5.23 ISOPROTHIOLANE (299)

TOXICOLOGY

Isoprothiolane is the ISO-approved name for diisopropyl 1,3-dithiolan-2-ylidenemalonate (IUPAC name), with the CAS number 50512-35-1. Isoprothiolane is a fungicide used on rice crops. Isoprothiolane belongs to the family of dicarboxylic acids and derivatives, organic compounds containing two carboxylic acid groups, which act by inhibition of phospholipid biosynthesis. Isoprothiolane has not previously been evaluated by the JMPR and was reviewed by the present Meeting at the request of the CCPR.

All studies evaluated in this monograph were performed by laboratories that were certified for GLP and that complied, where appropriate, with the relevant OECD test guidelines or similar guidelines of the European Union or USEPA, unless otherwise indicated.

Biochemical aspects

In rats orally administered radiolabelled isoprothiolane, the test compound was almost completely absorbed. C_{max} in blood was achieved within 6 hours of administration of a low dose (5 mg) and within 9 hours of administration of a high dose (500 mg), irrespective of sex. The radiolabel was widely distributed among tissues, with most found in the liver, kidney and gastrointestinal tract, irrespective of sex and dose administered. In the later phase, that is, 24 or 168 hours after the low and high dose, respectively, radioactivity in almost all organs and tissues was gradually eliminated, with elimination slowest from fur and skin. Radioactivity in these tissues appears to comprise amino acids incorporated into keratin. The radiolabel of orally dosed isoprothiolane was excreted mainly via urine (24–34% of low dose and 46–53% of high dose) and expired air (~30%). In both low and high dose groups, excretion of the radiolabel was rapid until 24 and 48 hours post dose, respectively, and became slower thereafter. After 168 hours post dose, the carcass still retained about 10% of the administered radiolabel.

Orally dosed isoprothiolane was metabolized in rats by hydroxylation and hydrolysis, and by cleavage of the dithiolane ring, resulting in carbon dioxide and other low molecular weight metabolites. The glucuronic acid conjugate of the monoester was the most prominent metabolite in excreta (urine), accounting for 6% and 15% of dosed radioactivity in low and high dose groups, respectively, irrespective of sex.

Toxicological data

In one study in rats, the oral LD_{50} was estimated to be between 300 and 2000 mg/kg bw. In another study, there were no mortalities below the dose level of 900 mg/kg bw. The dermal LD_{50} in rats was greater than 2000 mg/kg bw. Following inhalation, the LC_{50} in rats was greater than 2.32 mg/L. Isoprothiolane was a not an irritant to rabbit skin, but a mild irritant to the rabbit eye. Isoprothiolane was a dermal sensitizer in a Magnusson and Kligman maximization test in guinea-pigs.

Although findings in short- and long-term toxicity studies in mice, rats and dogs varied, the most consistently targeted organs were liver, kidney and the haematopoietic system.

In a 112–115 day oral toxicity study in mice using isoprothiolane at dietary concentrations of 0, 20, 100, 300, 900 or 2700 ppm (equal to 0, 3.32, 14.8, 48.0, 132 and 472 mg/kg bw per day for males and 0, 2.81, 14.3, 47.2, 140 and 444 mg/kg bw per day for females, respectively), the NOAEL was 900 ppm (equal to 140 mg/kg bw per day) based on decreases (by about 40%) in ovarian weight, the toxicological significance of which was equivocal because of the absence of specific histopathological findings, at 2700 ppm (equal to 444 mg/kg bw per day).

In a 90-day dietary study in rats, with isoprothiolane at dose levels of 0, 50, 300 or 3000 ppm (equal to 0, 3.4, 20.9 and 201 mg/kg bw per day for males and 0, 4.0, 23.4 and 223 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 3.4 mg/kg bw per day) based on

increased relative weights of liver (10%) and kidneys (7%) and increased gamma-glutamyltransferase (GGT) activity (1.5-fold compared to controls) in males at 300 ppm (equal to 20.9 mg/kg bw per day).

In a 52-week toxicity study in dogs, isoprothiolane was administered orally via gelatine capsule at dose levels of 0, 2.0, 10.0 or 50.0 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day based on reduced body weight gain in females (35%), increased ALP activity in both sexes (3.3-fold in males and 1.6-fold in females), increased absolute (37%) and relative (72%) thyroid/parathyroid weight in females and increased relative liver weight in males (17%) at 50 mg/kg bw per day.

In an 78-week carcinogenicity study in mice using isoprothiolane at dietary concentrations of 0, 200, 1000 and 5000 ppm (equal to 0, 20.0, 104 and 501 mg/kg bw per day for males and 0, 18.2, 95.6 and 558 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 95.6 mg/kg bw per day) based on reduced body weight in males at 5000 ppm (equal to 501 mg/kg bw per day). There were no treatment-related increases in tumour incidence.

In a 104-week combined chronic toxicity and carcinogenicity study in rats using isoprothiolane at dietary concentrations of 0, 50, 300 and 3000 ppm (equal to 0, 1.82, 10.9 and 115 mg/kg bw per day for males and 0, 2.06, 12.6 and 139 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 10.9 mg/kg bw per day) based on reduced body weight gain (12% in males and 36% in females), increased blood urea nitrogen in females (16–25%, significant at weeks 26 and 52) and increased relative weights of liver (30–40%) and kidneys (10–50%) in both sexes at the interim and final kills, at 3000 ppm (equal to 115 mg/kg bw per day). An increased incidence of benign dermal keratoacanthoma was observed in males in the highest dose group (13/80 compared to 3/79 in controls). No pre-neoplastic changes were observed in the skin. There were no compound-related increases in any other tumour incidence.

The Meeting concluded that isoprothiolane is not carcinogenic in mice but caused benign skin tumours in male rats at the highest dose.

Isoprothiolane was tested for genotoxicity in an adequate range of in vitro and in vivo assays. There was little evidence of genotoxicity in vitro and no evidence of genotoxicity in vivo.

The Meeting concluded that isoprothiolane is unlikely to be genotoxic in vivo.

In view of the lack of genotoxic potential in vivo, the absence of carcinogenicity in mice and the increase in benign skin tumours occurring only in male rats at the highest dose, the Meeting concluded that isoprothiolane is unlikely to pose a carcinogenic risk to humans at the levels occurring in the diet.

In a three-generation reproductive toxicity study in rats using isoprothiolane at dietary concentrations of 0, 30, 300 or 3000 ppm (equivalent to 0, 2, 20 and 200 mg/kg bw per day, respectively) for three generations, the NOAELs for parental and offspring toxicity were 300 ppm (equivalent to 20 mg/kg bw per day) based on decreased body weight gain at 3000 ppm (equivalent to 200 mg/kg bw per day). The NOAEL for reproductive toxicity was 3000 ppm (equivalent to 200 mg/kg bw per day), the highest dose tested.

In a two-generation reproductive toxicity study in rats using isoprothiolane at dietary concentrations of 0, 30, 300 or 3000 ppm (equal to 0, 1.9, 19.7 and 196 mg/kg bw per day in F_0 males; 0, 2.5, 25.0 and 242 mg/kg bw per day in F_0 females; 0, 2.3, 22.3 and 235 mg/kg bw per day in F_1 males; 0, 2.7, 27.6 and 276 mg/kg bw per day in F_1 females). the NOAEL for parental toxicity was 300 ppm (equal to 19.7 mg/kg bw per day) based on decreased body weights, body weight gains and feed consumption and other effects at 3000 ppm (equal to 196 mg/kg bw per day). The NOAEL for offspring toxicity was 300 ppm (equal to 22.3 mg/kg bw per day) based on delayed sexual maturation, delayed eye opening and other effects secondary to general toxicity at 3000 ppm (equal to 235 mg/kg bw per day) and secondary to effects on body weights in the dams. The NOAEL for reproductive toxicity was 3000 ppm (equal to 196 mg/kg bw per day), the highest dose tested.

In a developmental toxicity in rats, isoprothiolane was administered by oral gavage at doses of 0, 12, 50 or 200 mg/kg bw per day from gestation day 6 to 19. The NOAEL for maternal toxicity

was 50 mg/kg bw per day based on decreased body weight, body weight gain and feed consumption at 200 mg/kg bw per day. Isoprothiolane did not cause any fetal anomalies up to 200 mg/kg bw per day, the highest dose tested. The NOAEL for embryo/fetal toxicity in rats was 12 mg/kg bw per day, based on the significantly high incidence of incomplete ossification of the thoracic vertebral body and significantly low number of ossified cervical and total vertebral bodies at 50 mg/kg bw per day.

In a developmental toxicity study in rabbits, isoprothiolane was administered by oral gavage at doses of 0, 15, 80 or 400 mg/kg bw per day from gestation day 6 through 18. The NOAEL for maternal toxicity was 80 mg/kg bw per day based on decreased body weight gains and feed consumption at 400 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 400 mg/kg bw per day, the highest dose tested.

The Meeting concluded that isoprothiolane is not teratogenic.

Receptor-mediated effects identified from the literature

In in vitro studies of effects on estrogen or androgen receptors, isoprothiolane had little or no effect up to concentrations of 10^{-4} mol/L.

An in vitro study demonstrated that isoprothiolane is able to activate the pregnane X receptor (PXR) receptor. In vivo studies in rats showed that isoprothiolane can induce cytochrome CYP2B and UDPGT, characteristics of CAR activation.

Toxicological data on metabolites and/or degradates

The metabolites of importance in plant and animal commodities are 4-hydroxy isoprothiolane (M-3), 1-hydroxypropan-2-yl propan-2-yl 1,3-dithiolan-2-ylidenemalonate (M-5) and the monoester (M-2) glucuronide conjugate.

A structural comparison of these metabolites with isoprothiolane using Toxtree (version 2.6.13) identified no unique structural alerts that would not be covered by the toxicity tests on the parent. The Meeting therefore concluded that these metabolites are unlikely to be genotoxic.

In an acute toxicity study in mice, M-3 was less toxic than the parent, showing an LD_{50} equal or greater than 3290 mg/kg bw. In a metabolism study, M-3 was found in small amounts (< 1%) in rat faeces. Based on these observations and the close structural similarity between M-3 and isoprothiolane, the Meeting concluded that it was unlikely that the metabolite M-3 or its conjugates would be of greater toxicity than the parent, isoprothiolane.

M-5 was not found in rat metabolism studies. However, based on its structure the Meeting concluded that it was unlikely that M-5 or its conjugates would be of greater toxicity than the parent, isoprothiolane.

There were no specific data on the toxicity of the monoester (M-2) glucuronide conjugate. However, given its low lipid solubility and structure, the intact glucuronide is unlikely to be toxic by the oral route. Although intestinal hydrolysis may lead to formation of the monoester (M-2), the structural similarity of M-2 to isoprothiolane suggests that it would not be of greater toxicity than the parent, isoprothiolane.

The Meeting concluded that these metabolites are not of greater toxicological concern than the parent and considered that they would be covered by the ADI established for isoprothiolane.

Human data

A report on a field exposure study in human volunteers spraying an isoprothiolane formulation (FUJI ONE 40 EC) was provided. Human volunteers wearing the recommended protective clothing were exposed to spray drift during spraying. Subsequent monitoring for 3 consecutive days identified no effects on the health parameters assessed in this study (clinical examination, urine analysis and laboratory investigations on blood and plasma).

No adverse health effects were noted in reports on manufacturing plant personnel. No reports on accidental or intentional poisoning in humans were available.

The Meeting concluded that the existing database on isoprothiolane was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.1 mg/kg bw on the basis of a NOAEL of 10.9 mg/kg bw per day from a 2-year study of toxicity and carcinogenicity in rats, based on an increase in blood urea nitrogen in females and an increase in the relative weight of liver and kidneys in both sexes at 115 mg/kg bw per day. Although the NOAEL of 3.4 mg/kg bw per day in the 90-day oral rat study was lower, the LOAEL in this study was based on marginal effects. The Meeting therefore concluded that the NOAEL of the 2-year combined toxicity/carcinogenicity study was the more appropriate on which to establish the ADI.

This ADI was supported by a NOAEL of 10 mg/kg bw per day from a 52-week toxicity study in dogs. A safety factor of 100 was applied.

The Meeting concluded that it was unnecessary to establish an ARfD for isoprothiolane in view of its low acute oral toxicity and absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose.

A toxicological monograph was prepared

Levels relevant to risk assessment of isoprothiolane

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	1 000 ppm, equal to 95.6 mg/kg bw per day	5 000 ppm, equal to 501 mg/kg bw per day
		Carcinogenicity	5 000 ppm, equal to 501 mg/kg bw per day[b]	–
Rat	Ninety-day toxicity[a]	Toxicity	50 ppm, equal to 3.4 mg/kg bw per day	300 ppm, equal to 20.9 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 10.9 mg/kg bw per day	3 000 ppm, equal to 115 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 10.9 mg/kg bw per day (benign tumours)	3 000 ppm, equal to 115 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	3 000 ppm, equal to 196 mg/kg bw per day[b]	–
		Parental toxicity	300 ppm, equal to 19.7 mg/kg bw per day	3 000 ppm, equal to 196 mg/kg bw per day
		Offspring toxicity	300 ppm, equal to 22.3 mg/kg bw per day	3 000 ppm, equal to 235 mg/kg bw per day
	Three-generation study of reproductive toxicity[a]	Reproductive toxicity	3 000 ppm, equivalent to 200	–

			mg/kg bw per day[b]	
		Parental toxicity	300 ppm, equivalent to 20 mg/kg bw per day	3 000 ppm, equivalent to 200 mg/kg bw per day
		Offspring toxicity	300 ppm, equivalent to 20 mg/kg bw per day	3 000 ppm, equivalent to 200 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	50 mg/kg bw per day	200 mg/kg bw per day
		Embryo/fetal toxicity	12 mg/kg bw per day	50 mg/kg bw per day
Rabbit	Developmental toxicity study[c]	Maternal toxicity	80 mg/kg bw per day	400 mg/kg bw per day
		Embryo/fetal toxicity	400 mg/kg bw per day[b]	–
Dog	Fifty-two week study of toxicity[d]	Toxicity	10 mg/kg bw per day	50 mg/kg bw per day[b]

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

[d] Capsule administration.

Estimate of acceptable daily intake (ADI; applies to isoprothiolane and the metabolites M-2, M-3 and M-5)

0–0.1 mg/kg bw

Estimate of acute reference dose (ARfD)

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to isoprothiolane

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Almost completely absorbed; T_{max} at 6 h (low dose) or 9 h (high dose)
Dermal absorption	No data
Distribution	Widely distributed
Potential for accumulation	10% of dosed radioactivity in residual carcass at 168 h
Rate and extent of excretion	Excreted mainly into urine and expired air. Excretion is rapid until 24–48 h post dose and becomes slow afterwards
Metabolism in animals	Extensively metabolized through hydroxylation and hydrolysis
Toxicologically significant compounds in animals and plants	Isoprothiolane, M-2 glucuronide, M-3 and M-5

Acute toxicity	
Rat, LD_{50}, oral	≥300 mg/kg bw
Rat, LD_{50}, dermal	>2 000 mg/kg bw
Rat, LC_{50}, inhalation	>2.32 mg/L
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Dermal sensitizer (maximization test)
Short-term studies of toxicity	
Target/critical effect	Liver and kidneys
Lowest relevant oral NOAEL	3.4 mg/kg bw (rat)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver, kidney
Lowest relevant NOAEL	10.9 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in mice; benign dermal keratoacanthoma only in male rats at highest dose tested[a]
Genotoxicity	
	Unlikely to be genotoxic in vivo[a]
Reproductive toxicity	
Target/critical effect	No reproductive effects
Lowest relevant parental NOAEL	19.7 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	20 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	196 mg/kg bw per day (highest dose tested; rat)
Developmental toxicity	
Target/critical effect	Delayed ossification
Lowest relevant maternal NOAEL	50 mg/kg bw per day (rat)
Lowest relevant embryo/fetal NOAEL	12 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	No data
Studies on toxicologically relevant metabolites	
M-3	Acute oral toxicity data in mice: $LD_{50} \geq 3\ 290$ mg/kg bw
M-5	
	No data
Monoester (M-2) glucuronide conjugate	No data
Human data	

	No adverse health effects reported

[a] Unlikely to pose a carcinogenic risk to humans at the levels occurring as residues in the diet.

Summary

	Value	Study	Safety factor
ADI[a]	0–0.1 mg/kg bw	Two-year chronic toxicity/carcinogenicity (rat)	100
ARfD	Unnecessary	–	–

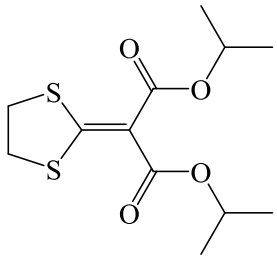
[a] Applies to isoprothiolane and M-2, M-3 and M-5.

RESIDUE AND ANALYTICAL ASPECTS

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.

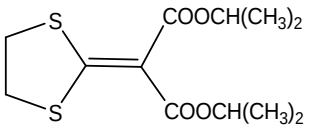
Registered authorisations exist for the use of isoprothiolane as foliar treatments on paddy fields in a number of countries including Japan and China. Submitted GAP information was available from Japan.

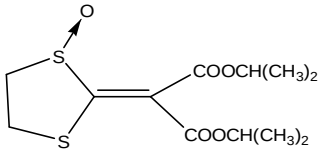
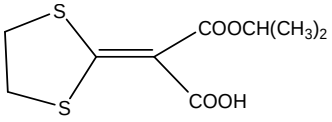
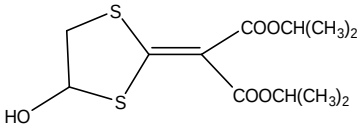
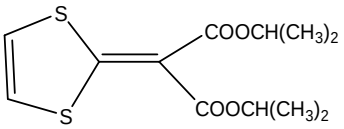
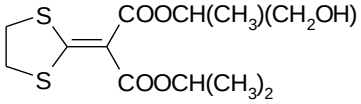


Isoprothiolane

(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）	

Chemical name (other names, codes)	Chemical structure
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)	
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 persistant 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

	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
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 in Annex 1 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.

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.