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Distribution and Metabolism of ^{3}H-2,5,2′,5′-tetrachlorobiphenyl in Rats[1] (38610)

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Recently, the effects of isomeric mixtures of polychlorinated biphenyls (PCBs) have received considerable attention. It has been reported (1, 2) that humans develop chloracne and subcutaneous edema and exhibit lethargy and nausea as a result of PCB intoxication. Nonhuman primates exhibit similar effects as well as pathological changes which include hypertrophy of the hepatic smooth endoplasmic reticulum (ER) and mucosal lining of the stomach (3–5). Alterations in protein levels and hepatic enzyme activity have also been observed in nonhuman primates after PCB intoxication. No gross abnormalities have been observed in rats, although similar hepatic ER and enzyme alterations have been noted (6, 7). In addition, it has been shown that the effect on hepatic function is increased as the chlorine content of the PCB isomers increases (8).

The necessity for further understanding of the distribution and metabolism of these compounds is evident. Since the complexity of the PCB mixtures makes such studies difficult, it has been necessary to use pure components. The compound 2,5,2′,5′-tetrachlorobiphenyl (TCB) has been shown to be one component of the PCB mixtures presently in use (9, 10).

Hutzinger *et al.* (11) were first to report hydroxylated metabolites of TCB in the excreta of rats and pigeons. However, structural confirmation of these findings was lacking. Yoshimura and coworkers (12) identified monohydroxy-tetrachlorobiphenyl among several phenolic metabolites of 2,4,3′,4′-tetrachlorobiphenyl in rat feces. Recently, Gardner *et al.* (13) have isolated and characterized three metabolites of TCB in rabbits. Two of the metabolites were identified as monohydroxy-TCB with the hydroxyl group located in the three or four position, while the third metabolite was identified as trans-3,4-dihydro-3,4-dihydroxy-TCB.

The presently reported study was undertaken to establish the distribution of TCB in the tissue and excreta of rats using ^{3}H-TCB. Further information was sought as to the amount of TCB metabolized and confirmative identification of these metabolites.

Materials and Methods. ^{3}H-TCB was prepared from 2,5,2′,5′-tetrachlorobenzidine by the method of Hutzinger and Safe (14). The compound was shown to be greater than 99% pure by gas liquid chromatography, with a specific activity of 1.13 μCi/mg after dilution with TCB. The compound was dissolved in corn oil as final preparation for dosage.

Four groups (three animals per group) of male Sprague–Dawley rats, weighing 92–103 g initially, were housed in metabolism cages and allowed to acclimate for 3 days prior to dosage. The rats were subsequently given a single dose of ^{3}H-TCB by gastric intubation. Each animal received 50.0 mg (56.5 μCi) of the compound in 0.5 ml corn oil. The animals were given access to unlimited food and water throughout the experiment. Feces and urine were collected at 24-hr intervals. The animals were sacrificed at 1, 3, 7, and 14 days and tissue samples collected for radioactivity analysis and histologic evaluation. All tissue and fecal samples (200–500 mg) were oxidized in a Packard Model 306 sample oxidizer, collected in Monophase 40 (Packard) scintillation cocktail, and the radioactivity measured in a Packard Tri-Carb liquid scintillation counter. Samples of blood and

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urine (0.5 ml) were treated similarly. In addition, subcellular fractions of the liver were prepared by the method of Hogeboom (15) and analyzed as described above.

Isolation and identification of metabolites were done on fecal and urine samples from the first 48 hr after dosage. Urine was freeze-dried and extracted with hexane and ethyl ether by two different methods. The first method involved hydrolyzing the sample after hexane extraction with glucuronidase–sulfatase (G–S) by the method of Reid *et al.* (16), followed by extraction with ethyl ether and a subsequent acid hydrolysis before a second ether extraction. The second procedure was similar to the above except no G–S hydrolysis was performed. Feces was extracted continuously first with acetone and then with methanol. TCB and metabolites were purified from the hexane extract of urine and acetone and methanol extracts of feces by thin layer chromatography and the samples analyzed by gas liquid chromatography, mass spectrometry, and infrared spectrometry. Ether extracts of urine could not be purified by TLC. These samples will be analyzed in future experiments upon development of a system for purification.

TLC samples were applied to silica gel 1B, flexible plates (J. T. Baker Chem. Co.) and developed with hexane, hexane:ethyl acetate (8:2), or hexane:acetone (1:1). The plates were scanned on a Model 7201 Radiochromatogram Scanner (Packard) and the peaks eluted with methanol for further analysis. GLC was done on a Model 7620A Hewlett-Packard gas liquid chromatograph fitted with an EC detector. Glass columns (⅛″ id × 6′) containing 2% Apiezone L on Gaschrom Q (100–120 mesh) at 215° were used. Argon-methane was used as carrier gas at approximately 40 cc/min. Samples for mass spectral analysis were collected from the GLC in capillary tubes which can be inserted directly into a MS-9 mass spectrometer (Associated Electrical Industries, Ltd.) equipped with a direct insertion probe. Samples for IR analysis were collected from the GLC on KBr in capillary tubes. A 1.5 mm KBr pellet was made with a KBr Ultra Micro Die (Perkin-Elmer Corp) and the IR spectrum measured on a Hitachi Model 247 Grating Infrared Spectrometer fitted with a beam condenser (Perkin-Elmer Corp.).

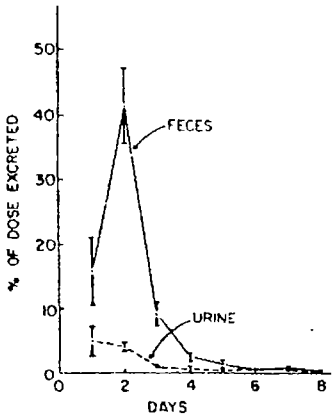

FIG. 1. Excretion of ^3H by rats for the first 8 days following a single oral dose of ^3H-2,5,2′,5′-tetrachlorobiphenyl. Each point represents mean ± 1 SD for three rats. Daily excretion values for feces and urine after 8 days were less than 0.2% of dose.

Results. A. *Distribution.* The experimental animals appeared normal, exhibited normal weight gain, and in general showed no deleterious effects from the TCB dosage. Microscopically, the tissues showed no abnormalities. The greatest percentage of the recovered tritium was found in the excreta (Fig. 1). Approximately 66% of the tritium was excreted in the feces by 72 hr with an additional 10% recovered in the urine. At 14 days the total tritium in the feces and urine was 72.5 ± 5.7% and 12.9 ± 4.0%, respectively. Through the first 7 days the largest internal concentrations of tritium were located in the adipose tissue, blood, liver, and skin (Table I). Significantly high concentrations of tritium were found in several other tissues at 24 hr (including the thymus, brain, lung, spleen, heart, small intestine, and muscle), but these values decreased rapidly and at 3 days were less than 2% of that present in the tissue containing the highest concentration, i.e., adipose tissue (0.243 ± 0.18% of dose/g). Although the specific activity of the blood (0.009 ± 0.002% of dose/g) was slightly higher, the levels throughout the body were generally constant (average: 0.004 ± 0.002% of dose/g) at 14 days.

In addition to the tritium present in the

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TABLE I. TISSUE CONCENTRATIONS[a] OF ^3H IN RATS AT 1, 3 AND 7 DAYS FOLLOWING A SINGLE ORAL DOSE OF ^3H-2,5,2',5'-TETRACHLOROBIPHENYL.

	1 day[b]	3 days[b]	7 days[c]
Adipose tissue	1.345 ±0.680	0.243 ±0.180	0.058 ±0.044
Blood[d]	0.593 ±0.193	0.126 ±0.017	0.044 ±0.019
Liver	0.281 ±0.055	0.035 ±0.004	0.008 ±0.002
Stomach	0.792 ±0.613	0.013 ±0.009	0.004 ±0.001
Kidney	0.540 ±0.082	0.010 ±0.002	0.005 ±0.00
Testes	0.143 ±0.037	0.012 ±0.006	0.005 ±0.003
Large intestine	0.445 ±0.147	0.011 ±0.004	0.003 ±0.001
Skin	0.344 ±0.144	0.026 ±0.007[c]	0.005 ±0.002

[a] Percent of dose per g tissue ± standard deviation.
[b] Mean for three rats.
[c] Mean for two rats.
[d] Percent dose per ml.

excreta and specific tissues evaluated, 2.1 ± 0.57% of the original radioactivity was detected in the remaining tissues of the animals on the 14th day of the experiment. Total recoveries for 1, 3, 7 and 14 days were 36.8 ± 6.4%, 79.9 ± 11.2%, 82.6 ± 4.0%, and 87.6 ± 7.8%, respectively. The low recovery obtained for the 24-hr animals was probably due to the large quantity of tritiated compounds in the intestinal tract which was not measured. This conclusion is substantiated by the fecal excretion value for the 24- to 48-hr period.

Subcellular fractions of the liver showed the largest specific activity of tritiated compounds in the microsomal fraction (Fig. 2). Significant specific activities were also found in the mitochondrial fraction with relatively low specific activity in the nuclei.

B. *Metabolites.* The procedures employed in metabolite extraction and TLC separation produced six purified compounds of metabolized TCB. Since complete identification of all these compounds was not possible, it is not known if any are identical and hence each will be termed a metabolite.

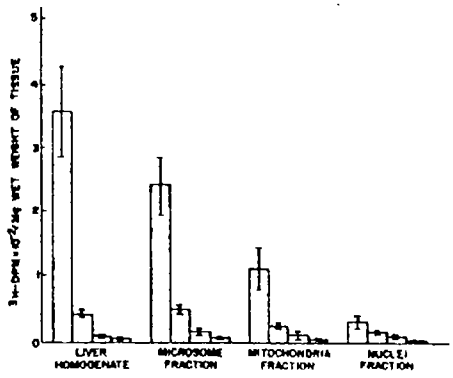

FIG. 2. Distribution of ^3H in liver homogenates and subcellular fractions of liver 1, 3, 7, and 14 days (L to R) following a single oral dose of ^3H-2,5,2',5'-tetrachlorobiphenyl. I signifies mean ± 1 SD.

TABLE II. DISTRIBUTION[a] OF TRITIUM IN EXTRACTS OF 24- AND 48-HOUR URINE FROM RATS GIVEN A SINGLE ORAL DOSE OF ^3H-2,5,2',5'-TETRACHLOROBIPHENYL.

Extract	24 hr G-S[b]	48 hr G-S[b]	24 hr	48 hr
Hexane	49.05	47.14	37.22	34.14
Ether-1[c]	33.71	20.67	11.11	7.37
Ether-2[d]	7.00	17.10	29.03	21.53
Aqueous	4.93	9.38	11.99	17.67

[a] Percent of freeze-dried urine.
[b] Glucuronidase-sulfatase-treated.
[c] Before acid hydrolysis.
[d] After acid hydrolysis.

Freeze-drying of the urine samples caused a loss of radioactivity between 50 and 65%. Table II shows the distribution of radioactivity remaining in the freeze-dried urine for the 24- and 48-hr urine samples. The percentage of radioactivity remaining after extraction is reported as percent in the aqueous phase. Glucuronidase–sulfatase treatment yielded a slightly improved recovery over the samples that were only acid hydrolyzed. No significant difference was noted between the 24- and 48-hr samples as to the amounts extracted or the metabolites found. A single metabolite (Metabolite I) was isolated from the hexane extracts of the urine samples. No unmetabolized TCB was found in these samples.

The tritium recoveries in the acetone and methanol extracts of feces were 77.4%

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TABLE III. CHROMATOGRAPHIC DATA FOR METABOLITES OF 2,5,2',5'-TETRACHLOROBIPHENYL ISOLATED FROM 24 AND 48 HOUR FECES AND URINE SAMPLES FROM RATS GIVEN A SINGLE ORAL DOSE OF ^{3}H-2,5,2',5'-TETRACHLOROBIPHENYL.

Metabolite	Origin	Fraction	TLC solvent[a]	R_f	GLC retention time (sec)
I	Urine	Hexane	A	0.8	120
II	Feces	Acetone	B	0.4	125
III	Feces	MeOH	B	0.0	
			C	0.4	124
IV	Feces	MeOH	B	0.25	
			C	0.34	122
V	Feces	MeOH	B	0.0	
			C	0.0	
			A	0.6	134
VI	Feces	MeOH	B	0.0	
			C	0.0	
			A	0.45	137

[a] TLC solvents were: A—hexane:acetone (1:1); B—hexane; C—hexane:ethyl acetate (8:2).

and 18.4% respectively. It was found that 8.7% of the acetone fraction was unmetabolized TCB, while no unmetabolized TCB was found in the methanol extract. Of the remaining activity in the acetone fraction only one metabolite was isolated (Metabolite II) which after purification comprised 47.3% of the total radioactivity in the fraction. TLC of the methanol extract of feces yielded four additional metabolites (Metabolites III–VI). The TLC and GLC data for the six metabolites are given in Table III. Mass spectra were identical for Metabolites I, II, IV and V with a molecular ion at 306 a.m.u. indicative of monohydroxy-TCB. Low quantities of Metabolites III and VI made it impossible to purify these samples in sufficient quantities for mass spectral analysis. Metabolite II was identified on the basis of the mass spectrum and IR spectrum as 3-OH-TCB. The IR spectrum compared extremely well with that for 3-OH-TCB reported by Gardner *et al.* (13), although minor differences occurred due to the use of KBr pellets instead of CCl_4 solutions of the metabolites. No other metabolites could be purified in sufficient quantity for IR analysis. The identical mass spectra, however, led to the conclusion that metabolites I, II, IV and V are all monohydroxy-TCB, although the positions of the hydroxyl groups were indeterminable. The mass spectrum and IR spectrum for Metabolite II are given in Fig. 3.

Discussion. The data indicate that over 95% of the recovered tritium was in the excreta of which over 90% was metabolized. The unmetabolized TCB found in the feces was probably the portion of the initial dose which was not absorbed. The loss of radioactivity in the urine from freeze-drying is probably due either to loss of tritium during hydroxylation of TCB or to a greater ability of the hydroxylated metabolites to exchange tritium with water. At least 60% and probably a considerably larger fraction of the metabolites are in the form of monohydroxy-TCB. However, the possibility of dihydroxy-TCB as a major metabolite as reported by Gardner *et al.* (13) cannot be ruled out from these data.

Allen *et al.* (17) have reported significantly higher concentrations of Aroclor 1248, a mixture of polychlorinated biphenyls, in the lipid of chronically treated rats than was observed for similarly treated rats given 2,5,2',5'-tetrachlorobiphenyl. Furthermore, the proliferation of the hepatic endoplasmic reticulum and alterations of other hepatic functions are more severe for Aroclor 1248 than for the single isomer.

The present study shows that TCB is rapidly metabolized to one or more hydroxylated metabolites and excreted. There is apparently no appreciable storage depot for the compound within the animal, as the specific activities in the tissues are all reduced to very low values after 14 days. The relatively high specific activity in the blood of the animals indicates that the compound remains mobile until metabolized and excreted.

Gardner *et al.* (13) has reported that the probable mechanism for formation of hydroxylated metabolites of TCB is through an epoxide intermediate. It is likely that the components of Aroclor 1248 with higher chlorine content which cannot form epoxide intermediates are the cause of these major differences between Aroclor 1248 and TCB.

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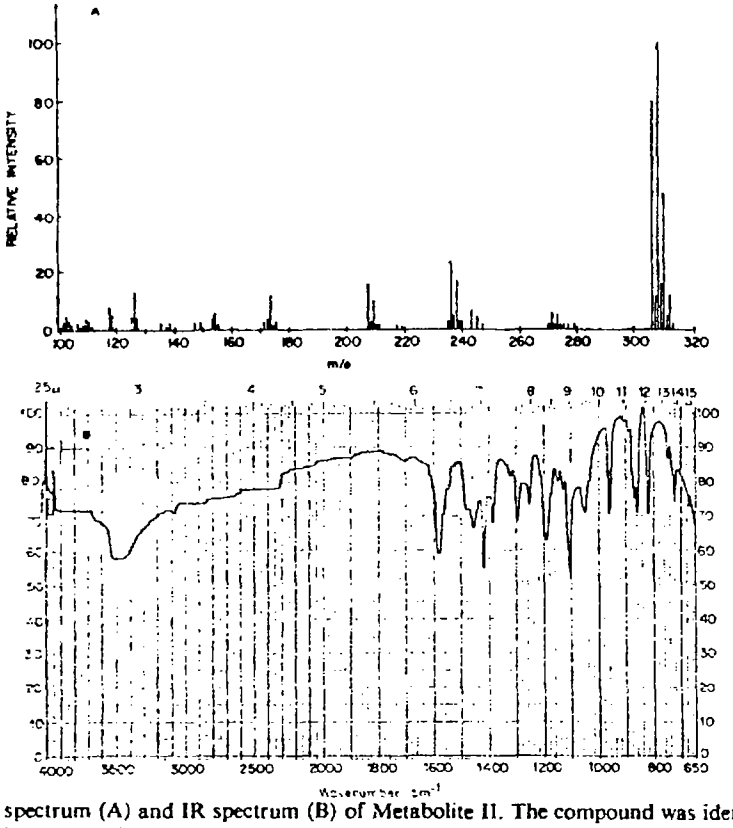

FIG. 3. Mass spectrum (A) and IR spectrum (B) of Metabolite II. The compound was identified as 3-OH-2,5,2′,5′-tetrachlorobiphenyl.

Acute lethal doses of TCB and Aroclor 1248 have been reported to be approximately 1.2–1.5 g/kg body wt. Further evidence of the importance of metabolism to toxicity is that both Aroclor 1248 and TCB were shown to be lethal to rats at significantly lower doses than 1.2 g/kg when metabolism was inhibited by administration of SKF-525A. Furthermore, no mortality was observed in rats at doses of 1.25 g/kg when metabolism was enhanced by treatment with phenobarbital (17). It thus appears that metabolism of polychlorinated biphenyls in rats is a method of detoxification of these compounds.

Summary. Distribution and metabolism of an isomeric polychlorinated biphenyl were determined in rats. Over 70% of a single dose of ^{3}H-2,5,2′,5′-tetrachlorobiphenyl was excreted in the feces and another 13% in the urine during a 14-day period. Adipose tissue, blood, skin, and liver were found to contain low levels of the compound. Over 90% of the excreted tritiated compound was found to be metabolized, the major metabolite being identified as 3-OH-2,5,2′,5′-tetrachlorobiphenyl. All of the unmetabolized 2,5,2′,5′-tetrachlorobiphenyl was eliminated by the alimentary route.

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