0.0060
12	0.13	7.2	0.041	0.027	0.016	0.0091
Average	0.14	6.9	0.046	0.029	0.017	0.011
RSD	14%	3.6%	9.5%	8.5%	6.7%	51%
Maximum	0.15	7.2	0.049	0.033	0.018	0.016

[1] Processing factor: residue in processed product ÷ residue in RAC (unpolished rice)

Processing of barley and rice grains, study 2. Barley grain (variety Clipper), unhusked paddy rice (variety Calrose), husked (brown) rice and (white) rice were fortified at 15 mg/kg (Desmarchelier *et al.*, 1980b) by dispersing 1000 g ai/l solution of fenitrothion in water and applying 2 ml of the dispersion by a dropping pipette to 3 x 2 kg samples of grain. The samples were stored for 3 or 6 months in sealed 3 litre glass jars at 25 ± 2°C. The relative humidity was 65% for barley and unhusked rice and 55% for husked and polished rice.

Barley samples were 'home malted' (sprouted without gibberellic acid and dried at <40°C) or malted using a commercial pilot scale process. The process was not described.

Husked and polished rice samples were cooked in a minimum of boiling water for 15 min (3 month samples) or 25 min (6 month samples). Unhusked rice samples (6 months storage) were milled into husked and polished rice and both were cooked in a minimum of boiling water for 25 min. Samples were analysed using GC-FPD method 2 (Desmarchelier, 1980a), and corrected for concurrent method recoveries (results not available). These results are summarized in Table 27.

Note. Some processing factors were derived from an already processed sample and not from the RAC (whole grain), concurrent method recoveries and sample storage conditions were not reported and no method validation was available.

Table 27. Residues of fenitrothion in stored rice and barley and their processed fractions.[1]

Storage (months)	Commodity	Fenitrothion (mg/kg)	Processing factor[2]
3	barley, whole grain home malt	7.2 1.7	- 0.24
6	barley, whole grain	3.4	-

Storage (months)	Commodity	Fenitrothion (mg/kg)	Processing factor[2]
	commercial malt	0.55	0.16
3	husked rice (brown rice)	7.7	-
	husked cooked rice	3.6	-
6	husked rice (brown rice)	4.5	-
	husked cooked rice	3.0	-
3	polished rice (white rice)	8.2	-
	polished cooked rice	3.2	-
6	polished rice (white rice)	5.5	-
	polished cooked rice	2.8	-
3	unhusked rice (paddy rice)	7.7	-
6	unhusked rice (paddy rice)	3.5	-
	husked rice	0.62	0.18
	polished rice	0.27	0.08
	husked cooked rice	0.39	0.11
	polished cooked rice	0.13	0.04

[1] Results corrected for concurrent method recovery (results not available)
[2] processing factor: residue in processed product ÷ residue in RAC (whole barley grain or paddy rice)

Processing of rice grains, study 3. Post-harvest treated samples of rice containing residues of fenitrothion were processed into various fractions (Hill, 2000) and analysed using method ALM/OP/1 (updated version 02.98 JP). Only the analytical report was submitted. The results are shown in Table 28. In control samples of pollard 0.08 mg/kg fenitrothion was found and therefore the LOQ for pollard has to be increased to a mimimum of 0.08 x 10/3 = 0.3 mg/kg, sufficient to quantify the high residue levels.

Note. Details of rice growing conditions, treatments, storage and processing were not submitted, concurrent method recoveries were not reported and no method validation was available. Sample storage conditions were not reported.

Table 28. The effect of processing on residues of fenitrothion in rice fractions.

Treatment	Residues (mg/kg)					Processing factors[1]			
	Paddy rice	Brown rice	White rice	Hulls	Bran	Brown rice	White rice	Hulls	Bran
6 g ai/t; day 0	0.28	0.73	0.06	18	2.6	2.6	0.21	64	9.1
6 g ai/t; day 2	3.7	0.36	<0.05	20	1.9	0.10	<0.01	5.4	0.51
6 g ai/t; day 7	2.7	0.43	<0.05	5.7	2.8	0.16	<0.02	2.1	1.0
6 g ai/t; day 14	3.8	0.43	<0.05	17	2.3	0.11	<0.01	4.5	0.60
6 g ai/t; 3 week	4.0	0.40	<0.05	0.50	1.1	0.10	<0.01	0.12	0.28
6 g ai/t; day 28	4.6	0.30	0.40	3.1	2.6	0.065	0.087	0.67	0.57
6 g ai/t; 6 weeks	3.1	1.0	<0.05	3.3	0.80	0.32	<0.02	1.1	0.26
6 g ai/t; 8 weeks	2.5	0.22	<0.05	2.2	0.94	0.089	<0.02	0.88	0.38
6 g ai/t; 10 weeks	2.1	0.30	<0.05	21	0.36	0.14	<0.02	10	0.17
6 g ai/t; 12 weeks	2.4	0.30	<0.05	11	0.90	0.12	<0.02	4.5	0.37
12 g ai/t; day 0	9.3	0.99	0.06	43	3.6	0.11	0.0065	4.7	0.39
12 g ai/t; day 2	11	1.0	<0.05	45	3.8	0.095	<0.005	4.3	0.36
12 g ai/t; day 7	10	0.91	<0.05	41	4.7	0.089	<0.005	4.0	0.46
12 g ai/t; day 14	6.4	0.61	<0.05	26	0.58	0.095	<0.008	4.0	0.090
12 g ai/t; 3 weeks	5.3	0.7/3.4[2]	<0.05	24	-	0.64	<0.009	4.4	-
12 g ai/t; day 28	23	0.70	<0.05	45	0.40	0.031	<0.002	2.0	0.018
12 g ai/t; 6 weeks	6.3	0.55	0.05	12	13	0.087	0.0079	1.8	2.0
12 g ai/t; 8 weeks	3.4	1.4	<0.05	35	2.8/3.0[2]	0.41	<0.02	10	0.91
12 g ai/t; 10 weeks	4.5	0.35	<0.05	35	-	0.077	<0.01	7.8	
12 g ai/t; 12 weeks	3.8	0.52	<0.05	24	2.7	0.14	<0.01	6.3	0.71
IPM 658-662	2.0	0.53	<0.05	12	3.2 (p)	0.27	<0.03	6.1	1.6
IPM 668-672	1.1	0.27	<0.05	5.2	1.1 (p)	0.24	<0.04	4.6	1.0
Mean[3]						0.17	0.018	4.3	0.61
RSD[3]						86%	102%	66%	84%

Treatment	Residues (mg/kg)					Processing factors[1]			
	Paddy rice	Brown rice	White rice	Hulls	Bran	Brown rice	White rice	Hulls	Bran
Minimum[3]						0.031	<0.002	0.12	0.018
Maximum[3]						0.64	0.087	10	2.0

p: pollard
[1] Processing factor: residue in processed rice commodity÷residue in RAC (paddy rice)
[2] Higher value taken for calculation of processing factor
[3] Results from trial at 6 g ai/t, day 0, not included in calculations since residue found in paddy rice unexpectedly low.

Residues in the edible portion of food commodities

No additional data submitted.

RESIDUES IN ANIMAL COMMODITIES

Direct animal treatments

Residues in livestock, soil and pasture after fenitrothion spray treatment of pastures

A trial was conducted in a 120 ha paddock in Wingadee, central-western New South Wales, Australia, in February 1998 (McDougall, 1998; Gilmour *et al.*, 1999). A 0.4 ha area was fenced off to exclude cattle and to study the degradation of fenitrothion on ungrazed pasture and soil, but cattle were on the rest of the pasture when it was treated.

Fenitrothion was applied by air as an ultra-low volume (ULV) spray at the rate of 0.508 kg ai/ha, the normal rate for the control of spur-throated locusts (*Austracis guttulosa*) and twice that used for plague locusts (*Chortoicetes terminifera*). The soil was heavy black clay and the weather hot and dry (maximum 29-40°C) with 1-7 mm rainfall on days 6 and 7.

Of the seventy 18-month old Hereford heifers with body weights 253-382 kg, average 311 ± 21 kg, twenty-eight were on the pastures when the application was made, and thirty-eight were returned to the treated pastures immediately after spraying. The remaining four heifers were controls. The cattle were allowed to graze the sprayed pasture for varying periods and some were slaughtered 2, 4, 7, 14 and 21 days after treatment (DAT). Samples of subcutaneous and perirenal fat, neck muscle and liver were taken for analysis. Others were removed from the treated pasture and grazed on clean pasture for 2, 4 and 7 days before being slaughtered to determine the effect on residues of this. Soil and pasture were sampled at days –3, 0, 1, 2, 4, 7, 10, 14, and 21. Fat samples were analysed immediately: all other samples were stored frozen (storage time and temperature not stated). Residues of fenitrothion were determined by HPLC method 3 (pasture), GC method 4 (animal commodities), and GC method 5 (soil) (Dubs *et al.*, 1985; Heath and Black, 1987; Gilmour *et al.*, 1999). The results for animal commodities and soil were corrected for recovery (uncorrected results not shown), and those for pasture were not. The residues in pasture ranged from <0.1-157 mg/kg dw and in soil were always below 1 mg/kg (Table 29). The pasture results indicate a half-life for fenitrothion of 1-2 days and that degradation was initially faster. About 50% degraded in the first 24 hours, 75% in 3 days, and 90% in 6 days. Residue data in the soil indicated a half-life of fenitrothion of 2-3 days.

Assuming cattle consumed 10 kg dry matter per day from the pasture, their initial intake of fenitrothion per day was 700-800 mg, and if they ingested about 500 g soil per day their intake from the soil was below 0.5 mg/day in the initial stages of the trial. Assuming that the footprint area of the cattle was about 1.5 m^2, the deposition of fenitrothion on sprayed cattle was about 76 mg/animal, and the worst-case estimate of total exposure on the day of spraying 800-900 mg fenitrothion/animal.

In the animal commodities residues were found only in the 2-7 day fat samples. They included control samples (Table 30), therefore the valid LOQ for fat should be increased to a minimum of 0.2 mg/kg (0.064 x 10/3). The residues in all animal commodities were below this LOQ. Removing cattle to unsprayed pastures for varying periods before slaughter had no impact on the residue levels since all residues that would have been there would have decayed to less than 0.05 mg/kg (the Australian MRL for meat or offal) in 14 days (the Australian pre-slaughter withholding period).

Table 29. Pasture and soil residues (average ± RSD%, range).

Interval after spraying (h)	Pasture area		Soil area	
	Ungrazed (mg/kg dw, n=9-10)	Grazed (mg/kg dw, n=9-10)	Ungrazed (mg/kg, n=2)	Grazed area (mg/kg, n=1)
0	-	-	<0.01	<0.01
14 (0 day)	81 ± 37% (52-157)	54 ± 56% (12-108)	0.68 (0.57-0.78)	0.41
24 (1 day)	35 ± 32% (19-56)	32 ± 44% (7.4-57)	0.51 (0.42-0.60)	0.32
48 (2 days)	24 ± 29% (13-36)	22 ± 54% (6.6-43)	0.41 (0.35-0.47)	0.27
70 (3 days)	17 ± 28% (9.7-24)	11 ± 89% (2.1-32)	-	-
92 (4 days)	10 ± 52% (3.6-20)	8.4 ± 61% (2.3-18)	0.28 (0.27-0.29)	0.20
165 (7 days)	2.2 ± 46% (<0.1-3.7)	1.6 ± 54% (0.8-3.8)	0.10 (0.097-0.10)	0.028
240 (10 days)	1.3 ± 28% (0.9-1.8)	1.3 ± 47% (0.5-2.4)	0.076 (0.063-0.088)	0.077
335 (14 days)	0.87 ± 64% (<0.1-1.8)	1.6 ± 62% (0.4-3.2)	0.040 (0.028-0.053)	0.024
384 (21 days)	-	-	0.020 (0.019-0.022)	0.019

Table 30. Residues in animal tissues after aerial application at 0.508 kg ai/ha.

Treatment	DAT	Parent (mg/kg)			
		Subcutaneous fat	Renal fat	Muscle	Liver
O	2	<0.02 (3), 0.021			
R	2	<0.02 (3), 0.020			
O	4	<0.02 (5), 0.036	<0.02 (2)		
R	4	<0.02 (6)			
O	7	<0.02 (4), 0.040, 0.043	<0.02 (4), 0.025, 0.037	<0.02 (6)	<0.02 (6)
R	7	<0.02 (5), 0.056	<0.02 (5), 0.044		
O	14	<0.02 (6)	<0.02 (6)	<0.02 (6)	<0.02 (6)
R	14	<0.02 (6)	<0.02 (6)	<0.02	<0.02
O	21	<0.02 (4)			
R	21	<0.02 (4)			
R, withdrawn 2 days before slaughter	7	<0.02 (4)	<0.02 (4)		
R, withdrawn 4 days before slaughter	7	<0.02 (4)	<0.02 (4)		
R, withdrawn 7 days before slaughter	14	<0.02 (4)	<0.02 (4)		
O, withdrawn 7 days before slaughter	14	<0.02 (2)	<0.02 (2)		
C	2	<0.02 (2)		<0.02 (2)	<0.02 (2)
C	4	<0.02, 0.064	<0.02, 0.025	<0.02 (2)	<0.02 (2)

O: over spray – cattle left in pasture during aerial spraying
R: returned - cattle removed from pasture during spraying, then returned.
C: control

Farm animal feeding studies

The 2003 JMPR evaluated the fate of fenitrothion residues in cattle grazing on fenitrothion-treated grass and in cattle fed with fenitrothion-treated maize. Feeding studies on lactating dairy cattle and poultry were reported to the present Meeting.

Dairy cattle. Groups of three lactating cows were dosed at 0, 10, 30 or 100 ppm in the feed for 28 days (Taylor, 1980a), equivalent to an average of 0.75, 1.80 and 9.6 mg/kg/bw. Blood samples were taken from all animals 1 and 2 weeks before the study commenced and then at 0, 7, 14 and 28 days. Any animals showing inhibition of cholinesterase activity were not fed the treated feed after the 28 days and monitored until levels had returned to normal (i.e. levels on days –14 and –7). All cows were milked twice daily, and composite samples of morning and afternoon milk taken from days –1, 0, 3, 7, 14, 21 and 28 were analysed.

Cream was analysed separately: cream and butterfat contents in the milk were 5–8% and 3–5% respectively. Residues of fenitrothion, fenitrooxon, aminofenitrothion and *p*-nitrocresol in milk, and of fenitrooxon and *p*-nitrocresol in cream, were below the limit of quantification of 0.01 mg/kg in all dose groups. Residues of fenitrothion and aminofenitrothion in cream are shown in Table 31.

Table 31. Residues in the cream of lactating cows dosed with fenitrothion at 10, 30 or 100 ppm in the diet for 28 days.

Feed group (ppm)	Residues (mg/kg)	
	Fenitrothion	Aminofenitrothion
Day 3		
10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
30	<0.01, <0.01, <0.01	<0.01, <0.01, 0.01
100	<0.01, <0.01, <0.01, <0.01, 0.01	<0.01, <0.01, 0.01, 0.02, 0.02
Day 7		
10	<0.01, <0.01, 0.01	<0.01, 0.01, 0.01
30	<0.01, 0.01, 0.01	0.01, 0.01, 0.02
100	<0.01, <0.01, <0.01, 0.01, 0.01	0.01, 0.01, 0.02, 0.02, 0.03
Day 14		
10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
30	<0.01, <0.01, 0.01	<0.01, <0.01, <0.01
100	<0.01, <0.01, <0.01, <0.01, 0.01	<0.01, 0.01, 0.01, 0.03, 0.04
Day 21		
10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
30	<0.01, <0.01, 0.01	0.01, 0.01, 0.01
100	<0.01, <0.01, <0.01, <0.01, <0.01	0.01, 0.01, 0.02, 0.02, 0.03
Day 28		
10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
30	<0.01, 0.01, 0.01	<0.01, <0.01, 0.01
100	<0.01, <0.01, <0.01, 0.01, 0.01	<0.01, 0.01, 0.01, 0.02, 0.02

Samples of liver, kidney, muscle (cardiac, hind-quarter and front-quarter) and fat (omental and perirenal) were taken. Residues above the limit of quantification of 0.05 mg/kg were found only in a single kidney sample (0.11 mg/kg aminofenitrothion, 100 ppm feed group).

Recoveries of fenitrothion, fenitrooxon and *p*-nitrocresol were within acceptable limits for all samples at all fortifications (0.05 to 0.3 mg/kg), but those of aminofenitrothion were generally below 60%.

Poultry. Layer and broiler hens were divided into four treatment groups, each receiving 0, 10, 30 or 100 ppm in the feed for 28 days (Taylor, 1980b), with average intakes of 0.72-2.63, 2.18-8.44 and

5.90-24.49 mg/kg/bw respectively. Eggs were collected twice a week from each group, composited within each group and frozen. Half of the hens from each group were killed on day 14 and the rest on day 28. Samples of red and white muscle, liver and fat were taken and analyses included fenitrothion and the two metabolites fenitrooxon and *p*-nitrocresol.

Residues in all tissues on both days 14 and 28 were below the LOQ of 0.05 mg/kg. Quantifiable residues of fenitrothion and the two metabolites were not found in any eggs throughout the study.

Recoveries were determined with fortification of fenitrothion, fenitrooxon and *p*-nitrocresol in tissues and eggs at concentrations of 0.05, 0.15 and 0.30 mg/kg, and were within acceptable limits.

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

No additional data were reported.

NATIONAL MAXIMUM RESIDUE LIMITS

No additional data were reported.

APPRAISAL

Fenitrothion was evaluated for residues within the periodic review programme of the CCPR by the 2003 JMPR, which recommended an MRL of 10 mg/kg (accommodating post-harvest treatment, Po) for cereals. The Meeting indicated that additional information on metabolism in cereals (including rice) after pre-harvest treatment, a validated analytical method for the determination of fenitrothion in animal commodities, freezer storage stability of residues in animal commodities, farm animal transfer studies and a processing study on rice were desirable. At the present Meeting, an undertaking was given to submit additional data to support uses on apple, pear and soya bean (dried seed), as these are important traded commodities.

The CCPR at its Thirty-sixth Session agreed to retain the existing MRLs for meat (0.05* mg/kg, fat), milks (0.002* mg/kg), unprocessed rice bran (20 mg/kg), polished rice (1 mg/kg), processed wheat bran (2 mg/kg), unprocessed wheat bran (20 mg/kg), wheat flour (2 mg/kg) and wheat wholemeal (5 mg/kg).

The data submitted to address the outstanding points are summarized below.

Metabolism

Animals

No additional data were submitted.

Plants

The 2003 JMPR evaluated the fate of fenitrothion after spray application to grapes and tomato (crop category fruits) and the fate of fenitrothion in stored rice. The Meeting received two additional studies on the fate of fenitrothion after pre-harvest spray application to rice under simulated paddy growing conditions.

In the first study, rice plants (variety Hatsushimo) were transplanted to a paddy field which had been prepared in a vinyl tent. The rice plants were sprayed once at 0.375 kg ai/ha with a ^{32}P-

fenitrothion emulsifiable concentrate formulation. At various intervals after application, rice plants were sampled at random by cutting down at the base and separating the leaf sheath and leaf blade. At normal harvest, mature grains were harvested and separated into bran and polished rice.

In mature rice grain, the main metabolite was phosphoric acid, while phosphorothionic acid, dimethylphosphorothioic acid and the parent were also found. The residue levels were 7–33 times higher in rice bran than in polished rice. Fenitrooxon, desmethylfenitrothion, desmethylfenitrooxon, dimethylphosphoric acid, monomethylphosphorothioic acid and monomethylphosphoric acid were not detected. In rice grains from plants (varieties Kin-nampu, Ginga, Aichi-asahi) treated with unlabelled fenitrothion, the parent compound could not be detected in rice bran or in polished rice (< 0.1 mg/kg), but 3-methyl-4-nitrophenol was found in rice bran of the varieties Kin-nampu and Aichi-asahi.

In a second study, rice (variety Nihonbare) was grown in a glasshouse under conditions simulating growth in a rice paddy. Rice seedlings were planted in pots filled with soil and flooded with 3–5 cm water throughout the study. The rice was treated with four spray applications of an emulsifiable concentrate formulation containing [phenyl-^{14}C]fenitrothion at a nominal dose rate of 0.75 kg ai/ha per application 81 (2 months after planting), 28, 21 and 14 days before mature harvest. Twelve days before harvest, water was withheld from the plants. Rice plants, soil and root samples were collected, and the plants were separated into unhulled whole grain and straw (leaf and stem). The unhulled whole grain was further processed to brown rice and chaff (hulls). The brown rice was further processed to give 90% (w/w) polished rice and 11% (w/w) bran.

A high proportion of the radioactivity was extractable (65–89% of the TRR). Fenitrothion was detectable in all rice fractions, at 0.003–0.3 mg/kg. The main metabolite in unhulled whole grain, brown rice, polished rice, chaff, bran and straw was 3-methyl-4-nitrophenol, at 0.09–3.9 mg/kg fenitrothion equivalents, and this compound was present in a mixture of free and conjugated forms. The conjugates were hydrolysed to the free form by enzymatic (β-glucosidase) and acid hydrolyses. Fenitrothion was present at levels of 0.30 mg/kg in unhulled whole grain rice, 0.027 mg/kg in brown rice and 0.003 mg/kg in polished rice.

The percentage transference would be equivalent to 8.9% for brown rice and 1.0% for polished rice. Fenitrooxon was present at levels of 0.14 mg/kg in unhulled whole grain rice and 0.009 mg/kg in brown rice; it was not detected in polished rice. Fenitrooxon was also found in bran at 0.042 mg/kg, chaff at 0.84 mg/kg and straw at 0.27 mg/kg. Most of the radioactivity in the post-extracted solids was released by acid or base hydrolysis. 3-Methyl-4-nitrophenol was detected in all the hydrolysates except that of polished rice. Minor amounts (< 2.5% of the TRR) of aminofenitrothion, fenitrooxon, fenitrothion S-isomer and fenitrothion were also released on hydrolysis. In polished rice, only fenitrothion S-isomer was found.

Environmental fate

No additional data were submitted.

Methods of analysis

The 2003 JMPR evaluated enforcement and monitoring methods for plant commodities. The Meeting received two additional analytical methods intended for enforcement and monitoring for animal commodities (RRC 78-32 and RRC 78-32A).

Method RRC 78-32 is used for the determination of fenitrothion (parent), fenitrooxon, aminofenitrothion and 3-methyl-4-nitrophenol in milk, cream and cattle tissues. Method RRC 78-32A is a modification for eggs and poultry tissues. In this appraisal, the focus is on the parent compound. Cattle milk, cream and tissue, and eggs and poultry tissues were extracted with acetone or acetonitrile:methanol:water and cleaned, and fenitrothion was determined by gas chromatography–flame photometry detection. The reported LOQ was 0.01 mg/kg for cattle milk and cream and 0.05 mg/kg for eggs and cattle and poultry tissues. The method is considered to be outdated because of the use of glass gas chromatography columns.

The Meeting received information on analytical methods for the determination of the parent in foodstuffs of plant, animal or environmental origin as used in various additionally submitted study reports (residue trials, storage stability of analytical samples, processing studies, feeding studies). Fenitrothion was determined by gas chromatography with flame photometry, nitrogen–phosphorus or FT detection. The reported LOQs ranged from 0.001 mg/kg to 0.1 mg/kg.

Stability of residues in stored analytical samples

The 2003 JMPR evaluated the stability in storage of residues in dry crops with starch and protein (grain of wheat, barley, rice) and in rice straw. The Meeting received additional data on the stability of residues in crops with a high water content (apple, green soya bean, green broad bean) and dry crops with starch and protein (dry seeds of soya bean, kidney bean, pea).

The Meeting concluded that fenitrothion residues are stable at –20°C for at least 192 days in apples, for at least 155 days in legumes, for at least 149 days in cereals and for at least 98 days in pulses. In several trials, however, samples were stored at –15°C, –10°C, +5°C or +10°C. Information on the stability of the samples under these conditions was not available.

Definition of the residue

The 2003 JMPR proposed fenitrothion as the residue definition for compliance with MRLs and for dietary intake, for both plant and animal commodities. The residue is not fat-soluble.

The supported uses of fenitrothion at that time were pre-harvest application on cereals and post-harvest application on stored cereal grains. The 2003 Meeting concluded that the available studies were adequate only for post-harvest uses on stored cereal grains; to support pre-harvest uses on cereals, relevant metabolism studies were required.

The manufacturer now wishes to support use on pome fruit and soya bean. The 2003 JMPR evaluated studies of metabolism in grape and tomato after pre-harvest use. The main metabolites were 3-methyl-4-nitrophenol and its conjugates. Two studies on metabolism in rice after pre-harvest use of fenitrothion were made available to the present Meeting.

The studies confirm that the main metabolite is 3-methyl-4-nitrophenol (free and conjugated). The parent compound was found in the raw agricultural commodity and in all processed fractions (unhulled whole rice grain, brown rice, polished rice, bran, hulls and rice straw). In addition, fenitrooxon was found in unhulled whole rice grain (0.14 mg/kg), brown rice (0.009 mg/kg), bran (0.042 mg/kg), hulls (0.84 mg/kg) and straw (0.27 mg/kg). Fenitrooxon should be considered for inclusion in the residue definition for dietary intake, as the 2000 JMPR concluded that it is the most important metabolite with respect to toxicity. As it is found in small amounts in brown rice and not at all in polished rice (the edible portions for humans), however, the Meeting decided not to include fenitrooxon in the residue definition for dietary intake.

Fenitrothion S-isomer and aminofenitrothion, metabolites that were found but not discussed previously, were present in such small amounts that the Meeting concluded that their inclusion in the residue definition was unnecessary.

Therefore, the Meeting concluded that the residue definition as proposed by the 2003 JMPR do not require alteration.

Definition of the residue for compliance with MRLs and for estimating dietary intake: fenitrothion, for both plant and animal commodities.

Results of supervised trials on crops

The Meeting received information on supervised trials on apple, pear, green broad bean, green soya bean, dry soya bean, dry beans and dry peas.

In 2003, the Meeting summarized the results of supervised trials of pre-harvest treatment on cereal grains (rice, wheat, barley, triticale) in Japan and Australia. In some trials, pre-harvest

treatment was combined with seed treatment before planting. The trials were not evaluated at that time, because information on metabolism after pre-harvest treatment was lacking.

Pome fruit

Nineteen trials on apple and two trials on pear were conducted in Canada in 1972–73. As there is no GAP in Canada or in the USA, the trials could not be evaluated.

In six of 24 trials on apple conducted in Japan in 1989–95, the analytical samples excluded hulls, pedicels, stylar scars and cores. As the Codex commodity for which the maximum residue level is estimated is the whole fruit, the trials could not be evaluated. In the remaining 18 trials, only pedicels, stylar scars and cores were removed. The Meeting decided that this deviation from the Codex commodity definition was acceptable, and it evaluated the trials. GAP in Japan is three applications of 0.025–0.050 kg ai/ha, with a PHI of 30 days. In two trials, residues were found in control samples, and those trials could not be evaluated.

The residue levels in trials meeting GAP were, in ranked order: < 0.01, 0.01 (three), 0.02 (two), 0.04, 0.08, 0.10 (two), 0.11, 0.12 and 0.41 mg/kg. The Meeting decided to withdraw the currently recommended maximum residue level for apple and replace it by 0.5 mg/kg, with an STMR of 0.04 mg/kg and a highest residue value of 0.41 mg/kg.

There were only two trials on pear, and neither complied with GAP. Furthermore, Japanese GAP allows six applications on pear and only three on apple. The Meeting decided to maintain withdrawal of the recommendation for pear.

Legume vegetables

Three trials on green broad beans (seeds only) and seven trials on green soya beans (seeds or beans with pods) were conducted in 1971–95 in Japan. There is no Japanese GAP for broad beans. Japanese GAP for soya beans (dry and green) is foliar spray treatment at 0.025–0.050 kg ai/ha, four applications, 21-day PHI. Two trials in which the portion analysed was green soya bean in the pod and which were conducted according to GAP yielded residue levels of 0.12 mg/kg and 0.18 mg/kg. Two trials is insufficient to estimate a MRL for soya bean, immature seeds.

Pulses

Nineteen trials on dry harvested soya beans, four trials on beans and two indoor trials on peas were carried out in 1971–90 in Japan. Japanese GAP is described above, except that when fenitrothion is applied by foliar spray from an unmanned helicopter, the dose is 0.50 kg ai/ha. In all but four trials, the PHI exceeded that specified for GAP. In the four trials, the residue levels were 0.004 (two) and < 0.01 (two) mg/kg. The Meeting decided that four trials is insufficient to estimate a MRL for soya bean, dry.

Cereal grains

The 2003 Meeting received information from supervised trials on pre-harvest treatment of cereal grains (rice, wheat, barley, triticale) in Australia and Japan. Because of lack of data on metabolism in cereal grains after pre-harvest treatment, the trials could not be evaluated at that time. As the present Meeting received the requested data, the trials can now be evaluated.

Sixteen trials on rice were conducted in Japan in 1993–96 (see 2003 JMPR). In the trials conducted in 1993–95, seeds were soaked in a fenitrothion solution for 24 h before planting and then given four applications of fenitrothion from a knapsack sprayer during the growing season. In the trials performed in 1996, fenitrothion was applied four times from an unmanned helicopter, without prior soaking of the seeds. Fenitrothion is registered in Japan for pre-harvest use on rice at a maximum of four foliar spray applications of 0.375–0.90 kg ai/ha and a PHI of 21 days. The residue levels in the trials complying with Japanese GAP were < 0.01 (four), 0.01 (two), 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10 and 0.12 mg/kg.

Four trials on *winter wheat* were conducted in Australia in 2001 and in Japan in 1993. In these trials, fenitrothion was applied three times from a knapsack sprayer or a boom sprayer. Fenitrothion is registered in Australia for pre-harvest use on cereals with a maximum of three applications of 0.27–

0.55 kg ai/ha (interval ≥ 14 days) and a PHI of 14 days; in Japan, it is registered for pre-harvest use on wheat with a single application of 0.45–0.60 kg ai/ha and a PHI of 7 days. The Australian trials were at Australian GAP and yielded residue levels of 0.10 and 0.21 mg/kg. The trials in Japan did not comply completely with Japanese GAP (three applications instead of one), but the results were evaluated. The residue levels were < 0.01 and 0.30 mg/kg.

Three trials on *winter barley* were conducted in Australia in 2001 and in Japan in 1993. Fenitrothion was applied three times from a knapsack sprayer or a hand-held boom sprayer. In Australia, fenitrothion is registered for pre-harvest use on cereals with a maximum of three applications of 0.27–0.55 kg ai/ha (interval ≥ 14 days) and a PHI of 14 days. In Japan, fenitrothion is not registered for use on barley. The Australian trial was at Australian GAP and yielded a residue level of < 0.06 mg/kg.

One trial on *winter triticale* was conducted in Australia in 2001, in which fenitrothion was applied three times from a hand-held boom sprayer. The trial was at the Australian GAP for cereal and yielded a residue levek of 0.08 mg/kg.

The Meeting decided to combine the results of all the trials on cereals residue, to yield residue levels, in ranked order, of: < 0.01 (five), < 0.06, 0.01 (two), 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08 (two), 0.09, 0.10 (two), 0.12, 0.21 and 0.30 mg/kg. As the residue levels resulting from pre-harvest treatment were lower than those after post-harvest treatment, the Meeting decided to maintain the current recommendations for cereal grain of 10 mg/kg (Po), an STMR of 5 mg/kg and a highest residue of 7.6 mg/kg.

Straw, fodder and forage of cereal grains and grasses

Two trials on *rice* were conducted in Japan in 1993 according to Japanese GAP. The residue levels in straw were 0.31 and 0.44 mg/kg.

Two trials on *winter wheat* were conducted in Australia in 2001 according to Australian GAP. The residue levels in straw were 1.2 and 4.1 mg/kg.

One trial on *winter barley* was conducted in Australia in 2001 according to Australian GAP. The residue level in straw was 0.41 mg/kg.

One trial on *winter triticale* was conducted in Australia in 2001 according to Australian GAP. The residue level in straw was 2.0 mg/kg.

The Meeting decided that there were insufficient data to estimate a maximum residue level in cereal straw.

Fate of residues during processing

The 2003 JMPR evaluated the fate of fenitrothion during simulated processing, in stored rice during polishing and cooking and in stored wheat during milling and baking. The Meeting received three additional studies on the fate of fenitrothion after post-harvest treatment of rice during polishing and cooking and in barley during malting.

Rice stored for up to 12 months after post-harvest treatment with fenitrothion at 2–15 g ai/t was separated into polished rice and bran. The polished rice was washed and cooked for 10–15 min at normal to 2.5 atm pressure. The parent compound was analysed in all processed products. The calculated processing factors were 0.11–0.15 for polished rice (mean, 0.14), 6.6–7.2 for bran (mean, 6.9), 0.041–0.049 for washed polished rice (mean, 0.046) and 0.0060–0.033 for cooked washed polished rice (mean, 0.020).

Paddy rice stored for up to 6 months after post-harvest treatment with fenitrothion at 15 g ai/t was processed into husked rice, polished rice and bran. Husked and polished rice were cooked for 25 min. The parent compound was analysed in all processed products. The calculated processing factors were 0.18 for husked rice, 0.08 for polished rice, 0.11 for cooked husked rice and 0.04 for cooked polished rice.

Paddy rice was processed into husked rice, polished rice, hulls and bran. The parent compound was analysed in all processed products. The calculated processing factors were 0.031–0.64 for husked rice (mean, 0.17), < 0.002–0.087 for polished rice (mean, 0.018), 0.12–10 for hulls (mean, 4.3) and 0.018–2.0 for bran (mean, 0.61).

Barley stored for up to 6 months after post-harvest treatment with 15 g ai/t fenitrothion was processed into malt and was analysed for the parent. The calculated processing factors were 0.16-0.24 for malt (mean 0.20).

The Tablebelow summarizes the processing factors for wheat, barley and rice commodities. The information on wheat was discussed by the 2003 JMPR. Three studies were available on the processing of rice, yielding different results. As details of the growing conditions, treatments, storage and processing were absent or partial, the Meeting could not judge which study was most representative of household processing. Therefore, maximum processing factors were used. Processing factors used for the calculation of maximum residue levels and STMR-Ps are underlined.

Commodity	Processing factor (range)	No. of trials	Processing factor (mean)	STMR-P (mg/kg)	HR-P (mg/kg)
Wheat bran	3.9–4.0	2	<u>3.95</u>	19.75	30.02
Wheat flour	0.21–0.26	2	<u>0.235</u>	1.175	1.786
White bread	0.089–0.11	2	<u>0.10</u>	0.5[1]	0.76
Wholemeal bread	0.33–0.43	2	<u>0.38</u>	1.9	2.888
Barley malt	0.16–0.24	2	<u>0.20</u>	1	1.52
Husked rice	0.031–<u>0.64</u>	22	0.17	3.2	4.864
Polished rice	< 0.002–<u>0.15</u>	26	0.039	0.75	1.14
Rice hulls	0.12–<u>10</u>	21	4.3	50	76
Rice bran	0.018–<u>7.2</u>	23	1.7	36	54.72
Cooked husked rice	<u>0.11</u>	1		0.55	0.836
Cooked polished rice	<u>0.04</u>	1		0.2	0.304
Washed polished rice	0.041–0.049	4	<u>0.046</u>	0.23	0.3496
Cooked, washed, polished rice	0.0060–0.033	13	<u>0.020</u>	0.1	0.152

[1] The Meeting noted that the 2003 Report incorrectly reported an STMR-P of 0.05 mg/kg in white bread.

Using the highest residue level for cereal grains (7.6 mg/kg) and the processing factors indicated above, the Meeting estimated maximum residue levels of 30 mg/kg in wheat bran and 60 mg/kg in rice bran. The Meeting decided to withdraw the current recommendations for 1 mg/kg in polished rice, 2 mg/kg in wheat flour, 1 mg/kg in white bread and 3 mg/kg in wholemeal bread (accommodating post-harvest treatment, PoP), because the MRL would be lower than that of the raw agricultural commodity.

Using the STMR for cereal grains (5 mg/kg), the Meeting estimated STMR-Ps for wheat bran, wheat flour, white bread, wholemeal bread, barley malt, husked rice, polished rice, rice hulls, rice bran, cooked husked rice, cooked polished rice, washed polished rice and cooked washed polished rice, as shown in the Tableabove.

Furthermore, using the highest residue level for cereal grains (7.6 mg/kg), the Meeting estimated HR-Ps for wheat bran, wheat flour, white bread, wholemeal bread, barley malt, husked rice, polished rice, rice hulls, rice bran, cooked husked rice, cooked polished rice, washed polished rice and cooked washed polished rice, as shown in the Tableabove.

The Meeting decided to use the STMR-P and HR-P values for cooked husked rice and cooked polished rice in calculating the dietary intake of fenitrothion from rice.

Residues in animal commodities

Dietary burden of farm animals

The Meeting estimated the dietary burden of fenitrothion residues in farm animals from the diets listed in Appendix IX of the *FAO Manual*. Only one feed commodity from each Codex Commodity Group is used; therefore, the calculation includes wheat grain, but no other cereals, and rice bran, but not rice hulls. Calculation from the highest residue values provides the concentrations in feed suitable for estimating MRLs for animal commodities, while calculation from the STMR values for feed is suitable for estimating STMR values for animal commodities. In the case of processed commodities, the STMR-P value is used for both intake calculations.

Estimated maximum dietary burden of farm animals

Commodity	Codex code	Residue (mg/kg)	Basis	Dry matter (%)	Residue, dry wt (mg/kg)	Dietary content (%)			Residue contribution of feeds (mg/kg)		
						Beef cattle	Dairy cattle	Poul-try	Beef cattle	Dairy cattle	Poul-try
Rice bran	CM	36	STMR-P	90	40						
Rice hulls	CM	50	STMR-P	90	55.6						
Wheat milled by-products[1]	CF	19.75	STMR-P	88	22.44	40	50	50	8.98	11.22	11.22
Wheat grain	GC	7.6	Highest residue	89	8.54						
Total						40	50	50	8.98	11.22	11.22

[1] Use of wheat bran

Estimated mean dietary burden of farm animals

Commodity	Codex code	Residue (mg/kg)	Basis	Dry matter (%)	Residue, dry wt (mg/kg)	Dietary content (%)			Residue contribution of feeds (mg/kg)		
						Beef cattle	Dairy cattle	Poul-try	Beef cattle	Dairy cattle	Poul-try
Rice bran	CM	36	STMR-P	90	40						
Rice hulls	CM	50	STMR-P	90	55.6						
Wheat milled by-products [1]	CF	19.75	STMR-P	88	22.44	40	50	50	8.98	11.22	11.22
Wheat grain	GC	5	STMR	89	5.62						
Total						40	50	50	8.98	11.22	11.22

[1] Use of wheat bran

Residues in grazing animals and feeding studies

The 2003 JMPR evaluated the fate of fenitrothion residues in cattle grazing on fenitrothion-treated grass and in cattle fed fenitrothion-treated corn. The Meeting received an additional study on cattle grazing on pasture treated with fenitrothion, a feeding study in lactating dairy cattle and a feeding study in laying hens.

Fenitrothion as technical material was applied by air to a 120-ha paddock by the ultra-low volume technique at the rate of 0.508 kg ai/ha. There were 28 *cattle* on the pastures when the application was made, and 38 were brought to the treated pastures immediately after spraying. Four cattle were maintained as controls, with no exposure to fenitrothion. The cattle were allowed to graze on the sprayed pasture for varying periods and were slaughtered within 1 day after removal from the paddock, 2, 4, 7, 14 or 21 days after treatment. Samples of subcutaneous and perirenal fat, meat (neck muscle) and liver were taken for analysis. Some cattle were removed from the treated pasture and were grazed on clean pasture for 2, 4 or 7 days before slaughter to determine the effect on the residue levels of this procedure. Soil and pasture were sampled on days –3, 0, 1, 2, 4, 7, 10, 14 and 21 and analysed for fenitrothion residues. The worst-case estimate of total exposure (pasture + soil + deposition) of the cattle on the day of spraying was 800–900 mg of fenitrothion per animal. As the total intake of dry matter from the pasture was assumed to be 10 kg/day, the exposure corresponds to a fenitrothion content of approximately 90 ppm.

Data on residues found in the pasture indicate a half-life for fenitrothion of 1–2 days, which decreased with time; the decay during the first period appeared to be faster than that during later periods on a per hour basis. About 50% of the fenitrothion was gone within 1 day, 75% within 3 days and 90% within 6 days. Data on residues in the soil indicated a half-life for fenitrothion of 2–3 days. The residue levels in all animal commodities were below the LOQ (< 0.2 mg/kg for fat, < 0.02 mg/kg for muscle and liver).

Groups of three *lactating cows* received feed containing fenitrothion at a concentration of 0, 10, 30 or 100 ppm for 28 days. All animals were milked twice daily, and composite milk samples from the morning and afternoon milkings on days –1, 0, 3, 7, 14, 21 and 28 were analysed for residues. Cream was analysed separately; the cream content of the milk was 5–8% and the butterfat content was 3–5%. The levels of residues of fenitrothion in milk were below the LOQ of 0.01 mg/kg for all groups. Residues were measured in cream at some times (scattered among groups), but the levels were never higher than 0.01 mg/kg. Samples of liver, kidney, muscle (cardiac, hind-quarter and front-quarter) and fat (omental and perirenal) were taken. Residue levels above the LOQ of 0.05 mg/kg were not found in any sample at any feed level.

Layer and broiler *hens* received feed containing fenitrothion at 0, 10, 30 or 100 ppm for 28 days. Egg samples were collected twice a week from each group and frozen until ready for analysis. Eggs from birds in the same dose group were combined. Half of the hens at each dietary concentration were killed on day 14, and the remaining hens were killed on day 28 of the study. Samples of red muscle, white muscle, liver and fat were taken at both times. The residue levels in all tissue samples taken on days 14 and 28 were below the limit of determination of 0.05 mg/kg. No residues of fenitrothion were found in eggs taken over the 28-day period.

Maximum residue levels

The calculated dietary burden of dairy cattle is 11.22 mg/kg, and that of beef cattle is 8.98 mg/kg. In the feeding study with cattle described above, no residues were found at levels above the LOQ (0.05 mg/kg) in muscle, fat, liver or kidney at dietary concentrations of 10, 30 and 100 mg/kg. Therefore, no residues at levels above the LOQ are to be expected at the calculated dietary burden. The levels of residues of fenitrothion in milk were below the LOQ of 0.01 mg/kg.

The calculated dietary burden of poultry is 11.22 mg/kg. In the feeding study in poultry, no residues were detected in muscle, liver, fat or eggs (< 0.05 mg/kg) at dietary concentrations of 10, 30 and 100 mg/kg.

The Meeting recommended maximum residue levels of 0.05* mg/kg in meat (from mammals other than marine mammals), in edible offal (mammalian), in poultry meat and in eggs. The Meeting also recommended a maximum residue level of 0.01 mg/kg in milks. The STMR and the highest residue values for muscle, fat, liver, kidney, poultry meat and fat are estimated to be 0 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 2004 CCPR meeting agreed to retain the existing Codex MRLs for meat (0.05* mg/kg (fat)), milks (0.002* mg/kg), unprocessed rice bran (20 mg/kg), polished rice (1 mg/kg), processed wheat bran (2 mg/kg), unprocessed wheat bran (20 mg/kg), wheat flour (2 mg/kg) and wheat wholemeal (5 mg/kg), awaiting the evaluation of new data.

Definition of the residue for compliance with MRL and for estimation of dietary intake:
Fenitrothion. The definition applies to plant and animal commodities.

CCN	Commodity	MRL (mg/kg)		STMR, STMR-P (mg/kg)	HR, HR-P (mg/kg)
		New	Previous		
FP 0226	Apple	0.5	W	0.04	0.41
GC 0080	Cereal grains	10 (Po)[1]	10 (Po)	5	7.6
MO 0105	Edible offal (mammalian)	0.05*	-	Liver 0 Kidney 0	Liver 0 Kidney 0
PE 0112	Eggs	0.05*	-	0	0
MM 0095	Meat (from mammals other than marine mammals)	0.05*	W	Muscle 0 Fat 0	Muscle 0 Fat 0
ML 0106	Milks	0.01	W	0	
FP 0230	Pear	W	W		
PM 0110	Poultry meat	0.05*	-	Muscle 0 Fat 0	Muscle 0 Fat 0
CM 1206	Rice bran, unprocessed	60	W	36	55
CM 0649	Rice, husked			3.2	4.9
CM 1205	Rice, polished	W	W	0.44	1.1
	Cooked husked rice			0.55	0.84
	Cooked polished rice			0.2	0.30
	Washed polished rice			0.23	0.35
	Cooked washed polished rice			0.1	0.15
CM 0654	Wheat bran (unprocessed)	30 PoP#	20 PoP	20	30
CF 1211	Wheat flour	W	W	1.2	1.8
CP 1211	White bread	W	W	0.5	0.76
CP 1212	Wholemeal bread	W		1.9	2.9

[1] proposal by 2003 JMPR, the recommendation is now considered to also cover pre-harvest use of fenitrothion

FURTHER WORK OR INFORMATION

None.

DIETARY RISK ASSESSMENT

Long-term intake

The IEDIs of fenitrothion, on the basis of the STMRs estimated for 12 commodities, for the five GEMS/Food regional diets represented 110–330% of the maximum ADI (0–0.005 mg/kg bw), see Annex 3. The information provided to the JMPR precludes an estimate that the dietary intake would be below the ADI.

The Meeting noted that the calculations of long-term intake were conservative, as they did not take into account the reduction in residue levels obtained by processing cereal grains, except for processing of wheat, barley and rice. The Meeting extrapolated processing data on wheat to rye.

Information on processing of barley (uses besides beer), maize, millet and sorghum would be particularly useful for refining the intake calculations.

Short-term intake

The IESTIs for fenitrothion were calculated for 25 food commodities for which maximum residue levels had been estimated and for which consumption data were available. The results are shown in Annex 4 of the Report.

The IESTI represented 0–100% of the ARfD (0.04 mg/kg bw) for the general population and 0–160% of the ARfD for children. The values 120%, 150% and 160% represent the estimated short-term intake of wholemeal bread, wheat bran (unprocessed) and maize (fresh, flour), respectively, by children. The Meeting concluded that the short-term intake of residues of fenitrothion from uses other than on these three commodities that have been considered by the JMPR is unlikely to present a public health concern.

The Meeting noted that the intake calculations were conservative, as they did not take into account the reduction in residue levels obtained by processing cereal grains, except for processing of wheat, barley and rice. Information on processing of maize would be particularly useful for refining the intake calculations. Nevertheless, the exceedences found for children in the consumption of wheat bran and wholemeal bread cannot be further refined.

The Meeting noted that the ADI and the ARfD for fenitrothion were established by the 2000 JMPR. At that time, the concepts of overall NOAEL and of compound-specific adjustment factors had not been fully developed. In addition, the process of establishing ARfDs was at an early stage of development. In view of these considerations, the Meeting concluded that a review of the toxicological database of fenitrothion, taking into account the new concepts, could lead to a refinement of the ADI and the ARfD.

REFERENCES

Desmarchelier JM. 1980a. Comparative study of analytical methods for bioresmethrin, fenothrin, d-fenothrin, pyrethrum I, carbaryl, fenitrothion, methacrifos, pirimiphos-methyl and dichlorvos in various grains. J. Pesticide Sci.;5:521-532. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Published.

Desmarchelier JM, Goldring M and Horgan R. 1980b. Predicted and observed residues of bioresmethrin, carbaryl, fenitrothion, d-fenothrin, methacrifos and pirimiphos-methyl on rice and barley after storage, and losses of these insecticides during processing. J. Pesticide Sci.;5:539-545. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Published.

Dubs A, Dove LG and Symmons PM. 1985. A method of investigating the effect of droplet size on pesticide collection by vegetation. Pestic. Sci.;15:217-226. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Published.

French IW. 1972. Sumithion residue study – apples. Ontario, Canada: Nucro Technics Ltd. Report no. HR-21-0004. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Unpublished.

Gilmour AR, McDougall KW and Spurgin P. 1999. The uptake and depletion of fenitrothion in cattle, pasture and soil following spraying of pastures for locust control. Australian Journal of Experimental Agriculture; 39:915-922. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Published.

Goto M. 1981. Pesticide residue analytical result report. Japan: The institute of environmental toxicology, report 56P-1. Provided by Sumitomo Chemical Co, Japan (Document no JB-007). Non-GLP. Unpublished.

Goto M. 1985. Agricultural chemical residue analytical result. Japan: Agricultural Chemical Residual Laboratory, report JB-014. Provided by Sumitomo Chemical Co, Japan (Document no JB-014). Non-GLP. Unpublished.

Goto M, Matano O and Kobayashi Y. 1991a. Agricultural chemical residue analytical result. Japan: Residual Agricultural Chemical Research Center, report JB-002. Provided by Sumitomo Chemical Co, Japan (Document no JB-002). Non-GLP. Unpublished.

Goto M, Matano O and Kobayashi Y. 1991b. Agricultural chemical residue analytical result. Japan: Residual Agricultural Chemical Research Center, report

JB-003. Provided by Sumitomo Chemical Co, Japan (Document no JB-003). Non-GLP. Unpublished.

Heath AB and Black RR. 1987. Assisted distillation cleanup of pesticide residues in animal fats: new beadless distillation tube. J. Assoc. Off. Anal. Chem.;70(5):862-865. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Published.

Herczog KJS. 2004. Metabolism of [14C]fenitrothion in rice. Concord OH, USA: Ricerca Biosciences, report number 015491-1. Provided by Sumitomo Chemical Co, Japan (Document no: HM-0201). GLP. Unpublished.

Hill D. 2000. Rice bran data for fenitrothion. Leeton, NSW, Australia: Ricegrowers Co-operative Ltd, Letter to J. Taylor of the National Registration Authority, Kingston, Australia, no reference number, dated 15 December 2000. Provided by Sumitomo Chemical Co, Japan (No Document number). Non-GLP. Unpublished.

Hirota M. 1994. Pesticide residue analysis report. Japan: Sumika Chemical Analysis Service, report JA-005. Provided by Sumitomo Chemical Co, Japan (Document no JA-005). Non-GLP. Unpublished.

Hirota M and Kikuchi T. 1994. Report on the analytical results of residual pesticides. Japan: Sumika Chemical Analysis Service, report JA-004. Provided by Sumitomo Chemical Co, Japan (Document no JA-004). Non-GLP. Unpublished.

Hirota M and Nakanishi T. 1995. Agricultural chemical residue special investigation analytical result. Japan: Sumika Chemical Analysis Service, report JB-019. Provided by Sumitomo Chemical Co, Japan (Document no JB-019). Non-GLP. Unpublished.

Hirota M and Umemoto T. 1993. Pesticide residue analysis report. Japan: Sumika Chemical Analysis Service, report JA-007. Provided by Sumitomo Chemical Co, Japan (Document no JA-007). Non-GLP. Unpublished.

Ishimaru J. 1996. Agricultural chemical residue special investigation analytical result. Japan: Ehime Agricultural Experiment Station, report JB-018. Provided by Sumitomo Chemical Co, Japan (Document no JB-018). Non-GLP. Unpublished.

Ito T, Kageyama Y and Hirose C. 1976. Fate of fenitrothion residues in rice grains. Hyogo, Japan: Sumitomo Chemical Co. Ltd, report no HR-60-0109. Provided by Sumitomo Chemical Co, Japan (Document no. HR-60-0109). Non-GLP. Unpublished.

Katague DB. 1978a. Determination of residues of sumithion, sumioxon, aminosumithion and 3-methyl-4-nitrocresol in milk, cream and cattle tissues. Richmond, CA, USA: Richmond Research Center, method no RRC 78-32, version 9/14/78. Provided by Sumitomo Chemical Co, Japan (Document no HA-81-0215). Non-GLP. Unpublished.

Katague DB. 1978b. Determination of residues of sumithion, sumioxon, aminosumithion and 3-methyl-4-nitrocresol in chicken eggs, liver, fat and muscle tissues. Richmond, CA, USA: Richmond Research Center, method no RRC 78-32A, version 11/22/78. Provided by Sumitomo Chemical Co, Japan (Document no HA-81-0216). Non-GLP. Unpublished.

Kato S. 1982. Agricultural chemical residue analysis report. Japan: Food Reseach Laboratories, report JB-010. Provided by Sumitomo Chemical Co, Japan (Document no JB-010). Non-GLP. Unpublished.

Komatsu K and Yabusaki T. 1991. Agricultural chemical residue analysis report. Japan: Food Research Laboratories, report JB-016. Provided by Sumitomo Chemical Co, Japan (Document no JB-016). Non-GLP. Unpublished.

Kuroda M and Araya K. 1981. Pesticide residue analysis report. Japan: Chemical Analysis Consultants, Inc., report JB-009. Provided by Sumitomo Chemical Co., Japan (Document no. JB-009). Non-GLP. Unpublished.

Kuroda M and Higuchi T. 1989. Pesticide residue analysis report. Japan: Chemical Analysis Consultant Co Ltd, report JA-010. Provided by Sumitomo Chemical Co, Japan (Document no JA-010). Non-GLP. Unpublished.

Kuroda M and Higuchi T. 1991a. Pesticide residue analysis report. Japan: Chemical Analysis Consultant Co Ltd, report JA-009. Provided by Sumitomo Chemical Co, Japan (Document no JA-009). Non-GLP. Unpublished.

Kuroda M and Higuchi T. 1991b. Agricultural chemical residue special investigation analytical result. Japan: Analytical Chemistry Consultant Co Ltd, report JB-004. Provided by Sumitomo Chemical Co, Japan (Document no JB-004). Non-GLP. Unpublished.

Kuroda M and Higuchi T. 1991c. Agricultural chemical residue special investigation analytical result. Japan: Analytical Chemistry Consultant Co Ltd, report JB-005. Provided by Sumitomo Chemical Co, Japan (Document no JB-005). Non-GLP. Unpublished.

Kuroda M and Higuchi T. 1991d. Agricultural chemical residue special investigation analytical result. Japan: Chemical Analysis Consultant Co Ltd, report JB-013. Provided by Sumitomo Chemical Co, Japan (Document no JB-013). Non-GLP. Unpublished.

Kuroda M and Higuchi T. 1991e. Agricultural chemical residue special investigation analytical result. Japan: Chemical Analysis Consultant Co Ltd, report JB-017. Provided by Sumitomo Chemical Co, Japan (Document no JB-017). Non-GLP. Unpublished.

Matano O and Kobayashi Y. 1991a. Report on the analytical results of residual pesticides. Japan: The Institute of Environmental Toxicology, report JA-008. Provided by Sumitomo Chemical Co, Japan (Document no JA-008). Non-GLP. Unpublished.

Matano O and Kobayashi Y. 1991b. Agricultural chemical residue analytical result. Japan: Residual Agricultural Chemical Research Center, report JB-012. Provided by Sumitomo Chemical Co, Japan (Document no JB-012). Non-GLP. Unpublished.

Matano O and Kobayashi Y. 1992. Report on the analytical results of residual pesticides. Japan: The Institute of Environmental Toxicology, report JA-006. Provided by Sumitomo Chemical Co, Japan (Document no JA-006). Non-GLP. Unpublished.

McDougall K. 1998. Animal Industries Report 6. Fenitrothion trial, Coonamble, NSW 1998. Lismore, NSW, Australia: NSW Agriculture and Australian Plague Locust commission, ISSN 1038-7633. Provided by Sumitomo Chemical Co, Japan (No document no). Non-GLP. Published.

Miyamoto J and Sato Y. 1965. Determination of insecticide residue in animal and plant tissue. II. Metabolic fate of Sumithion in rice plant applied at the preheading stage and its residue in harvested grains. Botyu-Kagaku; 30: 45-49. Provided by Sumitomo Chemical Co, Japan (No document number). Non-GLP. Published.

Miyamoto J, Sato Y, Kadota T, Fujinami A and Endo M. 1963. Studies on the mode of action of organophosphorus compounds. Part I. Metabolic fate of P^{32} labeled Sumithion and methyl parathion in guinea pig and white rat. Agr. Biol. Chem.; 27(5):381-389. Provided by Sumitomo Chemical Co, Japan (No document number). Non-GLP. Published.

Miyamoto J, Kawaguchi Y and Sato Y. 1965. Determination of insecticide residue in animal and plant tissue. I. Determination of Sumithion residue in bananas grown in Formosa. Botyu-Kagaku; 30:9-12. Provided by Sumitomo Chemical Co, Japan (No document number). Non-GLP. Published.

Nakajima T and Saito N. 1985. Agricultural chemical residue analysis report. Japan: Sumika Chemical Analysis Service, report JB-015. Provided by Sumitomo Chemical Co, Japan (Document no JB-015). Non-GLP. Unpublished.

Sato Y, Miyamoto J and Suzuki S. 1968. Determination of insecticide residue in animal and plant tissues. V. A device of preparing the specific detector of gas chromatograph highly sensitive to organophosphorus insecticides. Botyu-Kagaku; 33:8-12. Provided by Sumitomo Chemical Co, Japan (No document number). Non-GLP. Published.

Shimada M. 1981. Agricultural chemical residue analysis report. Japan: Sumika Chemical Analysis Service, report JB-011. Provided by Sumitomo Chemical Co, Japan (Document no JB-011). Non-GLP. Unpublished.

Suzuki R. 1995. Report on the analytical results of residual pesticides. Japan: Hodogaya Contract Laboratory Co Ltd, report JA-003. Provided by Sumitomo Chemical Co, Japan (Document no JA-003). Non-GLP. Unpublished.

Suzuki R. 1996a. Report on the analytical results of residual pesticides. Japan: Hodogaya Contract Laboratory Co Ltd, report JA-001. Provided by Sumitomo Chemical Co, Japan (Document no JA-001). Non-GLP. Unpublished.

Suzuki R. 1996b. Report on the analytical results of residual pesticides. Japan: Hodogaya Contract Laboratory Co Ltd, report JA-002. Provided by Sumitomo Chemical Co, Japan (Document no JA-002). Non-GLP. Unpublished.

Takimoto Y. 1972. Agricultural chemical residue analysis report. Japan: Research Department, Agricultural Chemical Division, Sumitomo Chemical, report JB-006. Provided by Sumitomo Chemical Co, Japan (Document no JB-006). Non-GLP. Unpublished.

Taylor R.E. 1980a. Sumithion meat and milk residue study in lactating dairy cattle. Nebraska, USA: Harris Laboratories, Inc. T-10416. Provided by Sumitomo Chemical Co, Japan (Document no HR-01-0083). GLP. Unpublished

Taylor R.E. 1980b. Sumithion poultry residue studies. Nebraska, USA: Harris Laboratories, Inc. T-1149 – Broilers, T-1150 – Layers. Provided by Sumitomo Chemical Co, Japan (Document no HR-01-0082). GLP. Unpublished.

Van Doornik A. 1974. Sumithion residue study – apples, pears and pPeaches. Ontario, Canada: Nucro Technics Ltd, report no HR-41-0006. Provided by Sumitomo Chemical Co, Japan (Document no HR-41-0006). Non-GLP. Unpublished.

Yamaya S. 1982. Pesticide residue analysis report. Japan: Akita Agricultural Experimental Station, report 56P-8. Provided by Sumitomo Chemical Co, Japan (Document no JB-008). Non-GLP. Unpublished.

CROSS-REFERENCES

JB-019. (Hirota and Nakanishi, 1995).
JB-018. (Ishimaru, 1996).
JB-017. (Kuroda and Higuchi, 1991e).
JB-016. (Komatsu and Yabusaki, 1991).
JB-015. (Nakajima and Saito, 1985).
JB-014. (Goto, 1985).
JB-013. (Kuroda and Higuchi, 1991d).
JB-012. (Matano and Kobayashi, 1991b).
JB-011: (Shimada, 1981)
JB-010: (Kato, 1982)
JB-009: (Kuroda and Araya, 1981).
JB-008: (Yamaya, 1982).
JB-006. (Takimoto, 1972).
JB-005. (Kuroda and Higuchi, 1991c).
JB-004. (Kuroda and Higuchi, 1991b).
JB-003. (Goto *et al.*, 1991b).
JB-002. (Goto *et al.*, 1991a).
JA-010. (Kuroda and Higuchi, 1989).
JA-009. (Kuroda and Higuchi, 1991a).
JA-008. (Matano and Kobayashi, 1991a).
JA-007. (Hirota and Umemoto, 1993).
JA-006. (Matano and Kobayashi, 1992;).

JA-005. (Hirota, 1994).
JA-004. (Hirota and Kikuchi, 1994).
JA-003. (Suzuki, 1995).
JA-002. (Suzuki, 1996b).
JA-001. (Suzuki, 1996a).
HR-21-0004. (French, 1972).
HR-41-0006. (Van Doornik, 1974).

FLUDIOXONIL (211)

First draft prepared by Stephen Funk, Health Effects Division, US Environmental Protection Agency, Washington, DC, USA

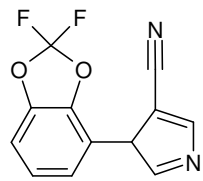
EXPLANATION

At the 33rd Session of the CCPR (ALINORM 01/24A), fludioxinil was identified as a priority for evaluation as a new pesticide by the 2004 JMPR. The manufacturer submitted information on physical and chemical properties, metabolism (plant and animal), environmental fate, analytical methods, freezer storage stability, good agricultural practices (GAP), supervised field trials, processing, and livestock feeding. Information on GAP was reported by the government of Australia, and on analytical methods by the government of the USA.

IDENTITY

ISO common name: fludioxonil
Chemical name:
IUPAC: 4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)pyrrole-3-carbonitrile
CA: 4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1*H*-pyrrole-3-carbonitrile
CAS no.: 131341-86-1
Syngenta code no.: CGA 173506

Structural formula:

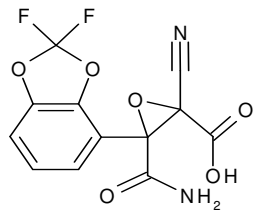
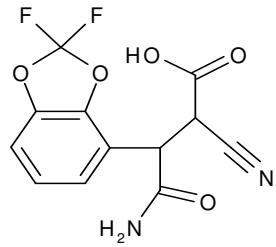
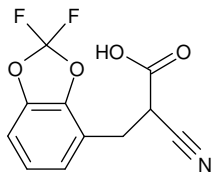
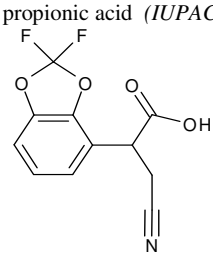


Molecular formula: $C_{12}H_6F_2N_2O_2$
Molecular mass: 248.2

PHYSICAL AND CHEMICAL PROPERTIES

Property	Results	Method Test material[1]	Reference
Melting point	199.8°C	EEC A.1 99.8% (PAI/1)	Rodler, 1992 Report EA169432
Boiling point	Thermal decomposition starts at about 306°C	EEC A.2 99.8% (PAI/1)	Das, 2000 Report 80806
Temperature of decomposition or sublimation	Thermal decomposition starts at about 306°C	EEC A.2 99.8% (PAI/1)	Das, 2000 Report 80806
	No thermal effect found between room temperature and 150°C	OECD 113 96.8%(TGAI)	Schürch, 1992 Report EA175120
Relative density	1.54-10^3 kg/m^3 at 20°C corresponding to a relative density of 1.54	OECD 109 99.8% (PAI/1)	Füldner, 1992 Report PP-92/11P.DES
Vapour pressure	Vapour pressure curve in the liquid state: ^{10}log P [Pa] = 16.8495-6936.15 · 1/T [K] from fit of measurements between 60.3°C and 186.5°C Vapour pressure at 25°C : 3.9 · 10^{-7} Pa (extrapolated)	EEC A.4 99.8% (PAI/1)	Nickler, 1992 Report PP92/11P.VPC
Volatility	Henry's law constant at 25°C 5.4 · 10^{-5} Pa · m³/mol	calculation	Burkhard, 1994 Archive CGA173506/0489

Property	Results	Method Test material1	Reference
Physical state and colour	Pure active substance : faintly yellow fine powder	visual 99.9% (PAI/2)	Das, 1998 Report EZA62940
	Technical grade active substance . light olive green powder	96.8%(TGAI)	Rodler, 1992 Report EA175120
Odour	Pure active substance : odourless	organoleptic 99.9% (PAI/2)	Das, 1998 Report EZA62940
	Technical grade active substance : odourless	96.8%(TGAI)	Rodler, 1992 Report EA175120
Spectra active substance	IR KBr pellet 3289, 2223, 1652, 1530, 1236 cm^{-1} ^{1}H-NMR (300 MHz, DMSO) chemical shift (ppm): 7.2-7.4 (3 protons), 7.5-7.6 (1 proton), 7.8 (1 proton), 12.2 (1 proton) ^{13}C-NMR (75 MHz, DMSO) chemical shift (ppm): 143, 139 (C1, C2), 135, 131, 127 (C12), 129, 120 (C8, C11), 125, 122, 108 (C4, C5, C6), 117 (C3, C10, C7), 90 (C9) mass spectra (quad EI): 248 (M+), 182, 154, 127 UV (methanol) Absorption Characteristics : Molar extinction coefficients (ε, l/mol.cm) were determined to be: solution wavelength (nm) ε (l/mol.cm) neutral 266 12384 acidic 265 12327 basic 271 11790 12384 (neutral solution), No absorption maximum between 340 nm and 750 nm was observed.	99.9% (PAI/2) UV/VIS OECD 101	Stulz, 1998 Report EZA63115 Stulz, 1998 Report EZA64462
Solubility in water including effect of pH	The solubility in pure water at 25°C was determined to be: 1.8 mg/l Fludioxonil has no dissociation within the range pH 2 to pH 12. pH has no effect on the water solubility of the compound in the pH range 4 to 10	OECD 105 99.8% (PAI/1)	Rodler, 1992 Report EA169432
Solubility in organic solvents	The solubility in different organic solvents at 25°C was determined to be : acetone 190 g/l dichloromethane 7.3 g/l ethyl acetate 86 g/l hexane 10 mg/l methanol 42 g/l octanol 20 g/l toluene 2.7 g/l	CIPAC MT 157.3 96.8% (TGAI)	Kettner, 2000 Archive CGA173506/5141
Partition coefficient n-octanol/water	The octanol/water partition coefficient (P_{ow}) and its logarithm to base 10 (log P_{ow}) was determined to be: P_{ow} = 13100 ± 472 at 25°C log P_{ow} = 4.12 ± 0.016	OECD 107 corresponding to EEC A.8 99.8% (PAI/1)	Rodler, 1992 Report EA169432
Hydrolysis rate	No degradation observed at pH 5, 7 and 9 respectively, during 32 days at 25°C	EPA 161-1 corresponding to EEC C.7 98% (PAI/3)	Kirkpatrick, 1991 Report HRC/CBG 487/891775

Property	Results	Method Test material1	Reference
Photochemical degradation	The experimental photolytic half-lives of fludioxonil at 25°C in sterile aqueous solutions buffered to pH 7 using xenon arc light were found to be : 8.7 summer sunlight days at 40°N ([phenyl-U-^{14}C]) 9.9 summer sunlight days at 30°N ([pyrrole-4-^{14}C]) Dark control experiments revealed negligible degradation of fludioxonil. Approximately 95.9% to 98.3% of the applied radioactivity was recovered. Up to 20% volatiles (CO_2, which indicates breakdown of the phenyl ring); three photoproducts >10% were found: CGA 339833  3-carbamoyl-2-cyano-3-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-oxirane-2-carboxylic acid *(IUPAC)* CGA 344623  2-cyano-3-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-succinamic acid *(IUPAC)* The third photoproduct >10% could be assigned either of the two isomeric structures:  2-cyano-3-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-propionic acid *(IUPAC)*  3-cyano-2-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-propionic acid *(IUPAC)*	EPA 161-2 is in accordance with European Community Commission Directive 95/36/EC of July 14, 1995 amending Council Directive 91/414/EEC 98% (PAI/3) 98% (PAI/4)	Kirkpatrick, 1994[a] Report CBG569A Kirkpatrick, 1994, Report CBG 49064 Kirkpatrick, 1994, Report HRC/CBG 516/901362 Kirkpatrick, 1996 Report CBG 720

Property	Results	Method Test material1	Reference
Quantum yield	The quantum yield of direct photolysis was found to be Φ = 0.026 Photolytic half-life in shallow waters was estimated for midspring time and mid summer time at the geographical latitudes of 40°N and 50°N. Under clear sky conditions half-lives between 160 days (at 40°N and midsummer time) and 1237 days (at 50°N and midspring time) are expected for fludioxonil.	ECETOC Guideline of the German Federal Environment Agency: "Phototransform-ation of Chemicals in Water; Part A : Direct Phototransfor-mation", Berlin, FRG, January 1990" 99.9% (PAI/2)	Abildt, 1994 Report 93UA02
Dissociation constant	The estimated dissociation constants of fludioxonil in water were found to be: pK_{a1} = <0 (basic) pK_{a2} = ~14.1 (acidic)	by estimation 99.8% (PAI/1)	Jäkel, 1992 Report FF92/11P.DCW
Stability in the air, photo-chemical degradation, identity of breakdown product(s)	Based on the calculation according to Atkinson the estimated half-life of fludioxonil in the atmosphere by hydroxyl radical oxidation is approximately 2-4 hours ($1.5 \cdot 10^6$ OH-radicals/cm³ and a 12 hour day)	calculation according to Atkinson, R., Environ. Toxicol. Chem., 7, 435 (1988)	Stamm, 1999 Report 95A99001SM
Flammability	Fludioxonil is not considered highly flammable	EEC A.10 96.8% (TGAI)	Schürch, 1992 Report EA 175120
Auto-flammability	No self-ignition	EEC A.16 96.8% (TGAI)	Schürch, 1992 Report PF 92/21T.AFS
Flash point	Not required, fludioxonil is a solid with a melting point >40°C		None
Explosive properties	Fludioxonil is not considered an explosive.	EEC A.14 96.8% (TGAI)	Schürch, 1992 Report PP 94/66 C.FLS
Surface tension	Surface tension of aqueous suspensions at 20°C by the Wilhelmy plate method was determined to be : σ = 47.7-48.5 mN/m (filtrates of 10 g/l suspension)	OECD 115 ≅ EEC A.5 96.8% (TGAI)	Ryser, 1992 Report PP 92/23T.SUR
Oxidizing properties	Fludioxonil is not considered an oxidizing substance	EEC A.17 96.8% (TGAI)	Schürch, 1992 Report PP92/23T.OXP

[1]PAI/1 . pure active substance, CGA 173506 pure; AMS 273/103, purity 99.8%; Details of purification method see Stulz 1994

PAI/2 . pure active substance, CGA 173506 pure;AMS 273/104, purity 99.9%

PAI/3 . [pyrrole-4-^{14}C] CGA 173506; radiochemical purity 98%;Batch K-736.3A

PAI/4 . [phenyl-U-^{14}C] CGA 173506;radiochemical purity 98%; Batch CFQ 7117

TGAI . technical grade active substance; Batch P.206006, purity 96.8%

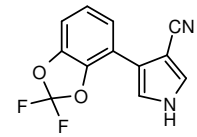
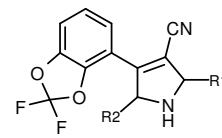
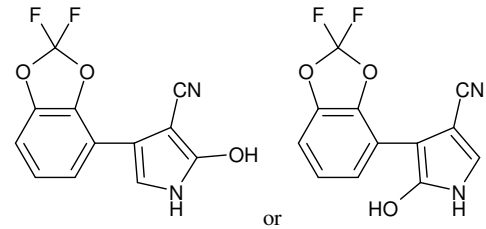
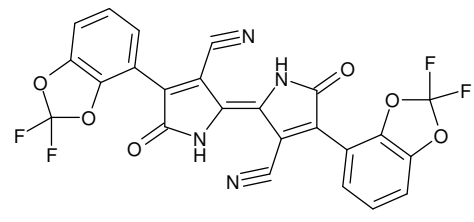
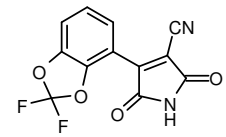
Formulations

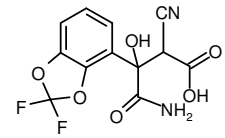
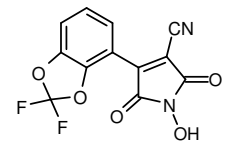
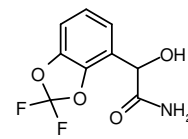
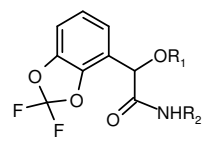
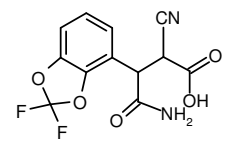
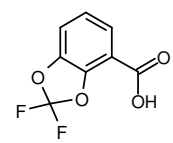
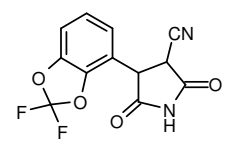
The formulations include DS (powder for dry seed treatment), ES (emulsion for seed treatment), FS (flowable concentrate for seed treatment), SE (suspo-emulsion), WG (water dispersible granules), and WP (wettable powder).

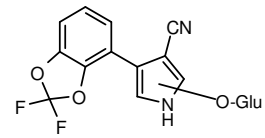
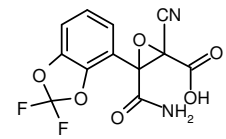
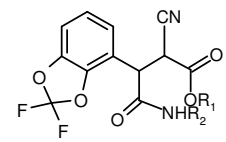
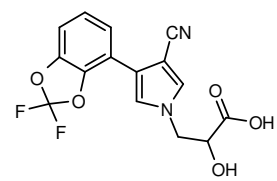
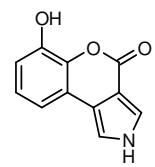
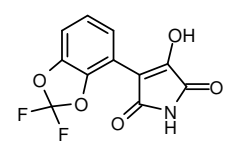
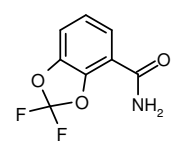
METABOLISM AND ENVIRONMENTAL FATE

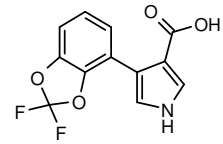
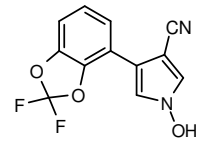
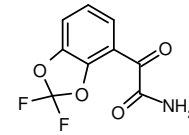
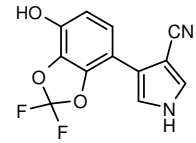
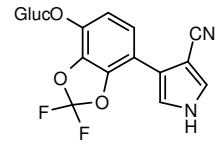
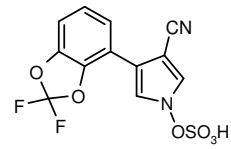
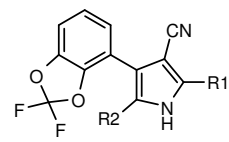
The following table links manufacturer code number, metabolite code, compound name,, and compound structure and applies to the various metabolism and rotational crop studies.

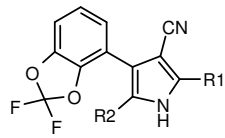
Table 1. Fludioxonil and metabolites: compound code, name, and structure

Code Number	Description / Chemical name	Plant/animal and metabolite code	Structure
Fludioxonil CGA 173506	Parent compound 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile	Grape (II_4), tomato (III_4), peach (P19), green onion (A: peak 11), lettuce (I_{15}), potato (I_{12}/II_4), wheat (II_4), goat (C), hen (E1)	
SYN 518579	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-5-hydroxy-2-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile or its tautomeric form 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-5-oxo-2,5-dihydro-1H-pyrrole-3-carbonitrile	Grape (II_2), tomato (III_2), peach (P15 and P16), green onion (P15), wheat (II_2),	 R1 = -OH, R2 = :O or R1 = :O, R2 = -OH
SYN 518577 or SYN 518578	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile or 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-5-hydroxy-1H-pyrrole-3-carbonitrile	Goat, Hen Rat	
SYN 518582	4,4'-bis-(2,2-difluorobenzo[1,3]dioxol-4-yl)-5,5'-dioxo-1,5,1',5'-tetrahydro-[2,2']bipyrrolylidene-3,3'-dicarbonitrile	Rat	
CGA 265378	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrole-3-carbonitrile	Grape (II_{3b}), green onion (B: peak 10), lettuce (I_{14}), wheat (II_{3b}), rotational wheat, radish and mustard, hen (E2)	

Code Number	Description / Chemical name	Plant/animal and metabolite code	Structure
SYN 518581	2-cyano-3-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-3-hydroxysuccinamic acid	Grape (I_{3b}), tomato (I_{3b})	
SYN 518580	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1-hydroxy-2,5-dioxo-2,5-dihydro-1H-pyrrole-3-carbonitrile	Grape (II_{3a}), tomato (III_3), wheat (II_3)	
CGA 308103	2-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxyacetamide	Grape (I_{11}), peach (P14), green onion (E peak 4), lettuce (I_{11}), wheat (I_{13}), rotational wheat radish and mustard,	
	Glucose conjugate of CGA 308103	Grape (I_6), tomato (I_6), lettuce (I_5)	 R_1 = Glu, R_2 = -H or R_1 = -H, R_2 = Glu
CGA 344623	2-cyano-3-(2,2-difluorobenzo[1,3]dioxol-4-yl)succinamic acid	Tomato (I_4), peach (P5), green onion (H: peaks 2/3), lettuce (I_4), wheat (I_4), hen (P2/P3)	
CGA 192155	2,2-difluorobenzo[1,3]dioxole-4-carboxylic acid	Potato (I_{10}), tomato (I_5), peach (P17), green onion (F: peak 1), lettuce (I_{10}), wheat (I_{10}), soybean, rotational wheat, radish and mustard, hen (P1)	
CGA 308565	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2,5-dioxo-pyrrolidine-3-carbonitrile	Peach (P18), rotational wheat, radish and mustard,	

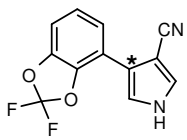
Code Number	Description / Chemical name	Plant/animal and metabolite code	Structure
	Glucose conjugate of oxidised CGA 173506 (pyrrole ring)	Peach (P10a, P10b P8b)	
	Sugar conjugate of oxidised CGA 173506 (pyrrole ring)	Peach (P8)	
CGA 339833	3-carbamoyl-2-cyano-3-(2,2-difluorobenzo[1,3]dioxol-4-yl)-oxirane-2-carboxylic acid	Peach (P6), green onion (I: peak 5), lettuce (I_{3b}), wheat (I_3), rotational wheat, radish and mustard,	
	N- or O-glucose conjugate of CGA 344623	Lettuce (I_2)	 R_1 = Glu, R_2 = H or R_1 = H, R_2 = Glu
	N-lactic acid conjugate of fludioxonil	Lettuce(I_{4b}+I_{4c})	
CGA-227731	6-hydroxy-2H-chromeno[3,4-c]pyrrol-4-one	Soybean, rotational spring wheat	
CGA-260766	3-(2,2-difluorobenzo[1,3]dioxol-4-yl)-4-hydroxy-pyrrole-2,5-dione	Soybean, rotational spring wheat	
CGA-340351	2,2-difluorobenzo[1,3]dioxole-4-carboxylic acid amide	Soybean, rotational spring wheat	

Code Number	Description / Chemical name	Plant/animal and metabolite code	Structure
CGA-257777	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carboxylic acid	Rotational spring wheat	
CGA-335892	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1-hydroxy-1H-pyrrole-3-carbonitrile	Hen (G)	
CGA 344624	2-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-oxo-acetamide	Hen (P7)	
SYN 518576	4-(2,2-difluoro-7-hydroxybenzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile	Hen (F)	
	Glucuronide conjugate of 4-(2',2'-difluoro-7'-hydroxy-1',3'-benzodioxol-4'-yl)-1H-pyrrole-3-carbonitrile	Goat (B2)	
	Sulfate conjugate of CGA-335892, 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1-hydroxy-1H-pyrrole-3-carbonitrile	Hen (I1)	
	Glucuronide conjugate of SYN 518577, 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile or SYN 518578, 4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5-hydroxy-1H-pyrrole-3-carbonitrile	Goat (B1, A) Hen (D)	 R1 = -OGluc, R2 = -H (B1) or R1 = -H, R2 = -OGluc (A)

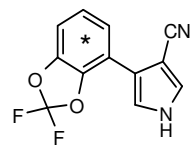
Code Number	Description / Chemical name	Plant/animal and metabolite code	Structure
	Sulfate conjugate of SYN 518577,4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile or SYN 518578, 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-5-hydroxy-1H-pyrrole-3-carbonitrile	Goat (G), Hen (I2)	 R1 = -OSO_3H, R2 = -H or R1 = -H, R2 = -OSO_3H

In studies of metabolism, fludioxonil was labelled with ^{14}C at one of two sites.

[Pyrrole-4-^{14}C]fludioxonil:



[Phenyl-U-^{14}C]fludioxonil:



*=Position of carbon-14 label

Animal metabolism

The Meeting received information on the fate of fludioxonil administered orally to lactating goats and laying hens.

Goats. ^{14}C-pyrrole-labelled fludioxonil was administered orally in gelatin capsules at 150 mg/day to two lactating goats for four consecutive days (Pfeffer, 1992a, Report F-00088), corresponding to a dietary intake of 94 and 111 ppm in the feed. The radioactive pesticide was stable in the capsule from the time of preparation through the administration period (TLC analyses).

Urine, faeces and milk were collected daily from day -2 through day 4 of dosing. Most of the radioactivity was excreted in the faeces (50% to 60%) and in the urine (15% to 23%). The total recovery (including the gastrointestinal contents) was 94% to 98%. Both goats were killed about six hours after the last dose, and samples of liver, kidneys, tenderloin muscle, thigh muscle, omental fat, perirenal fat, heart, bile, blood, and gastrointestinal contents were stored at approximately -20°C until analysis.

Liquid scintillation counting (LSC) was used to determine the total radioactive residues (TRR) in the samples. The results are shown in Table 2.

Table 2. [^{14}C]fludioxonil residues found in goat tissues and milk (Peffer, 1992a, Report F-00088).

Sample	mg/kg[1], animal no. 80	mg/kg[1], animal no. 78
blood	0.47	0.49

Sample	mg/kg[1], animal no. 80	mg/kg[1], animal no. 78
plasma	0.64	0.68
liver	6.18	5.37
kidney	2.92	2.89
heart	0.22	0.16
tenderloin	0.09	0.05
leg muscle	0.07	0.06
omental fat	0.26	0.11
perirenal fat	0.28	0.10
milk[2]		
day 1	1.1	1.2
day 2	1.1	1.8
day 3	1.2	2.0
day 4	1.6	2.9

[1] fludioxonil equivalents. Limits of detection for tissues ranged from 0.002 mg/kg(blood) to 0.01 mg/kg (liver, kidney, fat), typical aliquot 30 to 200 mg
[2] Day -2 and day -1 milk samples had dpm values below the limit of detection, 0.004 mg/kg, typical aliquot 100 mg.

The highest ^{14}C residues were in the liver and kidneys. Residues in the fat were 2–4 times those in muscle. Resides in the milk rose steadily during the four days, with maximum levels of 1.6 and 2.9 mg/kg on day 4.

The radioactivity in tissue and milk samples was extracted by two techniques. The first for liver, kidney, and tenderloin muscle, involved extraction of tissue aliquots with multiple volumes of acetonitrile, followed by multiple volumes of water. The multiple extracts were combined and the radioactivity determined in each by LSC. Weighed aliquots of the remaining solid pellets were combusted to determine the amount of unextracted radioactivity.

The second method, which involved mixing 0.5 g of homogenized tissue or milk with octadecyl (C-18) column packing (2 g) in a mortar and pestle, was used for milk, omental fat, and tenderloin (animal no. 80 only). The mixture was packed into a column and radioactive residues eluted with hexane, ethyl acetate/acetonitrile, methanol, and distilled water. The radioactivity in each fraction and in the exhausted C-18 packing material was determined by LSC. The results are summarized in Tables 3 and 4.

Table 3. Distribution of radioacativity in goat tissue extracts (Peffer, 1992a, Report F-00088). Values uncorrected for recoveries.

Sample (^{14}C as fludioxonil)	% of TRR			
	Extract		Post extraction solid (PES)	Extract + PES
	Acetonitrile	Water	Pellet	Total
liver #80 (6.2 mg/kg)	38	18	44	100
liver #78 (5.4 mg/kg)	32	14	44	90
kidney #80 (2.9 mg/kg)	47	7.6	39	94
kidney #78 (2.9 mg/kg)	52	6.2	29	87

Sample (^{14}C as fludioxonil)	% of TRR			
	Extract		Post extraction solid (PES)	Extract + PES
	Acetonitrile	Water	Pellet	Total
tenderloin muscle #80 (0.09 mg/kg)	76	5.7	29	111
tenderloin muscle #78 (0.05 mg/kg)	76	9.1	42	127

Table 4. Distribution of radioacativity in goat milk, fat, and muscle (Peffer, 1992a, Report F-00088). Values uncorrected for recoveries.

Sample (^{14}C as fludioxonil)	Hexane	Ethyl acetate/ acetonitrile	Methanol	Water	Remaining on Column	Total
milk, day 3						
#80 (1.2 mg/kg)	0.16	88.	19	1.4	7.0	116
#78 (2.0 mg/kg)	0.53	69	22	0.3	2.7	95
omental fat						
#80 (0.26 mg/kg)	13	86	1.3	0.1	2.5	103
#78 (0.11 mg/kg)	6.3	88	4.4	0.9	9.2	109
tenderloin muscle #80 (0.09 mg/kg)	2.6	70	7.0	0.7	11	91

Most of the radioactivity in the milk, omental fat and muscle (76 to 109%) was extractable with organic solvent, and small amounts with water (0.1 to 9.1%), whereas in liver and kidney moderate amounts (38 to 52%) were extracted with organic solvents and smaller amounts with water (6.2 to 18%), leaving 29 to 44% unextracted.

Before analysis the extracts were also treated by TLC and/or reverse-phase HPLC, and the organic phases were further treated with beta glucuronidase or sulfatase enzymes to hydrolyse any glucuronide or sulfate conjugates.

The unextracted residues in liver, kidney, and tenderloin tissue pellets (from acetonitrile and water extractions) were treated with protease, then at various pH levels with 2,4-dinitrofluorobenzene. Protease treatment liberated 75% to 91% of the bound activity from liver, kidney, and tenderloin. Treatment with dinitrofluorobenzene, DNFB, which reacts with the terminal amino groups of amino acids, derivatized only 24 to 41% of the protease-liberated activity. This suggests that the radioactive residues remaining in the liver and kidney are associated with biological molecules, in part proteins. Other treatments used to release bound or conjugated residues in the liver included treatment with Raney nickel, derivatization with *m*-toluoyl chloride followed by extraction and TLC, and lyophilization followed by extraction and HPLC. These additional treatments liberated very little activity.

To accomplish exhaustive extraction of the hitherto unextracted residues the liver and kidney pellets were also subjected to 24 h hot methanol Soxhlet extraction, followed by acid/base hydrolysis (6N HCL; 15%KOH; $\leq$95^{o}C), which resulted in the extraction of only slightly more radioactivity than the original acetonitrile/water extractions.

Qualitative TLC and HPLC profiles of the organic-phase extracts of kidney, milk, liver, and fat from the two goats were similar (typically containing one or two major components), indicating metabolism had proceeded similarly, although profiles in the ACN (acetonitrile) extracts of tenderloin differed because animal 78 had much lower ^{14}C residue levels. The HPLC profile of the ACN extract of animal 80 indicated the presence of one major (76% of activity) and one minor (7.6%) component while the HPLC profile of animal 78 indicated the same two components in differing quantities (34.5% and 31.9%). A qualitative comparison of the enzyme-treated with the untreated organic-phase extracts indicated the presence of conjugated residues from the shifts in retention times.

Attempts were made to identify the activity in the aqueous (polar) extracts of liver (initial extracts as well as acid/base hydrolysis products). Additional treatment included protease (kidney and tenderloin were treated with protease also) and analysis by 2 dimensional TLC. However no additional metabolites were isolated.

Metabolites were isolated from HPLC fractions or TLC bands from the tissue analyses where adequate levels of activity were present, analysed by GC/MS or HPLC/MS, and the spectra obtained compared to known standards. Metabolites identified in rat urine (see below) were co-chromatographed with goat metabolites by TLC and HPLC to help assign structures to the minor metabolites which had undergone biological transformations. Rat-bile metabolites were also co-chromatographed with the rat urine metabolites to determine the site of hydroxylation for glucuronide conjugates that had similar mass spectra. The main components were

component A: 4-(2',2'-difluoro-1',3'-benzdioxol-4'-yl)-1*H*-pyrrole-3-carbonitrile 5-*O*-glucuronide (present in rat urine)

component B-1: 4-(2',2'-difluoro-1',3'-benzodioxol-4'-yl)-1*H*-pyrrole-3-carbonitrile 2-*O*-glucuronide (also present in rat urine)

component C: fludioxonil

component G: 4-(2',2'-difluoro-1',3'-benzdioxol-4'-yl)-1*H*-pyrrole-3-carbonitrile, 2- or 5-*O*-sulfate (sulfate conjugate of SYN 518577 or SYN 518578)

Component B-2 was tentatively identified when isolated from the kidney as 4-(2',2'-difluoro-1',3'-benzdioxol-4'-yl)-1*H*-pyrrole-3-carbonitrile, 7'-*O*-glucuronide on the basis of similar chromatography to a biosynthetic standard from rat bile. HPLC retention times changed after isolation by TLC or HPLC of two labile metabolites in the liver, L-1 and L-2. Storage of a liver extract sample in HPLC mobile phase (formate buffer, pH 6.5) resulted in disappearance of these peaks. Attempts to stabilize them were unsuccessful so they could not be identified. They are presumed to be reactive intermediates.

The total radioactivity identified as components A, B-1, B-2, C, and/or G ranged from 14% in liver to 83% in fat (Table 5).

Table 5. Identified metabolites in the tissues and milk of goats dosed with 150 mg/day of [pyrrole-4-^{14}C]fludioxonil for four consecutive days (Peffer, 1992a, Report F-00088).

Sample	Mg/kg as fludioxonil and (% of TRR)								
	B-1	B-2	Total B	A	C	G	L-1	L-2	Total % of identified TRR
Kidney	0.67 (23)	0.23 (7.9)	0.90 (31)	0.44 (15)	0.05 (1.7)	0.02 (0.7)			48
Liver					0.86 (14)		0.6 (9.8)	0.33 (5.3)	29
Muscle (tenderloin)									
Animal 78			0.003 (5.6)	0.001 (2.3)	0.01 (24)	0.01 (22)			53
Animal 80					0.04 (43)	0.007 (7.2)			50
Omental fat					0.21 (83)				83
Milk, day 3	1.32 (65)	Trace	1.32 (65)			0.28 (14)			78

The metabolism of ^{14}C pyrrole-labelled CGA 173506 in ruminants appears to involve hydroxylation of the pyrrole ring at the 2- or 5- position, followed by the formation of either glucuronide or sulfate conjugates. The free hydroxylated-pyrrole compounds, themselves intermediates, were not observed, presumably owing to inherent instability.

To determine the stability of metabolites a sample of liver was extracted four times with acetonitrile (ACN) and the extract concentrated and analysed in two different TLC solvent systems, and a day-3 urine sample was filtered and analysed directly using the 2 TLC systems. Analysis was initially within one month of receipt of the samples at the laboratory (January 1990), and again after all tissue extracts had been profiled (January 1991). The profiles produced were sufficiently similar to suggest that the metabolites were stable throughout the study.

Laying hens. A study (Peffer, 1992, Report F-00089) on the metabolism of fludioxonil in White Leghorn hens was reported to the Meeting, with amendments subsequently issued (Peffer, 1994; 1996; Archive CGA173406/0237).

After a seven-day acclimatizing period, five laying hens were dosed with gelatine capsules (10 mg of ^{14}C-fludioxonil (^{14}C-pyrrole-labelled) per day) for eight consecutive days, with a sixth control hen. Based on feed consumption, the dose was equivalent to an average of 89 ppm (76 ppm to 106 ppm) in the diet (target dose: 100 ppm). Eggs and excreta were collected daily from day -2 to day 8 and the hens were killed 6 hours after the last dose. Blood samples were taken immediately before the hens were killed, and thereafter samples of lean meat (breast and thigh muscle), skin plus attached fat, peritoneal fat, and liver, gizzard, kidneys, and heart were taken.

The TRR in the tissues and excreta were determined by combustion and liquid scintillation counting (LSC) after homogenization in the presence of solid CO_2, in egg yolks and whites and plasma by LSC, and in whole blood by LSC after solubilization in a mixture of Soluene and ethanol and treatment with H_2O_2 and HCl.

Samples were extracted by several methods, similar to those used in the ruminant study. Liver and muscle samples were extracted four times with acetonitrile and then four times with water. In a second method used to extract larger amounts of radioactivity for characterisation, liver samples were extracted with acetonitrile followed by overnight Soxhlet extraction with methanol. The resultant

tissue pellet was divided into two portions and treated with either 6N HCl or 15% KOH aqueous solutions. The acid and base hydrolysates were extracted with ethyl acetate. Kidney samples were extracted four times with acetonitrile and then three times with water. Egg yolk samples were extracted by solid-phase dispersion: homogenised specimens were mixed with C-18 silica and packed into a glass syringe barrel and the mixture was then extracted by elution with hexane, ethyl acetate/acetonitrile (1:3), methanol and water. Egg white was extracted four times with acetonitrile. Skin samples with attached fat were extracted with hexane followed by five extractions with acetonitrile and two extractions with water.

Characterisation of extracted radioactivity was accomplished using TLC and HPLC. In some instances the extracts were treated with enzymes (β-glucuronidase or sulfatase) to characterise conjugated metabolites. The unextracted residue from the liver, kidney and muscle samples was further characterised by treatment with protease and extraction at different pHs with ethyl acetate, with and without reaction with 2,4-dinitrofluorobenzene.

Identifications were by co-chromatography (TLC and HPLC), NMR and MS.

A mean of 102% (88% to 112%) of the total administered radiolabelled dose was eliminated in the excreta.

The results are shown in Tables 6 and 7.

Table 6. Radioactive residues (as fludioxonil equivalents) found in tissues and blood of hens dosed with 10 mg/hen/day of [pyrrole-4-^{14}C]fludioxonil for eight consecutive days (Peffer, 1992, Report F-00089).

Sample	Residue (mg/kg)		Limit of detection (mg/kg)
	Mean (n = 5)	Std. dev.	
Liver	8.9	7.3	0.011
Peritoneal fat	0.17	0.06	0.013
Lean meat			
Breast muscle	0.11	0.02	0.005
Thigh muscle	0.12	0.03	0.006
Skin plus attached fat	0.25	0.08	0.008
Other			
Plasma	2.4	0.82	0.006
Whole blood	1.8	0.68	0.003
Gizzard	11.	4.0	0.009
Kidneys	5.3	2.3	0.011
Heart	1.09	0.78	0.014

Table7. Radioactive residues (as fludioxonil equivalents) in the egg yolks and whites of hens dosed with 10 mg/hen/day of [pyrrole-4-^{14}C]fludioxonil for eight consecutive days (Peffer, 1992, Report F-00089).

Day	Residue (mg/kg)			
	Egg yolks		Egg whites	
	Mean (n = 5)	Std. deviation	Mean (n = 5)	Std. deviation
-2	<0.005	-	<0.002	-
-1	<0.005	-	<0.002	-
1	<0.005	-	0.018	0.016
2	0.41	0.52	0.035	0.018
3	0.55	0.33	0.041	0.021
4	1.53	0.73	0.045	0.019
5	1.8 (n=3)	0.99	0.038	0.020
6	1.70	0.65	0.046	0.017
7	1.85	0.58	0.054	0.027
8	2.2 (n=2)		0.043	

Acetonitrile and methanol extracted 61% (65% corrected for recovery) of the TRR in liver. Acid and base solubilized the unextracted rsidues, yielding 9.6 and 16% of the TRR respectively as organosoluble material. ACN and water extracted 64%-69% of the TRR in lean meat and 46% in kidney. Acetonitrile extracted 74% of the TRR in egg whites, and solid-phase dispersion 88% of that in the yolks. The results for tissues, eggs, and excreta are shown in Table 8.

Table 8. Extraction of radioactive residues in the tissues, eggs and excreta of hens dosed with 10 mg/hen/day of [pyrrole-4-^{14}C]fludioxonil for eight consecutive days (Peffer, 1992, Report F-00089).

Sample and (mg/kg TRR as fludioxonil)	% of TRR										
	Hex-ane	Aceto-nitrile	EtOAc/ ACN	MeOH	Water	MeOH Soxhlet	Unex-tracted pellet	Base hydrolysis[1] (15% KOH, 95°C, overnight)		Acid hydrolysis (6N HCl, room temperature, 3 h)	
								organic	aqueous	organic	aqueous
Liver (8.9)		36				24.7	33	16	16	9.6	29
Kidney (5.3)		33			12		54				
Breast muscle (0.11)		62			2.3		36				
Thigh muscle (0.12)		62			6.3		31				
Skin with attached fat (0.15)	4.3	42			9.2		45				
Excreta				24	7.9						
Egg white (0.043)		74					26				
Egg yolk [2] (2.2)	2.6		68	15	2.4		12				

[1] Hydrolysate characterised as polar, acidic compounds by HPLC and TLC.
[2] Yolk mixed with C-18 material, transferred to syringe barrel and eluted sequentially with hexane, ethyl acetate/acetonitrile (1:3), methanol and water.

The radioactivity remaining in the tissue pellets after extraction of liver, kidney and breast muscle samples, 33%, 54%, and 36% of the TRR respectively, was solubilized with protease and characterised by treatment with DNFB and extraction with ethyl acetate at pH 2 and at pH 9. Approximately 54% and 63% of the previously unextracted radioactivity in liver and kidney samples and 67% of that in muscle was solubilized by protease, and of this, treatment with DNFB at pH 2 released 50%, 61% and 56% respectively extractable with ethyl acetate at pH 2 compared with 25%, 33% and 34% at pH 2 from the underivativised samples (Table 9). These results suggest that a portion of the radioactivity remaining in the pellet after solvent extraction represents covalent binding of reactive metabolite(s) to endogenous material, some of which is protein. HPLC showed the radioactivity in protease-treated liver to be highly polar and unresolved. Further identification was not pursued.

Table 9. Protease treatment of acetonitrile and water-extracted liver, kidney and muscle from hens dosed with 10 mg/hen/day of [pyrrole-4-^{14}C]fludioxonil for eight consecutive days (Peffer, 1992, Report F-00089).

Sample (extracted tissue pellets)	Protease treatment (radioactivitiy in extracted pellet)		Treated with DNFB (% of supernatant extracted into ethyl acetate, normalized for total recovery)		Untreated (% of supernatant extracted into ethyl acetate, normalized for total recovery)	
	% supernatant	% pellet	pH 9	pH 2	pH 9	pH 2
Liver	54	57	10	50	6.6	25
Kidney	63	43	41	61	23	33
Tenderloin muscle	67	-	14	56	24	34

Metabolites, isolated primarily from excreta, were identified by mass spectrometry (with GC or HPLC) and/or NMR. Additional information was obtained by glucuronidase or sulfatase incubations. The metabolites in the excreta were co-chromatographed with tissue and egg extracts, and in addition metabolites in rat urine identified by mass spectrometry were co-chromatographed with the poultry tissue and egg metabolites using TLC and HPLC. Biosynthetic standards were also co-chromatographed with the rat-urine metabolites to determine the site of hydroxylation in glucuronide conjugates that had similar mass spectra.. The following compounds were identified:

metabolite P1	CGA-192155: 2,2-difluorobenzo[1,3]dioxole-4-carboxylic acid
metabolite P2/P3	Form 1 SYN 518577: 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile
	Form 2: CGA 344623: 1 2-cyano-3-(2,2-difluoro-benzo[1,3]dioxol-4-yl)succinamic acid
metabolite P7	CGA 344624: 2-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-oxoacetamide
E1	parent fludioxonil
metabolite E2	CGA-265378: 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrole-3-carbonitrile
metabolite F	SYN 518576: 4-(2,2-difluoro-7-hydroxybenzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile
metabolite G	CGA-335892: 4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1-hydroxy-1H-pyrrole-3-carbonitrile
metabolite I-1	sulfate conjugate of CGA-335892
metabolite I-2	Sulfate conjugate of SYN 518577,4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile or SYN 518578, 4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-5-hydroxy-1H-pyrrole-3-carbonitrile

The analyses suggested the presence of additional polar metabolites P4, P5, P6, P7, P8, P9, P10, A, B, C, and D, although definitive identification was not possible, mainly because insufficient activity was present. Structures for P7, P10 and D were tentatively assigned.

Characterisation of the extracted residues by several HPLC systems yielded approximately twenty peaks, .the distribution and quantification of which are shown in Table 10.

Table 10. Identification and characterisation of radioactive residues in the tissues and eggs of hens dosed with 10 mg/hen/day (ca. 89 mg/kg diet) of [pyrrole-4-^{14}C]fludioxonil for eight consecutive days.

Compound or fraction		Egg white	Egg yolk	Egg total	Liver	Kidney	Breast muscle	Thigh muscle	Skin + attached fat
P1:CGA 192155	mg/kg	0.003	0.012	0.006	0.206	n.a.[1]	n.a.	n.a.	n.a.
	% TRR	4.6	0.7	0.9	2.3	n.a.	n.a.	n.a.	n.a.
P2/P3: CGA 344623	mg/kg	0.015	0.168	0.067	0.531	n.a.	n.a.	n.a.	n.a.
	% TRR	28	9.1	10.	5.9	n.a.	n.a.		n.a.
P4	mg/kg	0.001	0.002	0.001	0.074	n.a.	n.a.	n.a.	n.a.
	% TRR	1.1	0.1	0.2	0.8				
P5	mg/kg	0.002	0.015	0.006	0.091	n.a.	n.a.	n.a.	n.a.
	% TRR	3.4	0.8	0.9	1.0				
P6	mg/kg	0.001		0.001	0.110	n.a.	n.a.	n.a.	n.a.
	% TRR	2.1		0.1	1.2				
P7:CGA 344624	mg/kg	0.004	0.023	0.010	0.304	n.a.	n.a.	n.a.	n.a.
	% TRR	6.6	1.2	1.5	3.4				
P8	mg/kg				0.233	n.a.	n.a.	n.a.	n.a.
	% TRR				2.6				
P9	mg/kg	<0.001		<0.001	0.149	n.a.	n.a.	n.a.	n.a.
	% TRR	0.7		<0.1	1.7				
P10	mg/kg				0.154	n.a.	n.a.	n.a.	n.a.
	% TRR				1.7				

Compound or fraction		Egg white	Egg yolk	Egg total	Liver	Kidney	Breast muscle	Thigh muscle	Skin + attached fat
A	mg/kg	n.a.	n.a.	n.a.	n.a.	0.376			
	% TRR					7.1			
B	mg/kg	n.a.	n.a.	n.a.	n.a.	0.063	0.005		
	% TRR					1.2	4.3		
C	mg/kg	n.a.	n.a.	n.a.	n.a.	0.151			
	% TRR					2.9			
D: glucuronide conjugate of SYN 518577/518578	mg/kg				0.166	0.247			
	% TRR				1.9	4.7			
E1: fludioxonil	mg/kg		0.041			0.135	0.032	0.010	0.025
	% TRR		2.2			2.6	30	7.9	9.8
E2:CGA 265378	mg/kg				0.113				
	% TRR				1.3				
F:SYN 518576	mg/kg				0.235	0.146			
	% TRR				22.6	2.8			
G: CGA 335892	mg/kg	<0.001	0.025	0.008	0.073	0.121			
	% TRR	0.4	1.3	1.3	0.8	2.3			
I1: sulfate conjugate of CGA-335892	mg/kg	0.001	0.780	0.264	0.046	0.070	0.012	0.036	0.036
	% TRR	1.4	42	40	0.5	1.3	11	30	14
I2: sulfate conjugate of SYN 518577/518578	mg/kg	<0.001	0.258	0.087			0.004	0.008	0.012
	% TRR	0.4	14.0	13.3			3.9	6.9	4.9
% of TRR in analysed extracts		61	83.	n.a.	52	33.	62	62	33
% of TRR identified		42	71	69	24	14	44	45	29

[1] n.a.: not applicable

Fludioxonil was present in muscle (7.9-28% of the TRR) and skin plus attached fat (9.8%). It accounted for 1.2% of the TRR in the liver, 2.6% in kidney and 2.2% in egg yolk (equivalent to 2.1% of the egg TRR).

Unextracted radioactivity was 33% (35% corrected for recovery) of the TRR in liver, 5% of the TRR in kidney, 45% in skin with attached fat, and 31-36% of the TRR in muscle. Further treatment of liver, kidney and muscle with protease and with acid or base in the case of liver released a significant proportion (<50%). Lesser amounts of unextracted ^{14}C were found in egg whites (26%) and yolks (12%).

Rats. In four studies on the fate of fludioxonil (Thanei, 1992, Report 12/92; Bissig, 1990, Report 32/90; Thanei, 1995, Report 13/93, and Muller and Thanei, 1995, Report 4/95) six metabolites were identified in the urine, faeces and bile (see Table 11).

Table 11. Approximate amounts of fludioxonil metabolites in the rat (Thanei, 1992, Report 12/92; Bissig, 1990, Report 32/90; Muller and Thanei, 1995, Report 4/95; and Thanei, 1995, Report 13/93).

Designation	Identification	Fraction[1]	% of applied dose	
			male	female
SYN 518577	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-2-hydroxy-1H-pyrrole-3-carbonitrile			
MET 1G (glucuronyl-conjugate)	2-β-D-glucuronyl-4--(2,2-difluoro-benzo[1,3] dioxol-4-yl)-1H-pyrrole-3-carbonitrile	U11, G6	0.5-0.8	0.5-56
MET 2G (sulfate conjugate)	4-(2,2-difluorobenzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile-2-hydrogen sulfate	U10, G5	0.5-0.8	0.7-1.1
total			*1.2-1.6*	*1.5-56.7*

Designation	Identification	Fraction[1]	% of applied dose	
			male	female
SYN518578	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5-hydroxy-1H-pyrrole-3-carbonitrile			
MET 3G (glucuronyl-conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5-β-D-glucuronyl-1H-pyrrole-3-carbonitrile	U9, G4	0.6-0.9[3]	1.0[3]-2.8
MET 1U (sulfate conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile-5-hydrogen sulfate	U13	1.8-2.2	1.1-1.9
total			*2.4-2.8*	*2.5-3.9*
SYN 518576	4-(2,2-difluoro-7-hydroxy-benzo[1,3]dioxol-4-yl)-1H-pyrrole-3-carbonitrile			
MET 4G (glucuronyl-conjugate)	4-(2,2-difluoro-7--β-D-glucuronyl-benzo[1,3] dioxol-4-yl)-1H-pyrrole-3-carbonitrile	U6, G2	1.1-4.8	2.2 0.9-1.5
SYN 518582 [= MET 1G(G4)]	4,4'-bis-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5,5'-dioxo-1,5,1',5'-tetrahydro-[2,2']bipyrrolylidene-3,3'-dicarbonitrile	U19	0.2-0.4 (3.2)	0.1-0.7 (3.5)

[1] U: Urine, G: Bile

The postulated metabolic pathways of fludioxonil in goats, hens and rats are shown in Figure 1.

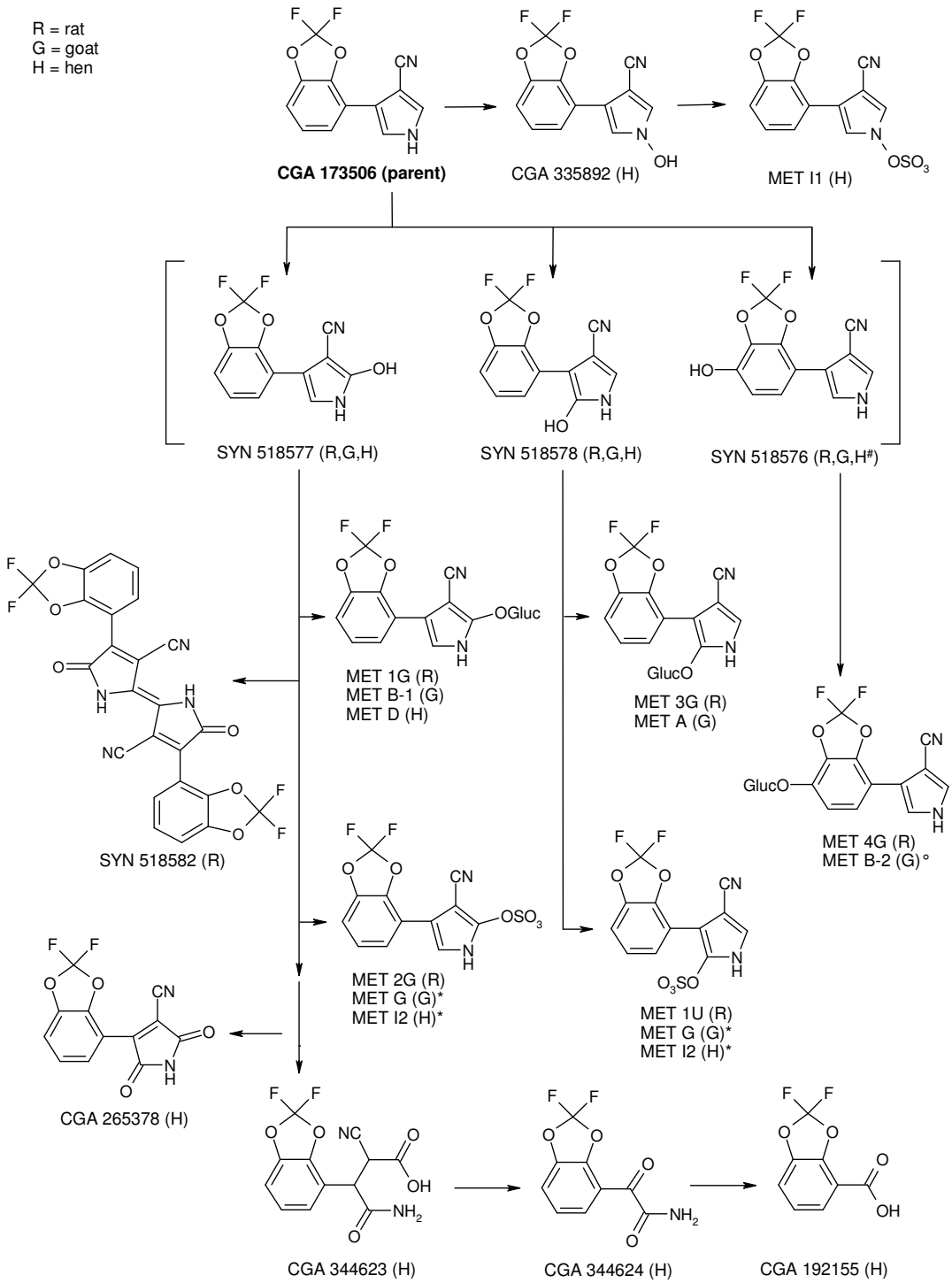


Figure 1: Postulated metabolic pathways of fludioxonil in rats, hens and goats.