ISOPROTHIOLANE (299)

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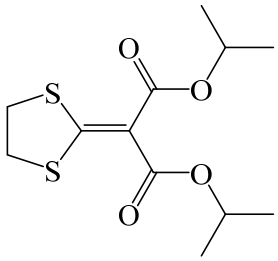
EXPLANATION

Isoprothiolane (chemical name: diisopropyl 1,3-dithiolan-2-ylidenemalonate) is a systemic fungicide with protective and curative action. It's used to control rice blast (*Pyricularia oryzae*), rice stem rot and Fusarium leaf spot on rice, also reducing plant-hopper populations following foliar applications. Isoprothiolane inhibits the penetration and elongation of infecting hyphae by inhibiting formation of infecting peg or cellulase secretion. Isoprothiolane was considered for the first time for toxicology and residues by the 2017 JMPR. Studies on metabolism, analytical methods, supervised field trials, processing, freezer storage stability and environmental fate in soil and water were submitted by the manufacturer.

IDENTITY

ISO common name	Isoprothiolane (published)
Chemical Name	
IUPAC	Diisopropyl 1,3-dithiolan-2-ylidenemalonate
CAS	bis(1-methylethyl) 2-(1,3-dithiolan-2-ylidene)propanedioate
CIPAC No.	456
CAS No.	50512-35-1
Structural Formula	
Molecular formula	$C_{12}H_{18}O_4S_2$
Molecular mass	290.4

PHYSICAL AND CHEMICAL PROPERTIES

Table 1 Physical and chemical properties of isoprothiolane pure active ingredient (99.8% purity)

Property	Guideline and method	Results	Reference/ Remarks
Color, Appearance, Odor	9-Nousan-5089, Notification by Director-General, 1997,Japan	White, crystalline powder, no odour	Study ID 98P001 PC-2034
Melting point	OECD test guideline 102	54.6–55.2 °C	Study ID 98P001/ PC-2034
Boiling point	OECD test guideline 103	175–177 °C/0.4 kPa	Study ID 98P001/ PC-2034
Water Solubility	OECD test guideline 105	48.5 mg/L (20 °C, pH6.0)	Study ID 98P001/ PC-2034
Partition Coefficient	OECD test guideline 117	log Pow 2.80 (HPLC Method)	Study ID 98P001/ PC-

Property	Guideline and method	Results	Reference/ Remarks
(octanol/water)			2034
Density	OECD 109	1.252 g/cm3 (20 °C)	Study ID 98P001 / PC-2034
Photolysis in water	9-Nousan-5089, Notification by Director-General, 1997, Japan	Over 96% of Isoprothiolane was remained in distilled and natural water after 168 hrs exposure of irradiation (25 °C, 17.2 W/m2, 280–500 nm)	Study ID 98P001/ PC-2034
Dissociation constant	OECD test guideline 105	No dissociation in water occurs. Solubility in water was not affected by pH.	Study ID 98P001/ PC-2034
Thermal stability	OECD test guideline 113	Stable at room temperature up to 150 °C(Endothermic peak 57.7 °C)	Study ID 98P001/ PC-2034

Isoprothiolane is registered as a 40.0% emulsifiable concentrate (EC) formulation and as a 12.0% granule (GR) formulation.

METABOLISM AND ENVIRONMENTAL FATE

Metabolism studies in paddy rice and lactating goatwere conducted with isoprothiolane labelled in the 3-dihydrofuranone moiety (**Error! Reference source not found.**). Environmental fate studies including soil photolysis study, anaerobic degradation in soil, adsorption on soil, hydrolysis in water, and anaerobic degradation of isoprothiolane in sediment/water systems were also performed with [dithiolane-4,5-^{14}C] isoprothiolane. Uptake and metabolism study on lettuce, radish and wheat for confined rotational crop studies were also conducted with this labelled isoprothiolane.

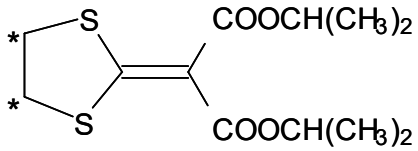
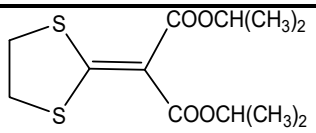
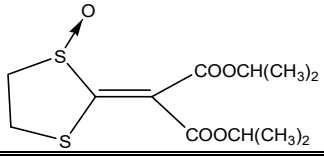
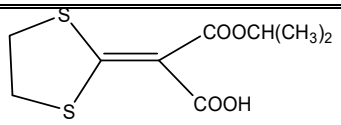
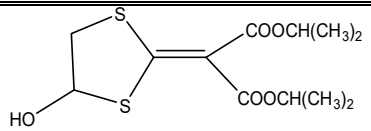
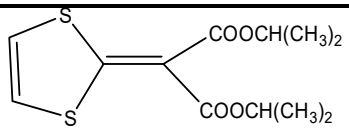
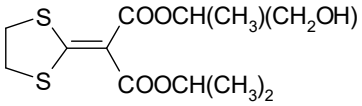


Figure 1 [^{14}C]Isoprothiolane, the labelled positions are C4 and C5 of the dithiolane ring

Chemical names, structures, and code names of metabolites and degradation products of isoprothiolane are shown below (Table 2). All of the compounds in Table 2 were identified in at least one matrix in studies with radiolabelled isoprothiolane.

Table 2 Known metabolites and degradation products of isoprothiolane

Chemical name (other names, codes)	Chemical structure	Found in
Diisopropyl 1,3-dithiolan-2-ylidenemalonate **（Isoprothiolane, Parent）**		Rice grain Rotational crops （lettuce, radish and wheat） Rat Mouse Goat
Diisopropyl 1- oxo-1,3- dithiolan-2-ylidenemalonate **(Isoprothiolane monosulfoxide, M-1)**		Rice grain Rotational crops （lettuce, radish and wheat） Mouse
Monoisopropyl, 1,3-dithiolan-2-ylidenemalonate **(Isoprothiolane monoester, M-2)**		Rice grain Rotational crops （lettuce, radish and wheat） Rat Mouse

Chemical name (other names, codes)	Chemical structure	Found in
		Goat
Diisopropyl 4- hydroxy-1,3- dithiolan-2-ylidenemalonate **(4-hydroxy isoprothiolane, M-3)**		Rice grain Rotational crops (lettuce, radish and wheat) Rat Mouse Goat
Didehydro isoprothiolane **(M-4)**		Rotational crops (lettuce, radish and wheat) Rat
Hydroxyl-Isopropyl isoprothiolane **(M-5)**		Rotational crops (lettuce, radish and wheat)

Plant metabolism

The Meeting received studies depicting the metabolism of isoprothiolane in paddy rice.

Paddy Rice

The metabolism of isoprothiolane in paddy rice was investigated by Takahashi (2006, Report R-2010). Isoprothiolane, radiolabelled at the 4,5-dithiolane carbon (specific activity 1.85 GBq/mmol), was prepared as a 40% emulsifiable concentrate formulation and applied twice to rice grown in a glasshouse at application rates of 600 g ai/ha. Conditions within the glasshouse were controlled to effectively represent paddy conditions. Individual plants were treated before ear emergence and harvested 7 (immature) and 28 days (mature stage) after the last application. Grain, stem and leaves, and roots were harvested separately and grains were subsequently separated into hulled grain and hull samples.

Selected leaf and stem samples were rinsed with methanol prior to extraction. All samples were then homogenized and sequentially extracted with methanol, and methanol/water (1/1, v/v). Samples with significant residual radioactivity were subjected to further extraction with methanol/1N HCl (1/1, v/v), methanol/1N NaOH aq. (1/1, v/v). Extraction was followed by solubilisation with cellulase treatment and 6N HCl hydrolysis. The thus obtained extracts and post-extraction solids (PES) were combusted and analysed by LSC. The sum of the radioactivity in all fractions was calculated as the TRR, and was expressed as mg/kg equivalents of the parent isoprothiolane. Methanol rinse, methanol extracts and methanol/water extracts containing more than 10% TRR were further analysed by TLC and HPLC to determine metabolite profiles following appropriate concentrating and purification procedure.

Total isoprothiolane residues were 0.21, 5.38, 1.91 and 0.03 mg/kg (parent equivalent) in the hulled grain, hulls, stems and leaves, and roots, respectively, at 7 days after treatment (DAT). Residues at 28 DAT were 0.20, 4.05, 1.36 and 0.02 mg/kg in the hulled grain, hulls, stems and leaves, and roots, respectively. Hulls contained higher concentration of residues than in the stems and leaves.

For grain, methanol and methanol/water extracted 41.8% TRR (0.07 mg/kg equivalents). Sequential treatment of PES using methanol/1N HCl, methanol/1N NaOH and 6N HCl hydrolysis released only up to a further 5% TRR (0.01 mg/kg equivalents) per treatment. Cellulase treatment released an additional 42.6% TRR (0.09 mg/kg equivalents) indicating a high degree of incorporation with biological macromolecules. Following treatment of PES with harsh procedures the radioactivity remaining in the solids was low (3.8% TRR, < 0.01 mg eq/kg).

For leaves and stems at the 28 DAT, methanol surface rinses, methanol and methanol/water extracts accounted for 76.9% TRR (1.04 mg/kg).

Table 3 Distribution of radioactivity in rice samples treated with [^{14}C] isoprothiolane

	7 DAT							
	Grain		**Hulls**		Stems/ **Leaves**		**Roots**	
	TRR (%)	**mg eq/kg**	**TRR (%)**	**mg eq/kg**	**TRR (%)**	**mg eq/kg**	**TRR (%)**	**mg eq/kg**
MeOH rinse	n.a.	n.a.	n.a.	n.a.	29.8	0.57	n.a.	n.a.
MeOH extract	27.0	0.06	73.9	3.97	44.4	0.85	48.2	0.02
MeOH/water extract	5.9	0.01	16.2	0.98	11.2	0.21	9.7	< 0.01
MeOH/HCl	3.5	< 0.01	n.a.	n.a.	1.9	0.04	n.a.	n.a.
MeOH/NaOH	3.5	< 0.01	n.a.	n.a.	4.4	0.08	n.a.	n.a.
Cellulase treatment	9.7	0.02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6N HCl treatment	46.2 *	0.10	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Remaining solids	5.1	0.01	7.8	0.42	8.3	0.16	42.1	0.01
TRR	100	0.21	100	5.38	100	1.91	100	0.03

	28 DAT							
	Grain		**Hulls**		Stems/ **Leaves**		**Roots**	
	TRR (%)	**mg eq/kg**	**TRR(%)**	**mg eq/kg**	**TRR (%)**	**mg eq/kg**	**TRR (%)**	**mg eq/kg**
MeOH rinse	n.a.	n.a.	n.a.	n.a.	20.2	0.27	n.a.	n.a.
MeOH extract	34.4	0.07	63.4	2.57	39.2	0.53	45.2	< 0.01
MeOH/water extract	7.4	0.02	25.6	1.04	17.5	0.24	9.3	< 0.01
MeOH/HCl	3.1	< 0.01	1.3	0.05	3.9	0.05	n.a.	n.a.
MeOH/NaOH	3.6	< 0.01	3.6	< 0.01	2.9	0.12	12.2 *	0.17
Cellulase treatment	5	0.01	n.a.	n.a.	1.5	0.02	n.a.	n.a.
6N HCl treatment	42.6 *	0.09	n.a.	n.a.	2.8	0.04	n.a.	n.a.
Remaining solids	3.8	< 0.01	6.8	0.28	2.7	0.04	45.6	< 0.01
TRR	100	0.2	100	4.05	100	1.36	100	0.02

*No single metabolite accounting for greater than 10%TRR

n.a. not applicable

Isoprothiolane was the major radioactive component identified in all matrices. At 7 DAT, isoprothiolane accounted for 16.4, 75.5, 50.9 and 42.4% TRR in the rice, hulls, stems and leaves, and root samples respectively. At 28 DAT, the proportion of unchanged parent in rice was 32.3, 61.6, 26.3 and 34.3% TRR in grain, hulls, stems and leaves and roots (respectively). Other than isoprothiolane, monosulfoxide was the most abundant component but was present at very low levels (< 0.01 mg/kg) in rice grain and was minor (< 10% TRR) in both hulls and stems and leaves at both 7 DAT and 28 DAT. Other identified metabolites including 4-hydroxy-isoprothiolane, the monoester and the didehydro were all below the LOQ (< 0.01 mg/kg) in grain samples. The identity of metabolites indicated by TLC was confirmed using radio-HPLC-UV. Table 4 gives the metabolic profile of isoprothiolane in the rice extracts.

In the TLC analysis, greater than 10% TRR was retained at the origin indicating the presence of highly polar metabolites. Further TLC analysis of the polar fractions using more highly polar development solvents demonstrated that this was made up of multiple components which individually accounted for less than 10% TRR. Further β-glucosidase treatment of the polar metabolites on TLC origin released 4-hydroxy and monoester, which suggested that the polar fraction contained glucose conjugates of these metabolites. Material at the origin was subjected to hydrolysis using 6*N* HCl and analysis by TLC, 15.3% of TRR was still retained on TLC origin.

Further analysis was also performed to characterise the radioactivity in PES. The radioactivity released by methanol/1*N* NaOH treatment of 28 DAT stems and leaves (12.2% TRR) was extracted by ethyl acetate and acetonitrile and analysed by TLC. TLC analysis revealed that less than 1% TRR was present as isoprothiolane, 4-hydroxy, monoester and monosulfoxide and the largest single unidentified component accounting for only 4.1% TRR.

The radioactivity released by cellulase treatment of PES from 7 and 28 DAT grain was extracted by ethyl acetate and acetonitrile and analysed by TLC. Radioactivity extracted by ethyl acetate was 8.1 and 13.1% TRR for 7 and 28 DAT respectively. Acetonitrile partition extracted 14.2 and 7.6% TRR in the same manner. The most prominent single unidentified component only accounted for 5.4% TRR (< 0.01 mg/kg).

Table 4 Summary of extractable radioactive residues in paddy rice following treatment with [dithiolane-4, 5-^{14}C] isoprothiolane

	7 DAT							
	Grain		Hulls		Stems/ Leaves		Roots	
	TRR %	mg eq/kg	**TRR %**	mg eq/kg	**TRR %**	mg eq/kg	**TRR %**	mg eq/kg
Total extract [a)]	32.9	0.07	82.2	4.96	85.4	1.63	48.2	0.02
Isoprothiolane	16.4	0.03	75.5	4.06	50.9	0.97	42.4	0.01
4-hydroxy-isoprothiolane (M-3)	0.1	< 0.01	1.0	0.05	1.4	0.03	n.d.	n.d.
Monoester (M-2)	< 0.1	< 0.01	n.d.	n.d.	0.6	0.01	n.d.	n.d.
Monosulfoxide (M-1)	0.3	< 0.01	6.5	0.35	8.1	0.15	n.d.	n.d.
Didehydro (M-4)	n.d.	n.d.	0.1	< 0.01	0.5	0.01	n.d.	n.d.
TLC-Origin	10.7 [b]	0.02	6.4	0.34	23.5 [b]	0.45	5.0	< 0.01
Unidentified Metabolites	5.4	0.01	2.7	0.14	0.5	< 0.01	0.8	< 0.01

	28 DAT							
	Grain		Hulls		Stems/ Leaves		Roots	
	TRR %	mg eq/kg	**TRR %**	mg eq/kg	**TRR %**	mg eq/kg	**TRR %**	mg eq/kg
Total extract [a)]	41.8	0.08	89.1	3.61	76.9	1.04	45.2	< 0.01
Isoprothiolane	32.2	0.06	61.6	2.5	26.3	0.36	34.3	< 0.01
4-hydroxy-isoprothiolane (M-3)	0.5	< 0.01	2.0	0.08	1.2	0.02	nd.	nd.
Monoester (M-2)	0.2	< 0.01	0.3	0.01	0.5	< 0.01	nd.	nd.
Monosulfoxide (M-1)	0.1	< 0.01	8.5	0.35	7.7	0.10	nd.	nd.
Didehydro (M-4)	0.2	< 0.01	0.3	0.01	0.6	< 0.01	nd.	nd.
TLC-Origin	7.5	0.01	13.5 [b]	0.55	40.0 [c]	0.54	9.8	< 0.01
Unidentified Metabolites	0.9	< 0.01	2.7	0.11	0.7	< 0.01	1.0	< 0.01

[a] Analysed fraction: methanol and methanol/water extract of brown rice and hull. For stem & leaves, sum of surface rinse, methanol and methanol/water extract were analysed, for root, methanol extract was analysed

[b] No single metabolite exceeded more than 10% TRR, mainly monoester and 4-hydroxy conjugates

[c] No single metabolite exceeded more than 10% TRR except highly polar origin on TLC

nd - not detected

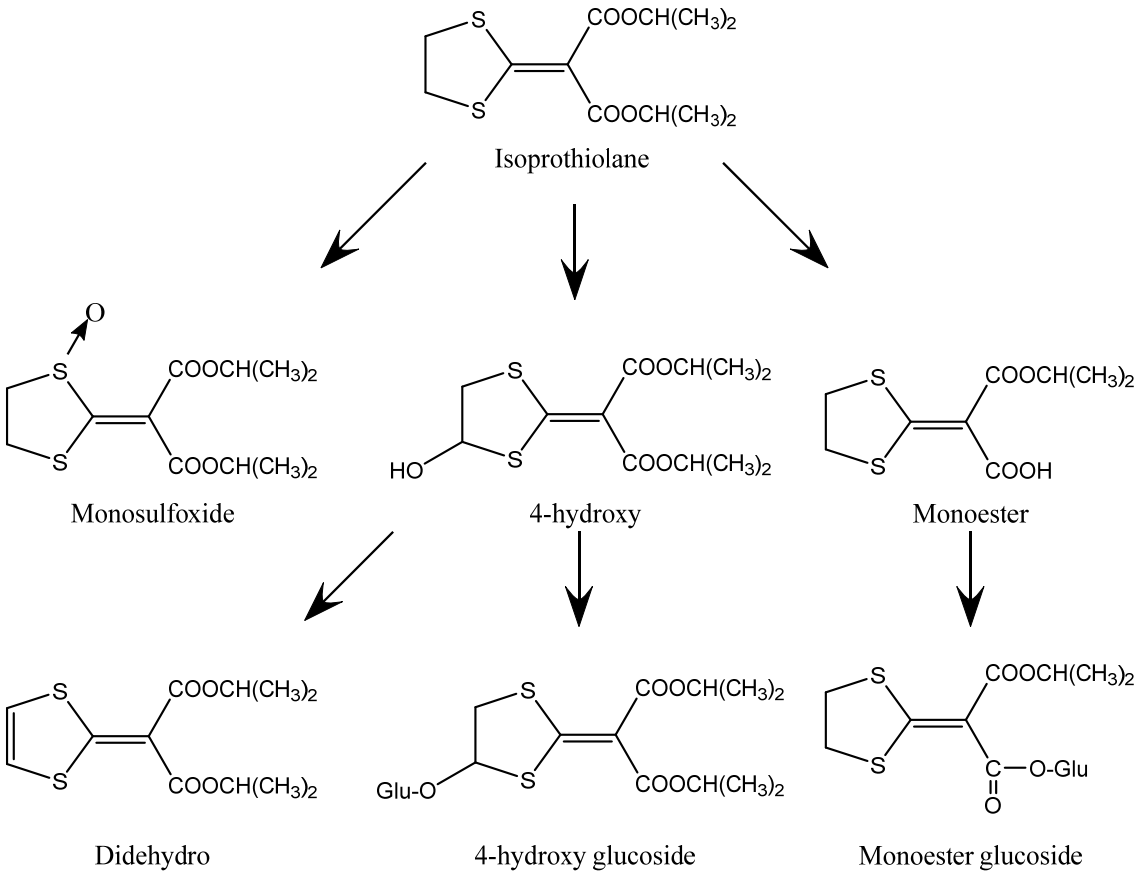


Figure 2 Proposed metabolic pathway of isoprothiolane in paddy rice.

Metabolism studies in paddy rice showed that isoprothiolane was the major residue in plant matrices. Metabolism of the active substance produced only low level of minor metabolites. Hydrolysis of the conjugated and unextractable residue released further low levels of the identified metabolites, with no single component in these accounting for >10% TRR.

Animal metabolism

The Meeting received animal metabolism studies on rats and lactating goats in laboratory conditions. All of the studies were conducted with radiolabelled [dithiolane-4,5-^{14}C] isoprothiolane.

Laboratory animals

Metabolism in laboratory animals was evaluated by the WHO Core Panel of the 2017 Meeting.

Lactating goats

The metabolism of isoprothiolane in lactating goats was investigated by Ahn, (2014, Study report R-2136). One lactating goat was dosed daily by gelatine capsule for five consecutive days with [dithiolane-4,5-^{14}C] isoprothiolane at a rate of 10.6 ppm diet/day as received, corresponding to12.3 ppm diet/day on a dry weight basis.

Urine and faeces were collected daily and each goat was milked twice daily (morning and evening). The animals were sacrificed on Day 5 approximately 6 hours after the last dose, and the following tissue samples collected: liver, kidney, flank muscle, loin muscle, subcutaneous fat, omental fat, renal fat, bile, blood and gastrointestinal tract with contents. Whole milk was centrifuged to separate milk fat and skim milk. Urine, cage wash and skim milk were counted directly by liquid scintillation counting (LSC). Faeces and gastrointestinal tract samples were combusted prior to LSC.

Radioactive residues in liver, kidney, muscle, fat and milk fat were solubilized, acidified with acetic acid and measured by LSC. Bile was analysed directly by LSC and blood was combusted.

Edible tissues and muscle were extracted with acetonitrile: water (1:1 v/v), followed by acetonitrile and centrifuged. The liquid phase samples were concentrated and analysed by HPLC and TLC. The post extraction solid (PES) was further extracted with potassium hydroxide overnight, then solubilized before quantification by LSC. Subsamples of PES from flank muscle, loin muscle, liver and kidney were subjected to pronase enzymatic digestion (*Streptomyces griseus*, 50 mg/g PES), incubated at 37 °C for 2 days, centrifuged, the precipitated solids extracted with methanol:water (1:1 v/v) and the resulting solid samples combusted or solubilized. The release of pronase (associated with protein) was calculated as the difference between control and treated extracts.

The hexane phases of fat extracts (omental, subcutaneous, renal and milk fat) were saponified by concentrating, diluting with potassium hydroxide in methanol: water (4: 1 v/v) and refluxed. The extracts were partitioned against hexane, the aqueous phase acidified and re-partitioned against ethyl acetate. All phases were analysed by LSC. Following quantification by LSC, characterization was performed by HPLC or TLC and elucidation by mass spectrometry (LC-MS).

Skim milk with higher radioactivity were extracted with acetone and acetone: water (1:1 v/v), centrifuged and the extract analysed by HPLC and TLC. The PES was combusted and quantified by LSC. Milk fat samples were extracted with hexane: acetone (4:1 v/v) followed by acetone and centrifuged. Acetone was removed by concentration, the sample reconstituted in hexane and partitioned against acetonitrile. The PES was treated with potassium hydroxide.

Urine and faeces were extracted with acetonitrile: water (2:1 and 4: 1 v/v for urine and 1:1 v/v for faeces) and the PES solubilized before quantification by LSC. Selected acetonitrile/water extracts of urine and kidney were subjected to β-glucoronidase hydrolysis. Phenolphthalein glucoronide was used as a standard to determine enzymatic activity. Mixtures were incubated at 37 °C overnight and analysed by HPLC or TLC. Further urine acetonitrile/water extracts were also subjected to acid hydrolysis using HCl (1N or 6N). The samples were incubated either a room temperature or 100 °C overnight. The samples were then partitioned with ethyl acetate and analysed by TLC.

The total recovery of administered dose after 5 consecutive days of treatment was 76.7%. As significant amounts of $^{14}CO_2$ (29–33%) were found using the same radiolabeled compound in the rat study, the low mass balance is may be attributed to unrecovered $^{14}CO_2$. Total radioactive residues expressed as mg/kg parent-equivalent, found in milk, excreta and tissue samples are presented in Table 5.

Table 5 Summary of TRRs in lactating goat after dosage of [^{14}C] isoprothiolane for five consecutive days

Day /Matrix		Radioactive excretion						
		Skim milk		Milk fat		Urine	Faeces	Daily total
		%TRR	mg/kg	% TRR	mg/kg	% TRR	% TRR	% TRR
1	am	n.d.	n.d.	n.d.	n.d.	0.00	n.d.	0.10
	pm	0.08	0.279	0.02	0.823			
2	am	0.17	0.223	0.05	1.206	12.27	1.56	14.25
	pm	0.15	0.424	0.05	1.611			
3	am	0.16	0.250	0.06	1.489	12.33	2.62	15.44
	pm	0.20	0.429	0.07	1.825			
4	am	0.15	0.208	0.06	1.362	12.53	1.94	14.88
	pm	0.15	0.420	0.05	1.792			
5	am	0.15	0.237	0.07	1.571	11.67	2.34	14.41
	pm	0.13	0.412	0.05	1.825			
5 at sacrifice (app. 6 hr after last dose)		—		—		5.91	0.95	6.86
Total excreted		1.84				54.71	9.41	**65.94**
Cage wash		0.01%						
G.I. tract [a]		6.37%						
Liver		1.06% (1.584 mg/kg)						

Day /Matrix	Radioactive excretion						
	Skim milk		Milk fat		Urine	Faeces	Daily total
	%TRR	mg/kg	% TRR	mg/kg	% TRR	% TRR	% TRR
Kidney	0.12% (1.204 mg/kg)						
Total muscle [b]	1.88%						
Flank muscle	0.03% (0.135 mg/kg)						
Loin muscle	0.05% (0.127 mg/kg)						
Omental fat	0.04% (0.296 mg/kg)						
Subcutaneous fat	0.02% (0.243 mg/kg)						
Renal fat	0.02% (0.320 mg/kg)						
Bile	0.03% (1.393 mg/kg)						
Blood [c]	1.23% (0.515 mg/kg)						
Total recovery	**76.7%**						

[a] Gastro-intestinal (G.I.) tract includes contents

[b] Calculated according to estimated muscle mass = 50% of goat weight

[c] Calculated according to estimated blood mass = 1/12 of total goat weight

The majority of radioactivity of administered dose was found in urine (54.7% total radioactive residues, TRR) with faeces only containing 9.4% TRR. Only 6.4% of the applied dose remained in the intestinal tract at sacrifice (6 hours after the final dose).

Radiolabel in the milk accounted for 1.84% of the administered dose. Milk residues reached plateau after 32 hours and peaked at 0.429 mg/kg for skim milk and 1.825 mg/kg for milk fat at day 3 (PM).

Liver tissues contained the highest residues in the tissues (1.584 mg/kg, equivalent to 1.1% of TRR). Kidney tissues contained only 0.12% of TRR (1.204 mg/kg). Residue levels were also relatively low in the muscle tissues (0.127 to 0.135 mg/kg), but higher in omental, subcutaneous and renal fats (0.243 to 0.320 mg/kg). The majority of the radioactivity was detected in the skim milk fraction (0.429 mg/kg on Day 3, PM), although the concentration in milk fat was higher (1.825 mg/kg on Day 3, PM).

Portions of all tissues (liver, kidney, muscle and fat) and the milk extract containing the highest level of residues (Day 3, PM) were analysed by HPLC or TLC within 42 days of sacrifice. Any metabolites found were identified by the use of reference standards. Characterizations of residues for non-fatty and fatty commodities in goat tissues are summarized in Table 6 and Table 7.

Table 6 Characterization of residue in goat tissues after dosage with [^{14}C] isoprothiolane for five consecutive days - % TRR (mg/kg): non-fatty commodities

Extract/metabolite	Liver		Kidney		Flank muscle		Loin muscle		Skim milk[a)]	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR	1.154	100	1.017	100	0.116	100	0.099	100	0.490	100
Initial extract [b)]	0.737	63.9	0.870	85.5	0.091	78.4	0.77	77.8	0.284	58.0
Didehydro isoprothiolane (M-4)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
Isoprothiolane	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
4-hydroxy isoprothiolane (M-3)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
Isoprothiolane monosulfoxide (M-1)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
Isoprothiolane monoester (M-2)	0.209	18.1	0.287	28.2	0.038	32.8	0.031	31.3	n.d.	n/a
Glucuronide conjugate of monoester (M-2)	n.d.	n/a	0.156	15.3	n.d.	n/a	n.d.	n/a	n.d.	n/a
Glucuronide conjugate of unknown	n.d.	n/a	0.087	8.6	n.d.	n/a	n.d.	n/a	n.d.	n/a
extractable proteins [c)]	0.528	45.8	0.340	33.4	0.053	45.7	0.046	46.5	0.284	58.0
PES	0.417	36.2	0.147	14.5	0.025	21.5	0.022	22.2	0.206	42.0
PES Characterization - Method 1: KOH Treatment										
0.1 N KOH Extract [d)]	0.021	1.8	0.094	9.2	0.015	12.9	0.012	12.1	0.206[e)]	42.0[e)]
4 N KOH Extract [d)]	0.385	33.4	0.050	4.9	0.010	8.6	0.01	10.1	n/a	n/a
Remaining solids	0.011	1.0	0.003	0.3	no PES		no PES		n/a	n/a
PES Characterization - Method 2: Pronase Treatment										
Solubilized/Hydrolysed Proteins	0.190	16.5	0.097	9.5	0.016	13.8	0.017	17.2	n/a	n/a
Soluble in Control	0.049	4.2	0.018	1.8	0.005	4.3	0.003	3.0	n/a	n/a

Extract/metabolite	Liver		Kidney		Flank muscle		Loin muscle		Skim milk[a)]	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
Remaining solids	0.178	15.4	0.032	3.1	0.004	3.4	0.002	2.0	n/a	n/a

[a] Day 3 pm

[b] Represents free residues from extraction with ACN/water (acetone/water for skim milk)

[c] Positive reaction with amino groups of proteins/peptides to purple colour by ninhydrin on TLC plate

[d] Additional extractions; shaking overnight at ambient temperature by Method 1 and shaking for 48 hr at 37 °C by Pronase

[e] PES was completely dissolved in 0.1N KOH

Table7: Characterization of residue in goat tissues after dosage with [^{14}C] isoprothiolane for five consecutive days - % TRR (mg/kg): fat containing commodities

Extract/metabolite	Milk fat[a)]		Omental fat		Subcut. fat		Renal fat	
	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR
TRR	1.492	100	0.295	100	0.218	100	0.291	100
Initial extract	1.471	98.6	0.255	86.4	0.193	88.5	0.253	86.9
Didehydro isoprothiolane (M-4)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
Isoprothiolane	n.d.	n/a	n.d.	n/a	n.d.	n/a	0.002	0.7
4-Hydroxy isoprothiolane (M-3)	n.d.	n/a	0.003	1.0	n.d.	n/a	0.002	0.7
Isoprothiolane monosulfoxide (M-1)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
Isoprothiolane monoester (M-2)	n.d.	n/a	0.036	12.2	0.052	23.9	0.036	12.4
Glucuronide conjugate of monoester (M-2)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
RT5 (Glucuronide conjugate of unknown)	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
RT22	n.d.	n/a	0.005	1.7	0.003	1.4	0.002	0.7
RT21.5	n.d.	n/a	n.d.	n/a	n.d.	n/a	0.002	0.7
RT21	n.d.	n/a	n.d.	n/a	0.003	1.4	0.001	0.3
RT4	n.d.	n/a	n.d.	n/a	0.002	0.9	n.d.	n/a
RT3 (extractable proteins)	0.150[b)]	10.1[b)]	0.003	1.0	0.006	2.8	0.005	1.7
Hexane soluble (triglycerides)[c)]	1.284	86.1	0.209	70.8	0.128	58.7	0.203	69.8
Saponification (Hydrolysis of triglycerides to fatty acids)								
Hexane phase after saponification	n.d.	n/a	n.d.	n/a	n.d.	n/a	n.d.	n/a
Aqueous phase after saponification/acidification	0.294	19.7	0.044	14.9	0.022	10.1	0.026	8.9
EtOAc phase (fatty acids) after saponification/acidification	0.990	66.4	0.165	55.9	0.106	48.6	0.177	60.8
PES		1.4		13.6		11.5		13.1
0.1 N KOH Extract[d)]	0.021	1.4	0.015	5.1	0.011	5.0	0.011	3.8
Remaining solids	-		0.025	8.5	0.014	6.4	0.027	9.3

n.d.: not detected; n/a: not applicable

[a] Day 3 pm

[b] Separated into RT3a (0.056 mg/kg; 3.8% TRR) and RT3b (0.094 mg/kg; 6.3% TRR) by TLC

[c] Represents ACN and hexane phases from ACN/hexane partition of fat extracts (ACN Phase analysed by HPLC) Radiolabel in hexane is mostly triglycerides confirmed by hydrolysis (saponification) to fatty acids

[d] Additional extractions; shaking overnight at ambient temperature

In tissues, the main metabolites were isoprothiolane monoester and unknowns at the solvent front (RT3), which following TLC and ninhydrin reaction investigation were concluded to be extractable proteins. β-glucoronidase treatment of extracts was shown to enhance the ratio of monoester, indicating the existence of a glucoronide conjugate of the monoester.

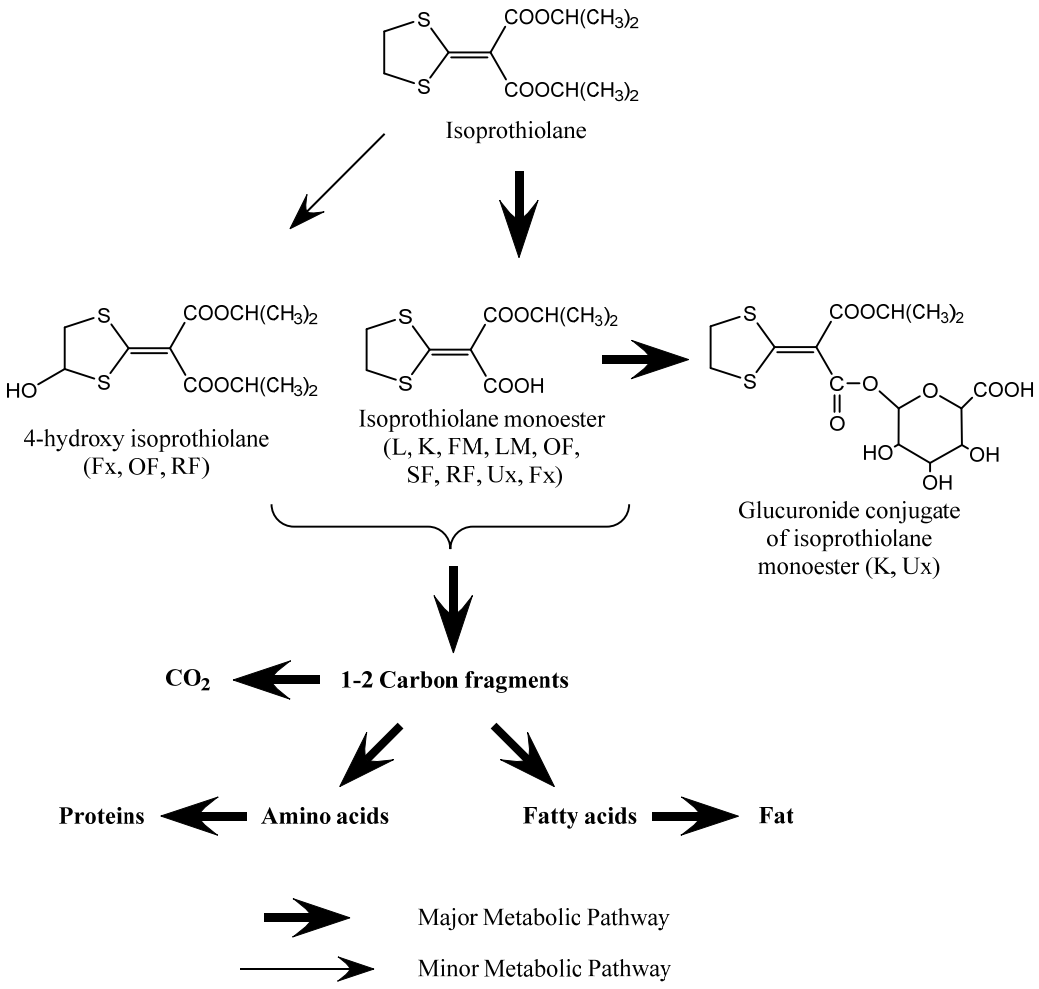
The potassium hydroxide treatment of the PES also showed that the radiolabel was incorporated into protein residues. Pronase treatment suggested that the radiolabel residues were comprised of proteins and was hydrolysed to individual amino acids by the enzyme digestion.

Skim milk and milk fat extracts with the highest residue (Day 3, PM) were analysed. Skim milk contained only the monoester and the polar residue (RT3) that had previously been shown to consist of extractable proteins. The PES was completely dissolved in potassium hydroxide and was shown to comprise precipitated biosolids as proteins. Milk fat contained the monoester at relatively low levels, with most of the radioactive residues associated with the hexane extract. The hexane

extract was saponified under alkaline conditions then acidified, prior to partition with ethyl acetate. Most of the radioactivity remained in the ethyl acetate phase, including fatty acids.

The main metabolite detected in urine was the isoprothiolane monoester and a glucoronide conjugate of the monoester. In faeces the monoester was again the major metabolite, with the remainder of the radioactivity found in the post extraction solids.

The proposed metabolic pathway for isoprothiolane in the lactating goat is shown in Figure 3.



L – liver
K-kidney
FM – flank muscle,
LM – loin muscle
OM – omental fat
SF – subcutaneous fat
RF – renal fat
Fx – faeces extract
Ux – urine extract

Figure 3 Proposed metabolic pathway of isoprothiolane in lactating goat

The major residue in tissues, fats, urine and faeces in the animal metabolism study was the isoprothiolane monoester. Isoprothiolane was mainly hydrolysed to isoprothiolane monoester, which is excreted through the kidney into urine as a glucoronide conjugate produced in the liver. Metabolites were further degraded to 1–2 carbon units, which were incorporated into natural products, such as

proteins and fats. The single carbon was considered likely to be expired as CO_2. A similar metabolic pathway was seen in metabolism of isoprothiolane in the rat.

ENVIRONMENTAL FATE

The FAO Manual on the Submission and Evaluation of Pesticide Residues Data for the Estimation of Maximum Residue Levels in Food and Feed (2009) explains the data requirements for studies of environmental fate. The focus should be on those aspects that are most relevant to MRL setting.The Meeting received studies depicting the environmental fate of isoprothiolane in soils and in water-sediments. Soil studies included a photolysis study, anaerobic soil metabolism and adsorption/desorption study. Aqueous studies included hydrolysis and anaerobic aquatic metabolism.Studies were also received on the behaviour of [^{14}C]-isoprothiolane in confined rotational crop situations.

Aqueous Hydrolysis

Solutions of aqueous buffers were prepared at pH 4, 7 and 9 at the test temperatures. [^{14}C]-isoprothiolane were applied to glass vials containing buffer to achieve final concentrations of *ca.* 1 µg/mL.

The recovery for the adsorption of the test substance to glassware was > 95% (pH 7 and 9) therefore there was no significant adsorption of isoprothiolane to these vials.

Radioactivity was determined by liquid scintillation counting (LSC). All samples were analysed for [^{14}C]-isoprothiolane and major degradation products by HPLC and selected samples were also analysed by TLC co-chromatography with authentic reference standards.

In Tier 1 test, isoprothiolane was hydrolytically stable at pH 4 and 7 after incubation for 5 days with a recovery > 92% of AR as unchanged isoprothiolane. No degradation products were detected. At pH 9 the mean recovery was 84% of AR as unchanged isoprothiolane, at 5 days with one degradation product detected at 10.2% AR.

The Tier 2 tests were conducted at pH 9. The percent of applied radioactivity as isoprothiolane, monoester, unknown fraction and unresolved background in sterile aqueous buffer (pH 9) incubated at different temperatures were presented in the submitted report.. The major degradation product observed was the monoester metabolite, which was formed to a maximum occurrence of 14.5% of AR (pH 9 at 40 °C, 22 days after treatment), 49.6% of AR (pH 9 at 50 °C, 30 days after treatment), and 81.9% of AR (pH 9 at 60 °C, 26 days after treatment).

The hydrolytic degradation rates of isoprothiolane in aqueous buffer (pH 9) are shown in Table 8. The activation energy value for hydrolysis at pH 9 was calculated to be 121.3 kJ/mol. The estimated hydrolytic degradation rates at 20 and 25 °C are shown in Table 9.

Table 8 Calculated hydrolytic DT_{50} and DT_{90} of isoprothiolane in sterile aqueous buffered at pH 9 using SFO kinetics

pH	Temperature (°C)	DT_{50} (days)	DT_{90} (days)	k (day^{-1})	Chi^2	Kinetics
9	40	147.3	489.4	0.0047	2.18	SFO
	50	25.7	85.4	0.0270	1.81	SFO
	60	9.0	30.0	0.0768	2.19	SFO

Table 9 Estimated hydrolytic DT_{50} and DT_{90} of isoprothiolane in sterile aqueous buffered at pH 9 at 20 and 25 °C

pH	Temperature (°C)	DT_{50} (days)	DT_{90} (days)	R^2
9	20 [a]	3217	10689	0.9841
	25 [a]	1395	4635	0.9841

[a] Extrapolated using Arrhenius plot and activation energy

The proposed degradation of isoprothiolane in aqueous solutions is shown in Figure 4.

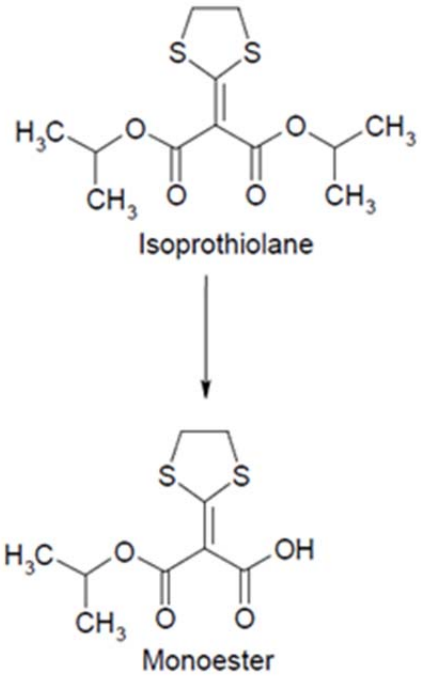


Figure 4 Proposed hydrolysis degradation route of isoprothiolane

In summary, isoprothiolane was hydrolytically stable at pH 4 and 7 over 5 days at 50 ± 0.5 °C. However, at pH 9 isoprothiolane degrades more than 10% after 5 days at 50 °C. The major hydrolytic degradate observed during the Tier 2 test was the monoester. Isoprothiolane degraded at all temperatures tested, (40, 50 and 60 °C) with DT_{50} values of 147.3, 25.7 and 9.0 days, respectively. The estimated DT_{50} values at 20 and 25 °C were > 1000 and > 3000 days, respectively.

Anaerobic aquatic metabolism

An anaerobic aquatic metabolism study was conducted with [dithiolane-4,5-^{14}C] isoprothiolane applied to two freshly collected sediment/water systems.

Individual sediment/water samples were treated with [^{14}C] isoprothiolane at a dose rate of approximately 5.0 mg/kg. The samples were incubated under anaerobic conditions at 20 ± 2 °C for 367 days and analysed at time 0, 7, 31, 62, 94, 122, 187, 276 and 367 days of incubation by duplicate. A pre-incubation period of 16 days was maintained for all samples.

Isoprothiolane and its metabolites were quantified by high performance liquid chromatography (HPLC) of the sediment extracts and water phases with co-injection of the analytical reference standards. The identity of isoprothiolane and degradates were confirmed by 2 dimensional thin layer chromatography (2D TLC).

Radiocarbon recoveries were calculated as a percent of applied radiocarbon (AR) and the averages ranged from 98.1 ± 1.8% to 98.7 ± 1.6% AR for the Goose River and Golden Lake respectively. The mass balances for both systems were presented in the submitted report. The product balance of isoprothiolane and degradates in the total system were also provided.

[^{14}C]Isoprothiolane represented an average of 94.8% AR (Goose River = GR) and 95.0% AR (Golden Lake = GL) at time 0 and declined to an average 69.7% AR and 75.3% AR respectively by the end of the study. Monoester was the only significant metabolite formed and reached an average of 6.6% AR (GR) and 13.6% AR (GL) at the end of the study. The decline of this metabolite was not observed in the length of the study. No other metabolite formed exceeded 1.8% AR at any time over the length of the study. Bound residues reached a maximum of 15.4% AR (GR) at day 276 and 6.5% AR (GL) at day 94. Radiocarbon in the NaOH traps reached a maximum of 7.4% AR (GR) and 3.8% AR (GL) at day 276. Organic volatiles did not exceed 0.7% AR at any point. Bound residues were further characterized by humic acid/fulvic acid fractionation from selected sediment samples. The dissipation rate of isoprothiolane in water was best determined using Double First Order in Parallel (DFOP). Results are shown in Table 10 for both systems. The proposed degradation pathway of isoprothiolane in soil under anaerobic conditions is shown in Figure 5.

Table 10 Degradation rates for [^{14}C]-isoprothiolane in two systems under anaerobic conditions

System	Statistic Model	DT_{50} (days)	DT_{90} (days)	χ^2	r^2	Rate constant ($days^{-1}$)			
						k SFO	**k_1** DFOP	**K_2** DFOP	g
Goose River Sediment									
GR water system	DFOP	6.5	140.5	2.758	0.999	n/a	0.161	0.007	0.751
GR total system	SFO	800.8	2660.1	2.847	0.815	0.001	n/a	n/a	n/a
Golden Lake Sediment									
GL water system	DFOP	35.8	414.4	2.321	0.997	n/a	0.082	0.004	0.449
GL total system	SFO	1117	3709	2.527	0.819	0.001	n/a	n/a	n/a

DFOP = Double First Order in Parallel

SFO = Single First Order

χ^2 = Chi-Squared

r^2 = Correlation coefficient

n/a not applicable

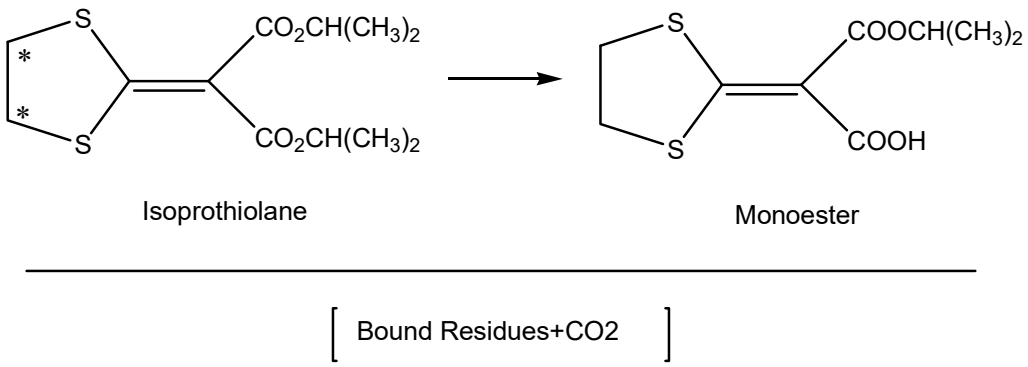


Figure 5 Proposed degradation pathway of isoprothiolane in soil under anaerobic conditions

In summary, isoprothiolane degraded very slowly in both test systems, representing at the end of the study still 69.7% AR and 75.3% AR in the Goose River and Golden Lake systems respectively. The dissipation rate of isoprothiolane in water was best fit using DFOP. The DT_{50} for isoprothiolane for the anaerobic water layers were estimated as 6.5 and 35.8 days in the Goose River and Golden Lake systems respectively. The degradation rate of isoprothiolane in total system under anaerobic conditions was determined using SFO. The DT_{50} for isoprothiolane for the anaerobic total system were estimated as 801 and 1117 days in the Goose River and Golden Lake systems respectively. The major degradate detected in the test systems was monoester, which increased steadily throughout the study reaching averages of 6.6% AR and 13.6% AR in the Goose River and Golden Lake systems respectively after 367 days. Therefore its degradation rate was not possible to be determined.

Mineralization to $^{14}CO_2$ represented a way for isoprothiolane degradation reaching averages of 6.7% AR and 3.4% AR in the Goose River and Golden Lake systems respectively at the end of the study.

Soil photolysis

A photo-degradation study on a sandy clay loam soil was conducted with [^{14}C]-Isoprothiolane for up to 16 days of continuous irradiation, at a dose rate of approximately 5.0 µg/g. Soil samples were prepared as thin layers (2 mm thick) in individual sample containers and maintained at 75% of field moisture capacity at 1/3 bar). The microbial biomass of the soil was determined prior to the experimental start as 296 µg C/g soil.

Samples were irradiated in quartz dishes with an apparatus equipped with a Xenon lamp with filters blocking infrared light and irradiation below 290 nm. The samples were subjected to continuous irradiation for up to 16 days. The average integrated intensity of the light source for the 300-800 nm range was 366 W/m^2. The average intensity for the 300–400 nm range was 41.7 W/m^2. The total irradiation period for the study was equivalent to 31 U.S. solar days (40 °N summer). Average mass balance was 101.2 ± 4.0% and 101.0 ± 3.0% AR for the irradiated and the control sets, respectively.

Isoprothiolane degraded significantly in light exposed samples and represented 85.7% AR after 72 hours of continuous irradiation, declining to an average of 57.7% AR at the end of the 16 days of exposure. The main degradation product observed in light exposed soil extracts was monosulfoxide, formed by photo-induced S-oxidation of isoprothiolane. Monosulfoxide appeared early in the study and increased to a maximum average of 9.1% AR after 24 hours of continuous irradiation, declining afterwards to an average of 4.2% AR by the end of the exposure period. Mineralization to CO_2 represented the second largest degradation product in light exposed samples and accounted for an average of 6.5% AR by the end of the study period. Bound residues represented an average of 17.2% AR in light exposed samples at the end of the study.

Representative post-extracted soil samples (both 16 days replicates) were subjected to humic acids/fulvic acids partition. The major fraction corresponded to the fulvic acids (11.1% to 12.3% AR) followed by the humic acids fraction (3.0% to 3.4% AR) and the humic residues (2.1% to 2.6% AR).

No significant degradation of isoprothiolane was observed in dark control soil extracts throughout the study period (< 1% AR). CO_2 was the main degradate observed in dark control samples and accounted for 0.6% AR at the end of the study. Formation of bound residues was the major degradation pathway in dark control samples, representing maximum average of 6.9% AR after 10 days of incubation.

The degradation rate of isoprothiolane in soils under artificial light irradiation and in dark controls was determined based on the percent isoprothiolane present in the extracts. The degradation rate of the metabolite monosulfoxide was also estimated. DT_{50} and DT_{90} values were calculated using the Single First Order (SFO) kinetics. The net photo-degradation of isoprothiolane (calculated as the degradation under irradiation minus the degradation occurred in dark conditions) was also calculated. The results are summarised in Table 11.

Table 11 Degradation rates for [^{14}C]-isoprothiolane and its metabolite, monosulfoxide

Sample set	k (hr^{-1})	DT_{50} (days)	DT_{90} (days)	r^2
[^{14}C] Isoprothiolane Irradiated	0.0012	24.1	80.0	0.9167
[^{14}C] Isoprothiolane Control	0.0002	185.3	615.1	0.4281
[^{14}C] Isoprothiolane Net degradation	0.0010	27.8	92.2	0.9167
Monosulfoxide Irradiated	0.0023	12.6	41.8	0.6398

The proposed degradation of isoprothiolane in soil under artificial light is shown in Figure 6.

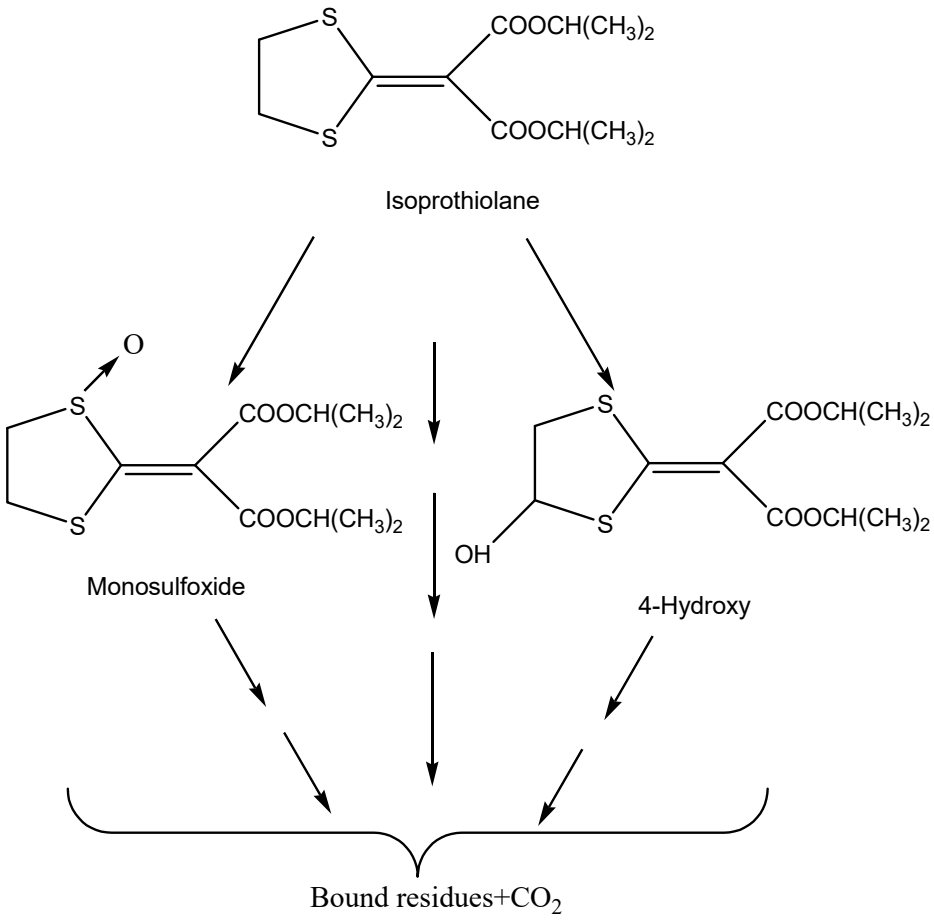


Figure 6 Proposed degradation pathway of isoprothiolane following photolysis in soil

In summary, isoprothiolane degraded moderately in light exposed samples with a half-life of 47 U.S. Summer Day at 40 °N Latitude (300–800 nm) or 40 Summer Day at 30–50°N Latitude (300-400 nm) and represented an average of 57.7% AR at the end of the 16 days of exposure. CO_2 and bound residues were major degradates in the photolysis of isoprothiolane in soil (6.5% AR and 17.2% AR respectively at the end of the irradiation period). Isoprothiolane also underwent photo-induced S-oxidation to monosulfoxide in light exposed samples (maximum of 9.1% AR at 24 hours). The decline of the monosulfoxide degradate was observed during the exposure period. The half-life of monosulfoxide was calculated as 24 U.S. Summer Day at 40°N Latitude (300–800 nm) or 21 Summer Day at 30–50°N Latitude (300-400 nm).

Isoprothiolane degraded very slowly in the dark control samples to mainly bound residues. At time zero monosulfoxide was present as result of the dose impurity and no additional formation of this or any other degradates was observed in dark control samples during the study period.

Anaerobic soil metabolism

An anaerobic soil metabolism study was conducted on four soils using [dithiolane-4,5-^{14}C] isoprothiolane and aged in the dark under aerobic conditions at 25 °C for 30 days. The target dose for all samples was 5 ppm based on dry soil weight. The final dose rates averaged 4.64 ppm for all soils tested. A foam plug and aqueous 10% NaOH solution trap were used to trap organic volatiles and CO_2, respectively.

Aerobic samples were collected immediately after treatment (Time 0) and after 30 days of incubation. Flooded samples were collected in duplicate following 7, 31, 61, 94, 121, 209, and 272 days after flooding.

Isoprothiolane and its degradates were quantified by high performance liquid chromatography (HPLC) of soil extracts and water phases with co-injection of the analytical reference standards. The identity of isoprothiolane and degradates were confirmed by two dimensional thin layer chromatography (2D TLC).

Radiocarbon recoveries were calculated as a percent of applied radiocarbon (AR) and the averages ranged from 93.9 ± 2.2% to 97.4 ± 1.5% AR for the four soils tested. Isoprothiolane degraded slowly during the aerobic portion of the study and ranged from an average of 68.1% to 79.2% AR in all soils after 30 days of aerobic incubation. Minor degradates observed in the soil extracts were isoprothiolane monoester and monosulfoxide, both present as < 4% AR in all test systems. Bound residues ranged from an average of 7.3% to 19.7% AR following the aerobic phase of the study. Radiocarbon in the NaOH traps ranged from 2.6% to 7.1% AR in all soils after 30 days of aerobic incubation.

Isoprothiolane degraded slowly over the course of the anaerobic phase of the experiment, ranging from 55.3% to 77.8% AR at the end of 4 months and from 29.2% to 68.7% AR at the end of the study. The primary degradates observed in soil extracts and water phases during the anaerobic phase study were isoprothiolane monosulfoxide (maximum average of 9.4% AR) and the monoester (maximum average of 18.2% AR). Major degradates in the study included CO_2 (up to a maximum average of 25.6% AR) and soil bound residues (up to 29.1% AR).

Bound residues in three of the soils were > 10%AR and were further characterized by humic acid/fulvic acid fractionation. In all cases, the smallest fraction was the humic acid fraction, which ranged from 1.4% to 2.4% AR. Radiocarbon associated with the fulvic acid fraction ranged from 5.6% to 13.5% AR while the radiocarbon remaining in the insoluble humins accounted for 6.3% to 13.2% AR.

The degradation rate of isoprothiolane in soil under aerobic conditions was determined based on the percent isoprothiolane in soil extracts during the aerobic incubation and the half-life ranged from 61.3 to 94.9 days. The DT_{50} for isoprothiolane under anaerobic flooded conditions ranged from 182 to 990 days for all soils tested. Table 12 summarises the results for all soils in the aerobic and anaerobic phase. DT_{50} and DT_{90} values were calculated using the Single First Order (SFO) kinetics.

The proposed degradation pathway of isoprothiolane in soil under anaerobic flooded conditions is shown in Figure 7.

Table12 Degradation rates for [^{14}C]-isoprothiolane in four soils under aerobic and anaerobic phase

Soil set	Rate constant ($days^{-1}$)	DT_{50} (days)	DT_{90} (days)	Correlation coefficient (r^2)
Aerobic soil				
Hanford	0.0073	95	315	0.938
MSL	0.0085	82	271	0.972
OE	0.0113	61	204	0.986
KD	0.0073	95	315	0.998
Anaerobic soil				
Hanford	0.0007	990	3289	0.735
MSL	0.0011	630	2093	0.720
OE	0.0022	315	1047	0.888
KD	0.0038	182	606	0.923

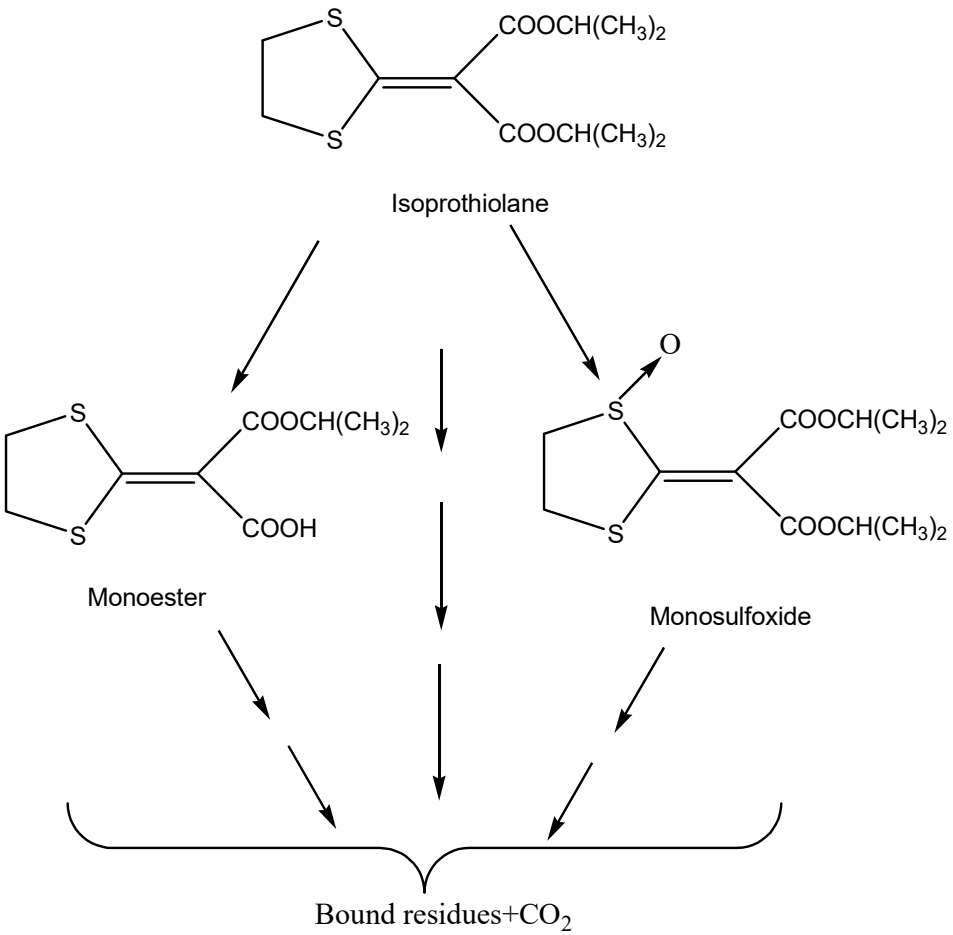


Figure 7 Proposed degradation pathway of isoprothiolane in soil under anaerobic conditions

In summary, an anaerobic soil metabolism study was conducted on four soils with [^{14}C]isoprothiolane at an average dose rate of 4.64 ppm based on the dry soil weight equivalent. Average material balance ranged from 93.9 ± 2.2% and 97.4 ± 1.5% AR for all soil sets. Isoprothiolane degraded slowly over the course of the experiment, ranging from 68.1% to 79.2% AR at the end of the aerobic phase of the study. Isoprothiolane ranged from 55.3% to 77.8% AR at the end of 4 months of anaerobic incubation and 29.2% to 68.7% AR at the end of the study (9 months). The primary degradates observed in the study were isoprothiolane monosulfoxide and monoester (maximum averages of 9.4% and 18.2% AR, respectively), CO_2 (up to a maximum average of 25.6% AR) and soil bound residues (up to 29.1% AR). The half-life of isoprothiolane during the aerobic phase ranged from 61.3 to 94.9 days, while the DT_{50} for isoprothiolane under anaerobic flooded conditions ranged from 182 to 990 days for all soils tested.

Confined Rotational Crops

The Meeting received data from a radiolabelled, confined study inlettuce, radish and wheat representing the rotational crop groups of leafy crops, root crops and cereal grains.

In uptake and metabolism study examining residues in confined rotational crops (Ahn, K.C., Report R-2139), [dithiolane-4,5-^{14}C]-isoprothiolane was investigated. Labelled isoprothiolanewas applied to outdoor sandy loam soil at a rate of 1937 g ai/ha. Rotational crops of lettuce, radish and wheat were planted into the treated soil 30, 120 and 365 days after application.

Onto the plots, lettuce, radish and wheat were directly seeded. Lettuce was collected at immature and mature growth stages, along with radish roots/tops and wheat forage, hay, straw and grain. Soil cores (top 7.6 cm, middle 7.6 cm and bottom 7.6–15,2cm inch layer) were also collected.

Crop samples were homogenized and combusted prior to liquid scintillation counting to quantify TRR.

No residues were detected in any of the control samples analysed. It's found that all samples from treated plots contained residues above 0.05 mg/kg. In general, residues declined with increasing plant-back intervals, the only exception being wheat forage, straw and grain, where higher levels of residues were detected in the 120 day samples than that in the 30 day samples.

The leafy vegetable sample contained the highest radioactive residues present (0.85 mg eq/kg) was the mature lettuce sample at the 30 day sampling interval. The highest residues in the root crops were 30 day radish tops (3.1 mg eq/kg). Generally, higher residues were found in the upper portions of the plant compared to the roots, indicating the uptake potential of isoprothiolane from soil to the plant.

Among all crops, the highest residues were found in wheat samples with up to 10 mg/kg found at 30 days and 18 mg/kg at 120 days for straw, decreasing significantly to 0.5 mg/kg around at the 365-days.

Extractability of residues in the initial extraction (acetonitrile/water and acetonitrile) varied from *ca.* 36 to 91% TRR. Extractability was low in radish roots and wheat commodities, suggesting bound residues in these matrices. Further extraction with weak acidic solvent extracted up to 10% TRR and a weak basic solvent system up to a further 5% TRR. The post extraction solids contained 7.5 to 57.3% TRR.

Parent isoprothiolane was found mainly at the earlier plant back intervals and generally at low levels in all matrices by 365 days, with the exception of radish roots. The extractable residues were predominantly glycoside conjugates of 4-hydroxy, hydroxyl isopropyl and polar components (solvent front) which were proposed to be protein/peptide residues, including free amines, which were not retained by reverse phase HPLC and gave positive ninhydrin reactions.

Residues in immature and mature lettuce were characterised as a mixture of components with retention times ranging between 23 to 29 minutes (up to 52% TRR). The identity of the components was confirmed by HPLC and TLC as monoester or hydroxyl isopropyl in the conjugates (cellulase hydrolysis). A significant amount of the detected radioactivity was also associated with the polar solvent front (up to 46% TRR at 365 days), along with smaller amounts of unassigned components. The post extraction solids (PES) accounted for bound residues up to a maximum of 21% TRR.

Parent isoprothiolane was detected in radish samples, occurring around 25% TRR at the 30 day interval in both roots and tops. The monoester of isoprothiolane was also detected in both sample types, but at lower levels (up to *ca.* 8% TRR). Hydroxyl isopropyl or 4-hydroxy metabolites were confirmed mainly in the radish roots. The polar front again accounted for a significant proportion of the radioactivity, with up to 47% TRR at 365 days in the radish tops. The PES accounted for around 35% TRR in the radish roots, but less in the radish tops, at around 8% TRR.

Isoprothiolane was found in wheat forage (up to 16% TRR) and straw (up to 6% TRR), but not in grain. The monoester was found in forage and straw, but at relatively low levels. Components were again found around retention time 21 to 29 minutes and were confirmed as either monoester or hydroxyl isopropyl in the forage, hay and straw, but not in the grain. Significant amounts of radioactivity were associated with the solvent front as for other matrices, with up to 65% TRR at 365 days (hay). Unassigned other peaks were noted in all wheat matrices, particularly at and beyond the 120 plant-back interval. The PES again accounted a significant proportion of the TRR, with up to 57% of the total radioactivity at 365 days in the grain extracts.

Isoprothiolane was mainly hydroxylated to 4-hydroxy and hydroxyl isopropyl, followed by conjugation to glycosides as major forms. Didehydro and monosulfoxide metabolites were only minor. Production of the monoester by hydrolysis was only seen in radish roots. Metabolites were further degraded and incorporated into natural products, such as proteins (indicated by ninhydrin reaction). Significant amounts of bound residues were present in post extraction solids (PES). TRR of

isoprothiolane in tested crops for 30, 120, and 365 plant back intervals (PBIs) were summarized in Table 13. Distribution of [^{14}C] isoprothiolane radioactivity in the extracts was shown in Table 14.

Table 13 Uptake and distribution of residues of isoprothiolane in rotational crops after bare soil application of [^{14}C] isoprothiolane

Sample Type	PBI (days)	TRR (mg/kg eq)[a]
Lettuce (immature)	30	0.647 (± 0.011)
	120	0.306 (± 0.011)
	365	0.156 (± 0.003)
Lettuce (mature)	30	0.851 (± 0.021)
	120	0.238 (± 0.011)
	365	0.066 (± 0.003)
Radish (roots)	30	1.069 (± 0.110)
	120	0.110 (± 0.008)
	365	0.100 (± 0.010)
Radish (tops)	30	3.102 (± 0.337)
	120	0.287 (± 0.010)
	365	0.124 (± 0.007)
Wheat (forage)	30	5.731 (± 0.111)
	120	6.005 (± 0.088)
	365	0.188 (± 0.005)
Wheat (hay)	30	8.811 (± 0.218)
	120	4.915 (± 0.140)
	365	0.120 (± 0.005)
Wheat (straw)	30	9.764 (± 0.693)
	120	17.701 (± 0.564)
	365	0.442 (± 0.024)
Wheat (grain)	30	4.022 (± 0.333)
	120	5.957 (± 0.072)
	365	0.139 (± 0.002)

[a] Mean ± standard deviation from five replicate analyses

Table 14 Distribution of radioactive residues following extraction in rotational crops after bare soil application of [^{14}C] isoprothiolane

Sample Type	PBI (days)	Combined ACN:H_2O extract		0.2N HCl/ACN (1:1) extract		0.2N NH_4OH/ACN (1:1) extract		PES		TRR
		mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Immature lettuce	30	0.567	88.5	0.005	0.8	0.008	1.2	0.061	9.5	0.641
	120	0.259	84.4	0.003	1.0	0.003	1.0	0.042	13.7	0.307
	365	0.115	79.9	0.002	1.4	0.002	1.4	0.025	17.4	0.144
Mature lettuce	30	0.669	81.1	0.014	1.7	0.013	1.6	0.129	15.36	0.825
	120	0.187	87.4	0.003	1.4	0.003	1.4	0.021	9.8	0.214
	365	0043	75.4	0.001	1.8	0.001	1.8	0.012	21.1	0.057
Radish roots	30	0.973	59.1	0.060	3.6	0.025	1.5	0.587	35.7	1.645
	120	0.070	65.4	0.000[1)]	0.0	0.002	1.9	0.035	32.7	0.107
	365	0.054	54.0	0.002	2.0	0.002	2.0	0.042	42.0	0.100
Radish tops	30	2.611	90.7	0.023	0.8	0.030	1.0	0.216	7.5	2.880
	120	0.239	88.8	0.006	2.2	0.003	1.1	0.021	7.8	0.269
	365	0.102	87.9	0.003	2.6	0.001	0.9	0.010	8.6	0.116
Wheat forage	30	4.670	82.2	0.138	2.4	0.078	1.4	0.794	14.0	5.680
	120	4.332	68.2	0.247	3.9	0.144	2.3	1.630	25.7	6.353
	365	0.128	71.5	0.008	4.5	0.005	2.8	0.038	21.2	0.179
Wheat hay	30	5.946	67.6	0.336	3.8	0.258	2.9	2.260	25.7	8.800
	120	3.461	67.6	0.201	3.9	0.102	2.0	1.356	26.5	5.120
	365	0.076	68.5	0.005	4.5	0.003	2.7	0.027	24.3	0.111

Sample Type	PBI (days)	Combined ACN:H_2O extract		0.2N HCl/ACN (1:1) extract		0.2N NH_4OH/ACN (1:1) extract		PES		TRR
		mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Wheat straw	30	6.712	62.7	0.584	5.5	0.217	2.0	3.200	29.9	10.713
	120	11.456	66.8	0.805	4.7	0.337	2.0	4.540	36.5	17.138
	365	0.236	54.3	0.044	10.1	0.023	5.3	0.132	30.3	0.435
Wheat grain	30	1.537	44.3	0.265	7.6	0.084	2.4	1.582	45.6	3.468
	120	2.300	35.8	0.320	5.0	0.123	1.9	3.686	57.3	6.429
	365	0.051	36.4	0.006	4.3	0.003	2.1	0.080	57.1	0.140

Identification of metabolites

All samples containing greater than 0.01 mg/kg were extracted in mixtures of acetonitrile/ water, followed by extraction in acid and basic mixtures (0.1*N* HCl, 0.1N ammonium hydroxide in acetonitrile). Extracts were analysed by reverse phase HPLC.

Summaries of the characterization of different sample extracts from the three rotational crops investigated are presented in Tables 15 to 22.

Table 15 Identification of metabolic profile of isoprothiolane in lettuce (immature)

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	0.641	100	0.307	100	0.144	100
Combined ACN/H_2O extract	0.567	88.5	0.259	84.4	0.115	79.9
Didehydro （M-4）	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	0.020	3.1	0.003	1.0	n.d.	n.d.
4-Hydroxy (M-3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Monosulfoxide (M-1)	n.d.	n.d.	0.002	0.7	n.d.	n.d.
Monoester （M-2） [a)]	n.d.	n.d.	0.007	2.3	0.001	0.7
Hydroxyl-Isopropyl (M-5) and its conjugate	0.40	62.7	0.126	41.1	0.023	16.0
Unassigned (RT 4.5)	n.d.	n.d.	0.027	8.8	n.d.	n.d.
Polar solvent front [b)]	0.107	16.7	0.051	16.6	0.057	39.6
Unassigned others	0.039	6.1	0.044	14.3	0.034	23.6
Max other single	0.014	2.2	0.009	2.9	0.007	4.9
0.2N HCl/ACN extract	0.005	0.8	0.003	1.0	0.002	1.4
0.2 N NH_4OH/ACN extract	0.008	1.2	0.003	1.0	0.002	1.4
PES	0.061	9.5	0.042	13.7	0.025	17.4

n.d. not detected

[a] Not confirmed due to a trace amount

[b] Extractable protein/peptide residues

Table 16 Identification of metabolic profile of isoprothiolane in lettuce (mature)

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	0.825	100	0.214	100	0.057	100
Combined ACN/H2O extract	0.669	81.1	0.187	87.4	0.043	75.4
Didehydro (M-4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	0.011	1.3	n.d.	n.d.	n.d.	n.d.
4-Hydroxy (M-3)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Monosulfoxide (M-1)	n.d.	n.d.	0.003	1.4	n.d.	n.d.
Monoester (M-2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
Hydroxyl-Isopropyl (M-5) conjugate	0.41	49.2	0.064	29.9	n.d.	n.d.
Polar solvent front [a)]	0.122	14.8	0.094	43.9	0.026	45.6
Unassigned others	0.131	15.9	0.026	12.1	0.017	29.8
Max other single	0.026	3.2	0.007	3.3	0.005	8.8
0.2N HCl/ACN extract	0.014	1.7	0.003	1.4	0.001	1.8
0.2 N NH4OH/ACN extract	0.013	1.6	0.003	1.4	0.001	1.8
PES	0.129	15.6	0.021	9.8	0.012	21.1

n.d. not detected

[a] Extractable protein/peptide residues

Table 17 Identification of metabolic profile of isoprothiolane in radish roots

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	1.645	100	0.107	100	0.100	100
Combined ACN/H2O extract	0.973	59.1	0.070	65.4	0.054	54.0
Didehydro (M-4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	0.469	28.5	0.014	13.1	0.017	17.0
4-Hydroxy (M-3)	n.d.	n.d.	n.d.	n.d.	0.002	2.0
Monosulfoxide (M-1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Monoester (M-2) [a)]	0.126	7.7	0.004	3.7	0.002	2.0
Unkown RT 23.2	0.071	4.3	n.d.	n.d.	n.d.	n.d.
Unkown RT 18.9	0.027	1.6	n.d.	n.d.	n.d.	n.d.
Polar solvent front [a)]	0.281	17.1	0.027	25.2	0.017	17.0
Unassigned others	n.d.	n.d.	0.024	22.4	0.016	16.0
Max other single	n.d.	n.d.	0.004	3.7	0.003	3.0
0.2N HCl/ACN extract	0.060	3.6	0.000	0.0	0.002	2.0
0.2 N NH4OH/ACN extract	0.025	1.5	0.02	1.9	0.002	2.0
PES	0.587	35.7	0.035	32.7	0.042	42.0

n.d. not detected

[a] Extractable protein/peptide residues

Table 18 Identification of metabolic profile of isoprothiolane in radish tops

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	2.880	100	0.269	100	0.116	100
Combined ACN/H2O extract	2.611	90.7	0.239	88.8	0.102	87.9
Didehydro (M-4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	0.689	23.9	0.15	5.6	0.002	1.7
4-Hydroxy (M-3) and it conjugates	1.265	43.9	0.082	30.4	0.018	15.6
Monosulfoxide (M-1)	0.112	3.9	0.006	2.32	0.001	0.9
Monoester (M-2) [a)]	0.115	4.0	0.005	1.9	0.002	1.7
Hydroxyl Isopropyl (M-5) conjugates	0.040	1.4	n.d.	n.d.	n.d.	n.d.
Polar solvent front [a)]	0.389	13.5	0.100	37.2	0.054	46.6
Unassigned others	n.d.	n.d.	0.030	11.2	0.025	21.6
Max other single	n.d.	n.d.	0.007	2.6	0.008	6.9
0.2N HCl/ACN extract	0.023	0.8	0.006	2.2	0.003	2.6
0.2 N NH4OH/ACN extract	0.030	1.0	0.003	1.1	0.001	0.9
PES	0.216	7.5	0.021	7.9	0.010	8.6

n.d. not detected

[a] Extractable protein/peptide residues

Table 19 Identification of metabolic profile of isoprothiolane in wheat forage

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	5.680	100	6.353	100	0.179	100
Combined ACN/H_2O extract	4.67	82.2	4.332	68.2	0.128	71.5
Didehydro (M-4)	0.103	1.8	0.035	0.6	n.d.	n.d.
Isoprothiolane	0.929	16.4	0.273	4.3	0.001	0.6
4-Hydroxy (M-3) and its conjugate	0.677	11.9	0.069	1.1	n.d.	n.d.
Monosulfoxide (M-1)	0.163	2.9	0.030	0.5	0.001	0.6
Monoester (M-2)	0.462	8.1	0.030	0.5	0.005	2.8
4-Hydroxy and hydroxyl isopropyl conjugates (M3+M5)	0.514	9.0	0.208	3.3	n.d.	n.d.
Polar solvent front [a)]	1.826	32.1	2.244	35.3	0.066	36.9
Unassigned others	n.d.	n.d.	1.209	19.0	0.056	31.3
Max other single	n.d.	n.d.	0.113	1.8	0.004	2.2
0.2N HCl/ACN extract	0.138	2.4	0.247	3.9	0.008	4.5
0.2 N NH4OH/ACN extract	0.078	1.4	0.144	2.3	0.005	2.8
PES	0.794	14.0	1.630	25.7	0.038	21.2

n.d. not detected

[a] Extractable protein/peptide residues

Table 20 Identification of metabolic profile of isoprothiolane in wheat hay

	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	8.800	100	5,120	100	0.111	100
Combined ACN/H2O extract	5.946	67.6	3.461	67.6	0.076	68.5
Didehydro (M-4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	0.547	6.2	0.062	1.2	n.d.	n.d.
4-Hydroxy (M-3)	n.d.	n.d.	0.038	0.7	n.d.	n.d.
Monosulfoxide (M-1)	0.309	3.5	0.059	1.2	n.d.	n.d.
Monoester (M-2)	0.155	1.8	0.031	0.6	n.d.	n.d.
4-Hydroxy (M-3) conjugate	0.731	8.3	0.190	3.7	n.d.	n.d.
4-Hydroxy and Hydroxyl Isopropyl conjugates (M3+M5)	0.886	10.1	0.163	3.2	n.d.	n.d.
Protein residue (RT 4.0-4.5)	0.333	3.8	0.128	2.5	n.d.	n.d.
Polar solvent front [a)]	2.985	33.9	2.174	42.5	0.072	64.9
Unassigned others	n.d.	n.d.	0.616	12.0	0.004	3.6
Max other single	n.d.	n.d.	0.066	1.3	0.002	1.8
0.2N HCl/ACN extract	0.336	3.8	0.201	3.9	0.005	4.5
0.2 N NH4OH/ACN extract	0.258	2.9	0.102	2.0	0.003	2.7
PES	2.260	25.7	1.356	26.5	0.027	24.3

n.d. not detected

[a] Extractable protein/peptide residues

Table 21 Identification of metabolic profile of isoprothiolane in wheat straw

TRR(mg/kg)[a)]	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	10.713	100	17.138	100	0.435	100
Combined ACN/H2O extract	6.712	62.7	11.456	66.8	0.236	54.3
Didehydro (M-4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	n.d.	n.d.	0.080	0.5	n.d.	n.d.
4-Hydroxy (M-3) and its conjugate	0.886	8.3	0.641	3.8	n.d.	n.d.
Monosulfoxide (M-1)	n.d.	n.d.	0.115	0.7	n.d.	n.d.
Monoester (M-2)	n.d.	n.d.	0.09	0.4	n.d.	n.d.
Unkown (RT 3.7)	0.651	6.1	n.d.	n.d.	n.d.	n.d.
Unknown (RT 3.5)	0.819	7.6	n.d.	n.d.	n.d.	n.d.
Polar solvent front [a)]	4.349	40.6	4.949	28.9	0.211	48.5
Unassigned others	n.d.	n.d.	5.602	32.7	0.025	5.7
Max other single	n.d.	n.d.	0.286	1.7	0.007	1.6
0.2N HCl/ACN extract	0.584	5.5	0.805	4.7	0.044	10.1
0.2 N NH4OH/ACN extract	0.217	2.0	0.337	2.0	0.023	5.3
PES	3.200	29.9	4.450	26.5	0.132	30.3

n.d. not detected

[a] Extractable protein/peptide residues

Table 22 Identification of metabolic profile of isoprothiolane in wheat grain

TRR(mg/kg)[a)]	Plant Back Interval (days)					
	30 DAT		120 DAT		365 DAT	
	mg eq/kg	%TRR	mg eq/kg	%TRR	mg eq/kg	%TRR
TRR (by extraction)	3.468	100	6.429	100	0.140	100
Combined ACN/H_2O extract	1.537	44.3	2.300	35.8	0.051	36.4
Didehydro （M-4）	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isoprothiolane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Hydroxy （M-3）	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Monosulfoxide (M-1)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Monoester (M-2)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Polar solvent front [a)]	1.537	44.3	1.594	24.8	0.051	36.4
Unassigned others	n.d.	n.d.	0.706	11.0	n.d.	n.d.
Max other single	n.d.	n.d.	0.131	2.0	n.d.	n.d.
0.2N HCl/ACN extract	0.265	7.6	0.320	0.006	0.006	4.3
0.2 N NH_4OH/ACN extract	0.084	2.4	0.123	0.003	0.003	2.1
PES	1.582	45.6	3.686	0.080	0.080	57.1

n.d. not detected

[a] Extractable protein/peptide residues

The metabolism of isoprothiolane in target and rotational crops appears to be similar. The proposed metabolic pathway for isoprothiolane in those crops is shown in Figure 8.

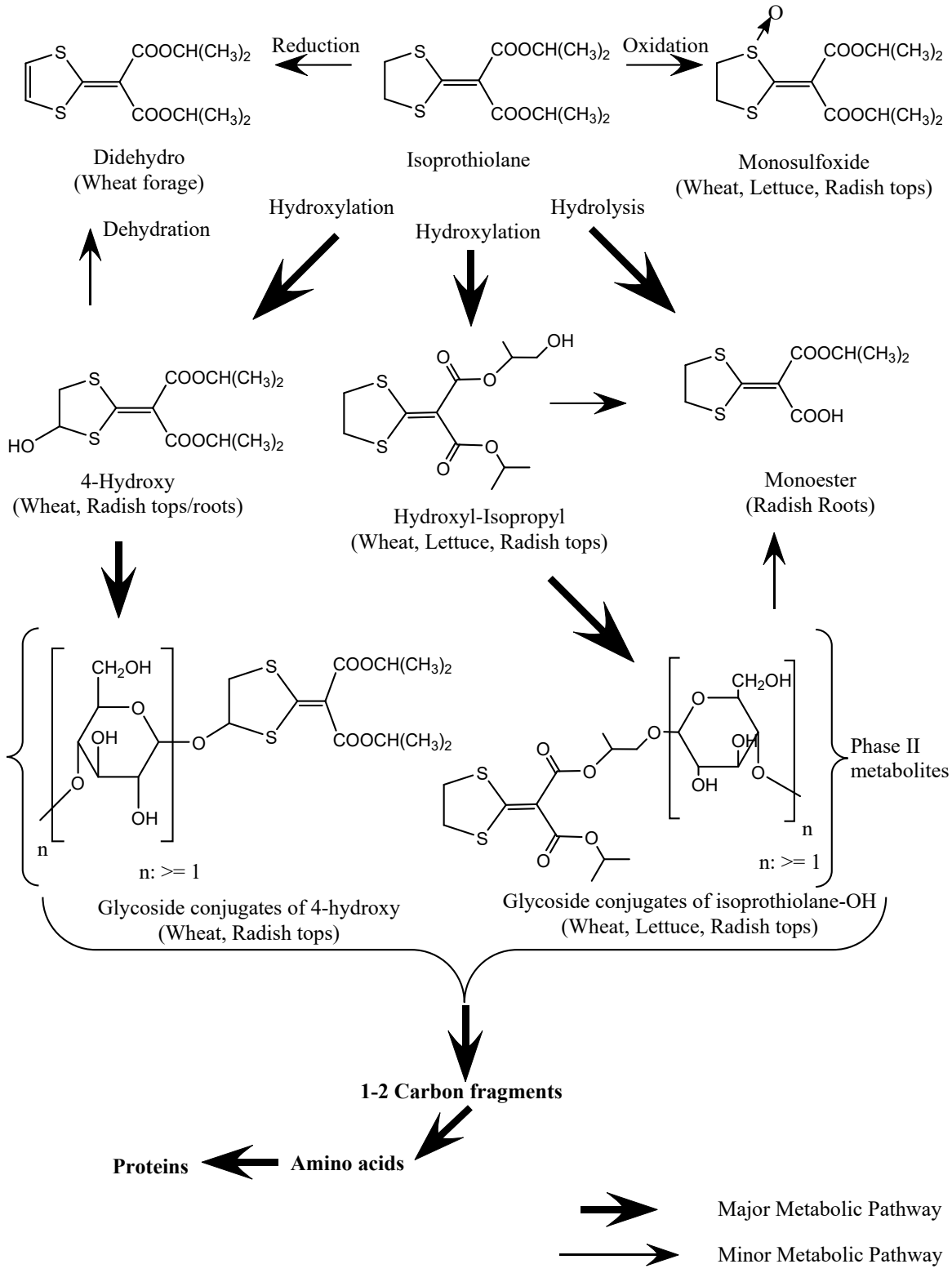


Figure 8 Proposed metabolic profile of isoprothiolane in rotational crops

In the above confined rotational crop trials, isoprothiolane was mainly hydroxylated to 4-hydroxy and hydroxyl isopropyl, followed by conjugation to glycosides as major forms. Didehydro and monosulfoxide metabolites were only minor in all matrices. Production of the monoester by hydrolysis was only seen in radish roots. Significant amounts of bound residues were present in post extraction solids (PES). All metabolites were further degraded and incorporated into natural products, such as proteins.

Environmental fatesSummary

Isoprothiolane could be expected to dissipate rather rapidly in the environment. It is susceptible to hydrolysis and photolysis, especially under acidic or alkaline conditions. Mineralisation to CO_2 appears to be a significant dissipation pathway in soil.

Isoprothiolane residues are not persistent in soils and it is unlikely that isoprothiolane residues in soils resulting from recommended uses make a significant contribution to the residues in succeeding crops.

METHODS OF RESIDUE ANALYSIS

Analytical methods

The Meeting received analytical method descriptions and validation data for isoprothiolane in plant and animal matrices and these are summarised below Table 23 to Table 29.

Table 23 Overview of the analytical methods submitted for isoprothiolane.

Report ID *Method ID*	Matrix	Analytes	Extraction	Clean-up	Separation/ Analysis/LOQ [a)]
Data Generation					
R-2024	Rice grain Rice straw	Isoprothiolane	benzene/acetone (1/1, v/v)	Partition against hexane/acetonitrile by liquid liquid extraction	GC-ECD LOD: 0.005 mg/kg for isoprothiolane in rice grain and 0.02 mg/kg for isoprothiolane in rice straw LOQ: not assessed; Lowest fortified level: 0.1 mg/kg for rice grain and 0.2 mg/kg for rice straw
R-2055 R-2082 R-2059	Rice grain	Isoprothiolane	acetonitrile	polymer cartridge column sequentially washed with acetonitrile and water	LC-MS LOQ: 0.01 mg/kg for isoprothiolane in rice grain
R-2007	Milk	Isoprothiolane	Methanol (with sodium oxalate)	Partition against hexane/acetonitrile by liquid liquid extraction	GC-ECD LOQ： 0.005 mg/kg
Enforcement					
Poulsen, M.E., 2010	Rice grain	Isoprothiolane	Cold water and acetonitrile	PSA based dispersive SPE clean-up	GC-MS/MS LOQ: 0.01 mg/kg
Poulsen, M.E., 2012	Wheat, oat, rye, barley, rice (grain)	Isoprothiolane	Buffered acetonitrile	PSA based dispersive SPE clean-up	LC-MS/MS （internal standard quantification） LOQ: 0.01 mg/kg
Poulsen, M.E., 2013	Wheat, oat, rye, barley, rice (grain)	Isoprothiolane	Buffered acetonitrile	PSA based dispersive SPE clean-up	GC-MS/MS LOQ: 0.01 mg/kg

[a] Defined by the lowest limit of method validation

Table 24 Method validation results for isoprothiolane using Method in Report R-2024

Sample material	Fortification level, mg/kg	Recovery, %	Mean recovery, %	RSD, %
Rice grain	0.1	91.2, 97.9, 96.4	95	3.7
Rice straw	0.2	78, 83	81	n.a.

Table25 Results of laboratory recovery test of isoprothiolane using method in Report 2055, R-2082. R-2059

Sample material	Fortification level, mg/kg	Recovery, % (replicates)	Mean recovery, %	RSD, %
Rice grain	0.01	86-111 (n=24)	100	7.2
	2	104-108 (n=12)	106	1.7
	4	96-111 (n=12)	103	5.0

Table 26 Results of laboratory recovery test of isoprothiolane using Method in Report R-2007

Sample material	Fortification level, mg/kg	Recovery, % (replicates)	Mean recovery, %	RSD, %
Milk	0.005	120 (n=1)	n.a.	n.a.
	0.01	80 (n=1)	n.a.	n.a.
	0.05	74 (n=1)	n.a.	n.a.
	0.1	84 (n=1)	n.a.	n.a.
	0.5	89 (n=1)	n.a.	n.a.
	1	82 (n=1)	n.a.	n.a.

Table 27 Method recoveries for isoprothiolane using enforcement Method: Poulsen, 2010

Sample material	Fortification level, mg/kg	Mean recovery, % (replicates)	RSD, %
Rice grain	0.01	105 (n=5)	7.8
	0.02	88 (n=5)	8.8
	0.1	88 (n=5)	6.7

Table 28 Method recoveries for isoprothiolane using enforcement Method: Poulsen, 2012

Sample material	Fortification level, mg/kg	Mean recovery, % (replicates)	RSD, %
Wheat (grain)	0.01	96 (n=5)	27
	0.02	106 (n=5)	6
	0.1	106 (n=5)	14
Oat (grain)	0.01	112 (n=5)	7
	0.02	100 (n=5)	6
	0.1	97 (n=5)	6
Rye (grain)	0.01	112 (n=5)	9
	0.02	106 (n=5)	5
	0.1	99 (n=5)	12
Barley (grain)	0.01	110 (n=5)	8
	0.02	101 (n=5)	8
	0.1	95 (n=5)	3
Rice (grain)	0.01	115 (n=5)	9
	0.02	103 (n=5)	6
	0.1	101 (n=5)	4

Table 29 Method recoveries for isoprothiolane using enforcement Method: Poulsen, 2013

Sample material	Fortification level, mg/kg	Mean recovery, % (replicates)	RSD, %
Wheat (grain)	0.01	105 (n=5)	24
	0.02	108 (n=5)	6
	0.1	101 (n=5)	12
Oat (grain)	0.01	96 (n=5)	3
	0.02	79 (n=5)	7
	0.1	92 (n=5)	4
Rye (grain)	0.01	106 (n=5)	6
	0.02	95 (n=5)	11
	0.1	76 (n=5)	10
Barley (grain)	0.01	83 (n=5)	17
	0.02	84 (n=5)	11
	0.1	77 (n=5)	6
Rice (grain)	0.01	119 (n=5)	10
	0.02	116 (n=5)	3
	0.1	113 (n=5)	4

The provided analytical methods are suitable for the analysis of isoprothiolane. Recoveries were within the generally acceptable range of 70–120% and relative standard deviations were less than 20%.

Stability of residues in stored samples

The Meeting received studies depicting the stability of residues of isoprothiolane in rice grain (Report R-2055, R-2082, R-2059).

Rice grain samples were homogenized and fortified at 0.5 mg/kg with isoprothiolane standard solution (5 µg/mL), stored in a freezer at -20 °C. Storage stability was determined concurrently with the residues trials. Procedural recoveries were made with the analyses for each of the trial sites and these demonstrated the effective performance of the method at the time that the analyses were performed. Two samples were analysed to demonstrate the stability of the isoprothiolane residues in samples from each site. All results demonstrated good stability under the conditions of the test.

Table 30 Stability of isoprothiolane residues in rice grain following storage at about -20 °C

Sample code (Report ID)	Spike level (mg/kg)	Storage Interval (days/months)	%remaining a
Hiroshima (R-2055, R-2059)	0.5	33/1	102, 100 (101)
Gifu (R-2059)	0.5	38/1	110, 109 (110)
Ishikawa (R-2055)	0.5	38/1	110, 104 (107)
Chiba (R-2082)	0.5	147/5	107, 95 (101)
Kouchi (R-2082)	0.5	148/5	98, 97 (98)
Ibaraki (R-2082)	0.5	159/5	94, 93 (94)
Miyazaki (R-2082)	0.5	195/6	100, 97 (98)

[a] Values in brackets are mean recoveries

Isoprothiolane residues are considered to be stable in rice for at least 6 months (195 days) when stored frozen at -20 °C or below.

No storage stability data for commodities of animal origin were submitted. No evidence of degradation of residues was seen in the metabolism study in the goat.

USE PATTERN

Isoprothiolane is registered for use on rice crops in Japan. Information on registered uses that were provided to the Meeting are summarized in Table 31.

Table 31 Summary of registered use patterns for isoprothiolane. n.s. = not specified; n.a. = not applicable.

Crop	Country	App. Method	Max No of Apps.	App. Interval (days)	Application		PHI (days)
					kg ai/ha	kg ai/hL	
Rice	Japan	Nursery box	1	n.a.	max 75 g/box (eq. 3600g ai/ha)	n.a.	n.a.
Rice	Japan	Foliar spray	2[a]	n.s.	0.6	0.04	14
Rice	Japan	Aerial	2[a]	n.s.	0.4	1.3 - 5	14

[a] Max. 2 applications in the field and 1 additional to the nursery box

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received data from supervised residue trials on rice conducted in paddy fields.

In the summary tables, values used for making maximum residue level recommendations are underlined and highest individual values for estimating dietary intake are bolded.

Supervised trials for Isoprothiolane:

Category	Crop	Table
Cereal grains	Rice (GC 0649)	32

Cereal grains

Rice

Two residue trials on rice were conducted in Japan in 1974. In both trials of study R-2024 up to four applications were performed as dust containing 25 g ai/kg of isoprothiolane applied at a rate of 750–1000 g ai/ha. All trials were performed as reversed decline trials with individual plots for each pre-harvest interval. The plots representing the PHI of 14 days received four applications; all other plots/PHIs received three applications. In the Fukushima trial all applications were done at 1000 g ai/ha, in the Tottori trial the first application was done at 750 g ai/ha and all following at 1000 g ai/ha.

Additional four residue trials on rice were conducted in Japan in 2007. In all trials a first application was performed, where the test item was applied as a granule formulation to the nursery box (broadcasting) at a rate of 9 g ai/box at the day of transplanting. In the two trials of study R-2055 the second and third applications were performed as an emulsion with water at 40 g ai/hL at a rate of 600 g ai/ha. In the two trials of study R-2059 the second and third applications were performed as dust containing 25 g ai/kg of dust applied at a rate of 1000 g ai/ha.

Additional four residue trials on rice were conducted in Japan in 2008. In a first application the test item was applied as a granule formulation to the nursery box (broadcasting) at a rate of 9 g ai/box at the day of transplanting. The second and third applications were performed as an emulsion with water at 40 g ai/hL at a rate of 600 g ai/ha.

All trials were performed as reversed decline trials with individual plots for each pre-harvest interval. Samples of brown (hulled) rice grain were harvested at commercial maturity at nominally 14, 30 and 60 days after the last application for the 2007/08 trials and at nominally 14, 20 and 30 days after the last application for the trials performed in 1974.

Brown (hulled) rice grain samples were analysed for isoprothiolane by laboratory internal validated residue analytical method with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels of 0.01 mg/kg, 2.0 mg/kg and 4.0 mg/kg were in the range of 86–111% for parent isoprothiolane.

Hulled rice samples were stored for 1–6 months (32–178 days) prior to analysis. This period is covered by storage stability data obtained within these studies for 195 days.

Summaries of the trial results are given in Table 32. Residues of isoprothiolane in rice grain at harvest were in the range of 0.066–3.54 mg/kg.

Table 32 Residues of isoprothiolane in rice field study (Japan)

Crop, Study, Trial, Country, Year (Variety)	Application			DAT (days)	Matrix	Isoprothiolane	Mean	Reference
	Formulation (g ai/kg)	Rate	No.					
GAP - Japan	120 g/kg GR	9g ai/box (3600 g ai/ha)	1	— broadcasting use				
	400 g/L EC	600 g ai/ha	2	14				
Rice, R-2024, R-2024-1 Fukushima prefecture, Japan, 1974 (Nohrin 21)	25 g/kg dust	1000 g ai/ha	4 3 3 4 3 3	14 22 31 14 22 31	grain (hulled) Straw	0.24, 0.21 0.11, 0.092 0.11,0.095 4.50, 4.71 0.64, 0.58	0.22 0.10 0.10 4.60 0.61 0.90	R-2024
Rice, R-2024, R-2024-2 Tottori prefecture, Japan, 1974 (Nohrin 21)	25 g/kg dust	750 g ai/ha 1000 g ai/ha	4 3 3 4 3 3	14 22 31 14 22 31	grain (hulled) Straw	0.45, 0.48 0.62, 0.62 0.32, 0.28 2.62,2.60 2.89, 3.03 1.32, 1.22	0.47 0.62 0.30 2.61 2.96 1.27	R-2024
Rice, R-2055, R-2055-1 Ishikawa prefecture plant, Japan, 2007 (Koshijhikari)	120 g/kg GR 400 g/L EC	9 g ai/box 600 g ai/ha	1 2	14 30 60	grain (hulled)	1.54, 1.53 0.62, 0.60 < 0.01,< 0.01	1.54 0.61 < 0.01	R-2055
Rice, R-2055, R-2055-2 Hiroshima prefecture plant, Japan, 2007 (Hinohikari)	120 g/kg GR 400 g/L EC	9 g ai/box 600 g ai/ha	1 2	14 30 60	grain (hulled)	1.47, 1.46 **3.55**, 3.53 0.05, 0.05	1.46 3.54 0.05	R-2055
Rice, R-2059, R-2059-1 Gifu prefecture plant, Japan, 2007 (Koshijhikari)	120 g/kg GR 25 g/kg dust	9 g ai/box 1000 g ai/ha	1 2	14 30 60	grain (hulled)	1.59, 1.52 0.61, 0.61 0.11,0.10	1.56 0.61 0.10	R-2059
Rice, R-2059, R-2059-2 Hiroshima prefecture plant, Japan, 2007 (Hinohikari)	120 g/kg GR 25 g/kg dust	9 g ai/box 1000 g ai/ha	1 2	14 30 60	grain (hulled)	2.33,2.28 2.50, 2.40 0.06, 0.05	2.30 2.45 0.06	R-2059
Rice, R-2082, R-2082-1 Ibaraki, prefecture plant, Japan, 2008 (Koshijhikari)	120 g/kg GR 400 g/L EC	9 g ai/box 600 g ai/ha	1 2	14 28 56	grain (hulled)	1.23, 1.18 1.54, 1.54 < 0.01,< 0.01	1.20 1.54 < 0.01	R-2082
Rice, R-2082, R-2082-2 Chiba prefecture plant, Japan, 2008 (Hinohikari)	120 g/kg GR 400 g/L EC	9 g ai/box 600 g ai/ha	1 2	14 28 56	grain (hulled)	1.20, 1.17 1.76, 1.61 < 0.01,< 0.01	1.18 1.68 < 0.01	R-2082

Crop, Study, Trial, Country, Year (Variety)	Application			DAT (days)	Matrix	Isoprothiolane	Mean	Reference
	Formulation (g ai/kg)	Rate	No.					
GAP - Japan	120 g/kg GR	9g ai/box (3600 g ai/ha)	1	— broadcasting use				
	400 g/L EC	600 g ai/ha	2	14				
Rice, R-2082, R-2082-3 Kouchi prefecture plant, Japan, 2008 (Hinohikari)	120 g/kg GR 400 g/L EC	9 g ai/box 600 g ai/ha	1 2	14 25 59	grain (hulled)	2.26,2.20 2.42,2.41 0.02,0.02	2.23 2.42 0.02	R-2082
Rice, R-2082, R-2082-4 Miyazaki prefecture plant, Japan, 2008 (Koshijhikari)	120 g/kg GR 400 g/L EC	9 g ai/box 600 g ai/ha	1 2	14 27 59	grain (hulled)	1.89, 1.88 1.07, 1.05 0.02, 0.02	1.88 1.06 0.02	R-2082

FATE OF RESIDUES IN STORAGE AND PROCESSING

Nature of the residue during processing

High-temperature hydrolysis

High-temperature hydrolysis of isoprothiolane was investigated by Button S.G. (2012, R-2134). In the study, [dithiolane-4,5-^{14}C]-isoprothiolane was spiked into buffered solutions at a target concentration of 5 mg/L. The spiked solutions were put into conditions simulating boiling (100 °C, pH 5, 60 min). Prior to and after processing, an aliquot from each sample was collected and analysed by LSC for total radioactivity and by radio-HPLC for determination of hydrolysis products. Mass balance of radioactivity after processing was 98.6% for condition of 100 °C/pH5.

The only residues identified in the high-temperature hydrolysis study were isoprothiolane and a small portion of unidentified metabolite (Table 33).Isoprothiolane was shown to be stable under conditions representative of cooking rice grains (pH 5 at 100 °C for 60 minutes).

Table 33 High-temperature hydrolysis radio-HPLC results for isoprothiolane

Conditions	% of Isoprothiolane content			
	Start		End	
	Isoprothiolane		Isoprothiolane	Unknown
100 °C, 60 minutes, pH 5	100		99.8	0.2

Residues after processing

The Meeting received data on residues of isoprothiolane in rice processing (Ikenaga O., 2007, English translation report only). This study reported the effects of polishing, sieving, washing and cooking on residue levels in rice. Experimental data from Tokai COOP on residue levels during polishing and washing/cooking processing was also provided.

Rice

The effect of processing including cooking on the residue levels of some pesticide chemicals including isoprothiolane was conducted by Food and Agricultural Materials Inspection Centre (FAMIC, Japan) in 2006 and 2007. The processing procedure especially washing and cooking significantly reduced the residue of isoprothiolane. Another processing study results from Tokai COOP was presented. Levels of residue and processing factors are summarized in Table 34. Residues of isoprothiolane are reduced during polishing (PF=0.25) and washing and cooking procedures (PF=0.10).

Table 34 Effect of polishing, sieving, washing and cooking on residues of isoprothiolane in rice

Study Year	Matrix	Isoprothiolane, mg/kg	Processing factor (PF)
FAMIC 2006-2007	Brown rice	n.a.	-
	Polished rice grain	0.77	-
	Sieving after polishing	0.67	-
	washing	0.36	-
	cooking	0.21	-
Tokai COOP n.a.	Brown rice	0.20	-
	Polishing	0.05	0.25
	Washing and cooking	0.02	0.10

n.a: not applicable

RESIDUES IN ANIMAL COMMODITIES

In the cattle feeding study (Ladd, R., Wingender, R.J., 1975; Report R-2007), two Holstein dairy cows were each daily administered isoprothiolane in 4.5 kg of cereal grain treated at nominal levels of 50 mg/kg and 500 mg/kg (37.5 mg/kg and 392 mg/kg measured) together with 13.5 kg of untreated clover-prairie hay for 28 consecutive days. During this period milk was sampled twice daily and morning and evening milking combined.

Milk was sampled twice daily with the morning and the evening milking combined. Two 100 mL sample aliquots were taken for each sampling event. Samples from day 1, 3, 7, 14, 21 and 28 were taken for analysis. No tissues were sampled or analysed.

Milk samples were analysed for isoprothiolane by laboratory internal validated residue analytical method with an LOQ of 0.01 mg/kg. Procedural recoveries at fortification levels in the range of 0.005–1.0 mg/kg were in the range of 74–120% for parent isoprothiolane.

The residue levels of isoprothiolane for milk samples from both dose levels (9.4 and 98 mg ai/kg diet as received) were found to be below the limit of quantification (< 0.001 mg/kg).

Table 35 Isoprothiolane residues in milk following feeding of dairy cattle for 28 consecutive days

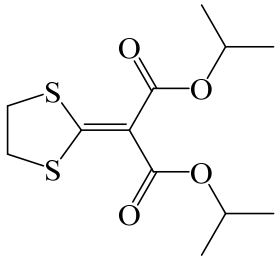
Dose / Group Number	9.4 mg/kg (Diet as received)	98 mg/kg (Diet as received)
Day of Test	Residues of isoprothiolane in milk (mg/kg)	
-3 (pre-dose)	< 0.001	< 0.001
-1 (pre-dose)	< 0.001	< 0.001
Day 1	< 0.001	< 0.001
Day 3	< 0.001	< 0.001
Day 7	< 0.001	< 0.001
Day 14	< 0.001	< 0.001
Day 21	< 0.001	< 0.001
Day 28	< 0.001	< 0.001
Depuration day 3	< 0.001	< 0.001
Depuration day 7	< 0.001	< 0.001
Depuration day 14	< 0.001	< 0.001

APPRAISAL

Isoprothiolane is a systemic fungicide with protective and curative action. It's used to control rice blast, rice stem rot and Fusarium leaf spot on rice, also reducing plant-hopper populations following foliar applications.

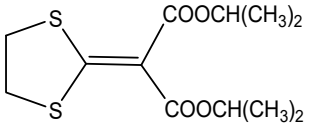
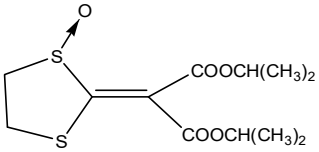
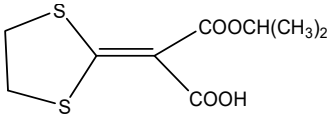
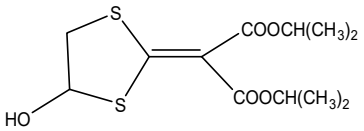
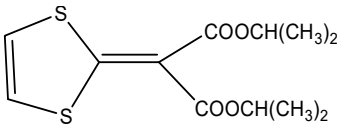
It was scheduled by the 48th Session of the CCPR (2016) as a new compound for consideration by the 2017 JMPR. The manufacturer submitted studies on metabolism, analytical methods, supervised field trials, processing, freezer storage stability and a feeding study.

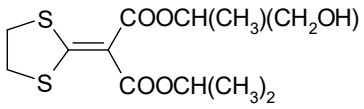
Registered authorisations exist for the use of isoprothiolane as foliar treatments on paddy fields in a number of countries including Japan and China. Submitted GAP information was available from Japan.



Isoprothiolane
(MW 290.4)

The following abbreviations are used for the major metabolites discussed below:

Chemical name (other names, codes)	Chemical structure
Diisopropyl 1,3-dithiolan-2-ylidenemalonate （Isoprothiolane, Parent）	
Diisopropyl 1- oxo-1,3- dithiolan-2- ylidenemalonate (Isoprothiolane monosulfoxide, M-1)	
Monoisopropyl, 1,3-dithiolan-2- ylidenemalonate (Isoprothiolane monoester, M-2)	
Diisopropyl 4- hydroxy-1,3- dithiolan-2- ylidenemalonate (4-hydroxy isoprothiolane, M-3)	
Diisopropyl 1,3- dithiol-2- ylidenemalonate (Didehydro isoprothiolane, M-4)	

Chemical name (other names, codes)	Chemical structure
1-Hydroxypropan-2-yl isopropyl 1,3-dithiolan-2-ylidenmalonate (Hydroxyl-Isopropyl isoprothiolane, M-5)	

Plant metabolism

The Meeting received plant metabolism studies on paddy rice following foliar applications of [^{14}C]-isoprothiolane.

Paddy rice – foliar applications

In a study on glasshouse grown rice 2 foliar treatments at 0.6 kg ai/ha ^{14}C-isoprothiolane were applied before ear emergence. Grain, stem and leaves, and roots samples were collected at 7 and 28 DALA. At 7/28 DALA, the TRR distributions were: grain (0.21/0.20 mg eq/kg), hulls (5.4/4.1 mg eq/kg), stems/leaves (1.9/1.4 mg eq/kg) and roots (0.03/0.02 mg eq/kg). Samples were surface rinsed, methanol extracted and the remaining solids (PES) were combusted and analysed by LSC. Surface rinsing of stems/leaves recovered 20–30% of the TRR. Solvent extractable residues in grains accounted for 33–42% TRR and more than 70% of the TRR in other samples. In grain samples 43–46% TRR remained in the PES and may comprise conjugated complexes based on acid and base hydrolysis.

Isoprothiolane parent was the major radioactive component in extractable parts in all plant parts with 16–32% TRR (0.03–0.06 mg eq/kg) in grain and 62–76% TRR (2.5–4.1 mg eq/kg) in hulls, 26–51% TRR (0.36–0.97 mg eq/kg) in stems/leaves. No metabolites exceeded 0.01 mg eq/kg in grain or roots at either 7 or 28 DALA. In hulls and stems/leaves, 4-hydroxy-isoprothiolane (M-3), monoester (M-2), monosulfoxide (M-1) and didehydro (M-4) metabolites were found. Of these, M-1 was the most abundant metabolite (up to 8.5% TRR (0.35 mg eq/kg in hulls). In these fractions significant radioactivity (6.4–40% TRR) remained at the TLC origin, which was shown to be conjugated forms of isoprothiolane, M-1, M-2 and M-3 by treatment with β-glucosidase and acid hydrolysis. No single metabolite in this polar fraction (at the TLC origin) exceeded 10% TRR.

In summary, metabolism studies in paddy rice showed that isoprothiolane was the major residue in rice matrices. Metabolism of the active substance produced only low levels of minor metabolites. Hydrolysis of the conjugated and unextractable residue released further low levels of the identified metabolites, with no single component in these accounting for >10% TRR.

Animal metabolism

The Meeting received animal metabolism studies on rats, lactating goats where animals were dosed with radiolabelled [dithiolane-4,5-^{14}C] isoprothiolane.

Lactating goats were orally dosed with ^{14}C-isoprothiolane capsules at rates equivalent to 12.3 ppm in the feed for 5 consecutive days and sacrificed 6 hours after the last dose.

The total recovery was 77% of the administered radioactivity (AR), with 64% being recovered from excreta (urine and faeces), 6.4% in the gastrointestinal tract, 1.8% in milk and 3.2% in edible tissues. Milk residues reached a plateau after 32 hours with a peak of 0.43 mg eq/kg in skim milk and 1.8 mg eq/kg in milk fat at day 3 (PM).

Total radioactive residue levels were 0.25–0.43 mg eq/kg in milk, 1.6 mg eq/kg in liver, 1.2 mg eq/kg in kidney, 0.13–0.14 mg eq/kg in muscle and 0.24–0.32 mg eq/kg in meat fats and 1.4–1.8 mg eq/kg in milk fat.

Solvent extractions with acetonitrile/water released 64–86% TRR from muscle, liver, and kidney, 58% TRR from skim milk with acetone/water. Subsequent enzymatic or alkali hydrolysis of the PES released another 20–35% TRR from liver, kidney and muscles. Acetonitrile/hexane extracted

86–98% TRR from skim milk and fat tissues. In tissues other than fat, soluble proteins extracted by acetonitrile/water accounted for >33% TRR. Protease treatment of the PES fractions released an additional 13–17% TRR in protein form.

Isoprothiolane was not found in goat matrices, except a trace detection (0.7% TRR, 0.002 mg eq/kg) in renal fat. Isoprothiolane monoester accounted for 18% TRR (0.21 mg eq/kg) in liver, 28% TRR (0.29 mg eq/kg) in kidney, 33% TRR (0.038 mg eq/kg) in flank muscle. No isoprothiolane monoester residues were found in skim milk or milk fat. In fat, 12–24% TRR (0.036–0.052 mg eq/kg) was present as isoprothiolane monoester. Analysis via protease treatments of non-fat matrices and saponification of fat matrices indicated that the majority of the radioactivity was associated with proteins and triglycerides.

The major metabolites in goat matrices were isoprothiolane monoester (M-2) with up to 33% TRR, 0.038 mg eq/kg in muscle and 18% TRR, 0.21 mg eq/kg in liver. Glucuronide conjugates of M-2 were only found in kidney with up to 15% TRR, 0.16 mg eq/kg. Monoester (M-2) and other minor metabolites including 4-hydroxy isoprothiolane (M-3) degraded to 1-2 carbon units, which were mainly incorporated into natural proteins.

Environmental fate

The Meeting received information on the environmental fate and behaviour of isoprothiolane, including photolysis, hydrolysis and anaerobic aquatic degradation. Studies were also received on the behaviour of [^{14}C]-isoprothiolane in several confined rotational crops.

Hydrolysis

Isoprothiolane was shown to be hydrolytically stable at pH 4 and 7 over 5 days at 50 ± 0.5 °C. At pH 9, isoprothiolane degrades above 10% after 5 days at 50 °C. Isoprothiolane monoester was the major hydrolytic degradate in basic solutions. DT_{50} values in solution at pH 9 of isoprothiolane at 40, 50 and 60 °C were 147, 26 and 9.0 days, respectively. Thus, the estimated DT_{50} values at 20 °C and 25 °C were > 1000 and > 3000 days, respectively. Isoprothiolane is considered to be hydrolytically stable at environmental temperatures at all pH values.

Anaerobic aquatic degradation

An anaerobic aquatic degradation study was conducted with [dithiolane-4,5-^{14}C] isoprothiolane applied to two freshly collected sediment/water systems.

The DT_{50} for isoprothiolane for the anaerobic water layers were estimated to be 6.5 and 36 days in two typical sediment/water systems. The degradation rate of isoprothiolane in the total system under anaerobic conditions was determined using SFO kinetics. The DT_{50} for isoprothiolane for the anaerobic total system were estimated as about 800–1100 days in the two test systems. The major degradate detected in the test systems was monoester, which increased steadily throughout the study reaching averages of 6.6–14% AR. Mineralization to $^{14}CO_2$ averaged 3.5–6.7% AR in the systems after one year. In conclusion, isoprothiolane is considered to be rather stable under anaerobic aquatic systems.

Soil Photolysis

A photodegradation study on a sandy clay loam soil was conducted with [^{14}C]-Isoprothiolane at a dose rate of approximately 5.0 mg/kg for up to 16 days of continuous irradiation.

The degradation rate of isoprothiolane in soils under artificial light irradiation and in dark controls was determined based on the percent isoprothiolane present in the extracts. DT_{50} and DT_{90} values for the net photodegradation of isoprothiolane were 28 and 92 days, respectively. The degradation rate of the metabolite monosulfoxide was as 13 and 42 days for the DT_{50} and $DT_{90,}$ respectively.

Isoprothiolane degraded moderately in light exposed samples with a half-life of 40 solar days (equivalent to natural sunlight at 40°N Latitude) or 47 solar days (equivalent to natural sunlight at 30–

50 °N Latitude) and represented an average of 58% AR at the end of the 16 days of exposure. CO_2 and bound residues were major degradates in the photolysis of isoprothiolane in soil (6.5% AR and 17.2% AR, respectively, at the end of the irradiation period). Isoprothiolane also underwent photo-induced S-oxidation to monosulfoxide (M-1) in light exposed samples (maximum of 9.1% AR at 24 hours). The decline of the monosulfoxide degradate was observed during the exposure period. The half-life of monosulfoxide was calculated as 21 (equivalent to natural sunlight at 40°N Latitude) or 24 solar days (equivalent to natural sunlight at 30–50 °N Latitude).

Aerobic and anaerobic degradation in soil

A soil degradation study was conducted on four soils with [^{14}C]isoprothiolane at an average dose rate of 4.64 ppm based on the dry soil weight equivalent. The primary degradates observed in the study were isoprothiolane monosulfoxide and monoester (maximum averages of 9.4% and 18.2% AR, respectively), CO_2 (up to a maximum average of 26% AR) and soil bound residues (up to 29.1% AR). The half-life of isoprothiolane during the aerobic phase ranged from 61 to 95 days, while the DT_{50} for isoprothiolane under anaerobic flooded conditions ranged from 182 to 990 days for all soils tested.

The Meeting considered that isoprothiolane was moderately persistent under aerobic conditions, and could be very persistent under anaerobic conditions.

Confined rotational crops

The Meeting received data from a radiolabelled confined study in lettuce, radish and wheat. Labelled isoprothiolane was applied to an outdoor sandy loam soil at a rate of 1937 g ai/ha. Plant-back intervals ranged from 30 to 365 days.

TRRs in tested crop samples were 0.14–6.0 mg eq/kg (wheat grain), 0.1–3.1 mg eq/kg (radish) and 0.067–0.85 mg eq/kg (lettuce). In animal feed items, TRRs were 0.44–18 mg eq/kg (wheat straw), 0.12–8.8 mg eq/kg (wheat forage and hay). Generally, higher residues were found in the upper portions of the plant compared to the roots, indicating the uptake potential of isoprothiolane from soil to the plant.

Extractability of residues in the initial extraction (acetonitrile/water and acetonitrile) varied from 36 to 91% TRR. Extractability was low in radish roots and wheat commodities, suggesting bound residues in these matrices. Further extraction with weak acidic solvent extracted up to 10% TRR and a weak basic solvent system up to a further 5% TRR. The post extraction solids contained 7.5 to 57% TRR.

At day 30 PBI parent isoprothiolane is found at 0.02 mg eq/kg (3.1% TRR) in immature lettuce, 0.011 mg eq/kg (1.3% TRR) in mature lettuce, 0.47 mg eq/kg (29% TRR) in radish roots, 0.69 mg eq/kg (24% TRR) in radish tops, 0.93 mg eq/kg （16% TRR） in wheat forage, 0.55 mg eq/kg (6.2% TRR) in wheat hay and generally not detected or at trace levels in most matrices at 365 days PBI.

Residues in immature and mature lettuce were characterised as monoester or hydroxyl isopropyl conjugates with up to 63% TRR.

Isoprothiolane was found in wheat forage (up to 16% TRR) and straw (up to 0.5% TRR), but not in grain. The monoester was found in forage, hay and straw, but at relatively low levels (from non-detected to 0.46 mg eq/kg). The main component in the grain samples analysed was at the solvent front. Monoester or hydroxyl isopropyl in the forage, hay and straw were confirmed.

Parent isoprothiolane was detected in radish samples, occurring around 25% TRR at the 30 day interval in both roots and tops, but generally decreasing over time. The monoester of isoprothiolane was also detected in both sample types, but at lower levels (from 0.002 to 0.13 mg eq/kg, less than 8% TRR). Hydroxyl isopropyl (M-5) or 4-hydroxy (M-3) metabolites were mainly detected in the radish tops.

The metabolism of isoprothiolane in rice and rotationally cropped wheat appears to be similar. In other confined rotational crop trials, isoprothiolane was mainly hydroxylated to 4-hydroxy

(M-3) and hydroxyl isopropyl (M-5), followed by conjugation to glycosides as major forms. Didehydro and monosulfoxide metabolites were only minor in all matrices. Production of the monoester by hydrolysis was only seen in radish roots. Significant amounts of bound residues were present in post extraction solids. All metabolites were considered to be further degraded and incorporated into natural products, such as proteins.

The Meeting agreed that residues of isoprothiolane will possibly occur in succeeding crops especially at short PBIs.

Methods of analysis

Analytical methods have been reported and validated for the analysis of isoprothiolane in plant and in the case of animal commodities, only for milk.

Data generation methods involved extraction with benzene/acetone or acetonitrile for rice grain/straw, methanol with sodium oxalate for milk, and separation of isoprothiolane by GC or LC, with detection methods of GC-ECD or LC-MS. For GC-ECD detection, partition against hexane/acetonitrile by liquid-liquid extraction was used. Polymer cartridge column clean-up was used for LC-MS detection of rice grain samples. The LOQs for rice grain and milk could reach 0.01 mg/kg (LC-MS method), 0.005 mg/kg, respectively. It's noted that the GC-ECD method could reach a LOD of 0.005 and 0.02 mg/kg for rice grain and rice straw, respectively.

For MRL-compliance, multi-residue methods are available for plant-origin commodities based on QuEChERS extraction/clean-up and GC-MS/MS, LC-MS/MS detections. The QuEChERS multi-residue methods were evaluated for measuring residues of isoprothiolane in wheat, oat, rye, barley and rice grain with LOQs of 0.01 mg/kg.

The Meeting concluded that suitable data generation methods are available to measure isoprothiolane in commodities including matrices of rice grain/straw, milk and the multi-residue methods based on QuEChERS are suitable for monitoring residues of isoprothiolane in cereal grains.

The Meeting also noted that there is no method available for isoprothiolane monoester or the parent compound in animal commodities, except for the parent compound in milk.

Stability of residues in stored analytical samples

In general, residue storage stability was shown for at least 6 months in rice grain. Residues of isoprothiolane were stable in analytical samples stored frozen (-20 °C) for at least the storage intervals used in the supervised residue trials. The Meeting also noted that no storage stability data for commodities of animal origin were submitted.

Definition of the residue

Plant commodities

In the plant metabolism studies involving foliar applications on paddy field, isoprothiolane parent was the predominant residue, accounting for 26–76% TRR in rice grain, hulls and stems/leaves. In the supervised field trials, isoprothiolane was also detected at residue levels of 0.07–3.5 mg/kg.

In the confined rotational crop study, isoprothiolane was observed in most matrices at the 30-day plant-back interval; however, the metabolites M-3 and M-5 generally made up the majority of the residue. Isoprothiolane was not observed in wheat straw and wheat grain.

Methods have been validated for the analysis of isoprothiolane, but not for M-3 or M-5 in plant commodities. The Meeting agreed that for enforcement purposes, a residue definition of parent isoprothiolane is suitable for rice and other crop commodities.

For assessing dietary risk, the meeting noted that isoprothiolane was the only quantifiable residue in rice grain. As no other metabolism studies were provided, the meeting relied on information from the confined rotational crop study to ascertain the nature of the residue in plants other than rice. Metabolites M-3 and M-5 (and their conjugates) were observed at levels greater than isoprothiolane in

rotational crops at short plant back intervals, especially M-5 in leafy crop matrices. The information available to the meeting indicated that M-3 is not more toxic than the parent compound and that M-5 is likely to have toxicity equivalent to M-3 by SAR analysis.

The Meeting decided that for assessing dietary risk, the residue definition for rice is isoprothiolane and for other crops is the sum of isoprothiolane, M-3 (free and conjugated), and M-5 (free and conjugated).

Animal commodities

Aside from a trace amount (0.002 mg/kg) in renal fat, isoprothiolane was not observed in milk or tissues from the goat metabolism study. In this study, isoprothiolane monoester (M-2) accounted for nearly all of the radioactivity not otherwise associated with proteins or triglycerides. Therefore, the Meeting decided that the residue definition for compliance is the sum of isoprothiolane and its M-2 metabolite, expressed as isoprothiolane. Residues of M-2 occurred at approximately equal concentrations in muscle and fat matrices in the metabolism study. The Meeting decided that the residue is not fat soluble.

In considering dietary risk assessment, the Meeting noted that M-2 was observed in the rat metabolism study and concluded that it is not more toxic than the parent compound. The only other identified compound that was observed as significant levels in the goat metabolism study was glucuronide conjugate of M-2, which was observed only in kidney and at approximately half the level of isoprothiolane. Given the low exposure expected from consumption of kidney, the Meeting decided not to include the glucuronide conjugate of M-2 in the definition for assessing dietary risk. Thus, the Meeting determined that the residue definition for assessing dietary risk for animal commodities is the sum of isoprothiolane and M-2, expressed as isoprothiolane.

The Meeting noted that residue methods exists to measure isoprothiolane residues in plants and milk.

Definition of the residue (for compliance with MRLs) for plant commodities*: isoprothiolane*

Definition of the residue (for estimation of dietary intake)for rice*: isoprothiolane.*

Definition of the residue (for estimation of dietary intake) for plants other than rice*: sum of isoprothiolane, diisopropyl-4-hydroxy-1,3-dithiolan-2-ylidenemalonate (M-3); free and conjugated, and 1-hydroxypropan-2-yl propan-2-yl 1,3-dithiolan-2-ylidenemalonate (M-5); free and conjugated, expressed as isoprothiolane*

Definition of the residue (for compliance with MRLs and estimation of dietary intake) for animal commodities: *sum of isoprothiolane and 2-(1,3-dithiolan-2-ylidene)-3-oxo-3-(propan-2-yloxy)propanoic acid (M-2), expressed as isoprothiolane.*

The residue is not fat soluble.

Results of supervised residue trials on crops

The Meeting received residue trials on paddy rice.

Cereal grains

Rice

The critical GAP for rice in Japan is a 9 g ai/box application with a granule formulation, and followed by 2 foliar applications of 600g ai/ha, with a PHI of 14 days. Six trials matched this GAP. Two trials with a 9 g ai/box application with granule formulation, and followed by 2 foliar applications of 1000g ai/ha were not considered to be within the ±25% cGAP, but still within 5 times cGAP, and hence the final residues of these trials were scaled, with a scale factor of 0.6 which related to the application rate ratio.

In eight independent trials conducted in Japan, isoprothiolane residues in husked rice were 0.94 (scaled from 1.56), 1.5 (scaled from 2.45), 1.5, 1.5, 1.7, 1.9, 2.4 and 3.5 mg/kg.

The Meeting estimated an STMR of 1.6 mg/kg and a maximum residue level of 6 mg/kg for isoprothiolane on rice, husked.

Rice straw

The critical GAP for rice in Japan is a 9 g ai/box application with granule formulation, and followed by 2 foliar applications of 600g ai/ha, with a PHI of 14 days.

In 2 independent trials conducted in Japan at 3–4 applications of 1000 g ai/ha dosage with a dust formulation, isoprothiolane residues in rice straw were 3.0 and 4.6 mg/kg.

The Meeting concludes that no recommendation will be made for rice straw due to insufficient data.

Fate of residues during processing

In a high-temperature hydrolysis study, isoprothiolane was shown to be stable under conditions representative of cooking rice grains at pH 5, 100 °C for 60 minutes.

The Meeting agreed that no processing factors could be derived from one of the processing studies as the residue in the RAC was not reported. In another processing study, husked rice grain was obtained from husking using a commercial machine with dried grain from a residue field trial. Fine rice bran was removed by sieving after polishing. Processing studies showed that residues of isoprothiolane decreased in most commodities. Isoprothiolane residues decreased significantly after rice washing and cooking.

For the commodities considered at this Meeting (rice), estimated processing factors and STMR-Ps for their processed food or feed commodities are summarised below.

Summary of selected processing factors and STMR-P values for isoprothiolane

Husked rice (STMR)	Matrix	Isoprothiolane [a]		STMR-P (mg/kg)
		Calculated processing factors	PF median	
1.6 mg/kg	Husked rice	-	-	
	Polished rice	0.25	0.25	0.4
	Cooked rice *	0.10	0.10	0.16

* made from polished rice

[a] Each value represents a separate study where residues were above the LOQ in the husked rice. The factor is the ratio of isoprothiolane residues in the processed item divided by the residue of isoprothiolane in the husked rice.

The meeting estimated a maximum residue level for polished rice of 1.5 (0.25×6) mg/kg.

Residue in animal commodities

Farm animal feeding studies

A dairy cattle feeding study was provided. Two dairy cows were each administered with daily doses of isoprothiolane. The residue levels of isoprothiolane parent in milk samples from both dose levels (9.4 and 98 mg ai/kg diet as received) were found to be below the limit of quantification (< 0.001 mg/kg).

In the metabolism study, a lactating goat was dosed with ^{14}C-labelled isoprothiolane at a rate of 12.3 ppm in the diet/day on a dry weight basis. At this rate, residues of parent and identified metabolites including isoprothiolane monoester along with its conjugates were found in liver, kidney, muscle and fat. The highest residue in tissues or milk was 0.29 mg eq/kg for isoprothiolane monoester in kidney. In milk samples, isoprothiolane and its monoester metabolite were not detected.

No poultry feeding studies or metabolism studies were provided.

Estimation of livestock dietary burdens

Dietary burden calculations for beef cattle and dairy cattle and poultry are provided below. The Meeting estimated the dietary burden of isoprothiolane in farm animals using the OECD diets listed in Appendix IX of the 2016 edition of the FAO Manual.

Livestock feed commodities considered by the Meeting were husked rice, using the entry for rice grain in OECD Feeding Table.

Summary of livestock dietary burden (ppm isoprothiolane equivalents of dry matter diet)

	US-Canada		EU		Australia		Japan	
	Max	mean	Max	mean	max	Mean	Max	Mean
Beef cattle	0.36	0.36	-	-	0.73[a]	0.73[c]	-	-
Dairy cattle	0.36	0.36	-	-	0.36[b]	0.36[d]	-	-
Broilers	0.36	0.36	-	-	0.91	0.91	-	-
Layers	0.36	0.36	-	-	0.91[e]	0.91[f]	-	-

[a] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat

[b] Highest maximum dairy cattle dietary burden suitable for MRL estimates for mammalian milk

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

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

[e] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs

[f] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs

Animal commodity maximum residue levels

The calculations used to estimate highest total residues for use in estimating maximum residue levels, STMR and HR values are shown below.

	Feed level (ppm) for milk residues	Residues (mg/kg) in milk	Feed level (ppm) for tissue residues	Residues (mg/kg) in			
				Muscle	Liver	Kidney	Fat
MRL beef or dairy cattle							
Feeding study [a c]	12.3	ND	12.3	ND	ND	ND	0.002
	-	-	-	-	-	-	-
Dietary burden and high residue	0.36	0	0.73	0	0	0	0
STMR beef or dairy cattle							
Feeding study [b c]	12.3	ND	12.3	ND	ND	ND	0.002
Dietary burden and median residue estimate	0.36	0	0.73	0	0	0	0

[a] highest residues for tissues and mean residues for milk

[b] mean residues for tissues and mean residues for milk

[c] based on the animal metabolism study on lactating goat

ND: not detected (LOD at 0.001 mg/kg)

The Meeting estimated a maximum residue level and STMR at 0.01* mg/kg, 0 mg/kg, respectively, for milks, mammalian meat, mammalian fats (except milk fats) and mammalian edible offal. The Meeting recommended no maximum residue levels for other animal commodities.

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/IESTI assessment.

Definition of the residue (for compliance with MRLs) for plant commodities: *isoprothiolane.*

Definition of the residue (for estimation of dietary intake) for rice: *isoprothiolane.*

Definition of the residue (for estimation of dietary intake) for plants other than rice: *sum of isoprothiolane, diisopropyl-4-hydroxy-1,3-dithiolan-2-ylidenemalonate (M-3); free and conjugated, and 1-hydroxypropan-2-yl propan-2-yl 1,3-dithiolan-2-ylidenemalonate (M-5); free and conjugated, expressed as isoprothiolane.*

Definition of the residue (for compliance with MRLs and estimation of dietary intake) for animal commodities: *sum of isoprothiolane and 2-(1,3-dithiolan-2-ylidene)-3-oxo-3-(propan-2-yloxy)propanoic acid (M-2), expressed as isoprothiolane.*

The residue is not fat soluble.

CCN	Commodity Name	Proposed MRL (mg/kg)	STMR or STMR-P (mg/kg)	HR or HR-P (mg/kg)
		New		
CM 0649	Rice, husked	6	1.6	
CM 1205	Rice, Polished	1.5	0.4	
MM 0095	Meat (from mammals other than marine mammals)	0.01 *	0	
ML 0106	Milks	0.01 *	0	
MF 0100	Mammalian fats (except milk fats)	0.01 *	0	
MO 0105	Edible offal (mammalian)	0.01 *	0	

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The International Estimated Daily Intake (IEDI) for isoprothiolane was calculated for the food commodities for which STMRs were estimated and for which consumption data were available. The results are shown in Annex 3.

The International Estimated Daily Intakes of isoprothiolane for the 17 GEMS/Food cluster diets, based on estimated STMRs were 0–2% of the maximum ADI of 0.1 mg/kg bw, expressed as isoprothiolane.

The Meeting concluded that the long-term dietary exposure to residues of isoprothiolane from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term dietary exposure

The Meeting decided that an ARfD is unnecessary and concluded that the short-term dietary exposure to residues of isoprothiolane from uses considered by the Meeting is unlikely to present a public health concern.

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