Metabolism of Tolclofos-methyl in Rats and Mice

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On single oral administration of 5 mg/kg of ^{14}C -tolclofos-methyl [Rizolex®, *O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl)phosphorothioate] labeled at the 4-methyl group to both sexes of rats and mice, approximately 87 to 91% of the radiocarbon administered was excreted into the urine and feces within 1 week. The total ^{14}C -residue in the animal bodies 1 week after administration was less than 1% of the dose. The results of tissue analysis showed very low ^{14}C -residue in the tissues, although a somewhat higher amount was found in the hair. More than 14 metabolites were detected in the excreta of both animal species. Of these, 13 metabolites were found in common in both rats and mice. The amounts of these metabolites differed between rats and mice, although no significant differences were found between male and female in either animal species. In both animals, tolclofos-methyl was mainly metabolized via oxidative desulfuration of the *P=S* group to *P=O* group, oxidation at the 4-methyl group and cleavage of the *P-O*-aryl and *P-O*-methyl linkages. The main metabolite was 3,5-dichloro-4-hydroxybenzoic acid, which was excreted as free form in rats, but further conjugated with glycine in mice.

INTRODUCTION

Tolclofos-methyl, Rizolex®, [*O,O*-dimethyl *O*-(2,6-dichloro-4-methylphenyl)phosphorothioate], is effective for control of soil borne diseases caused by *Rhizoctonia solani* and *Corticium rolfsii*,¹⁾ and is now under development as an agricultural fungicide. The acute toxicity of this compound to mammals is very low, the oral LD_{50} for rats being ca. 5,000 mg/kg.²⁾ This compound is found to vaporize easily from water solution and soil surface. Moreover, on exposure to sunlight, it is rapidly decomposed to 2,6-dichloro-4-methylphenol and 3,5-dichloro-4-hydroxybenzoic acid in air and water,³⁾ and on surfaces of soils and plants.⁴⁾ The present paper deals with the metabolism of tolclofos-methyl in rats and mice.

MATERIALS AND METHODS

1. Chemicals

^{14}C -Tolclofos-methyl labeled at the 4-methyl group was prepared by Yoshitake *et al.*⁵⁾ of the Institute for Biological Science, Sumitomo Chemical Co., Ltd., Takarazuka,

Hyogo, Japan. The preparation had a specific activity of 16.2 mCi/mmol and radiochemical purity of more than 99%, as determined by radio-thin layer chromatography in solvent systems of *n*-hexane/ether (5 : 1, v/v), *n*-hexane/acetone (4 : 1, v/v), benzene and chloroform. Non-radioactive tolclofos-methyl (TM) and the following compounds were synthesized in the Research Department, Pesticide Division, Sumitomo Chemical Co., Ltd. 2,6-Dichloro-4-methylphenol (Ph-CH_3) and 3,5-dichloro-4-hydroxybenzaldehyde (Ph-CHO) were prepared by chlorination of *p*-cresol and 4-hydroxybenzaldehyde, respectively, according to Biltz.⁶⁾ *O,O*-Dimethyl *O*-(2,6-dichloro-4-formylphenyl)phosphorothioate (TM-CHO) was prepared as follows: To a mixture of 3,5-dichloro-4-hydroxybenzaldehyde (1.9 g), CuCl (1 g) and K_2CO_3 (1.4 g) in 20 ml of toluene, 1.6 g of *O,O*-dimethyl phosphorothioate was added dropwise at room temperature over 0.5 hr. Then the reaction mixture was further stirred for 3 hr at 60 to 80°C.⁷⁾ After cooling the mixture, water was added to dissolve the precipitated inorganic compounds, and the

toluene solution was washed with water and dried over anhydrous sodium sulfate. After distilling off toluene *in vacuo*, 2 g of the crude product was obtained and purified on a silica gel column using benzene as the eluting solvent. *O,O*-Dimethyl *O*-(2,6-dichloro-4-formyl-phenyl)phosphate (TM-CHO) was prepared as follows:⁸⁾ To a stirring mixture of dimethylphosphite (5.5 g) and Ph-CHO (9.5 g) in 10 ml of carbon tetrachloride cooled to 5°C, 8.4 ml of triethylamine was added dropwise. The reaction mixture was stirred at room temperature for 3 hr and then filtered off. The filtrate was washed with water and dried over anhydrous sodium sulfate. After concentration, 11 g of the crude product was obtained and purified on a silica gel column eluted with a 2 : 9 mixture of benzene and ether. *O,O*-Dimethyl *O*-(2,6-dichloro-4-methylphenyl)phosphate (TMO) was also prepared from dimethylphosphite and Ph-CH₃ in the same manner as with TM-CHO. 3,5-Dichloro-4-hydroxybenzyl alcohol (Ph-CH₂OH) was prepared as follows: To 1.9 g of Ph-CHO in 20 ml of methanol, 2 g of NaBH₄ was added slowly at 0°C by stirring.⁹⁾ The mixture was stirred at 5°C for 30 min. Then water was added to the mixture, which was extracted with benzene. The benzene layer was dried over anhydrous sodium sulfate. After concentration of the benzene solution, the crude Ph-CH₂OH was obtained, and purified by column chromatography using silica gel and benzene saturated with formic acid as the solvent. Similarly, *O,O*-dimethyl *O*-(2,6-dichloro-4-hydroxymethylphenyl)phosphorothioate (TM-CH₂OH) and *O,O*-dimethyl *O*-(2,6-dichloro-4-hydroxymethylphenyl)-phosphate (TMO-CH₂OH) were prepared from the corresponding aldehydes. *O,O*-Dimethyl *O*-(2,6-dichloro-4-carboxyphenyl)phosphorothioate (TM-COOH) was prepared as follows: To 3.15 g of TM-CHO in 5 ml of methanol, 4 g of potassium permanganate dissolved in 10 ml of water was added dropwise at room temperature by stirring.¹⁰⁾ After an additional 1 hr of stirring, the reaction mixture was adjusted to pH 1 with 1 N HCl solution, and extracted with 30 ml of ether. The ether layer was dried over anhydrous sodium sulfate. After evaporation of the solvent *in vacuo*, the

crude TM-COOH was obtained and purified by silica gel column chromatography using benzene saturated with formic acid as the solvent. Similarly, *O,O*-dimethyl *O*-(2,6-dichloro-4-carboxyphenyl)phosphate (TMO-COOH) was prepared from the corresponding aldehyde. *O*-Methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl)phosphorothioate (DM-TM), *O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-methylphenyl)phosphate (DM-TMO), *O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-hydroxymethylphenyl)phosphorothioate (DM-TM-CH₂OH), *O*-methyl-*O*-hydrogen *O*-(2,6-dichloro-4-hydroxymethylphenyl)phosphate (DM-TMO-CH₂OH), *O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl)phosphorothioate (DM-TM-COOH) and *O*-methyl *O*-hydrogen *O*-(2,6-dichloro-4-carboxyphenyl)phosphate (DM-TMO-COOH) were prepared from TM, TMO, TM-CH₂OH, TMO-CH₂OH, TM-COOH, respectively, by reaction with 1.2-fold moles of potassium dimethyldithiocarbamate in methanol at room temperature for 5 hr.¹¹⁾ Each crude product was purified by repeated thin-layer chromatography (*tlc*) in toluene/ethyl acetate/isopropanol/acetic acid (8 : 12 : 5 : 3, v/v). The structure of these compounds was confirmed by nuclear magnetic resonance (NMR) spectrometry (Hitachi R-40B, 90 MHz, or JEOL-JNM-FX-100, 100 MHz) and/or mass (MS) spectrometry (Shimadzu LKB-9000, 70 eV). Data on NMR and MS spectra of the authentic compounds are listed in Table 1 and 2, respectively. *p*-Cresol, 4-hydroxybenzaldehyde and 3,5-dichloro-4-hydroxybenzoic acid (Ph-COOH) were purchased from Aldrich Co., Milwaukee, Wis.

2. Analysis

Precoated silica gel chromatoplates (silica gel 60 F₂₅₄, 0.25 mm thickness, E. Merck) were used for both analytical and preparative *tlc*. *R_f* values for major metabolites and solvent systems used are listed in Table 3. Radioactive regions on the developed *tlc* plates were detected by radioautography using industrial X-ray films (IX-50, Fuji Photo Film Co., Ltd., Tokyo), and spots of unlabeled reference compounds were visualized under ultraviolet light irradiation.

Table 1 NMR spectra of tolclofos-methyl and reference compounds.

Compound	Solvent	Spectral signal (90 MHz, δ^{TMS} ppm)
TM	CDCl_3	2.29 (methyl, 3H, s), 3.93 (methoxy, 6H, d, $J_0=14$ Hz), 7.15 (aromatic, 2H, s)
TM- CH_2OH	CDCl_3	3.96 (methoxy, 6H, d, $J_0=14$ Hz), 4.63 (hydroxymethyl, 2H, s), 7.37 (aromatic, 2H, s)
TM-COOH	$(\text{CD}_3)_2\text{CO}$	3.99 (methoxy, 6H, d, $J_0=14$ Hz), 8.07 (aromatic, 2H, s)
TMO	CDCl_3	2.30 (methyl, 3H, s), 3.97 (methoxy, 6H, d, $J_0=12$ Hz) 7.17 (aromatic, 2H, s)
TMO- CH_2OH	CDCl_3	3.95 (methoxy, 6H, d, $J_0=12$ Hz), 4.62 (hydroxymethyl, 2H, s), 7.45 (aromatic, 2H, s)
TMO-COOH	$(\text{CD}_3)_2\text{CO}$	4.00 (methoxy, 6H, d, $J_0=12$ Hz), 8.07 (aromatic, 2H, s)
Ph- CH_3	CDCl_3	2.20 (methyl, 3H, s), 7.00 (aromatic, 2H, s)
Ph- CH_2OH	$(\text{CD}_3)_2\text{CO}$	4.57 (hydroxymethyl, 2H, s), 7.35 (aromatic, 2H, s)
Ph-COOH	$(\text{CD}_3)_2\text{CO}$	7.96 (aromatic, 2H, s)
DM-TM	CD_3OD	2.27 (methyl, 3H, s), 3.81 (methoxy, 3H, d, $J_0=14$ Hz), 7.15 (aromatic, 2H, s)
DM-TM- CH_2OH	CD_3OD	3.75 (methoxy, 3H, d, $J_0=14$ Hz), 4.48 (hydroxymethyl, 2H, s), 7.23 (aromatic, 2H, s)
DM-TM-COOH	CD_3OD	3.76 (methoxy, 3H, d, $J_0=14$ Hz), 7.80 (aromatic, 2H, s)
DM-TMO	CD_3OD	2.26 (methyl, 3H, s), 3.76 (methoxy, 3H, d, $J_0=12$ Hz), 7.18 (aromatic, 2H, s)
DM-TMO- CH_2OH	CD_3OD	3.74 (methoxy, 3H, d, $J_0=12$ Hz), 4.48 (hydroxymethyl, 2H, s), 7.25 (aromatic, 2H, s)
DM-TMO-COOH	CD_3OD	3.75 (methoxy, 3H, d, $J_0=12$ Hz), 7.83 (aromatic, 2H, s)
Ph-CHO	$(\text{CD}_3)_2\text{CO}$	7.93 (aromatic, 2H, s), 9.93 (formyl, 1H, s)
TM-CHO	CDCl_3	3.99 (methoxy, 6H, d, $J_0=14$ Hz), 7.89 (aromatic, 2H, s), 9.93 (formyl, 1H, s)
TMO-CHO	CDCl_3	4.00 (methoxy, 6H, d, $J_0=12$ Hz), 7.89 (aromatic, 2H, s), 9.93 (formyl, 1H, s)

Table 2 Mass spectra of tolclofos-methyl and reference compounds.

Compound	m/e (at 70 eV)
TM	267 (M+2-Cl), 265 (M-Cl), 175 [$\text{OC}_6\text{H}_2\text{Cl}_2\text{CH}_3$], 125 [$(\text{CH}_3)_2\text{P}(\text{S})$]
TM- CH_2OH	283 (M+2-Cl), 281 (M-Cl), 125 [$(\text{CH}_3)_2\text{P}(\text{S})$]
TM-COOH	297 (M+2-Cl), 295 (M-Cl), 205 [$\text{OC}_6\text{H}_2\text{Cl}_2\text{CH}_3$], 125 [$(\text{CH}_3)_2\text{P}(\text{S})$]
TMO	288 (M+4), 286 (M+2), 284 (M), 215 (M+2-Cl), 249 (M-Cl), 234 [M-50(Cl, CH_3)], 109 [$(\text{CH}_3)_2\text{P}(\text{O})$]
TMO- CH_2OH	267 (M+2-Cl), 265 (M-Cl), 109 [$(\text{CH}_3)_2\text{P}(\text{O})$]
TMO-COOH	281 (M+2-Cl), 279 (M-Cl), 109 [$(\text{CH}_3)_2\text{P}(\text{O})$]
Ph- CH_3	180 (M+4), 178 (M+2), 176 (M), 143 (M+2-Cl), 141 (M-Cl)
Ph- CH_2OH	196 (M+4), 195 (M+3), 194 (M+2), 193 (M+1), 192 (M), 191 (M-Cl), 177 (M+2-OH), 175 (M-OH), 165 (M+2-Cl), 163 (M-CHO), 159 (M+2-Cl), 157 (M-Cl)
Ph-COOH	210 (M+4), 208 (M+2), 206 (M), 193 (M+4-OH), 191 (M+2-OH), 189 (M-OH)
Ph-CHO	194 (M+4), 193 (M+3), 192 (M+2), 191 (M+1), 190 (M), 189 (M-1), 163 (M+2-CHO), 161 (M-CHO)
TM-CHO	281 (M+2-Cl), 279 (M-Cl), 125 [$(\text{CH}_3)_2\text{P}(\text{S})$]
TMO-CHO	302 (M+4), 300 (M+2), 298 (M), 265 (M+2-Cl), 263 (M-Cl), 109 [$(\text{CH}_3)_2\text{P}(\text{O})$]

Table 3 *R_f* values of tolclofos-methyl and its ¹⁴C-metabolites in the urine and feces from rats and mice with six solvent systems in thin layer chromatography.

Metabolite	<i>R_f</i> in the solvent system*					
	A	B	C	D	E	F
S 1 TM	0.66	0.73	0.84	0.75	0.69	0.58
S 2 Ph-CH ₃	0.58	0.75	0.82	0.69	0.70	0.47
S 3 TM-CH ₂ OH	0.38	0.73	0.73	0.69	0.49	0.33
S 4 Ph-CH ₂ OH	0.23	0.67	0.53	0.57	0.50	0.13
S 5 TM-COOH	0.03	0.70	0.63	0.39	0.30	0.29
S 6 Ph-COOH	0.05	0.69	0.55	0.33	0.40	0.26
S 7 TMO-COOH	0.00	0.60	0.39	0.25	0.07	0.13
S 8 Ph-CO-glycine	0.00	0.54	0.33	0.10		
S 9 DM-TM	0.00	0.45	0.25	0.21		0.03
S10 DM-TM-COOH	0.00	0.37		0.13		
S11 DM-TM-CH ₂ OH	0.00	0.35		0.13		
S12 DM-TMO	0.00	0.26	0.12	0.10		
S13 DM-TMO-COOD	0.00	0.20		0.00		
S14 DM-TMO-CH ₂ OH	0.00	0.10		0.07		

* Solvent systems used; A: chloroform/acetone (9 : 1, v/v), B: toluene/ethyl acetate/isopropanol/acetic acid (8 : 12 : 5 : 3, v/v), C: toluene/ethyl formate/formic acid (5 : 7 : 1, v/v), D: methanol/chloroform (1 : 3, v/v), E: benzene/ether (2 : 9, v/v), F: toluene/acetic acid (7 : 1, v/v).

Radioactivity in urine, organo-soluble extracts and silica gel regions scraped from *tlc* plates was quantitated by liquid scintillation counting (*lsc*) on a Packard Tri-Carb 3385 with automatic external standard, as reported previously.¹²⁾ Counting efficiency and background levels were more than 85% and 40 to 50 cpm, respectively, in low potassium glass vials containing 15 ml of a dioxane scintillation fluid (5 g of 2,5-diphenyl oxazole and 100 g of naphthalene in 1 liter of dioxane). Aliquots (0.2 to 0.6 g) of feces, tissues and organo-insoluble residues were combusted with a sample oxidizer (Packard Tri-Carb 306) before *lsc*. For combustion analysis, a mixture of 15 ml of ¹⁴CO₂-counting cocktail [OXI-PREP^{TM-2}, New England Nuclear (NEN), Boston] and 9 ml of ¹⁴CO₂-absorber (OXISORBTM-CO₂, NEN) was used. Counting efficiency and background levels were more than 73% and 45 to 55 cpm, respectively. Radioactivity in alkaline solution (0.5 N NaOH) was quantitated by *lsc* after neutralization with 0.5 N HCl solution and then mixing with 10 ml of Aquasol 2®(NEN). In this case, counting efficiency and background levels were more than 83% and 45 to 55 cpm, respectively.

3. Treatment of Animals

Five-week old Sprague-Dawley rats weighing approximately 190 g (male) and 140 g (female) were supplied by Nihon Dobutsu Co., Osaka. Seven-week old ICR mice weighing 28 g (male) and 24 g (female) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka. Rats and mice were placed in polycarbonate cages in an air-conditioned room of 24±2°C and allowed to adjust to the environment for 1 week before administration. The animals were supplied with a diet (CE-2 Clea Japan, Inc.) and water *ad libitum* during the experiment. Both rats and mice were individually treated with a single oral dose of 5 mg/kg of ¹⁴C-tolclofos-methyl suspended in 10 ml of corn oil (Yamakei Sangyo, Osaka) by stomach intubation. The treated rats and mice were individually housed in metabolism cages (Metabolica CO-2®, Sugiyamagen Iriki Co., Ltd., Tokyo), which enabled separate collection of urine, feces and expired air. Radioactive carbon dioxide expired was trapped in 250 ml of 0.5 N NaOH solution. During a sampling period, urine and feces were kept at 0 to 5°C to prevent further alteration of metabolites. Seven days after administration, the

treated rats were sacrificed, and tissues were dissected out and homogenized by a multi-blender mill BL-3 (Nihon Seiki Co., Tokyo), aliquots (0.2 to 0.6 g) of which were subjected to combustion analysis prior to *lsc*.

Whole-body autoradiography was conducted according to the methods of Ullberg¹³⁾ 1,6 and 24 hr after administration of 5 mg/kg of the ¹⁴C-compound to male rats.

4. Extraction, Analysis and Identification of Metabolites

The one-day pooled urine from the treated rats or mice was adjusted to pH 1 with 2 N HCl and then extracted 3 times with 3-fold volumes of ethyl ether. The one-day pooled feces was homogenized in an equal volume of distilled water using a Waring blender. The homogenate was adjusted to pH 1 and extracted 3 times with 3-fold volumes of ethyl ether. The combined ethyl ether extracts from urine and feces were each concentrated, and the residues were dissolved in small volumes of methanol and subjected to one-dimensional *tlc* cochromatography with authentic standards in solvent systems A), C), E) and F). After extraction, the remaining aqueous layers from urine and feces were each neutralized with 2 N NaOH and then lyophilized. The residues were each suspended in a small volume of methanol, and subjected to one-dimensional *tlc* cochromatography with authentic standards in solvent systems B) and D).

To obtain larger amounts of ¹⁴C-metabolites, ¹⁴C-tolclofos-methyl with specific activity of 1.62 mCi/mmol was orally administered to 30 male mice at the rate of 50 mg/kg, and urine was collected during 1 day post-treatment. The collected urine (46 ml) was subjected to column chromatography (column size; 185×34 mm ϕ) on Amberite XAD-2 resin, and eluted with 270 ml of water, followed by 150 ml of methanol. The methanol fraction was concentrated *in vacuo* and the residue was subjected to preparative *tlc* in solvent systems B) and D). The separated fractions on *tlc* plates were each extracted with methanol and then subjected to *tlc* cochromatography and/or spectroanalysis (¹H-Fourier transform-NMR, 100 MHz and EI-MS, 70

eV).

RESULTS

1. Distribution and Tissue Residues of Radio-carbon

After single oral administration of ¹⁴C-tolclofos-methyl to male rats at the rate of 5 mg/kg, whole-body autoradiography was conducted at various time intervals. As shown in Fig. 1, the highest radioactivity was located in digestive organs including stomach and intestines 1 hr and 6 hr after treatment, followed by kidney and liver. Other tissues such as brain, spleen, eye-ball, testis, thymus, heart, lung, muscle, salivary gland, spinal cord and bone contained lower radioactivity. Twenty four hr after dosing, very low radio-

Table 4 Contents of total ¹⁴C in tissues of male and female rats 7 days after oral administration of 5 mg/kg of tolclofos-methyl.

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

after 1 hr

after 6 hr

after 24 hr

Fig. 1 Autoradiograms showing distribution of radioactivity in the whole body of male rats 1 hr, 6 hr and 24 hr after single oral administration of ^{14}C -tolclofos-methyl at 5 mg/kg.

activity was present in the whole body of rats.

Seven days after single oral administration, 27 tissues of male and female rats were each examined for ^{14}C residues. As shown in Table 4, the ^{14}C residues were less than 6 ppb tolclofos-methyl equivalents in most of the tissues analyzed, except that hair contained 52 to 60 ppb. The ^{14}C residues in hair were not removed by rinsing with methanol.

2. Excretion

On single oral administration of ^{14}C -tolclo-

fos-methyl to both sexes of rats and mice, 74 to 83% of the administered radioactivity was excreted into urine, feces and expired air within 1 day after treatment, as shown in Table 5. During the 7 days after treatment, approximately 87 to 91% of the dose was recovered in the excreta. Of this, 65 to 70% of the dose, 17 to 22% and 0.3 to 0.7% was eliminated into urine, feces and expired air, respectively, of both sexes of rats. On the other hand, 82 to 83% of the dose, 5 to 7% and 0.6 to 0.9% was recovered in urine, feces

Table 5 Cumulative excretion of radiocarbon into the urine, feces and expired air after oral administration of 5 mg/kg of ^{14}C -tolclofos-methyl to rats and mice.

Mammals	Sex	Excreta	% of the administered radiocarbon			
			Days after administration			
			1	2	4	7*
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

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

and expired air, respectively, of both sexes of mice. The total ^{14}C residue remaining in the whole body of rats and mice was less than 1% of the dose at 7 days after administration. In both animals approximately 9 to 13% of the dose was not recovered.

3. Metabolites

Tlc analysis with solvent systems A), B), C), D), E) and F) indicated that at least 13 metabolites were also detected in organosoluble extracts of urine and feces from mice, and 14 metabolites were found in the extracts of urine and feces from rats. All of the 13 metabolites found in male mice were detected in the excreta of male rats, although there were differences in the amounts of these metabolites between the two animals. Also, *tlc* analysis of the metabolites revealed that no significant differences in the nature and amounts of metabolites were found between male and female of both animal species. The data on male animals are listed in Table 6. Among 14 metabolites detected on *tlc*, 13 metabolites, from S1 to S14 except S8,

were tentatively identified by *tlc* cochromatography with the authentic standards by increasing numerical orders as TM, Ph-CH₃, TM-CH₂OH, Ph-CH₂OH, TM-COOH, Ph-COOH, TMO-COOH, DM-TM, DM-TM-COOH, DM-TM-CH₂OH, DM-TMO-COOH and DM-TMO-CH₂OH, respectively. In addition, the NMR and MS spectra of S6 coincided well with those of the authentic Ph-COOH. The NMR spectrum (100 MHz) in CD₃OD of S8 indicated the presence of two methylene protons ($\delta^{\text{TMS}} = 3.92$ ppm, s) and two aromatic protons ($\delta^{\text{TMS}} = 7.93$ ppm, s). The metabolite S8 was methylated by reaction with diazomethane for 12 hr at room temperature to give one product, which was purified by preparative *tlc* in solvent systems A) and B) (*Rf*=0.40 and 0.67, respectively). The methylated S8 gave NMR (100 MHz) spectral signals in CDCl₃ at $\delta^{\text{TMS}} = 3.82$ ppm (s, COOCH₃), 3.95 ppm (s, OCH₃), 4.22 ppm (d, *J*=5 Hz, NCH₃), 6.50 ppm (broad s, NH) and 7.76 ppm (s, aromatic 2H). The EI-MS fragments of the methylated S8 appeared at *m/e* 293 (*M*+2), 291 (*M*), 234 (*M*+2-

Table 6 Relative amounts of tolclofos-methyl metabolites excreted into urine and feces during 24 hr after oral administration of ^{14}C -tolclofos-methyl to male rats and mice.

Metabolite		Amounts (% of the administered radiocarbon)			
		Rats		Mice	
		Urine	Feces	Urine	Feces
S 1	TM	nd**	5.0	nd	1.3
S 2	Ph-CH ₃	7.9	3.5	7.9	0.8
S 3	TM-CH ₂ OH	nd	trace	nd	0.1
S 4	Ph-CH ₂ OH	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***	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 ₂ OH	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-TM-CH ₂ OH	0.4	trace	4.0	trace
Others*		1.9	0.6	5.0	0.2
Total		66.7	16.4	75.9	5.8

* methanol-insoluble ^{14}C , **nd: not detected, *** trace: less than 0.1%.

COOCH₃), 232 (M-COOCH₃), 205 (M+2-NHCH₂COOCH₃) and 203 (M-NHCH₂COOCH₃). Based on these data, S8 was identified as 3,5-dichloro-4-hydroxybenzyl glycine, Ph-CO-glycine. Ph-CO-glycine was one of the major metabolites in mice, but a minor product in rats. The metabolite DM-TM-COOH (S10) was found in rats, but not in mice. The 12 other metabolites were formed in both animal species, although these amounts differed in the two animals. Large amounts of metabolites S6, S2, S7, S13, S1 and S4 were found in rats, whereas, metabolites S8, S6, S13, S7 and S2 were formed to large extents in mice.

Figure 2 shows the proposed metabolic pathways for ^{14}C -tolclofos-methyl in rats and mice.

DISCUSSION

Upon single oral administration of 5 mg/kg of ^{14}C -tolclofos-methyl to male rats, the radiocarbon was readily absorbed and distributed mainly into stomach, intestines, liver and kidney, as shown by whole-body autoradiography. ^{14}C -Tolclofos-methyl was rapid-

Fig. 2 Proposed metabolic pathways for tolclofos-methyl in rats and mice.

ly metabolized in rats and mice of both sexes and the metabolites were excreted mainly into the urine and feces, and also into the expired air to a minor extent. Total recovery of the radiocarbon was about 87 to 91%. However, the ^{14}C residue in most of the tissues were very low, and the total ^{14}C residue in the whole animal body was less than 1% of the administered radiocarbon 7 days after treatment. Therefore, it is presumed that a part of tolclofos-methyl and/or metabolites may disappear from the excreta by evaporation during the collection of urine and feces, since tolclofos-methyl and Ph-CH_3 have high vapor pressure.^{3,4)}

As expected from the results of many studies on metabolism of organophosphorus insecticides in mammals, tolclofos-methyl underwent oxidation of the P=S group to P=O group, oxidation at the 4-methyl group, and cleavage of the P-O-aryl and P-O-methyl linkages. In general, the oxidation reactions and cleavage of the P-O-aryl linkage of the P=S compound are mediated by liver microsomal oxidases, so-called mixed function oxidases. The cleavage of P-O-methyl (alkyl) and/or P-O-aryl linkage of both P=S and P=O compounds is catalyzed by GSH S-transferases. Also, the P-O-aryl linkage of P=O compounds is hydrolyzed by A-esterase. It was reported that liver microsomal oxidase and GSH S-transferase activities were higher in mouse liver than in rat liver, whereas A-esterase activity was higher in rat liver than in mouse liver, when fenitrothion and fenitrooxon were used as substrates.¹⁴⁾ In rats and mice administered tolclofos-methyl, cleavage of the P-O-aryl linkage occurred to a greater extent in rats than in mice. In contrast, oxidations of the P=S group to the P=O group and the 4-methyl group, and desmethylation of the P-O-methyl group were more predominant in mice than in rats. Ph-COOH was the major metabolite with both animal species; however, its conjugation with glycine occurred in mice to a larger extent, but to a trace extent in rats.

When metabolism of tolclofos-methyl in rats and mice is compared with that of fenitrothion which has 3-methyl-4-nitrophenyl group and $\text{O,O-dimethyl phosphorothioate}$

group in the molecule, there were marked differences in the relative amounts of metabolites. The oxidation of the 4-methyl group constitutes a major metabolic reaction with tolclofos-methyl, but a minor with fenitrothion.¹²⁾ On the other hand, desmethylation products accounted for 35 to 43% of the dose with fenitrothion, but only 18 to 24% of the dose with tolclofos-methyl.¹²⁾ The cleavage of P-O-aryl linkage was one of major metabolic pathways with both fenitrothion and tolclofos-methyl. The phenolic metabolites produced from fenitrothion were subsequently conjugated to form the majority of the corresponding glucuronides and sulfates.¹²⁾ On the other hand, the phenolic metabolites from tolclofos-methyl were mostly present as free form. It appears that the phenolic OH group of 2,6-dichlorophenols may have steric hindrance related to 2 ortho-chlorine atoms, which may restrict conjugation reactions with uridine diphosphate glucuronic acid and/or 3'-phosphoadenosine-5'-phospho sulfate.

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要 約

トルクロホスメチルのラットおよびマウスにおける代謝

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¹⁴C-4-メチル標識トルクロホスメチル (リゾレックス®) を 5 mg/kg の割合でラットおよびマウスの雌雄ラットにそれぞれ 1 回経口投与すると, 投与した ¹⁴C の

87~91% が 1 週間以内に糞尿中に排泄された。投与 1 週間後, 体内の ¹⁴C 残存量は投与量の 1% 以下であり, しかも各種組織における残存量は 0.06ppm 以下であり, 組織内に ¹⁴C が蓄積する傾向は認められなかった。両動物の排泄物中には少なくとも 14 個の代謝物が見いだされ, そのうち 13 個は両動物で共通したものであった。

トルクロホスメチルはおもに P=S 基の P=O 基への酸化的脱硫化, 4-メチル基の酸化および P-O-アシル結合と P-O-メチル結合の開裂を経て代謝され, 主要代謝物は 3,5-ジクロロ-4-ヒドロキシ安息香酸であった。この代謝物はラットではほとんど遊離で存在したが, マウスでは 55% がグリシン抱合をうけていた。