Original Article

Comparative Metabolism of Stereoisomers of Cyphenothrin and Phenothrin Isomers in Rats

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On single oral or subcutaneous administration of each of the ^{14}C -acid, alcohol- and CN-labeled preparations of [1R, *trans*]- and [1R, *cis*]-cyphenothrin ((RS)- α -cyano-3-phenoxybenzyl-(1RS, *trans/cis*, 8/2)chrysanthemate) at 2 to 4 mg/kg, the radiocarbon from the acid and alcohol moieties was almost completely eliminated from rats and the ^{14}C tissue residues were generally very low. On the other hand, the radiocarbon from the CN group was somewhat slowly excreted and the hair, skin and stomach contents showed relatively higher ^{14}C residues. The major metabolic reactions of these isomers were oxidation at the 2'- and 4'-positions of the alcohol moiety, and at the isobutenyl group and *gem*-dimethyl group of the acid moiety, cleavage of ester linkage and conversion of the CN group to SCN^- and CO_2 . The [1R, *trans*]-isomer underwent ester cleavage to a larger extent than the [1R, *cis*]-isomer, which afforded the metabolites retaining ester linkage in larger amounts. There were no significant differences in the ^{14}C tissue residues and the nature of the metabolites between the oral and subcutaneous doses, except that larger amounts of ester-cleaved metabolites were obtained with the subcutaneous than with oral doses. When the *in vivo* metabolic fates of [1R, *trans*]- and [1R, *cis*]-cyphenothrin were compared with the corresponding fates of [1R, *trans*]- and [1R, *cis*]-phenothrin, [1R, *cis*]-cyphenothrin showed higher biodegradability than [1R, *cis*]-phenothrin, but [1R, *trans*]-cyphenothrin did not differ significantly in the biodegradability from that of [1R, *trans*]-phenothrin. These features were in accordance with the results from the *in vitro* ester hydrolysis of these compounds by hepatic esterase(s). The hepatic esterase(s) hydrolyzed these compounds in the following order; [1R, *trans*]-phenothrin $\geq$ [1R, *trans*]-cyphenothrin > [1R, *cis*]-cyphenothrin > [1R, *cis*]-phenothrin. On the other hand, plasma esterase(s) did not show any *trans/cis* preference and hydrolyzed phenothrin to a larger extent than cyphenothrin.

INTRODUCTION

The pyrethroid insecticide, cyphenothrin[S-2703, (RS)- α -cyano-3-phenoxybenzyl-[1RS, *trans/cis*, 8/2] chrysanthemate] has a strong potential for controlling the medical pests as well as agricultural pests and is now coming to a final stage of development. To meet this situation, metabolism study in animals is needed. The chemical structure of this compound resembles that of phenothrin [S-2539, 3-phenoxybenzyl-[1RS, *trans/cis*, 8/2] chrysanthemate], except for the presence of the α -

cyano group in the alcohol moiety. The metabolism of the [1R, *trans*]- and [1R, *cis*]-phenothrin isomers was studied extensively in rats¹⁻³⁾ and the major metabolic pathways were established to be the oxidation at the 4'-position of the alcohol moiety, and at the isobutenyl and *gem*-dimethyl groups of the acid moiety, and cleavage of ester linkage. The *trans* isomer was reported to undergo ester hydrolysis to a larger extent than the *cis*-isomer, which afforded larger amounts of metabolites retaining ester linkage in the feces. It would be interesting to study the metabolism of cypheno-

thrin so as to distinguish this compound from phenothrin in their metabolism and to elucidate the influence of the α -cyano group on the metabolic fates or biodegradation, besides the above requirements.

This report deals with metabolism in rats and *in vitro* hydrolysis of [1R, *trans*]- and [1R, *cis*]-cyphenothrin, and with the comparison of behaviors between cyphenothrin and phenothrin.

MATERIALS AND METHODS

1. Designation of Compounds

The following abbreviations were used; *t* and *c* for [1R, *trans*] and [1R, *cis*], respectively; *ot* and *oc* for the *trans* and *cis* methyl positions of the isobutenyl group relative to the double bond, respectively; 2-OH(*t*) for the hydroxymethyl at the *trans* methyl of the *gem*-dimethyl group relative to the carbonyl; 2'- or 4'-OH for the hydroxy at 2'- or 4'-position of the phenoxy group; CA for chrysanthemic acid; PBalc for 3-phenoxybenzyl alcohol; PBald for 3-phenoxybenzaldehyde; PBacid for 3-phenoxybenzoic acid.⁸⁾

2. Chemicals

t- and *c*-Cyphenothrin labeled with ^{14}C at the benzylic carbon ($^{14}\text{C}\alpha$) of the alcohol moiety, at the CN group (^{14}CN) and at the carbonyl group (^{14}CO) of the acid moiety and *t*- and *c*-phenothrin labeled with ^{14}C at the benzylic carbon ($^{14}\text{C}\alpha$) of the alcohol moiety were prepared in this laboratory.⁴⁾ The labeled position and specific activities of these labeled preparations are listed in Table 1.

The preparation of the unlabeled authentic standards used in this study were reported previously.^{3,5-7)}

3. Spectroscopy

Proton magnetic resonance spectra (PMR) were determined in deuteriomethanol or deuteriochloroform using tetramethylsilane (TMS) as an internal standard on a Hitachi R-40 spectrometer at 90 MHz or on a JEOL FX 100 spectrometer at 100 MHz. Electron impact mass spectra (EI-MS) and chemical ionization mass spectra (CI-MS) were obtained on a Hitachi M-80 mass spectrometer using a direct insertion probe. With EI-MS, samples were

Table 1 Labeled positions and specific activities of ^{14}C labeled preparations of *t*- and *c*-cyphenothrin(cyphe) and *t*- and *c*-phenothrin(phe).

	Specific activity (mCi/mmol)		
	^{14}CO	$^{14}\text{C}\alpha$	^{14}CN
<i>t</i> -Cyphe	27.8	29.4	44.4
<i>c</i> -Cyphe	27.8	29.4	44.4
<i>t</i> -Phe		7.3	
<i>c</i> -Phe		7.3	

analyzed at 70 eV. The conditions of CI-MS were as follows; reagent gas, CH_4 ; ion source temperature, 100°C; probe temperature, 120–185°C.

4. Thin-layer Chromatography (TLC)

Precoated silica gel 60 F₂₅₄ chromatoplates (20×20 cm, 0.25 mm layer thickness, E. Merck) were used for both analysis and preparation of the metabolites. The following solvent systems were used;

- n*-hexane–toluene–acetic acid (3/15/2, two developments)
- benzene saturated with formic acid–ether (10/3, two developments)
- n*-hexane–chloroform–acetic acid (4/1/1)
- n*-butanol–acetic acid–water (6/1/1)

R_f values for authentic standards of various metabolites common to cyphenothrin and phenothrin were reported previously.^{3,5-7)} *R_f* values for cyphenothrin and their ester metabolites are shown in Table 2. Unlabeled standard chemicals and radioactive spots on developed TLC plates were detected in the same manners as reported previously.^{5,7)}

5. Radioanalysis and Autoradiography

Liquid scintillation counting (LSC), combustion analysis, $^{14}\text{CO}_2$ determination, TLC auto-

Table 2 The *Rf* values for cyphenothrin isomers and their ester metabolites.

Compound	<i>Rf</i> values	
	A	B
<i>t</i> -Cyphe	0.88	0.93
<i>c</i> -Cyphe	0.88	0.93
<i>ωc</i> -Acid- <i>c</i> -cyphe	0.64	0.57
<i>ωt</i> -Acid- <i>c</i> -cyphe	0.60	0.51
<i>ωc</i> -Alc- <i>c</i> -cyphe	0.38	0.44
<i>ωt</i> -Alc- <i>c</i> -cyphe	0.36	0.43
<i>ωt</i> -Acid,4'-OH- <i>c</i> -cyphe	0.24	0.19
<i>ωt</i> -Acid,2-OH(<i>t</i>)- <i>c</i> -cyphe	0.21	0.14

A: *n*-hexane-toluene-acetic acid (3/15/2, two developments), B: benzene saturated with formic acid-ether (10/3, two developments).

radiography were conducted as reported previously.⁵⁾ Also, whole-body autoradiography of male rats to which single oral or subcutaneous doses of 2.5 mg/kg of ¹⁴CN-*t*- and *c*-cyphenothrin were given was conducted as reported previously.⁵⁾

6. Treatment of Animals

6.1 *In vivo* study

Sprague-Dawley male rats weighing 200–230 g were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan and used for this study. Each of three labeled preparations of *t*- and *c*-cyphenothrin was suspended in 10% Tween 80 solution and the rats were treated with single oral or subcutaneous doses of these labeled preparations at the rate of 2–4 mg/kg. The administered volumes were 2 ml for oral doses and 0.4–0.5 ml for subcutaneous ones. The treated animals were individually held in all-glass metabolism cages (Metabolica CO-2®, Sugiyamagen Iriki Co., Ltd., Tokyo, Japan), and the urine and feces were collected separately for 7 days. Expired ¹⁴CO₂ was also trapped in 400 ml of 10% NaOH solution. The rate of air-flow through the cage was 200 to 300 ml/min. The animals were supplied with a diet (CE-2, Clea Japan, Inc., Japan) and water *ad libitum* during the experiment. The treated animals were sacrificed 7 days after administration and 22 to 23 tissues were removed to be analyzed for total radiocarbon.

Tissue residue levels were expressed as parts per billion equivalents (ppb) of the administered ¹⁴C-labeled preparations, based on wet tissue weight.

After each of ¹⁴Cα-*t*- and *c*-cyphenothrin and ¹⁴Cα-*t*- and *c*-phenothrin was administered orally to rats at about 2.5 mg/kg, the liver, brain and blood were removed at appropriate intervals for determination of total tissue levels.

6.2 *In vitro* study

The liver, stomach, intestine with the contents, kidney, brain, lung and spleen removed after decapitation were homogenized in 50 mM phosphate buffer (pH 7.4) at a concentration of 10% (fresh weight/volume). In addition, 10% liver microsome, supernatant and nuclei/cell debris fractions were prepared as follows: the liver homogenate was centrifuged at 9000 *g* for 15 min, the recovered supernatant at 100,000 *g* for 60 min and the pellet was resuspended in buffer & centrifuged at 100,000 *g* for 60 min. Each of 1 μg ¹⁴Cα-labeled preparations of *t*- and *c*-cyphenothrin and *t*- and *c*-phenothrin in 5 μl of ethanol was applied to 2 ml of the 10% tissue homogenate or 10% plasma in a 25 ml Erlenmeyer flask and incubated for 30 min at 37°C. To inhibit esterase, TEPP (tetraethylphosphorophosphate, 100 μg) in 10 μl of acetone was poured into a flask, the acetone was completely evaporated and the liver or plasma fractions were added and pre-incubated for 10 min at 37°C. Thereafter, substrate and/or NADPH (4–5 mg) were added and incubated for 30 min at 37°C. Each reaction mixture was acidified to pH 2–3 with 1 N HCl after incubation and extracted twice with 3 fold volumes of ether/ethanol (3/1, v/v) after addition of 1 g of (NH₄)₂SO₄. The ¹⁴C content was determined for the organosoluble and the remaining aqueous fractions.

7. Identification of Metabolites

The metabolites were tentatively identified by TLC cochromatography with appropriate authentic standards in the same manners as reported previously.^{3,4,6)} The methanol extract of the feces was subjected to TLC in solvent system A. Each of the metabolites detected on TLC plates was extracted with methanol and subjected to TLC cochromatography before and/or after methylation with

diazomethane at 5°C for 15 min.

For preparation of sufficient amounts of the ester metabolites from the *c*-isomer, ^{14}CN -*c*-cyphenothrin dissolved in corn oil was administered every day for 6 days to 20 male rats at the rate of 60 mg/kg. The pooled feces was extracted as described above, and the concentrated methanol-water extracts were dissolved in acidic water (pH 1) and the metabolites were extracted 3 times with ethylacetate. The ethylacetate layer was concentrated and partitioned between *n*-hexane and acetonitrile. The acetonitrile portion was concentrated and subjected to column chromatography on silica gel using the following sequence of solvents: *n*-hexane/acetone (5/1, 3 l, (4/1, 3 l) and (1/1, 3 l), acetone (2 l) and methanol (2 l). Each fraction was concentrated and purified by repetitive preparative TLC in the following solvent systems: A, B, *n*-hexane-acetone-acetic acid (4/1/1) and ethylacetate-methanol (3/1), and finally subjected to spectroscopy. Moreover, for confirmation of the acid and alcohol moiety of the ester metabolites, fecal metabolites from ^{14}CO - and $^{14}\text{C}\alpha$ -preparations were hydrolyzed in 10% NaOH methanol solution and 10% NaOH ethanol containing NaBH_4 at 5 mg/ml, respectively, and reaction mixture was extracted 3 times with ether after being adjusted to pH 2 with conc. HCl and then the acid moiety or reduced alcohol moiety was tentatively identified by TLC cochromatography with the appropriate authentic standards.

After TLC analysis of methanol extracts of urine and feces in solvent systems A or B, a fraction at the origin of TLC plates was scraped out, extracted with methanol and subsequently analyzed by TLC in solvent systems A, B and C. Individual conjugates extracted from the TLC plates were subjected to enzyme hydrolysis, as reported previously.^{3,4)}

RESULTS

1. ^{14}C Excretion

Single oral or subcutaneous administration of ^{14}CO - and $^{14}\text{C}\alpha$ -*t*- and *c*-cyphenothrin preparations to male rats at 2 to 4 mg/kg resulted in almost complete elimination of the radiocarbon from the animal body. Major excretion routes with ^{14}CO - and $^{14}\text{C}\alpha$ -labelings were the

Table 3 Radiocarbon recovered in the urine, feces and expired air 7 days after single oral (PO) and subcutaneous (SC) administration of three labeled preparations (^{14}CO , $^{14}\text{C}\alpha$ and ^{14}CN) of each of *t*- and *c*-cyphenothrin at 2 to 3 mg/kg.

	% of the dosed $^{14}\text{C}^a)$			
	<i>Trans</i>		<i>Cis</i>	
	PO	SC	PO	SC
^{14}CO -Labeling				
Urine	42.8	51.2	33.7	53.9
Feces	49.2	50.3	70.9	45.7
Expired air	0.7	— ^{b)}	0.2	—
Total	92.7	101.5	104.8	99.6
$^{14}\text{C}\alpha$ -Labeling				
Urine	83.1	87.7	50.4	76.1
Feces	13.1	5.7	47.2	18.7
Expired air	<0.1	—	<0.1	—
Total	96.1	93.7	97.6	94.8
^{14}CN -Labeling				
Urine	28.2	52.9	22.1	36.2
Feces	28.4	21.7	52.1	23.8
Expired air	3.7	5.8	3.5	4.4
Total	60.3	80.4	77.7	64.4

^{a)} Data show the average of 2 to 3 experiments.

^{b)} not analyzed.

urine and feces (Table 3). The total recovery of the ^{14}C within 7 days after administration of these labeled preparations was more than 93%. On the other hand, the ^{14}C from the ^{14}CN -labelings was more slowly excreted than the ^{14}C from ^{14}CO - and $^{14}\text{C}\alpha$ -labelings and was mainly excreted into the urine and feces. In addition, 4 to 6% of the ^{14}C was expired as $^{14}\text{CO}_2$. The total recovery of the radiocarbon was 60 to 80% for the *t*- and *c*-isomers. The three labeled preparations of *t*- and *c*-isomers showed more urinary excretion of the ^{14}C with subcutaneous than with oral administration.

2. Tissue Residues

Table 4 shows ^{14}C residue levels in 22 to 23 tissues 7 days after single oral or subcutaneous administration of each of ^{14}C -labeled preparations of *t*- and *c*-cyphenothrin. With ^{14}CO - and $^{14}\text{C}\alpha$ -preparations of *t*- and *c*-isomers, the tissue residue levels were generally very low. However, the fat showed slightly higher residue level compared with other tissues. Administration of the CN-labelings of *t*- and *c*-isomers

Table 4 Tissue residues 7 days after single oral (PO) or subcutaneous (SC) administration of three labeled preparations (^{14}CO , $^{14}\text{C}\alpha$ and ^{14}CN) of each of *t*- and *c*-cyphenothrin at 2 to 4 mg/kg.

Tissues	ng cyphenothrin eq/g wet tissue ^{a)}											
	^{14}CO				$^{14}\text{C}\alpha$				^{14}CN			
	<i>Trans</i>		<i>Cis</i>		<i>Trans</i>		<i>Cis</i>		<i>Trans</i>		<i>Cis</i>	
	PO	SC	PO	SC	PO	SC	PO	SC	PO	SC	PO	SC
Adrenal	9	16	13	5	5	6	10	7	325	403	176	520
Blood	3	3	7	5	4	4	5	6	742	687	913	771
Bone	2	3	2	2	<1 ^{b)}	4	1	4	357	273	191	302
Brain	<1	1	1	1	<1	<1	<1	2	41	34	23	43
Caecum	3	6	10	37	2	4	3	7	112	50	61	81
Fat	32	26	107	100	73	59	264	113	109	119	245	159
Hair	21	14	11	3	8	89	14	48	3033	6831	1399	5970
Heart	1	2	8	12	<1	<1	<1	2	225	210	160	296
Intestine	8	5	2	26	2	3	5	6	315	46	243	139
Intestine content	20	54	5	61	1	3	3	6	531	381	455	649
Kidney	3	3	12	10	6	10	6	8	421	397	334	399
Liver	8	4	84	68	8	6	8	8	360	333	216	382
Lung	2	1	4	2	<1	<1	1	2	475	498	336	607
Muscle	1	1	2	5	<1	<1	<1	<1	129	108	82	121
Pancreas	2	2	3	4	1	2	2	3	324	333	224	374
Sciatic nerve	2	1	4	5	5	7	8	12	404	106	321	445
Skin	1	2	3	3	2	6	4	12	893	1238	617	920
Spinal cord	<1	1	2	1	<1	1	<1	3	49	114	33	52
Spleen	2	2	3	2	<1	1	<1	1	328	325	237	341
Stomach	2	2	4	9	<1	2	1	4	2288	1694	1157	1606
Stomach content	<1	7	1	16	<1	5	<1	1	6188	3478	2239	4867
Testis	1	1	1	2	<1	<1	<1	2	291	244	186	272
Carcass	3	— ^{c)}	6	—	3	—	8	—	587	—	264	—

^{a)} Data show the average of 2 to 3 animals, ^{b)} the detection limit (1 ng/g wet tissue), ^{c)} not analyzed.

resulted in somewhat higher tissue residues compared with ^{14}CO - and $^{14}\text{C}\alpha$ -labelings. Relatively higher residues were found in the hair, skin, stomach, stomach contents, blood and fat. No apparent differences in the ^{14}C tissue residues were observed between oral and subcutaneous administration.

3. Whole-Body Autoradiography

A whole-body autoradiogram taken 168 hr after a single oral dose of 2.5 mg/kg of ^{14}CN -*c*-cyphenothrin to rats showed that radioactivity was located in the hair, skin and stomach contents, but hardly detected in other tissues (Fig. 1). This ^{14}C distribution pattern was nearly the same as that of rats to which a single oral dose of ^{14}CN -*t*-cyphenothrin and a

single subcutaneous dose of ^{14}CN -*t*- and *c*-cyphenothrin were given.

4. ^{14}C Elimination from Tissues

Table 5 shows the half-life ($T_{1/2}$) values and maximum ^{14}C levels in the blood, liver and brain after single oral administration of $^{14}\text{C}\alpha$ -*t*- and *c*-cyphenothrin and $^{14}\text{C}\alpha$ -*t*- and *c*-phenothrin to rats. The $T_{1/2}$ values of ^{14}C in three tissues with the four $^{14}\text{C}\alpha$ -labelings ranged from 2.5 to 6.9 hr. The $T_{1/2}$ values for the *t*-isomers of cyphenothrin and phenothrin in three tissues were smaller compared with those for the corresponding *c*-isomers. Also, *c*-cyphenothrin showed smaller $T_{1/2}$ values in three tissues than *c*-phenothrin whereas there were no apparent differences in $T_{1/2}$

Fig. 1 A whole-body autoradiogram of a rat 168 hr after single oral administration of ^{14}C -*c*-cyphenothrin at the rate of 2.5 mg/kg.

Table 5 Half-life values and maximum level of ^{14}C in the blood, liver and brain after single oral administration of $^{14}\text{C}\alpha$ -*t*- and *c*-cyphenothrin (cyphe) and $^{14}\text{C}\alpha$ -*t*- and *c*-phenothrin(phe) isomers to rats at 2.5 mg/kg.

^{14}C -Compound	Blood	Liver	Brain
Half-life values ($T_{1/2}$), hr			
<i>t</i> -Cyphe	2.5	2.5	3.0
<i>c</i> -Cyphe	4.2	4.8	3.8
<i>t</i> -Phe	2.8	3.7	3.1
<i>c</i> -Phe	6.2	6.9	4.5
Maximum ^{14}C tissue level, μg eq/g wet tissue			
<i>t</i> -Cyphe	2.4	2.4	0.1
<i>c</i> -Cyphe	2.0	3.0	0.1
<i>t</i> -Phe	2.0	2.0	0.2
<i>c</i> -Phe	1.0	2.6	0.2

Data show average of 3 experiments.

values between *t*-isomers of cyphenothrin and phenothrin. *c*-Phenothrin showed the largest $T_{1/2}$ values in three tissues among the four $^{14}\text{C}\alpha$ -labelings.

The ^{14}C levels in three tissues reached maxima within 1 to 2 hr after administration. The maximum ^{14}C level of the brain with the four $^{14}\text{C}\alpha$ -labelings was 1/10 to 1/26 of that of the blood and liver. The four $^{14}\text{C}\alpha$ -labelings show no remarkable differences in the maximum ^{14}C tissue levels.

5. Identification of Metabolites

The TLC patterns of the urinary and fecal metabolites from the three labeled (^{14}CO , ^{14}CN and $^{14}\text{C}\alpha$) preparations were both similar with oral and subcutaneous doses, based on TLC radioautograms. The ester-cleaved me-

tabolites in free and conjugated forms from the acid and alcohol moieties were identified as those described in previous studies on tetramethrin⁷⁾ and fenvalerate.³⁾ More than six ester metabolites of the *c*-isomer were found in the feces whereas the *t*-isomer gave few, if any, the ester metabolites being in trace amount. The ester metabolites were confirmed by spectroanalysis and by alkali hydrolysis and/or reduction followed by tentative identification of the liberated acid and alcohol moieties on the basis of TLC cochromatography. The acid and alcohol moieties of six ester metabolites and their analytical data were as follows; 1) *wt*-acid-*c*-cyphenothrin: EI-MS (methylated), m/z , 419 (M), 388, 181 (base peak), 167; CI-MS, m/z , 405 (M), 388, 181, 53 (base peak); *wt*-acid-*c*-CA and PBalc were identified. 2) *oc*-acid-*c*-cyphenothrin: EI-MS (methylated) and CI-MS were the same as those of *wt*-acid-*c*-cyphenothrin; *oc*-acid-*c*-CA and PBalc were identified; 3) *wt*-alc-*c*-cyphenothrin: CI-MS, m/z , 391 (M), 374, 330, 181, 139, 69 (base peak) and the PMR showed the methylene protons at 4.0 ppm, indicating the presence of hydroxymethyl in the *trans* position of the isobutenyl group;³⁾ *wt*-alc-*c*-CA and PBalc were identified; 4) *oc*-alc-*c*-cyphenothrin: CI-MS were the same as those of *wt*-alc-*c*-cyphenothrin and the PMR showed the methylene protons at 4.20 ppm, indicating the presence of hydroxymethyl at the *cis* position of the isobutenyl group;³⁾ *oc*-alc-*c*-CA and PBalc were identified; 5) *wt*-acid, 4'-OH-*c*-cyphenothrin: EI-MS, m/z , 421 (M), 197, 153 (base peak); *wt*-acid-*c*-CA and 4'-OH-PBalc were identified; 6) *wt*-acid, 2-OH (*t*)-*c*-cyphenothrin: EI-MS, m/z , 421 (M), 181,

153 (base peak) and the PMR showed the absence of the multiplet of the olefin proton at 6.42 ppm and the presence of the doublet of methylene protons at 3.6 ppm, indicating the presence of carboxyl group at the *trans* position of the isobutenyl group and the hydroxymethyl at the *trans* position of *gem*-dimethyl group relative to carbonyl group; PBalc were identified.

6. Amounts of Metabolites

Table 6 shows the relative amounts of metabolites in the urine and feces 2 days after single oral and subcutaneous doses. The parent *t*- and *c*-cyphenothrin and six ester metabolites from the *c*-isomer were found in the feces. The amounts of parent compounds ranged from 2 to 8% of the oral dose and ester metabolites amounted to 1 to 3%. The subcu-

Table 6 Relative amounts of the identified metabolites in the urine and feces 2 days after single oral and subcutaneous administration of three labeled preparations ($^{14}\text{C}\alpha$, ^{14}CO and ^{14}CN) of *t*- and *c*-cyphenothrin (cyphe) to rats.

Metabolites		% of the dosed ^{14}C							
		<i>Trans</i>				<i>Cis</i>			
		PO		SC		PO		SC	
		Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
Ester metabolites									
<i>t</i> - or <i>c</i> -Cyphe			1.9-4.0	0.3	0.1-0.2		4.5-8.3		<0.1-0.7
<i>wt</i> -Acid- <i>c</i> -cyphe							0.3-3.1		0.3-1.0
<i>wc</i> -Acid- <i>c</i> -cyphe							1.9-2.2		0.3-1.2
<i>wc</i> -Alc- <i>c</i> -cyphe							0.9-2.1		0.4-1.2
<i>wt</i> -Alc- <i>c</i> -cyphe							1.4-1.6		0.2-0.7
<i>wt</i> -Acid,4'-OH- <i>c</i> -cyphe							0.3-3.3		0.3-2.7
<i>wt</i> -Acid,2-OH(<i>t</i>)- <i>c</i> -cyphe							0.5-2.3		0.3-1.2
Alcohol moiety									
$^{14}\text{C}\alpha$ -Labeling									
PBald		0.2		0.6				0.7	
PBacid	Free	5.6	0.5	5.7	0.3	2.9	0.3	4.6	0.7
	Glu.	1.7		0.5		1.4		0.7	
	Gly.	0.9		0.8		0.4		0.8	
2'-OH-PBacid	Free	0.2		0.2		0.3		0.1	
	Sul.	0.8		0.6	0.2	1.2	0.2	0.7	0.5
4'-OH-PBacid	Free	2.7	1.8	3.5	1.4	2.1	6.3	2.4	2.8
	Sul.	62.9	0.3	66.2	0.4	33.5	2.0	57.7	1.7
	Glu.	0.4		1.8		0.5		1.4	
CN Group									
^{14}CN -Labeling									
SCN $^-$		10.8	0.3	28.2	0.4	7.0	0.7	16.6	0.5
$^{14}\text{CO}_2$		1.2		1.9		1.3		1.2	
Acid moiety									
^{14}CO -Labeling									
<i>t</i> - or <i>c</i> -CA	Free	0.3	1.0	0.7	0.3	0.2	1.8	0.8	
	Glu.	0.4		0.5				0.7	
<i>wt</i> -Acid- <i>t</i> -CA		11.9	8.0	14.9	5.4	0.9	1.0	2.2	1.6
<i>wc</i> -Acid- <i>t</i> -CA		7.3	4.7	10.0	4.5				1.6
<i>wt</i> -Alc- <i>t</i> -CA		3.6	0.6	4.9	0.5	2.7	3.0	3.5	2.6
<i>wc</i> -Alc- <i>t</i> -CA		1.8	2.5	3.6	1.7	0.6		1.0	
<i>wt</i> -Acid- <i>c</i> -CA						5.9		4.8	
<i>wc</i> -Acid- <i>c</i> -CA			0.3		0.8	0.5		0.2	
<i>wt</i> -Alc- <i>c</i> -CA						5.7	0.7	7.4	
$^{14}\text{CO}_2$		0.7				0.2			

Glu: glucuronide, Gly: glycine, Sul: sulfate.

taneous dose led to less amounts of the parent compounds and ester metabolites than the oral dose.

The major metabolites from the alcohol moiety were PBacid and 4'-OH-PBacid in free and conjugated forms. 4'-OH-PBacid-sulfate accounted for 63 to 66% with *t*-cyphenothrin and 34 to 58% with *c*-cyphenothrin. This conjugate was obtained with the *t*-isomer and subcutaneous dose to a greater extent compared with the *c*-isomer and oral dose, respectively. From the ^{14}C labeling, thiocyanate and carbon dioxide were produced as the major metabolites. Thiocyanate was obtained with the *t*-isomer and subcutaneous dose to a greater extent compared with the *c*-isomer and oral dose. As the acid moiety, *c*- or *t*-CA was found in the feces and urine in the amounts ranging from 0.2 to 1.8% of the dose. With the *t*-isomer, *t*-CA, *wt*-acid-*t*-CA, *wc*-acid-*t*-CA, *wt*-alc-*t*-CA and *wc*-alc-*t*-CA were detected in urine and feces. Among them, the predominant one was *wt*-acid-*t*-CA. The glucuronide of *t*-CA was found in urine, but not in the feces. The *c*-isomer produced the ester-cleaved metabolites to less extents than the *t*-isomer. The major metabolites were *wt*-acid-*t*-CA, *wt*-alc-*t*-CA, *wt*-acid-*c*-CA and *wt*-alc-*c*-CA. As for the *c*-isomer, the metabolites receiving the isomerization of *c*-isomer to *t*-isomer were found to a considerable extent.

7. In Vitro Study

Figure 2 shows the ester hydrolysis of *t*- and *c*-cyphenothrin and *t*- and *c*-phenothrin in 10% homogenates of several tissues. The intestine, spleen, pancreas and brain showed less activities to ester hydrolysis than the lung, kidney, liver and plasma. The hepatic microsome, precipitate (9000*g*) and supernatant (100,000*g*) fractions showed the same substrate specificity and hydrolyzed the *t*-isomers of cyphenothrin and phenothrin more rapidly than the corresponding *c*-isomers. Also, their fractions hydrolyzed *c*-cyphenothrin more rapidly than *c*-phenothrin whereas no significant differences were observed in the extent of the hydrolysis of these fractions between *t*-phenothrin and cyphenothrin. On the other hand, the plasma showed a different substrate specificity from the liver, hydrolyzed *t*- and *c*-isomers of both cyphenothrin and phenothrin at almost the same rates, and hydrolyzed phenothrin to a larger extent than cyphenothrin. The lung and kidney showed same substrate specificities as the liver. The hydrolytic activity in liver homogenate was almost completely inhibited by the presence of TEPP, even though the system of liver microsome-NADPH-TEPP (oxidase system) led to produce the ester-cleaved metabolites such as PBalc and PBacid. Their amounts were very small compared with those of metabolites produced in the esterase

Fig. 2 *in vitro* Degradation of *t*- and *c*-cyphenothrin(cyphe) and *t*- and *c*-phenothrin(phe) in several 10% tissue homogenate.

system.

DISCUSSION

t- and *c*-Cyphenothrin were readily metabolized in rats and the acid and alcohol moieties were almost completely eliminated from the bodies. The tissue residues with ^{14}C - and $^{14}\text{C}\alpha$ -labeled preparations were very low. These findings were nearly the same as those of pyrethroids such as permethrin,⁸⁾ phenothrin,³⁾ cypermethrin,⁹⁾ fenvalerate^{5,6)} and deltamethrin,¹⁰⁾ although both the cyphenothrin isomers showed less ^{14}C residue levels in the fat than pyrethroids as described above. However, the ^{14}C from the CN group showed somewhat slower excretion and higher tissue residue levels than that from the alcohol and acid moieties. These findings were also similar to those with the CN-labelings of fenvalerate,^{5,6)} cypermethrin,⁹⁾ and deltamethrin.¹⁰⁾

The ^{14}C excretion patterns of *t*-cyphenothrin did not remarkably differ from that of *t*-phenothrin (urine 75.1%; feces 20.9%),³⁾ but *c*-cyphenothrin showed less ^{14}C excretion into the feces in comparison with *c*-phenothrin (urine 22.4%; feces 73.5%)³⁾ after single oral

administration.

The elimination rates of the ^{14}C from the blood, brain and liver with *c*-cyphenothrin were greater than those of *c*-phenothrin by 1 to 2 times. On the other hand, there were no significant differences in the elimination rates between both the *t*-isomers of cyphenothrin and phenothrin.

Whole body radioautograms of rats treated orally with ^{14}CN -*t*- and *c*-cyphenothrin were very similar to those of ^{14}CN -fenvalerate.⁵⁾ It is likely that the ^{14}C residue in the stomach content, hair and skin of rats given ^{14}CN -*t*- and *c*-cyphenothrin were mainly SCN^- , as shown in the study on ^{14}CN -labeling of fenvalerate,⁶⁾ because the CN group of both cyphenothrin and fenvalerate⁶⁾ was mostly converted to SCN^- in rats and appeared to undergo the same metabolic fate.

Figure 3 shows metabolic pathways proposed for cyphenothrin isomers. The both isomers underwent the following major metabolic reactions; oxidation at 2'- and 4'-phenoxy positions of the alcohol moiety, and at the isobutenyl and *gem*-dimethyl groups, cleavage of ester linkage, conversion of CN group to

Fig. 3 Proposed metabolic pathways for *t*- and *c*-cyphenothrin(cyphe) in rats.

Table 7 Comparison of *in vivo* ester hydrolysis and *in vivo* oxidation at 4'-position between cyphenothrin and phenothrin isomers after oral administration.

Compound	% of the dose	
	Ester hydrolysis	Oxidation
<i>t</i> -Cyphe	78	68
<i>t</i> -Phe	74	55
<i>c</i> -Cyphe	51	37
<i>c</i> -Phe	17	40

Data show the summation of the ester-cleaved metabolites and metabolites oxidized at 4'-position from the $^{14}\text{C}\alpha$ -labeling in 0-2 days excreta.

SCN^- and CO_2 , and conjugation of the resultant carboxylic acids and phenols with glucuronic acid, sulfuric acid and glycine.

Table 7 shows the data of *in vivo* ester hydrolysis and *in vivo* oxidation at 4'-position estimated by summation of ester-cleaved metabolites in excreta of rats treated with $^{14}\text{C}\alpha$ -*t*- and -*c*-cyphenothrin and -phenothrin. Both the *t*-isomers showed no significant differences in the extents of ester hydrolysis and oxidation. However, *c*-cyphenothrin underwent ester hydrolysis as 3 times as larger extent compared with *c*-phenothrin. Thus, the larger amounts of ester metabolites was obtained from *c*-phenothrin than from *c*-cyphenothrin.

The results of the above *in vivo* study were in accordance with those from the *in vitro* study. The *t*-isomers of cyphenothrin and phenothrin were hydrolyzed more rapidly in liver homogenates than those in the corresponding *c*-isomers, and *c*-cyphenothrin was hydrolyzed to a larger extent than *c*-phenothrin. In contrast, in many previous studies,¹¹⁻¹³⁾ the ester bond of pyrethroids with secondary alcohol was generally considered not to be hydrolyzed easily as compared with the corresponding one with primary alcohol. Soderlund and Casida¹⁴⁾ reported that cyanopyrethroids were hydrolyzed much more slowly than the corresponding primary alcohol analogues. Hutson *et al.*⁹⁾ suggested that the ester linkage of α -cyano pyrethroids was hydrolyzed not by microsomal esterase, but by NADPH-dependent oxidase system.

The substrate concentrations in the above

in vitro studies,¹¹⁻¹⁴⁾ however, seem to be rather high as compared with that in liver actually occurring after administration in *in vivo* experiments. Thus, in this study the substrate concentrations were fixed at approximately 3 to 4 times higher than the *in vivo* ^{14}C maximum level, corresponding to 1/40 to 1/100 of the substrate levels applied in the above studies. It is most likely that in the *in vitro* ester hydrolysis using liver homogenates, the esterases were under most favorable conditions as in *in vivo* experiment in the low substrate concentrations, whereas NADPH-dependent oxidase system contributed majorly in the high substrate concentrations. On the other hand, plasma esterases showed different substrate specificity from the liver esterases and hydrolyzed the *t*- and *c*-isomers of cyphenothrin and phenothrin nearly to the same extents. However, they were less active to cyphenothrin than to phenothrin. This feature of plasma esterases not distinguishing *trans* from *cis* configuration is the same as that of bioresmethrin and cismethrin reported by White *et al.*¹⁵⁾

From the results of the present *in vivo* and *in vitro* studies, the CN group introduced into the molecule did not affect biodegradability of *t*-cyphenothrin, but rather it made *c*-cyphenothrin more biodegradable than *c*-phenothrin. These *in vivo* characteristics may be determined by the activity and/or substrate specificity of the liver esterases.

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要 約

Cyphenothrin および phenothrin 立体異性体のラットにおける比較代謝

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Cyphenothrin [S-2703, (RS)- α -cyano-3-phenoxybenzyl-(1RS, *trans/cis*, 8/2)chrysanthemate] の [1R, *trans*]-および [1R, *cis*]-体の 3 種 ^{14}C 標識体 (酸側, アルコール側および CN 基) をそれぞれラットに 2~4 mg/kg の割合で 1 回経口または皮下投与すると酸側およびアルコ

ール側由来の ^{14}C は速やかにかつほぼ完全に排泄され組織残留量もきわめて低かった。一方, CN 基由来の ^{14}C 排泄は不完全で被毛, 皮膚および胃内容物中に若干高い ^{14}C 残留量が認められた。両異性体の主要代謝経路はアルコール側の 2' および 4' 位の水酸化, 酸側のイソブテニル基および *gem*-ジメチル基の酸化, エステル結合の開裂, CN 基の SCN^- および CO_2 への変換およびこれらの代謝反応を経て生成したフェノール, アルコールおよびカルボン酸類と硫酸, グルクロン酸およびアミノ酸との抱合反応であった。[1R, *trans*]-体は [1R, *cis*]-体よりエステル結合の開裂を多く受け, 一方, [1R, *cis*]-体からはエステル結合を有する代謝物が [1R, *trans*]-体より多く生成した。投与経路が異なっても組織残留量および代謝物の性質には差が認められなかったが, 皮下投与は経口投与に比べてエステル結合が開裂した代謝物を多く与えた。一方, 上記の結果を phenothrin [3-phenoxybenzyl (1RS, *trans/cis*) chrysanthemate] と比較すると [1R, *trans*]-cyphenothrin および phenothrin 間では生分解性に関してほとんど差は認められなかったが, [1R, *cis*]-cyphenothrin は [1R, *cis*]-phenothrin に比べて CN 基を導入することによって著しく生分解性が高められた。また *in vivo* での生分解性と肝エステラーゼの基質特異性は一致し次のようであった。 *trans*-phenothrin $\geq$ *trans*-cyphenothrin > *cis*-cyphenothrin > *cis*-phenothrin。一方, 血漿エステラーゼは *trans/cis* の選択性を示さず phenothrin を cyphenothrin より速やかに加水分解した。