5.26 Tolclofos-methyl (191)

TOXICOLOGY

Tolclofos-methyl is the ISO-approved common name for *O*-2,6-dichloro-*p*-tolyl *O*,*O*-dimethyl phosphothioate (IUPAC) with the CAS number 57018-04-9.

Tolclofos-methyl is used for the control of soil-borne fungal diseases of potatoes but may also be used for the treatment of lettuce and other crops. Unlike other organophosphorous pesticides that are used as insecticides, tolclofos-methyl is a fungicide and its pesticidal MOA is via inhibition of phospholipid biosynthesis.

Tolclofos-methyl was last evaluated by JMPR in 1994. At that time, an ADI of 0–0.07 mg/kg bw was established on the basis of reduced brain cholinesterase activity in a two-year study of toxicity and carcinogenicity in mice. New studies submitted since the last JMPR evaluation include in vitro comparative metabolism (human and rat microsome), one-generation reproduction toxicity, neurotoxicity, immunotoxicity, phototoxicity, and in vitro endocrine toxicity studies.

Tolclofos-methyl was re-evaluated by the present Meeting within the periodic review programme of CCPR. The majority of studies considered by the current Meeting were evaluated during the 1994 JMPR meeting and, where appropriate, the text from the previous monograph has been adopted here verbatim. Some of the critical studies do not comply with GLP, as the data were generated before the implementation of GLP regulations. Overall, however, the database was considered adequate for the risk assessment.

Biochemical aspects

After oral administration [^{14}C-4-methyl]-tolclofos-methyl was rapidly absorbed from the GI tract of mice and rats and was extensively distributed throughout the body.

In mice exposed to a single dose of tolclofos-methyl at 5 mg/kg bw, 74–83% of the administered radiolabel was recovered in urine, faeces, and expired air within the first day of exposure. The major route of excretion was the urine, accounting for 69–76% of the administered dose, while faecal excretion accounted for < 6%.

In a study in rats exposed to a single 5 mg/kg bw dose of [^{14}C-4-methyl]-tolclofos-methyl, absorption and elimination was rapid with radioactivity recovery reaching 83% one day after exposure. The majority of the radioactivity was recovered in the urine (> 62%) followed by faeces (> 16%). For most tissues, peak concentration was reached within two hours of administration. The oral absorption was at least 63% within 48 hours. The highest concentration of radioactivity was localized to the kidney (3–5 times higher than plasma and liver). For the remainder of the tissues, radioactivity concentration was < 29% of plasma. By 72 hours post-exposure, < 3% of the administered dose remained in the tissues and carcass.

Tolclofos-methyl undergoes extensive metabolism in mammals, proceeding through a pathway involving stepwise oxidative desulfuration to an oxon and related derivatives, oxidation of the 4-methyl group to alcohols and acids, cleavage of P–O–aryl and P–O–methyl linkages, and conjugation of the resultant acid with glycine. In rats, the major metabolites were Ph-CH_3 (12%) and Ph-COOH (29%). In mice, the major metabolites were Ph-COOH (12%), Ph-CO-glycine (13% ; unique to mice) and DM-TMO-COOH (12%). An in vitro metabolism study comparing the metabolic profile of rat microsomes to human microsome preparations, indicated that the rodent and human metabolic pathways are virtually identical with only two unique minor metabolites (< 5% of radioactivity) identified in rat, but not in human microsome preparations.

Toxicological data

The acute LD_{50} of tolclofos-methyl was > 5000 mg/kg bw in rats, > 3500 mg/kg bw in mice and

> 1000 mg/kg bw in dogs. The dermal LD_{50} was > 5000 mg/kg bw in rats and mice and > 2000 mg/kg bw in rabbits. The LC_{50} for inhalation in rats was > 2.07 mg/L. No signs of ocular irritation were noted. Slight dermal irritation in rabbits was observed at 500 mg/kg bw. There was evidence of dermal sensitization as assessed by the Magnusson and Kligman methodology.

In general, tolclofos-methyl exhibited relatively low toxicity. Body weight decrements, decreases in cholinesterase activity and liver weight changes were the most commonly observed effects.

In a 32–34-day toxicity study in rats exposed to dietary concentrations of 0, 200, 1000, 5000, or 20 000 ppm (equal to 0, 16, 79, 414, and 1635 mg/kg bw per day for males, 0, 18, 88, 452, and 1830 mg/kg bw per day for females), the NOAEL was 1000 ppm, (equal to 79 mg/kg bw per day), on the basis of increased relative kidney weights and reduced brain cholinesterase activity at 5000 ppm, (equal to 414 mg/kg bw per day).

In rats exposed for 13 weeks to tolclofos-methyl at dietary concentrations of 0, 100, 1000 or 10 000 ppm (equal to 0, 6.5, 66 or 653 mg/kg bw per day for males, 0, 7.1, 71 or 696 mg/kg bw per day for females), the NOAEL was 1000 ppm (equal to 66 mg/kg bw per day), based on marginal reduction in erythrocyte cholinesterase activity and changes in clinical chemistry parameters at 10 000 ppm (equal to 653 mg/kg bw per day).

In a 26-week study, dogs were exposed to tolclofos-methyl at dietary concentrations of 0, 200, 600 or 2000 ppm (equal to 0, 7.4, 23 or 69 mg/kg bw per day in males, 0, 4.1, 21 or 65 mg/kg bw per day in females). The NOAEL was 600 ppm (equal to 21 mg/kg bw per day), on the basis of reduced body weight gain at 2000 ppm (equal to 65 mg/kg bw per day).

In a 52-week study, dogs were exposed to tolclofos-methyl at dietary concentrations of 0, 80, 400 or 2000 ppm (equal to 0, 2.2, 11 or 59 mg/kg bw per day for males, 0, 2.6, 11.2, or 62 mg/kg bw per day for females). The NOAEL was 400 ppm (equal to 11 mg/kg bw per day), on the basis of reduced body weight gain at 2000 ppm, equal to 59 mg/kg bw per day.

The overall NOAEL for dogs after short-term exposure was 600 ppm (equal to 21 mg/kg bw per day), on the basis of reduced body weight gain at 2000 ppm (equal to 59 mg/kg bw per day).

In a nine-month study, mice were exposed to tolclofos-methyl at dietary concentrations of 0, 10, 30, 100 or 3000 ppm (equal to 0, 1.2, 3.8, 12 and 510 mg/kg bw per day for males, 0, 1.4, 4.1, 14 and 560 mg/kg bw per day for females), the NOAEL was 100 ppm (equal to 12 mg/kg bw per day), based on decreased body weight as well as decreased erythrocyte and brain cholinesterase activity at 3000 ppm (equal to 510 mg/kg bw per day).

In a 104-week toxicity study, mice were exposed to tolclofos-methyl at dietary concentrations of 0, 10, 50, 250 or 1000 ppm (equal to 0, 1.3, 6.5, 32 and 134 mg/kg bw per day in males, 0, 1.3, 6.8, 34 and 137 mg/kg bw per day in females). The NOAEL was identified at 50 ppm (equal to 6.5 mg/kg bw per day), based on reduced brain and erythrocyte cholinesterase activity and an increase in kidney weights at 250 ppm (equal to 32 mg/kg bw per day). The carcinogenicity NOAEL was 1000 ppm (equal to 134 mg/kg bw per day), the highest dose tested.

In rats exposed to tolclofos-methyl for 28 weeks at dietary concentrations of 0, 10, 30, 1000, 3000 or 10 000 ppm (equal to 0, 16, 51, 164 and 540 mg/kg bw per day for males, 0, 18, 65, 184, and 623 mg/kg bw/day for females), the NOAEL was 1000 ppm (equal to 51 mg/kg bw per day) on the basis of bile duct proliferation and oval cell proliferation at 3000 ppm (equal to 164 mg/kg bw per day).

In a two-year chronic and carcinogenicity toxicity study rats were exposed to dietary concentrations of 0, 100, 300 or 1000 ppm (equal to 0, 4.2, 12, and 42 mg/kg bw for males, 0, 4.8, 15, and 49 mg/kg bw for females) for either 122 weeks (males) or 129 weeks (females). A systemic NOAEL could not be identified due to the variability in the cholinesterase activity data. Although an increase in the incidence of follicular cell carcinomas was noted at 1000 ppm (equal to 42 mg/kg bw per day), in the absence of any indication of thyroid toxicity at higher doses in the remainder of the tolclofos-methyl database this observation was considered to be a spurious finding, based on the weight of the evidence. The carcinogenicity NOAEL was 1000 ppm (equal to 42 mg/kg bw per day), the highest dose tested.

In a two-year study in rats with concentrations of 0, 100, 300, or 1000 ppm (equivalent to 0, 5, 15, or 50 mg/kg bw per day), the systemic NOAEL was 1000 ppm (equivalent to 50 mg/kg bw per day), the highest dose tested. The carcinogenic NOAEL was 1000 ppm (equivalent to 50 mg/kg bw per day), the highest dose tested.

The Meeting concluded that tolclofos-methyl is not carcinogenic in rats or mice.

Tolclofos-methyl was tested for genotoxicity in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was found.

The Meeting concluded that tolclofos-methyl is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that tolclofos-methyl is unlikely to pose a carcinogenic risk to humans.

In a multigeneration toxicity study of rats exposed to dietary concentrations of 0, 100, 300, or 1000 ppm (equal to 0, 6.9, 20.5, and 70.6 mg/kg bw per day for F_0 males, 0, 8.9, 26.2, and 90.5 mg/kg bw per day for F_0 females) no evidence of parental, reproductive, or offspring toxicity was observed at any dose, The parental, offspring, and reproductive NOAEL was 1000 ppm (equal to 70.6 mg/kg bw per day), the highest dose tested.

In a one-generation reproduction toxicity study rats were exposed to dietary concentrations of 0, 2500, 5000, or 10 000 ppm (equal to 0, 173, 338, and 680 mg/kg bw per day for males, 0, 178, 353, or 668 mg/kg bw per day for females). The parental NOAEL was 5000 ppm (equal to 338 mg/kg bw per day) on the basis of body, ovarian, uterine, and liver weight changes at 10 000 ppm (equal to 680 mg/kg bw per day). The reproductive NOAEL was 10 000 ppm (equal to 680 mg/kg bw per day), the highest dose tested. The offspring NOAEL was 2500 ppm (equal to 173 mg/kg bw per day), on the basis of decreased body weight, body weight gain, and food consumption at 5000 ppm (equal to 338 mg/kg bw per day).

In a developmental toxicity study in rats, tolclofos-methyl was administered via gavage at doses of 0, 100, 300 or 1000 mg/kg bw per day from GD 6–15. The NOAEL for maternal toxicity was 300 mg/kg bw per day based on decreased body weight gain at 1000 mg/kg bw per day. The embryo/fetal NOAEL was 1000 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, rabbits were administered 0, 300, 1000 or 3000 mg/kg bw per day tolclofos-methyl via gavage on days GD 6–18. The maternal toxicity NOAEL was 300 mg/kg bw per day on the basis of decreased body weight gain and food consumption at 1000 mg/kg bw per day. The embryo/fetal NOAEL was 3000 mg/kg bw per day, the highest dose tested.

The Meeting concluded that tolclofos-methyl is not teratogenic.

The neurotoxic potential of tolclofos-methyl was evaluated in a series of neurotoxicity studies including a time-to-peak-effect study for cholinesterase activity, acute and subchronic neurotoxicity studies in rats, and a delayed neuropathology study in hens.

In a time-to-peak-effect study designed to investigate the effects of tolclofos-methyl exposure on cholinesterase activity, tolclofos-methyl was administered by gavage at a single dose of 0 or 2000 mg/kg bw. The NOAEL was 2000 mg/kg bw, the highest dose tested. As a result of this study, cholinesterase activity was not assessed in a subsequent acute neurotoxicity study.

In an acute neurotoxicity study, rats were given a single oral dose of 0, 200, 700, or 2000 mg/kg bw tolclofos-methyl by gavage. The systemic NOAEL was 200 mg/kg bw on the basis of decreased motor activity at 700 mg/kg bw.

In a subchronic neurotoxicity study tolclofos-methyl was administered to rats in their diet for 90 days at concentrations of 0, 300, 1800, or 10 000 ppm (equal to 0, 20.6, 122, 736 mg/kg bw per day for males, 0, 23.1, 136, and 763 mg/kg bw per day for females). The Meeting noted that at 10 000 ppm erythrocyte cholinesterase activity was slightly reduced from week five onwards, while brain cholinesterase activity was slightly, and inconsistently, reduced at certain time points only. The systemic NOAEL was 1800 ppm (equal to 122 mg/kg bw per day) on the basis of decreased body

weight, body weight gain, and food utilization as well as decreases in motor activity at 10 000 ppm (equal to 736 mg/kg bw per day).

In a delayed neuropathy study, Leghorn hens were administered 0 or 8000 mg/kg bw tolclofos-methyl. Hens treated with tolclofos-methyl had no signs of leg weakness or paralysis and no histopathological changes to their nervous tissues.

The Meeting noted that the small decreases in cholinesterase activity recorded in several studies, particularly in mice, were never associated with the typical signs of the cholinergic syndrome. Even at high single doses, from 1500 mg/kg bw to > 3500 mg/kg bw (the LD_{50} for mice), which caused lethality, such signs were not observed.

Therefore, the Meeting concluded that the clinical observations in these studies are not indicative of specific toxicity to the nervous system but rather a generalized toxic effect, and that the slightly reduced erythrocyte and brain cholinesterase activity observed at doses above the LOAEL in repeated dose studies is likely not due to direct inhibition by tolclofos-methyl.

The immunotoxic potential of tolclofos-methyl was investigated in an immunotoxicity study with mice exposed to concentrations of 0, 500, 1500, or 4500 ppm (equal to 0, 91, 273, and 811 mg/kg bw per day) for 28 days. The immunotoxicity NOAEL was 4500 ppm (equal to 811 mg/kg bw per day), the highest concentration tested. The systemic NOAEL was 1500 ppm (equal to 273 mg/kg bw per day) on the basis of decreased body weight, body weight gain, and food consumption at 4500 ppm (equal to 811 mg/kg bw per day).

The Meeting concluded that tolclofos-methyl is not immunotoxic.

Four in vitro assays were conducted to evaluate tolclofos-methyl's potential impact on estrogen activity or pregnane X receptor (PXR) agonism. None of the assays suggested endocrine activity in relation to estrogen or PXR.

Toxicological data on metabolites and/or degradates

No toxicological data specific to the metabolites or degradates identified as residues in crops or livestock (goat) are available. However, all the residues identified (ph-CH_3, TMO-COOH, ph-COOH, TMO, TM-CH_2OH, DM-TM, DM-TM-CH_2OH and TMO-CH_2OH) are also major rat metabolites (> 10%). Hence, the Meeting concluded that the toxicity of these metabolites would be covered by that of tolclofos-methyl.

Microbiological data

No data are available to assess the potential impact of tolclofos-methyl exposure on the microbiome.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted. There are no reports of poisoning incidents and no epidemiological studies available for tolclofos-methyl.

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

Toxicological evaluation

The Meeting reaffirmed the ADI for tolclofos-methyl of 0–0.07 mg/kg bw based on a NOAEL of 6.5 mg/kg bw per day based on reduced erythrocyte and brain cholinesterase activity along with increased kidney weights in a two-year study of toxicity and carcinogenicity in mice.

The Meeting concluded that it was not necessary to establish an ARfD for tolclofos-methyl in view of its low acute oral toxicity and the absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose. The Meeting noted that although brain cholinesterase activity is decreased in mice after 28 weeks of exposure, the oral LD_{50} for mice is > 3500 mg/kg bw suggesting that acute exposure would not elicit a decrease in cholinesterase activity.

Furthermore, the toxic effects reported (for example, decreased motor activity, dyspnea, irregular respiration) were not typical of a cholinergic syndrome and were only noted at doses ≥ 1500 mg/kg bw.

A toxicological monograph was prepared.

Levels relevant to risk assessment of tolclofos-methyl

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 6.5 mg/kg bw per day	250 ppm, equal to 32 mg/kg bw per day
		Carcinogenicity	1000 ppm, equal to 134 mg/kg bw per day[b]	-
Rat	13-week toxicity study[a]	Toxicity	1000 ppm, equal to 66 mg/kg bw per day	10 000 ppm, equal to 653 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a,c]	Toxicity	1000 ppm, equivalent to 50 mg/kg bw per day[b]	-
		Carcinogenicity	1000 ppm, equivalent to 50 mg/kg bw per day[b]	-
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1000 ppm, equal to 70.6 mg/kg bw per day[b]	-
		Parental toxicity	1000 ppm, equal to 70.6 mg/kg bw per day[b]	-
		Offspring toxicity	1000 ppm, equal to 70.6 mg/kg bw per day[b]	-
	One-generation study of reproductive toxicity[a]	Reproductive toxicity	10 000 ppm, equal to 680 mg/kg bw per day[b]	-
		Parental toxicity	5000 ppm, equal to 338 mg/kg bw per day	10 000 ppm, equal to 680 mg/kg bw per day
		Offspring toxicity	2500 ppm, equal to 173 mg/kg bw per day	5000 ppm, equal to 338 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	1000 mg/kg bw per day[b]	-
	Acute neurotoxicity study[d]	Toxicity[e]	200 mg/kg bw	700 mg/kg bw
	Subchronic neurotoxicity[a]	Toxicity[e]	1800 ppm, equal to 122 mg/kg bw per day	10 000 ppm, equal to 736 mg/kg bw per day
	Immunotoxicity study[a]	Immunotoxicity	4500 ppm, equal to 811 mg/kg bw per day[b]	
Rabbit	Developmental toxicity study[d]	Maternal toxicity	300 mg/kg bw per day	1000 mg/kg bw per day
		Embryo and fetal toxicity	3000 mg/kg bw per day[b]	
Dog	26-week toxicity study[a]	Toxicity	600 ppm, equal to 21 mg/kg bw per day	2000 ppm, equal to 59 mg/kg bw per day

One-year study of toxicity[a]	Toxicity	400 ppm, equal to 11 mg/kg bw per day	2000 ppm, equal to 59 mg/kg bw per day

[a] Dietary administration
[b] Highest dose tested
[c] Three studies combined
[d] Gavage administration
[e] Generalized toxicity not associated with neurotoxicity

Acceptable daily intake (ADI), applies to tolclofos-methyl, ph-CH3, TMO-COOH, ph-COOH, TMO, TM-CH2OH, DM-TM, DM-TM-CH2OH and TMO-CH2OH, expressed as tolclofos-methyl

0–0.07 mg/kg bw

Acute reference dose (ARfD)

Unnecessary

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

Results from epidemiological studies of human exposure.

Critical endpoints for setting guidance values for exposure to tolclofos-methyl

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; > 75% at 5 mg/kg bw (mouse and rat)
Dermal absorption	No data
Distribution	Extensive; highest concentration found in the kidney
Potential for accumulation	Low
Rate and extent of excretion	Rapid; largely complete within the first 24 h after dose administration
Metabolism in animals	Converted primarily to Ph-CH_3 (12%) and Ph-COOH (29%) in rats and Ph-COOH (12%), Ph-CO-glycine (13%, unique to mice), and DM-TMO-COOH (12%) in mice
Toxicologically significant compounds in animals and plants	Tolclofos-methyl
Acute toxicity	
Mouse LD_{50}, oral	≥ 3500 mg/kg bw
Rat LD_{50}, oral	> 5000 mg/kg bw
Rat LD_{50}, dermal	> 5000 mg/kg bw
Rat LC_{50}, inhalation	> 3.32 mg/L after 4 h exposure
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Slightly irritating
Guinea pig, dermal sensitization	Sensitizer (Magnusson & Kligman assay)
Short-term studies of toxicity	
Target/critical effect	Decreased body weight gain (dog)
Lowest relevant oral NOAEL	11 mg/kg bw per day
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rabbit; highest dose tested)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced erythrocyte and brain cholinesterase activity and increased kidney weights
Lowest relevant NOAEL	6.5 mg/kg bw per day (mouse)

Carcinogenicity	Not carcinogenic in rat or mouse[a]
Genotoxicity	Not genotoxic [a]
Reproductive toxicity	
Target/critical effect	Decreased body, thymus, kidney, brain, ovarian, uterine, seminal vesicles, epididymal, and liver weights
Lowest relevant parental NOAEL	338 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	173 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	680 mg/kg bw per day (rat; highest dose tested)
Developmental toxicity	
Target/critical effect	No embryo/fetal effects; decreased body weight gains in maternal animals (rats and rabbits)
Lowest relevant maternal NOAEL	300 mg/kg bw per day (rats and rabbits)
Lowest relevant embryo/fetal NOAEL	1000 mg/kg per day (rat; highest dose tested)
Neurotoxicity	
Acute neurotoxicity NOAEL	Not neurotoxic
Subchronic neurotoxicity NOAEL	Not neurotoxic
Developmental neurotoxicity NOAEL	No data
Immunotoxicity	
Immunotoxicity NOAEL	811 mg/kg bw per day (rat; highest dose tested)
Human data	No poisoning incidents or adverse effects have been reported as part of the medical surveillance data collection

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet

Summary

	Value	Study	Safety Factor
ADI	0–0.07 mg/kg bw [a]	Two-year study of toxicity and carcinogenicity (mouse)	100
ARfD	Unnecessary		

[a] applies to tolclofos-methyl, ph-CH3, TMO-COOH, ph-COOH, TMO, TM-CH2OH, DM-TM, DM-TM-CH2OH and TMO-CH2OH, expressed as tolclofos-methyl

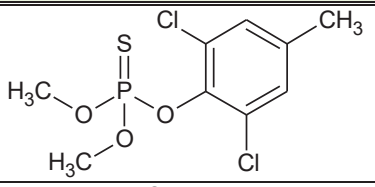
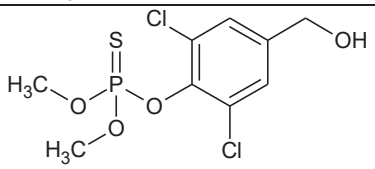
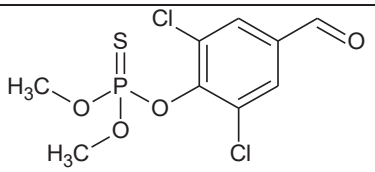
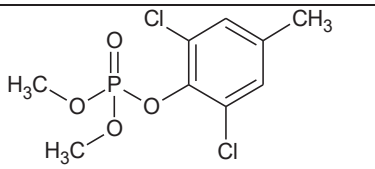
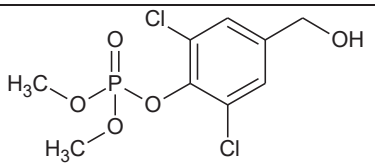
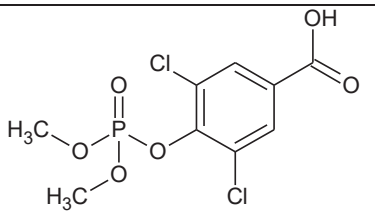
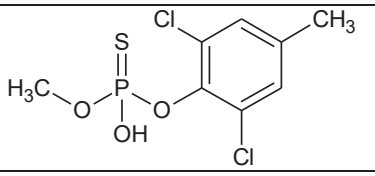
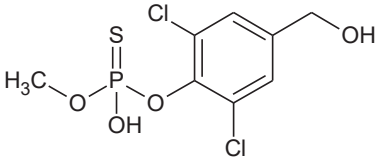
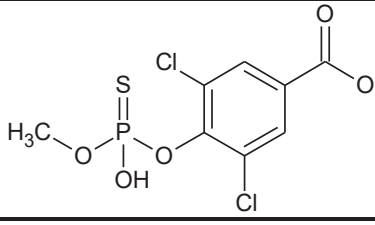
RESIDUE AND ANALYTICAL ASPECTS

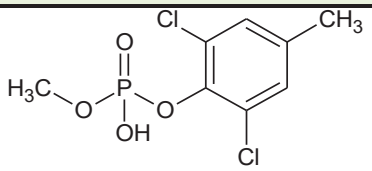
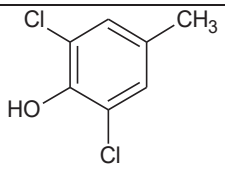
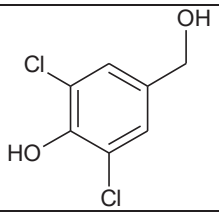
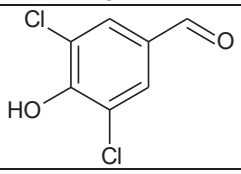
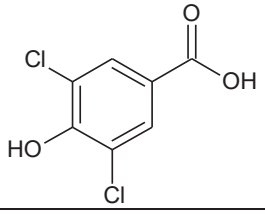
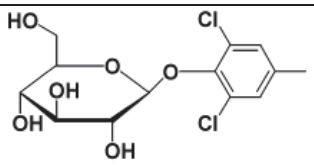
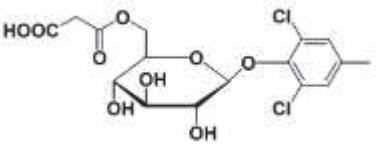
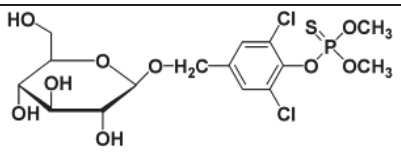
Tolclofos-methyl is a non-systemic contact organophosphorus fungicide used for control of soil-borne diseases caused by *Rhizoctonia solani*. The IUPAC name for tolclofos-methyl is *O*-2,6-dichloro-*p*-tolyl *O,O*-dimethyl phosphorothioate. Tolclofos-methyl was first evaluated for toxicology and residues by the JMPR in 1994.

Tolclofos-methyl was scheduled at the Fiftieth Session of the CCPR for periodic review by the 2019 JMPR. The Meeting received information on identity, physical and chemical properties, plant and animal metabolism, environmental fate, methods of residue analysis, storage stability, GAP information and supervised trials.

The following abbreviated names were used for the metabolites referred to in this appraisal.

Table 1 Abbreviated names used for the metabolites referred to in this appraisal

Abbreviation	Matrix found	Structure
Tolclofos-methyl TM (parent)	Goat (liver, kidney), Hen (egg yolk, liver, fat, skin, muscle), Sugar beet (leaves, shoots, roots) Peanut (leaves, hull), Potato (foliage, shoots, roots, parent tubers, daughter tubers), Lettuce (plants)	
TM-CH_2OH	Sugar beet (leaves), Peanut (leaves, stem), Potato (foliage, roots, parent tubers, daughter tubers)	
TM-CHO	Hen (liver)	
TMO	Goat (milk), Sugar beet (leaves, shoots, roots), Peanut (leaves, stem)	
TMO-CH_2OH	Goat (kidney), Hen (liver), Sugar beet (leaves) Peanut (stem)	
TMO-COOH	Goat (kidney, milk), Hen (liver, muscle, skin) Sugar beet (leaves, shoots, roots), Peanut (stem)	
DM-TM	Goat (kidney), Sugar beet (leaves), Potato (foliage, shoots, roots, parent tubers, daughter tubers)	
DM-TM-CH_2OH	Goat (, kidney), Potato (foliage, shoots, roots, parent tubers, daughter tubers)	
DM-TM-COOH	Goat (milk), Potato (foliage, roots, parent tubers, daughter tubers)	

Abbreviation	Matrix found	Structure
DM-TMO	Goat (kidney), Sugar beet (leaves) Peanut (hull), Potato (foliage, roots, parent tubers, daughter tubers)	
ph-CH_3	Goat (liver), Hen (liver), Sugar beet (leaves, roots), Peanut (leaves)	
ph-CH_2OH	Goat (liver, kidney), Hen (skin), Sugar beet (leaves), Peanut (leaves, stem)	
ph-CHO	Goat (liver), Peanut (leaves)	
ph-COOH	Goat (milk, kidney, liver), Hen (liver, kidney, muscle, fat, skin), Potato (foliage, shoots, roots, parent tubers, daughter tuber)	
Glucose conjugate of ph-CH_3	Lettuce	
Malonylglucose conjugate of ph-CH_3	Lettuce	
Glucose conjugate of TM-CH_2OH	Lettuce	

Tolclofos-methyl is of low volatility (0.88 mPa at 20 °C). The log K_{ow} value (3.8 at 25 °C) suggests that tolclofos-methyl has the potential to partition into fat. Hydrolysis is unlikely to be a significant route of degradation in the environment, but may be significant at higher temperatures during food processing.

Plant metabolism

The Meeting received plant metabolism studies for tolclofos-methyl radiolabelled in the phenyl ring after foliar, soil, or seed tuber application on leafy vegetables (lettuce), root and tuber vegetables (sugar beet, potato) and oilseeds (cotton, peanut).

Lettuce

[phenyl-^{14}C]-Tolclofos-methyl was applied once to lettuce seedlings (3–4 leaf stage; BBCH 14) and soil in crates grown in a greenhouse at rates of 2 or 10 kg ai/ha. Lettuce grown to maturity in a greenhouse was harvested 34 days after the application.

TRRs in mature lettuce were 0.23 and 0.77 mg eq/kg for the 2 and 10 kg ai/ha experiments, respectively. Aqueous acetone extracted 66% of the total radioactivity from the lettuce matrices, and a subsequent extraction with methanol added 16–20%, thus, total extractability was 82–86%TRR. After hydrolyses of PES with acid and base, only 0.5–1.7% TRR remained in the solids.

Parent was a major component of the residue, accounting for 37–40% TRR (0.084–0.30 mg/kg). The malonylglucose conjugate of ph-CH_3 (M22 fraction) was found at 20–23% TRR (0.052–0.15 mg eq/kg). Glucose conjugate of TM-CH_2OH (M35 fraction) was found at 14–15% TRR (0.032–0.11 mg eq/kg). These metabolites were found in aqueous acetone extracts. In addition, unidentified fractions of 8–9% TRR (0.020–0.059 mg eq/kg) in total were present in the extracts. In the acid and base hydrolysates, unidentified fractions were present at 14% TRR (0.031–0.11 mg eq/kg) in total. Meanwhile, it was observed that TM-CH_2OH sugar conjugate may be transformed to TMO-COOH under acidic conditions (1 M HCl at 80 °C for 2 hours).

The conjugates were further identified in another study, where the radiolabelled substance was topically applied once to lettuce leaves grown in a greenhouse at rates of 75 g ai/ha and 750 g ai/ha. Lettuce leaves were harvested at 2 and 7 days after the application. In the study, the major conjugated metabolite was identified as a malonylglucose conjugate of ph-CH_3.

Sugar beet foliar treatment

A metabolism study was performed on sugar beet plants in a greenhouse with foliar treatment. The radiolabelled substance was topically applied to the third leaf of potted six-month old sugar beets grown in a greenhouse at a rate equivalent to 3.3 kg ai/ha. Sugar beet plants harvested at 3, 7, 14, 21, 28, 35 and 50 days after treatment (DAT; 28, 35 and 50 DAT, relevant to harvest practice) were sectioned into treated leaf, untreated leaves, and root portions. The treated leaves were rinsed with methanol.

Total recovery of applied radiocarbon (AR) from leaves and roots was in the range of 8.4–40% AR over the study period. The radioactivity comprised 7.1–40% AR in treated leaves, 0.3–1.6% AR in untreated leaves and 0.3–0.6% AR in roots, indicating limited translocation of radiocarbon into untreated leaves and roots.

At 28–50 DAT, surface wash accounted for 3.9–4.2% of total radioactivity in the treated leaves. Organic solvents (MeOH/chloroform) extracted 60–83% of the total radioactivity in washed leaves, untreated leaves and roots at 28–50 DAT. Partitioning with acidified solvents may result in conversion of TM-CH_2OH to TMO-COOH.

In treated leaves (28–50 DAT), metabolite DM-TMO was a major component, accounting for 38–42% TRR. Parent was present at 8.4–9.2% TRR (including 1.4–2.6% TRR from surface wash). TMO-COOH was detected at 3.9–6.5% TRR. Other minor components (ph-CH_3, ph-CH_2OH, TM-CH_2OH, TMO-CH_2OH, TMO, DM-TM) were also found individually at up to 3.9% TRR. Unidentified fractions were present at up to 3–12% TRR in total.

In untreated leaves (28–50 DAT), parent was the predominant residue, accounting for 40–47% TRR. TMO-COOH was found at up to 13% TRR. Unidentified fractions were present at 13–20% TRR in total.

For roots (28–50 DAT), parent was found at 17–33% TRR. TMO-COOH was found at 17–33% TRR. Unidentified fractions were present at 33–50% TRR in total; no characterisation of these fractions was provided.

Sugar beet soil treatment

Six-month old sugar beets were planted in loamy sand soil, grown in a greenhouse and treated at a rate of 20 mg/kg soil on a dry weight basis. Sugar beets (roots and leaves) were harvested at 3, 7, 14, 21, 28, 35 and 75 DAT (28, 35 and 75 DAT, relevant to harvest practice).

Total recovery of applied radiocarbon from leaves, roots and soil was in the range of 48–63% AR over the study period. The radioactivity comprised 0.1–1.0% AR in leaves, 0.1–1.5% AR in roots and 47–63% AR in soil, indicating very limited uptake of radioactive carbon from soil into plants.

TRRs (28–75 DAT) were 0.24–0.33 mg eq/kg in leaves and 0.44–0.49 mg eq/kg in roots. Organic solvent (MeOH/chloroform) extracted 33–75% TRR in leaves and roots.

In leaves (28–75 DAT), parent was the predominant residue, present at levels of 17–33% TRR and 0.05–0.07 mg/kg. Metabolites TMO and ph-CH_3 were found at ≤0.1% AR. Unidentified fractions were present at 17–33% TRR (0.041–0.11 mg eq/kg) in total.

For roots (28–75 DAT), parent was a major component found at residue levels of 17–50% TRR (0.07–0.18 mg/kg). TMO was also a major component found at 17–25% TRR (0.075–0.12 mg eq/kg). pH-CH_3 was found, but at < 0.1% AR. Unidentified fractions were present at < 0.1% AR in total.

Potato

Seed potatoes were surface treated once with the radiolabelled substance, immediately prior to planting at a rate of 125 g ai/tonne of tubers. Potato plants were grown in a glasshouse and harvested at an immature stage (27 days after planting) and at full maturity stage (129 days after planting). The harvested plant material was separated into foliage, parent and daughter tubers (only at mature stage).

TRRs in immature potato plants were 0.25 mg eq/kg in foliage and 56 mg eq/kg in parent tubers. For mature potato plants, TRRs were 0.040 mg eq/kg in shoots, 1,890 mg eq/kg in parent tubers and 0.048 mg eq/kg in daughter tubers.

Organic solvents (acetone and aqueous acetone) extracted 76–96% TRR in foliage, 98–99% TRR in parent tubers and 66% TRR in daughter tubers (unextracted, 33% TRR; 0.016 mg eq/kg).

In foliage at the immature stage, parent was found at 1.3% TRR (0.003 mg/kg). The largest component, metabolite DM-TM-CH_2OH was found at 31% TRR (0.076 mg eq/kg). Five unidentified fractions were present individually at 4.4–11% TRR (0.011–0.028 mg eq/kg). At the mature stage, parent was not detected in foliage. Metabolite ph-COOH (37% TRR, 0.015 mg eq/kg) was the predominant residue. One unidentified fraction (19% TRR, 0.008 mg eq/kg) was observed.

In parent tuber at immature and mature stages, parent was the predominant residue accounting for 97% TRR (55 mg/kg) and 95% TRR (1,790 mg/kg), respectively. Metabolites were not found at either the immature or mature harvest timing.

In daughter tubers, parent was not detected. Metabolite DM-TM-CH_2OH was a major component found at 27% TRR (0.013 mg eq/kg). DM-TM-COOH was also found but at a lower level, 6.0% TRR (0.003 mg eq/kg). Three unidentified fractions were observed at 4.3–12% TRR (0.002–0.006 mg eq/kg).

Another study with a seed potato treatment was conducted at rates of 250 g ai/t tuber and 1,250 g ai/t tuber. A single application with the radiolabelled substance was made immediately prior to planting. Plants were grown outside in a caged enclosure and harvested at maturity (118 days after planting). At harvest, parent tubers, daughter tubers and foliage were collected.

TRRs were 40–180 mg eq/kg in parent tubers, 0.032–0.067 mg eq/kg in daughter tubers, and 0.13–0.36 mg eq/kg in foliage.

Organic solvents extracted 95–98% TRR in parent tuber, 78–79% TRR in daughter tubers (unextracted, 21–22%; 0.007–0.015 mg eq/kg) and 63–86% TRR in foliage.

In parent tubers, parent was the predominant residue, accounting for 89–96% TRR (35–172 mg/kg). Metabolites DM-TM-CH_2OH, DM-TM-COOH, TM-CH_2OH, ph-COOH, DM-TMO and DM-TM were found at very low levels, each up to 0.1% TRR and 0.18 mg eq/kg. Unidentified fractions were at less than 4%, in total, with individual components of ≤ 1% TRR.

In daughter tubers, parent was detected, but at very low levels of 2.6–8.3% TRR (0.002 mg/kg). The largest component was DM-TM-CH_2OH present at 11–12% TRR (< 0.01 mg eq/kg). Metabolite DM-TM-COOH was found at 6.0–10% TRR. Other metabolites TM-CH_2OH, ph-COOH, DM-TMO and DM-TM were present at levels of less than 6% TRR each. Unidentified multiple fractions were present individually at less than 6% TRR, totaling < 29% TRR.

Foliage contained parent residues at levels of 6.6–9.7% TRR (0.012–0.025 mg eq/kg). DM-TM-CH_2OH and DM-TM-COOH were found at 5.2–15% TRR (0.018–0.019 mg eq/kg) and 8.9–13% TRR (0.017–0.032 mg eq/kg), respectively. Metabolites TM-CH_2OH, ph-COOH, DM-TMO and DM-TM were found at individually less than 8% TRR (≤ 0.025 mg eq/kg). Unidentified multiple fractions were observed at less than 0.06 mg eq/kg in total.

Cotton seed and peanuts

Cotton and peanut plants grown under field conditions were treated with a single soil application at a rate of 5.2 or 15.7 kg ai/ha. For peanuts, an additional foliar application was made 75 days after the soil treatment, at a rate of 5.2 kg ai/ha (in total, 10 kg ai/ha) or 15.7 kg ai/ha (in total, 31 kg ai/ha). Cotton and peanut plants were harvested 150 days after the soil treatment. Cotton (bolls, squares, leaves, stems and seeds) and peanut (hull, leaves, stems and nutmeat) samples were taken. The surface of peanut leaves was rinsed with methanol.

In cotton samples, radioactivity was not detected (< 0.003–< 0.008 mg eq/kg), except in the stem (0.008–0.010 mg eq/kg at 5.2 kg ai/ha; 0.015–0.026 mg eq/kg at 15.7 kg ai/ha) and in the leaf (0.015 mg eq/kg at 15.7 kg ai/ha). In peanuts, TRR levels (10, 31 kg ai/ha) were 0.016–0.052 mg eq/kg in hulls, 1.4–3.8 mg eq/kg in leaves, 0.044–0.079 (10 kg ai/ha)/0.090–0.38 (31 kg ai/ha) mg eq/kg in stems and 0.010 mg eq/kg (both rates) in nutmeat. In peanut leaves, parent was detected only in the surface wash (0.1% TRR), and TM-CH_2OH and ph-CH_2OH and the conjugates were found. Overall, characterisation of residues was not sufficient (low extraction and low recovery in TLC analysis) to draw conclusions.

Conclusions

In plants, tolclofos-methyl was non-systemic and mostly recovered in directly treated parts. Parent was present at various levels in the edible parts of the plants and the metabolite profiles were dependent on the mode of application.

In lettuce with seedling and soil treatment, major metabolites were sugar conjugates of ph-CH_3 and TM-CH_2OH, generated via cleavage of the P-O aryl bond or oxidation of the 4-methyl group and further, their conjugation with sugar. In potato with seed tuber treatment, a major metabolite was DM-TM-CH_2OH, generated via demethylation and oxidation of the 4-methyl group.

Environmental fate

The Meeting received soil and aqueous photolysis, aqueous hydrolysis and aerobic soil metabolism studies for tolclofos-methyl.

Hydrolysis

Hydrolytic degradation of [phenyl-^{14}C]-tolclofos-methyl was mostly dependent on pH and temperature in the sterile aqueous buffered solution. At pH 4–9, the half-lives calculated from experiments at higher temperatures were 97–126 days at 20 °C and 50–68 days at 25 °C. A single hydrolysis product DM-TM

occurred (up to 81% AR at 62 °C, pH 7 after 50 hours). Another metabolite ph-CH_3, produced only at pH 9, was observed at much lower levels (up to 13% AR at 62 °C after 50 hours).

Therefore, it was considered that hydrolysis is unlikely to be a significant route of degradation under environmental conditions.

Photochemical degradation

Aqueous photolysis

Aqueous photolysis is not a significant environmental degradation pathway for tolclofos-methyl, as shown by an aqueous photolysis study which determined half-lives of 8.2–48.5 days at latitudes of 20°N–50°N.

Soil photolysis

On irradiated soil under natural sunlight, the half-life of tolclofos-methyl was 113 days. Photolysis was not a significant degradation pathway of tolclofos-methyl on soil.

Aerobic soil metabolism

In three studies, a total of 11 soils were treated with [phenyl-^{14}C]-tolclofos-methyl. Tolclofos-methyl degraded rapidly in the tested soils. DT_{50} values for tolclofos-methyl ranged from 2 to 30 days (geometric mean: 9.2 days). The DT_{90} values ranged from 6.9 to 100 days. Major degradation products were DM-TM and ph-CH_3 (up to 13% and 8% of the applied radioactivity). Other identified metabolites ph-COOH, ph-CH_2OH, TM-COOH, TMO and DM-TMO were found at low levels of < 2–7% of the applied radioactivity.

The Meeting considered that tolclofos-methyl is not persistent in soil.

Rotational crop metabolism

No information was provided.

Animal metabolism

The Meeting received animal metabolism studies on rats, lactating goats and laying hens.

Rats

The metabolism of tolclofos-methyl in rats was reviewed within the framework of the toxicological evaluation by the WHO Core Assessment Group of the 2019 JMPR.

Goats

One goat was orally dosed, once daily, for 4 consecutive days at a rate equivalent to 250 ppm in the feed (10 mg/kg bw per day). Milk was collected twice daily. The goat was sacrificed 7 hours after the final dosing.

Of the total dose, only 27% was recovered, and most (26% of the total dose) was recovered in urine and a small amount (0.6% of the total dose) was recovered from faeces. TRRs were 0.2 mg eq/kg in muscle, 1.1 mg eq/kg in fat, 3.0 mg eq/kg in liver, 4.3 mg eq/kg in kidney. For milk, the TRR was 0.41 mg eq/kg at 48 hours after the first dosing. Residue levels in milk reached an equilibrium of about 0.8 mg eq/kg within 4 days after the first dosing.

Muscle, liver and kidney samples were extracted with acidified organic solvent (diethyl ether after adjusting to pH 1) and refluxed with diethyl ether at acid and base conditions followed by extraction with water. The extraction process may hydrolyse conjugates and oxidise TM-CH_2OH to TMO-COOH. For milk and fat, acidified organic solvent was not used.

Acidified organic solvent extracted 37% TRR in liver, 28% TRR in kidney, and 13% TRR in muscle. Further extractions (acid- and base-reflux followed by extraction with diethyl ether) released 13% TRR in liver, 39% TRR in kidney and 25% TRR in muscle. Water extract contained 21% TRR (0.62 mg eq/kg) in liver, 44% TRR (1.9 mg eq/kg) in kidney and 54% TRR (0.11 mg eq/kg) in muscle. Final unextracted radioactivity was 30% TRR in liver, 4% TRR in kidney and 8% TRR in muscle. For milk and fat, organic solvent extracted 74% TRR and 118% TRR, respectively; and the final unextracted radioactivity was 21% TRR and 8% TRR, respectively. For muscle and fat, further investigations for identification of metabolites were not performed.

In liver, parent was not detected. Metabolite ph-COOH was a major component found at 18% TRR (17% free+conj. form, 1.6% base released, total: 0.55 mg eq/kg). Another major component ph-CH_3 was found at 15% TRR (11% free+conj., 4.0% acid released, total: 0.45 mg eq/kg). Metabolite ph-CH_2OH and one unknown fraction, free+conj., were present at 5.4% TRR (0.16 mg eq/kg) and 4.4% TRR (0.13 mg eq/kg), respectively. Acid- and base-released two other fractions that were observed, individually, at less than 5.1% TRR (0.15 mg eq/kg). Some 21% TRR (0.62 mg eq/kg) in water extract was not further investigated.

In kidney, parent was not detected. TMO-COOH was a major component found at 21% TRR (8.7% free+conj., 8.1% acid released, 4.4% base released, total: 0.91 mg eq/kg). Another major component ph-COOH was found at 21% TRR (5.7% free+conj., 7.4% acid released, 8.0% base released, total: 0.91 mg eq/kg). TMO-CH_2OH was found at a lesser extent of 11% TRR (3.1% free+conj., 4.0% acid released, 3.9% base released, total 0.47 mg eq/kg). DM-TM-CH_2OH, DM-TMO and two unknown fractions, free+conj., were present at levels of less than 3.8% TRR (0.16 mg eq/kg). Acid- and base-released four fractions that were observed individually at less than 3.2% TRR (0.14 mg eq/kg). Some 44% TRR (1.9 mg eq/kg) in water extract was not further investigated.

In milk, parent was not detected. Metabolite TMO was the predominant residue accounting for 42% TRR (0.17 mg eq/kg). Metabolite ph-COOH was found at a level of 9.0% TRR (0.037 mg eq/kg). DM-TM-COOH and one unknown fraction were present individually at less than 6.9% TRR (0.028 mg eq/kg).

In another study, a goat was dosed twice daily with [phenyl-^{14}C]-tolclofos-methyl for 6 consecutive days at a rate equivalent to 11 ppm in the feed (0.39 mg/kg bw per day). Milk was collected twice daily. The goat was sacrificed 7 hours after the last dosing.

The majority (85%) of the radiolabelled tolclofos-methyl was excreted in urine (46% of the total dose) and faeces (39% of the total dose). Residue levels in muscle and fat were near or below the limit of quantification. TRR levels in liver and kidney were 0.25 mg eq/kg and 0.22 mg eq/kg, respectively. TRR levels in milk reached a plateau of 0.014–0.019 mg eq/kg at approximately one day after the first dosing. A ratio of 8.1% of the total radioactivity in whole milk was distributed into milk fat.

In liver, acid and base conditions were used for extraction and partitioning with organic solvents. For milk and kidney samples, extraction was conducted under neutral conditions and partitioning steps were conducted at neutral, acidic and basic conditions. The extraction conditions may hydrolyse conjugates. Further, TMO-COOH found in the matrices may be an artefact produced under acidic conditions by oxidation of TM-CH_2OH.

Extraction efficiency of radioactivity was 66% TRR in liver and 93% TRR in kidney. Further treatments for liver released additional residues of 24% TRR (18% TRR by acid hydrolysis and 6.3% TRR by pronase incubation), and 9.9% TRR remained unextracted. Acetone extracted 87% TRR from the whey.

Parent was not detected in milk (milk whey). Metabolite TMO-COOH was found but at a low level of 6.7% TRR (0.001 mg eq/kg). Two unidentified fractions were observed individually at less than 12% TRR (0.002 mg eq/kg) in the organic phase. The radioactivity in the aqueous phase (66% TRR, 0.01 mg eq/kg) was not further investigated.

In liver, parent was present at 4.4% TRR (0.011 mg/kg). Metabolite ph-COOH was the largest component, accounting for 10% TRR (0.026 mg eq/kg). Five unidentified fractions were observed individually at less than 8.8% TRR (0.022 mg eq/kg) in the organic phase. The 28% TRR (0.069 mg eq/kg) in the aqueous phase consisted of nine fractions, individually at less than 8.2% TRR (0.021 mg eq/kg).

In kidney, parent was present at 12% TRR (0.029 mg/kg). Metabolite ph-COOH was the largest component, accounting for 13% TRR (0.031 mg eq/kg). TMO-COOH was found at 5.4% TRR (0.013 mg eq/kg). ph-CH_2OH and DM-TM were also found but at levels of less than 2% TRR (0.005 mg eq/kg). Five unidentified fractions were observed individually at less than 5.9% TRR (0.014 mg eq/kg) in the organic phase. The 43% TRR (0.096 mg eq/kg) in the aqueous phase consisted of six fractions, individually at less than 19% TRR (0.045 mg eq/kg).

Laying hens

The radiolabelled substance was orally administered to three laying hens daily for four consecutive days at a rate equivalent to 167 ppm in the diet (10 mg/kg bw per day). Eggs and excreta were collected daily. Hens were sacrificed 7 hours after the last dosing.

The majority (86%) of the administered total dose was recovered from excreta. TRR levels were 0.11 mg eq/kg in muscle, 1.0 mg eq/kg in fat, 3.4 mg eq/kg in liver, 6.0 mg eq/kg in kidney, up to 0.37 mg eq/kg in egg yolk and up to 0.07 mg eq/kg in egg white.

Liver and kidney samples were extracted with acidified organic solvent (diethyl ether after adjusting to pH 1), conditions that may hydrolyse conjugates.

Acidified organic solvent extracted 20% TRR and 40% TRR in liver and kidney, respectively. Further extractions (acid- and base-reflux followed by extraction with diethyl ether) released 8.1% TRR and 19% TRR in liver and kidney, respectively. Final unextracted radioactivity was 70% TRR and 40% TRR in liver and kidney, respectively.

In liver, parent was not detected. Metabolite TM-CHO was found at 3.4% TRR (free+conj., 0.12 mg eq/kg). Five unidentified fractions were present individually at less than 5.2% TRR (0.018 mg eq/kg). Acid- and base-released residues were not analysed.

In kidney, parent was not detected. Metabolite ph-COOH was found at 9.3% TRR (free+conj., 0.56 mg eq/kg). Eight unidentified fractions were present individually at less than 7.7% TRR (0.46 mg eq/kg). Acid- and base-released residues were not analysed.

In another study on laying hens (ten animals), the radiolabelled substance was orally administered for fourteen days at a dose level equivalent to 11 ppm in the feed (0.92 mg/kg bw per day). Eggs were collected daily prior to dosing. Hens were sacrificed 7 hours after the last dosing, and liver, muscle, fat, and skin were taken.

The majority (89%) of the radiolabelled tolclofos-methyl was eliminated in excreta. TRR levels were 0.008 (breast)–0.013 (thigh) mg eq/kg in muscle, 0.045 mg eq/kg in fat, 0.073 mg eq/kg in skin, 0.42 mg eq/kg in liver. In egg white and yolk, maximum TRR levels were 0.006 mg eq/kg and 0.059 mg eq/kg, respectively, with a plateau level of 0.057–0.059 mg eq/kg after 8–9 days in yolk.

For muscle and liver samples, acid and base conditions were used for extraction and partitioning with organic solvents. Egg yolk samples were extracted at neutral conditions with organic solvent and partitioned with organic solvent at neutral, acidic and basic conditions. Fat and skin samples were not treated with acid in extraction and partitioning. The extraction conditions used may hydrolyse conjugates. Further, TMO-COOH found in the matrices may be an artefact produced from TM-CH_2OH under acidic conditions.

Extractability of the radioactivity was 60–93% TRR in muscle, liver, fat, skin and yolk. For liver with the lowest extraction efficiency, 38% of the radioactivity was further extracted by acid, base and pronase hydrolyses (unextracted residue, 1.6% TRR).

In muscle (thigh), parent was detected at a level of 5.0% TRR (0.001 mg/kg). The largest component was metabolite ph-COOH found at 12% TRR (0.001 mg eq/kg). TMO-COOH was found at a level of 2.0% TRR (< 0.001 mg eq/kg). Six unidentified fractions in the organic phase were present individually at less than 16% TRR (0.002 mg eq/kg). Some 22% TRR (< 0.01 mg eq/kg) in the aqueous phase was not further investigated.

In fat, parent was the predominant residue, accounting for 76% TRR (0.034 mg/kg). Metabolite ph-COOH was found at a level of 3.7% TRR (0.002 mg eq/kg). Four unidentified fractions were observed individually at less than 4.0% TRR (0.002 mg eq/kg). TMO-COOH was not detected.

For skin, parent was found at 29% TRR (0.021 mg/kg). Metabolite ph-COOH was found at 11% TRR (0.008 mg eq/kg). Metabolites ph-CH_2OH and TMO-COOH were found at levels of less than 5.4% TRR (0.004 mg eq/kg in TMO-COOH). Four unidentified fractions were present at less than 6.8% TRR (0.005 mg eq/kg).

In liver, parent was detected at a level of 0.5% TRR (0.002 mg/kg). The largest component was ph-COOH found at 18% TRR (0.076 mg eq/kg; 15.6% TRR in the organic phase; 2.7% TRR in the aqueous phase). Other metabolites ph-CH_3, TMO-CH_2OH and TMO-COOH (0.7% TRR, 0.003 mg eq/kg) were found in the organic phase individually at less than 3.5% TRR (0.014 mg eq/kg). Eleven unidentified fractions in the organic and aqueous phases were present individually at less than 9.9% TRR (0.041 mg eq/kg).

In egg yolk, parent was the predominant residue, accounting for 35% TRR (0.021 mg eq/kg). TMO-COOH was not detected. Five unidentified fractions were present individually at less than 13% TRR (0.007 mg eq/kg). Some 13% TRR in the aqueous phase was not further investigated.

Conclusions

In general, the metabolism between goat, hen and rat is qualitatively similar. The Meeting concluded that, in all species investigated (goats, hens and rats), the total administered radioactivity was predominantly eliminated in excreta.

The routes and products of metabolism were similar across all animals. Tolclofos-methyl undergoes oxidative desulfuration, demethylation and hydrolysis of the P-O aryl bond to form ph-CH_3. The ph-CH_3 is further metabolized to its alcohol (ph-CH_2OH) and acid analogue (ph-COOH).

Methods of analysis

The Meeting received information on analytical methods for tolclofos-methyl in plant and animal matrices.

Single-residue analytical methods based on GC-NPD or GC-FPD involving extraction and partitioning with various organic solvents tested with potato or lettuce matrices were generally suitable to measure tolclofos-methyl. The multi-residue methods DFG S-19 (GC-FPD) and QuEChERS (LC-MS/MS) for the determination of tolclofos-methyl were sufficiently validated with potato in the former method and with lettuce, orange, cotton seed and dried beans in the latter method. In both single- and multi-residue methods, LOQ values were 0.01 mg/kg.

Regarding the determination of tolclofos-methyl in animal matrices, one multi-residue method was provided. This method involved use of the QuEChERS technique and LC-MS/MS, and was sufficiently validated in animal matrices (milk, bovine meat and liver, eggs and fat) with LOQs of 0.01 mg/kg. Further, the extraction efficiency for tolclofos-methyl was also validated relating with extraction of radiolabelled tolclofos-methyl in goat liver, hen's egg and fat.

The Meeting concluded that the presented methods were sufficiently validated and are suitable to measure tolclofos-methyl in animal and plant commodities.

Storage of pesticide residues in stored analytical samples

The Meeting received information on storage stability of tolclofos-methyl in lettuce and potato tuber

commodities.

Tolclofos-methyl was stable for at least 18 months in lettuce and 22 months in potatoes, when stored frozen at -18 °C.

The Meeting agreed that the demonstrated storage stability in the high water and the high starch commodities covered the residue sample storage intervals used in the field trials considered by the current Meeting.

Definition of the residue

Plant commodities

Parent tolclofos-methyl was present at various levels in the edible parts of the plants at up to 40% TRR in lettuce, up to 8.3% TRR in potato tubers and up to 50% TRR in sugar beet roots. In cotton and peanuts, the TRRs were too low to permit identification of residue components in the edible parts. Different metabolic profiles were observed for different application methods in lettuce with seedlings and soil treatment and in potato with seed tuber treatment.

In most primary crop commodities in the metabolism studies, tolclofos-methyl is found in significant proportions (8.3–40% TRR) and is a suitable marker compound. In supervised field trials on potatoes, parent was frequently found above the LOQ. The Meeting noted that suitable analytical methods exist to measure tolclofos-methyl in plant commodities. The Meeting defined the residue for compliance with the MRL in plant commodities as tolclofos-methyl.

Regarding dietary risk assessment, major metabolites were DM-TM-CH_2OH found at 11–27% TRR in potatoes, ph-CH_3 sugar conjugate (malonylglucose conjugate) found at 20–23% TRR and TM-CH_2OH sugar conjugate (glucose conjugate) found at 14–15% TRR in lettuce. DM-TM was a major processing degradate of tolclofos-methyl, which occurred at 24–87% applied radioactivity in a high temperature hydrolytic study and could be detected in heated potatoes and lettuce. The metabolites were identified in the rodent metabolism studies at significant levels (> 10% of TRR), and hence are covered by the risk assessment for parent compound. The Meeting concluded that these metabolites potentially add significantly to the dietary exposure to tolclofos-methyl in plant commodities and should be included in the residue definition for dietary risk assessment in plant commodities.

In plant commodities, the residue definition for dietary risk assessment is the sum of tolclofos-methyl, ph-CH_3 (including conjugates), TM-CH_2OH (including conjugates), DM-TM-CH_2OH and DM-TM, expressed as tolclofos-methyl.

To convert residues of tolclofos-methyl from the supervised trials to values for total residue (sum of tolclofos-methyl and the metabolites), the Meeting derived the following adjustment factors from the ratios of total residue to parent residues observed in the metabolism studies (lettuce, potato).

Leafy greens (seedlings and soil treatment): 2.0 (lettuce).

Potato (seed tuber treatment): 6.0 (potato seed tuber).

Animal commodities

Metabolism studies in lactating goats and laying hens were conducted at two dose levels (250 ppm and 11 ppm in goats; 167 ppm and 11 ppm in hens). The Meeting noted that the livestock dietary burden based on uses considered by the Meeting was very low and considered the lower dose level more representative. The Meeting decided to use the results from the metabolism study performed at the lower dose level.

In goat, tolclofos-methyl was found at 4.4% TRR (0.011 mg/kg) in liver, 12% TRR (0.029 mg/kg) in kidney and was not detected in milk. In hens, tolclofos-methyl was found at 0.5% TRR (0.002 mg/kg) in liver, 5.0% TRR (0.001 mg/kg) in muscle, 35% TRR (0.021 mg/kg) in yolk, 29% TRR (0.021 mg/kg) in skin and 76% TRR (0.034 mg/kg) in fat.

Tolclofos-methyl was found in most animal commodities, was the most significant residue in hen fat, skin and yolk and is therefore a suitable marker compound. The Meeting noted that a suitable analytical method exists to measure tolclofos-methyl in animal commodities. The Meeting defined the residue for compliance with the MRL in animal commodities as tolclofos-methyl.

The log K_{ow} value of tolclofos-methyl indicates lipophilic properties (3.8 at 25 °C). Residues of tolclofos-methyl in fatty matrices were at least 30-fold higher than residues in non-fatty matrices (egg white/egg yolk: ND/0.021 mg/kg; hens muscle/fat: 0.001/0.034 mg/kg). Therefore, the Meeting concluded that the residue is fat-soluble.

Regarding dietary risk assessment, metabolite ph-COOH (incl. conjugate) was a major metabolite found at 10% TRR (0.026 mg eq/kg) in goat liver, 13% TRR (0.031 mg eq/kg) in goat kidney, 18% TRR (0.076 mg eq/kg) in hen liver, 12% TRR (0.001 mg eq/kg) in hen muscle, 11% TRR (0.008 mg eq/kg) in hen skin and 3.7% TRR (0.002 mg eq/kg) in hen fat. The metabolite was identified at significant levels (> 10% of TRR) in the rodent metabolism studies, and hence is covered by the risk assessment for the parent compound. The Meeting concluded that the metabolite adds significantly to the dietary exposure arising from animal commodities and decided to include ph-COOH for dietary risk assessment for animal commodities.

Metabolite TMO-COOH (incl. conjugate), which can be produced by oxidation of TM-CH_2OH under acidic conditions during the analytical extraction process, was also found at 5.4% TRR (0.013 mg eq/kg) in goat kidney, 6.7% TRR (0.001 mg eq/kg) in goat milk, 2.0% TRR (< 0.001 mg eq/kg) in hen muscle, 1.3% TRR (0.001 mg eq/kg) in hen skin, and 0.7% TRR (0.003 mg eq/kg) in hen liver. The Meeting noted that TMO-COOH is less than 10% of TRR in all matrices and < 0.01 mg eq/kg in all matrices except goat kidney. The Meeting further noted that the dose level in the goat metabolism study (11 ppm) is 24-fold higher than the maximum dietary burden for beef cattle calculated by the current Meeting. The interval between the last dose and sacrifice in the goat study (6–7 hours) is significantly shorter than the interval between last feeding and slaughter of mammalian livestock in normal commercial practice (typically 20–24 hours), and the Meeting therefore considered that the metabolism study is likely to overestimate the level of TMO-COOH found in animal commodities in practice. The Meeting considered that there is little possibility of significant levels of TMO-COOH being detected in animal commodities, and decided not to include TMO-COOH in the definition for dietary risk assessment for animal commodities.

The Meeting defined the residue for dietary risk assessment for animal commodities as the sum of tolclofos-methyl and 3,5-dichloro-4-hydroxybenzoic acid (ph-COOH), expressed as tolclofos-methyl.

The Meeting recommended the following residue definitions for tolclofos-methyl:

Definition of the residue for compliance with the MRL for plant commodities: *tolclofos-methyl*.

Definition of the residue for dietary risk assessment for plant commodities: *sum of tolclofos-methyl, ph-CH_3 (incl. conjugates), TM-CH_2OH (incl. conjugates), DM-TM-CH_2OH and DM-TM, expressed as tolclofos-methyl.*

Definition of the residue for compliance with the MRL for animal commodities: *tolclofos-methyl*.

Definition of the residue for dietary risk assessment for animal commodities: *sum of tolclofos-methyl and ph-COOH, expressed as tolclofos-methyl*.

The residue is fat-soluble.

Results of supervised residue trials on crops

Supervised trials were available for the use of tolclofos-methyl on potato and lettuce. Product labels were available from Belgium, Germany, Italy and the Netherlands. The Meeting withdrew its previous recommendation on radish.

Leafy vegetables

Lettuce

The critical GAP for tolclofos-methyl on protected lettuce and other salad greens in Italy is a single spray application at a rate of 2 kg ai/ha when transplanting with a 28 days PHI. Five independent trials conducted in Belgium, France and Italy in 2000 and 2005 matched the Italian GAP.

Tolclofos-methyl residues in head lettuce were (n = 5): 0.08, 0.16, 0.18, 0.24 and 0.39 mg/kg.

As application was made at BBCH 18–19 or 12–16 (2–6 true leaves), no difference in residue levels between head lettuce and leafy lettuce is expected. Therefore, the Meeting decided to estimate a maximum residue level for head lettuce and leafy lettuce.

The Meeting estimated maximum residue levels of 0.7 mg/kg for tolclofos-methyl in head lettuce and leafy lettuce. Based on the adjustment factor of 2.0 for total residues (parent plus metabolites), the Meeting estimated STMRs of 0.36 mg/kg (0.18 mg/kg × 2.0) for head lettuce and leafy lettuce.

The GAP covers use on crops in the subgroup of leafy greens, except spinach, purslane and chard. Therefore, the Meeting estimated a maximum residue level of 0.7 mg/kg and a STMR of 0.36 mg/kg for tolclofos-methyl in the Subgroup 013A Leafy greens except spinach, purslane and chard.

Root and tuber vegetables

Potato

The critical GAP for tolclofos-methyl on potato seed tuber in Italy is a single seed dressing before planting at a rate of 0.25 kg ai/t tubers. Thirty-one independent trials conducted in France, Germany, Greece, Italy, Spain and the UK, conducted between 1980 and 2013, matched the Italian GAP.

Tolclofos-methyl residues in potato were (n = 31): < 0.01 (10), 0.01 (6), 0.02 (5), 0.03, 0.04, < 0.05 (2), 0.05, 0.08, 0.08, 0.12, 0.18 and 0.21 mg/kg.

The Meeting estimated a maximum residue level of 0.3 mg/kg for tolclofos-methyl in potato. Based on the adjustment factor of 6.0 for total residues, the Meeting estimated a STMR of 0.060 mg/kg (0.010 mg/kg × 6.0) for potato.

Fate of residues during processing

Tolclofos-methyl was converted into DM-TM under processing hydrolysis conditions. At pH 4 and 90 °C (20 min), pH 5 and 100 °C (60 min), and pH 6 and 120 °C (20 minutes) conditions, tolclofos-methyl/DM-TM occurred at 75%/24%, 47%/53% and 13%/87%, respectively.

In the processing studies on potatoes, no information was provided on the fate of tolclofos-methyl metabolites during processing.

Table 2.Tolclofos-methyl processing factors (PF) for livestock dietary burden estimation

Raw commodity	Processed commodity	Individual PF	Mean or best estimate PF
Potato	Potato wet peel	1.5, 2.0, 2.5, 2.9, 3.3, 3.6, 3.7, 4.0, 4.0, 4.1, 4.4, 4.5, 5.0, 5.3, 5.3, 6.0, 6.0, 6.6, 6.7, 7.1 and 7.2 (n = 21)	4.4

Residues in animal commodities

Farm animal feeding studies

No information was provided.

Farm animal dietary burden

In the current Meeting, potato cull and potato process waste (wet peel) were feed items relevant to estimate animal dietary burdens. Based on potato field residue data, median and highest residue values for tolclofos-methyl in potatoes were 0.01 mg/kg and 0.21 mg/kg, respectively. The median residue of tolclofos-methyl in potato wet peel (potato process waste) was calculated as 0.044 mg/kg by applying the processing factor of 4.4 (0.01 mg/kg × 4.4).

Dietary burden calculations for cattle and poultry are provided below. The dietary burdens were estimated using the 2018 OECD Feed diets listed in Appendix XIV Electronic attachments to the 2016 edition of the FAO manual[17].

To estimate maximum residue levels for tolclofos-methyl in animal commodities, maximum dietary burdens for tolclofos-methyl from potato feed items were estimated. Further, to estimate STMRs and HRs for the sum of tolclofos-methyl and ph-COOH in animal commodities, mean and maximum dietary burdens for the sum of tolclofos and the metabolites convertible to ph-COOH (DM-TM-CH_2OH, DM-TM-COOH, TM-CH_2OH, ph-COOH and DM-TMO found in a metabolism study on potato) were estimated by multiplying maximum and mean dietary burdens for tolclofos-methyl with a factor of 6. The factor 6 was calculated by 0.012 mg eq/kg (sum) divided by 0.002 mg/kg (parent) based on residue levels shown in a potato metabolism study: parent 0.002 mg/kg, DM-TM-CH_2OH 0.004 mg eq/kg, DM-TM-COOH 0.002 mg eq/kg,TM-CH_2OH 0.002 mg eq/kg, ph-COOH < 0.001 mg eq/kg and DM-TMO < 0.001 mg eq/kg.

Table 3 Estimated animal dietary burden

	Animal dietary burden: tolclofos-methyl, ppm of dry matter diet					
	US-Canada		**EU**		**Australia**	
	max	**mean**	**max**	**mean**	**max**	**mean**
Beef cattle	0.43	0.13	0.46[a]	0.16	0.12	0.023
			(2.8)[A]	(0.97)[B]		
Dairy cattle	0.14	0.042	0.43[b]	0.13	0.11	0.005
			(2.6)[A]	(0.75)[B]		
Poultry – broiler			0.11[c]	0.005		
			(0.63)[A]	(0.030)[B]		
Poultry – layer			0.11[d]	0.005		
			(0.63)[A]	(0.030) [B]		

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

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

[c] Highest maximum poultry dietary burden suitable for MRL estimates for poultry tissues.

[d] Highest maximum poultry dietary burden suitable for MRL estimates for poultry eggs.

[A] Values in bracket are burdens for estimates of animal HRs (6×parent)

[B] Values in bracket are burdens for estimates of animal STMRs (6×parent)

Animal commodity maximum residue levels

Feeding studies (goat, hen) were not available. The Meeting decided to use the goat and hen metabolism studies conducted at a feeding level of 11 ppm to evaluate residue levels in mammalian and poultry commodities.

The residue definition for compliance with MRLs in animal commodities is tolclofos-methyl. Residues of tolclofos-methyl in the goat metabolism study were not detected in milk, < 0.01 mg/kg in fat, < 0.005 mg/kg in muscle, 0.011 mg/kg in liver and 0.029 mg/kg in kidney. When scaled to the dietary burden for estimating maximum residue levels (0.46 ppm beef cattle, 24-fold lower than the dose in the metabolism study/0.43 ppm dairy cattle), the anticipated residues are < 0.01 mg/kg in all

[17] http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmpr/jmpr-docs/en/

commodities. The Meeting estimated maximum residue levels of 0.01(*) mg/kg for all mammalian commodities.

The residue definition for dietary risk assessment in animals is the sum of tolclofos-methyl and ph-COOH, expressed as tolclofos-methyl. Residues corresponding to the risk assessment definition from the goat metabolism study were: not detected in milk, < 0.01 mg/kg in fat, < 0.005 mg/kg in muscle, 0.037 mg/kg in liver and 0.060 mg/kg in kidney. When scaled to the dietary burden for risk assessment (mean 0.97 ppm in beef cattle; mean 0.75 ppm in dairy cattle STMR estimates are 0 mg/kg for fats (except milk fats), 0 mg/kg for meat (from mammals other than the marine mammals), 0.0055 mg/kg for edible offal (mammalian; based on kidney) and an STMR of 0 mg/kg in milks.

For poultry, residues of tolclofos-methyl in the metabolism study were 0.001 mg/kg in muscle, 0.034 mg/kg in fat, 0.021 mg/kg in skin, 0.002 mg/kg in liver and 0.021 mg/kg in yolk. When scaled to the dietary burden for estimating maximum residue levels (0.11 ppm poultry, broiler and layer, 105-fold lower than the dose of the metabolism study), the anticipated residues are < 0.01 mg/kg in all commodities. The Meeting estimated maximum residue levels of 0.01(*) mg/kg for all poultry commodities.

Residues corresponding to the risk assessment definition from the hen metabolism study were: 0.002 mg/kg in muscle, 0.036 mg/kg in fat, 0.029 mg/kg in skin, 0.078 mg/kg in liver and 0.021 mg/kg in yolk. When scaled to the dietary burden for risk assessment (mean 0.030 ppm in poultry broiler and layer), STMR estimates are 0 mg/kg for muscle, 0 mg/kg for fat, 0 mg/kg in skin, 0 mg/kg for edible offal (based on liver) and 0 mg/kg in egg yolk.

RECOMMENDATIONS

On the basis of the data obtained from supervised trials, the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IEDI assessment.

Definition of the residue for compliance with the MRL for plant and animal commodities: *tolclofos-methyl*

Definition of the residue for dietary risk assessment for plant commodities: *sum of tolclofos-methyl, 2,6-dichloro-4-methylphenol (ph-CH_3, incl. conjugates), O,O-dimethyl O-2,6-dichloro-4-(hydroxymethyl) phenylphosphorothioate (TM-CH_2OH, incl. conjugates), O-methyl O-hydrogen O-2,6-dichloro-4-(hydroxymethyl) phenylphosphorothioate (DM-TM-CH_2OH) and O-methyl O-hydrogen O-(2,6-dichloro-4-methylphenyl) phosphorothioate (DM-TM), expressed as tolclofos-methyl*

Definition of the residue for dietary risk assessment for animal commodities: *sum of tolclofos-methyl and 3,5-dichloro-4-hydroxybenzoic acid (ph-COOH), expressed as tolclofos-methyl*

The residue is fat-soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for tolclofos-methyl is 0–0.07 mg/kg bw. The International Estimated Daily Intakes (IEDIs) for tolclofos-methyl were estimated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2019 JMPR Report.

The IEDIs ranged from 0–1% of the maximum ADI. The Meeting concluded that long-term dietary exposure to residues of tolclofos-methyl from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The 2019 JMPR decided that an ARfD for tolclofos-methyl was unnecessary. The Meeting therefore concluded that the acute dietary exposure to residues of tolclofos-methyl from the uses considered is

unlikely to present a public health concern.