TOLCLOFOS-METHYL (191)

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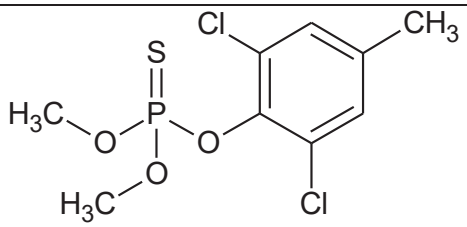
EXPLANATION

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.

IDENTITY

ISO common name:	Tolclofos-methyl
Chemical name	
IUPAC:	O-2,6-dichloro-p-tolyl O,O-dimethyl phosphorothioate
CAS:	O-(2,6-dichloro-4-methylphenyl) O,O-dimethyl phosphorothioate
CAS Registry No.:	57018–04–9
CIPAC No.	479
Molecular formula:	C9H11Cl2O3PS
Structural formula:	
Molecular weight:	301.1 g/mol

PHYSICAL AND CHEMICAL PROPERTIES

Pure active ingredient

Chemical/physical property	Findings	Reference
Appearance (TM 97.9%, technical ai)	White crystalline solid; white solid at 22 °C	Asada, 1996a, QP-0072, 1996b, QP-0073; Reitz, 2010, QP-0118
Appearance (TM 100%, purified ai)	White crystalline solid at 20 °C	Walker, 2014a, QP-0131
Melting point (TM 99.3%)	78.1–79.3 °C	Reitz, 2010, QP-0118
Boiling point and thermal decomposition (TM 99.7%)	Decomposed before boiling; decomposition temperature, 120–220 °C	Bates, 2001, QP-0094
Density (TM 100%)	1.53×10^3 kg/m^3 at 24 °C	Walker, 2014, QP-0131
Vapour pressure (TM 99.7%)	8.77×10^{-4} Pa at 20 °C and 1.82×10^{-3} Pa at 25 °C (by interpolation)	Hayes, 2001, QP-0100
Solubility in water (TM 99.7%)	0.708 mg/L at 20 °C	Concha, 2001, QP-0095
Solubility in organic solvents	At 20 °C,	Walker, 2014, QP-0131

Chemical/physical property	Findings	Reference
(TM 100%)	10–14 g/L n-heptane, 200–250 g/L toluene, 167–200 g/L dichloromethane, 25–29 g/L methanol, > 250 g/L acetone, > 250 g/L ethyl acetate	
Partition co-efficient n-octanol/water (TM 99.3%)	log K_{ow}= 3.8 at 25 °C and pH 5.7	Bondarenko, 2010, QP-0117
Dissociation in water; pKa values	Not applicable (Concerning the structure of the test material)	Shigenaga, 1989, QP-90-0038
Hydrolytic stability [phenyl-^{14}C]TM 98.9%	Half-life values (at pH 4, 7 and 9, respectively): 97, 61 and 76 hours at 50 °C, 32, 17 and 24 hours at 62 °C and 9.6, 5.1 and 7.3 hours at 74 °C. Calculated half-lives (at pH 4, 7 and 9, respectively) 126, 97 and 102 days at 20 °C and 68, 50 and 55 days at 25 °C	Lewis, 2001a, QM-0051
Photolysis in sterile water [phenyl-^{14}C]TM 99.0% (sterilised water at 25°C and pH 7),	Half-life value, 38.3 days (76.6 days under dark condition) under artificial light (at least 30 days outdoor conditions)	Takahashi and Katagi, 1988, QM-80-0024
Photolysis in sterile water [Phenyl-^{14}C] TM 99.7% (sterilised water at 25°C)	Half-lives estimated for direct photolysis, 8.2–48.5 days at any latitude and seasons	Curtis-Jackson, 2014a, QM-0074

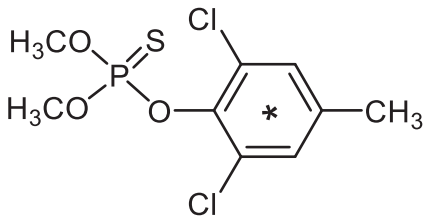
Formulations

FAO specifications for tolclofos-methyl have not been developed. The formulations are as follows:

Type of formulation	Active ingredient content
DS (powder for dry seed treatment)	100 g/L (10%, w/w)
SC (suspension concentrate)	500 g/L (42%, w/w)
WP (wettable powder) or WPS (wettable powder in water-soluble bags)	50 g/100 g (50%, w/w)
Flowable concentrate for seed treatment (FS)	250 g/L (25%, w/w)

METABOLISM AND ENVIRONMENTAL FATE

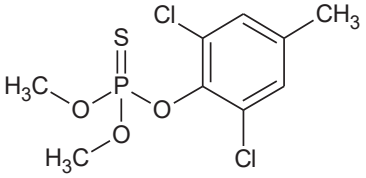
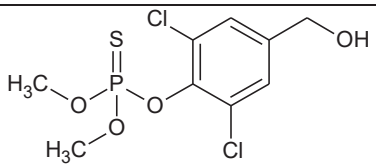
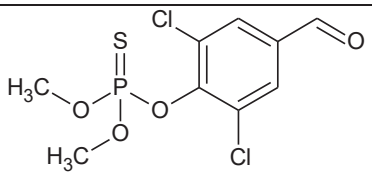
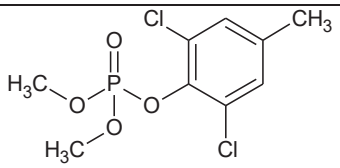
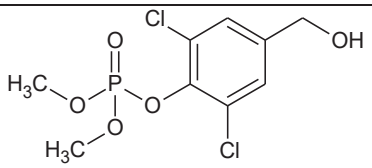
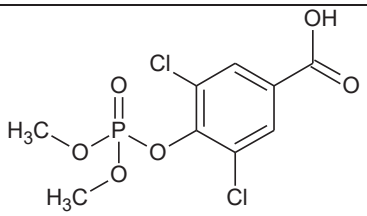
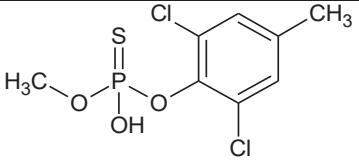
The fate and behaviour of tolclofos-methyl in animals, plants and soils were investigated using tolclofos-methyl labelled in the phenyl ring.

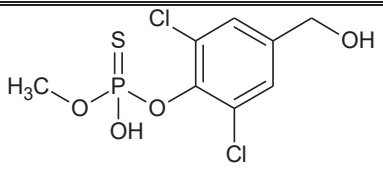
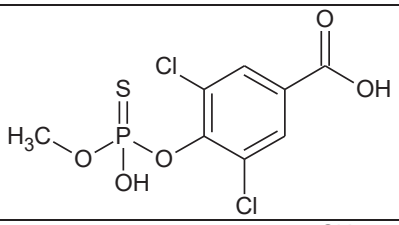
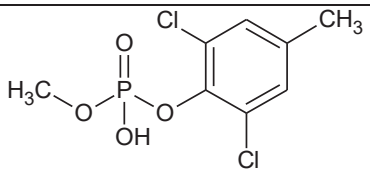
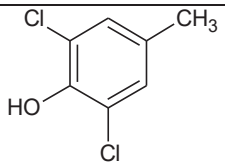
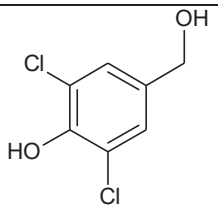
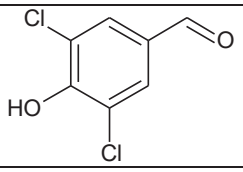
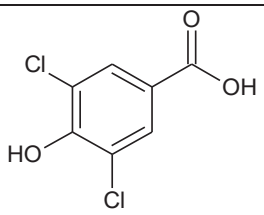
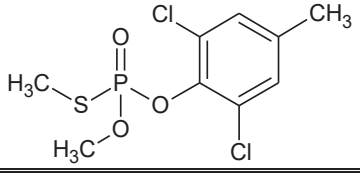


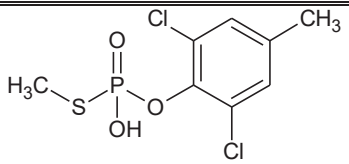
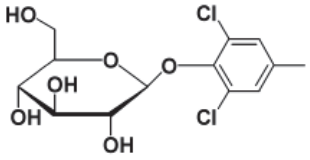
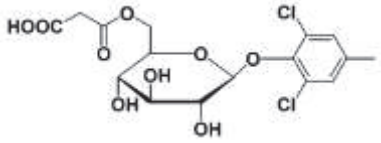
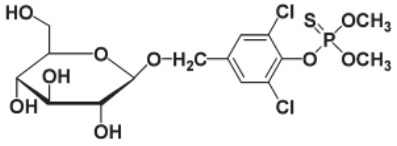
(*) position of radiolabel

The chemical structures of the major degradation compounds arising from the metabolism of tolclofos-methyl are presented in Table 1.

Table 1 Degradation compounds from metabolism of tolclofos-methyl in plants, animals and the environment

Compound name	Chemical name	Structure	Found in
Tolclofos-methyl TM (parent)	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate MW: 301.12 g/mol		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	*O,O*-dimethyl *O*-2,6-dichloro-4-(hydroxymethyl) phenylphosphorothioate MW: 317.13 g/mol		Sugar beet (leaves) Peanut (leaves, stem) Potato (foliage, roots, parent tubers, daughter tubers) Lettuce (as TM-CH_2OH conjugate)
TM-CHO	*O,O*-dimethyl *O*-(2,6-dichloro-4-formylphenyl) phosphorothioate MW: 315.11 g/mol		Hen (liver)
TMO	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphate MW: 285.06 g/mol		Goat (milk) Sugar beet (leaves, shoots, roots) Peanut (leaves, stem), Aqueous photolysis (irradiated, control), Aerobic soil
TMO-CH_2OH	*O,O*-dimethyl *O*-2,6-dichloro-4-(hydroxymethyl) phenylphosphate MW: 301.06 g/mol		Goat (kidney) Hen (liver) Sugar beet (leaves) Peanut (stem)
TMO-COOH	*O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl) phosphate MW: 315.04 g/mol		Goat (kidney, milk) Hen (liver, muscle, skin) Sugar beet (leaves, shoots, roots) Peanut (stem), Aerobic soil
DM-TM	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate MW: 287.10 g/mol		Goat (kidney) Sugar beet (leaves) Potato (foliage, shoots, roots, parent tubers, daughter tubers), Aqueous hydrolysis, Aqueous photolysis (irradiated, control), Aerobic soil

Compound name	Chemical name	Structure	Found in
DM-TM-CH_2OH	*O*-methyl *O*-hydrogen *O*-2,6-dichloro-4-(hydroxymethyl) phenylphosphorothioate MW: 303.10 g/mol		Goat (kidney) Potato (foliage, shoots, roots, parent tubers, daughter tubers)
DM-TM-COOH	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphorothioate MW: 317.08 g/mol		Goat (milk) Potato (foliage, roots, parent tubers, daughter tubers)
DM-TMO	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphate MW: 271.03 g/mol		Goat (kidney) Sugar beet (leaves) Peanut (hull) Potato (foliage, roots, parent tubers, daughter tubers), Aqueous photolysis (irradiated), Aerobic soil
ph-CH_3	2,6-dichloro-4-methylphenol MW: 177.03 g/mol		Goat (liver) Hen (liver) Sugar beet (leaves, roots) Peanut (leaves), Aqueous hydrolysis (pH 9: irradiated, control), Aerobic soil
ph-CH_2OH	3,5-dichloro-4-hydroxybenzyl alcohol MW: 193.03 g/mol		Goat (liver, kidney) Hen (skin) Sugar beet (leaves) Peanut (leaves, stem), Aerobic soil
ph-CHO	3,5-dichloro-4-hydroxybenzaldehyde MW: 191.01 g/mol		Goat (liver) Peanut (leaves)
ph-COOH	3,5-dichloro-4-hydroxybenzoic acid MW: 207.01 g/mol		Goat (milk, kidney, liver) Hen (liver, kidney, muscle, fat, skin) Potato (foliage, shoots, roots, parent tubers, daughter tuber), Aerobic soil
SM-TM (TM-SCH_3)	O-methyl S-methyl O-(2,6-dichloro-4-methyl-phenyl) phosphorothioate MW: 301.13 g/mol		Aqueous photolysis, Soil photolysis (irradiated, control)

Compound name	Chemical name	Structure	Found in
DM-SM-TM (DM-TM-SCH_3)	O-hydrogen S-methyl O-(2,6-dichloro-4-methyl-phenyl) phosphorothioate MW: 287.1 g/mol		Soil photolysis (irradiated, control)
Glucose conjugate of ph-CH_3			Lettuce
Malonylglucose conjugate of ph-CH_3			Lettuce
Glucose conjugate of TM-CH_2OH			Lettuce

PLANT METABOLISM

The Meeting received information on the fate of tolclofos-methyl following foliar or soil application or seed treatment on leafy vegetables (lettuce), root and tuber vegetables (sugar beet, potato) and oilseeds (cotton, peanut).

Lettuce

Study 1

The metabolism of [phenyl-^{14}C]-tolclofos-methyl was investigated in greenhouse grown lettuce plants following a single application to seedlings and soil in crates [Croucher, 2002, QM-0053]. Lettuce seed (Winter lettuce; var. Nixon) was sown in seed trays and seedlings grown to the 3–4 leaf stage (BBCH 14) were transplanted and on the next day, the radiolabelled tolclofos-methyl was applied. [phenyl-^{14}C]-tolclofos-methyl, prepared with wettable powder formulation, was once sprayed evenly over the soil surface (UK, loamy sand or sand soil; USDA, sand soil) at a rate of 2 kg ai/ha (actual, 1.9 kg ai/ha) and at an exaggerated rate of 10 kg ai/ha (actual, 9.2 kg ai/ha). The plants were grown to maturity in a greenhouse and harvested 34 days after the application. The roots and any decaying leaves were removed and discarded. Lettuce samples were chopped, stored at -20 °C and for analysis, homogenised with dry ice. Soil samples were taken at harvest.

Radioactive residues in lettuce were extracted with acetone/water (1:1, v/v), thus acetone/water extract and post extraction solid (PES) were produced. The PES was further extracted with methanol and then sequentially, treated with 1M HCl at 60 °C for 1 hour and 5M NaOH at 80 °C for 3 hours.

The acetone/water extract was concentrated and resulted in two fractions, "concentrated extract" fraction and "condensate" fraction (during evaporation using rotary evaporator, acetonitrile was added to the concentrated extract to aid the removal of the water from the extract, without using excessive temperature; the condensate trapped during the evaporation of the extract was also collected). The "concentrated extract" was centrifuged (supernatant and pellet) and the pellet radioactivity was extracted with acetonitrile. The "condensate fraction" was fractionised to top

(hexane), middle (acetonitrile/water) and bottom (aqueous) layers by partitioning with hexane/water (1:2, v/v).

Radioactivity was by LSC or combustion/LSC. HPLC analyses were performed by using the following reference standards: TM, TM-CH_2OH, TM-COOH, TMO, TMO-CH_2OH, TMO-COOH, DM-TM, DM-TM-CH_2OH, DM-TM-COOH, DM-TMO, DM-TMO-CH_2OH, DM-TMO-COOH, ph-CH_3, ph-CH_2OH and ph-COOH. TLC analysis was carried out for confirmation of TM, TM-CH_2OH and ph-CH_3 identity.

Unknown fractions, M22 and M35, were subjected to enzyme hydrolyses as follows and analysed by HPLC. The metabolite fractions were treated separately with cellulase and β-glucosidase enzyme at 39 °C for 16 hours (0.01 M pH 5 phosphate buffer). For cellulase enzyme, a prolonged incubation (16 hours and 4 days at 39 °C 0.01 M pH 5 phosphate buffer) was further made. In addition, M22 and M35 fractions were subjected to acid hydrolysis with 1M HCl at 80 °C for 2 hours and analysed by HPLC. For M22 fraction treated with cellulase, LC/MS was also used for identification.

For soil, radioactivity was determined by combustion/LSC analysis and soil samples were subjected to Soxhlet extraction using acetone/glacial acetic acid (98:2, v/v). Radioactivity in extracts was analysed by LSC and HPLC. TLC was used for confirmation for tolclofos-methyl identity. The results are shown in Tables 2–3.

TRR levels were in mature lettuce were 0.23 mg eq/kg and 0.77 mg eq/kg at a rate of 2 kg ai/ha (1× rates) and 10 kg ai/ha (5× rates), respectively.

From the lettuce matrices (1–5× rates), 50% aqueous acetone extracted 66% of the total radioactivity. From the PES, methanol extracted additionally 16–20% of the total radioactivity in the lettuce. Thus, in total, 82–86% of the total radioactivity in lettuce was extracted. By acid and base hydrolyses on the remained residue, 3.3–3.5% TRR by acid hydrolysis and 10.6–12.7% TRR by base hydrolysis were released. Finally, remaining residue was 0.5–1.7% TRR.

In acid hydrolysis experiments on the two unknown fractions, the M22 and M35 were almost intact with *β*-glucosidase enzyme, but significantly hydrolysed with cellulase enzyme (16 hours incubation).

The M22 treated with cellulase (4 days incubation) was completely hydrolysed to 37 min peak, which was ph-CH_3 (confirmed by TLC and LC/MS). Meanwhile, in 16 hours incubation, thoursee peaks, M22, 19 min and 37 min, occurred. The 19 min peak, an intermediate product between M22 and ph-CH_3, was demonstrated as ph-CH_3-glucose by LC-MS (exact structure, not determined). Further, under acid hydrolysis condition, M22 was almost completely converted to ph-CH_3. Thus, the unknown M22 fraction was identified as a sugar conjugate of ph-CH_3.

Table 2 Extraction of radioactive residues in lettuce following a single application of [phenyl-^{14}C]-tolclofos-methyl to seedlings and soil

Fraction	Lettuce, 2 kg ai/ha		Lettuce, 10 kg ai/ha	
	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)
Acetone/water extract	66.0	0.15	65.6	0.50
Concentrated extract	54.2	0.12	51.3	0.39
Supernatant*	47.5	0.11	46. 5	0.36
Pellet	6.7	0.016	4.9	0.037
ACN extract*	5.0	0.011		
Pellet	1.8	0.004		
Condensate	9.9	0.023	12.5	0.096
Hexane phase*	4.7	0.011	5.3	0.040
Bottom phase	0.5	0.001	0.9	0.007
Middle phase*	4.7	0.011	6.1	0.047
Losses in partition	0.4	0.001	0.4	0.003
Losses during concentration	1.8	0.004	1.7	0.013

Fraction	Lettuce, 2 kg ai/ha		Lettuce, 10 kg ai/ha	
	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)
PES	34.0	0.078	34. 5	0.26
MeOH extract*	16.2	0.037	20.1	0.15
Pellet	17.9	0.041	14.3	0.11
Supernatant after acid hydrolysis (1M HCl at 60 °C for 1 hours)*	3.5	0.008	3.3	0.025
Supernatant after base hydrolysis (5M NaOH at 80 °C for 3 hours)	12.7	0.029	10.6	0.081
Unextracted	1.7	0.004	0.5	0.003
Total	100	0.23	100	0.77

* Analysed by HPLC

Table 3 Parent and its metabolites in lettuce following a single application of [phenyl-^{14}C]-tolclofos-methyl to seedlings and soil

Fraction	Lettuce, 2 kg ai/ha (0.23 mg eq/kg)		Lettuce, 10 kg ai/ha (0.77 mg eq/kg)	
	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)
Acetone/water extract	66.0	0.15	65.6	0.49
Parent	17.9	0.041	19.6	0.15
TM-CH_2OH conjugate (M35)	13.7	0.032	14.7	0.11
ph-CH_3 conjugate (M22)	22.5	0.052	19.9	0.15
Polar	6.8	0.016	2.9	0.022
Others	1.8	0.004	4.9	0.037
Total	62.8		63.9	
PES	34.0	0.078	34.5	0.26
Methanol extract	16.2	0.037	20.1	0.15
Parent	16.2	0.037	20.1	0.15
Acid hydrolysate (1M HCl at 60 °C for 1 hours)	3.5	0.0081	3.3	0.025
Parent	2.7	0.0061		
Polar	0.9	0.0020		
Others	0.01	0.0000		
Base hydrolysate (5M NaOH at 80 °C for 3 hours)	12.7	0.029	10.6	0.081
Remaining	1.7	0.0038	0.5	0.003
Total	96.1		94.5	

Polar, not retained on HPLC column

Parent: 36.7% TRR (0.084 mg/kg; 17.9% TRR+16.2% TRR+2.7% TRR) at 2 kg ai/ha; 39.7% TRR (0.30 mg/kg; 19.6% TRR +20.1% TRR) at 10 kg ai/ha

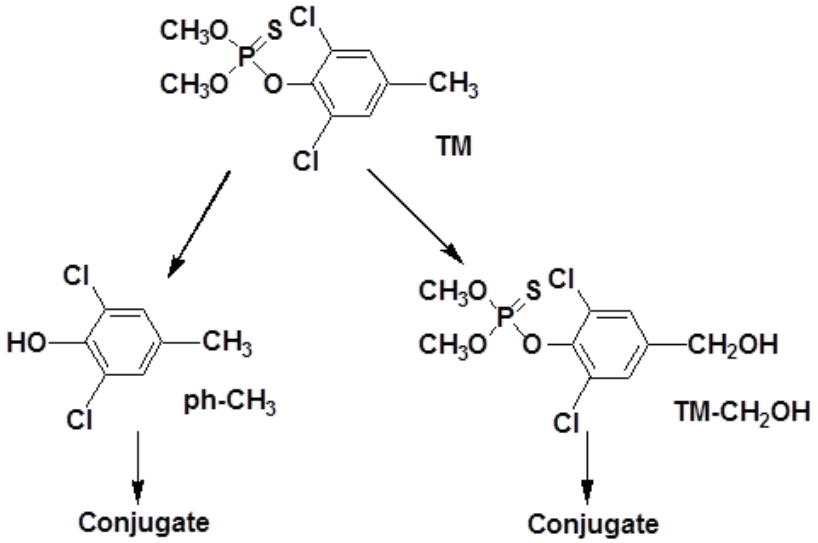


Figure 1 Proposed metabolic pathway of tolclofos-methyl in lettuce following seedlings and soil application

The M35 treated with cellulase (16 hours incubation) was completely converted to 40 min peak, which was TM-CH_2OH (confirmed by TLC). Under acid hydrolysis condition, M35 was almost completely converted to 28 min peak (co-choursomatographed with TMO-COOH reference standard), therefore, it was assumed that the acidic conditions not only de-conjugated the TM-CH_2OH conjugate but also further transformed to TMO-COOH. Thus, M35 was identified as a sugar conjugate of TM-CH_2OH (LC-MS analysis, not performed).

In lettuce (1–5× rates), parent was a main component, accounting for 36.7–39.7% TRR (0.084–0.30 mg/kg). The unknown M22, identified as a sugar conjugate of ph-CH_3, was present at 19.9–22.5% TRR (0.052–0.15 mg eq/kg). The unknown M35, identified as a sugar conjugate of TM-CH_2OH, was present at 13.7–14.7% TRR (0.032–0.11 mg eq/kg). Polar unidentified components were present at 2.9–7.7% TRR (0.018–0.022 mg eq/kg) in total. Other minors were present at 1.8–4.9% TRR (0.004–0.037 mg eq/kg) in total.

Soil (1–5× rates) was shown to contain only parent compound with TRR levels of 0.70–4.3 mg eq/kg.

Based on the identified metabolites in lettuce (seedlings and soil application), it was shown that tolclofos-methyl is metabolized via cleavage of the P-O-aryl linkage to form ph-CH_3 and oxidation of the 4-methyl group to form TM-CH_2OH. These metabolites subsequently undergo conjugation with sugars.

A proposed metabolic pathway of tolclofos-methyl in lettuce is shown in Figure1.

Study 2

The JMPR found a study of metabolism on tolclofos-methyl in lettuce available from open literature. The study was conducted to investigate the metabolic pathway of [phenyl-^{14}C]-tolclofos-methyl in lettuce following a foliar application [Ichise-Shibuya, *et al*., 2004]. Seedlings of lettuce (Lactuca sativa, cv. King crown) grown until the 3 to 4-leaf stage in a seed tray filled with Takarazuka soil were transplanted to wagner pots. The plants were grown in a greenhouse until harvest (for approximately 3 months). The radiolabeled substance in acetonitrile was topically applied once at rates of 75 g ai/ha and 750 g ai/ha (regular rate) to the lettuce leaves using a microsyringe. Lettuce leaves were harvested at 2 and 7 days after treatment and stored in a freezer (<-20 °C) until analysis. Meanwhile, leaf discs

were incubated in aqueous solution containing [phenyl-^{14}C]-tolclofos-methyl or [phenyl-^{14}C]-ph-CH3 for 3 or 4 days in order to collect enough conjugated metabolites.

The leaves were rinsed with acetonitrile and then extracted with (3×) with acetone/water (4:1, v/v). Radioactivity was determined by LSC or combustion/LSC. HPLC and TLC were used for analysis. Reference standards used were: TM, ph-CH_3, TM-CH_2OH, TMO, DM-TM and glucose conjugate of ph-CH_3. Metabolites that did not match with any reference compound were subjected to acid hydrolysis in 1 M HCl at 80 °C for 2 hours and enzymatic hydrolysis by cellulase at 37 °C overnight in 10 mM phosphate buffer at pH 5. The hydrolysates were subjected to HPLC and TLC analyses. Furthermore, the metabolites (malonylglucose conjugate of ph-CH_3 and glucose conjugate of TM-CH_2OH) produced and isolated using leaf discs were subjected to LC/MS and NMR. The study results are show in Tables 4 and 5.

Recovery of radioactivity in the treated leaves was 26.8–28.7% of the applied radioactivity at 75 g ai/ha and 67.1–90.3% at 750 g ai/ha; the loss was considered due to vaporisation.

Most of the radioactivity (68.7–91.7% TRR) in the leaves was present in surface rinse. Organic solvent extracted most (7.7–27.1% TRR) of the radioactivity in the rinsed leaf and then 0.6–4.2% TRR was remained in the unextracted residues.

In lettuce leaves, total radioactive residues were present at levels of 1.8–2.6 mg eq/kg (1× rate) and 46–81 mg eq/kg (10× rate).

Parent was present at 68.5–90.4% TRR (1× rate; 1.2–2.0 mg/kg) and 92.5–99.4% TRR (1× rate; 41–75 mg/kg) in the leaves. Most of the radioactivity in surface rinse was present as parent. The largest metabolite a malonylglucose conjugate of ph-CH_3 (U1) was found at 3.6–11% TRR (1× rate; 0.09–0.20 mg eq/kg; 11% TRR at 7 DAT) and nd–2.4% TRR (10× rate; nd–1.1 mg eq/kg; 2.4% TRR at 7 DAT). Other metabolite glucose conjugate of ph-CH_3 and glucose conjugate of TM-CH_2OH (U2) were found at up to 3.0% TRR. Other unidentified metabolites were present at less than 11% TRR in total.

For the unknown metabolite (U1), no aglycon was released in cellulase hydrolysis. U1 was converted to a glucose conjugate of ph-CH_3 when stored at 4 °C for two weeks. Furthermore, acid hydrolysis of U1 quantitatively gave ph-CH_3. These findings suggested that a malonylglucose conjugate of ph-CH_3 is a conjugated metabolite of a glucose conjugate of ph-CH_3 with other natural components. LC-MS and NMR analyses showed U1 has a malonylglucose moiety and its aglycon ph-CH_3. For the unknown metabolite (U2), all of that was quantitatively converted to TM-CH_2OH with cellulase and acid hydrolysis treatments, clearly demonstrating that U2 is a glucose conjugate of TM-CH_2OH. Spectroscopic identification of U2 was not completed. Based on the results, the proposed metabolic pathway of tolclofos-methyl in lettuce following a foliar application is shown in Figure 2.

Table 4 Extraction of radioactive residues in lettuce in lettuce following a foliar application of [phenyl-^{14}C]-tolclofos-methyl to lettuce leaves

Lettuce	Surface rinse		Acetone/Water extract		Unextracted residue		Total	
	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)
75 g ai/ha								
2 DAT	75.8	2.0	22.9	0.59	1.3	0.03	100	2.6
7 DAT	68.7	1.2	27.1	0.48	4.2	0.07	100	1.8
750 g ai/ha								
2 DAT	91.7	75	7.7	6.3	0.6	0.51	100	81
7 DAT	88.9	41	9.6	4.4	1.5	0.71	100	46

Table 5 Parent and its metabolites in lettuce following a foliar application of [phenyl-^{14}C]-tolclofos-methyl to lettuce leaves

Component	Lettuce, 75 g ai/ha				Lettuce, 750 g ai/ha			
	2 DAT (2.6 mg eq/kg)		7 DAT (1.8 mg eq/kg)		2 DAT (81 mg eq/kg)		7 DAT (46 mg eq/kg)	
	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)	TRR (%)	TRR (mg eq/kg)
Surface								
Parent	75.8	2.0	66.6	1.2	91.7	75	88.9	41
Others	nd	nd	2.1	0.04	nd	nd	nd	nd
Extract	(22.9)	(0.59)	(27.1)	(0.48)	(7.7)	(6.3)	(9.6)	(4.4)
Parent	14.6	0.38	1.3	0.02	7.7	6.3	3.6	1.7
ph-CH_3 glucose conj.	nd	nd	3.0	0.05	nd	nd	0.5	0.24
U1 (ph-CH_3 malonylglucose conj.)*	3.6	0.09	11.4	0.20	nd	nd	2.4	1.1
U2 (TM-CH_2OH glucose conj.) **	1.9	0.05	3.0	0.05	nd	nd	0.5	0.25
Others	2.8 (2 fr. each < 1.4% TRR, 0.04 mg eq/kg)	0.07	8.4 (5 fr. each < 2.5% TRR, 0.04 mg eq/kg)	0.15	nd	nd	2.5 (3 fr. each < 1.2% TRR, 0.57 mg eq/kg	1.1
Unextracted	1.3	0.03	4.2	0.07	0.6	0.51	1.5	0.71
Total	100		100		100		100	

nd: not detected

* Identified by LC-MS and NMR

** Identified by LC-MS and NMR was not completed.

U: unknown metabolite

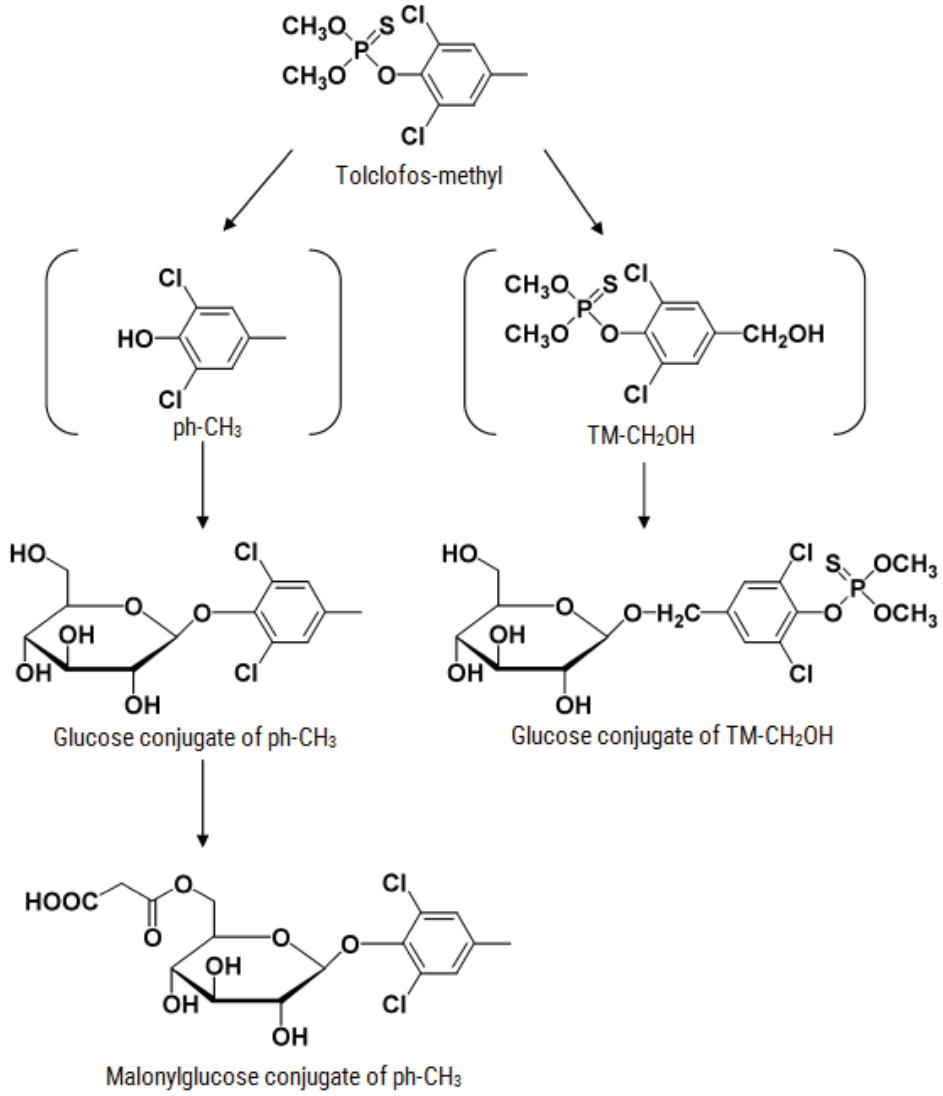


Figure 2 Proposed metabolic pathway of tolclofos-methyl in lettuce following a foliar application

Sugar beet

The metabolic fate of [phenyl-^{14}C]-tolclofos-methyl in sugar beet plants was studied with two application methods of foliar treatment and soil treatment [Mikami, 1980, report QM-00-0003]. For the foliar treatment, the radiolabelled substance was evenly applied to the upper surface (ca. 60 cm^2) of the third leaves of potted six-month old sugar beets, at the rate of 2 mg in 1 ml methanol per leaf by a pipette (equivalent to 3.3 kg ai/ha). On 3, 7, 14, 21, 28, 35 and 50 days after treatment (DAT), sugar beets were harvested and sectioned into leaf, shoot (except leaf), and root portions. The leaves were rinsed with methanol.

For the soil treatment, the sugar beets were grown in pots containing loamy sand soil in a greenhouse at 25 °C. After 6-month cultivation, the soil approximately 5 cm in depth (ca. 700 g) from soil surface was removed, to which radiolabelled substance in 1 ml of methanol was applied at a rate of 20 mg/kg on a dry weight basis to simulate the practical field applications. The treated soil was mixed well, and then returned to each pot in a greenhouse. On 3, 7, 14, 21, 28, 35 and 75 DAT, sugar beets were harvested and the roots were thoroughly washed with water. The harvested plants were separated into root and shoot portions.

Radioactive residues in the leaves or washed leaves, shoots and roots were extracted thoursee times with a solvent mixture of methanol/chloroform (2:1, v/v). Pooled extract was partitioned with ethyl acetate after adding 1N HCl. For soil, residues were extracted with a solvent mixture of ethyl acetate/1N HCl (3:2, v/v) and further with ethyl acetate.

Radioactivity in each fraction (aqueous and ethyl acetate) and unextracted residues was determined by LSC or combustion/LSC. TLC analysis was performed using unlabelled reference standards synthesised in the laboratory. The results are shown in Tables 6–7.

Foliar treatment

After treatment on sugar beet leaves, total recovery of applied radiocarbon (AR) (the sum of radioactivity in treated leaves, shoots and roots) was in the range of 8.4%–40.3% AR. The radioactivity comprised 7.1–39.5% AR in leaves, 0.3–1.6% AR in shoots and 0.3–0.6% AR in roots, indicating a little translocation of radiocarbon into shoots and roots.

Surface wash of the treated leaves accounted for 38% of the total radioactivity in the leaves at 3 DAT and decreased to 4% at the end of study. Extraction efficiencies of the total radioactivity in washed leaves, shoots and roots were 66–95%, 60–85% and 67–100%, respectively.

Parent was a major component in treated leaves at all harvest intervals, present at 7.4–87% TRR (including 1.4–37% TRR from surface wash; parent, 33–97% of radioactivity in surface wash). Surface wash contained 2.5–38% TRR of the radioactivity in leaves. Metabolite TMO (< 0.1% AR at all harvest intervals) and other components (0.5–2.8% TRR in treated leaves) were also shown in surface wash. Metabolite DM-TMO (≥ 1.4% AR at all harvest intervals) was another major component found at up to 42% TRR (35 DAT). TMO-COOH (≥ 0.3% AR at all harvest intervals) was found at up to 14% TRR (21 DAT). TMO and DM-TM (both, ≥ 0.1% AR at all harvest intervals) were present at less than 10% TRR at all harvest intervals. ph-CH_3 (≥ 0.2% AR at 3–14 DAT) and TMO-CH_2OH (≥ 2.3% AR at 7–14 DAT) were present at up to 20% TRR (7 DAT) and up to 18% TRR (14 DAT), respectively. Other unidentified components were present at up to 13% TRR (28 DAT) in total.

In shoots, parent was the predominant residue found at all harvest intervals, accounting for 39–67% TRR. TMO-COOH (≥ 0.1% AR at 21–35 DAT) and TMO (≥ 0.1% AR only at 14 DAT) were found at up to 19% TRR (21 DAT) and 7.7% TRR (14 DAT), respectively. Other unidentified components were present at up to 39% TRR in total.

For roots, parent was found at up to 33% TRR (≥ 0.1% AR at 3 and 21–50 DAT). TMO-COOH (≥ 0.1% AR at 7 and 21–35 DAT) was also a major component present at up to 33% TRR (28 DAT). Other unidentified components were present at up to 80% TRR in total.

Soil treatment

After soil treatment, total recovery of applied radiocarbon (sum of the radioactivity from shoots, roots and soil) was 47.7–62.9% AR. The radioactivity comprised 0.1–1.0% AR in shoots, 0.1–1.5% AR in roots and 46.5–62.7% AR in soil, indicating very limited uptake of radioactive carbon from soil into plants.

TRR levels were 0.12–0.82 mg eq/kg in shoots and 0.11–1.8 mg eq/kg in roots. Organic solvent extracted approximately 33–80% and 33–100% of the total radioactivity in shoots and roots, respectively.

Table 6 Parent and its metabolites in sugar beets following a foliar treatment with [phenyl-^{14}C] tolclofos-methyl

Fraction	3 DAT		7 DAT		14 DAT		21 DAT		28 DAT		35 DAT		50 DAT	
	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR
Treated leaves	39.5	100	22.9	100	20.2	100	8.0	100	7.7	100	7.6	100	7.1	100
Surface wash	15.0	38	2.8	12	0.5	2.5	0.4	5.0	0.3	3.9	0.3	3.9	0.3	4.2
Parent	14.7	37	2.6	11	0.4	2.0	0.2	2.5	0.2	2.6	0.2	2.6	0.1	1.4
TMO	< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1	
Others	0.3	0.8	0.2	0.9	0.1	0.5	0.2	2.5	0.1	1.3	0.1	1.3	0.2	2.8
Washed leaves	24.5	62	20.1	88	19.7	97	7.6	95	7.4	96	7.3	95	6.8	95
Extract	23.2	59 (95)	17.8	78 (89)	17.4	86 (88)	5.6	70 (74)	5.4	70 (73)	5.2	68 (71)	4.5	63 (66)
Parent	19.7	50	3.9	17	1.1	5.4	1.4	18	0.5	6.5	0.5	6.6	0.5	7.0
TMO	0.2	0.5	1.4	6.1	0.3	1.5	0.1	1.3	0.1	1.3	0.1	1.3	0.1	1.4
TM-CH_2OH	< 0.1		< 0.1		< 0.1		0.1	1.3	0.2	2.6	< 0.1		< 0.1	
TMO-CH_2OH	< 0.1		2.3	10	3.7	18	< 0.1		< 0.1		< 0.1		< 0.1	
TMO-COOH	0.5	1.3	0.4	1.7	0.5	2.5	1.1	14	0.5	6.5	0.3	3.9	0.4	5.6
DM-TM	0.6	1.5	0.9	3.9	1.4	6.9	0.6	7.5	0.3	3.9	0.3	3.9	0.2	2.8
DM-TMO	1.4	3.5	1.9	8.3	7.8	39	1.9	24	2.9	38	3.2	42	2.8	39
ph-CH_3	0.2	0.5	4.6	20	1.3	6.4	< 0.1		< 0.1		< 0.1		< 0.1	
ph-CH_2OH	< 0.1		0.4	1.7	< 0.1		< 0.1		< 0.1		< 0.1		< 0.1	
Others	0.6	1.5	2.0	8.7	1.3	6.4	0.4	5.0	0.9	12	0.8	11	0.5	7.0
Unextracted	1.3	3.3	2.3	10	2.3	11	2.0	25	2.0	26	2.1	27	2.3	32
Shoots	0.3	100	1.3	100	1.3	100	1.6	100	1.5	100	1.5	100	1.0	100
Extract	0.2	67	1.0	77	1.1	85	1.3	81	1.2	80	1.1	73	0.6	60
Parent	0.2	67	0.7	54	0.5	39	0.7	44	0.7	47	0.7	47	0.4	40
TMO	< 0.1		< 0.1		0.1	7.7	< 0.1		< 0.1		< 0.1		< 0.1	
TMO-COOH	< 0.1		< 0.1		< 0.1		0.3	19	0.2	13	0.2	13	< 0.1	
Others	< 0.1		0.3	23	0.5	39	0.3	19	0.3	20	0.2	13	0.2	20
Unextracted	0.1	33	0.3	23	0.2	15	0.3	19	0.3	20	0.4	27	0.4	40
Roots	0.5	100	0.4	100	0.5	100	0.6	100	0.6	100	0.6	100	0.3	100
Extract	4	80	0.4	100	0.4	80	0.5	83	0.5	83	0.5	83	0.2	67
Parent	0.1	20	< 0.1		< 0.1		0.1	17	0.1	17	0.1	17	0.1	33
TMO-COOH	< 0.1		0.1	25	< 0.1		0.1	17	0.2	33	0.1	17	< 0.1	
Others	0.3	60	0.3	75	0.4	80	0.3	50	0.2	33	0.3	50	0.1	33
Unextracted	0.1	20	< 0.1		0.1	20	0.1	17	0.1	17	0.1	17	0.1	33
Total	40.3		24.6		22.0		10.2		9.8		9.7		8.4	

% AR, percent of the applied radiocarbon

Extract, using a solvent mixture of $MeOH/CHCl_3$

Value in parenthesis means solvent extraction efficiency from the washed leaves

Parent in treated leaves (sum of radioactivity in surface wash and washed leaves): 34.4% AR(87% TRR) at 3 days; 6.5% AR (28% TRR) at 7 days; 1.5% AR (7.4% TRR) at 14 days; 1.6% AR (20.5% TRR) at 21 days; 0.7% AR (9.1% TRR) at 28 days; 0.7% AR (9.2% TRR) at 35 days; 0.6% AR (8.4% TRR) at 50 days

Table 7 Parent and its metabolites in sugar beets and soil following a soil treatment with [phenyl-^{14}C]-tolclofos-methyl

Fraction	3 DAT		7 DAT		14 DAT		21 DAT		28 DAT		35 DAT		75 DAT	
	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR	% AR	% TRR
Shoots	0.1 (0.12)	100	0.2 (0.13)	100	1.0 (0.82)	100	0.5 (0.46)	100	0.3 (0.33)	100	0.4 (0.27)	100	0.6 (0.24)	100
Extract	< 0.1		0.1	50	0.8	80	0.3	60	0.2	67	0.2	50	0.2	33
Parent	< 0.1 (0.02)	17	< 0.1 (0.03)	23	0.7 (0.60)	70	0.2 (0.20)	40	0.1 (0.07)	33	0.1 (0.07)	25	0.1 (0.05)	17
TMO	< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1	
ph-CH_3	< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1	
Others	< 0.1		0.1	50 (0.065)	0.1	10 (0.082)	0.1	20 (0.094)	0.1	33 (0.11)	0.1	25 (0.068)	0.1	17 (0.041)
Unext.	0.1		0.1	50	0.2	20	0.2	40	0.1	33	0.2	50	0.4	67
Roots	0.1 (0.11)	100	0.4 (0.38)	100	1.5 (1.8)	100	0.5 (0.50)	100	0.4 (0.49)	100	0.5 (0.48)	100	0.6 (0.44)	100
Extract	0.1	100	0.3	75	1.3	87	0.3	60	0.3	75	0.3	60	0.2	33
Parent	< 0.1 (0.03)	30	0.2 (0.22)	50	1.0 (1.2)	67	0.2 (0.19)	40	0.2 (0.18)	50	0.2 (0.17)	40	0.1 (0.07)	17
TMO	< 0.1		0.1	25 (0.095)	0.2	13 (0.23)	0.1	20 (0.10)	0.1	25 (0.12)	0.1	20 (0.096)	0.1	17 (0.075)
ph-CH_3	< 0.1		< 0.1		0.1	6.7 (0.12)	< 0.1		< 0.1		< 0.1		< 0.1	
Others	< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1		< 0.1	
Unext.	< 0.1	0	0.1	25	0.2	13	0.2	40	0.1	25	0.2	40	0.4	67
Soil	62.7 (2.3)	100	61.4 (2.1)	100	57.5 (1.9)	100	49.9 (1.6)	100	50.4 (1.6)	100	47.2 (1.6)	100	46.5 (1.5)	100
Extract	44.6	71	40.8	66	37.7	66	28.1	56	28.4	56	24.5	52	23.0	50
Parent	34.0 (1.3)	54	31.5 (1.1)	51	29.1 (0.94)	51	24.1 (0.79)	48	20.7 (0.69)	41	19.0 (0.65)	40	15.3 (0.51)	33
TMO	1.2	1.9	0.9	1.5	0.8	1.4	0.5	1.0	< 0.1		< 0.1		< 0.1	
ph-CH_3	3.9	6.2	3.9	6.4	3.6	6.3	0.9	1.8	2.3	4.6	1.4	3.0	4.4	9.5
Others	5.5	8.8	4.5	7.3	4.2	7.3	2.6	5.2	5.4	11	4.1	8.7	3.3	7.1
Unext.	18.1	29	20.6	34	19.8	34	21.8	44	22.0	44	22.7	48	23.5	51
Total	62.9		62.0		60.0		50.9		51.1		48.1		47.7	

% AR: percent of the applied radiocarbon

Extract, using a solvent mixture of MeOH/$CHCl_3$

Value in parenthesis means residue level of radioactivity, expressed as mg/kg for TM and mg eq/kg for the others.

In shoots, parent was the predominant residue found at all harvest intervals, present at levels of 17–70% TRR and 0.02–0.60 mg/kg. Metabolites TMO and ph-CH_3 were ≤0.1% AR at all harvest intervals. Other unidentified components were present at up to 50% TRR and 0.11 mg eq/kg in total.

For roots, parent was a major component found at all harvest intervals at residue levels of 17–67% TRR and 0.03–1.2 mg/kg. TMO was also a major component found at up to 25% TRR (7, 28 DAT) and 0.23 mg eq/kg (14 DAT). ph-CH_3 was present at up to 6.7% TRR (0.12 mg eq/kg). Other unidentified components were present at < 0.1% AR in total.

TRR levels in soil were 1.5–2.3 mg eq/kg. Parent, TMO, and pH-CH_3 were present at up to 54% TRR, 1.9% TRR and 9.5% TRR, respectively.

In sugar beet plant, following foliar treatment (leaves, shoots and roots) or soil treatment (shoots and roots), parent was a major component in the matrices. In leaves (foliar treatment), another major component DM-TMO and minor components (TMO-COOH, ph-CH_3, TMO-CH_2OH, TMO and DM-TM) were observed. The metabolite patterns of tolclofos-methyl indicated that it is degraded via pathways including oxidation form P=S to P=O, oxidation at 4-methyl group and cleavage of P-O-alkyl and P-O-aryl linkages. In roots by soil treatment, another major component TMO was observed.

Potato

Study 1

The metabolism of [phenyl-^{14}C]-tolclofos-methyl was investigated in potato plants following a single application to the surface of seed potato [Goodyear, 1995, report QM-510041]. Surface of seed potatoes (var., Maris Piper) were treated immediately prior to planting with phenyl radiolabelled tolclofos-methyl formulated as a suspension concentrate at a rate of 125 g ai/t tuber. 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 shoots (top growth), roots, parent tubers, and daughter tubers (only in mature stage). Root and daughter tuber material were rinsed with water to remove soil particles. Samples were stored at -20 °C until analysis.

Residues in potato tubers (parent and daughter) and roots (immature and mature) were extracted with (1–3×) acetone and (2–3×) acetone/water (1:1, v/v). The organic solvent extracts (acetone extract and acetone/water extract) each were analysed by HPLC, except acetone/water extracts from mature tuber (parent and daughter) due to the low level of radioactivity, 1% TRR and 6% TRR, respectively.

Residues in shoots (immature and mature) were extracted with (3–4×) acetone and (3×) acetone/water (1:1, v/v). For immature shoots, acetone extract was evaporated and then separated into aqueous fraction and organic fraction (organic fraction: solid residue on evaporation flask, dissolved in acetonitrile), which were analysed by HPLC. Acetone/water extract were also analysed by HPLC. For mature shoots, acetone extract (63% TRR) was concentrated and purified by partitioning with hexane (hexane phase, discarded) and the aqueous phase (containing 98% of radioactivity) was analysed by HPLC. The acetone/water extract (0.0052 mg eq/kg) was not analysed further.

Radioactivity were analysed by LSC or combustion/LSC. Un-radiolabelled reference standards used in HPLC analysis were as follows: TM, TMO, DM-TM, ph-CH_3, DM-TMO, SM-TM, TM-COOH, TM-CH_2OH, ph-COOH, ph-CH_2OH, DM-TM-CH_2OH, DM-TMO-CH_2OH, DM-TM-COOH and DM-TMO-COOH. The results are shown in Tables 8-10.

Additionally, radiovalidation of the residue analytical method with potato matrices of immature parent tuber and mature daughter tuber was performed. Radioactive residues in immature parent tuber, washed with water, were extracted with acetone and then by Soxhlet extraction overnight using the acetone extract. Extract (99% TRR) was concentrated and partitioned twice with hexane. The hexane phase (93% TRR) was cleaned up using solid phase extraction (silica) and analysed by HPLC and GG-NPD. As results, most of the radioactivity was tolclofos-methyl (aqueous phase, not analysed). For mature daughter tuber, it was washed with water and then residues were extracted with acetone by Soxhlet extraction overnight. Extract (78% TRR) was partitioned with hexane. The hexane phase (24% TRR) was not further analysed, due to insufficient radioactivity. The aqueous phase was not analysed.

TRR levels in immature potato plants were 0.25 mg eq/kg in shoots, 16 mg eq/kg in roots, 56 mg eq/kg in parent tuber. For mature potato plants, TRR levels were 0.040 mg eq/kg in shoots, 6.5 mg eq/kg in roots, 1,886 mg eq/kg in parent tuber and 0.048 mg eq/kg in daughter tuber.

Table 8 Extraction of radioactive residues in potato plant following a single application of [phenyl-^{14}C]-tolclofos-methyl to seed potatoes at a rate of 125 g ai/t tuber

Sample	Acetone extract		Acetone/water extract		Total extract	Unextracted residue		Total residue
	mg eq/kg	% TRR	mg eq/kg	% TRR	% TRR	mg eq/kg	% TRR	mg eq/kg
Immature stage (27 days after planting)								
Shoots (foliage)	0.21	84*	0.029	12*	96	0.016	6	0.25
Roots	12	72*	2.2	13*	85	2.4	14	16
Parent tuber	53	95*	2. 5	4*	99	0.61	1	56
Mature stage (129 days after planting)								
Shoots	0.025	63*	0.0052	13	76	0.0092	23	0.040
Roots	3.2	50*	1.7	26*	76	1.6	24	6.5
Parent tuber	1,828	97*	26	1	98	32	2	1,886
Daughter tuber	0.029	60	0.0031	6	66	0.016	33	0.048

Total residue is the sum of radioactivity in extracts and unextracted residue.

* Characterisation was performed.

Table 9 Parent and its metabolites in immature potato plants following a single application of [phenyl-^{14}C]-tolclofos-methyl to seed potatoes at a rate of 125 g ai/t tuber

Components	TRR, mg eq/kg (% TRR)						
	Shoots (foliage), 0.25 mg eq/kg			Roots, 16 mg eq/kg		Parent tuber, 56 mg eq/kg	
	Acetone ext.		Acetone/ Water ext.	Acetone ext.	Acetone/ Water ext.	Acetone ext.	Acetone/ Water ext.
	Org. phase	Aq. phase					
Parent	nd	0.002 (0.9)	0.001 (0.4)	12 (71.8)	0.074 (0.4)	52 (93.5)*	2.4 (3.9)
TMO	nd	nd	nd	nd	nd	nd	nd
DM-TM	0.001 (0.3)	0.004 (1.5)	nd	nd	0.049 (0.3)	nd	nd
DM-TMO	nd	nd	nd	nd	0.060 (0.4)	nd	nd
Unretained [a]	0.002 (0.6)	0.009 (3.6)	0.004 (1.6)	nd	nd	nd	nd
Unresolved	< 0.001 (0.1)	0.003 (1.4)	< 0.001 (0.1)	0.029 (0.2)	0.021 (0.1)	0.84 (1.5)	0.019 (< 0.1)
Polar metabolites	0.027 (10.0)	0.16 (65.7) [b]	0.024 (10.0)	nd	2.0 (11.8) [b]	nd	0.049(0.1)
DM-TM-CH_2OH		0.076 (30.7)†			0.52 (3.1) †		
DM-TM-COOH		nd			0.31† (1.8)		
ph-COOH		nd			nd		
U1		0.028 (11.2)			0.15 (0.9)		
U2		0.004 (1.8)			1.0 (6.0)		
U3		0.024 (9.7)			nd		
U4		0.018 (7.4)			nd		
U5		0.011 (4.4)			nd		
Unresolved		0.001 (0.5)			0.003 (< 0.1)		
Total	0.03 (11)	0.18 (73)	0.029 (12)	12 (72)	2.2 (13)	53 (95)	2. 5 (4)

[a] Unretained radioactivity on the HPLC column

[b] Polar metabolite fraction further analysed by another HPLC system

* Confirmed by LC-MS

† Confirmed by LC-MS/MS

U, unidentified metabolite

nd, not detected

Table 10 Parent and its metabolites in mature potato plants following a single application of [phenyl-^{14}C]-tolclofos-methyl to seed potatoes at a rate of 125 g ai/t tuber

Components	TRR, mg eq/kg (% TRR)				
	Shoots (foliage), 0.040 mg eq/kg	Roots 6.5 mg eq/kg		Parent tubers 1,886 mg eq/kg	Daughter tubers 0.048 mg eq/kg
	Acetone ext. Aq. phase[c]	Acetone ext.	Acetone/water ext.	Acetone ext.	Acetone ext.
Parent	nd	2.6 (40.2)	nd	1,792 (95.1)	nd
TMO	nd	nd	nd	nd	nd
DM-TM	nd	nd	nd	nd	nd
DM-TMO	nd	0.15 (2.3)	nd	nd	nd
Unretained[a]	001 (2.7)	0.056 (0.9)	nd	nd	0.002 (3.4)
Unresolved	0.001 (2.9)	0.011 (0.2)	0.025 (0.4)	36 (1.9)	< 0.001 (0.3)
Polar metabolites	0.023 (57.4) [b]	0.42 (6.4)	7 (25.6) [b]	nd	0.027 (56.3)
DM-TM-CH_2OH	nd		0.29 (4.3) †		0.013 (26.7)
DM-TM-COOH	nd		0.51 (7.7) †		0.003 (6.0)
ph-COOH	0.015 (37.2)		0.12 (1.8)		nd
U1	nd		nd		nd
U2	nd		0.78(11.8%)		0.002 (4.3)
U3	nd		nd		0.002 (4.8)
U4	nd		nd		nd
U5	0.008 (19.4)		nd		0.006 (12.4)
Unresolved	< 0.001 (0.8)		0.007 (0.1)		0.001 (2.1)
Total	0.025 (63)	3.2 (50)	1.7 (26)	1,827 (97)	0.029 (60)

[a] Unretained radioactivity on the HPLC column

[b] Polar metabolite fraction further analysed by another HPLC system

[c] Acetone extract was concentrated and partitioned with hexane. The resultant aqueous phase (including 98% of radioactivity)

was analysed by HPLC and the hexane phase was discarded.

† Confirmed by LC-MS/MS

U, unidentified metabolite

nd, not detected

From the potato matrices of immature and mature stages, organic solvents (acetone and then a mixture of acetone/water) extracted in total 76–96% of the total radioactivity in shoots, 76–85% in roots, 98–99% in parent tuber and 66% (unextracted, 33% TRR) in daughter tubers.

In shoots at immature stage, parent was found at a very low level of 1.3% TRR (0.003 mg/kg). The largest component, metabolite DM-TM-CH_2OH was found at 30.7% TRR (0.076 mg eq/kg). At a less extent, two unidentified components were found at levels of 9.7–11.2% TRR (0.024–0.028 mg eq/kg). In addition, minor components were present at 1.8–7.4% TRR (0.004–0.018 mg eq/kg). At maturity, parent was not detected in shoots. Metabolite ph-COOH was the predominant residue accounting for 37.2% TRR (0.015 mg eq/kg). One unidentified component was present at 19.4% TRR (0.008 mg eq/kg).

For roots at immature stage, parent was the predominant residue accounting for 72.2% TRR (12 mg/kg). Metabolites DM-TM, DM-TMO, DM-TM-CH_2OH, DM-TM-COOH were found at levels of 0.3–3.1% TRR (0.049–0.52 mg/kg). Two unidentified components were present at 0.9–6.0% TRR (0.15–1.0 mg eq/kg). At mature stage, parent was the predominant residue accounting for 40.2% TRR (2.6 mg/kg). Metabolites DM-TMO, DM-TM-CH_2OH, DM-TM-COOH, ph-COOH were found at 1.8–7.7% TRR (0.12–0.51 mg eq/kg). One largest metabolite component accounting for 11.8% TRR (0.78 mg eq/kg) was found in mature roots, but not identified.

In parent potato tuber at immature and mature stages, parent was the predominant residue accounting for 97.4% TRR (55 mg/kg) and 95.1% TRR (1,792 mg/kg), respectively. Metabolites were not found in parent tubers at both stages.

In daughter tuber, parent was not detected. Metabolite DM-TM-CH2OH was a major component found at 26.7% TRR (0.013 mg eq/kg). DM-TM-COOH was found at 6.0% TRR (0.003 mg eq/kg). Thoursee unidentified components were present at 4.3–12.4% TRR (0.002–0.006 mg eq/kg).

Study 2

The metabolism of [phenyl-^{14}C]-tolclofos-methyl was investigated in potato plants following a single application to the surface of seed potatoes (var. Desiree) at a rate of 250 g ai/t tuber (1× rate) and 1,250 g ai/t tuber (5× rate) [Swales, 2005, report QM-0060]. Seed potatoes were treated immediately prior to planting in sandy loam soil (UK) with [phenyl-^{14}C]-tolclofos-methyl formulated as a suspension concentrate. Treated potatoes were grown outside in a caged enclosure and to full maturity (BBCH 97; 118 days after planting). The plants harvested were sectioned into parent tuber, roots, daughter tubers and foliage. Tubers and roots were rinsed in water to remove the soil and stored frozen at <-10 °C until analysis. For analysis, samples were homogenised to powder in dry ice.

Radioactive residues in plant sample (parent tuber, daughter tubers, roots and foliage) were extracted one time sequentially with acetonitrile, acetonitrile/water (9:1, v/v), acetonitrile/water (1:1, v/v) and acetone. Radioactivity in extracts and unextracted residue was counted by LSC or combustion/LSC. Most extracts were analysed by HPLC and/or TLC using the following un-radiolabelled reference standards: DM-TM, DM-TM-CH_2OH, DM-TM-COOH, DM-TMO, ph-COOH, ph-CH_3 and TM-CH_2OH. Unextracted residues in roots were subjected to sequential extraction with (2×) water, (2×) 0.1M HCl, (2×) 0.1M NaOH, (1×) 2M HCl reflux and (1×) 2M NaOH reflux. The results are shown in Tables 11 to 14.

With both application rates (1–5×), TRR levels were 40–179 mg eq/kg in parent tuber, 0.032–0.067 mg eq/kg in daughter tubers, 5.0–14 mg eq/kg in roots and 0.13–0.36 mg eq/kg in foliage.

Organic solvents, using acetonitrile, acetonitrile/water (9:1, v/v), acetonitrile/water (1:1, v/v) and acetone, extracted 95–98% of the radioactivity in parent tuber, 78–79% from daughter tubers (unextracted, 21–22%, 0.007–0.015 mg eq/kg), 71–77% from roots and 63–86% from foliage. For roots, further extractions (using water, mild acid/base, and strong acid/base) released 0.4–9.4% TRR by each extraction, which were not analysed by HPLC or TLC.

In parent tuber (1–5× rates), parent was the predominant residue, accounting for 88.9–96.1% TRR (35–172 mg/kg). Metabolites DM-TM-CH_2OH, DM-TM-COOH, TM-CH_2OH, ph-COOH (only 5× rate), DM-TMO and DM-TM were found at very low levels, less than 0.1% TRR and 0.18 mg eq/kg. In addition, a number of unidentified minor metabolites were observed at ≤ 1% TRR each.

In daughter tubers (1–5× rates), parent was detected but at very low levels of less than 0.01 mg/kg (2.6–8.3% TRR). The largest component was DM-TM-CH_2OH present at 11.0–11.5% TRR (< 0.01 mg eq/kg). Metabolite DM-TM-COOH was found at 6.0–10.4% TRR. Other metabolites TM-CH_2OH, ph-COOH, DM-TMO and DM-TM were present at levels of less than 6% TRR. In addition, a number of unidentified minor metabolites were observed at <10% TRR each (< 0.01 mg eq/kg).

For roots (1–5× rates), parent was present at 13.6–31.6% TRR (0.68–4.5 mg/kg). DM-TM-CH_2OH and DM-TM-COOH were found at 3.9–9.0% TRR (0.45–0.56 mg eq/kg) and 5.6–8.5% TRR (0.42–0.80 mg eq/kg), respectively. Metabolites TM-CH_2OH, ph-COOH, DM-TMO and DM-TM were found at levels of less than 4% TRR (≤ 0.52 mg eq/kg). Other numerous minor metabolites were present at mostly < 10% TRR each.

Table 11 Extraction of radioactive residues in potato plants following a single application of [^{14}C]-tolclofos-methyl to seed potatoes at a rate of 250 g ai/t tuber

Sample	Radioactive residues											
	ACN extract		ACN: water (9:1) extract		ACN: water (1:1) extract		Acetone extract		Total ext.	Unextracted residue		Total residue
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	% TRR	mg eq/kg	% TRR	mg eq/kg
Foliage	0.085	66.7	0.016	12.3	0.009	6.8*	0.000	0.0	85.8	0.018	14.1	0.13
Roots	2.7	53.6	0.76	15.2	0.35	6.9	0.073	1.5	77.2	1.1	22.8	5.0
PES												
Water										0.094	1.9*	
0.1M HCl										0.023	0.5*	
0.1M NaOH										0.13	2.6*	
2M HCl reflux										0.15	3.0*	
2M NaOH reflux										0.37	7.3*	
Parent tuber	32	79.9	4.5	11.3	1.3	3.3	0.17	0.4*	94.9	2.0	5.1	40
Daughter tubers	0.017	53.0	0.005	17.1	0.003	9.3	0.000	0.0*	79.4	0.007	20.6	0.032

Total residue was calculated from the sum of the radioactivity in the extracts and unextracted residue.

Unextracted residues from roots were subjected to sequential extraction with water, 0.1M HCl, 0.1M NaOH, 2M HCl reflux and 2M NaOH reflux; in total 15.3% TRR released and 7.5% TRR unextracted (calculated by subtraction).

* Not further analysed

Table 12 Extraction of radioactive residues in potato plants following a single application of [^{14}C]-tolclofos-methyl to seed potatoes at a rate of 1,250 g ai/t tuber

Sample	Radioactive residues											
	ACN extract		ACN: water (9:1) extract		ACN: water (1:1) extract		Acetone extract		Total ext.	Unextracted residue		Total residue
	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	% TRR	mg eq/kg	% TRR	mg eq/kg
Foliage	0.14	39.5	0.043	11.8	0.038	10.6	0.005	1.4*	63.3	0.13	36.7	0.36
Roots	7.5	52.3	1.7	11.8	0.79	5.5	0.14	1.0	70.6	4.2	29.3	14
PES												
Water										0.23	1.6*	
0.1M HCl										0.051	0.4*	
0.1M NaOH										0.59	4.1*	
2M HCl reflux										0.63	4.4*	
2M NaOH reflux										1.3	9.4*	
Parent tuber	159	88.7	13	7.4	2.7	1.5	0.42	0.2*	97.8	3.9	2.2	179
Daughter tubers	0.028	41.4	0.013	18.6	0.012	18.2	0.000	0.0*	78.2	0.015	21.8	0.067

Total residue was calculated from the sum of the radioactivity in the extracts and unextracted residue.

Unextracted residues from roots were subjected to sequential extraction with water, 0.1M HCl, 0.1M NaOH, 2M HCl reflux and 2M NaOH reflux; in total 19.9% TRR released and 9.4% TRR unextracted (calculated by subtraction).

* Not further analysed

Table 13 Parent and its metabolites in potato plants following a single application of [^{14}C] -tolclofos-methyl to seed potatoes at a rate of 250 g ai/t tuber (1× rate)

Metabolite fraction	Foliage (0.13 mg eq/kg)		Roots (5.0 mg eq/kg)		Parent tuber (40 mg eq/kg)		Daughter tubers (0.032 mg eq/kg)	
	%TRR	TRR (mg eq/kg)	%TRR	TRR (mg eq/kg)	%TRR	TRR (mg eq/kg)	%TRR	TRR (mg eq/kg)
Total extracted	85.8	0.110	92.5	4.6	94.9	37	79.4	0.025
Parent	9.7	0.012	13.6	0.68	88.9	35	8.3	0.002
DM-TM	7.8	0.010	4.0	0.20	0.1	0.055	1.1	< 0.001
DM-TM-CH_2OH	15.3	0.019	9.0	0.45	0.1	0.026	11.0	0.004
DM-TM-COOH	12.9	0.017	8.5	0.42	0.1	0.032	6.0	0.002

Metabolite fraction	Foliage (0.13 mg eq/kg)		Roots (5.0 mg eq/kg)		Parent tuber (40 mg eq/kg)		Daughter tubers (0.032 mg eq/kg)	
	%TRR	TRR (mg eq/kg)	%TRR	TRR (mg eq/kg)	%TRR	TRR (mg eq/kg)	%TRR	TRR (mg eq/kg)
DM-TMO	1.7	0.002	2.2	0.11	< 0.05	0.012	1.6	< 0.001
ph-COOH	7.7	0.010	2.2	0.11	nd	nd	3.1	< 0.001
TM-CH_2OH	nd	nd	0.8	0.040	0.1	0.030	6.3	0.002
Unretained/origin	nd	nd	3.8[a]	0.19	nd	nd	13.3[a]	0.004
Unresolved	2.2	0.002	0.9	0.045	0.8	0.32	0.6	< 0.001
Unknowns	21.7[b]	0.028	32.2[c]	1.6	3.5[d]	1.4	28.2[e]	0.008
Total identified	55.1	0.070	40.3	2.0	89.3	35	37.4	0.010
Total characterized	79.0	0.101	77.2	3.9	93.6	37	79.4	0.025
Unextracted	14.1	0.018	7.5	0.37	5.1	2.0	20.6	0.007

[a] The unretained/origin (material not retained on HPLC or staying at the origin on TLC) consisted of multiple components

(each <10 % TRR), based on analysis made on 5× rate daughter tubers.

[b] Multiple components, based on analysis made on 5× rate foliage

[c] Of 32.2% TRR, 28.1% TRR (23.1% TRR in acetone extract and 5.0% TRR in acetone/water, 9:1 extract) was analysed and consisted of multiple components of ≥ 11 (each ≤5.3% TRR, each ≤0.26 mg eq/kg).

[d] Each ≤1% TRR (each ≥ 0.05 mg eq/kg)

[e] Multiple components, each ≤6% TRR (each ≤0.002 mg eq/kg), based on analysis made on 5× rate daughter tubers

"Total extracted" is the sum of all solvent extracts (for roots, acid/base extracts are also included). Individual component was found mainly in acetonitrile extract and next, acetonitrile/water (9:1) extract.

nd, not detected

Table 14 Parent and its metabolites in potato plants following a single application of [^{14}C] -tolclofos-methyl to seed potatoes at a rate of 1,250 g ai/t tuber (5× rate)

Metabolite fraction	Foliage (0.36 mg eq/kg)		Roots (14 mg eq/kg)		Parent tuber (179 mg eq/kg)		Daughter tubers (0.067 mg eq/kg)	
	% TRR	TRR (mg eq/kg)	% TRR	TRR (mg eq/kg)	% TRR	TRR (mg eq/kg)	% TRR	TRR (mg eq/kg)
Total extracted	63.3	0.23	90.5	13	97.8	175	78.2	0.053
Parent	6.6	0.025	31.6	4.5	96.1	172	2.6	0.002
DM-TM	4.1	0.015	3.6	0.52	0.1	0.18	0.7	< 0.001
DM-TM-CH_2OH	5.2	0.018	3.9	0.56	< 0.05	0.056	11.5	0.008
DM-TM-COOH	8.9	0.032	5.6	0.80	0.1	0.11	10.4	0.008
DM-TMO	3.8	0.014	2.9	0.42	< 0.05	0.050	2.1	0.002
ph-COOH	6.9	0.025	2.9	0.41	< 0.05	0.026	4.0	0.003
TM-CH_2OH	0.7	0.002	1.8	0.26	< 0.05	0.031	0.2	< 0.001
Unretained/Origin	8.3	0.030	3.8[a]	0.55	0.1	0.10	15.3[a]	0.011
Unresolved	1.0	0.004	0.4	0.063	0.3	0.46	1.2	< 0.001
Unknowns	16.6[b]	0.058	13.4	1.9	1.0	1.7	22.0[c]	0.013
Total identified	36.2	0.13	52.3	7.5	96.5	172	31.5	0.025
Total characterised	61.9	0.22	69.8	10	97.7	175	70.0	0.047
Unextracted*	36.7	0.13	9.4	1.4	2.2	3.9	21.8	0.015

[a] The unretained/origin (material not retained on HPLC or staying at the origin on TLC) consisted of multiple components (each <10 % TRR).

[b] Of 16.6% TRR, 14.2% TRR (10.0% TRR in acetone extract and 4.2% TRR in acetone/water, 9:1 extract) was analysed and consisted of multiple components of ≥5 (each ≤3% TRR, each ≤0.010 mg eq/kg).

[C] Of 22.0% TRR, 18.6% TRR (16.2% TRR in acetone extract and 2.4% TRR in acetone/water, 9:1 extract) was analysed and consisted of multiple components of ≥8 (each ≤4% TRR, each ≤0.003 mg eq/kg).

"Total extracted" is the sum of all solvent extracts (for roots, acid/base extracts are also included). Individual component was found mainly in acetonitrile extract and next, acetonitrile/water (9:1) extract.

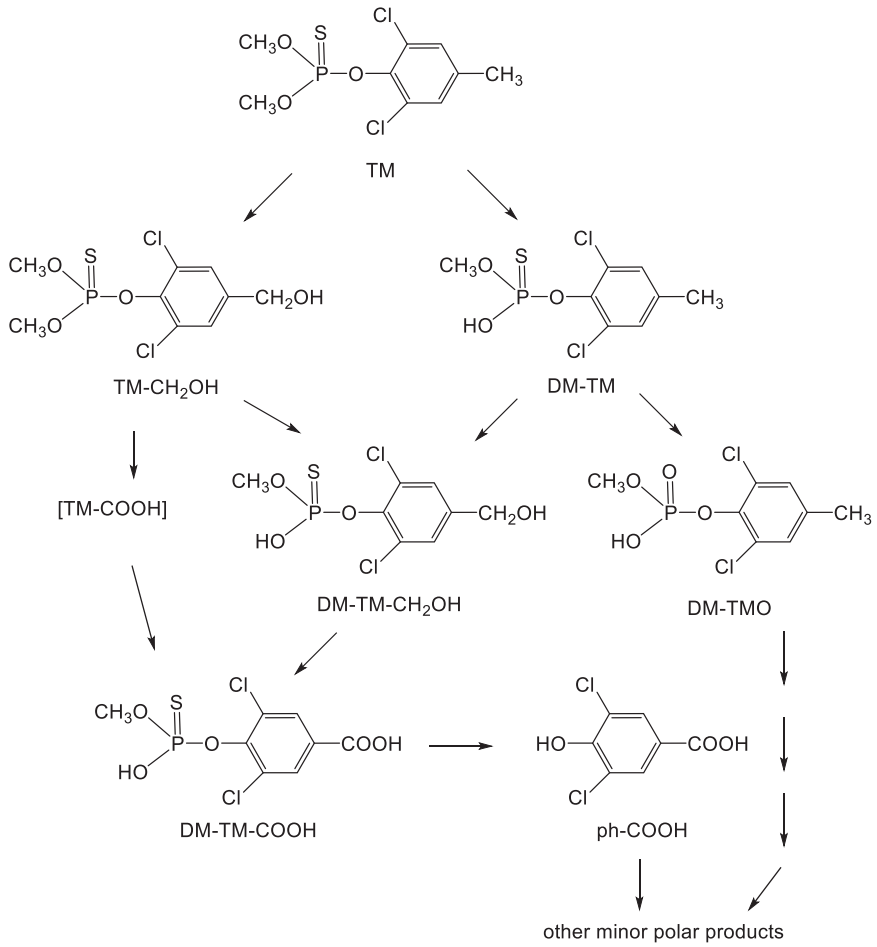


Figure 3 Proposed metabolic pathway of tolclofos-methyl in potatoes following seed tuber treatment

(Compound names in brackets refer to compounds not identified in the study but being logical intermediates in the proposed pathway)

Foliage (1–5× rates) contained parent at a level of 6.6–9.7% TRR (0.012–0.025 mg eq/kg). DM-TM-CH_2OH and DM-TM-COOH were found at 5.2–15.3% TRR (0.018–0.019 mg eq/kg) and 8.9–12.9% TRR (0.017–0.032 mg eq/kg), respectively. Metabolites TM-CH_2OH (only 5× rate), ph-COOH, DM-TMO and DM-TM were found at levels of less than 8% TRR (each ≤0.025 mg eq/kg). Other numerous minor metabolites were present at < 0.03 mg eq/kg.

From the two studies on potato (seed treatments; 0.125, 0.25 and 1,250 g ai/t tuber), in the daughter tubers (edible commodity), parent was found at up to 8.3% TRR and the concentrations were very low (< 0.01 mg/kg even at five exaggerated rate, 1,250 g ai/t). Major components were DM-TM-CH_2OH and DM-TM-COOH found at up to 26.7% TRR and up to 10.4% TRR. However, the residue levels of two metabolites were less than 0.01 mg/kg as well as parent.

Based the results, it was considered that tolclofos-methyl in potatoes following seed treatment is metabolized via demethylation and oxidation of 4-methyl group.

A proposed metabolic pathway is shown in Figure 3.

Cotton and peanut

The metabolism of [phenyl-^{14}C]-tolclofos-methyl was investigated in cotton and peanut plants grown under field conditions [Savidge, *et al*., 1986, report QM-61-0021]. Cotton seeds (var. Stoneville 213) were planted 15–30 min after a single soil treatment with the radiolabelled substance at a rate of 5.2

kg ai/ha (1× rate) and an exaggerated rate of 15.7 kg ai/ha (3× rate). For peanuts (var. Florigiants), soil treatments were identical to cotton with an additional single foliar application made 75 days after soil treatment (at the pegging stage) at rates of 5.2 kg ai/ha (in total, 10 kg ai/ha 1× rate) and 15.7 kg ai/ha (in total, 31 kg ai/ha; 3× rate). Cotton and peanut plants were harvested 150 days after soil treatment (150 DAT). Stems were taken from stem regions 10, 20, 30 cm from base of plant. Harvest was sectioned into bolls, squares, leaves, stems and seeds for cotton, and hull, leaves, stems and nutmeat for peanut. All samples were frozen until shipment to analysis laboratory, where stored at -20 °C until analysis. Radioactivity in the collected plant samples was measured by combustion/LSC. Surface of peanut leaves was rinsed with methanol. Characterisation was performed with samples treated at exaggerated rates

Radioactive residues in peanut samples (3× rate hull, leaf, stem and nutmeat, except methanol rinse of peanut leaves) and cotton samples (3× rate) were extracted with methanol/chloroform (2:1, v/v). Radioactivity was determined by LSC or combustion/LSC. TLC analysis was made for characterisation of metabolic components, using the following reference standards: TM, ph-CH_3, ph-CHO, TM-CH_2OH, TMO, ph-CH_2OH, TMO-COOH, TMO-CH_2OH, DM-TMO and DM-TM. The polar residues (radiocarbon remained at the origin during TLC analysis) in methanol/chloroform extract of peanut leaves were treated with cellulase enzyme at 37 °C for 24 hours. The hydrolysate was analysed by TLC. The study results are shown in Tables 15–16.

In whole cotton plants and seeds, radioactivity was not detected (< 0.003–< 0.008 mg eq/kg). Stems (0.008–0.010 mg eq/kg at 1× rate; 0.015–0.026 at 3× rate) and leaves (0.015 mg eq/kg at 3× rate) showed TRR levels slightly above the LOQ. In TLC analysis for cotton samples (3× rate; solvent extraction ratios, not reported), parent and any metabolites were not detected in leaf and seed, and the radioactivity in boll, square and stem was remained at the origin during TLC.

In peanut, TRR levels (1–3× rates) were 0.016–0.052 mg eq/kg in hull, 1.4–3.8 mg eq/kg in leaf, 0.044–0.079 (1× rate)/0.090–0.38 (3× rate) mg eq/kg in stem and 0.010 mg eq/kg (both rates) in nutmeat.

Methanol rinse from peanut leaves (3× rate) contained only 2.6–3.7% TRR (two replicates). Organic solvent extracted only 47–53% (radioactivity in methanol rinse, subtracted) of the radioactivity in the washed leaves, with unextracted residues of 36.6–52.6% TRR. Further, in TLC analysis for the extract, recovery of radioactivity was only 45–72%. Thus, characterisation performance was not sufficient. Obtained results were as follows: parent was detected only in methanol rinse at a very low level of 0.1% TRR (0.002–0.006 mg/kg, two replicates). The largest component was polar residue fraction at the origin during TLC analysis, accounting for 6.5–11% TRR (0.26–0.53 mg eq/kg), which comprised mainly ph-CH_2OH conjugate (51–58% ratio) and TM-CH_2OH conjugate (27–31% ratio). Other components ph-CHO, TM-CH_2OH, ph-CH_2OH, ph-CH_3 and TMO, and thoursee unknown fractions were also found.

In peanut hull, nutmeat and stem samples (3× rate), the solvent extraction ratios were not reported. TM, DM-TMO and one polar fraction (largest component) were observed in the hull and ph-CH_2OH, TM-CH_2OH, TMO, TMO-COOH, TMO-CH_2OH and one polar fraction (largest component) were observed in stem. In nutmeat, the all radioactivity was remained at the origin in TLC.

Table 15 Total radioactive residues in cotton and peanut plants following an application of [phenyl-^{14}C]-tolclofos-methyl

Cotton	TRR (mg eq/kg)		Peanut.	TRR (mg eq/kg)	
	5.2 kg ai/ha (1× rate, soil)	15.7 kg ai/ha (3× rate, soil)		10 kg ai/ha (1× rate): 1st 5.2 kg ai/ha (soil), 2nd 5.2 kg ai/ha (foliar)	31 kg ai/ha (3× rate): 1st 15.7 kg ai/ha (soil), 2nd 15.7 kg ai/ha (foliar)
Boll	< 0.004	< 0.004	Hull	0.016	0.052
Square	< 0.004	< 0.004	Leaf	0.030 in surface 1.4 in internal part total 1.4	0.18 in surface 3.6 in internal part total 3.8
Leaf	< 0.008	0.015	Stem	0.044-0.079	0.090-0.38

Cotton	TRR (mg eq/kg)		Peanut.	TRR (mg eq/kg)	
	5.2 kg ai/ha (1× rate, soil)	15.7 kg ai/ha (3× rate, soil)		10 kg ai/ha (1× rate): 1st 5.2 kg ai/ha (soil), 2nd 5.2 kg ai/ha (foliar)	31 kg ai/ha (3× rate): 1st 15.7 kg ai/ha (soil), 2nd 15.7 kg ai/ha (foliar)
Stem	0.008-0.010	0.015-0.026	Nutmeat	0.010	0.010
Seed	< 0.003	< 0.004			

Cotton and peanut were harvested 150 days after soil treatment

Stems, collected at different heights (10, 20, 30 cm) from bottom

Table 16 Parent and its metabolites in peanut leaves following soil (15.7 kg ai/ha) and foliar treatments with [phenyl-^{14}C]-tolclofos-methyl

Fraction	Rep 1			Rep 2		
	DPM/gm	TRR (mg eq/kg)	% TRR	DPM/gm	TRR (mg eq/kg)	% TRR
Peanut leaves, combusted	244,097	4.9	100	200,351	4.0	100
MeOH rinse	9,042	0.18	3.7	5,271	0.11	2.6
Parent	286	0.006	0.1	112	0.002	0.1
TM-CH_2OH[a]	644	0.013	0.3	370	0.007	0.2
ph-CH_2OH	1,688	0.034	0.7	754	0.015	0.4
Origin[a]	4,534	0.091	1.9	3,629	0.072	1.8
TLC recovery	7,152		3.0 79.1%[c]	4,865		2.5 92.3%[c]
MeOH/$CHCl_3$ extract	125,909	2.5	51.6	91,797	1.8	45.8
ph-CH_3	nd	nd		1,605	0.032	0.8
TM-CH_2OH[a]	11,317	0.23	4.6	2,233	0.044	1.1
TMO	nd	nd		1,569	0.032	0.8
ph-CHO	14,771	0.30	6.1	6,624	0.13	3.3
Unknown	nd	nd		1,703	0.036	0.9
Unknown	11,206	0.22	4.6	nd	nd	
Unknown	nd	nd		7,954	0.16	4.0
Origin[a]	22,105	0.44	9.1	9,417	0.19	4.7
Between bands	31,548	0.63	12.9	9,876	0.20	4.9
TLC recovery	90,947		37.3 72%[c]	40,981		20.5 45%[c]
Unextracted	128,352	2.6	52.6	73,332	1.5	36.6
Total recovery[b]	263,303		108	170,400		85.0

[a] Fractions found in both MeOH rinse and extract: origin 6.5-11% TRR (0.26-0.53 mg eq/kg) and TM-CH_2OH 1.3-4.9% TRR

[b] Total recovery is the sum of radioactivity in MeOH rinse, extract and unextracted residues

[c] % Recovery of TLC based on DPM/gm in rinse or extract

TRR values (mg eq/kg) for peanut leaves were calculated by using a specific radioactivity of 50,000 DPM/ μg. TRR values (mg eq/kg) for the others were derived from leaves TRR value and % TRR of each fraction; nd, not detected

Summary: 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 seedlings and soil treatment, major metabolites were sugar conjugates of ph-CH_3 and TM-CH_2OH, generated via cleavage of P-O aryl bond or oxidation of 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 4-methyl group.

To convert residues of tolclofos-methyl from the supervised trials to values for total residue (sum of tolclofos-methyl and the metabolites), the following adjustment factors from the ratios of total residue to parent residues observed in the metabolism studies (lettuce, potato) could be derived, as shown in Table 17.

Table 17 Conversion factors for estimation of STMRs and HOURSs for plant commodities

Crop group	Tolclofos-methyl	Tolclofos-methyl, ph-CH_3 (incl. conjugates) TM-CH_2OH (incl. conjugates), DM-TM-CH_2OH and DM-TM, calculated as tolclofos-methyl	Conversion factor
Leafy crops (seedlings and soil treatment)	Lettuce Parent: 0.084mg/kg	Lettuce [a] Parent: 0.084 mg/kg ph-CH_3 (incl. conj.): 0.052 mg eq/kg TM-CH_2OH (incl. conj): 0.032 mg eq/kg DM-TM-CH_2OH: ND DM-TM: ND Total: 0.17 mg eq/kg	2.0
	Lettuce Parent: 0.30 mg/kg	Lettuce [b] Parent: 0.30 mg/kg ph-CH_3 (incl. conj.): 0.15 mg eq/kg TM-CH_2OH (incl. conj.): 0.11 mg eq/kg DM-TM-CH_2OH: ND DM-TM: ND Total: 0.56 mg eq/kg	1.9
			Mean: 2.0
Root and tuber vegetables (seed tuber treatment)	Potato (daughter tubers) Parent: 0.002 mg/kg	Potato (daughter tubers) [c] Parent: 0.002 mg/kg ph-CH_3 (incl. conj.): ND TM-CH_2OH (incl. conj.): < 0.001mg eq/kg DM-TM-CH_2OH: 0.008 DM-TM: < 0.001 mg eq/kg Total: 0.012 mg eq/kg	6.0

[a] Croucher, 2002, QM-0053, 2 kg ai/ha

[b] Croucher, 2002, QM-0053, 10 kg ai/ha

[c] Swales, 2005, report QM-0060, 1.25 kg ai/t tuber

Residues in succeeding or rotational crops

No information was provided.

ANIMAL METABOLISM

Rat

Metabolism studies on laboratory animal including rats were reviewed within the framework of the toxicological evaluation by the current JMPR.

Lactating goats

Study 1

A metabolism study on the lactating goat (Nubian), was performed to investigate the residue behaviour of [phenyl-^{14}C]-tolclofos-methyl [Yu, 1987, report QM-71-0027]. The test substance was administered to one goat by oral gavage in gelatine capsules for 4 consecutive days (times 0, 24, 48 and 72 hours) at a dose level of 250 ppm feed (10 mg/kg bw/day). Urine and faeces were taken at 7 and 24 hours and daily thereafter; milk was collected twice daily. The goat was sacrificed 7 hours after the last dosing: muscle, liver, kidney and fat were excised and frozen prior to analysis. Radioactivity in collected samples and extracts was determined by LSC or combustion/LSC.

Radioactive residues in muscle, kidney and liver (homogenised with water and then adjusted to pH 1) were extracted thoursee times (3×) with diethyl ether. Further extractions with diethyl ether were made after reflux (1 hours) under acidic condition (acid-released fraction), and subsequently after reflux (1 hours) under basic condition (pH 12) (base-released fraction). The remains were centrifuged and the resultant water soluble fraction was lyophylised and extracted with methanol and then with water. The aqueous fraction was adjusted to pH 7, lyophilysed and extracted with methanol and then water.

Fat sample was homogenised with hexane and then the hexane fraction was partitioned using acetonitrile. For milk, residue was extracted with diethyl ether. The diethyl ether fraction was concentrated and partitioned with acetonitrile and hexane.

TLC analysis was carried out using the following reference standards: TM, TM-CHO, TM-CH_2OH, TM-COOH, TMO, TMO-CHO, TMO-CH_2OH, TMO-COOH, DM-TM, DM-TM-CH_2OH, DM-TM-COOH, DM-TMO, DM-TMO-CH_2OH, DM-TMO-COOH, ph-CH_3, ph-CHO, ph-CH_2OH, ph-COOH, SM-TM, DM-SM-TM, ph-CO-gly. The study results are shown in Tables18-21.

Of the total dose, only 27.2% was recovered, and most (26% of total dose) was detected in urine. Small amount was recovered from faeces (0.6%), milk (0.004%) and tissues (0.6% from muscle, fat, liver, and kidney). The steady increase in the concentration of radiocarbon in faeces indicated that passage thoursough the GI tract of the goat was very slow, which made considerable amounts of the dose remained in the GI tract at the end of study period (not quantified) and the longer study period could show more excretion in faeces.

TRR levels 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. Residue levels in milk reached equilibrium of about 0.8 mg eq/kg within 4 days after dosing.

Acidified organic solvent extracted 37.4% TRR in liver, 28.4% TRR in kidney, and 12.6% TRR in muscle. Further extractions (acid- and base-reflux followed by extraction with diethyl ether) released 12.9% TRR in liver and 39.0% TRR in kidney and 25.3% TRR in muscle. Water extract contained 20.6% TRR in liver, 43.8% TRR in kidney and 54.2% TRR in muscle. Final unextracted radioactivity was 29.8% TRR in liver, 3.8% TRR in kidney and 8.0% 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 extracts, TLC analysis was not performed or not succeeded.

In liver, parent was not detected. Metabolite ph-COOH was a major component found at 18.1% TRR (16.5% TRR free form, 1.6% TRR base released, total: 0.55 mg eq/kg). Another major component ph-CH_3 was also found at 15.1% TRR (11.1% TRR free form, 4.0% TRR acid released, total: 0.45 mg eq/kg). Metabolite ph-CH_2OH and one unknown fraction were present at 5.4% TRR (0.16 mg eq/kg) and 4.4% TRR (0.13 mg eq/kg), respectively. Other components from acid- and base-released fractions were present at less than 5.1% TRR (0.15 mg eq/kg).

In kidney, parent was not detected. TMO-COOH was a major component found at 21.2% TRR (8.7% TRR free form, 8.1% TRR acid released, 4.4% base released, total: 0.91 mg eq/kg). Another major component ph-COOH was found at 21.1% TRR (5.7% TRR free form, 7.4% TRR acid released, 8.0% TRR base released, total: 0.91 mg eq/kg). TMO-CH_2OH was found at a lesser extent of 11.0% TRR (3.1% free form, 4.0% acid released, 3.9% base released, total 0.47 mg eq/kg). DM-TM-CH_2OH, DM-TMO and two unknown fractions were present at levels of less than 3.8% TRR (0.16 mg eq/kg). Other components from acid- and base-released fractions were present at less than 3.2% TRR (0.14 mg eq/kg).

In milk, parent was not detected. Metabolite TMO was the predominant residue, accounting for 42.4% 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 at 6.8–6.9% TRR (0.028 mg eq/kg).

Table 18 Total radioactive residues in a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for four days at a dose level of 250 ppm diet

Sample	Collection time (hours)	% of total dose	TRR (mg eq/kg)
Urine	7	4.2	180
	24	3.8	430
	48	10.6	514
	72	5.0	401
	79	2.3	485
		Total: 26	
Faeces	7	0.02	4
	24	0.03	24
	48	0.36	44
	72	0.12	81
	79	0.10	143
		Total: 0.6	
Milk	7	< 0.001	0.32
	24	0.001	0.77
	31	0.001	0.67
	48	0.001	0.41
	55	< 0.001	0.42
	72	0.001	0.80
	79	< 0.001	0.87
		Total: 0.004	
Kidney	79	0.035	4.3
Fat	79	0.14	1.1
Muscle	79	0.25	0.2
Liver	79	0.12	3.0
		Total: 0.6	
Bile	79	0.01	9.4
Total		27.2	

Table 19 Extraction of radioactive residues in kidney, liver, muscle, urine and faeces of a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for four days at a dose level of 250 ppm diet

Fraction	% TRR		
	Liver (3.0 mg eq/kg)	Kidney (4.3 mg eq/kg)	Muscle (0.2 mg eq/kg)
Diethyl ether extract	37.4*	28.4*	12.6
Aq./solids			
Acid-released	9.1*	22.7*	17.9
Base-released	3.8*	16.3*	7.4
Subtotal	50.3	67.4	37.9
Remains			
Water soluble			54.2
MeOH extract	10.3	37.5	
Water extract	10.3	6.3	
Solids (unextracted)	29.8	3.8	8.0
Total	101	115	100

Ethyl ether extract: extracted with diethyl ether after adjusting to pH 1

Acid released: reflux for 1 hours (acidic), and then extracted with diethyl ether.

Base-released: reflux for 1 hours (pH 12), and then extracted with diethyl ether (pH 7)

* Further analysed by TLC

Table 20 Extraction of radioactive residues in milk and fat of a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for at a dose level of 250 ppm diet

Fraction	% TRR	
	Milk (48 hours, 0.41 mg eq/kg)	Fat (1.1 mg eq/kg)
Diethyl ether extract (milk) or hexane extract (fat)		
ACN phase	65.1*	98.9
Hexane phase	9.3	18.8
Subtotal	74.4	118
Unextracted	21.4+	8.2
Total	95.9	126

* Further analysed by TLC.

+ Water soluble 3.1% TRR; final remains, 18.3% TRR

Table 21 Parent and its metabolites in a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for four days at a dose level of 250 ppm diet

Components	% TRR in liver (3.0 mg eq/kg)				% TRR in kidney (4.3 mg eq/kg)				Milk (0.41 mg eq/kg)
	Free+conj.	Acid-rel.	Base-rel.	Total	Free+conj.	Acid-rel.	Base-rel.	Total	Free form
Parent									
ph-CH_3	11.1 (0.33)	4.0 (0.12)	0.0	15.1 (0.45)					
TMO-COOH					8.7 (0.37)	8.1 (0.35)	4.4 (0.19)	21.2 (0.91)	
ph-CHO	0.0	0.0	0.9	0.9 (0.027)					
ph-COOH	16.5 (0.50)	0.0	1.6 (0.048)	18.1 (0.55)	5.7 (0.25)	7.4 (0.32)	8.0 (0.34)	21.1 (0.91)	9.0 (0.037)
TMO									42.4 (0.17)
ph-CH_2OH	5.4 (0.16)	0.0	0.0	5.4 (0.16)					
TMO-CH_2OH					3.1 (0.13)	4.0 (0.17)	3.9 (0.17)	11.0 (0.47)	
DM-TM-CH_2OH					3.8 (0.16)	0.0	0.0	3.8 (0.16)	
DM-TM-COOH									6.8 (0.028)
DM-TMO					3.2 (0.14)	0.0	0.0	3.2 (0.14)	
Identified				39.5				60.3	58.2
Characterised, unknowns	1 fr. 4.4 (0.13)	1 fr. 5.1 (0.15)	1 fr. 1.3 (0.039)	10.8 (0.32)	2 frs. 1.1-3.0 (0.047-0.13)	2 frs. 0.0-3.2 (0.14)	2 frs. 0.0	7.3 (0.74)	1 fr. 6.9 (0.028)
Total#				50.3	28.6			67.6	65.1

Value in parenthesis means TRR (mg eq/kg).

Total means radioactivity identified and characterised by TLC/HPLC or TLC analyses.

Study 2

Metabolism of [phenyl-^{14}C]-tolclofos-methyl on a lactating goat was investigated [Burri, 2014b, report QM-0073]. A goat (Strain, Saanen) was orally administered in gelatine capsules with [phenyl-^{14}C]-tolclofos-methyl for 7 consecutive days (twice daily; repeatedly 14×) at dose level of 10 ppm dry matter diet (actual, 10.75 ppm dry feed; 0.388 mg/kg bw/day). The goat was sacrificed at approximately 6 hours after the last administration, and then muscle, fat, liver, kidney, bile and blood

were collected. Milk (twice daily), urine and faeces were collected during the dosing period. Samples and extracts were stored at approximately -20 °C and the initial analytical investigations on all samples were completed within approximately 5 months after beginning of sample work-up. Radioactivity in collected samples and extracts were determined by LSC, directly or after digestion with scintillation mixture. Metabolite patterns were determined in milk, liver, kidney, urine and faeces.

Radioactive residues in milk whey (milk fat, removed by centrifugation) were extracted thoursee times with acetone (protein precipitated during 1st extraction). The extract was partitioned twice with dichloromethane at neutral, acidic and basic condition, respectively (acid and base conditions of pH and temperature, not reported). The organic phase was analysed by TLC.

Radioactive residues in liver were extracted (each 1-3×) using the following solvents: acetonitrile/water, methanol/water, acidic and basic acetonitrile/water, basic acetonitrile/water and Soxhlet extraction with methanol (acid and base conditions of pH and temperature, not reported). The extract was partitioned with hexane after adding acetonitrile. The acetonitrile/water phase was further partitioned with (each 2–4×) dichloromethane, ethyl acetate and, at acidic and basic condition, dichloromethane (acid and base conditions of pH and temperature, not reported).The organic and aqueous phases were analysed by TLC.

The unextracted residues from liver was subjected to acid hydrolyses with 1N HCl (washed with methanol), 6N HCl (washed with methanol/6N HCl) and pronase treatment. The extract from the 6N HCl hydrolysis was partitioned with (each 2×) dichloromethane at acidic, neutral and basic condition.

For kidney, residues were extracted with (each 1–3×) acetonitrile/water and methanol/water. The extract was partitioned with hexane after adding acetonitrile. The acetonitrile/water phase was further partitioned with (each 2×) dichloromethane, ethyl acetate and, at acidic and basic condition, dichloromethane (acidic and basic conditions of pH and temperature, not reported). The organic phase was analysed by both TLC and HPLC. The aqueous phase was analysed by TLC. The reference standards used were TM, TMO-CH_2OH, TMO-COOH, DM-TM, DM-TM-CH_2OH, DM-TM-COOH, DM-TMO, DM-TMO-CH_2OH, DM-TMO-COOH, ph-CH_3, ph-CH_2OH and ph-COOH. The study results are shown in Tables 22 to 26.

Majority (85%) of the radiolabelled tolclofos-methyl was excreted in urine (45.7% of total dose) and faeces (39.3% of total dose). Transfer to milk was a level of 0.08% of the total dose and 0.24% of the total dose was detected in edible tissues (muscle, fat, liver and kidney).

Residue levels in muscle and fat were near or below the limit of quantification (muscle, 0.005 mg eq/kg; fat, < 0.0098 mg eq/kg). In liver and kidney, TRR were 0.25 mg eq/kg and 0.22 mg eq/kg, respectively. TRR in milk (0–166 hours) was 0.009–0.019 mg eq/kg, with a plateau at 0.014–0.019 mg eq/kg at 16–24 hours after the first dosing.

A ratio of 8.1% of the total radioactivity in whole milk was distributed into milk fat.

Extraction efficiency of radioactivity was 65.6% TRR in liver and 93.1% TRR in kidney. Further treatments for liver released additional residues of 24.2% TRR (17.9% TRR by acid hydrolysis and 6.3% TRR by pronase incubation), and 9.9% TRR remained unextracted. Acetone extracted 87.2% TRR from the whey.

Parent was not detected in milk (milk whey). Metabolite TMO-COOH was found at a level of 6.7% TRR (0.001 mg eq/kg). Other two unidentified components found in organic phase were present at up to 12.4% TRR (0.002 mg eq/kg)

Table 22 Total radioactive residues in a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for seven days (twice daily) at a dose level of 10.75 ppm diet

Goat samples	% of total dose	TRR (mg eq/kg)
Urine	45.7	
Faeces	39.3	
Milk	0.08	0.009-0.019
Cage wash	0.50	
Bile	< 0.01	0.22
Liver	0.12	0.25
Kidney	0.02	0.22
Fat (pool)	0.03	< 0.0098
Fat omental		< 0.0098
Fat renal		< 0.0098
Fat subcutaneous		0.006
Muscle (pool)	0.07	0.005
Muscle fore-leg		0.004
Muscle rump		0.005
Blood at sacrifice	0.09	0.029 (0.010-0.026, taken prior to each administration)
Plasma at sacrifice	0.08	0.041 (0.012-0.034, taken prior to each administration)
Content in intestinal tract	8.8	
Total	94.7	

Table 23 Radioactivity in milk of in a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for seven days (twice daily) at a dose level of 10.75 ppm diet

Time period after first admin. (hours)	% of total dose	Radioactivity (mg eq/kg)
Prior to administration	< LOQ	< 0.0023
0-16	0.005	0.009
16-24	0.004	0.015
24-40	0.008	0.016
40-48	0.004	0.015
48-64	0.007	0.014
64-72	0.004	0.014
72-88	0.007	0.014
88-96	0.004	0.015
96-112	0.008	0.017
112-120	0.004	0.016
120-136	0.009	0.019
136-144	0.004	0.017
144-160	0.007	0.015
160-166 (characterised)	0.002	0.015
Total	0.08	

Table 24 Extraction of radioactive residues in milk of a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for seven days (twice daily) at a dose level of 10.75 ppm diet

Extraction	Milk (0.015 mg eq/kg)	
	% TRR	mg eq/kg
Fat	8.1	0.001
Whey-acetone extract	87.2	0.013
Org. phase (CH_2Cl_2)	21.1*	0.003
Aq. phase	66.1	0.010
Unextracted (proteins)	4.7	0.001
Total	100	

Milk sample collected at 160 -166 hourss. Extraction was conducted under neutral conditions and partitioning steps were conducted at neutral, acidic and basic conditions.

* Further analysed by TLC

Table 25 Extraction of radioactivity in liver and kidney of a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for seven days (twice daily) at a dose level of 10.75 ppm diet

Extraction	Liver (0.25 mg eq/kg)		Kidney (0.22 mg eq/kg)	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Extract	65.6	0.17	93.1	0.20
Hexane phase	3.1	0.008	6.1	0.013
ACN/water phase				
Org. phase (ethyl acetate/CH_2Cl_2)	35.0*	0.088	43.4*+	0.093
Aq. phase	27.5*	0.069	43.6*	0.094
Unextracted	(34.1)		6.9	0.015
Hydrolysis (1N HCl)	1.5	0.004		
Hydrolysis (6N HCl)	16.4	0.041		
Org. phase (CH_2Cl_2)	3.5	0.009		
Aq. phase	12.9	0.033		
Pronase incubation	6.3	0.016		
Unextracted (final)	9.9	0.025		
Total	100		100	

For liver, acid and base conditions were used for extraction and partitioning with organic solvents. For kidney, extraction was conducted under neutral conditions and partitioning steps were conducted at neutral, acidic and basic conditions.

* Further analysed by TLC

+ Further analysed by HPLC

Table 26 Parent and its metabolites in a lactating goat administered with [phenyl-^{14}C]-tolclofos-methyl for seven days (twice daily) at a dose level of 10.75 ppm diet

Components	Milk (0.015 mg eq/kg)		Liver (0.25 mg eq/kg)		Kidney (0.22 mg eq/kg)	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Org. phase	21.1	0.003	35.0		43.4	
Parent			4.4	0.011	11.9	0.029
ph-COOH			10.2	0.026	12.5	0.031
ph-CH_2OH					1.5	0.004
TMO-COOH	6.7	0.001			5.4	0.013
DM-TM					0.8	0.002
Identified	6.7		14.6		32.1	
Characterised, unknowns	14.4% TRR, 2 frs. (each 2.0–12.4% TRR, < 0.001–0.002 mg eq/kg)		20.4% TRR, 5 frs. (each 0.5–8.8% TRR, 0.001–0.022 mg eq/kg)		11.4% TRR, 5 frs. (each 0.4–5.9% TRR, 0.001–0.014 mg eq/kg)	
Aq. phase	66.1		27.5%		43.6	
Characterised, unknowns			27.5% TRR, 9 frs. (each 0.8–8.2% TRR, 0.002–0.021 mg eq/kg)		42.8% TRR, 6 frs (each 0.4–18.5% TRR, 0.001–0.045 mg eq/kg)	
Total#	21.1		62.5		86.3	

Total means radioactivity identified and characterised by TLC/HPLC or TLC analyses.

In liver, parent was present at 4.4% TRR (0.011 mg/kg). Metabolite ph-COOH was the largest component, accounting for 10.2% TRR (0.026 mg eq/kg). Other many unidentified components were present: five components found in organic phase, at less than 8.8% TRR (0.022 mg eq/kg); nine components found in aqueous phase, at less than 8.2% TRR (0.021 mg eq/kg).

In kidney, parent was present at 11.9% TRR (0.029 mg/kg). Metabolite ph-COOH was the largest component, accounting for 12.5% TRR (0.031 mg eq/kg). TMO-COOH, ph-CH_2OH and DM-TM were also found but at less than 5.4% TRR (0.013 mg eq/kg). Other many unidentified

components were present: five components found in organic phase, at less than 5.9% TRR (0.014 mg eq/kg); six components found in aqueous phase, at less than 18.5% TRR (0.045 mg eq/kg).

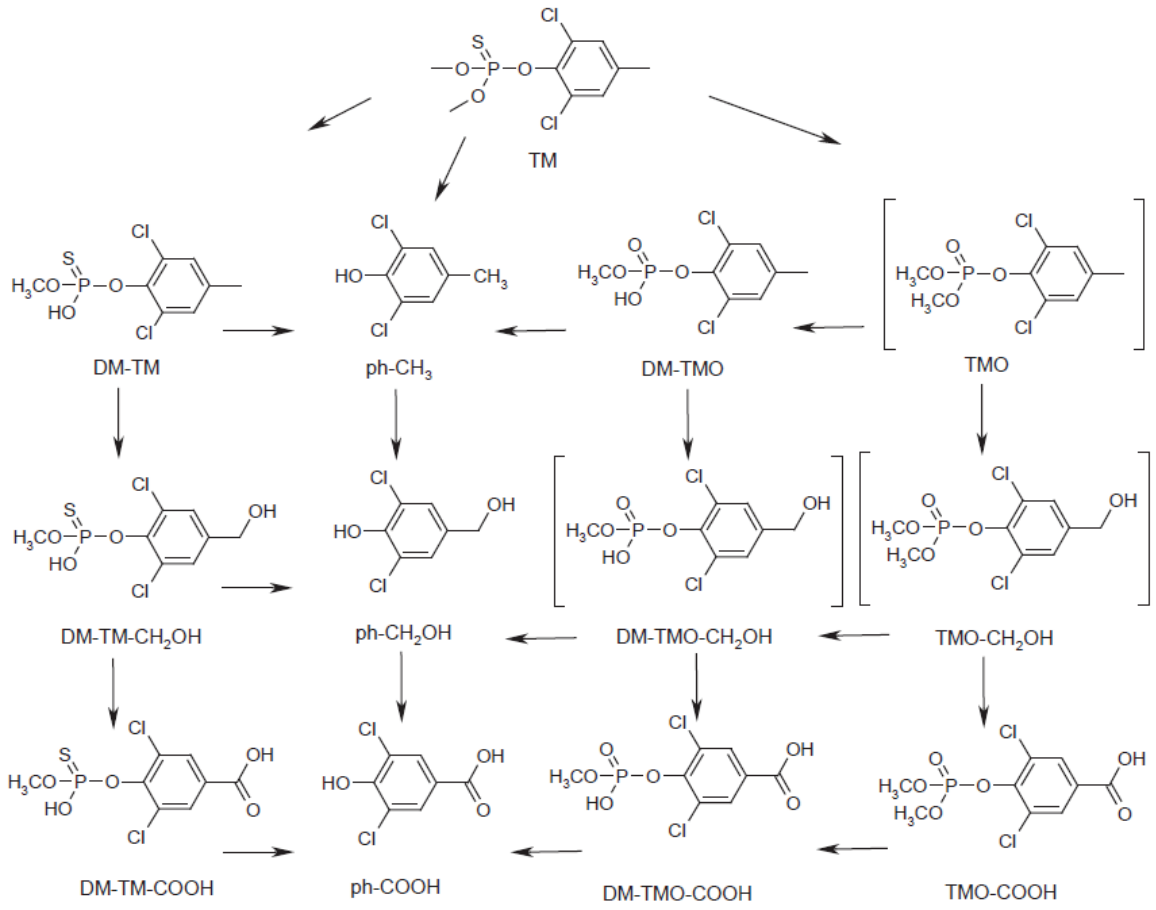


Figure 4 Proposed metabolic pathway for tolclofos-methyl in lactation goats

(Compounds in brackets refer to compounds not identified in the study but being logical intermediates in the proposed pathway)

From the study results, it was shown that tolclofos-methyl in goat undergoes oxidative desulfuration, demethylation and hydrolysis of the P-O bond to ph-CH_3. The ph-CH_3 is further metabolized to its alcohol (ph-CH_2OH) and acid analogue (ph-COOH).

A proposed metabolic pathway for tolclofos-methyl in lactating goat is shown in Figure 4.

Laying hens

Study 1

Metabolism of [phenyl-^{14}C]-tolclofos-methyl on laying hens was investigated [Yu and Guirguis, 1987, report QM-71-0028]. The radiolabelled test substance was orally administered to white leghorn laying hens (control 3 hens; treated, 3 hens) daily for four consecutive days (0, 24, 48 and 72 hours) at 10 mg/kg bw/day (calculated to be 167 ppm in diet). Eggs (twice daily) and excreta were collected daily. Hens were sacrificed 7 hours after the last dosing and tissues and organs were excised. All samples were frozen prior to analysis. Radioactivity in collected samples was determined by combustion/LSC. Excreta, liver and kidney samples were subjected to solvent extraction and further analysis.

Radioactive residues in liver were extracted (3×) with diethyl ether after adjusting to pH 1. The remaining (aqueous and solid fraction) was refluxed for 1 hour and then extracted (3×) with diethyl ether (acid-released fraction). The remaining (aqueous and solid fraction) was centrifuged: the solid fraction (adjusted to pH 12) was refluxed for 1 hour, lyophilised and extracted with methanol (base-released fraction); the aqueous fraction (neutralised) was freeze-dried and extracted with methanol.

For kidney, residues were extracted (3×) with diethyl ether after adjusting to pH 1. The remaining (aqueous and solid fraction) was refluxed for 1 hour and then extracted (3×) with diethyl ether (acid-released fraction). The remaining (aqueous and solid fraction) was refluxed for 1 hour, adjusted to pH 12, and then extracted (3×) with diethyl ether (base-released fraction).

TLC analysis was performed with reference standards: TM, TM-CH_2OH, TM-CHO, TM-COOH, TMO, TMO-CH_2OH, TMO-CHO, TMO-COOH, DM-TM, DM-TM-CH_2OH, DM-TM-COOH, DM-TMO, DM-TMO-CH_2OH, DM-TMO-COOH, ph-CH_3, ph-CH_2OH, ph-CHO, ph-COOH, ph-CO-gly, SM-TM and DM-SM-TM. Identity of found metabolites was confirmed by GC analysis (metabolite compound, methylated). The study results are shown in Tables 27 to 29.

Excretion was rapid and 85.9% of the total dose was excreted in excreta. Eggs and edible tissues (muscle, fat, liver and kidney) contained 0.03% and 0.7% of the total dose, respectively.

TRR levels were 0.11 mg eq/kg in muscle, 1.0 mg eq/kg in fat, 3.4 mg eq/kg in liver and 6.0 mg eq/kg in kidney. In eggs, TRR were 0.01–0.37 mg eq/kg in egg yolk and 0.03–0.07 mg eq/kg in egg white.

Acidified organic solvent extracted 19.8% TRR and 40.0% TRR in liver and kidney, respectively. Further extractions (acid- and base-reflux followed by extraction with diethyl ether) released 8.1% TRR and 18.5% TRR in liver and kidney, respectively. Final unextracted radioactivity was 69.9% TRR and 40.3% TRR in liver and kidney, respectively.

In liver, parent was not detected. Metabolite TMO-CHO was found at 3.4% TRR (0.12 mg eq/kg). Other components (five, unidentified) were present at 0.28–5.2% TRR (0.010–0.018 mg eq/kg). Acid- and base-released residues were not analysed by TLC.

In kidney, parent was not detected. Metabolite ph-COOH was found 9.3% TRR (0.56 mg eq/kg). Other components (eight, unidentified) were present at 0.5–7.7% TRR (0.030–0.46 mg eq/kg). Acid- and base-released residues were not analysed by TLC.

Table 27 Total radioactive residues in laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for four days at a dose level of 167 ppm dry feed

Sample	Collection time (hours)	% of total dose	TRR (mg eq/kg)	
Excreta	7	17.8 (71)		
	24	4.0 (16)		
	48	23.8 (95)		
	72	18.8 (75)		
	79	21.6 (86)		
		Total: 85.9		
Eggs			Yolk	Albumin
	7		0.04	0.05
	48		0.01	0.03
	72		0.37	0.07
	79		0.27	0.06
		Total: 0.03		
Edible tissues				
Kidney	79	0.11	6.0	
Liver	79	0.20	3.4	
Muscle	79	0.16	0.11	
Fat	79	0.23	1.0	
		Total 0.7		

Sample	Collection time (hours)	% of total dose	TRR (mg eq/kg)
Blood		0.58	0.76 in plasma, 0.24 in blood cell
Heart	79	< 0.01	0.18
Lung	79	< 0.01	0.44
Spleen	79	< 0.01	0.12
Ovary	79	0.04	0.47
Stomach content	79	1.2	
Intestine	79	0.76	
Total		89.3	

Value in parenthesis, based on single dose

Table 28 Extraction of radioactive residues in liver and kidney of laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for four days at a dose level of 167 ppm dry feed

Liver (3.4 mg eq/kg)		Kidney (6.0 mg eq/kg)	
Fraction	% TRR	Fraction	% TRR
Diethyl ether extract	19.8*	Diethyl ether extract (free form)	40.0*
Aq/solids		Aq/solids	
Acid-released	6.2	Acid-released	9.1
Remaining	(73.4)	Base-released	9.4
Aqueous		Remaining	(40.3)
MeOH extract	1.6	Aq. fr.	15.1
Unextracted	28.9	Solid fr. (unextracted)	25.2
Solids			
Base-released	1.9		
Unextracted	41.0		
Total	99.4		98.8

Ethyl ether extract: extracted with diethyl ether after adjusting to pH 1

Acid released: refluxed for 1 hours (acidic), and then extracted with diethyl ether

Base-released: refluxed for 1 hours (pH 12), and then extracted with methanol for liver and diethyl ether for kidney

* Further analysed by TLC

Table 29 Parent and its metabolites in liver and kidney of laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for four days at a dose level of 167 ppm dry feed

Metabolites*	Liver (3.4 mg eq/kg)		Kidney (6.0 mg eq/kg)	
	% TRR	mg eq/kg	% TRR	mg eq/kg
Parent	nd		nd	
TM-CHO	3.4	0.12		
ph-COOH	nd		9.3	0.56
Identified	3.4		9.3	
Characterised, unknowns	14.5% TRR, 5 frs (0.28-5.2% TRR, 0.010-0.018 mg eq/kg)		30.6% TRR, 8 frs (0.5-7.7% TRR, 0.030-0.46 mg eq/kg)	
Total#	17.9		39.9	

*Detected in diethyl ether extract (free form): TM-CHO and ph-COOH, confirmed by GC analysis

Total means radioactivity identified and characterised by TLC analysis.

Study 2

Metabolism of [phenyl-^{14}C]-tolclofos-methyl was investigated in laying hens [Burri, 2014a, report QM 0075]. The ten hens (Strain, white Lohmann LSL-Classic) were orally administered (repeatedly, 14×) via capsules with the radiolabelled test substance for fourteen days at a dose level of 10 ppm diet (actual, 10.97 ppm dry feed; 0.915 mg/kg bw/day). Eggs were collected daily prior to dosing. Hens were sacrificed 7 hours after the last dosing. Liver, muscle (breast and thigh), fat (peritoneal), and skin

(including subcutaneous fat), excreta and blood were taken. Radioactivity in collected samples was determined by LSC, directly, or after digestion or combustion. Samples and extracts were stored at approximately -20 °C until analysis. The initial analytical investigations on all samples were completed within 5 months after beginning of sample work-up.

For muscle (thigh) and liver, radioactive residues were extracted using the following solvents: (each 1–3×) with acetonitrile/water, methanol/water, acetonitrile/water (acidic), acetonitrile/water (basic) and Soxhlet extraction with methanol. The extract was partitioned twice with hexane adding acetonitrile and water. The acetonitrile/water phase was further partitioned (each 1–3×) with dichloromethane, ethyl acetate, dichloromethane (acidic) and dichloromethane (basic) (acidic and basic conditions of pH and temperature, not reported). For liver, the remaining residues after solvent extraction were further processed: acid hydrolyses (1N HCl, 6N HCl), pronase incubation, and base hydrolysis (refluxed with 10N NaOH at 105 °C for 6 hours); after each hydrolysis, washed with methanol. The methanol wash from the base hydrolysis was partitioned (each 2×) with dichloromethane at basic, acidic and neutral condition.

Radioactive residues in fat and skin were extracted (each 3×) with dichloromethane and ethyl acetate. For skin, further extraction (2nd extraction) was conducted (each 1–2×) with acetonitrile/water at neutral, acidic and basic condition (acid and base conditions of pH and temperature, not reported). The extract (for skin, 1st extract only) was partitioned four times with hexane after adding acetonitrile.

For egg yolk (collected at 216–240 hours and 288–312 hours), residues were extracted using the following solvents: (each 1–3×) acetone, methanol, acetonitrile, acetonitrile/water and Soxhlet extraction with methanol. The extract was partitioned with (each 2–3×) dichloromethane at neutral, acidic, basic condition and diethyl ether (acid and base conditions of pH and temperature, not reported). The organic phase (CH_2Cl_2/ethyl acetate) was cleaned-up by partitioning with hexane after adding acetonitrile.

TLC was used for analysis. For liver organic phase (CH_2Cl_2/ethyl acetate), HPLC was further used. Reference standards were TM, TMO-CH_2OH, TMO-COOH, DM-TM, DM-TM-CH_2OH, DM-TM-COOH, DM-TMO, DM-TMO-CH_2OH, DM-TMO-COOH, ph-CH_3, ph-CH_2OH and ph-COOH. The study results are shown in Tables 30 to 34.

The majority (89.0%) of the radiolabelled tolclofos-methyl was excreted in excreta. Eggs and edible tissues (muscle, fat, skin and liver) contained 0.06% and 0.22% of the total dose, respectively.

TRR levels were 0.008 mg eq/kg in breast muscle, 0.013 mg eq/kg in thigh 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 was 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 192–216 hours (8–9 days) in yolk.

Extractibility of the radioactivity was 72.7% TRR in muscle, 60.3% TRR in liver, 92.6% TRR in fat, 91.6% TRR in skin and 73.0% TRR in yolk. For liver with the lowest extraction efficiency, 38.2% 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 12.0% TRR (0.001 mg eq/kg). TMO-COOH was found at 2.0% TRR (< 0.001 mg eq/kg). Other components (six, unidentified) were present at less than 16.2% TRR (0.002 mg eq/kg).

In fat, parent was the predominant residue, accounting for 75.9% TRR (0.034 mg/kg). Metabolite ph-COOH was found at 3.7% TRR (0.002 mg eq/kg). Other components (five, unidentified) were present at less than 4.0% TRR (0.002 mg eq/kg).

For skin, parent was found at 28.8% TRR (0.021 mg/kg). Metabolite ph-COOH was found at 10.6% TRR (0.008 mg eq/kg). Other metabolites ph-CH_2OH and TMO-COOH were found at 5.4%

TRR (0.004 mg eq/kg) and 1.3% TRR (0.001 mg eq/kg), respectively. Other components (four, unidentified) 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 18.3% TRR (0.076 mg eq/kg). Other metabolites ph-CH_3, TMO-CH_2OH and TMO-COOH, were found at 3.5%TRR (0.014 mg eq/kg), 1.6% TRR (0.007 mg eq/kg) and 0.7% TRR (0.003 mg eq/kg), respectively. Other components (ten, unidentified) were present at up to 9.9% TRR (0.041 mg eq/kg).

In egg yolk, parent was the predominant residue, accounting for 35.1% TRR (0.021 mg eq/kg). Any reference metabolites were not detected. Unidentified components (five) were detected at less than 12.6% TRR (0.007 mg eq/kg).

Table 30 Total radioactive residues in laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for fourteen days at a dose level of 10.97 ppm dry feed

Sample	Collection time (hours)	% of total dose	TRR (mg eq/kg)
Excreta	0-318, 324	89.0	
Cage wash	318	1.3	
Eggs	0-318	0.06 (0.04 in white, 0.01 in yolk)	0.002-0.006 in white 0.001-0.059 in yolk
Edible tissues			
Muscle	324	0.04	0.008 (breast), 0.013 (thigh)
Fat	324	0.04	0.045
Skin	324	0.06	0.073
Liver	324	0.08	0.42
		Total: 0.22	
Blood	324	0.06	0.096 (cell)
Plasma	324		0.13
Total		90.6	

Table 31 Radioactivity in hen eggs administered with [phenyl-^{14}C]-tolclofos-methyl for fourteen days at a dose level of 10.97 ppm dry feed

Collection time (hours)	TRR in egg white (mg eg/kg)	TRR in egg yolk (mg eg/kg)
0-24	0.002	0.001
24-48	0.005	0.009
48-72	0.005	0.019
72-96	0.005	0.031
96-120	0.005	0.040
120-144	0.005	0.050
144-168	0.006	0.052
168-192	0.006	0.053
192-216 (8-9 days)	0.006	0.057
216-240	0.006	0.058
240-264	0.006	0.059
264-288	0.006	0.059
288-312	0.006	0.058
312-318	no eggs laid	no eggs laid

TRR, mean value of radioactivity determined in eggs from 10 hens

Table 32 Extraction of radioactive residues in muscle and liver of laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for fourteen days at a dose level of 10.97 ppm dry feed

Fraction	Muscle (0.013 mg eq/kg)		Liver (0.42 mg eq/kg)	
	% TRR	TRR (mg eq/kg)	% TRR	TRR (mg eq/kg)
Extract	72.7	0.009	60.3	0.25
ACN/water phase				
Org. phase (CH_2Cl_2/ethyl acetate)	41.9*	0.005	29.3*+	0.12

Fraction	Muscle (0.013 mg eq/kg)		Liver (0.42 mg eq/kg)	
	% TRR	TRR (mg eq/kg)	% TRR	TRR (mg eq/kg)
Aq. phase	21.6	0.003	28.8*	0.12
Hexane phase	9.1	0.001	2.2	0.009
Unextracted	(27.3)		(39.8)	
Acid hydrolysis (1N HCl)			0.7	0.003
Acid hydrolysis (6N HCl)	1.3	< 0.001	1.2	0.005
Pronase incubation			6.1	0.025
Base hydrolysis (10N NaOH, at 105℃ for 6 hours)			30.2	0.13
Org. phase (CH_2Cl_2)			16.6*	0.069
Aq. phase			13.6	0.057
Unextracted	26.0	0.003	1.6	0.007
Total	100		100	

Acid and base conditions were used for extraction and partitioning with organic solvents.

Total, the sum of extracted and unextracted radioactivity

* Further analysed by TLC + Further analysed by HPLC

Table 33 Extraction of radioactive residues in egg yolk, skin and fat of laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for fourteen days at a dose level of 10.97 ppm dry feed

Fraction	Fat (0.045 mg eq/kg)		Skin (0.073 mg eq/kg)		Fraction	Egg yolk (0.058 mg eq/kg)	
	% TRR	TRR (mg eq/kg)	% TRR	TRR (mg eq/kg)		% TRR	TRR (mg eq/kg)
Extract	92.6	0.042	72.5, 19.1	0.053, 0.014	Extract	73.0	0.043
ACN phase	89.0*	0.040	67.2*	0.049	Org. phase (CH_2Cl_2/ethyl acetate)	(59.6)	0.032
Hexane phase	3.6	0.002	5.3	0.004	ACN phase	55.2*	0.032
Unextracted	7.4	0.003	8.4	0.006	Hexane phase	(4.4)	
					Aq. phase	13.4	0.008
					Unextracted	27.1	0.016
Total	100		100			100	

Egg yolk: mean value of 216-240 hours and 288-312 hours samples; of the 73.0% TRR, only 68.6% TRR was partitioned (Soxhlet extract of 4.3% TRR, not included).

Egg yolk samples were extracted at neutral condition 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.

Skin: only 72.5% TRR (1st extract, 0.053 mg eq/kg) was partitioned. 19.1% TRR (2nd extract) was not processed.

Total: the sum of extracted and unextracted radioactivity * Further analysed by TLC

Table 34 Parent and its metabolites in laying hens administered with [phenyl-^{14}C]-tolclofos-methyl for fourteen days at a dose level of 10.97 ppm dry feed

Components	Egg yolk (0.058 mg eq/kg)		Liver (0.42 mg eq/kg)						Muscle (0.013 mg eq/kg)		Fat (0.045 mg eq/kg)		Skin (0.073 mg eq/kg)	
	ACN phase		Org. phase		Aq. phase		Total		Org. phase		ACN phase		ACN phase	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Parent	35.1	0.021	0.5	0.002			0.5	0.002	5.0	0.001	75.9	0.034	28.8	0.021
ph-CH_3			3.5	0.014			3.5	0.014						
ph-COOH			15.6	0.065	2.7	0.011	18.3	0.076	12.0	0.001	3.7	0.002	10.6	0.008
ph-CH_2OH													5.4	0.004
TMO-COOH			0.7	0.003			0.7	0.003	2.0	< 0.001			1.3	0.001
TMO-CH_2OH			1.6	0.007			1.6	0.007						
Identified	35.1						24.6		19.0		79.6		46.1	

Components	Egg yolk (0.058 mg eq/kg)		Liver (0.42 mg eq/kg)						Muscle (0.013 mg eq/kg)		Fat (0.045 mg eq/kg)		Skin (0.073 mg eq/kg)	
	ACN phase		Org. phase		Aq. phase		Total		Org. phase		ACN phase		ACN phase	
	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg	% TRR	mg eq/kg
Characterised, unknown	20.1% TRR 5 frs. (0.9-12.6% TRR; 0.001-0.007 mg eq/kg)		7.5% TRR 5 frs. (0.2-3.4% TRR; 0.001-0.014 mg eq/kg)		26.1% TRR 6 frs. (2.1-9.9% TRR; 0.009-0.041 mg eq/kg)				22.9% TRR 6 frs. (0.6-16.2% TRR; < 0.001-0.002 mg eq/kg)		9.4% TRR 4 frs. (1.2-4.0% TRR; 0.001-0.002 mg eq/kg)		21.1% TRR 4 frs. (3.7-6.8% TRR; 0.003-0.005 mg eq/kg)	
Total[#]	55.2						74.7[*]		41.9		89.0		67.2	

Egg yolk: mean value of 216-240 hours and 288-312 hours samples

[*] 16.6% TRR (base hydrolysed organic phase of unextracted residue, see Table 32) included, which consisted of unknown 7 frs, individually 0.4-5.8% TRR (0.002-0.024 mg eq/kg).

[#] Total means radioactivity identified and characterised by TLC/HPLC or TLC analyses.

Based on the study results, it was shown in laying hens that tolclofos-methyl undergoes oxidative desulfuration, demethylation and hydrolysis of the P-O bond to ph-CH_3. The ph-CH_3 is further metabolized to its alcohol (ph-CH_2OH) and acid analogue (ph-COOH). A proposed metabolic pathway for tolclofos-methyl in laying hens is shown in Figure 3 (the metabolic pathway in lactating goat).

Summary: In general, the metabolism between goat and hen was qualitatively similar. The routes and products of metabolism were similar between the species. Tolclofos-methyl underwent oxidative desulfuration, demethylation and hydrolysis of P-O aryl bond to form ph-CH_3. The ph-CH_3 was further metabolized to its alcohol (ph-CH_2OH) and acid analogue (ph-COOH).

ENVIRONMENTAL FATE

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

Hydrolysis

The hydrolytic degradation of [phenyl-^{14}C]-tolclofos-methyl was studied at pH 4, 7 and 9, and at 50, 62 and 74 °C [Lewis, 2001a, report QM-0051]. Aqueous buffer solutions prepared at pH 4, 7 and 9 were sterilised and then the test substance was added to achieve a concentration of 0.2 mg/L. The test solutions were incubated at 50, 62 and 74 °C for up to 5 days. Radioactivity in sample was extracted with acetonitrile. HPLC was used for analysis. For confirmation of identity of hydrolysis products TLC was used. The study results are shown in Table 35–36.

Degradation of tolclofos-methyl was dependent on pH and more temperature. At pH 4–9, the half-life values (calculated based on first order kinetics) were 61–97 hours at 50 °C, 17–32 hours at 62 °C and 5.1–9.6 hours at 74 °C. The half-life values (pH 4–9) at 20 °C and 25 °C (calculated using Arrhenius equation) were 97–126 days and 50–68 days, respectively.

A single hydrolysis product DM-TM was produced (46–65% AR at pH 4–9, 50 °C and last time points), corresponding to degradation of tolclofos-methyl. Another metabolite ph-CH_3 was produced only at pH 9, at up to *ca*. 12% AR (at last time points; at 50, 62 and 74 °C). Both products increased thoursoughout the incubation period.

Table 35 Degradation of [phenyl-^{14}C]-tolclofos-methyl in aqueous buffer solution

Temperature (°C)	pH	Time (hours)	TM (% AR)	DM-TM (% AR)	ph-CH_3 (% AR)
50	4	2.4 50.0 120	93.6 66.1 41.6	0.7 25.5 48.0	nd nd nd
	7	2.4 46 120.2	93.3 54.8 25.5	2.6 35.2 65.4	nd nd nd

Temperature (°C)	pH	Time (hours)	TM (% AR)	DM-TM (% AR)	ph-CH_3 (% AR)
	9	2.4	94.5	1.5	nd
		47.2	61.1	23.2	5.6
		120.9	33.1	45.7	11.5
62	4	15.1	65.5	26.6	nd
		25.5	53.8	38.6	nd
		51.2	30.4	62.1	nd
	7	9.5	61.2	30.9	nd
		16.6	46.2	45.6	nd
		50.4	13.5	80.9	nd
	9	10.6	66.5	19.2	2.9
		21.4	51.4	33.5	5.9
		50.6	21.1	56.6	12.6
74	4	4.0	68.7	22.3	nd
		8.1	51.7	41.5	nd
		14.2	34.8	57.8	nd
	7	3.0	62.8	31.4	nd
		5.0	48.7	45.6	nd
		8.2	30.7	64.0	nd
	9	3.6	68.5	21.3	3.4
		8.6	42.4	41.9	8.5
		11.2	32.2	48.7	11.6

Time, selected

nd, not detected

Table 36 Half-life value of tolclofos-methyl in aqueous buffer solution

Temperature (°C)	Half-life, days		
	pH 4	pH 7	pH 9
50	4.0 (97 hours)	2.5 (61 hours)	3.2 (76 hours)
62	1.3 (32 hours)	0.7 (17 hours)	1.0 (24 hours)
74	0.4 (9.6 hours)	0.2 (5.1 hours)	0.3 (7.3 hours)
20*	126	97	102
25*	68	50	55

* Calculated using the Arrhenius equation and data form the 50, 62 and 74 °C studies

Photochemical degradation

Aqueous photolysis

Study 1

The photolysis of [phenyl-^{14}C]-tolclofos-methyl in water was investigated using artificial light [Takahashi, 1988, report QM-80-0024]. Sterile aqueous buffered solution (pH 7) with [phenyl-^{14}C]-tolclofos-methyl at 0.2 mg/L (sterilised) was irradiated continuously by a Xenon arc lamp (290–750 nm) for periods of 30 days, at 25 °C with continuous stirring. The light intensity was equivalent to sunlight in November at latitude 40°N. Samples were taken immediately after dosing, and at 1, 3, 5, 7, 14, 21 and 30 days after treatment. Prior to sampling the test solution, carbon dioxide-free air was passed over the water surface to trap the volatiles. The test solution was acidified to pH 2 and extracted with ethyl acetate. Radioactivity was determined by LSC. TLC was used for analysis. For photoproducts occurring at > 10 % of the applied radioactivity, HPLC analysis was made.

Mass balance in irradiated samples and dark control samples (control) were 93–107% AR and 93–106% AR in all samples, respectively. Extraction was 87–106% AR and 91–105% AR in irradiated and control samples, respectively. Amounts of volatiles were ≤0.1% AR in all samples.

Tolclofos-methyl gradually decreased to 60% AR (irradiated) and 80% AR (control) by the study end, indicating enhanced degradation by irradiation. In both conditions, DM-TM was a main

degradation product found at maximum 12.6% AR (day 30) in irradiated condition and, at lesser extent, maximum 7.3% AR (day 21) in the dark condition. TMO and ph-CH_3 also occurred but at individually ≤1.5% AR in both conditions. DM-TMO and SM-TM were observed only in irradiated condition, at levels of maximum 0.9% AR (day 30) and 0.2% AR (day 30), respectively. Other unknowns were detected at, in total, maximum 11.1% AR (day 30) and 8.2% AR (day 21) in irradiated and dark condition, respectively.

Half-lives of tolclofos-methyl (based on first order kinetics) were 38.3 days ($r^2 = 0.94$) under irradiated conditions and 76.6 days ($r^2 = 0.74$) under dark conditions.

Study 2

Photolytic degradation of tolclofos-methyl was investigated in sterilised water using simulated sunlight [Curtis-Jackson, 2014a, Report QM-0074]. Tolclofos-methyl (not radioactive), dissolved in sterilised water at 0.317 mg/L 1% acetonitrile and at 0.332 mg/L 10% acetonitrile as co-solvent, was exposed under light from Xenon arc lamp (below 290 nm, removed). The mean light intensity reached the surface of the aqueous solutions was 50.24 W/m^2 within the visual light spectrum (300 to 400 nm). Temperature and pH were monitored thoursoughout the study, which were at 24.4–24.9 °C (mean) and pH 5.4–7.1. Aliquots were removed from the test solutions at 0, 3, 7, 10, 14 and 17 days for 1% acetonitrile solutions and at 0, 4, 7, 11, 14 and 18 days for 10% acetonitrile solutions. The simulated light intensity of 17 days was equivalent to 48.7 days at 50º N and 46.7 days, at 30–40 ºN. Tolclofos-methyl was measured by LC/MS analysis after dilution. Degradation rate constants of tolclofos-methyl were determined after correction for the dark control. The results are shown in Tables 37 to 39.

The rate constant corrected for any non-photolytic processes accounts in 0.036 d^{-1} and 0.034 d^{-1} for 10% and 1% acetonitrile solution, respectively. The respective half-lives were 19.3 days and 20.4 days under continuous irradiation. Quantum yield (Φ) of tolclofos-methyl in 10% and 1% acetonitrile solution were 3.70×10^{-6} and 3.49×10^{-6}, respectively, and the average value was 3.6×10^{-6}. From the average quantum yield of tolclofos-methyl, photolytic rate constants (K_{pE}, day^{-1}) and subsequently, half-lives were estimated at any latitude and season. The estimated half-lives of tolclofos-methyl for direct photolysis were 8.2–48.5 days at any latitude and seasons.

Table 37 Experimental photodegradation rate constants and corresponding half-lives

Xenon arc lamp	TM (10% ACN)	TM (1% ACN)
$k_{irradiated\ vessel}$ (1/d)	0.058	0.063
$k_{dark\ control\ vessel}$ (1/d)	0.022	0.029
$k_{photolysis}$ (1/d)	0.036	0.034
$t_{1/2\ LAB}$ (d)	19.3	20.4
$t_{90\ LAB}$ (d)	64.0	67.7

$k_{irradiated\ vessel}$ = Rate constant of irradiated sample

$k_{dark\ control\ vessel}$ = Rate constant of dark control

$k_{photolysis}$ = Rate constant corrected for any non-photolytic processes which occurred in the dark control vessels

$t_{1/2\ LAB}$ = Photolysis half-life under the solar simulator

$t_{90\ LAB}$ = 90% photochemical degradation under the solar simulator

Table 38 Estimated photolytic rate constants and half-lives at Northern hemisphere latitudes

Latitude	$k_{pE\ (spring)}$ (day^{-1})	$t_{1/2\ (spring)}$ (day)	$k_{pE\ (summer)}$ (day^{-1})	$t_{1/2\ (summer)}$ (day)	$k_{pE\ (autumn)}$ (day^{-1})	$t_{1/2\ (autumn)}$ (day)	$k_{pE\ (winter)}$ (day^{-1})	$t_{1/2\ (winter)}$ (day)
50°N	0.065266	10.6	0.082823	8.4	0.034407	20.1	0.014286	48.5
40°N	0.072474	9.6	0.082789	8.4	0.044932	15.4	0.029038	23.9
30°N	0.076449	9.1	0.084319	8.2	0.056082	12.4	0.042875	16.2
20°N	0.078253	8.9	0.082762	8.4	0.064926	10.7	0.054850	12.6

Soil photolysis

The photolytic degradation of [phenyl-^{14}C]-tolclofos-methyl was studied on a sandy loam soil [Concha, *et al*., 1992, report QM-21-0036]. Soil treated at a rate of 17.9 kg ai/ha was incubated at natural sunlight (daily light energy of avg. 8.90 W.min/cm^2) and 24–25 °C. Soil layers contained moisture of 75% of the soil field moisture capacity. Soil samples were taken at 0, 3, 7, 18, 25 and 30 days after treatment. Volatiles were also collected. Soil samples were extracted with acetone/water (5:1, v/v). Radioactivity was determined by LSC or combustion/LSC. HPLC was used for analysis. Selected sample were further analysed by TLC and GC/MS.

In control and irradiated soils, mass balance was 88–101% and 91–102% of the applied radioactivity (AR), respectively. Extracted radioactivity was 86–101% AR in control soil and 89–101% AR in irradiated soil. CO_2 of 1% AR was generated at the study end in both soils. \

At the study end, tolclofos-methyl represented 75% AR in control and 83% AR in irradiated soil. In a supplementary test, it represented 90% AR in both soils. Metabolite SM-TM was found in irradiated soil at maximum 6.2% AR (day 30). In control soil, SM-TM was found at lower level, maximum 2.5% AR (day 30). DM-SM-TM was also found in both soils, at maximum 1.0% AR (day 25) in irradiated soil and maximum 7.6% AR (day 30) in control soil. Other degradation products were found in both soils (day 25 and 30), which did not exceed, in total, 4% AR. DT_{50} values of tolclofos-methyl in control and irradiated soils were 71.9 days and 113.1 days, respectively. The DT_{90} values in control and irradiated soils were 238.9 days and 376.0 days, respectively. The results indicated that photolysis is not a significant degradation pathway of tolclofos-methyl on soil.

Aerobic soil metabolism

Study 1

The aerobic metabolism and degradation of tolclofos-methyl was studied in thoursee soils for 112 days [Lewis, 1995, report QM-51-0042; Kodaka, *et al*., 1999, report QM-0046]. [phenyl-^{14}C]-Tolclofos-methyl was applied to the following soils at a rate of 2 kg ai/ha: Abington (clay loam), Shuttleworth (sandy loam) and Terling (silty clay lacom). The soils were incubated in the dark at 10 °C, with moisture content of 40% MWHC. Carbon dioxide-free air was drawn over the surface of the soils. Soil samples were taken at 0, 3, 7, 14, 28, 55, 84 and 112 days after treatment. In addition, Shuttleworth soil (sandy loam) was treated with [Phenyl-^{14}C]-tolclofos-methyl at a rate of 15 kg ai/ha and incubated in the dark and at moisture content of pF 2.5 (19.3% w/w; 40% MWHC) and at 15 °C. Soil samples were taken at 0, 7, 14, 28, 55 and 112 days after treatment. In all soil experiments, organic volatiles and $^{14}CO_2$ were collected over the incubation period.

Soil was Soxhlet extracted with acetone:glacial acetic acid (98:2, v/v). Radioactivity was determined by LSC or combustion/LSC. Unextracted residues (> 20% of the applied radioactivity) from Abington soil samples were further extracted with acetone: 0.5M HCl (5:1, v/v), as results, < 2% of the applied radioactivity was extracted, then not further analysed.

For Shuttleworth and Terling soils incubated at 10 °C, parent was choursomatographed with the extracts in the TLC analysis; other reference standards, not used. For Shuttleworth soil incubated at 15 °C, parent and other reference standards were used in the TLC analysis; further HPLC analysis was made for selected soil extracts (7, 28, 112 days after treatment).

In all four tests, mass balance was between 87 and 100% of the applied radioactivity (AR) over time (majority, > 90% AR). Extracted residues decreased from 95–100% (day 0) to 7–18% (day 112) of the AR. Unextracted residue increased from 4–19% (day 7) to 42–58% (day 112) of the AR. By the end of study, CO_2 was generated 20–44% of the AR (polar volatile degradates, < 0.3% of the AR in all samples).

In the soils, tolclofos-methyl was declined rapidly and steadily (93–98% AR at day 0; 4–8% AR at day 112), and metabolized to unextracted residues and CO_2. TLC and HPLC analyses for

Shuttleworth soil (15 °C incubation) showed up to 12 degradation products (in total, <5% AR; each <3% AR), six of which were ph-COOH, ph-CH_2OH, DM-TM, TM-COOH, TMO and DM-TMO.

DT_{50} values of tolclofos-methyl in aerobic soils, based on single first-order kinetics, were 28 days in Abington soil, 23 days in Shuttleworth soils (incubated at 10 °C and 15 °C) and 30 days in Terling soil. DT_{90} values were 93 days in Abington soil, 77 days in Shuttleworth soils (incubated at 10 °C and 15 °C) 100 days in Terling soil.

Study 2

Degradation of [phenyl-^{14}C]-tolclofos-methyl was investigated in four soils under an aerobic condition [Lewis, 2001b, report QM-0049]. The radiolabelled test substance was applied to the following four soils at a rate of 2 kg ai/ha: PT102 (sandy loam), PT103 (sandy loam), SK15556090 (clay loam) and SK960087 (clay loam); UK textural class was applied. The soils were maintained in the dark and at 20 °C, with moisture content of 45% MWHC. Carbon dioxide-free air was drawn over the surface of the soils. Soil samples were taken at 1, 3, 7, 15, 30, 62 and 90 days after treatment. Organic volatiles and $^{14}CO_2$ were also collected over the incubation period. Residues in soil were extracted by Soxhlet extraction with acetone:glacial acetic acid (98:2, v/v). Extracts were analysed by HPLC. Confirmation of identity for found metabolites were by TLC. Radioactivity was determined by LSC or combustion/LSC. Unextracted residues from the four soil samples with the highest level were fractionated into humin, humic acids and fulvic acids. The study results are shown in Tables 39 and 40.

Mass balance was 91–101% of the applied radioactivity (AR) in all samples. Radioactivity extracted from soils decreased from 97–100% AR (day 0) to 3% AR (day 90). Unextracted radioactivity increased from 1% AR (day 0) to 49–64% AR (day 30) and then slightly decreased to 45–56% AR (day 90). The unextracted radioactivity (49–64% AR) was fractionated into fulvic acids at 13–23% AR, humic acids at 9–25% AR and humin at 17–26% AR. By the end of study, CO_2 was generated 37–43% of the AR (polar volatile degradates, < 0.5% AR in all samples).

Table 39 Degradation rates of [^{14}C]-tolclofos-methyl in different soils

	Soil			
	PT 102	PT 103	SK 15556090	SK 960087
DT_{50} (days)	3.1	2.0	5.3	5.4
DT_{90} (days)	15.0	6.9	19.5	20.1
Correlation coefficient R^2	0.996	0.992	0.999	0.996
C_0 (%)	98.2	99.7	98.9	95.9

Based on a two phase exponential model

Table 40 Accumulation and degradation of DM-TM and ph-CH_3 in soil treated with [^{14}C]-tolclofos-methyl

	Soil			
	PT 102	PT 103	SK 15556090	SK 960087
DM-TM				
DT_{50} (days)	*	6.1	7.4	*
DT_{90} (days)	*	14.0	17.1	*
Correlation coefficient R^2	*	0.969	0.973	*
C_{max} [%]	1.1*	12.8	3.6	0.6*
t_{max} (days)	1*	3.7	4.4	15*
Ph-CH_3				
DT_{50} (days)	*	6.9	9.5	10.2
DT_{90} (days)	*	16.7	22.8	23.9
Correlation coefficient R^2	*	0.983	0.915	0.981
C_{max} (%)	1.3*	7.9	2.8	4.4
t_{max} (days)	3*	4.0	5.3	6.1

Based on a single phase exponential decline model with accumulation phase

* concentrations too low for computer modelling. C_{max} and t_{max} are the observed values, not calculated

In the four soils, tolclofos-methyl declined from 96–100% AR (day 1) to 1–3% AR by the end of the study. Metabolite DM-TM was found at a maximum 13% AR (day 3) in soil PT103, and at maximum 0.6–4% AR levels in the other thoursee soils. ph-CH_3 was found, at lower levels, at a maximum 8% AR (day 3) in soil PT103, and at maximum 1–4% AR levels in the other thoursee soils. In all four soils, unknown metabolites did not exceed 2% of the applied radioactivity. The DT_{50} and DT_{90} values of TM, DM-TM and ph-CH_3 were calculated as shown in Tables 39 and 40.

In aerobic soils, tolclofos-methyl was rapidly degraded and metabolized to carbon dioxide. The degradation products DM-TM and ph-CH_3 were also rapidly degraded. Bound residues decreased toward the end of the study and the final product was carbon dioxide. In the four soils, the DT_{50} values of TM, DM-TM and ph-CH_3 were as follows:

DT_{50} values (days): 2.0, 3.1, 5.3 and 5.4 for TM; 6.1 and 7.4 for DM-TM; 6.9, 9.5 and 10.2 for ph-CH_3

DT_{90} values (days): 6.9, 15.0, 19.5 and 20.1 for TM; 14.0 and 17.1 for DM-TM; 16.7, 22.8 and 23.9 for ph-CH_3

Study 3

Degradation of [^{14}C]-tolclofos-methyl was investigated in four soils under an aerobic condition [Graham and Strachan, 2008, report QM-0065]. The radiolabelled test substance was applied to the following four soils at a rate of 16 kg ai/ha: PT102 (sandy loam), TL78517228 (sand), SK920191 (loam) and SK104691(silt loam); USDA classification was applied. The soils were maintained in the dark and at 20 °C, with mean of water holding capacity at pF 2 and pF 2.5 (WHC 5.7-33.5% at pF 2.5). Carbon dioxide-free air was drawn over the surface of the soils.

In two soils of PT102 and SK920191, samples were taken at 0, 3, 7, 14, 28 and 56 days after treatment. For soil TL78517228, soil sample of 91 days was added and for SK104691, soil samples of 91 days and 120 days. Organic volatiles and $^{14}CO_2$ were also collected over the incubation period. Residues in soil were extracted with acetone:glacial acetic acid (98:2, v/v) by Soxhlet extraction. Extracts (for extract including ≥ 5% of applied radioactivity) were analysed by HPLC. Confirmation of identity for found metabolites was by TLC. Radioactivity was determined by LSC or combustion/LSC. Unextracted residues from the four soil samples (study end) were fractionated into humin, humic acids and fulvic acids.

Table 41 Degradation rates of [^{14}C]-tolclofos-methyl in different soils

Soil	Kinetics	DT_{50} (days)	DT_{75} (days)	DT_{90} (days)	X^2 error
PT102	FOMC	6.5	15.1	30.7	1.50
TL78517228	SFO	19.4	38.8	64.5	4.05
SK920191	DFOP	6.3	25.3	39.0	7.10
SK104691	SFO	15.3	30.5	50.7	2.87

SFO, single first order model; FOMC, first order multi-compartment; DFOP, double first order in parallel

Mass balance was 94–102% of the applied radioactivity (AR) in all samples. Radioactivity extracted from soils decreased from 101–102% AR (day 0) to 4–6% AR (study end). Unextracted radioactivity increased to 48–63% AR (study end), which were fractionated into fulvic acids (10–16% AR), humic acids (8–24% AR) and humin (18–32% AR). By the end of study, CO_2 was generated 27–38% of the AR (other volatile organics, not detected in all samples).

Tolclofos-methyl declined from 100–101% AR (day 0) to 3–4% AR by the end of the study. No metabolites were observed to be present at ≥5% AR with the exception of ph-CH_3, which was detected at 7% AR in soil TL 78571228 at 28 days (one replicate, 10% AR; the other replicate, 3% AR). Besides ph-CH_3 (detected in 4 soils), DM-TM was detected in four soils at ≤4% AR. DM-TMO

and TM-COOH were detected in thoursee and one soils, respectively, at ≤ 1% AR. The DT_{50} and DT_{90} values of TM were calculated, shown in Table 41.

In aerobic soil, tolclofos-methyl was rapidly degraded and metabolized to carbon dioxide. Metabolites ph-CH_3, DM-TM, DM-TMO and TM-COOH were observed but at low levels. The DT_{50} values of tolclofos-methyl were 6.3, 6.5, 15.3 and 19.4 days, and the DT_{90} values were 30.7, 39.0, 50.7 and 64.5 days. A proposed metabolic pathway is shown in Figure 5 below.

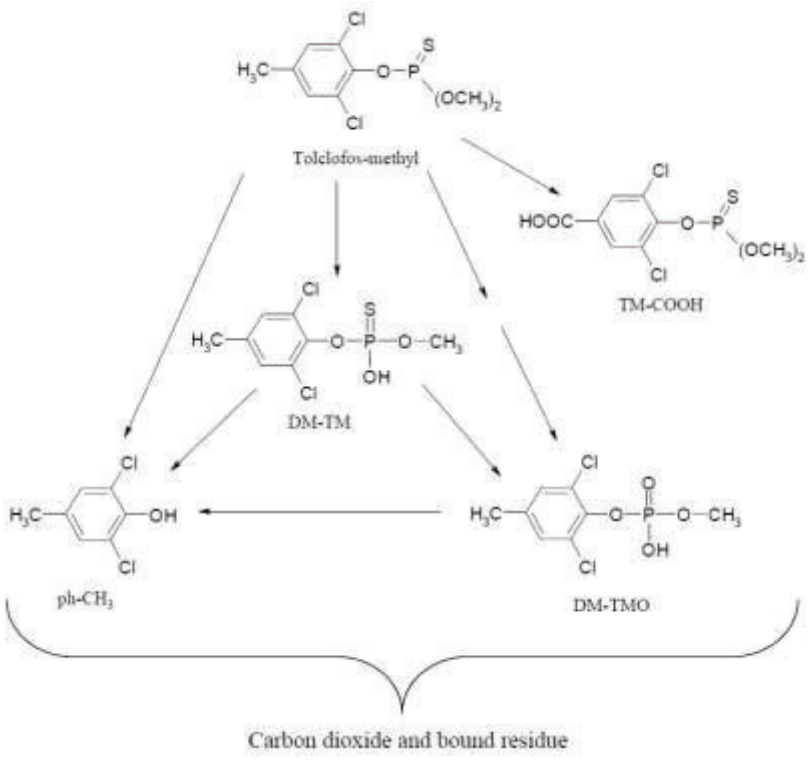


Figure 5 Proposed degradation pathway of tolclofos-methyl in aerobic soil

METHODS OF RESIDUE ANALYSIS

Analytical methods

Analytical methods have been developed for the determination of tolclofos-methyl in plant and animal commodities (Table 42). In addition, radiovalidation data was provided.

Table 42 Overview of analytical methods for tolclofos-methyl

Method: Report No.	Matrix	Extraction	Clean-up	Detection	LOQ
QA-0063	Potato	Dichloromethane/methanol (9:1, v/v)	Partitioning with 10% NaCl solution, silica gel column	GC-TSD	0.02
QA-31-0062	Potato	Soxhlet extraction with acetone	Partitioning with hexane, silica cartridge	GC-NPD	0.05
QR-0119	Potato	Hexane/acetone (1:1, v/v)	Partitioning with 5% NaCl solution	GC-FPD	0.02
QR-31-0104	Potato	Water/methanol/acetonitrile (2:1:4, v/v)	Partitioning with dichloromethane, florisil cartridge	GC-ECD	0.01
QR-0126	Lettuce	Acetone	Partitioning with dichloromethane	GC-ECD	0.01
QA-0070	Potato	Acetone	Partitioning with ethyl acetate/cyclohexane (1:1, v/v), gel permeation choursomatography	GC-FPD	0.01

Method: Report No.	Matrix	Extraction	Clean-up	Detection	LOQ
QA-0091 Multi-residue method	Lettuce, Orange, cotton seed, dried bean	QuEChERS		LC-MS/MS	0.01
QA-0098: ILV to QA-0091	Lettuce, cotton seed	QuEChERS		LC-MS/MS	0.01
QA-0093 Multi-residue method	Milk, meat, liver, egg, fat	QuEChERS		LC-MS/MS	0.01
QA-0100 ILV to QA-0093	Liver, fat	QuEChERS		LC-MS/MS	0.01

Plant commodities

Method in the study QA-0063

Potatoes were homogenised and extracted with dichloromethane/methanol (9:1, v/v) [Cron, 1982b, report QA-0063]. The extract was partitioned with 10% aqueous sodium chloride and purified using a silica gel column. GC-TSD (thermionic specific detector) was used for determination of TM. The detector response was linear over 0.05-2 µg/mL. Limit of determination and LOQ values for TM were 0.002 mg/kg and 0.02 mg/kg, respectively. This method was used in residue trial studies, QR-01-0068 and QR-21-0069.

Method in the study QA-31-0062

Homogenised potatoes were extracted with acetone by Soxhlet extraction [Burden, 1994d, report QA-31-0062]. Extract was partitioned with hexane and then cleaned-up using a pre-packed silica cartridge. For determination of TM, GC-NPD was used. The linearity of the detector response and specificity were acceptable. The LOQ for TM was 0.05 mg/kg. This method was used in residue trial studies, QR-41-0101, QR-41-0102 and QR-41-0103.

Methods used in residue trial studies

QR-0119

Homogenised potatoes were extracted with hexane/acetone (1:1, v/v) [Lindner and Giesau, 2013, report QR-0119]. Extract was partitioned with 5% aqueous sodium chloride. TM was determined by GC-FPD. The linearity of the detector response and specificity were acceptable. The LOQ for TM was 0.02 mg/kg.

QR-31-0104

Homogenised potatoes were extracted with water/methanol/acetonitrile (2:1:4, v/v) [Holmgaard, 1994, report QR-31-0104]. Extract was partitioned with dichloromethane and sodium chloride, cleaned up using florisil cartridge, and determined using GC-ECD. The LOQ for TM was 0.01 mg/kg.

QR-0126

Homogenised lettuce were extracted with acetone [Pigeon, 2001, report QR-0126]. Extract was partitioned with dichloromethane. GC-ECD was used for determination of TM. The linearity of the detector response and specificity were acceptable. The LOQ for TM was 0.01 mg/kg in lettuce matrix.

Table 43 Validation of single-residue analytical methods for tolclofos-methyl in plant commodities

Reference	Matrix	Fortification level (mg/kg)	n	Recovery (%)		RSD (%)	LOQ
				Range	Mean		
QA-0063	Potato	0.02	5	70-104	87	18	0.02
		0.04	6	63, 67, 71-85 (n = 4)	75	12	
		0.06	3	84-97	89	8	
		0.08	1	-	85	-	
		0.10	3	96-98	97	1	
		0.20	2	81-90	86		
QA-31-0062	Potato	0.05	3	75-100	86	15	0.05
		0.25	3	88-100	95	7	
		0.5	3	91-105	97	8	
QR-0119*	Potato	0.02	2	97-98	98	-	0.02
		0.1	2	101	101	-	
QR-31-0104 *	Potato	0.01	6	70-118	91	23	0.01
		0.1	2	81-96	89	-	
		0.5	3	98-105	102	4	
QR-0126	Lettuce	0.01	5	63, 96-107 (n = 4)	92	18	0.01
		0.5	5	81- 98	90	7	

*Procedural recoveries

Multi-residue method

Method in the study QA-0070 (validation for the method DFG S 19 extended version)

Potato (tuber) were homogenised and then stored deep frozen [Pelz and Weeren, 2000, report QA-0070]. The samples were extracted with acetone, partitioned with ethyl acetate/cyclohexane (1:1, v/v), cleaned-up by gel permeation choursomatography. GC-FPD was used for determination of TM. The linearity of the detector response covered the working range of 0.0101 to 2.03 µg/mL (n = 9; R^2=≥ 0.99). Specificity was acceptable. The LOQ for TM was 0.01 mg/kg in potato tuber matrix. This method was used in residue trial studies, QR-0136, QR-41-0107, QR-0123 and QR-0125.

Table 44 Validation of multi-residue analytical methods for tolclofos-methyl in plant commodities

Reference	Matrix	Fortification level (mg/kg)	Mass transitions (m/z)	n	Recovery (%)		RSD (%)	LOQ
					Range	Mean		
QA-0070	Potato	0.01	not related	5	87-98	91	5	0.01
		0.1	not related	5	85-89	87	2	
QA-0091	Lettuce	0.01	301→175	5	98-102	100	2	0.01
		0.01	301→125	5	93-98	95	2	
		0.1	301→175	5	90-95	92	2	
		0.1	301→125	5	92-93	93	0.5	
	Orange (whole)	0.01	301→175	5	88-95	91	4	0.01
		0.01	301→125	5	96-103	99	3	
		0.1	301→175	5	95-99	97	2	
		0.1	301→125	5	90-94	92	2	
	Cotton seeds	0.01	301→175	5	81-93	87	5	0.01
		0.01	301→125	5	84-94	88	6	
		0.1	301→175	5	75-80	78	3	
		0.1	301→125	5	75-78	77	2	
	Dried	0.01	301→175	5	93-102	97	3	0.01

Reference	Matrix	Fortification level (mg/kg)	Mass transitions (m/z)	n	Recovery (%)		RSD (%)	LOQ
					Range	Mean		
	beans	0.01	301→125	5	80-95	86	7	
		0.1	301→175	5	74-92	87	9	
		0.1	301→125	5	74-93	86	9	

Method in the study QA-0091

Specimens of head lettuce, orange (whole fruit), cotton seed and dried beans were homogenised with dry ice [Lindner and Giesau, 2013, report QA-0091]. For extraction of residues and clean-up, QuEChERS sample preparation technique was used: extraction with acetonitrile, centrifugation after addition of citrate buffered salt mixture and clean-up by dispersive solid phase extraction using primary secondary amines (PSA). LC-MS/MS (electrospray positive ion mode) was used. Two mass transitions for TM determinations were 301 *m/z*→175 *m/z* and 301 *m/z*→125 *m/z* (used interchangeably for quantitation and confirmation). The linearity of the detector response in the working range of 0.06–6.0 ng/mL pure solvent solution was confirmed. Specificity was acceptable. No significant matrix effects of above 17% were detected. The LOQ for TM was 0.01 mg/kg in the all matrices. This method was used in residue trial study, QR-0293.

Study QA-0098: ILV to the method in the study QA-0091

Independent laboratory validation for the method in the study QA-0091 was conducted [De Vos, 2014, QA-0098]. Minor modifications in LC-MS/MS analysis were employed: use of mass transition 303 m/z→125 m/z for confirmation (for quantification, 301 m/z→125 m/z) and use of matrix-matched standards (matrix effects, observed). The LOQ for TM was 0.01 mg/kg for lettuce and cotton seed matrices. The method in the study QA-0091 was considered independently validated and acceptable for determination of TM in foodstuff of plant origin.

Table 45 Independent laboratory validation of analytical method in the study QA-0091: study QA-0098

Matrix	Fortification level (mg/kg)	n	*m/z* 301→125 (quantification)			*m/z* 303→125 (confirmation)		
			Recovery (%)		RSD (%)	Recovery (%)		RSD (%)
			Range	Mean		Range	Mean	
Lettuce, head	0.01	5	97-108	102	4	91-115	104	8
	0.1	5	90-98	94	4	89-99	96	4
Cotton seeds	0.01	5	70-98	81	13	94-98	96	2
	0.1	5	84-96	91	5	89-97	92	3

Animal commodities

Regarding the analysis of tolclofos-methyl in animal matrices, one multi-residue method along with its ILV was provided. In addition, one radiovalidation study was provided.

Method in the study QA-0093

The analytical method was derived from the QuEChERS (EN 15662) multi-residue method [Richter, 2013, report QA-0093]. Homogenized specimens (bovine meat and liver, eggs, fat) were extracted with acetonitrile. After addition of $MgSO_4$, NaCl and buffering citrate salts, the mixture was shaken intensively and centrifuged for phase separation. After freezing out fat, an aliquot of the organic extract was cleaned-up by dispersive SPE with PSA and $MgSO_4$. Determination was made by LC-

MS/MS in the positive ionization mode monitoring the *m/z* 301 parent ion and its daughter ions, i.e. *m/z* 175 and *m/z* 111, in addition, *m/z* 303 parent ion of TM with its *m/z* 113 daughter ion.

The method was highly specific. Good linearity ($R^2 \geq 0.99$) was observed in the range of 0.1–10.0 ng/mL standard solutions for milk, meat, egg and liver and 0.075-10 ng/mL matrix-matched standards for fat (matrix effects observed). The LOQ for TM was 0.01 mg/kg in all matrices.

Table 46 Validation of analytical method for tolclofos-methyl in animal commodities: method in the study QA-0093 (enforcement)

Matrix	Fortification level (mg/kg)	n	Recovery of TM, %								
			m/z 301→175			*m/z* 301→111			*m/z* 303→113		
			Range	Mean	RSD	Range	Mean	RSD	Range	Mean	RSD
Milk	0.01	5	86-93	88	4	81-91	84	5			
	0.1	5	85-91	88	3	84-89	87	3			
Meat	0.01	5	79-85	82	3	78-83	80	3			
	0.1	5	80-85	83	2	78-83	80	3			
Liver	0.01	5				87-89	88	1	88-89	88	1
	0.1	5				90-92	92	1	88-92	90	1
Egg	0.01	5				87-90	88	1	85-89	87	2
	0.1	5				90-93	91	1	87-90	89	1
Fat	0.01	5				78-83	81	3	80-84	82	3
	0.1	5				80-86	83	3	82-88	84	3

m/z 301→175, for quantification in milk and meat

m/z 301→111, for quantification in liver, egg and fat and for confirmation in milk and meat

m/z 303→113, for confirmation in liver, egg and fat

Study QA-0100: ILV to the method in the study QA 0093

Independent laboratory validation to the method in the study QA-0093 was conducted using matrices of bovine liver and fat [Weir, 2014, report QA-0100]. In this validation study, matrix-matched standards were used for liver sample as well as fat. The LOQ for TM was 0.01 mg/kg in the matrices. The method in the study QA-0093 was considered independently validated and acceptable for determination of TM in animal matrices.

Table 47 ILV of analytical method in the study QA-0093: Study QA-0100

Matrix	Fortification level (mg/kg)	n	Recovery of TM, %					
			m/z 301→111 (Quantification)			*m/z* 303→113 (Confirmation)		
			Range	Mean	RSD	Range	Mean	RSD
Liver	0.01	5	94-103	99	3	88-105	94	7
	0.1	5	92-96	94	2	92-99	95	3
Fat	0.01	5	71-87	79	8	76-90	84	7
	0.1	5	67, 78-82 (n = 4)	78	8	66-80	75	7

Radiovalidation study

A radiovalidation study on extraction efficiency in analysis of tolclofos-methyl was conducted [Townley, 2015, report QA-0104]. Analytical matrices with [phenyl-^{14}C]-tolclofos-methyl were goat liver, hen's egg yolk and fat samples, which were obtained from the metabolism studies [Burri, 2014b, report QM-0073] and [Burri, 2014a, report QM-0075]. Samples were extracted using the QuEChERS method [Richter, 2013, report QA-0093] and the extract (supernatant) was analysed by TLC.

Re-analysis was made according to the metabolism study methods. For goat liver, radioactive residues were extracted (each 1–3×) with acetonitrile/water, methanol/water, acidic and basic acetonitrile/water, basic acetonitrile/water and Soxhlet extraction with methanol (acidic and basic conditions of pH and temperature, not reported). The extract was partitioned with hexane after adding acetonitrile. The acetonitrile/water phase was further partitioned with (each 2–4×) dichloromethane, ethyl acetate and, at acidic and basic condition, dichloromethane (acid and base conditions of pH and temperature, not reported). The organic phase was analysed by TLC. For hen fat, radioactive residues were extracted (each 2–3×) with dichloromethane and ethyl acetate. The extract was partitioned four times with acetonitrile after dissolving in hexane. The acetonitrile phase was analysed by TLC. For egg yolk, radioactive residues were extracted (each 1–3×) with acetone, methanol, acetonitrile, acetonitrile/water and Soxhlet extraction with methanol. The extract was partitioned with (each 2–3×) dichloromethane at neutral, acidic, basic condition (acid and base conditions of pH and temperature, not reported) and diethyl ether. The organic phase (CH_2Cl_2/ethyl acetate) was cleaned-up by partitioning with hexane after adding acetonitrile. The acetonitrile phase was analysed by TLC. Radioactivity was determined by LSC. The result is shown in Table 48.

In re-analysis, TRR values were overall lower than those of the original study. When QuEChERS method was applied, amounts of tolclofos-methyl in the animal matrices were greater (>131%) than those obtained by re-analysis. Therefore, the extraction efficiencies of tolclofos-methyl by QuEChERS method were considered comparable with those of metabolism studies.

Table 48 Extraction efficiency of radioactive residues by QuEChERS preparation technique

Sample[a] (TRR, mg eq/kg)	Original metabolism study			Re-analysis			Use of QuEChERS		
	TLC analysis[b] (% TRR)	TM (% TRR)	Ph -COOH (% TRR)	TLC analysis[b] (% TRR)	TM (% TRR)	Ph -COOH (% TRR)	TLC analysis[b] (% TRR)	TM (% TRR)	Ph -COOH (% TRR)
Goat liver (0.25)	38.1[c]	4.4	10.2	18.6[*]	nd	4.4-14.2	9.6	0.2-1.2	2.4-5.1
Hen egg yolk (0.058)	55.2	35.1	nd	25.1	3.1-7.4	nd	30.1	6.4-9.8 (159)	nd-1.1
Hen fat (0.045)	89.0	75.9	3.7	52.9	38.6-50.5	nd-2.0	60.9	56.6-59.9 (131)	nd

[a] Samples obtained from metabolism studies QM-0073 (2014b) and QM-0075 (2014a)

[b] Radioactivity subjected to TLC

[c] Hexane layer, included

Hyphen, range of results obtained from TLC 3 solvent systems

Value in parenthesis means a ratio (%) of tolclofos-methyl (% TRR) obtained by QuEChERS method over re-analysis

Storage of pesticide residues in stored analytical samples

Study 1

Lettuce head were cut and homogenised prior to fortification. The homogenised samples were fortified with tolclofos-methyl in acetone at a fortification at a level of 0.1 mg/kg [Anspach and Pelz, 2002, report QR-0127]. After fortification, the samples were stored at ≤-18 °C until analysis. Samples were analysed immediately (day 0) and at regular intervals of 3, 6, 12 and up to 18 months after freezing. Tolclofos-methyl was measured by the analytical method of the study QA-0070 (DFG Method S 19 extended version). The results are shown in Table 49. Recoveries of the stored samples were 79–103% and the procedural recoveries were 83–99%. The study showed that tolclofos-methyl is stable in lettuce up to 18 months under frozen conditions (≤-18 °C).

Table 49 Tolclofos-methyl recoveries in frozen lettuce samples

Storage interval (months)	Fortification level (mg/kg)	After frozen storage		Procedural recovery (%)
		Concentration (mg/kg)	Percent remaining	
0	0.1	0.0895, 0.0897	90, 90	95
3	0.1	0.0919, 0.0946	92, 95	99
6	0.1	0.0868, 0.0860	87, 87	83
12	0.1	0.0786, 0.0871	79, 87	94
18	0.1	0.1028, 0.1012	103, 101	91

Study 2

Homogenised potato samples were fortified with tolclofos-methyl at a concentration of 0.50 mg/kg and then stored in a freezer (ca. -18 °C) until analysis [Burden, 1996, QR-0122]. Samples were analysed immediately (day 0) and at regular intervals of 3, 6, 12 and up to 22 months after freezing. Tolclofos-methyl was measured by the analytical method of the study QA-31-0062. The results are shown in Table 50. Recoveries of the stored samples were 86–108% and the procedural recoveries were 88–104%. The study showed that tolclofos-methyl is stable in potatoes up to 22 months under frozen conditions (≤-18 °C).

Table 50 Tolclofos-methyl recoveries in frozen potato samples

Storage interval (months)	Fortification level (mg/kg)	After frozen storage		Procedural recovery (%)
		Concentration (mg/kg)	Percent remaining	
0	0.50	0.49, 0.51, 0.52	98, 102, 104	102, 103
3	0.50	0.50, 0.54, 0.53	100, 108, 106	97, 104
6	0.50	0.43, 0.51, 0.46	86, 102, 92	88, 92
12	0.50	0.48, 0.51, 0.48	96, 102, 96	92, 99
22	0.50	0.54, 0.45, 0.51	108, 90, 102	95, 98

USE PATTERN

Tolclofos-methyl is a non-systemic contact fungicide with preventive activity. Tolclofos-methyl inhibits phospholipid biosynthesis which leads to disturbance of the formation of cell membranes in the fungus. Germination of the spores and mycelium growth are slowed down and contamination of the plants via the roots and bottom of the stalk is prevented. The fungicide is used for control of damping-off/bottom rot (*Rhizoctonia solani*) in potatoes (black scurf, root rot, whiteleg or stem rot), lettuce, brassicas, radishes, tobacco, ornamental crops, and so on.

The Meeting received information on its registered use in Belgium, Germany, the Netherlands and Italy, as shown in Table 51.

Table 51 Registered uses of tolclofos-methyl on food crops

Crop	Country	Form.	Application					PHI (days)
			Method	Timing	No.	Water (L/ha)	Rate (kg ai/ha)	
Brassica vegetable seedbeds (protected)	NL	SC 500 g/L 42% w/w	Spray over the soil	Just before sowing	1		2	
Lettuce species and lamb's lettuce (protected)	BE	SC 500 g/L	Spray	The week following planting[a]; directly after sowing[b]	1		2	
Lettuce (*Lactuca* spp.) and lamb's lettuce (protected)	NL	SC 500 g/L	Spray	Up to one week after transplanting	1	1,000	2	4 or 8 weeks[c]
Lettuce and other salad greens (greenhouse)	IT	WP 500 g/kg	Spray	When transplanting	1	100-1,000	2	28

Crop	Country	Form.	Application					PHI (days)
			Method	Timing	No.	Water (L/ha)	Rate (kg ai/ha)	
Potato	BE	DS[d] 100 g/L 10% w/w	Seed treatment	Before planting or during planting	1		0.15 kg ai/t tubers	
Potato	DE	FS 250 g/L	Spray onto the seed potatoes	Before planting in the warehouse	1	[e]	0.15 kg ai/t tubers	
				When planting outdoor		80	0.15 kg ai/t tubers	
Potato	IT	WP 500 g/kg	Seed dressing	Before planting	1	165-200 L/t tubers	0.25 kg ai/t tubers	
Radish[f]	BE	SC 500 g/L	Spray	Directly after sowing	1		2.5	
Radish crops (protected)	NL	SC 500 g/L	Spray	Up to one week after sowing	1		2.5	

[a] For lettuce species [b] For lamb's lettuce

[c] 4 weeks during from 1 March to 1 November (summer lettuce) or 8 weeks during from1 November to 1 March (winter lettuce)

[d] The formulation can be applied manually on potatoes. However, a powder dosing dispenser mounted on the conveyor belt is recommended for distribution of the product on potatoes. The product can also be applied when planting potatoes with a powder dispenser mounted on the planter.

[e] Sprayed undiluted at the application rate of 60 mL/100 kg seed when using ULV application methods

[f] Including radish (open field and protected crops), horseradish, radish root (open field)

WP (wettable powder), SC (suspension concentrate), FS (flowable concentrate for seed treatment), DS (powder for dry seed treatment)

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

Supervised residue trial data on potato and lettuce were provided.

CODEX Group	Commodity	Table No.
013A Leafy greens	VL 0482 Lettuce, head	Table 52
	013H Baby leaves	Table 53
016B Tuberous and corm vegetables	VR 0589 Potato	Table 54

Lettuce

QR-0126

Eight residue trials were conducted in Belgium during the year of 2000. Four decline curve trials (two, summer lettuce; two, winter lettuce) and four at harvest trials (two, summer lettuce; two, winter lettuce) were carried out in lettuce growing in greenhouse. A single application was made at rates of 1.9-2.1 kg ai/ha with a spraying volume of about 1,000 L/ha, one week after planting (BBCH 17-20). The application was done on August for summer lettuce (trials 1, 3, 5, 7) and on October for winter lettuce (trials 2, 4, 6, 8). Lettuce was harvested 27 or 28 days (0, 14, 27 or 28 days in decline trials) for summer lettuce, and 53 or 56 days (0, 42, 56 days in decline trials) for winter lettuce. Tolclofos-methyl was analysed by the analytical method in the study QR-0126, described in residue analysis section, sufficiently validated. Procedural recoveries were satisfactory. Samples were frozen stored to analysis for maximum 180 days.

QR-0136

Four trials were conducted in France (1 trial) and Italy (3 trials) in the year of 2005. Two decline curve trials and two at harvest trials were carried out in lettuce (head-forming cultivars) growing in non-heated glasshouse. Protected lettuces were transplanted at BBCH 12 to 16 (2 to 6 true leaves) and a single application was made 7-8 days after transplanting at rates of 2.0-2.1 kg ai/ha (spraying volume, about 1,500 L/ha). Lettuce was harvested 28 days (0, 14, 26 or 27 days in decline trials). Tolclofos-methyl was analysed by the analytical method in the study QA-0070, described in the residue analysis section, sufficiently validated. Procedural recoveries were satisfactory. Samples were frozen stored for maximum 61 days until analysis.

Table 52 Residues of tolclofos-methyl following spraying on lettuce in greenhouse

Country, Year Location (Variety) Trial ID	Application					DAT	Portion analysed	TM (mg/kg)
	Form.	Method	Growth stage	No.	Rate (kg ai/ha)			
GAP: IT			transplanting	1	2	28		
Belgium, 2000 Kruishoutem (Flandria) Summer lettuce QR-0126/20079/1a	SC 500 g/L	Spraying	BBCH 18-19	1	2.1	27	head (leaves)	0.39
Belgium, 2000 Kruishoutem (Brigade) Winter lettuce QR-0126/20079/2b	SC 500 g/L	Spraying	BBCH 19-20	1	2.1	53	head (leaves)	0.10
Belgium, 2000 Kruishoutem (Flandria) Summer lettuce QR-0126/20079/3a	SC 500 g/L	Spraying	BBCH 18-19	1	2.0	0	head (leaves)	182
						14	head (leaves)	1.3
						27	head (leaves)	0.25
Belgium, 2000 Kruishoutem (Brigade) Winter lettuce QR-0126//20079/4b	SC 500 g/L	Spraying	BBCH 18-19	1	2.0	0	head (leaves)	201
						42	Head (leaves)	1.3
						56	head (leaves)	0.41
						70	head (leaves)	0.17
Belgium, 2000 Sint-Katelijne-Waver (Flandria) Summer lettuce QR-0126/20079/5c	SC 500 g/L	Spraying	BBCH 18-19	1	2.2	28	head (leaves)	0.24
Belgium, 2000 Sint-Katelijne-Waver (Brigade) Winter lettuce QR-0126/20079/6d	SC 500 g/L	Spraying	BBCH 17	1	2.1	56	head (leaves)	0.09
Belgium, 2000 Sint-Katelijne-Waver (Rex) Summer lettuce QR-0126/20079/7c		Spraying	BBCH 18-19	1	1.9	0	head (leaves)	213
						14	head (leaves)	0.98
						28	head (leaves)	0.23

Country, Year Location (Variety) Trial ID	Application					DAT	Portion analysed	TM (mg/kg)
	Form.	Method	Growth stage	No.	Rate (kg ai/ha)			
Belgium, 2000 Sint-Katelijne-Waver (Troubadour)Winter lettuce QR-0126/20079/8d	SC 500 g/L	Spraying	BBCH 17	1	2.0	0	head (leaves)	149
						42	head (leaves)	0.14
						56	head (leaves)	0.06
France, 2005 Villetelle Languedoc-Roussilon (Ceralexan) QR-0136/X-05-080-712 FR01	WP 500 g/kg	Foliar appl.	BBCH 16	1	2.1	27	head	0.18
Italy, 2005 Sealza di Vintimiglia Liguria (Canasta) QR-0136/X-05-080-712 IT01*	WP 500 g/kg	Foliar appl.	BBCH 14-16	1	2.0	26	Head (leaves)	0.04
Italy, 2005 Dolceaqua Liguria (Canasta) QR-0136/X-05-080-712 IT02*	WP 500 g/kg	Foliar appl.	BBCH 14-16	1	2.0	28	Head (leaves)	0.08
Italy, 2005 Ceriale Liguria (Dinana) QR-0136/X-05-080-712 IT03	WP 500 g/kg	Foliar appl.	BBCH 12-14	1	2.0	28	Head (leaves)	0.16

QR-0126

Application, made one week after planting

[a, b, c, d] Not independent trials, the same site and the same or close application time

QR-0136

Application, application made 7-8 days after transplanting (BBCH 12-16: 2 to 6 true leaves)

* Not independent trials, close site and application time (8 days apart)

Table 53 Residues of tolclofos-methyl following spraying on baby leaves in greenhouse

Country, Year Location (Variety) Trial ID	Application					DAT	Portion analysed	TM (mg/kg)
	Form.	Method	Growth stage	No.	Rate (kg ai/ha)			
France, 2005 Villetelle Languedoc-Roussilon (Ceralexan) QR-0136/X-05-080-712 FR01	WP 500 g/kg	Foliar appl.	BBCH 16	1	2.1	0	immature plant w/o roots	198
						14	immature plant w/o roots	4.3
Italy, 2005 Sealza di Vintimiglia Liguria (Canasta) QR-0136/X-05-080-712 IT01*	WP 500 g/kg	Foliar appl.	BBCH 14-16	1	2.0	0	immature plant w/o roots	133
						14	immature plant w/o roots	0.42

Potato

QR-01-0068

Eleven trials were conducted in the UK during 1980 and 1981. Applications were done in the following thoursee ways: dust application (DP formulation at 0.25 kg ai/t tuber) or spray application (EC, WP, SC formulation at 0.06–0.5 kg ai/t tuber) onto tubers before planting; spray application (WP, EC, SC formulation) in-furrow at planting at 5 to 80 g ai/100 m row (0.5 to 8 kg ai/ha); spray application (EC at 0.13 kg ai/t tuber) onto tubers and in-furrow application at planting (20 g ai/100 row, 2 kg ai/ha). Potatoes were harvested at 110–196 days after treatment. Tolclofos-methyl was measured by the analytical method of the study QA-0063, described in the residue analysis section with validation data. Recoveries (overall mean recovery 85% at 0.02–0.20 mg/kg) were satisfactory. All residue values were corrected for the recovery (85%). Residues in control samples were < 0.01 mg/kg in all, but 3 cases. Samples were frozen stored for maximum 641 days until analysis.

QR-21-0069

Ten trials were conducted in the UK during the 1982 growing season. Seed potatoes were treated by hopper application of DP formulation except one trial (EC formulation), at rates of 0.13–0.25 kg ai/t tuber. Potato samples were harvested at 89–216 days after treatment. In two trials, flesh and peel factions were analysed. The weight ratio of flesh and peel were 66–83% (mean, 71%) and 17–34% (mean, 29%), respectively. Tolclofos-methyl was measured by the analytical method of the study QA-0063, described in the residue analysis section with validation data. Procedural recovery test results were satisfactory (overall mean recovery of 94%, n = 12, RSD 19%, at 0.02–0.20 mg/kg fortification levels in whole potato or flesh) except for a 0.02 mg/kg fortification level in whole potato (a recovery of 142%, n = 1). All residue values were corrected for the recovery (94%). Residues in control samples were < 0.01 mg/kg in all, but 2 trials (0.015, 0.016 mg/kg). Samples were frozen stored for maximum 364 days until analysis.

QR-61-0036, QR-61-38, QR-61-40, QR-61-42 and QR-61-44

Five trials (QR-61-0036, QR-61-38, QR-61-40, QR-61-42 and QR-61-44) were conducted in Germany during the 1986 growing season. Seed potatoes were treated once with SC formulation at rates of 0.15 kg ai/t tuber. Potatoes were harvested at 123–165 days after treatment. Tolclofos-methyl was determined by GC-NPD. Recovery was 95% and the limit of determination was 0.002 mg/kg. Other analytical information (extraction method) was not reported. A maximum storage period of the frozen samples was 153 days until analysis.

QR-0119

Two trials were conducted in the UK during the 1992 growing season. Seed potatoes were treated once with SC formulation at rates of 0.13 kg ai/t tuber. Potatoes were harvested at 84 days after treatment. Analytical method for tolclofos-methyl was described in the residue analysis section with validation data. A maximum storage period of the frozen samples was 62 days until analysis.

QR-31-0104

Two trials were conducted in Denmark during the 1993 growing season. Seed potatoes were treated once with SC formulation in one trial and DP formulation in the other trial. The application was made just pre-planting as a tuber dressing at a rate of 0.15 kg ai/t tuber. Potatoes were harvested 60, 109 and 148 days after treatment. Analytical method for tolclofos-methyl was described in the residue analysis section with validation data. A maximum storage period for the frozen samples until analysis was 206 days.

QR-41-0101

Four trials were conducted in the UK during the 1993 growing season. Two varieties were cultivated: early variety (Cleopatra and Dundrod) in two trials and maincrop variety (Desiree and Record) in the other two trials. Application on the seed potatoes was made once at planting with DP formulation at rates of 0.13 or 0.25 kg ai/t tuber. Harvest (daughter tuber) in thoursee trials was done 96–149 days after treatment. In the other one trial, immature daughter tubers were taken at 83 and 111 days and mature daughter tubers, at 156 days. Tolclofos-methyl was analysed by the method of the study QA-31-0062, described in the residue-analysis section with validation data. Procedural recoveries (corrected for control sample) were 80–101% (overall mean, 94%). Residue values above LOQ (0.05 mg/kg) were corrected for the overall mean recovery (94%). A maximum storage period of the frozen samples was 219 days until analysis.

QR-41-0102

Two trials were conducted in the UK during the 1993 growing season. Application on the seed potatoes was made once at planting or pre-planting (17 days) with SC formulation at rates of 0.13 or 0.063 kg ai/t tuber. Daughter tubers were harvested 0–155 days after treatment. Tolclofos-methyl was analysed by the method of the study QA-31-0062, described in the residue-analysis section with validation data. Procedural recoveries (corrected for control sample) were 70–103% (overall mean, 91%). Residue values above LOQ (0.05 mg/kg) were corrected for the overall mean recovery (91%). A maximum storage period of the frozen samples was 386 days until analysis.

QR-41-0103

Two trials were conducted in the UK during the 1993 growing season. Application on the seed potatoes was made once at planting with SC formulation at rates of 0.13 kg ai/t tuber. Immature daughter tubers were harvested 84 days after treatment. Parent tubers (0 days) were also taken. Analysis of tolclofos-methyl was done by the method of the study QA-31-0062, described in the residue-analysis section with validation data. Procedural recoveries, corrected for control sample, were 70–103% (overall mean, 91%). Residue values above LOQ (0.05 mg/kg) were corrected for the overall mean recovery (91%). A maximum storage period of the frozen samples was 386 days until analysis.

QR-41-0107

Two trials were conducted in Germany during the 1993 growing season. Application on the seed potatoes was made once at rates of 0.14 (SC form.) or 0.20 (DP form) kg ai/t tuber. Daughter tubers were harvested 149 days after treatment. Tolclofos-methyl was analysed by the method of the study QA-0070 (multi-residue method), described in the residue-analysis section, sufficiently validated. Procedural recovery test was not carried out. In the supplementary experiment, recoveries were 109 and 111%. Residue values were not corrected with the recoveries. In control samples, residue values did not exceed the LOQ, 0.02 mg/kg. A maximum storage period of the frozen samples was 196 days until analysis.

QR-0125

Six trials were conducted in the UK during the 2000 growing season. A seed dressing application (SC formulation) to tubers was made once prior to planting (a few hours or the day before planting), at a rate of 0.13 kg ai/t tuber in an ultralow spray volume of 2 liters/t tuber. Potato tubers were collected on commercial harvest date, 80–95 days after application. Tolclofos-methyl was analysed by the method of the study QA-0070 (multi-residue method), described in the residue-analysis section, sufficiently validated. Procedural recoveries were satisfactory. A maximum storage period of the frozen samples was 198 days until analysis.

QR-0123

Six trials were conducted in France (Northern, 2 trials), Italy (2 trials) and Greece (2 trials) during the 2001 growing season. A seed dressing application (SC formulation) to tubers was made once 11-29 days before planting, at rates of 0.22-0.26 kg ai/t tuber in an ultralow spray volume of 2 liters/t tuber. Potato tubers were collected on commercial harvest date, 96-125 days after application. Tolclofos-methyl was measured by the method of the study QA-0070 (multi-residue method), described in the residue-analysis section (sufficiently validated). Procedural recoveries were satisfactory. A maximum storage period of the frozen samples was 172 days until analysis.

QR-0293

Four trials were conducted in France (1 trial), Spain (2 trials) and Italy (1 trial) during the 2013 growing season. Application with SC formulation was made once on potato tubers at BBCH 01 (early varieties), 3–14 days before planting. Application was made at a rate of 0.25 kg ai/t tuber by misting equipment mounted over roller table with spray volume of ca. 2 litres/t tuber. Potato tubers were collected on commercial harvest date, 81–87 days after planting. Tolclofos-methyl was measured by the method of the study QA-0091 (multi-residue method), described in the residue-analysis section, sufficiently validated. Procedural recoveries were satisfactory. A maximum storage period of the frozen samples was 41 days until analysis.

Table 54 Residues of tolclofos-methyl following treatment on potato tuber

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
GAP: IT		Seed dressing	1	0.25			
UK, 1980 Shelford (Pentland Crown and King Edward) QR-01-0068/Shelford II	50% WP	Seed dressing	1	0.13	153	Tuber	< 0.01
			1	0.25	153	Tuber	0.01
			1	0.50	153	Tuber	0.01
	20% EC	Seed dressing	1	0.25	153	Tuber	< 0.01
	50% WP	In-furrow	1	2 kg ai/ha	153	Tuber	0.01
UK, 1980 Brightwell (Pentland Crown) QR-01-0068/Brightwell	50% WP	Seed dressing	1	0.13	155	Tuber	< 0.01 [control 0.005, 0.015]
			1	0.25	155	Tuber	< 0.01
			1	0.50	155	Tuber	0.04
	20% EC	Seed dressing	1	0.25	155	Tuber	0.12
	50% WP	In-furrow	1	2 kg ai/ha	155	Tuber	0.12
UK, 1980 Chesterford Park (King Edward) QR-01-0068/Chesterford Park	50% WP	Seed dressing	1	0.13	162	Tuber	0.07
			1	0.25	162	Tuber	0.08
			1	0.50	162	Tuber	0.62
	20% EC	Seed dressing	1	0.25	162	Tuber	0.04
	50% WP	In-furrow	1	2 kg ai/ha	162	Tuber	0.02

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
UK, 1980 Levington (Pentland Crown) QR-01-0068/Levington	50% WP	Seed dressing	1	0.13	167	Tuber	0.01 [control 0.002, 0.018]
			1	0.25	167	Tuber	0.01
			1	0.50	167	Tuber	0.02
	20% EC	Seed dressing	1	0.25	167	Tuber	0.01
	50% WP	In-furrow	1	2 kg ai/ha	167	Tuber	0.01
UK, 1980 Shelford (King Edward) QR-01-0068/Shelford I	50% WP	Seed dressing	1	0.13	196	Tuber	0.03
			1	0.25	196	Tuber	0.01
			1	0.50	196	Tuber	0.05
	20% EC	Seed dressing	1	0.25	196	Tuber	< 0.01
	50% WP	In-furrow	1	2 kg ai/ha	196	Tuber	< 0.01
UK, 1981 Hollesley (Pentland Javelin) QR-01-0068, Hollesley	50% WP	Seed dressing	1	0.25	110	Tuber	0.06
				0.50	110	Tuber	0.20
	25% SC	Seed dressing	1	0.25	110	Tuber	0.21
	20% EC	Seed dressing	1	0.25	110	Tuber	0.07
			1	0.40	110	Tuber	0.05
	20% EC	In-furrow	1	4 kg ai/ha	110	Tuber	0.12
	25% SC	In-furrow	1	8 kg ai/ha	110	Tuber	0.20
	20% EC	Seed dressing plus in-furrow	1 + 1	0.13 + 2 kg ai/ha	110	Tuber	0.33
UK, 1981 Chesterford Park (Maris Piper) QR-01-0068/Chesterford Park	50% WP	Seed dressing	1	0.06	132	Tuber	0.02
			1	0.50	132	Tuber	0.04
	25% SC	Seed dressing	1	0.25	132	Tuber	0.04
	20% EC	Seed dressing	1	0.06	132	Tuber	< 0.01
			1	0.25	132	Tuber	0.05
			1	0.4	132	Tuber	0.05
		In-furrow	1	0.5 kg ai/ha	132	Tuber	0.01
	25% SC	In-furrow	1	8 kg ai/ha	132	Tuber	0.99
	20% EC	Seed dressing plus in-furrow	1 +1	0.13 + 2 kg ai/ha	132	Tuber	0.08
UK, 1981 Shelford (Pentland Crown) QR-01-0068/Shelford*	50% WP	Seed dressing	1	0.06	183	Tuber	< 0.01

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
			1	0.50	183	Tuber	0.05
	25% SC	Seed dressing	1	0.25	183	Tuber	0.01
	20% EC	Seed dressing	1	0.06	183	Tuber	< 0.01
			1	0.25	183	Tuber	0.01
			1	0.40	183	Tuber	0.02
	20% EC	In-furrow	1	0.50 kg ai/ha	183	Tuber	< 0.01
			1	4 kg ai/ha	183	Tuber	0.12
			1	8 kg ai/ha	183	Tuber	0.36
	20% EC	Seed dressing plus in-furrow	1 +1	0.13 + 2 kg ai/ha	183	Tuber	0.05
UK, 1981 Shelford (Desiree) QR-01-0068/Shelford*	50% WP	Seed dressing	1	0.25	189	Tuber	0.02
			1	0.50	189	Tuber	0.12
	25% SC	Seed dressing	1	0.25	189	Tuber	0.04
	20%EC	Seed dressing	1	0.25	189	Tuber	0.03
			1	0.40	189	Tuber	0.04
		In-furrow	1	4 kg ai/ha	189	Tuber	0.05
	25% SC	In-furrow	1	8 kg ai/ha	189	Tuber	0.53
	20% EC	Seed dressing plus in-furrow	1 +1	0.13 + 2 kg ai/ha	189	Tuber	0.05
UK, 1981 Iken (Pentland Javelin) QR-01-0068/Iken	10% DP	Seed dressing	1	0.25	nr	Tuber	0.02
UK, 1981 Wissington (Maris Piper) QR-01-0068/Wissington	10% DP	Seed dressing	1	0.25	145	Tuber	0.03 [control 0.029]
UK, 1982 Saffron Walden, Essex (Pentland Javelin) QR-21-0069/Saffron Walden	5% DP	Seed dressing	1	0.13	134	Tuber	0.01
	10% DP	Seed dressing	1	0.25	134	Tuber	0.02
UK, 1982 Brightwell, Suffolk (Pentland Javelin) QR-21-0069/Brightwell (P.J.)	5% DP	Seed dressing	1	0.13	99	Tuber	0.02 [control: 0.015]
	10% DP	Seed dressing	1	0.25	99	Tuber	0.08
UK, 1982 Shouldham Thorpe, Norfolk (Home Guard) QR-21-0069/Shouldham Thorpe	5% DP	Seed dressing	1	0.13	119	Tuber	0.02 / 0.02 (0.02)

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
UK, 1982 Wissington, Norfolk (Maris Piper) QR-21-0069/Wissington	5% DP	Seed dressing	1	0.13	133	Tuber	< 0.01
	10% DP	Seed dressing	1	0.25	133	Tuber	< 0.01
UK, 1982 Iken, Norfolk (King Edward) QR-21-0069/ Iken	5% DP	Seed dressing	1	0.13	196	Tuber	< 0.01
	10% DP	Seed dressing	1	0.25	196	Tuber	0.02
UK, 1982 Swaffham, Prior Cambs (Record) QR-21-0069/Swaffham	5% DP	Seed dressing	1	0.13	151	Tuber	< 0.01
	10% DP	Seed dressing	1	0.25	151	Tuber	< 0.01
UK, 1982 Brightwell, Suffolk (Cara) QR-21-0069/Brightwell	5% DP	Seed dressing	1	0.13	216	Tuber	< 0.01
	10% DP	Seed dressing	1	0.25	216	Tuber	< 0.01
UK, 1982 Shelford, Cambs (King Edward) QR-21-0069/Shelford, Cambs	20% EC	Seed dressing	1	0.13	183	Tuber	< 0.01
	20% EC	Seed dressing	1	0.25	183	Tuber	< 0.01
UK, 1982 Bawdsey, Suffolk (Pentland Javelin) QR-21-0069/ Bawdsey	5% DP	Seed dressing	1	0.13	98	Tuber	0.02/< 0.01/< 0.01 [Control: 0.016, nd, nd]
						Flesh	< 0.01/< 0.01/0.01
					98	Peel	0.04/0.06 / 0.04
	10% DP	Seed dressing	1	0.25	98	Tuber	0.01/0.02 / 0.06 (0.03)
						Flesh	< 0.01/< 0.01/< 0.01
					98	Peel	0.05/0.08 / 0.09
UK, 1982 Hollesley, Suffolk (Pentland Javelin) QR-21-0069/Hollesley	5% DP	Seed dressing	1	0.13	89	Tuber	0.01
						Flesh	< 0.01
					89	Peel	0.06
	10% DP	Seed dressing	1	0.25	89	Tuber	0.18
					89	Flesh	0.01
					89	Peel	1.2

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
Germany, 1986 Mutterstadt (Berolina) QR-61-0038G/Mutterstadt	25% SC	Seed dressing	1	0.15	147	Tuber	< 0.002
					165	Tuber	< 0.002
Germany, 1986 Natendorf (Berolina) QR-61-0040G/Natendorf	25% SC	Seed dressing	1	0.15	123	Tuber	< 0.002
					136	Tuber	< 0.002
Germany, 1986 Choursistinenthal (Berolina) QR-61-0042G/Choursistinenthal	25% SC	Seed dressing	1	0.15	136	Tuber	0.007
					147	Tuber	< 0.002
Germany, 1986 Dirmstein (Berolina) QR-61-0044G/Dirmstein	25% SC	Seed dressing	1	0.15	150	Tuber	< 0.002
					165	Tuber	< 0.002
Germany, 1986 Eddesse (Berolina) QR-61-0036G/Eddesse	25% SC	Seed dressing	1	0.15	125	Tuber	< 0.002
					139	Tuber	< 0.002
UK, 1992 Mont au Prêtre, St Helier (Jersey Royal) QR-0119/PP3-92H1300	SC 500 g/L	Seed dressing	1	0.13	84	Tuber	0.11/ 0.01/ 0.01 (0.04)
UK, 1992 Vinchelez, St. Ouen (Jersey Royal) QR-0119/PP4-92 O764	SC 500 g/L	Seed dressing	1	0.13	84	Tuber	0.06/ 0.03 / 0.09 (0.06)
Denmark, 1993 Middelfart (Sava) QR-31-0104/12920101	DP 100 g/kg	Seed treatment	1	0.15	60	Tuber	0.07
					109	Tuber	0.04
					148	Tuber	0.04
	SC 500 g/L	Seed treatment	1	0.15	60	Tuber	0.05
					109	Tuber	< 0.01
					148	Tuber	0.02

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
UK, 1993 Ancaster (Cleopatra) QR-41-0101/9F9308	DP100 g/kg	Seed treatment at planting	1	0.13	110	Tuber	0.08
					124	Tuber	< 0.05
UK, 1993 Long Eaton (Desiree) QR-41-0101/9F9308	DP 100 g/kg	Seed treatment at planting	1	0.25	149	Tuber	< 0.05
UK, 1993 Cressage (Record) QR-41-0101/9F9308	DP 100 g/kg	Seed treatment at planting	1	0.25	83	Tuber (immature)	< 0.05
					111	Tuber (immature)	< 0.05
					156	Tuber	< 0.05
UK, 1993 Hadnall (Dundrod) QR-41-0101/9F9308	DP 100 g/kg	Seed treatment at planting	1	0.13	96	Tuber	< 0.05
					115	Tuber	< 0.05
UK, 1993 Ancaster (Cleopatra) QR-41-0102/ Ancaster	SC 500 g/L	Seed treatment before planting (17 days)	1	0.13	0	Tuber (seed)	123
					141	Tuber	0.20
					155	Tuber	0.06
UK, 1993 Hadnall (Dundrod) QR-41-0102/ Hadnall	SC 500 g/L	Seed treatment at planting	1	0.063	0	Tuber (seed)	16
					120	Tuber	< 0.05
					139	Tuber	< 0.05
UK, 1993 St. Clement (Jersey Royal) QR-41-0103/ PP9A/93	SC 500 g/L	Seed treatment at planting	1	0.13	0	Tuber (seed)	46
					84	Tuber (immature)	0.10
UK, 1993 St. Peter (Jersey Roya) QR-41-0103/ PP9B/93	SC 500 g/L	Seed treatment at planting	1	0.13	0	Tuber (seed)	41
					84	Tuber (immature)	0.07

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
Germany, 1993 Heuchelheim (Saturna) QR-41-0107/310761	SC 236 g/L	Seed treatment	1	0.14	149	Tuber	< 0.02
Germany, 1993 Heuchelheim (Saturna) QR-41-0107/310861	DP 100 g/kg	Seed treatment	1	0.20	149	Tuber	0.02
UK, 2000 Rewe EX5 4ER Devon (Maris Bard) QR-0125/ EA000110 UK01*	SC 500 g/L	Seed dressing	1	0.13	93	Tuber	< 0.01
UK, 2000 Sowton EX5 2AF Devon (Maris Bard) QR-0125/ EA000110 UK02*	SC 500 g/L	Seed dressing	1	0.13	93	Tuber	< 0.01
UK, 2000 Revesby PE22 7NH Lincolnshire (Saxone) QR-0125/ EA000110 UK03	SC 500 g/L	Seed dressing	1	0.13	85	Tuber	0.01
UK, 2000 Kings Newnham CV23 OJT Warwickshire (Wilja) QR-0125/ EA000110 UK04	SC 500 g/L	Seed dressing	1	0.13	95	Tuber	< 0.01
UK, 2000 Stratton Audley OX6 9BW Oxfordshire (Wilja) QR-0125/ EA000110 UK05**	SC 500 g/L	Seed dressing	1	0.13	95	Tuber	< 0.01
UK, 2000 Stratton Audley OX6 9BW Oxfordshire (Nadine) QR-0125/EA000110 UK06**	SC 500 g/L	Seed dressing	1	0.13	80	Tuber	< 0.01
France (Northern), 2001 Terminiers Centre (Charlotte) QR-0123/EA010119 FR01	SC 250 g/L	Seed dressing before planting (15 days)	1	0.23	125	Tuber	0.02
France (Northern), 2001 Talcy Centre (Mona-Lisa) QR-0123/EA010119 FR02	SC 250 g/L	Seed dressing before planting (14 days)	1	0.26	120	Tuber	0.01
Greece, 2001 63073 Galatista Halkidiki (Spunta) QR-0123/ EA010119 GR01	SC 250 g/L	Seed dressing before planting (22 days)	1	0.23	99	Tuber	0.01

Country, Year Location (Variety) Trial ID	Application				DAT	Portion analysed	TM (mg/kg)
	Form.	Method	No.	Rate (kg ai/t tuber)			
Greece, 2001 66033 Perithori Drama (Spunta) QR-0123/EA010119 GR02	SC 250 g/L	Seed dressing before planting (29 days)	1	0.23	99	Tuber	< 0.01
Italy, 2001 37040 Roveredo di Gua Veneto (Agata) QR-0123/EA010119 IT01	SC 250 g/L	Seed dressing before planting (12 days)	1	0.22	98	Tuber	< 0.01
Italy, 2001 Alba Adriaca Abruzzo (Agata) QR-0123/ EA010119 IT02	SC 250 g/L	Seed dressing before planting (11 days)	1	0.23	96	Tuber	0.01
France (Southern), 2013 Marsillargues Languedoc-Roussillon (Artemis) QR-0293/JCB-13-15474 FR01	SC 500 g/L	Tuber before planting (3 days)	1	0.25	82	Tuber+	< 0.01, < 0.01, < 0.01, < 0.01
Spain, 2013 Vilarino Galicia (Spunta) QR-0293/JCB-13-15474 ES02	SC 500 g/L	Tuber before planting (10 days)	1	0.25	81	Tuber+	< 0.01, < 0.01, < 0.01, < 0.01
Spain, 2013 Os Barreiros Galicia (Monalisa) QR-0293/JCB-13-15474 ES03	SC 500 g/L	Tuber before planting (10 days)	1	0.25	87	Tuber+	< 0.01, < 0.01, < 0.01, < 0.01
Italy, 2013 Settala Lombardy (Artemis) QR-0293/JCB-13-15474 IT04	SC 500 g/L	Tuber before planting (14 days)	1	0.25	87	Tuber+	< 0.01, < 0.01, < 0.01, < 0.01

QR-01-0068

Dust or spray application onto tubers one day before planting except Chesterford Park trial (1980), 7 days and Hollesley trial (1981), 3 days; in-furrow spray application, the day at planting

All residue values were corrected for overall mean recovery (85%) and residues in control samples were < 0.01 mg/kg in all but 3 cases.

* Not independent trials, close site and application date (6 days apart)

QR-21-0069

All residue values were corrected for overall mean recovery (94%) and residues in control samples were < 0.01 mg/kg in all but 2 trials.

QR-41-0101, QR-41-0102, QR-41-0103

Procedural recoveries were corrected for control. And residue values were corrected for the overall mean recovery.

QR-0125

* Not independent trials

** Not independent trials

QR-0293

+Analysed samples: hand-picked/unwashed, hand-picked/washed under water, mechanically harvested/unwashed, and mechanically harvested/washed under water

FATE OF RESIDUES IN STORAGE AND PROCESSING

Nature of the residue during processing

A processing hydrolysis study was conducted on [phenyl-^{14}C]-tolclofos-methyl in buffered water using thoursee sets of conditions representative of processing of raw agricultural commodities [Curtis-Jackson, 2014b, report QM-0072]. The application solution was prepared by dissolving radiolabelled test substance in acetonitrile. The application solution was added to the sterile buffer solution and incubated at 90 °C for 20 min in a solution at pH 4 (simulation of pasteurisation), at 100 °C for 60 min in a solution at pH 5 (simulation of baking, brewing and boiling) and at 120 °C for 20 min in a solution at pH 6 (simulation of sterilisation). Following incubation, LSC, HPLC and TLC analyses were performed. The results are shown in Table 55.

For all non-incubated control samples, > 97% of the TRR was attributed to tolclofos-methyl. Tolclofos-methyl degraded increasingly to DM-TM with increased temperature and pH. At 20 min incubation at pH 4 and 90 °C, 60 min incubation at pH 5 and 100 °C, and 20 min incubation at pH 6 and 120 °C, tolclofos-methyl occurred at 74.8%, 47.3% and 12.6%, respectively and DM-TM occurred at 23.6%, 52.7% and 87.0%, respectively. Under simulated processing conditions, tolclofos-methyl was converted into DM-TM.

Table 55 Radioactivity distribution after simulating pasteurisation, baking/brewing/boiling and sterilisation for Tolclofos-methyl

Compound	Mean radioactivity distribution after Heating					
	pH 4 and 90 °C (20 min)		pH 5 and 100 °C (60 min)		pH 6 and 120 °C (20 min)	
	%	mg/L	%	mg/L	%	mg/L
TM	74.8	0.60	47.3	0.40	12.6	0.097
DM-TM	23.6	0.19	52.7	0.45	87.0	0.67
Total	98.4	*0.79*	100	*0.85*	99.6	*0.77*

Residues after processing

Study 1

Distribution of tolclofos-methyl in potato flesh and peel was investigated using field trial samples [Jongstra, 1980, report QR-01-0004]. Residue trials were performed in 1979 in the Netherlands. Soil was treated with tolclofos-methyl and incorporated to a depth of 15 cm. Potatoes (var. Bintje E 35/45 cultivatornumber 5-1772) were planted 1, 20 and 0 days after soil treatment for the trials in Emmeloord (sandy clay), Uithuizen (clay) and Kloosterburen (clay), respectively. Potatoes were harvested 106, 113 and 84 days after treatment in Emmeloord, Uithuizen and Kloosterburen, respectively. Residues of tolclofos-methyl in washed potatoes, flesh and peel of washed potatoes were extracted with methanol/acetonitrile (1:4, v/v), partitioned with dichloromethane and cleaned up using silica gel. GC-FPD was used for determination of tolclofos-methyl. All data was corrected for procedural recovery 83% (< 0.002 mg/kg, limit of determination, in controls). The results are shown in Table 56.

The weight percent of potato peelings was 12.5-17.2% (mean, 15.2%). In potato flesh, level of tolclofos-methyl was below the limit of determination, < 0.002 mg/kg. The processing factors for tolclofos-methyl in peeled potatoes were < 0.05, < 0.06, < 0.11, < 0.11, < 0.17, < 0.20, < 0.20, < 0.25 (3), < 0.29 and 0.50 (n = 12). The processing factors for tolclofos-methyl in potato peels were 2.5, 2.9, 3.3, 3.6, 3.7, 4.0, 4.1, 5.3, 6.0, 6.6, 7.1 and 7.2 (n = 12).

Table 56 Tolclofos-methyl residues in potato tuber and peel (soil treatment; report QR-01-0004)

Trial	TM in whole potato tuber (mg/kg)	TM in peeled potato (mg/kg)	Processing factor to potato flesh	TM in potato peel (mg/kg)	Processing factor to potato peel
Emmeloord	0.010	< 0.002	< 0.20	0.029	2.9
	0.019	< 0.002	< 0.11	0.101	5.3
	0.038	< 0.002	< 0.05	0.140	3.7
	0.018	< 0.002	< 0.11	0.064	3.6
Uithuizen	0.008	< 0.002	< 0.25	0.053	6.6
	0.010	< 0.002	< 0.20	0.025	2.5
	0.008	< 0.002	< 0.25	0.048	6.0
	0.012	< 0.002	< 0.17	0.039	3.3
Kloosterburen	0.008	< 0.002	< 0.25	0.058	7.2
	0.031	< 0.002	< 0.06	0.123	4.0
	0.004	< 0.002	< 0.50	0.028	7.1
	0.007	< 0.002	< 0.29	0.029	4.1

Study 2

Distribution of tolclofos-methyl in potato flesh and peel was investigated in field trials [Smith and Brunt, 1989, report QR-11-0072]. Residue trial on potato (var. Elkana) was performed in 1984 in Rolde of the Netherlands (sandy soil). Prior to planting, the soils were treated with tolclofos-methyl WP formulation by spraying and incorporating into soil at rates of 7.5, 15 and 30 kg ai/ha. Harvest potatoes were washed with water to remove adhering soil. Tolclofos-methyl residues in whole tuber, flesh and peel were extracted with methanol/acetonitrile (1:4, v/v), partitioned with dichloromethane and cleaned up using silica gel, and then determined by GC-FPD. Procedural recoveries were satisfactory. Limit of determination was 0.001 mg/kg. All residue values were corrected for the overall mean recovery (75%). The results are shown in Table 57.

In potato flesh, no residues of tolclofos-methyl were found. The processing factors for tolclofos-methyl in peeled potatoes were < 0.01, < 0.03 and < 0.05 (n = 3). The processing factors for tolclofos-methyl in potato peels were 4.4, 4.5 and 5.3 (n = 3).

Table 57 Tolclofos-methyl residues in potato tuber and peel (soil treatment, report QR-11-0072)

Application rate (kg ai/ha)	TM in whole potato tuber (mg/kg)	TM in potato flesh (mg/kg)	Processing factor to potato flesh	TM in potato peel (mg/kg)	Processing factor to potato peel
15	0.019	< 0.001	< 0.05	0.083	4.4
30	0.029	< 0.001	< 0.03	0.15	5.3
60	0.086	< 0.001	< 0.01	0.38	4.5

Limit of determination, < 0.001

Study 3

Method and results of this study were described in the residue trial section [Longland and Churchill, 1983, report QR-21-0069]. Residue trials in potatoes were performed in 1982 in the UK. Potatoes were seed treated at a rate of 0.13 or 0.25 kg ai/ton tuber using DP formulation of tolclofos-methyl. Potatoes were harvested after 98 and 89 days. Residues of tolclofos-methyl were analysed in whole potato tubers, potato flesh and potato peel (peelings, mean 29%). The results are shown in Table 58.

The processing factors for tolclofos-methyl in peeled potatoes were < 0.50, < 0.50, 0.06, < 0.17, < 1 and <1 (n = 6). The processing factors for tolclofos-methyl in potato peels were 1.5, 2.0, 4.0, 5.0, 6.0 and 6.7 (n = 6).

Table 58 Tolclofos-methyl residues in potato tuber and peel (seed treatment; report QR-21-0069)

Trial	Rate (kg ai/t)	TM in whole potato tuber (mg/kg)	TM in peeled potato (mg/kg)	Processing factor to potato flesh	TM in potato peel (mg/kg)	Processing factor to potato peel
Bawdsey	0.13	0.02	< 0.01	< 0.50	0.04	2.0
	0.25	0.01	< 0.01	<1	0.05	5.0
	0.25	0.02	< 0.01	< 0.50	0.08	4.0
	0.25	0.06	< 0.01	< 0.17	0.09	1.5
Hollesley	0.13	0.01	< 0.01	<1	0.06	6.0
	0.25	0.18	0.01	0.06	1.2	6.7

From the thoursee studies, the processing factors for tolclofos-methyl in peeled potatoes were < 0.01, < 0.03, < 0.05, < 0.05, < 0.06, 0.06, < 0.11, < 0.11, < 0.17, < 0.17, < 0.20, < 0.20, < 0.25 (3), < 0.29, < 0.50 (3), <1 and <1 (n = 21). The processing factors for tolclofos-methyl in potato peels were 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).

RESIDUES IN ANIMAL COMMODITIES

Farm animal feeding studies

No information was provided.

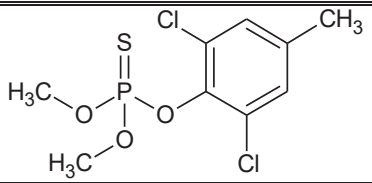
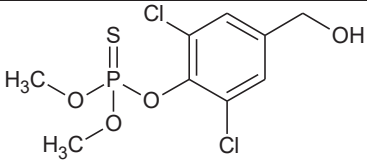
APPRAISAL

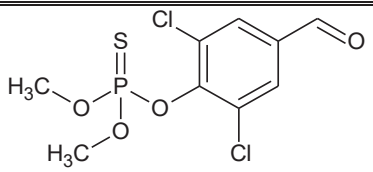
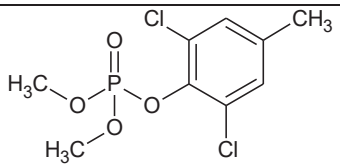
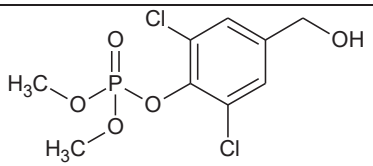
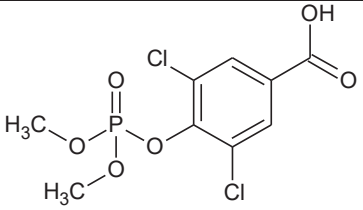
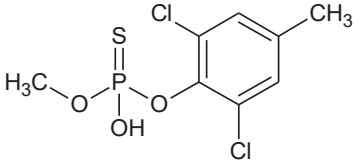
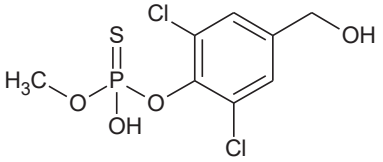
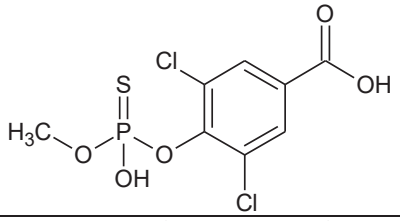
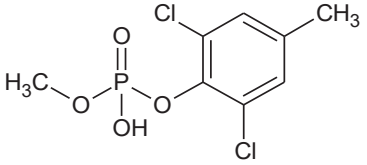
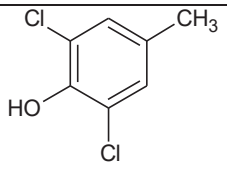
Tolclofos-methyl is a non-systemic contact organophosphorus fungicide used for control of soil-borne diseases caused by *Rhizoctonia solani*. The IUPAC name for tolclofos-methyl is *O*-2,6-dichloro-*p*-tolyl *O,O*-dimethyl phosphorothioate. Tolclofos-methyl was first evaluated for toxicology and residues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

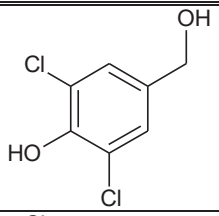
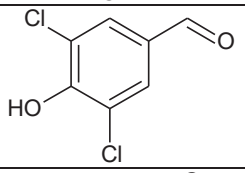
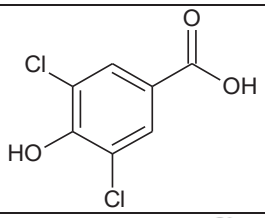
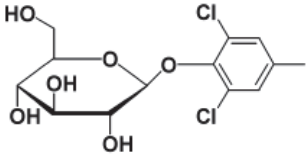
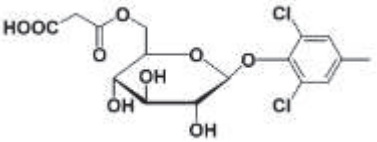
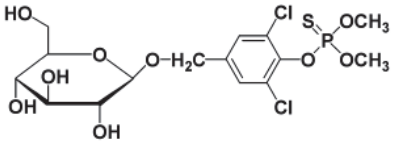
Tolclofos-methyl was scheduled at the Fiftieth Session of the CCPR for periodic review by the 2019 JMPR. The Meeting received information on identity, physical and chemical properties, plant and animal metabolism, environmental fate, methods of residue analysis, storage stability, GAP information and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

Abbreviation	Matrix found	Structure
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)	
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)	

Abbreviation	Matrix found	Structure
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[6].

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] (2.8)[A]	0.16 (0.97)[B]	0.12	0.023
Dairy cattle	0.14	0.042	0.43[b] (2.6)[A]	0.13 (0.75)[B]	0.11	0.005
Poultry – broiler			0.11[c] (0.63)[A]	0.005 (0.030)[B]		
Poultry – layer			0.11[d] (0.63)[A]	0.005 (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.

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

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 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 below 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.

Table 4 Residue levels suitable for establishing maximum residue limits and for IEDI assessments

CCN	Commodity	Recommended maximum residue level (mg/kg)		STMR or STMR-P mg/kg	HR or HR-P mg/kg
		New	Previous		
VL 0482	Lettuce, head	w	2		
VL 0483	Lettuce, leaf	w	2		
VL 2050	Leafy greens except spinach, purslane and chard	0.7		0.36	
VR 0589	Potato	0.3	0.2	0.060	

CCN	Commodity	Recommended maximum residue level (mg/kg)		STMR or STMR-P mg/kg	HR or HR-P mg/kg
		New	Previous		
MO 0105	Edible offal (Mammalian)	0.01*		0.0055 (kidney) 0.0033 (liver)	
PE 0112	Eggs	0.01*		0	
MF 0100	Mammalian fats (except milk fats)	0.01*		0	
MM 0095	Meat (from mammals other than marine mammals)	0.01*		0	
ML 0106	Milks	0.01*		0	
PF 0111	Poultry fats	0.01*		0	
PM 0110	Poultry meat	0.01*		0	
PO 0111	Poultry, Edible offal of	0.01*		0	
VR 0494	Radish	W	0.1		

Table 5 Additional values used in estimating livestock dietary burdens.

Codex classification	Commodity	Median residue (-P) (mg/kg)	Highest residue (-P) (mg/kg)
	Potato culls	0.01	0.21
	Potato process waste	0.044	

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.

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