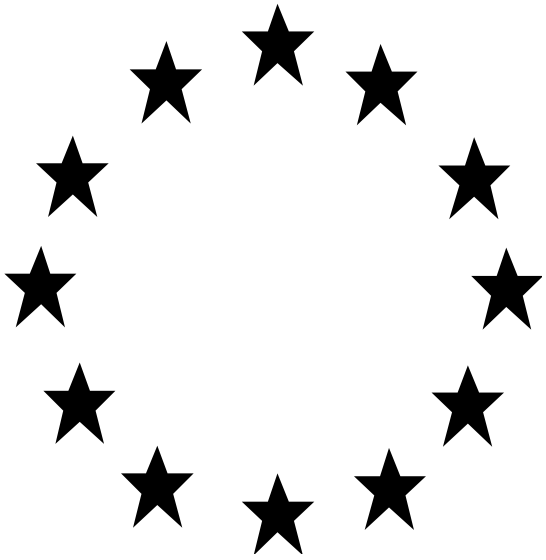

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Sweden for the existing active substance

TOLCLOFOS-METHYL

of the second stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, B.6, part 1

January 2005

Draft Assessment Report

Tolclofos-methyl

Volume 3
Annex B.6
Toxicology and Metabolism

Rapporteur Member State: Sweden

October 2003

KEMI
NATIONAL CHEMICALS INSPECTORATE

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B.6 Toxicology and metabolism

Tolclofos-methyl is an organophosphorus fungicide, which is used to control *Rhizoctonia solani* in soil in which potatoes and lettuce are planted. Below follows the evaluation of the toxicological properties of Tolclofos-methyl.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

B.6.1.1 Single dose (one dose level) in rats and mice (4-methyl-label)

Reference	: Mihara, K. et al., 1980	**Exposure**	: Single exposure
Title of study	: Metabolism of tolclofos-methyl in rats and mice	**Dose**	: Rats and mice: 5 mg/kg bw Additional mice: 50 mg/kg bw
Test substance	: [4-methyl-^{14}C]tolclofos-methyl, batch No.: not specified, radiochemical purity: >99%, specific radioactivity: 16.2 mCi/mmol) Radiochemically diluted [4-methyl-^{14}C]tolclofos-methyl with specific activity of 1.62 mCi/mmol was also used as test material	**Vehicle**	: Corn oil (10 ml)
Administration way	: Oral (stomach intubation)	**GLP statement**	: No
Species	: Sprague-Dawley rats and ICR mice	**Guideline**	: In-house method, in accordance with 88/302/EEC, part B, Toxicokinetics except that only one single dose was used one
Group size	: Number not indicated, later 30 male mice	**Acceptability**	: Yes

Materials and methods

The animals were administered a single oral dose of 5 mg/kg bw of [4-methyl-^{14}C]tolclofos-methyl 16.2 mCi/mmol. Free access was given to feed and water. [4-methyl-^{14}C]tolclofos-methyl 1.62 mCi/mmol was administered at 50 mg/kg to additional mice to obtain larger amounts of metabolites.

Whole body autoradiography was performed on male rats 1, 6 and 24 hours after dosing. Excreta and expired air were collected in rats and mice for up to 7 days after treatment. Rat tissues were analysed for level of radioactivity 7 days after treatment. Radioassay was performed using liquid scintillation counting.

According to guidelines, two dose levels should be used. However, the study adequately fulfils the criteria of guidelines together with B.6.1.2.

Findings

Absorption and distribution:

Autoradiography in male rats showed that 1 and 6 hours after dosing, most of the radioactivity was present in the digestive organs, followed by kidney and liver. In both sexes of rats and mice, 74 to 83% of the administered dose was recovered in the excreta within 24 hours of treatment (Table B.6.1.1-1).

Excretion:

Recoveries at 7 days ranged from 87 to 91%. Excretion via the urine was the predominant route (65 to 70% in rats, 82 to 83% in mice). Faecal excretion accounted for 17 to 22% of the dose in rats, and 5 to 7% in mice. In both species, 0.3 to 0.9% was excreted in the expired air. It is presumed that the unrecovered label (9 to 13% of dose) was lost from the excreta by evaporation, due to the high vapour pressure of tolclofos-methyl and its metabolite 2,6-dichloro-4-methylphenol ($phCH_3$).

Table B.6.1.1-1: Metabolism of [4-methyl-^{14}C]tolclofos-methyl in rats and mice: Radiocarbon excretion over 7 days (dose level: 5 mg/kg)

Mammals	Sex	Excreta	% of the administered radiocarbon (days after administration)			
			1	2	4	7[a]
Rats	Male	Urine	66.7	69.2	69.6	69.8
		Feces	16.4	17.0	17.1	17.2
		Expired air	0.2	0.3	0.3	0.3
		Total	83.3	86.5	87.0	87.3
	Female	Urine	62.1	64.1	64.8	65.1
		Feces	20.5	21.5	21.7	21.9
		Expired air	0.6	0.7	0.7	0.7
		Total	83.2	86.3	87.2	87.7
Mice	Male	Urine	75.9	83.1	83.2	83.3
		Feces	5.8	6.6	6.7	6.7
		Expired air	0.7	0.9	0.9	0.9
		Total	82.4	90.6	90.8	90.9
	Female	Urine	69.3	76.7	81.0	81.5
		Feces	4.4	4.9	5.1	5.2
		Expired air	0.5	0.6	0.6	0.6
		Total	74.2	81.2	86.7	87.3

a: The residues remaining in the animal body 7 days after administration were less than 1 % of the dose.

Residue levels:

Very low levels of radioactivity were present after 7 days (Table B.6.1.1-2). Higher ^{14}C was found in the hair. Less than 1% of the dose was retained in the tissues after 7 days.

Table B.6.1.1-2: Metabolism of [4-methyl-^{14}C]tolclofos-methyl in rats and mice: Radiocarbon distribution in rat body after 7 days

Tissues	Total ^{14}C (ng tolclofos-methyl eq. /g tissue)		Tissues	Total ^{14}C (ng tolclofos-methyl eq. /g tissue)	
	Male	Female		Male	Female
Plasma	1	2	**Fat**	6	6
Red blood cell	4	5	**Spinal cord**	1	1
Brain	1	1	**Sciatic nerve**	3	1
Eye-ball	1	3	**Skin**	6	6
Thyroid	< 1	< 1	**Hair**	52	60
Thymus	1	2	**Testis**	1	-
Lung	2	3	**Ovary**	-	4
Heart	1	1	**Uterus**	-	3
Kidney	4	4	**Stomach**	2	2
Liver	4	5	**Duodenum**	2	2
Pancreas	1	1	**Jejunum**	2	2
Spleen	1	2	**Caecum**	2	2
Adrenal	3	4	**Colon and rectum**	3	3
Muscle	1	2			

Metabolism:

Radiocarbon characteristics and identity:

Of the 14 metabolites identified in rats, all except DM-TM-COOH were also identified in mice (Table B.6.1.1-3). The major urinary metabolite in both species was ph-COOH. The amounts of the metabolites were different between rats and mice, although no significant differences were found between males and females of both animal species (Table B.6.1.1-4). In both species, tolclofos-methyl was mainly metabolised via oxidative desulfuration, oxidation of 4-methyl group, and cleavage of P-O-aryl and P-O-methyl linkages.

Table B.6.1.1-3: Metabolism of [4-methyl-^{14}C]tolclofos-methyl in rats and mice: Identity of metabolites identified

Designation	Chemical name
TM (parent compound)	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
DM-TM	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
TM–CH_2OH	*O,O*-dimethyl *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl]phosphorothioate
DM-TM–CH_2OH	*O*-methyl *O*-hydrogen *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl] phosphorothioate
TM-COOH	*O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl) phosphorothioate
DM-TM-COOH[a]	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphorothioate
ph-CH_3	2,6-dichloro-4-methylphenol
ph-COOH	3,5-dichloro-4-hydroxybenzoic acid
TMO-CH_2OH	*O,O*-dimethyl *O*-2,6-dichloro-4-(hydroxymethyl)phenylphosphate
ph-CH_2OH	3,5-dichloro-4-hydroxybenzyl alcohol
TMO-COOH	*O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl) phosphate
ph-CO-glycine[b]	3,5-dichloro-4-hydroxyhippuric acid
DM-TMO	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphate
DM-TMO-CH_2OH	*O*-methyl *O*-hydrogen *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl] phosphate
DM-TMO-COOH	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphate

a: Rats only
b: Mice only

Table B.6.1.1-4: Metabolism of [4-methyl-^{14}C]tolclofos-methyl in rats and mice: Identity and amounts of radiocarbon in the urine and feces during 24 hours after administration

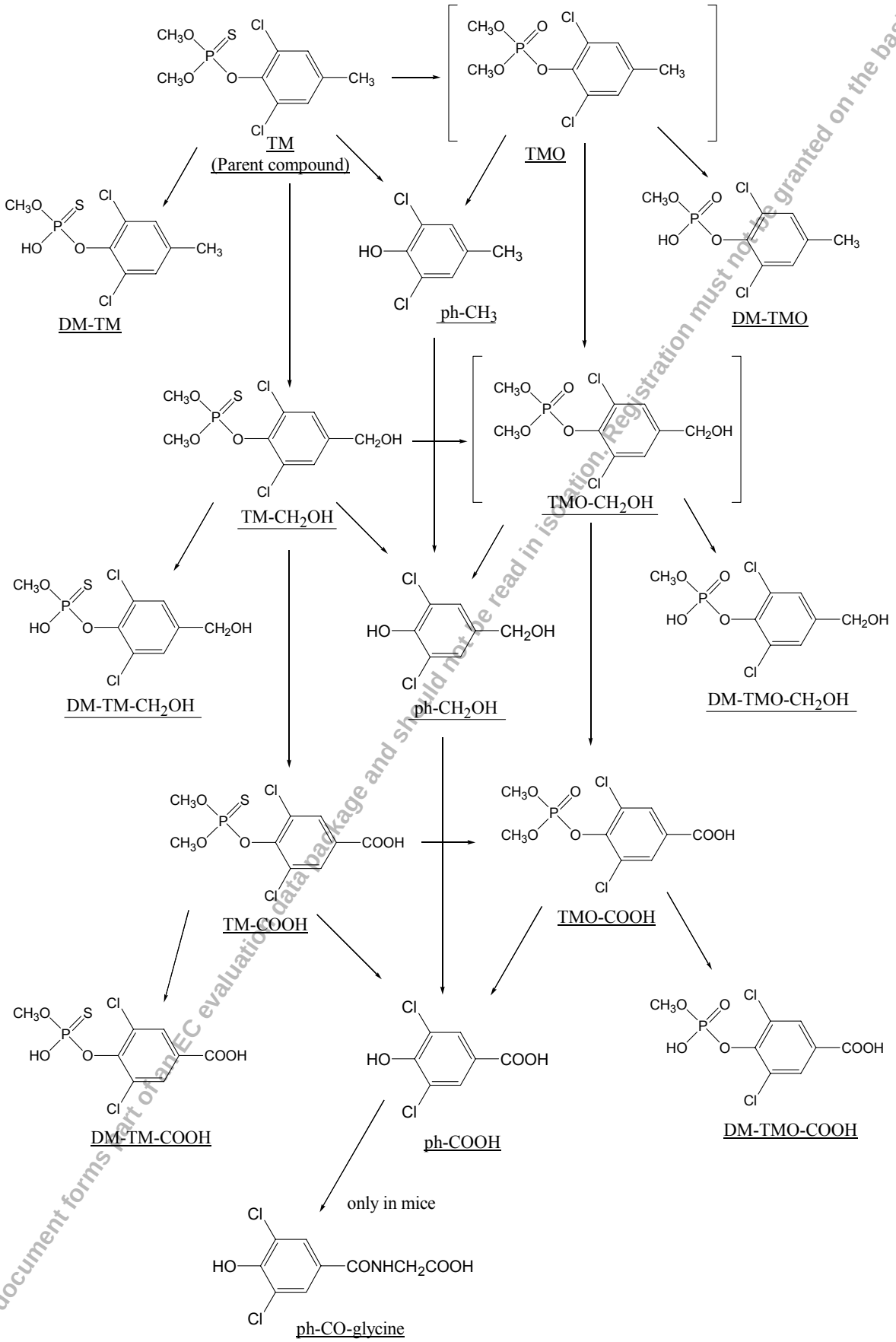
Metabolite	Amounts (% of the administered radiocarbon [1])			
	Rats		Mice	
	Urine	Feces	Urine	Feces
S 1 TM	Nd[a]	5.0	nd	1.3
S 2 Ph-CH_3	7.9	3.5	7.9	0.8
S 3 TM-CH_2OH	nd	trace	nd	0.1
S 4 Ph-CH_2OH	3.0	1.6	2.5	0.2
S 5 TM-COOH	3.3	0.7	3.0	1.0
S 6 Ph-COOH	26.1	3.2	10.4	1.2
S 7 TMO-COOH	7.8	0.7	9.9	0.7
S 8 Ph-CO- glycine	trace[b]	nd	13.2	nd
S 9 DM-TM	4.3	trace	0.9	trace
S10 DM-TM-COOH	3.3	0.3	nd	nd
S11 DM-TM-CH_2OH	1.3	0.2	3.8	0.1
S12 DM-TMO	1.9	0.3	3.3	0.1
S13 DM-TMO-COOH	5.5	0.3	12.0	0.1
S14 DM-TMO-CH_2OH	0.4	trace	4.0	trace
Others[c]	1.9	0.6	5.0	0.2
Total	66.7	16.4	75.9	5.8

1: Samples taken during 24 hours after administration.
a: nd: Not detected
b: trace: Less than 0.1 %
c: Methanol-insoluble ^{14}C

Conclusions

Tolclofos-methyl was rapidly excreted in rats and mice, mainly in the urine (~80%). Tissue residues 7 days after dosing were generally very low although somewhat higher radioactivity level was found in the hair. Less than 1% of the dose was retained in the tissues after 7 days. In both species, tolclofos-methyl was mainly matabolised by oxidative desulfuration, oxidation of 4-methyl group, and cleavage of P-O-aryl and P-O-methyl linkages. The major metabolites found in rats and mice were ph-COOH, ph-CH3, TMO-COOH, DM-TMO-COOH and ph-CO-glycine (only in mice) (Figure B.6.1.1-1).

Figure B.6.1.1-1: Proposed metabolic pathway for tolclofos-methyl in rats and mice



B.6.1.2 Single dose (two dose levels) and repeated dose in rats (phenyl-label)

Reference	: Krautter, G.R. et al., 1988a; 1988b	**Exposure**	: Single and repeated administration
Title of study	: Metabolism of tolclofos-methyl in the rat (1988a); Final report amendment: metabolism of tolclofos-methyl in the rat (1988b)	**Dose**	: Group 1: 5 mg/kg bw Group 2: 200 mg/kg bw Group 3: 14 consecutive 5 mg/kg bw/day unlabelled TM followed by a dose of 5 mg/kg bw labelled TM
Test substance	: [phenyl-^{14}C]tolclofos-methyl, batch No.: C-86-125, radiochemical purity: not specified, specific radioactivity: 45.0 mCi/mmol, concentration of 0.408 mCi/ml at 2.72 mg/ml in benzene-hexane solution, and unlabelled tolclofos-methyl, batch No.: 20503, purity: 100%	**Vehicle**	: Corn oil (volume not indicated)
		GLP statement	: Yes
Administration way	: Stomach intubation		
Species	: Sprague-Dawley CD rats	**Guideline**	: FIFRA § 85-1, equivalent to 88/302/EEC, part B, Toxicokinetics
Group size	: 5/sex/dose, control 3/sex	**Acceptability**	: Yes

Materials and methods

Sprague-Dawley CD rats were allocated to four groups as follows:

Group 1) Treated low dose (5 males/females): single dose of 5 mg/kg of [phenyl-^{14}C]tolclofos-methyl; mean actual radiolabelled dose = 69.79µCi/male, 48.31µCi/female.

Group 2) Treated high dose (5 males/females): single dose of 200 mg/kg of [phenyl-^{14}C]tolclofos-methyl; mean actual radiolabelled dose = 65.25µCi/male, 47.26µCi/female.

Group 3) Treated consecutive dose (5 males/females): treatment for 14 consecutive days with 5 mg/kg unlabelled tolclofos-methyl, followed by a single dose of 5 mg/kg of [phenyl-^{14}C]tolclofos-methyl; mean actual radiolabelled dose = 69.50µCi/male, 50.77µCi/female.

Group 4) Control (3 males/females): single dose of corn oil (vehicle).

Bodyweights were measured at time of treatment and at termination. Daily food and water consumption was measured during treatment and collection periods. The elimination of radiocarbon in feces and urine was quantitated at 0.5, 1, 2, 3, 4-5 and 6-7 days post-treatment. Carbon dioxide trapping solution was quantitated at the same collection times until less than 0.1% of the administered dose was present. All animals were sacrificed 7 days post-treatment and residue levels quantitated in blood, bone (femur), brain, fat (visceral), heart, kidney, liver, lungs, muscle (thigh), ovary, skin (clipped), spleen, testes, uterus and the residual carcass. Quantitation of radiocarbon was performed using liquid scintillation counting (lsc).

Findings

Absorption, distribution and excretion:

For all three treated groups, administered radiocarbon was readily excreted, with greater than 95% of the dose eliminated in the urine and feces within 48 hours, where the overall recovery of total radiocarbon after 7 days was 103.8% (Table B.6.1.2-1). Based on the elimination data, high dose males eliminated about two times more radiocarbon in the feces than females. When comparing the effects of a single dose (low dose group) with consecutive doses (at the same daily dosage rate), a shift away from fecal elimination is evident where the average elimination in feces is two-times higher in the low dose group compared to the consecutive dose group. Given the similarities in elimination rates for all three treated groups with the fact that urinary excretion is the primary route of elimination for all groups indicate that the processes of absorption and elimination are relatively unaffected at dosages up to 200 mg/kg bw of tolclofos-methyl.

Table B.6.1.2-1: Metabolism of [phenyl-^{14}C]tolclofos-methyl in the rat: Radiocarbon excretion in urine and feces after 7 days (% of the dose administered)

Group	Low dose		High dose		Consecutive dose	
Excreta	Male	Female	Male	Female	Male	Female
Urine	87.5	91.1	85.4	87.7	88.9	90.2
Feces	20.1	19.3	19.9	11.9	9.3	11.5

Metabolism:

Residue levels:

Total residue levels in tissues of high dose group animals accounted for less than 1.0% of the dose administered.

Radiocarbon characteristics and identity:

More than 10 metabolites were detected in the excreta of male and female rats (Table B.6.1.2-2 and B.6.1.2-3). Generally, no major differences were noted between sexes or between dosage groups. In all cases, tolclofos-methyl was metabolised via oxidative desulfuration of the P=S group to P=O, oxidation at the 4-methyl group, and cleavage of the P-O-aryl and P-O-methyl linkages. The four major metabolites found in excreta were DM-TMO, DM-TM-CH_2OH, DM-TM-COOH and DM-TM (Table B.6.1.2-4).

A proposed metabolic pathway for tolclofos-methyl in the rat is presented in Figure B.6.1.2-1.

Table B.6.1.2-2: Metabolism of [phenyl-^{14}C]tolclofos-methyl in the rat: Identity of metabolites identified

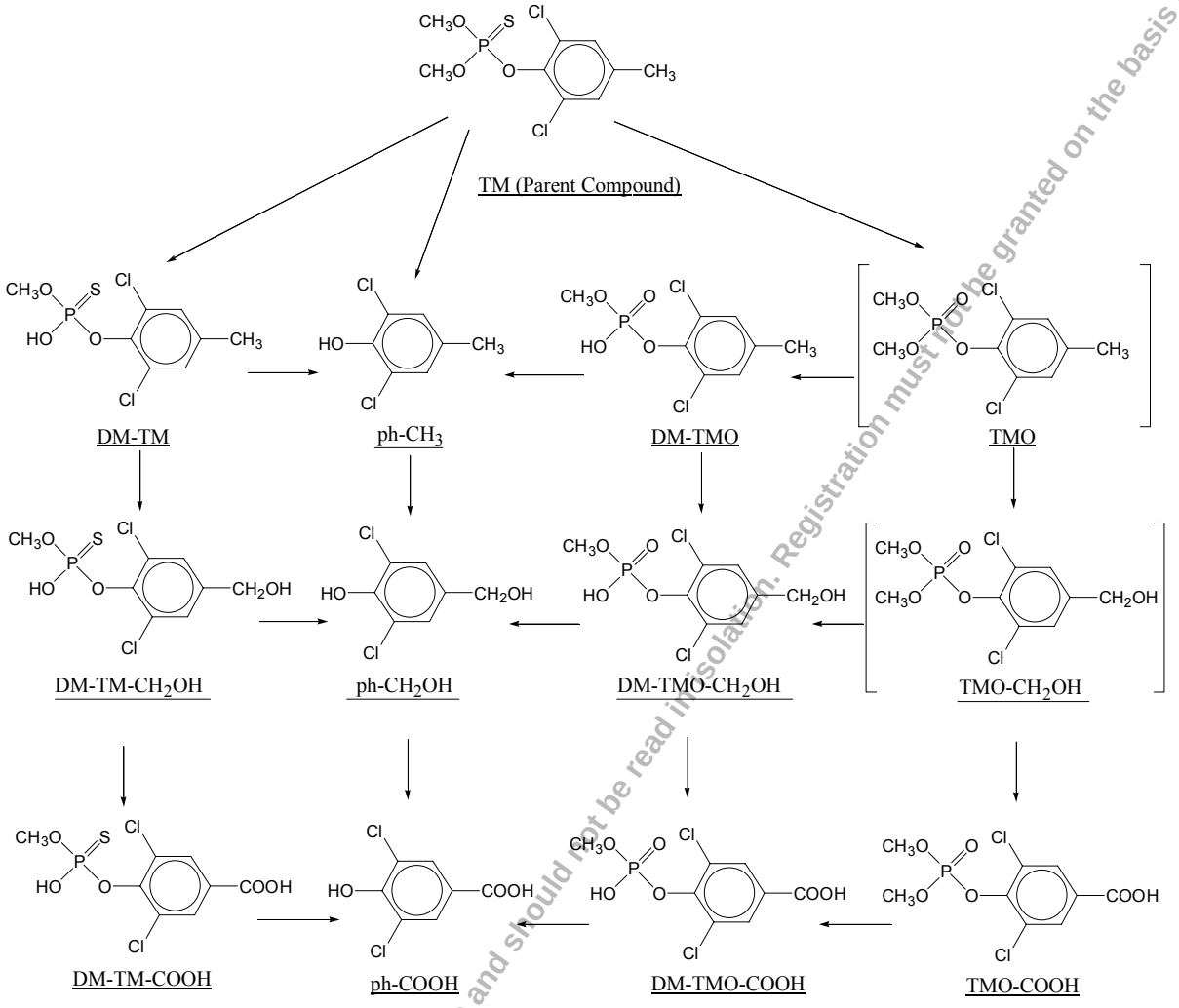
Designation	Chemical name
TM (parent compound)	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
DM-TM	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
TM–CH_2OH	*O,O*-dimethyl *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl]phosphorothioate
DM-TM–CH_2OH	*O*-methyl *O*-hydrogen *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl] phosphorothioate
DM-TM-COOH	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphorothioate
ph-CH_3	2,6-dichloro-4-methylphenol
ph-COOH	3,5-dichloro-4-hydroxybenzoic acid
TMO	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphate
ph-CH_2OH	3,5-dichloro-4-hydroxybenzyl alcohol
TMO-COOH	*O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl) phosphate
DM-TMO	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphate
DM-TMO-CH_2OH	*O*-methyl *O*-hydrogen *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl] phosphate
DM-TMO-COOH	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphate

Table B.6.1.2-3: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Identity and amounts of radiocarbon in excreta (% of the dose administered)

Metabolite	Low dose		High dose		Consecutive dose	
	Male	Female	Male	Female	Male	Female
Urine						
ORIGIN	1.5	1.4	2.0	1.4	2.2	2.3
DM-TMO-CH_2OH	8.6	6.2	5.9	2.3	4.9	6.5
DM-TMO-COOH	8.9	5.7	7.7	4.1	6.9	5.2
DM-TMO	18.8	26.4	10.1	9.8	15.1	23.9
DM-TM-CH_2OH	23.1	19.4	25.3	12.2	25.1	16.9
DM-TM-COOH	13.3	13.5	17.4	34.5	10.5	13.4
DM-TM	5.3	8.3	18.2	27.6	8.4	8.4
TMO-COOH	10.0	9.1	6.8	3.1	8.5	10.0
Ph-COOH	6.4	6.4	3.6	1.8	9.9	5.2
SOLVENT FRONT	2.5	1.4	0.5	0.6	1.1	1.0
REMAINDER [a]	1.7	2.1	2.6	2.7	7.3	7.1
Mean Percent Recovery	91.8	89.6	95.4	99.2	95.6	99.2
Feces						
ORIGIN [b]	53.4	58.8	51.0	37.7	58.9	69.3
DM-TM	6.4	4.0	17.5	15.9	6.5	6.8
TMO-COOH	2.6	1.9	2.3	1.2	2.9	4.1
Ph-CH_2OH	2.1	2.5	2.0	1.4	3.4	3.1
Ph-COOH	5.7	4.8	4.1	2.0	7.6	7.7
UNKNOWN [c]	2.1	1.3	1.5	1.0	1.5	0.9
Ph-CH_3	0.2	0.8	ND [d]	ND	0.5	ND
TM	17.8	16.0	11.8	35.3	10.2	0.8
REMAINDER [a]	10.3	10.4	10.2	6.2	8.8	7.6
Mean Percent Recovery	96.8	98.4	98.4	93.2	99.0	97.6

a: Radioactivity not associated with authentic standards
b: Based on TLC System II analysis of representative sample (animal #80), mixture of DM-TMO, DM-TM-COOH, ph-COOH, DM-TM, TMO-COOH, DM-TMO-COOH, and unresolved material
c: Radioactive band (Rf = 0.54) without a corresponding authentic standard
d: ND: Not detected

Figure B.6.1.2-1: Proposed metabolic pathway for tolclofos-methyl in rats



Conclusions

Tolclofos-methyl was rapidly excreted in rats, mainly in the urine (>85%). Tissue residues 7 days after dosing were generally very low. Less than 1% of the high dose was retained in the tissues after 7 days.

Tolclofos-methyl was mainly metabolised by oxidative desulfuration of the P=S group to P=O, oxidation of 4-methyl group, and cleavage of P-O-aryl and P-O-methyl linkages. The major metabolites found in the rat were DM-TMO, DM-TM-CH_2OH, DM-TM-COOH, DM-TM and TMO-COOH.

B.6.1.3 Single dose (one dose level) in rats (phenyl-label)

Reference	: Esumi, Y., 1989	**Exposure**	: Single administration
Title of study	: Study on metabolism of tolclofos-methyl in rats	**Dose**	: 5 mg/kg bw
Test substance	: [phenyl-^{14}C]tolclofos-methyl, batch No.: C-85-127, radiochemical purity: ≥ 99%, specific radioactivity: 5.55 MBq (150μCi)/mg), and unlabelled tolclofos-methyl, batch No.: 20503, purity: 100%).Unlabelled tolclofos-methyl was added to [phenyl-^{14}C]tolclofos-methyl so that the specific radioactivity became 740 kBq (20 μCi)/mg.	**Vehicle**	: Corn oil (5 ml/kg bw)
Administration way	: Stomach intubation	**GLP statement**	: No
Species	: Sprague-Dawley rats	**Guideline**	: In-house method, in accordance with 88/302/EEC, part B, Toxicokinetics
Group size	: Tissue distribution study: 3/time point/sex, 7 time points, Biliary excretion study (bile-duct cannulated rats): 3/sex (biliary excretion), 3/sex (biliary excretion and metabolite characteristics)	**Acceptability**	: Yes

Materials and methods

The radioactivity given was 3.63 to 3.85 MBq/kg (98 to 104 μCi)/kg. Free access was given to feed and water. Bile, urine and feces were sampled from bile-duct cannulated rats.

Tissues were analysed for level of radioactivity at 30 min, 1, 2, 4, 8, 24 and 72 hours after administration. Radioactivity in bile was measured at 1, 2, 4, 6, 8, 12, 24 and 48 hours after administration, urine and feces was analysed at 6 (urine only), 12, 24 and 48 hours after administration. Radioassay was performed using liquid scintillation counting. Metabolites were determined by TLC.

Findings

Absorption and distribution

Tissue concentrations and percentage distribution:

The radioactivity level reached a peak in 2 hours in almost all tissues; therefore the absorption from the gastrointestinal tract was supposed to be relatively rapid and is estimated to be about 60-70% on the basis of the results of the biliary excretion study. At 2 hours, the concentration in kidneys was the highest and was 3 to 4 times that in plasma, followed by the liver and blood (Table B.6.1.3-1). The level of radioactivity in various organs at 72 hours after administration was 5% or less of the respective peak concentrations.

Table B.6.1.3-1: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Radiocarbon distribution in tissues (dose level: 5 mg/kg)

Tissue	Tissue content (% of dose)						
	30 min	1 hr	2 hr	4 hr	8 hr	24 hr	72 hr
Males							
Blood	0.47	0.67	0.94	0.58	0.35	0.00	0.00
Brain	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eyeball	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Submaxillary gland	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Heart	0.01	0.01	0.02	0.01	0.00	0.00	0.00
Lung	0.01	0.02	0.03	0.02	0.01	0.00	0.00
Liver	0.55	0.94	1.09	0.68	0.47	0.03	0.01
Kidney	0.34	0.57	0.78	0.39	0.23	0.01	0.00
Spleen	0.00	0.01	0.01	0.01	0.00	0.00	0.00
Pancreas	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Adrenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fat	0.04	0.06	0.19	0.29	0.31	0.08	0.00
Muscle	0.32	0.47	0.96	0.45	0.24	0.00	0.00
Skin	0.46	0.80	1.74	1.34	0.76	0.20	0.08
Testis	0.01	0.01	0.02	0.02	0.01	0.00	0.00
Epididymis	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Females							
Blood	0.70	0.62	1.07	0.71	0.39	0.04	0.00
Brain	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Thyroid	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Eyeball	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Submaxillary gland	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Heart	0.01	0.01	0.02	0.01	0.01	0.00	0.00
Lung	0.02	0.02	0.03	0.03	0.02	0.00	0.00
Liver	0.89	0.64	1.02	0.81	0.37	0.05	0.01
Kidney	0.74	0.41	0.56	0.53	0.20	0.03	0.00
Spleen	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Pancreas	0.00	0.00	0.01	0.01	0.00	0.00	0.00
Adrenal	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fat	0.04	0.05	0.25	0.29	0.48	0.11	0.01
Muscle	0.43	0.34	0.86	0.54	0.27	0.00	0.00
Skin	0.70	0.56	1.53	1.63	0.95	0.18	0.06
Uterus	0.01	0.01	0.01	0.01	0.00	0.00	0.00
Ovary	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data are expressed as the mean values for three animals.
Blood, fat, muscle and skin volumes were assumed to be 6.4, 5, 40 and 22 % of body weight, respectively.

Excretion

Excretion into bile:

Excretion into bile in the bile-duct cannulated rats is shown in Table B.6.1.3-2. Cumulative excretion over 48 hours was 5.8 to 11.7% of the dose of radiocarbon in the bile, 46.7 to 59.4% in the urine and 42.3 to 23.7% in the feces, in males and females, respectively.

Table B.6.1.3-2: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Cumulative excretion of radioactivity in bile, urine and feces in bile-duct cannulated rats (dose: 5 mg/kg)

Time (hr)	Excretion of radioactivity (% of dose)					
	Male			Female		
	Bile	Urine	Feces	Bile	Urine	Feces
0 – 1	0.2	/	/	0.4	/	/
2	0.6	/	/	1.1	/	/
4	1.6	/	/	2.7	/	/
6	2.5	6.6	/	3.8	16.6	/
8	3.3	/	/	5.0	/	/
12	4.3	23.8	7.3	7.4	40.0	0.7
24	5.5	43.6	37.2	10.7	56.2	21.2
48	5.8	46.7	42.3	11.7	59.4	23.7

Radioactivity in gastro-intestinal contents at 48 hr: 0.6 ±0.3 % of dose (male); 1.8 ±1.9 % of dose (female)
Radioactivity in carcass at 48 hr: 3.3 ±1.0 % of dose (male); 2.1 ±1.6 % of dose (female)
Data are expressed as the mean values for three animals.
/: Not assayed.

Table B.6.1.3-3: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Radioactivity in bile, urine and feces (0-24 hr) in bile-duct cannulated rats (for metabolite characteristics)

Sample	Excretion of radioactivity (% of dose)	
	Male	Female
Bile	4.6	5.6
Urine	27.0	41.5
Feces	29.4	26.6

Data are expressed as the mean values for three animals.

Metabolism

Radiocarbon characteristics and identity:

Two hours after administration, at least 7 metabolites were detected in the blood, liver and kidney (Table B.6.1.3-4). The main metabolites at 2 hours were TM-COOH, TMO-COOH, Ph-CH_3, Ph-CH_2OH, Ph-COOH, DM-TM and DM-TM-CH_2OH. Only a small amount of the parent compound was detected in the liver. In blood, no parent compound was detected. The main metabolite in the liver and kidney was TM-COOH or Ph-COOH produced by oxidation of the 4-methyl group and cleavage of the P-O-aryl bond.

In bile collected from 0 to 24 hours after administration, were detected the parent compound, TM-COOH, Ph-COOH and DM-TM, although their percentages were low and most of the metabolites were polar, glucuronide of DM-TM-CH2OH and glucuronide of Ph-CH_3 being the representative metabolites (Tables B.6.1.3-4, 5 and 6). Only the parent compound was detected in the feces collected from 0 to 24 hours after administration (Table B.6.1.3-7) as did for the bile, therefore the unabsorbed tolclofos-methyl was supposed to be excreted without being degraded in the gastrointestinal tract.

There was no remarkable sexual difference in regard to radiocarbon concentrations in tissues and identity of the metabolites. Tolclofos-methyl was mainly metabolised via oxidation of the P=S group, oxidation of the 4-methyl group, and cleavage of P-O-aryl and/or P-O-methyl linkages. A proposed metabolic pathway for tolclofos-methyl in rats is presented in Figure B.6.1.3-1.

Table B.6.1.3-4: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Identity of metabolites identified

Designation	Chemical name
TM (parent compound)	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
DM-TM	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphorothioate
DM-TM–CH_2OH	*O*-methyl *O*-hydrogen *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl] phosphorothioate
TM-COOH	*O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl) phosphorothioate
DM-TM-COOH	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphorothioate
ph-CH_3	2,6-dichloro-4-methylphenol
ph-COOH	3,5-dichloro-4-hydroxybenzoic acid
TMO	*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl) phosphate
ph-CH_2OH	3,5-dichloro-4-hydroxybenzyl alcohol
TMO-COOH	*O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl) phosphate
DM-TMO	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl) phosphate
DM-TMO-CH_2OH	*O*-methyl *O*-hydrogen *O*-[2,6-dichloro-4-(hydroxymethyl)phenyl] phosphate
DM-TMO-COOH	*O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl) phosphate

Table B.6.1.3-5: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Identity and amounts of radiocarbon in bile (0-24hr) - dose level 5 mg/kg

Metabolite	Composition of radioactivity (% in bile)			
	Male		Female	
	Solvent 1	Solvent 2	Solvent 1	Solvent 2
TM	4.7	3.4	5.2	3.5
TM-COOH + Ph-COOH	2.7	1.8	2.0	1.3
TMO	ND	ND	ND	ND
TMO-COOH	ND	ND	ND	ND
DM-TM	2.6	1.0	3.6	2.6
DM-TM-CH_2OH	ND	ND	ND	ND
DM-TM-COOH	ND	ND	ND	ND
DM-TMO	ND	ND	ND	ND
Ph-CH_3	ND	ND	ND	ND
Ph-CH_2OH	ND	ND	ND	ND
Ph-CH_3glucuronide	24.3	/	21.5	/
Unknown A	2.1	/	1.7	/
Unknown B	4.6	/	3.6	/
Unknown C	12.2	/	7.9	/
Origin	40.5	86.6[a]	43.2	83.9[a]
Others	6.3	7.2	8.4	5.8
Recovery (%)	100.01		97.1	
Unextractable ^{14}C (%)	0		2.9	

Data are expressed as the mean values for three animals.
a: This fraction contains metabolites whose amounts are shown as /.
ND: Not detected.
Solvent system: Solvent 1: toluene:ethyl acetate:2-propanol:acetic acid (8:12:5:3);
Solvent 2: chloroform:methanol (3:1).

Table B.6.1.3-6: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Identity and amounts of radiocarbon in bile (0-24hr) - dose level 5 mg/kg

Metabolite	Composition of radioactivity (% in bile)	
	Male	Female
	Solvent 3	Solvent 3
DM-TM-CH_2OH glucuronide	30.6	35.1
Origin	ND	ND
Others**	69.4	61.9
Recovery (%) *	100.1	97.1
Unextractable ^{14}C (%)	0	2.9

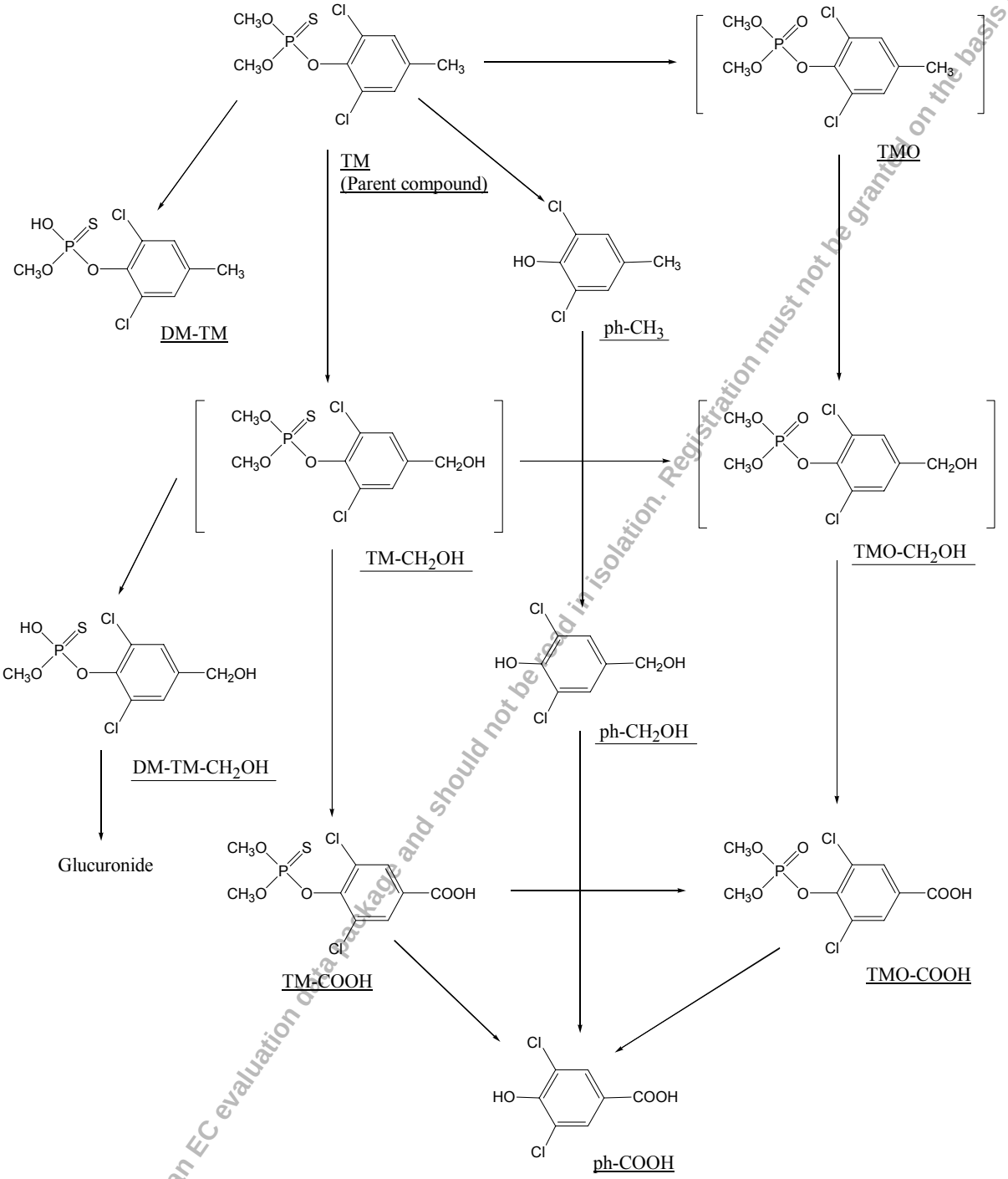
***: Other metabolites are contained in this fraction. (Rf 0.36~0.80).*
ND: Not detected
Solvent Data are expressed as the mean values for three animals.
**: Bile was extracted with methanol after lyophilization.*
system: Solvent 3: 1-butanol:acetic acid:water (5:1:1)

Table B.6.1.3-7: Metabolism of [phenyl-^{14}C]tolclofos-methyl in rats: Identity and amounts of radiocarbon in feces (0-24hr) - dose level 5 mg/kg

Metabolite	Composition of radioactivity (% in feces)			
	Male		Female	
	Solvent 1	Solvent 2	Solvent 1	Solvent 2
TM	96.0	95.6	96.9	96.2
TM-COOH	ND	ND	ND	ND
TMO	ND	ND	ND	ND
TMO-COOH	ND	ND	ND	ND
DM-TM	ND	ND	ND	ND
DM-TM-CH_2OH	ND	ND	ND	ND
DM-TM-COOH	ND	ND	ND	ND
DM-TMO	ND	ND	ND	ND
Ph-CH_3	ND	ND	ND	ND
Ph-CH_2OH	ND	ND	ND	ND
Ph-COOH	ND	ND	ND	ND
Origin	ND	ND	ND	ND
Others	0.7	1.1	0.5	1.2
Recovery (%) *	96.7		97.4	
Unextractable ^{14}C (%)	3.3		2.6	

Data are expressed as the mean values for three animals.
**: Feces were extracted with methanol.*
ND: Not detected
Solvent system: Solvent 1: toluene:ethyl acetate:2-propanol:acetic acid (8:12:5:3).
Solvent 2: chloroform:methanol (3:1).

Figure B.6.1.3-1: Proposed metabolic pathway for tolclofos-methyl in rats



Conclusions

The absorption from the gastrointestinal tract was supposed to be relatively rapid and is estimated to be about 60-70% under the conditions of this study. Tolclofos-methyl and its metabolites were found in a concentration of 5% or less of the administrated dose in rat tissue. These results indicated that distribution of tolclofos-methyl into tissues is small and that persistence of the compound over a long period is unlikely.

Only the parent compound was detected in the feces collected from 0 to 24 hours after administration; therefore the unabsorbed tolclofos-methyl was supposed to be excreted without being degraded in the gastrointestinal tract.

Tolclofos-methyl was mainly metabolised via oxidation of the P=S group, oxidation of the 4-methyl group, and cleavage of P-O-aryl and/or P-O-methyl linkages. The major metabolites of tolclofos-methyl found in the bile were ph-CH_3glucuronide and DM-TM-CH_2OH glucuronide.

B.6.1.4 Summary and conclusions on absorption, distribution, excretion and metabolism studies in mammals

Absorption

The results of the rats and mice metabolism studies indicate that most of the ^{14}C tolclofos-methyl-derived radioactivity was rapidly eliminated by male and female rats at both high (200 mg/kg) and low (5 mg/kg) dose levels, and by male and female mice at the low dose level. Most of the radioactivity was eliminated within the first 48 hours. The similarities in elimination rates for all groups and the fact that urinary excretion is the primary route of elimination, indicate that the processes of absorption and elimination are relatively unaffected at dosages up to 200 mg/kg bw. The absorption from the gastrointestinal tract was supposed to be relatively rapid and is estimated to be higher than 78% based on the biliary excretion study, which indicated that the unabsorbed tolclofos-methyl was excreted without any metabolic transformation in the gastrointestinal tract.

Distribution

Distribution of tolclofos-methyl into tissues is small and persistence of the compound over a long period is unlikely.

Excretion

Tolclofos-methyl was readily excreted in rats and mice, mainly in the urine. Less than 1% of the dose was retained in the tissues after 7 days. In another study, excretion into bile in the bile-duct cannulated rats showed that cumulative excretion over 48 hours was 5.8 to 11.7% of the dose of ^{14}C in the bile, 46.7 to 59.4% in the urine and 42.3 to 23.7% in the feces, in males and females respectively (dose level: 5 mg/kg). Only the parent compound was detected in feces and bile when collected 0 to 24 hours after administration. The unabsorbed tolclofos-methyl was therefore supposed to be excreted without being degraded in the gastrointestinal tract.

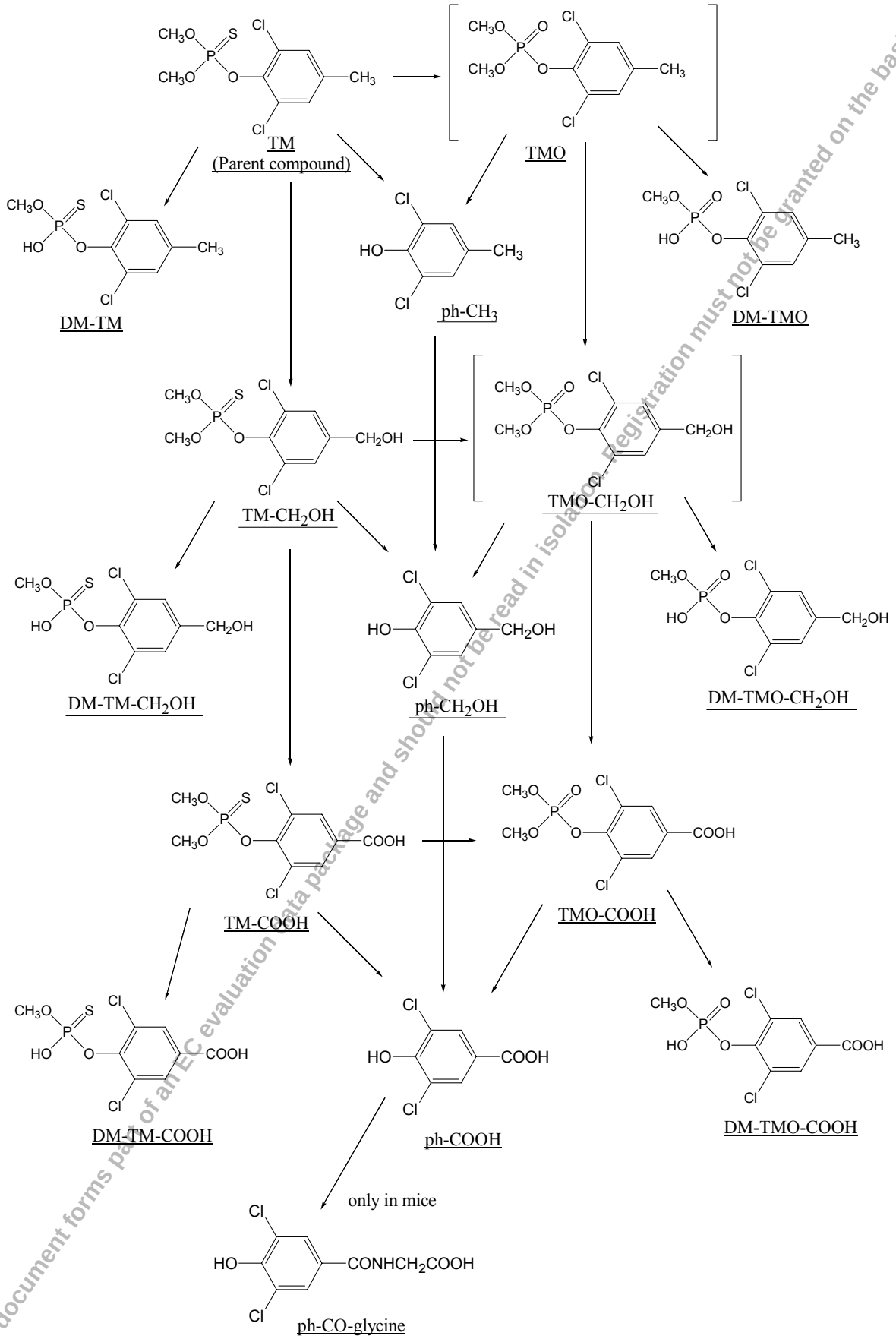
Metabolism

In both rats and mice, tolclofos-methyl was mainly metabolised via oxidative desulfuration of the P=S group to P=O, oxidation of 4-methyl group, and cleavage of P-O-aryl and P-O-methyl linkages.

The major metabolite in both rats and mice was 3,5-dichloro-4-hydroxybenzoic acid. This metabolite was excreted as the glycine conjugate in mice and as the free form in rats.

The proposed metabolic pathway for tolclofos-methyl in rats and mice is shown in Figure B.6.1.2-1

Figure B.6.1.2-1: Proposed metabolic pathway for tolclofos-methyl in rats and mice



B.6.2 Acute toxicity including irritancy and skin sensitization (Annex IIA 5.2)

B.6.2.1 Oral

B.6.2.1.1 Rat and mouse

Reference	: Segawa, T., 1978	**Exposure**	: Single administration
Title of study	: Acute toxicity of S-3349 in rats and mice	**Dose**	: Rats: 1000, 2500, 3750, 5000 mg/kg bw Mice: 1000, 1500, 2000, 3000, 4000 mg/kg bw
Test substance	: Test material: Tolclofos-methyl, batch No.: 524, purity: 97.0%, specification No. 01	**Vehicle**	: Corn oil (5 ml/kg bw (rats), 25 ml/kg bw (mice))
Administration way	: Oral *via* gavage	**GLP statement**	: No
Species	: Sprague-Dawley rats and dd mice	**Guideline**	: In-house method, in accordance with 92/69/EEC, B.1
Group size	: 10/sex/dose	**Acceptability**	Yes
		LD_{50}	: Rats: 5000 mg/kg bw/day Mice: 3500 mg/kg bw/day

Materials and methods

See above.

According to guidelines too much vehicle has been administered to the mice. No more than 10 ml/kg bw should be given.

Findings

At the highest dose level of 5000 mg/kg bw in rats, dead animals, five for male and six for female, were found within 1-5 days post-treatment (Table B.6.2.1.1-1). Toxic symptoms such as decrease of spontaneous motor activity, irregular respiration, dyspnea, piloerection, incontinence of urine and ataxia of hind limb or whole body developed 3-4 hours after administration. The minimum toxic dose level was 3750 mg/kg bw for both sexes. At gross necropsy, no visible lesions were observed.

In mice, the toxic symptoms were essentially similar to those of rats. The minimum toxic dose level in mice was 1500 mg/kg bw for both sexes.

Table B.6.2.1.1-1: Acute oral toxicity of tolclofos-methyl

	Males			Females		
	Dose (mg/kg)	Cumulative Mortality	Time of death, No of animals	Dose (mg/kg)	Cumulative Mortality	Time of death No of animals
Rat	1000	0	-	1000	0	-
	2500	0	-	2500	0	-
	3750	0	-	3750	0	-
	5000	5/10*	Day3: 4; Day5: 1	5000	6/10*	Day1: 1; Day2: 2; Day3: 3
Mouse	1000	0	-	1000	0	-
	1500	1/10*	Day2: 1	1500	0	-
	2000	2/10*	Day1: 1; Day2: 1	2000	1/10*	Day1: 1
	3000	3/10*	Day 1: 3	3000	2/10*	Day1: 2
	4000	6/10*	Day1: 5; Day2: 1	4000	6/10*	Day1: 2; Day2: 4

**: Number of animals which died/number of animals used*

Conclusions The oral LD_{50} values of tolclofos-methyl were about 5000 mg/kg bw in male and female rats, and 3500 mg/kg bw (male) or 3600 mg/kg bw (female) in mice.
In accordance with the provisions of Council Directive 67/548/EEC, classification of tolclofos-methyl is not required.

B.6.2.1.2 Rat

Reference	: Kynoch, S. R., 1985a	**Exposure**	: Single exposure
Title of study	: Acute oral toxicity to rats of Rizolex technical	**Dose**	: 500, 1000, 5000 mg/kg bw (initial study), 5000 mg/kg bw (main study)
Test substance	: Test material: Tolclofos-methyl, batch No.: 40810, purity: 97.7%, specification No. 01	**Vehicle**	: Corn oil (<10 ml/kg bw)
Administartion way	: Oral *via* gavage	**GLP statement**	: Yes
Species	: Sprague-Dawley rats	**Guideline**	: FIFRA § 81-1 in accordance with 92/69/EEC, B.1bis
Group size	: 2/sex/dose (initial study) 5/sex/dose (main study)	**Acceptability**	: Yes
		LD_{50}	: > 5000 mg/kg bw/day

Materials and methods

No abnormal sign or death was found in the initial study at 5000 mg/kg bw in both sexes. Therefore a limit test at 5000 mg/kg bw was performed using a group of five male and five female rats.

Findings

Pilo-erection and abnormal body carriage (hunched posture) were observed in all rats shortly after dosing (Table B.6.2.1.2-1). Recovery as judged by external appearance and behaviour was apparently complete by day 5. Bodyweight gains were observed in all rats on days 8 and 15. Terminal autopsy findings were normal.

Table B.6.2.1.2-1: Reactions to treatment observed in rats dosed orally with tolclofos-methyl (5000 mg/kg)

Signs	Number of dead animals/number of rats showing signs/number of rats used	
	Male	Female
Pilo-erection	0/5/5	0/5/5
Abnormal body carriage (hunched posture)	0/5/5	0/5/5

Conclusions The acute oral LD_{50} value of tolclofos-methyl was found to be greater than 5000 mg/kg bw in rats (there were no mortalities).
In accordance with the provisions of Council Directive 67/548/EEC, classification of tolclofos-methyl is not required.

B.6.2.1.3 Rat (cholinesterase estimation)

Reference	: Parcell, B. I. et al., 1994	**Exposure**	: Single administration (2 mg/kg way was administered at four occasions (4*0.5mg/kg bw) two hours apart)
Title of study	: Tolclofos-methyl technical, Code CR21214, rat acute oral toxicity with cholinesterase estimations	**Dose**	: 2, 200, 5000 mg/kg bw
Test substance	: Tolclofos-methyl, batch No.: CR21214/01/93071, purity: 98.6%, specification No. 01	**Vehicle**	: Corn oil (10 ml/kg bw)
Administration way	: Oral *via* gavage	**GLP statement**	: Yes
Species	: Sprague-Dawley rats	**Guideline**	: In-house method, in accordance with 92/69/EEC, B.1
Group size	: 10 females/dose	**Acceptability**	: Yes
		LD_{50}	: >5000 mg/kg bw

Materials and methods

Surviving animals were killed 8 hours after dosing. Blood samples (1.0 ml) were withdrawn from the orbital sinus of all rats during the week prior to dosing and placed into heparinised tubes. A similar amount was removed 3 hours after dosing and 8 hours after dosing (at termination). For animals treated at 2 mg/kg bw, this was 3 hours and 8 hours respectively, after the fourth dose. At termination, the animals were killed and the brain was removed before brain cholinesterase determination. For actylcholinesterase determinations each animal served as its own control.

Findings

General observations:

One animal treated at 200 mg/kg bw died from the effects of ether inhalation during the blood sampling procedure three hours after dosing (Table B.6.2.1.3-1). This animal only showed piloerection prior to death. Macroscopic examination revealed petechiae of the lungs. Death was not considered to be treatment-related.

Table B.6.2.1.3-1: Acute oral toxicity of tolclofos-methyl in female rats: Mortality

Dose (mg/kg)	Cumulative Mortality	Time of death
2	0	-
200	1*/10	3 hours
5000	0	-

**: The death of this rat was not considered to be treatment related, but to be attributable to ether inhalation during blood sampling procedure.*

Piloerection was observed in all rats one hour after dosing and throughout the remainder or the majority of the remainder day. This sign was accompanied three hours after dosing or later by: hunched posture in all surviving rats from all groups, ungroomed appearance and greasy fur in all rats at 2 mg/kg bw, liquid faeces in all rats at 2 mg/kg bw and all surviving rats at 200 mg/kg bw, partially closed eyes in all rats at 5000 mg/kg bw. Clinical signs were still evident at study termination.

Bodyweights:

There were no marked differences between the groups or group mean bodyweights.

Blood cholinesterase determination:

Group mean acetylcholinesterase levels (plasma, RBC and brain) were comparable for all three treated groups.

Macroscopic pathology:

The macroscopic examination performed at termination revealed no changes attributable to treatment with technical tolclofos-methyl. Brain weights were similar for all treated groups.

Conclusions A single oral dose of 5000 mg/kg technical tolclofos-methyl was well tolerated by female rats with only minimal clinical signs of toxicity. The acute oral LD_{50} value of tolclofos-methyl was found to be greater than 5000 mg/kg bw.
There was no evidence of acetylcholinesterase inhibition.

B.6.2.1.4 Dog

Reference	: Pence. D.H., 1978	**Exposure**	: Single administration
Title of study	: Acute oral toxicity study in male and female dogs – S3349	**Dose**	: Fasted dogs: 100, 1000 mg/kg bw Non fasted dogs: 215, 464 mg/kg bw
Test substance	: Tolclofos-methyl, batch No.: 4, purity: 98.7%, specification No. 01	**Vehicle**	: Gelatine capsules
Administration way	: Oral *via* gavage	**GLP statement**	: No
		Guideline	: In-house method, in accordance with 92/69/EEC, B.1
Species	: Beagle dog		
Group size	: 2/sex/dose	**Acceptability**	Yes
		LD_{50}	: >1000 mg/kg bw

Materials and methods

See above.

According to guidelines, dog is not a required species.

Findings

There were no deaths during the study. In addition there were no treatment-related effects on cholinesterase activities at any of the dose levels tested. Emesis occurred in all dogs receiving 1000 mg/kg bw and soft stools or diarrhea was observed in many of the dogs that received lower doses.

Conclusions The LD_{50} of tolclofos-methyl in dogs is estimated to be greater than 1000 mg/kg bw.

B.6.2.2 Percutaneous

B.6.2.2.1 Acute dermal toxicity to rats and mice

Reference	: Segawa, T., 1978	**Exposure**	: 24 hours
Title of study	: Acute oral toxicity of S-3349 in rats and mice	**Dose**	: Rats: 1000, 2500, 5000 mg/kg bw Mice: 1000, 2500, 5000 mg/kg bw
Test substance	: Test material: Tolclofos-methyl, batch No.: 524, purity: 97.0%, specification No. 01	**Vehicle**	: Corn oil (10 ml/kg bw)
Administration way	: Dermal	**GLP statement**	: No
Species	: Sprague-Dawley rats and dd mice	**Guideline**	: In-house method, in accordance with 92/69/EEC, B.3
Group size	: 10/sex/dose	**Acceptability**	: Yes
		LD_{50}	: >5000 mg/kg bw/day

Materials and methods

Tolclofos-methyl was spread over a sheared area of 30 cm^2 and 3 cm^2 in rats and mice, respectively. The treatred area was covered with surgical tape for 24 hours.

Findings

Neither toxic symptoms nor death was observed at any dose level. At gross necropsy, no visible lesions were observed.

Conclusions The dermal LD_{50} values of tolclofos-methyl in rats and mice were determined to be greater than 5000 mg/kg bw.

In accordance with the provisions of Council Directive 67/548/EEC, classification of tolclofos-methyl is not required.

B.6.2.2.2 Acute dermal toxicity to rabbits

Reference	: Kynoch, S. R., Parcell, B. I., 1985b	**Exposure**	: 24 hours
Title of study	: Acute dermal toxicity to rabbits of Rizolex technical	**Dose**	: 2000 mg/kg bw
Test substance	: Tolclofos-methyl, batch No.: 40810, purity: 97.7%, specification No. 01	**Vehicle**	: Test material moistened with distilled water (1 ml/kg)
Administration way	: Dermal	**GLP statement**	: Yes
Species	: New Zealand White rabbit	**Guideline**	: FIFRA § 81-2, in accordance with 92/69/EEC, B.3
Group size	: 5/sex/dose	**Acceptability**	: Yes
		LD_{50}	: > 2000 mg/kg bw

Materials and methods

Tolclofos-methyl was applied to the shaved skin and held in place with an occlusive wrapping. At the end of the 24-hour exposure period, the dressings were carefully removed and the treated area of skin decontaminated by washing in warm (30-40°C) water and blotting dry with absorbent paper. The animals were observed during 15 days following exposure.

Findings

One female rabbit was found dead on day 5. In view of the delayed onset of clinical signs and the absence of clinical signs in any of the other treated rabbits at the time it is thought that the death of this rabbit was unlikely to be related to tolclofos-methyl although the exact cause of death was not established (Table B.6.2.2.2-1). One female rabbit did not eat normally during days 9 to 13 and showed pilo-erection on day 11. Recovery was complete by day 14. It is not clear if this has a connection to the test substance.

Table B.6.2.2.2-1: Acute dermal toxicity of tolclofos-methyl

Males			Females		
Dose (mg/kg)	Cumulative Mortality	Time of death	Dose (mg/kg)	Cumulative Mortality	Time of death
2000	0	-	2000	1/5*	Day 5

**: Number of animals which died/number of animals used*

Conclusions

The dermal LD_{50} value of tolclofos-methyl in rabbits was found to be greater than 2000 mg/kg bw.

In accordance with the provisions of Council Directive 67/548/EEC, classification of tolclofos-methyl is not required.

B.6.2.3 Inhalation

B.6.2.3.1 Inhalation: rat

Reference	: Hardy, C.J. et al., 1986	**Exposure**	: 4 h
Title of study	: Tchnical Rizolex – Acute inhalation toxicity in rats – 4-hour exposure	**Dose**	: 0, 1350, 3320 mg/m^3
Test substance	: Tolclofos-methyl, batch No.: 40810, purity: 97.7%, specification No. 01	**Vehicle**	: Air
Administration way	: Inhalation	**GLP statement**	: Yes
Species	: Wistar albino rats	**Guideline**	: In.house method, in accordance with 92/69/EEC, B.2
Group size	: 5/sex/dose	**Acceptability**	: Yes
		LC_{50}	: >3320 mg/m^3

Materials and methods

Rats (whole body) were exposed to tolclofos-methyl was administered by inhalation of a test atmosphere containing dust generated from the test substance. The rats were observed for a 14 day period following exposure.

Findings

There were no deaths during the study. Signs consistent with exposure to high concentration of a mildly irritant dust were noted, including closing or partial closing of the eyes, abnormal body position and abnormal breathing. Abnormal respiratory pattern was observed immediately after exposure. No other significant signs were noted during the observation period. Small losses of body weight or a reduction in rate of gain over 3 days following exposure to tolclofos-methyl were observed, with marginal reduction in food consumption. There were no observable abnormalities at gross necropsy. The lung weights to body weight ratios were within normal limits. The microscopic examination showed no treatment-related changes.

Conclusions The acute LC_{50} by inhalation of tolclofos-methyl in albino rats was determined to be greater than 3320 mg/m^3 (maximum concentration which can be tested).
In accordance with the provisions of Council Directive 67/548/EEC, classification is not required.

B.6.2.4 Skin irritation

B.6.2.4.1 Rabbit (1st study)

Reference	: Matsubara, T. et al., 1978	**Exposure**	: 4 h
Title of study	: Eye and skin irritation test of S-3349 in rabbits	**Dose**	: 500 mg/animal
Test substance	: Tolclofos-methyl, batch No.: 524, purity 97.0%, specification No. 01	**Vehicle**	: Vaseline
Administration way	: Dermal	**GLP statement**	: No
Species	: Albino rabbits	**Guideline**	: U.S. Federal Register, vol. 37, 1972, in accordance with 92/69/EEC, B.4
Group size	: 6/dose (males)	**Acceptability**	: Yes
		Result	: Not irritant

Materials and methods

Tolclofos-methyl was applied to each abraded and intact area of skin; each treatment site (approximately 38.1 mm x 38.1 mm) was covered with an occlusive tape. Examination was performed at 1, 24, 48, 72 hours and finally after 7 days post application.

Findings

Tolclofos-methyl did not produce any irritant reactions, such as erythema, eschar and oedema.

Conclusions In accordance with the criteria specified in Council Directive 67/548/EEC, tolclofos-methyl is not classified as a skin irritant.

B.6.2.4.2 Rabbit (2nd study)

Reference	: Liggett, M.P., Parcell, B.I 1985a	**Exposure**	: 4 h
Title of study	: Irritant effects on rabbit skin of Technical Rizolex	**Dose**	: 500 mg/animal
Test substance	: Tolclofos-methyl, batch No.: 40810, purity 97.7%, specification No. 01	**Vehicle**	: Not indicated
Administration way	: Dermal	**GLP statement**	: Yes
Species	: New Zealand white rabbit	**Guideline**	: FIFRA § 81-5, in accordance with 92/69/EEC, B.4
Group size	: 6/dose (female)	**Acceptability**	: Yes
		Result	: Not irritant

Materials and methods

Tolclofos-methyl was applied under a 2.5 cm square gauze pad moistened with 0.5 ml of distilled water, to the shaved skin of the rabbits. Each treatment area was covered with elastic adhesive dressing (semi-occlusive).

Findings

Examination of the treated skin was made on day 1 (i.e. approximately 30 minutes after removal of the patches) and on days 2, 3 and 4. None of the animals showed any observable response to treatment throughout the four-day observation period.

Conclusions On the basis of the degree of skin reaction and the criteria specified in Council Directive 67/548/EEC, tolclofos-methyl is not classified as a skin irritant.

B.6.2.5 Eye irritation

B.6.2.5.1 Rabbit (1st study)

Reference	: Matsubara, T. et al., 1978	**Exposure**	: Group I: 5 minutes Group II: 24 hours
Title of study	: Eye and skin irritation test of S-3349 in rabbits	**Dose**	: 50 mg/animal
Test substance	: Tolclofos-methyl, batch No.: 524, purity 97.0%, specification No. 01	**Vehicle**	:
Administration way	: Eye	**GLP statement**	: No
Species	: Albino rabbits	**Guideline**	: U.S. Federal Register, vol. 37, 1972, in accordance with 92/69/EEC, B.5
Group size	: 5/dose (groupI), 3/dose (groupII) (males)	**Acceptability**	: Yes
		Result	: Not irritating to eyes

Materials and methods

Tolclofos-methyl/animal was applied into the left eye of 8 rabbits; right eye served as a control.
Group I (5 rabbits): 5 min after application, the eyes were washed with 300 ml of physiological saline for 2 min;
Group II (3 rabbits): 24 h after application, the eyes were washed as described above.

Findings

Examination of the ocular responses was made 1, 24, 48, 72 hours and 7 days after exposure. There were no irritant reactions, such as hyperaemia, swelling, opacity, ulceration, necrosis etc. observed in conjunctiva, cornea and iris in eyes treated with tolclofos-methyl.

Conclusions On the basis of the reactions observed and the criteria specified in Council Directive 67/548/EEC, classification is not required.

B.6.2.5.2 Rabbit 2nd study

Reference	: Liggett, M.P., Parcell, B.I., 1985a	**Exposure**	: Singel instillation
Title of study	: Irritant effects on the rabbit eye of Technical Rizolex	**Dose**	: 75 mg/animal
Test substance	: Tolclofos-methyl, batch No.: 40810, purity 97.7%, specification No. 01	**Vehicle**	:
Administration way	: Eye	**GLP statement**	: Yes
Species	: New Zealand white rabbit	**Guideline**	: FIFRA § 81-5, in accordance with 92/69/EEC, B.5
Group size	: 6/dose (female)	**Acceptability**	: Yes
		Result	: Not irritating to eyes

Materials and methods

Tolclofos-methyl occupying a volume of 0.1 ml was applied into the lower everted lid of the left eye of each rabbit; the eyelids were then gently held together for one second before releasing. The contralateral eye remained untreated and served as control. Examination of the eyes was made after 1 hour and 1, 2, 3, 4 and 7 days after instillation.

Findings:

No corneal damage or iridial inflammation was observed in any of the animals. Transient mild conjunctival reactions with slight to moderate discharge were observed in the six animals (Table B.6.2.5.2-1). The eyes were normal one or two days after instillation.

Table B.6.2.5.2-1: Ocular reactions elicited by tolclofos-methyl in rabbits

	Cornea						Iris						Conjunctiva																	
													Redness						Chemosis						Discharge					
time/rabbit	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
1 hour	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1
Day 1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Day 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mean score Day 1-3	0.0						0.0						0.06						0.0						0.0					

Conclusion: On the basis of the eye reactions observed and the criteria specified in Council Directive 67/548/EEC, tolclofos-methyl is not classified as an eye irritant.

B.6.2.6 Skin sensitization

B.6.2.6.1 Skin sensitization test in guinea pigs (Buehler test)

Reference	: Seaber, J. A., 1985	**Exposure**	: 9 x 6 hours (induction), 6 hours (challenge)
Title of study	: Delayed contact hyper-sensitivity in the guinea-pig with technical Rizolex	**Dose**	: 0.5 ml 50% w/w/animal
Test substance	: Tolclofos-methyl, batch No.: 40810, purity 97.7%, specification No. 01	**Vehicle**	: Acetone
Administration way	: Dermal	**GLP statement**	: Yes
Species	: Albino guinea pig (Hartley/Duncin strain)	**Guideline**	: 92/69/EEC, B.6
Group size	: 10/dose (female)	**Acceptability**	: Yes
		Result	: Not sensitizising

Materials and methods

Tolclofos-methyl was applied to the skin and the treated area was covered by impermeable plastic adhesive tape and secured by elastic adhesive bandage for six-hour exposure. Nine induction applications were performed three times a week during a three-week period. The treated animals were challenged topically for 6 hours two weeks after the last induction using tolclofos-methyl. The control animals were treated similarly with the exception that the test compound was omitted from the induction applications.
The challenge sites were evaluated 24, 48 and 72 hours after removal of the patch.
According to guidelines unnecessary many inductions have been performed.

Findings

Range finding for induction and challenge:

The topical irritancy of a range of dilutions of tolclofos-methyl in acetone was investigated to identify (a) a slightly irritant concentration, where possible, suitable for the induction phase of the main study and (b) a non-irritant concentration for the challenge phase. The following concentrations were selected: induction: 50% w/w in acetone; challenge: 50% w/w in acetone.

Observations during the main study:

After several applications of tolclofos-methyl during induction, some irritation was noted on the induction site in the test group; no irritation occurred with the vehicle in the control group. After challenge, no dermal reactions were seen in any of the test or control animals.

Conclusions In accordance with the provisions of Council Directive 67/548/EEC, classification is not required. In this study tolclofos-methyl did not produce evidence of delayed hypersensitivity in any of the treated animals.

B.6.2.6.2 Skin sensitisation test in guinea pigs (Maximization)

Reference	: Nakamura, Y., 2001	**Exposure**	: 48 hours (induction), 24 hours (challenge)
Title of study	: Skin sensitisation test of tolclofos-methyl in guinea-pigs (Maximization test)	**Dose**	: Dose finding study: 0.1, 0.2, 0.5, 1, 2, 5% (induction), 5, 10 , 25% (challenge). Main study: 5% (induction), 10% (challenge)
Test substance	: Tolclofos-methyl, batch No.: 90437G, purity: 98.0%, specification No. 01	**Vehicle**	: Corn oil (0.1 ml ; intradermal injection), acetone (0.2 ml; topical application)
Administration way	: Intradermal injection, topical and epidermal application	**GLP statement**	: Yes
Species	: Hartley guinea pigs	**Guideline**	: 92/69/EEC B.6, OECD 406, JMAFF 12 Nohsan 8147
Group size	: 3/dose (dose finding study), 20/dose (main study), 10 (negative control), 5 (positive control) (females)	**Acceptability**	: Yes
		Result	: Sensitizising

Materials and methods

Induction was performed by intradermal injections of Freund Complete Adjuvant (maximization test) and the test material (day 1), then topical application of the test material for 48 hours (day 8) (See Table B.6.2.6.2-1). Challenge was performed for 24 hours two weeks after the second sensitization, and the sites were examined 24 and 48 hours after removal of the patches. A dose finding study was performed and the doses used are listed in the above table.

Table B.6.2.6.2-1: Group treatment during the sensitization test in guinea-pigs

Treatment	Group			
	Tolclofos-methyl sensitized	**Tolclofos-methyl control**	**HCA sensitized**	**HCA control**
Number of animals	20	10	5	5
Induction: intradermal injections	5% tolclofos-methyl in corn oil	corn oil	5% HCA in corn oil	corn oil
Induction: 48 hr epidermal application	25% tolclofos-methyl in acetone	acetone	100% (undiluted) HCA	-
Challenge: 24 hr epidermal application	10% tolclofos-methyl in acetone	10% tolclofos-methyl in acetone	10% HCA in acetone	10% HCA in acetone

HCA: alpha-hexylcinnamaldehyde

Findings

Range finding for induction and challenge: The irritancy potential of a range of dilutions of tolclofos-methyl in corn oil or acetone was investigated to identify (a) a slightly irritant concentration, if possible, by intradermal injections and by topical application, suitable for the induction phase of the main study and (b) a non-irritant concentration by topical application for the challenge phase. The following concentrations were selected: induction: 5% in corn oil for the intradermal injections and 25% for the topical application; challenge: 10% in acetone.

Observations during the main study: After challenge, slight to moderate erythema and swelling were seen in 7 and 5 out of 20 animals, respectively (Table B.6.2.6.2-2). No skin reactions were seen in any of control animals. Slight to moderate erythema and swelling were observed in the HCA-treated animals.

Table B.6.2.6.2-2: Results of the skin sensitization test

Group		**Tolclofos-methyl sensitized**				**Tolclofos-methyl control**				**HCA sensitized**				**HCA control**			
Material used for the induction treatment		tolclofos-methyl				-				HCA				-			
Material used for challenge treatment		tolclofos-methyl				tolclofos-methyl				HCA				HCA			
Concentration (%)		10				10				10				10			
Number of animals used		20				10				5				5			
Observation time		24 hrs		48 hrs		24 hrs		48 hrs		24 hrs		48 hrs		24 hrs		48 hrs	
Skin reaction*		E	S	E	S	E	S	E	S	E	S	E	S	E	S	E	S
Grade**	**0**	13	16	13	15	10	10	10	10	0	1	0	1	5	5	5	5
	1	5	3	5	3	0	0	0	0	2	3	4	3	0	0	0	0
	2	2	1	2	2	0	0	0	0	3	1	1	1	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

** E: Erythema, S: Swelling*
*** 0: No reaction, 1: Slight, 2: Moderate, 3: Severe*

Conclusions Tolclofos-methyl showed a sensitizing potential in a maximization test.
In accordance with the provisions of Council Directive 67/548/EEC, tolclofos-methyl is classified as sensitising and assigned the symbol Xi'. Based on the toxicological sensitisation properties tolclofos-methyl should be labelled with the risk phrase **"May cause sensitisation by skin contact" R43**.

B.6.2.7 Summary and conclusions on acute toxicity studies

Study	Dose levels	Results
Segawa, 1978 Acute oral toxicity study in rats and mice.	Rats: 1000, 2500, 3750 or 5000 mg/kg bw Mice: 1000, 1500, 2000, 3000 or 4000 mg/kg bw	Rats: LD_{50} around 5000 mg/kg bw Mice: LD_{50} 3500 mg/kg (male), 3600 mg/kg (female)
Kynoch, 1985a Acute oral toxicity study in rats	500*, 1000* or 5000 mg/kg bw	LD_{50} > 5000 mg/kg bw
Pence 1978 Acute oral toxicity study in dogs	100, 215, 464 or 1000 mg/kg bw	LD_{50} > 1000 mg/kg bw
Parcell et al., 1994 Acute oral toxicity study in rats (Cholinesterase estimations)	2, 200 or 5000 mg/kg bw	LD_{50} > 5000 mg/kg bw No effect on cholinesterase levels
Segawa 1978 Acute dermal LD_{50} in rats and mice	Rats: 1000, 2500 or 5000 mg/kg bw Mice: 1000, 2500 or 5000 mg/kg bw	LD_{50} > 5000 mg/kg LD_{50} > 5000 mg/kg
Kynoch et al., 1985b Acute dermal LD_{50} in rabbits	2000 mg/kg bw	LD_{50} > 2000 mg/kg
Hardy et al., 1986 Acute inhalation LC_{50} (4 hours) in rats	0, 1.35 or 3.32 mg/l	LC_{50} > 3.32 mg/l
Matsubara et al., 1978 Acute skin irritation in rabbits	500 mg	Non irritant
Ligget et al., 1985a Acute skin irritation in rabbits	500 mg	Non irritant
Matsubara et al., 1978 Acute eye irritation in rabbits	50 mg	Non irritant
Ligget et al., 1985a Acute eye irritation in rabbits	75 mg	Non irritant
Seaber 1985 Skin sensitization in guinea pigs - Buehler test	0.5 ml of 50% (induction), 0.5 mL of 50% (challenge)	Non sensitizing
Nakamura 2001 Skin sensitization in guinea pigs - Maximization test	0.1 mL of 5% (intradermal induction), 0.4 mL of 25% (demal induction), 0.2 mL of 10% (challenge)	Sensitizing **R43**

** used only in preliminary study*

Tolclofos-methyl has low acute toxicity when administered orally, dermally and via inhalation to rats. It is not a skin or eye irritant. It is not a skin sensitizer by Buehler test, but a skin sensitizer by Maximization test and should be labelled with the risk phrase **"May cause sensitisation by skin contact" R43**.

After oral administration, toxic symptoms in rats and mice were decrease of spontaneous motor activity, irregular respiration, dyspnea, piloerection, incontinence of urine and ataxia of hind limb or whole body 3-4 hours after administration. The minimum toxic dose level was 3750 mg/kg bw and 1500 mg/kg bw in rats and mice, respectively. At gross necropsy, no visible lesions were observed.

Emesis and soft stools or diarrhea was observed in many of the dogs.

After inhalation, signs consistent with exposure to high concentration of a mildly irritant dust were noted, including closing or partial closing of the eyes, abnormal body position and abnormal breathing. Abnormal respiratory pattern was observed immediately after exposure.

Tolclofos-methyl did not produce any irritant reactions on the skin nor in the eyes.

B.6.3 Short-term toxicity (Annex IIA 5.3)

B.6.3.1 Oral

B.6.3.1.1 Oral 28-day study: rat

Reference	: Colley, J. et al., 1982	**Exposure**	: 32 days (males), 34 days (females)
Title of study	: S-3349 toxicity to rats by dietary administration for 4 weeks	**Dose**	: Males/females: 0, 200, 1000, 5000, 20000 ppm (0, 16, 79, 414, 1635/ 0, 18, 88, 452, 1830 mg/kg bw/day)
Test substance	: Tolclofos-methyl, batch No.: 10901, purity: not specified, specification No. 01	**Vehicle**	: Pulverised basal Spratts' Laboratory animal diet No. 2
Administration way	: Oral *via* the diet	**GLP statement**	: Yes
Species	: Sprague-Dawley rats	**Guideline**	: In-house method, in accordance with 92/69/EEC, B.7
Group size	: 10/sex/dose	**Acceptability**	: Yes
		NOAEL	: 5000 ppm (414 mg/kg bw/day)
		LOEL	: 200 ppm (16 mg/kg bw/day)

Materials and methods

See above

Findings

General observations:

One female receiving 20000 ppm was sacrificed following accidental injury. Patchy hair loss possibly related to treatment was noted for 6 females treated at 20000 ppm.

Lower food intake was noted in the 20000 ppm group, particularly in week 1 (Table B.6.3.1.1-1).

A suppression of bodyweight gain was noted for males and females at 20000 ppm (Table B.6.3.1.1-2).

Table B.6.3.1.1-1 Group mean food consumption (g/rat/week)

Week	Group (ppm)									
	Males					Females				
	Control	200	1000	5000	20000	Control	200	1000	5000	20000
-1	175	180	180	174	176	137	140	141	142	131
1	176	175	170	172	128	125	127	125	123	100
2	187	185	186	180	167	129	132	130	130	129
3	188	191	185	185	170	131	135	129	127	130
4	173	188	186	181	143	116	136	128	131	110
1-4	724	739	727	718	608**	501	530*	512	511	469*
% of control		102	100	99	84		106	102	102	94

** Significantly different from control ($p < 0.05$)*
*** Significantly different from control ($p < 0.01$)*

Table B.6.3.1.1-2: 4-week oral toxicity study in rats: Body weights (g)

Dose level	0 ppm	200 ppm	1000 ppm	5000 ppm	20000 ppm
Males					
Body weight	407	418	423	394	317
Weight gain Week 0.0 - 4.0	194	202	194	178	106***
% of control	100	104	100	92	55
Females					
Body weight	247	257	248	240	203
Weight gain Week 0.0 - 4.0	73	88**	76	66	46***
% of control	100	121	104	90	63

***: Significantly different from control ($p < 0.01$)*
****: Significantly different from control ($p < 0.001$)*

Haematology, clinical chemistry and cholinesterase activity:

There were no changes in haematology which were considered to be related to treatment. The clinical chemistry showed higher cholesterol levels for males and females treated at 20000 ppm; higher total protein and albumin values were noted for males receiving 20000 ppm and higher inorganic phosphorus levels were noted for females of the same group (Table B.6.3.1.1-3).

Lower plasma cholinesterase values were noted for both sexes at 20000 ppm (-39% in females, -14% in males). Lower brain cholinesterase values were noted for males in all dose groups, and for females in the 5000 and 20000 ppm groups (-31% and –21% in males and females, respectively, at 20000 ppm) (Table B.6.3.1.1-4).

Table B.6.3.1.1-3: 4-week oral toxicity study in rats: Clinical chemistry

	0 ppm	200 ppm	1000 ppm	5000 ppm	20000 ppm
Males					
Cholesterol (mg/dl)	32	37	40*	36	69***
Total protein (g/dl)	6.9	6.8	6.9	6.7	7.3***
Albumin	3.5				3.7*
α 1	1.2				1.2
α 2	0.6				0.6
β	1.4				1.6*
γ	0.2				0.1***
Globulin (g/dl)	3.4				3.6*
Phosphorus (mEq/l)	4.3	4.4	4.3	4.4	4.4
Females					
Cholesterol (mg/dl)	34	31	31	37	81***
Phosphorus (mEq/l)	3.6	3.8	3.9	3.9**	4.1***

**: $p < 0.05$ in comparison with controls **: $p < 0.01$ in comparison with controls ***: $p < 0.001$ in comparison with controls*

Table B.6.3.1.1-4: 4-week oral toxicity study in rats: Cholinesterase activity (µmol/ml/min)

Week 5	0 ppm	200 ppm	1000 ppm	5000 ppm	20000 ppm
Males					
Erythrocytes	2.01	1.81* (90%)[a]	1.84 (92%)	1.64*** (82%)	1.63*** (81%)
Plasma	0.43	0.38 (88%)	0.41 (95%)	0.41 (95%)	0.37* (86%)
Brain	7.27	6.40** (88%)	5.99*** (82%)	6.06*** (83%)	5.01*** (69%)
Females					
Erythrocytes	2.01	1.79* (89%)	1.88 (94%)	1.62*** (81%)	1.83 (91%)
Plasma	1.17	1.16 (99%)	1.09 (93%)	1.01 (86%)	0.71*** (61%)
Brain	8.98	8.84 (98%)	8.90 (99%)	7.15*** (80%)	7.12*** (79%)

**: p < 0.05 in comparison with control*
***: p < 0.01 in comparison with control*
****: p < 0.001 in comparison with control*
[a] *Figures in brackets indicate % of control*

Gross pathology, organ weights and histopathology:

An increased incidence of fur thinning was noted for females treated at 20000 ppm. Higher liver weights were recorded for males and females receiving 20000 ppm and for females receiving 5000 ppm. Hepatocyte enlargement was noted in 7/10 male and 9/9 female rats treated at 20000 ppm. Kidney weights fluctuated and higher and lower kidney weights (Table B.6.3.1.1-5) were noted for males in the 1000, 5000 and 20000 ppm dose groups, and females at 5000 ppm. Ophthalmic examinations afforded no evidence for treatment related changes of the eyes.

Table B.6.3.1.1-5: 4-week oral toxicity study in rats: Organ weights (g)

Dose level	0 ppm	200 ppm	1000 ppm	5000 ppm	20000 ppm
Males					
Kidneys	3.5	3.8	4.0*	3.9**	3.2*
Liver	20.3	20.9	20.1	20.5	21.0***
Females					
Kidneys	2.4	2.6	2.3	2.6**	2.2
Liver	11.1	11.2	11.4	11.9*	12.8***

****: p < 0.05** in comparison with controls*
*****: p < 0.01** in comparison with controls*
******: p < 0.001** in comparison with controls*

Conclusions

NOAEL: 5000 ppm (414 mg/kg bw/day) - based on decrease of body weight gain and increased liver weight accompanied by hepatocyte enlargement at 20000 ppm.

NOEL: <200 ppm

LOEL: 200 ppm (16 mg/kg bw/day) - based on decreased brain cholinesterase activity

B.6.3.1.2 Oral 90-day toxicity (rat)

Reference	: Kimura, J., 1990	**Exposure**	: 90 days
Title of study	: 90-day oral toxicity study of S-3349 in rats	**Dose**	: Males/females: 0, 100, 1000, 10000 ppm (0, 6.46, 66.1, 653/ 0, 7.13, 71.0, 696 mg/kg bw/day)
Test substance	: Tolclofos-methyl, batch No.: 90437-M, purity: 96.6%, specification No. 01	**Vehicle**	: Pulverised basal diet
Administration way	: Oral *via* the diet	**GLP statement**	: Yes
Species	: Sprague-Dawley rats	**Guideline**	: FIFRA § 82-1, equivalent to 88/302/EEC part B
Group size	: 12/sex/dose	**Acceptability**	: Yes
		NOAEL	: 1000 ppm (66.1 mg/kg bw/day)
		NOEL	: 100 ppm (6.46 mg/kg bw/day)

Materials and methods

See above

Findings

General observations:

No deaths were noted during the study. Loss of hair was noted for one male and two or three females receiving 10000 ppm from week 1 to 2. Significant decrease of body weight (Table B.6.3.1.2-1) and related decreases of food consumption (83% of control at the end of treatment) were noted for both sexes receiving 10000 ppm.

Table B.6.3.1.2-1: 90-day oral toxicity study in rats: Body weights (g)

	0 ppm	100 ppm	1000 ppm	10000 ppm
Males				
Day 1	147	147	147	145
Day 91	521	515	520	424** (81%)[a]
Females				
Day 1	123	123	122	123
Day 91	286	287	275	243** (85%)

*** Significantly different from control (p < 0.01)*
[a] *Figures in brackets indicate % of control*

Ophthalmology, haematology, clinical chemistry, urinalysis and cholinesterase activity:

No changes were found in the ophthalmologic examination. Haematological examinations and clinical chemistry revealed several significant changes compared to the control group. Some of the parameters showed only minor deviations while other show large deviations compared to the control (Table B.6.3.1.2-2). Plasma, erythocyte and brain cholinesterase levels were reduced in the 10000 ppm dose group in both sexes (Table B.6.3.1.2-3).

Table B.6.3.1.2-2: 90-day oral toxicity study in rats: Haematology and clinical chemistry

	0 ppm	100 ppm	1000 ppm	10000 ppm
Males				
Activated partial thromboplastin time (sec)	23.5	23.4	23.6	25.5** (109%)[a]
Blood urea nitrogen (g/l)	0.15	0.16* (107%)	0.15	0.18** (120%)
α_2-Globulin (%)	5.2	5.2	5.9* (113%)	6.6** (127%)
β-Globulin (%)	17.3	17.2	17.8	18.9** (109%)
Cholesterol (g/l)	0.58	0.6	0.59	0.86** (148%)
Phospholipids (g/l)	0.82	0.85	0.9	1.12** (137%)
Inorganic phosphorus (g/l)	0.06	0.059	0.062	0.064* (107%)
γ-Glutamyl transpeptidase (U/l)	1	1	1	4** (400%)
Glutamic-oxaloacetictransaminase (U/l)	138	118* (86%)	115** (83%)	112** (81%)
Triglycerides (g/l)	0.37	0.37	0.38	0.19* (51%)
Females				
Lymphocytes (10^3/µl)	4.47	4.03	4.83	5.92** (132%)
Leukocytes (10^3/µl)	5.16	4.78	5.66	6.59* (128%)
α_2-Globulin (%)	4.9	5.2	4.8	6.6** (135%)
β-Globulin (%)	15.7	15.5	16.7	17.5** (111%)
γ-Globulin (%)	7.7	7.3	6.6* (86%)	5.8** (75%)
Cholesterol (g/l)	0.66	0.73	0.69	1.33** (202%)
Phospholipids (g/l)	1.07	1.15	1.08	1.76** (164%)
Inorganic phosphorus (g/l)	0.044	0.049	0.05* (114%)	0.054** (123%)
Triglycerides (g/l)	0.08	0.07	0.09	0.22** (275%)
Alkaline phosphatase (U/l)	68	60	66	44** (65%)
Glucose (g/l)	1.43	1.33	1.27** (89%)	1.21** (85%)

**: p < 0.05 in comparison with controls*
***: p < 0.01 in comparison with controls*
[a] *Figures in brackets show % of control*

Table B.6.3.1.2-3: 90-day oral toxicity study in rats: Cholinesterase activity (µmol/ml/min)

	0 ppm	100 ppm	1000 ppm	10000 ppm
Males				
Erythrocytes	1.98	2.01	1.91	1.60** (81%)[a]
Plasma	0.52	0.54	0.46	0.43* (84%)
Brain (µmol/g/min)	11.9	12.0	11.8	10.9** (92%)
Females				
Erythrocytes	1.92	1.87	1.73* (90%)	1.54** (80%)
Plasma	3.55	4.56* (129%)	3.48	1.67** (47%)
Brain (µmol/g/min)	12.9	13.0	12.5	11.7** (91%)

**: p < 0.05 in comparison with control*
***: p < 0.01 in comparison with control*
[a] *Figures in brackets indicate % of control*

Gross pathology, organ weights and histopathology:

Dark red discoloration of the liver was noted for both sexes at 10000 ppm. Increased weight of the liver was noted for both sexes at 10000 ppm and males at 1000 ppm. Increased relative kidney weight was noted for both sexes at 10000 ppm and in females at 1000 ppm. On the contrary, the absolute kidney weights were not changed. This effect may be related to the decrease of body weight in the treated groups (Table B.6.3.1.2-4).

Histopathological examination revealed slight hypertrophy of the hepatocytes in both sexes at 10000 ppm. The increased liver and kidney weight ratios observed at 1000 ppm were not accompanied by histopathological changes.

Table B.6.3.1.2-4: 90-day oral toxicity study in rats: Organ weights

	0 ppm	100 ppm	1000 ppm	10000 ppm
Males				
Liver				
Absolute (g)	11.98	12.23	12.44	13.07
Relative (%)	2.42	2.51	2.52*	3.25**
Kidneys				
Absolute (g)	3.57	3.41	3.58	3.37
Relative (%)	0.73	0.7	0.73	0.84**
Females				
Liver				
Absolute (g)	6.66	6.62	6.51	7.72**
Relative (%)	2.49	2.46	2.54	3.41**
Kidneys				
Absolute (g)	1.9	2.0	2.0	2.01
Relative (%)	0.71	0.75	0.78**	0.89**

**: p < 0.05 in comparison with control*
***: p < 0.01 in comparison with control*

Conclusions NOAEL: 1000 ppm, equivalent to 66.1 mg/kg bw/day - based on increased weight of liver at 10000 ppm in both sexes accompanied by dark red discoloration of the liver, hypertrophy of hepatocytes and several changes of haematological and clinical chemistry parameters at 10000 ppm.
NOEL: 100 ppm, equivalent to 6.46 mg/kg bw/day – based on changes of haematological and clinical chemistry parameters at 1000 and 10000 ppm.

B.6.3.1.3 6-month oral toxicity (rat)

Reference	: Hiromori, T. et al., 1978; Takatsuka, M., 1985	**Exposure**	: 6 months
Title of study and amendments	: Six-month oral toxicity study of S-3349 in rats (Hiromori et al); Quality assurance statement (Takatsuka)	**Dose**	: Males/females: 0, 300, 1000, 3000, 10000 ppm (0, 16, 51, 164, 540/ 0, 18, 65, 184, 623 mg/kg bw/day)
Test substance	: Tolclofos-methyl, batch No.: 524, purity: 97.0%, specification No. 01	**Vehicle**	: Pulverised basal diet CE 2
Administration way	: Oral *via* the diet	**GLP statement**	: No (later reviewed according to GLP)
Species	: Sprague Dawley rats	**Guideline**	: In-house method
Group size	: 15/sex/dose	**Acceptability**	: Yes
		NOAEL	: 3000 ppm (164 mg/kg bw/day)
		NOEL	: 300 ppm (16 mg/kg bw/day)

Materials and methods

See above

Findings

General observations:

No abnormal clinical sign or any treatment-related mortality was noted during the study. Body weight and body weight gain were lower (15% and 18%, resoectively) in the 10000 ppm group when compared to controls (Table B.6.3.1.3-1). Food consumption of the treated groups was similar to controls.

Table B.6.3.1.3-1: 6-month oral toxicity study in rats: Body weights (g)

	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Males					
Initial body weight	161	160	162	159	144**
Final body weight	581	596	595	562	537
Body weight gain	420	436	433	404	393
Females					
Initial body weigth	134	129*	135	138	127*
Final body weight	342	320	344	339	292**
Body weight gain	208	199	209	201	171**

**: Significantly different from control ($p < 0.05$)*
***: Significantly different from control ($p < 0.01$)*

Ophthalmology, haematology, clinical chemistry, urinalysis and cholinesterase activity:

No remarkable changes were found in any dose groups in ophthalmologic examination. A slight decrease of haemoglobin concentration was observed in 10000 ppm females. A moderate inhibition of plasma cholinesterase was noted in 10000 ppm females and a slightly lower concentration of uric acid was observed from 3000 ppm upwards in females (Table B.6.3.1.3-2). No abnormality was found in urinalysis.

Table B.6.3.1.3-2: 6-month oral toxicity study in rats: Clinical chemistry

	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Males					
Haemoglobin (g/l)	15.9	15.8	16.1	16.2	15.8
Uric acid (mg/dl)	1.80	1.69	1.73	1.85	1.85
Plasma cholinesterase (µmol/ml/min)					
Week 2	0.55	0.57	0.49	0.45	0.42
Week 4	0.48	0.51	0.50	0.49	0.42
Week 13	0.65	0.62	0.54*	0.58	0.61
Week 28	0.66	0.61	0.52*	0.48	0.50
Females					
Haemoglobin (g/l)	15.2	15.1	14.8	14.7	14.5**
Uric acid (mg/dl)	1.77	1.73	1.66	1.54*	1.39**
Plasma cholinesterase (µmol/ml/min)					
Week 2	1.45	1.51	1.60	1.26	1.01**
Week 4	1.73	1.60	1.62	1.36*	1.03**
Week 13	3.47	3.06	3.12	3.16	2.66*
Week 28	3.16	2.51	3.12	3.29	2.31

**: Significantly different from control ($p < 0.05$)*
***: Significantly different from control ($p < 0.01$)*

Gross pathology, organ weights and histopathology:

Gross pathology and histopathological examinations did not reveal any treatment-related abnormalities. Slightly higher relative weight of liver was observed in 10000 ppm males and from 1000 ppm in females and slightly higher relative weight of testes was observed in 10000 ppm males (Table B.6.3.1.3-3). Higher weight of kidneys was observed in 10000 ppm males and from 1000 ppm upwards in females. The change of relative weight of kidney in 300 ppm females may not be an effect of the test compound, because of the correlation between the kidney weight and the body weight in female rats.

Table B.6.3.1.3-3: 6-month oral toxicity study in rats: Organ weights

	0 ppm	300 ppm	1000 ppm	3000 ppm	10000 ppm
Males					
Liver					
Absolute (g)	13.9	13.7	13.6	13.7	14.2
Relative (%)	2.41	2.31	2.30	2.45	2.64*
Kidneys					
Absolute (g)	3.22	3.31	3.24	3.36	3.58
Relative (%)	0.56	0.56	0.62	0.65	0.67**
Testes					
Absolute (g)	3.48	3.38	3.67*	3.62	3.60
Relative (%)	0.60	0.57	0.62	0.65	0.67**
Females					
Liver					
Absolute (g)	7.22	6.70	7.92*	8.10*	7.50
Relative (%)	2.12	2.09	2.30**	2.39**	2.57**
Kidneys					
Absolute (g)	1.84	1.91	2.30**	2.23**	2.16**
Relative (%)	0.54	0.60*	0.67**	0.66**	0.74**

**: $p < 0.05$ in comparison with control*
***: $p < 0.01$ in comparison with control*

Conclusions

NOAEL: 3000 ppm, equivalent to 164 mg/kg bw/day - based on decreased body weight and increased liver and kidney weights at 10000 ppm. The organ weight changes do not show a clear dose response ant are therefore not considered to be adverse.

NOEL: 300 ppm, equivalent to 16 mg/kg bw/day- based on changes in the weight of liver, kidney and testes and changes in chlinical chemistry parameters at doses from 1000 ppm.

B.6.3.1.4 6-month oral toxicity (dog)

Reference	: Pence, D. H. et al., 1979b; Cox, R. H., 1987	**Exposure**	: 6 months
Title of study	: Subacute dietary administration in dogs – S-3349 (Pence et al), Amendment I to final report (Cox)	**Dose**	: Males/females 0, 200, 600, 2000 ppm (0, 6.6, 24, 70/ 0, 6.0, 21, 63 mg/kg bw/day)
Test substance	: Tolclofos-methyl, batch No.: not specified, purity: 98.7%, specification No. 01	**Vehicle**	: Pulverised basal diet
Administration way	: Oral *via* the diet	**GLP statement**	: No. Quality assurance according to GLP performed later
Species	: Beagle dog	**Guideline**	: In-house method
Group size	: 6/sex/dose	**Acceptability**	: Yes
		NOAEL	: 600 ppm (21 mg/kg bw/day)
		NOEL	: 600 ppm (21 mg/kg bw/day)

Materials and methods

See above

Findings

General observations:

None of the animals died and no compound-related toxic or pharmacologic signs were noted for any treated dog. Body weight changes and total food consumption values did not reveal any significant differences between the control and treated groups. However the 2000 ppm group exhibited a suppression of body weight gains (Table B.6.3.1.4-1).

Table B.6.3.1.4-1: 6-month feeding study in dogs: Body weights (kg)

Dose level	0 ppm	200 ppm	600 ppm	2000 ppm
Males				
Body weight (g)	11400	11800	11466	10283
Weight change Week 0-26	2.43	3.63	2.85	1.12
Females				
Body weight (g)	10166	9566	9633	8983
Weight change Week 0-26	2.10	1.77	2.17	1.13

Haematology, clinical chemistry, urinalysis and cholinesterase activity:

The following alterations were noted in the clinical laboratory data:

- Decreased mean haemoglobin values, and mean erythrocyte counts in the treated groups (Table B.6.3.1.4-2).
- Increased mean alkaline phosphatase values in the high dose group (Table B.6.3.1.4-3), which may be indicative in intra-hepatic cholestasis.
- Plasma cholinesterase inhibition in the female high dose group, most evident in the high dose group (Table B.6.3.1.4-3).

Urinalysis was not remarkable at any interval.

Table B.6.3.1.4-2: 6-month feeding study in dogs: Haematology

Dose level	0 ppm	200 ppm	600 ppm	2000 ppm
Males				
Haemoglobin (g/dl)				
Week 24	15.92	16.00	14.72	13.83*
Erythrocytes (mills)				
Week 24	6.428	6.142	5.870	5.473*
Females				
Haemoglobin (g/dl)				
Week 24	16.43	15.78	15.48	14.07*
Erythrocytes (mills)				
Week 24	6.572	6.437	6.335	5.785*

**: $p < 0.05$ in comparison with controls*

Table B.6.3.1.4-3: 6-month feeding study in dogs: Clinical chemistry and cholinesterase activity

Dose level	0 ppm	200 ppm	600 ppm	2000 ppm
Males				
Alkaline phosphatase (UI/l)				
Week 24	46.33	49.83	67.83	154.67*
Plasma cholinesterase (Δ pH/min)				
Week 26	1.89	1.89	1.84	1.77
Females				
Alkaline phosphatase (UI/l)				
Week 24	56.00	57.17	64.33	197.83*
Plasma cholinesterase (Δ pH/min)				
Week 26	1.91	1.99	1.78	1.55*

**: $p < 0.05$ in comparison with controls*

Gross pathology, organ weights and histopathology:

Statistically significantly higher than control mean absolute and relative liver weights were noted in the 2000 ppm group (Table B.6.3.1.4-4). Although the organ weight and clinical laboratory data suggest possible liver disease in the 2000 ppm group, there were no compound-related gross pathological or histopathological alterations. The incidence and severity of the spontaneous disease lesions noted histopathologically were essentially comparable in all groups.

Table B.6.3.1.4-4: 6-month feeding study in dogs: Liver weights

Week 26	0 ppm	200 ppm	600 ppm	2000 ppm
Males				
Liver absolute weight (g)	275.7	295.7	300.2	437.2*
Liver relative weight (%)	2.4	2.5	2.6	4.3*
Females				
Liver absolute weight (g)	237.5	231.5	265.2	340.3*
Liver relative weight (%)	2.3	2.4	2.8	3.8*

**: Significantly different from control ($p < 0.05$)*

Conclusions NOAEL/NOEL: 600 ppm, equivalent to 21 mg/kg bw/day - based on increased liver weights and increased ALP activity at 2000 ppm.
NOEL is also based on decreased plasma cholinesterase activity at 2000 ppm.

B.6.3.1.5 Oral 1 year toxicity (dog)

Reference	: Cox, R. H., 1988, 1993; Moore, M. R., 1993	**Exposure**	: 1 year
Title of study and amendments	: Chronic toxicity study in dogs with S-3349 (Main study); Supplement to the final report; Comments on the final report	**Dose**	: Males/females: 0, 80, 400, 2000 ppm (0, 2.2, 11.4, 59/ 0, 2.6, 11.2, 62 mg/kg bw/day)
Test substance	: Tolclofos-methyl, batch No.: 40807, purity: 96.7 and 97.6%, specification No. 01	**Vehicle**	: Purina certified lab Canine diet #5007
Administration way	: Oral *via* the diet	**GLP statement**	: Yes
Species	: Beagle dog	**Guideline**	: FIFRA § 83-1 and Japanease MAFF, 59 NohSan No. 4200
Group size	: 6/sex/dose	**Acceptability**	: Yes
		NOAEL	: 400 ppm (11 mg/kg bw/day)
		NOEL	: 80 ppm (2.6 mg/kg bw/day)

Materials and methods

See above

Findings

General observations:

There were no treatment-related clinical signs noted in any of the dogs. The only significant nutritional effect observed was reduced food consumption in females of the 2000 ppm dose group (Table B.6.3.1.5-2).

Table B.6.3.1.5-1: 12-month feeding study in dogs: Body weights (kg)

Dose level	0 ppm	80 ppm	400 ppm	2000 ppm
Males				
Week -1	6.9	6.7	6.3	6.7
Week 52	10.4	11.9	10.4	9.1
Body Weight Gains				
Week 0-52	3.1	4.7	3.3	2.1
Females				
Week -1	6.4	6.4	6.4	6.3
Week 52	9.5	9.3	9.3	8.0
Body Weight Gains				
Week 0-52	2.8	2.7	2.7	1.3

Table B.6.3.1.5-2: 12-month feeding study in dogs: Food consumption (kg/week)

Dose level	0 ppm	80 ppm	400 ppm	2000 ppm
Males				
Total Food Consumption				
Week 1-52	101.3	105.4	101.6	91.9
Females				
Total Food Consumption				
Week 1-52[T]	101.3	a	89.5	85.0*

a: Total food consumption not available due to invalidation of week 33 values.
Food consumption invalidated as a result of water-soaked feed due to a ruptured waterline.
**: Significantly different from control ($p \leq 0.05$)*
[T]: Significant trend ($p \leq 0.05$)

Haematology, clinical chemistry, urinalysis and cholinesterase activity:

Haematological changes included decreased erythrocyte count, hematocrit, and haemoglobin values for the 2000 ppm group when compared to control values of the same group (Table B.6.3.1.5-3).

Table B.6.3.1.5-3: 12-month feeding study in dogs: Haematology

Dose level	0 ppm	80 ppm	400 ppm	2000 ppm
Males				
Hematocrit (%)				
Week 52	49.3	48.4	48.5	41.1*
Haemoglobin (g/dl)				
Week 52	17.6	17.4	17.4	14.6*
Erythrocytes (MI/UL)				
Week 52	7.42	7.35	7.30	6.01*
Females				
Hematocrit (%)				
Week 52	46.6	48.5	48.5	43.0
Haemoglobin (g/dl)				
Week 52	16.7	17.4	17.3	15.3
Erythrocytes (MI/UL)				
Week 52	7.04	7.33	7.25	6.39

**: Significantly different from control ($p \leq 0.05$)*

Serum chemistry changes included elevated alkaline phosphatase values and decreased albumin values with corresponding decreases in total protein (Table B.6.3.1.5-4).

Table B.6.3.1.5-4: 12-month feeding study in dogs: Clinical chemistry

Dose level	0 ppm	80 ppm	400 ppm	2000 ppm
Males				
Albumin (g/dl)				
Week 52[T]	3.4	3.5	3.3	3.2
Total protein (g/dl)				
Week 52	6.8	7.1	6.7	6.5
Alkaline Phosphatase (U/l)				
Week 52	32	29	47	201*
Females				
Albumin (g/dl)				
Week 52	3.5	3.4	3.4	3.1*
Total protein (g/dl)				
Week 52[T]	6.9	6.6	6.3	6.1*
Alkaline Phosphatase (U/l)				
Week 52	47	46	59	229*

**: Significantly different from control ($p \leq 0.05$)*
[T]: Significant trend ($p \leq 0.05$)

Urinalysis differences revealed an increased amount of reducing substances for the 2000 ppm group. No biologically significant changes were seen in the cholinesterase values (plasma, erythrocyte and brain cholinesterase activity).

Ophthalmology, gross pathology, organ weights and histopathology:

There were no ophthalmologic findings associated with the treatment. At necropsy, dark coloration of the liver (4 animals at 2000 ppm) and prostate small in size (1 male at 2000 ppm) were considered to be treatment-related. The small prostate, although only occurring in one dog, is consistent with the decreased prostate weights noted for 2000 ppm males. Analysis of the organ weights obtained at necropsy revealed increased mean absolute and relative weights, as compared to respective control values, for the liver and pancreas in the high dose group, as well as decreased prostate weights (Table B.6.3.1.5-5).

Table B.6.3.1.5-5: 12-month feeding study in dogs: Organ weights

	0 ppm	80 ppm	400 ppm	2000 ppm
Males				
Liver[a]				
Absolute (g)	227	258*	251	366*
Relative (%)	2.3	2.2	2.5	4.0*
Pancreas				
Absolute (g)	22.2	21.8	20.9	25.8
Relative (%)	0.22	0.19	0.21	0.28*
Prostate				
Absolute (g)	6.2	7.7	6.6	3.4*
Relative (%)	0.063	0.067	0.064	0.038
Females				
Liver[b]				
Absolute (g)	223	209	226	295*
Relative (%)	2.5	2.4	2.5	3.8*
Pancreas				
Absolute (g)	18.4	20.8	18.2	23.5
Relative (%)	0.20	0.24	0.21	0.30*

**: Significantly different from control ($p \leq 0.05$)*
[a]: Significant trend for absolute and relative ($p \leq 0.05$)
[b]: Significant trend for absolute ($p \leq 0.05$)

Microscopic examination of the livers revealed an increased incidence of hepatocytic hypertrophy, intracytoplasmic homogeneous material (Table B.6.3.1.5-6), and an increased amount of hepatocytic pigment in the high dose dogs. The significance and/or nature of intracytoplasmic homogeneous material is not known. The hepatocytic pigment was present in all dogs including controls, indicating that it was endogenous, but the amount present increased with dose in 400 and 2000 ppm dogs compared to controls (Table B.6.3.1.5-7). Prussian blue and Hall's bilirubin stains in the control and high dose groups revealed no detectable amounts of iron and bilirubin, respectively, in the hepatocytes. No other treatment-related histomorphologic lesions were observed.

Table B.6.3.1.5-6: 12-month feeding study in dogs: Histopathology of the liver, incidence summary

	Number of animals affected							
	Male				Female			
Dose level (ppm)	**0**	**80**	**400**	**2000**	**0**	**80**	**400**	**2000**
Liver:								
-number examined	6	6	6	6	5	6	5	6
-not remarkable	0	0	0	0	0	0	0	0
-findings:								
hepatocyte, hypertrophy	0	0	0	6	0	0	0	6
intracytoplasmic homogeneous material	0	0	0	6	0	0	0	6
hepatocyte, pigment	6	6	6	6	5	6	5	6
foci of mononuclear cells	4	4	6	6	5	4	3	6
Inflammation, chronic active	0	1	0	0	0	0	0	0

Table B.6.3.1.5-7: 12-month feeding study in dogs: Histopathology of the liver, graded findings

		Number of animals affected							
		Male				Female			
Dose level (ppm)		**0**	**80**	**400**	**2000**	**0**	**80**	**400**	**2000**
Liver:									
Number examined		6	6	6	6	6	6	6	6
Not remarkable		0	0	0	0	0	0	0	0
Hepatocyte, hypertrophy	-	6	6	6	0	6	6	6	0
	1	0	0	0	0	0	0	0	1
	2	0	0	0	6	0	0	0	5
	Total	6	6	6	6	6	6	6	6
	Mean	0.0	0.0	0.0	2.0	0.0	0.0	0.0	1.8
Intracytoplasmic homogeneous material	-	6	6	6	0	6	6	6	0
	2	0	0	0	4	0	0	0	2
	3	0	0	0	2	0	0	0	4
	Total	6	6	6	6	6	6	6	6
	Mean	0.0	0.0	0.0	2.3	0.0	0.0	0.0	2.7
Hepatocyte, pigment	-	0	0	0	0	1	0	0	0
	1	5	3	0	0	5	5	0	0
	2	0	3	5	1	0	1	5	0
	3	1	0	1	2	0	0	1	2
	4	0	0	0	3	0	0	0	4
	Total	6	6	6	6	6	6	6	6
	Mean	1.3	1.5	2.2	3.3	0.8	1.2	2.2	3.7
Foci of mononuclear cells	-	2	2	0	0	1	2	2	0
	1	4	4	6	6	4	4	4	5
	2	0	0	0	0	1	0	0	1
	Total	6	6	6	6	6	6	6	6
	Mean	0.7	0.7	1.0	1.0	1.0	0.7	0.7	1.2
Congestion	-	6	6	6	6	5	6	6	6
	4	0	0	0	0	1	0	0	0
	Total	6	6	6	6	6	6	6	6
	Mean	0.0	0.0	0.0	0.0	0.7	0.0	0.8	0.0
Inflammation, chronic active	-	6	5	6	6	6	6	6	6
	3	0	1	0	0	0	0	0	0
	Total	6	6	6	6	6	6	6	6
	Mean	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0

The grading system generally, but not necessarily, reflects the functional impact of the particular change on the organ function
The five grading steps used are:
1 = Minimal, 2 = Slight (mild), 3 = Moderate
4 = Moderately severe (marked), 5 = Severe

Conclusions

NOAEL: 400 ppm (11.2 mg/kg bw/day) - based on changes in relative and absolute liver weights with corresponding histopathological changes. Changes were also observed in relative pancreas weights in both sexes and prostate weight changes were observed in males. An increased ALP activity was also noted. All these effects ocurred at 2000 ppm (59 mg/kg bw/day).

NOEL: 80 ppm (2.2 mg/kg bw/day) – based on non adverse effects on hepatocytic pigment at 400 ppm and changes in haematological parameters at 2000 ppm

B.6.3.1.6 Nine-month oral toxicity (mouse)

Reference	: Suzuki, T. et al., 1978	**Exposure**	: 9 months
Title of study	: Nine-month feeding study of S-3349 in mice	**Dose**	: Males/females: 0, 10, 30, 100, 3000 ppm (0, 1.2, 3.8, 12.2, 513/ 0, 1.4, 4.1, 13.8, 564 mg/kg bw/day)
Test substance	: Tolclofos-methyl, batch No.: 524, purity: 97.0%, specification No. 01	**Vehicle**	: Mixed with corn oil and pulverised basal diet CE-2
Administration way	: Oral *via* the diet	**GLP statement**	: No
Species	: ddY mice	**Guideline**	: In-house method
Group size	: 15/sex/dose + 3 satellite groups of 5/sex/dose	**Acceptability**	: Yes
		NOAEL	: 100 ppm (12.2 mg/kg bw/day)
		LOEL	: 10 ppm (1.2 mg/kg bw/day)
		NOEL	: < 10 ppm

Materials and methods

In addition of the main dose groups, each group had three satellite groups to determine time-course changes of plasma, erythrocyte and brain cholinesterase activities at 2nd, 4th and 13th weeks.

Findings

General observations:

No abnormal clinical sign nor any treatment-related mortality were noted during the study. A lower body weight was observed in 3000 ppm males and females as well as their lower body weight gain (Table B.6.3.1.6-1). Lower body weights were found in 10 and 30 ppm females, but were not dose-related. The amounts of consumed food in 3000 ppm males and females were higher than that of the other groups in most weeks of the feeding period (Table B.6.3.1.6-2). Water intake was increased in 3000 ppm males.

Table B.6.3.1.6-1: 9-month feeding study in mice: Body weights

	0 ppm	10 ppm	30 ppm	100 ppm	3000 ppm
Males					
Initial body weight (g)	14.6	16.2*	15.5	16.4*	16.1*
Final body weight (g)	52.7	50.7	50.6	50.9	41.7**
Gain (g)	38.1	34.5	35.1	34.6	25.6**
Food consumption (g/kg bw/day)	119	120	126	122	171
Females					
Initial body weight (g)	15.4	15.1	15.0	15.2	14.5
Final body weight (g)	46.6	41.0**	40.0**	44.0	36.1**
Gain (g)	31.2	26.0**	25.0**	28.8	21.7**
Food consumption (g/kg bw/day)	131	142	138	138	188

**: Significantly different from control ($p < 0.05$)*
***: Significantly different from control ($p < 0.01$)*

Ophthalmology, haematology, clinical chemistry and cholinesterase activity:

No remarkable changes were found in any dose groups in ophthalmologic, haematological and biochemical examination. The most evident effect observed was inhibition of plasma and erythrocyte cholinesterase activities at 100 and 3000 ppm (Table B.6.3.1.6-2).

Table B.6.3.1.6-2: 9-month feeding study in mice: Cholinesterase activity

	0 ppm	10 ppm	30 ppm	100 ppm	3000 ppm
Males					
Plasma cholinesterase (µmol/ml/min)					
Week 40	7.45	8.92	6.24	4.14** (56%)	0.363** (5%)
Erythrocyte cholinesterase (µmol/ml/min)					
Week 40	1.08	0.93	1.04	0.86* (80%)	0.49** (45%)
Brain cholinesterase (µmol/g/min)					
Week 40	7.25	5.31** (73%)	6.77	8.62* (119%)	5.46** (75%)
Females					
Plasma cholinesterase (µmol/ml/min)					
Week 40	12.62	9.60* (76%)	7.99** (63%)	5.36** (42%)	1.51** (12%)
Erythrocyte cholinesterase (µmol/ml/min)					
Week 40	0.98	0.96	0.97	0.85* (87%)	0.64** (65%)
Brain cholinesterase (µmol/g/min)					
Week 40	6.77	6.48	7.70	7.36	7.80

**: Significantly different from control ($p < 0.05$)*
***: Significantly different from control ($p < 0.01$)*
[a] *Figures in brackets indicate % of control*

Gross pathology, organ weights and histopathology:

Gross pathology, histopathological examinations and organ weights as well as their ratios to body weight did not reveal any treatment-related abnormalities.

Conclusions

NOAEL: 100 ppm, equivalent to 12 mg/kg bw/day - based on the decrease of erythrocyte cholinesterase activity and decrease of body weight and body weight gain at the highest dose.

LOEL: 10 ppm, equivalent to 1.2 mg/kg bw/day – based on decreased plasma cholinesterase levels.

NOEL: < 10 ppm

B.6.3.1.7 21-day dermal toxicity (rabbits)

Reference	: Gargus, J. L., 1986	**Exposure**	: 6 h/day for 3 weeks
Title of study	: 21-day dermal toxicity study in rabbits with Rizolex, technical 97.7%	**Dose**	: 0, 30, 300, 1000 mg/kg bw/day
Test substance	: Tolclofos-methyl, batch No.: 40810, purity: 97.7%, specification No. 01	**Vehicle**	: Acetone
Administration way	: Dermal	**GLP statement**	: Yes
Species	: New Zealand White rabbit	**Guideline**	: FIFRA § 82-2, in accordance with 92/69/EEC, B.9
Group size	: 5/sex/dose	**Acceptability**	: Yes
		NOAEL (systemic)	: ≥1000 mg/kg bw/day
		NOEL (systemic)	: 30 mg/kg bw/day
		NOEL (local)	: <30 mg/kg bw/day

Materials and methods

Tolclofos-methyl was applied 5 days per week. The doses of 30 and 300 mg/kg bw/day constituted a solution while 1000 mg/kg bw/day constituted a suspension.

Findings

General observations:

There were no mortalities and no compound related clinical signs or changes in body weights or food consumption. Dermal irritation was evident in all compound-treated groups in the form of very slight erythema, beginning on day 6 and continuing sporadically at all measured intervals until study termination in three males and four females treated at 30 mg/kg bw/day, and in one male and four females treated at 300 mg/kg bw/day. Only one male treated at 1000 mg/kg bw/day displayed this sign. At the end of the study all rabbits showed "compound build-up" with the exception of one male from the lowest dose group (Table B.6.3.1.7-1).

Table B.6.3.1.7-1: 21-day dermal toxicity study in rabbits: Incidence of dermal irritation scores

Dose	**0 mg/kg bw**			**30 mg/kg bw**			**300 mg/kg bw**			**1000 mg/kg bw**		
Type of reaction	normal	very slight erythema	compound build-up	normal	very slight erythema	compound build-up	normal	very slight erythema	compound build-up	normal	very slight erythema	compound build-up
Males												
Day 21	5	0	0	1	0	4	0	0	5	0	0	5
Females												
Day 21	5	0	0	0	0	5	0	1	5	0	0	5

Haematology, clinical chemistry and cholinesterase activity:

Evaluation of clinical haematology parameters revealed a significantly increased mean eosinophil value in males treated at 1000 mg/kg bw/day (Table B.6.3.1.7-2). All other haematology parameters were comparable for all groups. Significantly lower plasma cholinesterase activity in females treated at 300 and 1000 mg/kg bw/day were observed (Table B.6.3.1.7-3). All other trends were considered incidental in nature.

Table B.6.3.1.7-2: 21-day dermal toxicity study in rabbits: Haematology and clinical chemistry values at termination of study

Week 4	0 mg/kg bw	30 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
Males				
Eosinophil (10^3/µl)	0.1	0.1	0.1	0.2*
Females				
Eosinophil (10^3/µl)	0.1	0.1	0.1	0.1

**: Significantly different from control ($p < 0.05$)*

Table B.6.3.1.7-3: 21-day dermal toxicity study in rabbits: Cholinesterase activity at termination of the study

Week 4	0 mg/kg bw	30 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
Males				
Erythrocyte (µmol/ml)	11.6	11.7	8.4	9.9
Plasma (µmol/ml)	3.2	2.9	2.5	2.3
Brain (µmol/g)	96.6	89.5	80.0	82.3
Females				
Erythrocyte (µmol/ml)	10.7	11.8	11.3	9.5
Plasma (µmol/ml)	2.8	2.8	2.0*	2.1*
Brain (µmol/g)	83.9	82.4	99.2	90.2

**: Significantly different from control ($p < 0.05$)*

Gross pathology, organ weights and histopathology:

Macroscopic pathology suggestive of a treatment effect consisted of compound build-up and erythema on the treated skin of all groups exposed to the test material. Organ-to-body weight ratios were significantly increased for the kidneys (females treated at 1000 mg/kg bw/day) (Table B.6.3.1.7-4).

Table B.6.3.1.7-4: 21-day dermal toxicity study in rabbits: Kidney weight

Week 4	0 mg/kg bw	30 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
Males				
Kidney absolute weight (g)	17.9	19.6	20.4	19.1
Kidney relative weight (%)	0.57	0.68	0.69	0.66
Females				
Kidney absolute weight (g)	16.2	16.0	16.8	19.0
Kidney relative weight (%)	0.54	0.54	0.57	0.65*

**: Significantly different from control ($p < 0.05$)*

Histopathology evaluation revealed compound related alterations resulting in hyperkeratosis, acanthosis, and subepidermal pleocellular infiltration in all treated groups except one male and one female treated at 30 mg/kg.

Conclusions

NOAEL (systemic): ≥1000 mg/kg bw/day.

NOEL (local): <30 mg/kg bw/day - based on the slight dermal irritation noted in all test levels

NOEL (systemic): 30 mg/kg bw/day - based on increased mean eosinophil value (males treated at 1000 mg/kg bw/day); lower plasma cholinesterase activity (females treated at 300 and 1000 mg/kg bw/day) with a negative trend for both sexes; and an increased kidney-to-body weight ratio (females treated at 1000 mg/kg bw).

B.6.3.2 Summary and conclusions on short-term toxicity studies

Study	Dose levels	NOEL/NOAEL	Targets/main effects
Colley et al., 1982 Oral 4-week toxicity study in rats	0, 200, 1000, 5000, 20000 ppm M/F: 0, 16, 79, 414, 1635 0,18, 88, 452, 1830 mg/kg bw/day	**NOAEL** 5000 ppm (414 mg/kg bw/day) LOEL 200 ppm (16 mg/kg bw/day) NOEL < 200 ppm	**↓Body weight gain** **↑Liver weights** **Hepatocyte enlargement** ↓Cholinesterase (dose response for brain) levels ↑Cholesterol, total protein, albumin, inorganic phosphorus ↓Food consumption Changes in kidney wights
Kimura 1990 Oral 90-day toxicity study in rats	0, 100, 1000, 10000 ppm M/F: 0, 6.46, 66.1, 653/ 0, 7.13, 71.0, 696 mg/kg bw/day	**NOAEL** 1000 ppm (66.1 mg/kg bw/day) NOEL 100 ppm (6.46 mg/kg bw/day)	**↑Liver weight** **Hypertrophy of hepatocytes** ↓Body weight gain ↓Cholinesterase levels ↓Food consumption Several changes of haematological and chlinical chemistry parameters
Hiromori et al., 1978; Takatsuka, 1985a Oral 6-month toxicity study in rats	0, 300, 1000, 3000, 10000 ppm M/F: 0, 16, 51, 164, 540/ 0, 18, 65, 184, 623 mg/kg bw/day	**NOAEL** 3000 ppm (164 mg/kg bw/day) NOEL 300 ppm (16 mg/kg bw/day)	**↓Body weight, body weight gain** **↑Kidney, liver and testes weights** ↓Haemoglobin concentration ↓Plasma cholinesterase, uric acid
Pence et al., 1979b; Cox, 1987 Oral 6-month toxicity study in dogs	0, 200, 600, 2000 ppm M/F: 0, 6.6, 24, 70/ 0, 6.0, 21, 63 mg/kg bw/day	**NOAEL** 600 ppm (21 mg/kg bw/day) NOEL 600 ppm (21 mg/kg bw/day)	**↓Body weight gain** **↑Alkaline phosphatase** **Liver weights** ↓Hematocrit, erythrocyte count and haemoglobin values ↓Plasma cholinesterase
Cox, 1988, 1993; Moore, 1993 Oral 12-month toxicity study in dogs	0, 80, 400, 2000 ppm M/F: 0, 2.2, 11.4, 59/ 0, 2.6, 11.2, 62 mg/kg bw/day	**NOAEL** 400 ppm (11 mg/kg bw/day) NOEL 80 ppm (2.2 mg/kg bw/day)	**↑Liver and pancreas weight** **↓Prostate weight** **↑Hepatocytic hypertrophy** **↑Alkaline phophatase** Based on non-adverse effects on hepatocytic piment at 400 ppm and changes at 2000 ppm: ↓Erythrocyte count, hematocrit, and haemoglobin values F: ↓Albumin, total protein ↑Reducing substances in urine
Suzuki et al., 1978 Oral 9-month toxicity study in mice	0, 10, 30, 100, 3000 ppm M/F: 0, 1.2, 3.8, 12.2, 513/ 0, 1.4, 4.1, 13.8, 564 mg/kg bw/day	**NOAEL** 100 ppm (12.2 mg/kg bw/day) LOEL 10 ppm (1.2 mg/kg bw/day) NOEL < 10 ppm	**↓Body weight, body weight gain** **↓Cholinesterase levels**

Study	Dose levels	NOEL/NOAEL	Targets/main effects
Gargus et al., 1986 Percutaneous 21-day in rabbits	0, 30, 300, 1000 mg/kg bw/day	**NOAEL (systemic)** ≥1000 mg/kg bw/day NOEL (systemic) 30 mg/kg bw/day NOEL (local) <30 mg/kg bw/day	 ↓Cholinesterase levels ↑Kidney weights ↑Eosinophil values ↑Dermal irritation

M: Males, F: females

Following repeated oral administration of high doses of tolclofos-methyl, no evidence for cumulative toxicity was seen in rats, mice or dog..

In the three species, reduced body weight development and reduced food intake were noted at the highest dose levels, except for food intake in mice.

Reduced cholinesterase levels were seen in several studies and in all species.

Rats: Increased levels of cholesterol, total proteins and inorganic phosphorus were noted, as well as decreased cholinesterase activity (mainly in brain and plasma). Target organs were kidneys and liver, with increased weights. Hypertrophy of the hepatocytes was noted in rats after 4-week or 90-day treatment. It was not observed in the 6-month study.

Mice: Only inhibition of plasma, erythrocyte and/or brain cholinesterase activities was observed, without any other relevant modifications in haematological or clinical chemistry parameters, or in organ weights.

Dogs: Haematological changes consisted of decreased erythrocyte count, hematocrit and haemoglobin values. Clinical chemistry showed elevated alkaline phosphatase values. Decreased albumin values were also noted. Liver was the target organ with increased weight and hypertrophy of the hepatocytes. Changes in the weight of prostate and pancreas were also noted.

Rabbits: Dermal irritation was evident in many rabbits, in the form of very slight erythema. A significantly increased mean eosinophil value were noted in the males treated at 1000 mg/kg bw/day. Plasma cholinesterase levels were reduced. Increased weight of kidneys was seen in the females. Histopathology evaluation only revealed hyperkeratosis, acanthosis, and subepidermal pleocellular infiltration in most animals of all treated groups.