A Comparative Study on Accumulation and Elimination of Tetrachlorobiphenyl Isomers in Mice

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INTRODUCTION

The presence and persistence of polychlorinated biphenyls (PCB's) in various global ecosystems have been well recognized (FISHBEIN 1972). Considerable papers have dealt with the PCB accumulation and elimination during a long term oral exposure (CURLEY et al. 1971; BURSE et al. 1974). Whereas only limited data are available on the bioaccumulation of individual chlorobiphenyl isomers of known structure (JAN et al. 1975; GAGE et al. 1976). This study was undertaken to obtain the systematic information on the accumulation and elimination of all the possible isomers of symmetrically substituted tetrachlorobiphenyl (TCB) in connection with their structures.

MATERIALS AND METHOD

Symmetric TCB's were unambiguously synthesized from the respective dichloroiodobenzenes by a modified Ullmann procedure (KOLNBLUM and KENDALL 1952). All the isomers were estimated to be over 99.9% pure when examined by gas chromatography with electron-capture detection (EC-GC) and by thin layer chromatography on fluorescent silica gel layers. The physical data of the isomers used in this study are shown in Table 1.

Experiment A: A group of 20 female mice weighing 20-22 g was fed ad libitum a diet containing 10 ppm of each of the TCB isomers listed in Table 1 for 20 days. At the end of dosing period the treated diets were replaced with a control diet. Five mice from each group were sacrificed at the intervals scheduled according to a preliminary experiment. The liver was removed and analysed separately from the remainder of carcass.

Experiment B: A group of 20 mice from the same source as in experiment A was fed a diet containing 200 ppm of phenobarbital (PB) plus 10 ppm of 2,4,2',4'-, 2,5,2',5'-, or 3,5,3',5'-TCB for 20 days. After the

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TABLE 1

Physical data of TCB isomers

Compound	mp (°C)	Rt[a]	Rf[b]
2,3,2',3'-TCB	116 - 120	60	0.57
2,4,2',4'-TCB	39 - 40	49	0.73
2,5,2',5'-TCB	83 - 85	46	0.71
2,6,2',6'-TCB	197	33	0.56
3,4,3',4'-TCB	177	106	0.63
3,5,3',5'-TCB	166 - 168	70	0.77

[a] Relative to p,p'-DDE (=100) on an OV-1 column at 190°

[b] On commercially prepared TLC plate (Wakogel FM) developed with hexane

end of this period a diet containing 200 ppm of PB alone was fed and 5 mice from each group were sacrificed at intervals. The whole body of mice was subjected to residue analysis.

Food consumption of each group was measured during the TCB-dosing periods in both experiments.

For residue analysis, each sample was digested by the method of STANLEY and LEFAVOURE (1965) using a mixture of acetic acid and 60% perchloric acid (1:1). The digest was extracted with n-hexane. The extract was treated with fuming sulfuric acid to remove lipids and, after centrifugation if neccessary, the organic layer was analysed by EC-GC on columns of OV-1 and/or OV-17.

RESULTS AND DISCUSSION

The dosage level of TCB's, 10 ppm, was chosen to be high enough to permit precise and accurate determination of residue levels but low enough to have minimal effect on the activity of microsomal drug-metabolizing enzymes in mice. The dosage level of PB in experiment B was chosen so as to cause a significant increase in microsomal enzyme activity. The validity of the adopted dosage levels was confirmed by a separate experiment, where the enzyme activity was followed by the measurements of pentobarbital-sleeping times of mice.

The concentrations of TCB isomers at various times after the end of exposure in experiments A and B are

TABLE 2

Concentrations of TCB isomers at various times after end of exposure in experiment A, mean ± SEM for 5 mice, μg/g of wet tissue

Compound	Time after end of exposure (days)							
	1	2	3	4	5	7	12	19
2,3,2',3'-TCB								
Carcass	0.079 ±0.018	0.025 ±0.006	0.010 ±0.001	0.006 ±0.001				
Liver	Not detected							
2,4,2',4'-TCB								
Carcass	10.35 ±1.91				7.63 ±0.95		3.53 ±0.90	2.99 ±0.58
Liver	4.14 ±0.25				3.74 ±0.09		1.71 ±0.18	0.98 ±0.17
2,5,2',5'-TCB								
Carcass	3.20 ±0.52		2.96 ±0.29		1.98 ±0.66	1.09 ±0.25		
Liver	0.67 ±0.08		0.38 ±0.04		0.27 ±0.06	0.16 ±0.05		
3,4,3',4'-TCB								
Carcass	0.113 ±0.030	0.116 ±0.038	0.021 ±0.005	0.016 ±0.005				
Liver	Not detected							
3,5,3',5'-TCB								
Carcass	3.40 ±0.29		1.41 ±0.30		1.06 ±0.41	0.67 ±0.29		
Liver	0.58 ±0.04		0.20 ±0.11		0.15 ±0.05	0.11 ±0.03		

TABLE 3

Concentrations of TCB isomers at various times after end of exposure in experiment B, mean ± SEM for 5 mice, μg/g of wet tissue

Compound	Time after end of exposure (days) 1	3	5	7	8	15	22
2,4,2',4'-TCB + PB							
Carcass	2.87 ±0.66				0.66 ±0.16	0.28 ±0.06	0.17 ±0.08
Liver				Not detected			
2,5,2',5'-TCB + PB							
Carcass	0.24 ±0.05	0.20 ±0.02	0.04 ±0.01	0.07 ±0.02			
Liver		Not detected					
3,5,3',5'-TCB + PB							
Carcass	1.42 ±0.30	0.78 ±0.13	0.49 ±0.18	0.09 ±0.03			
Liver		Not detected					

shown in Table 2 and 3, respectively. The results given in Table 2 show that the degree of accumulation varies widely from compound to compound irrespective of the same degree of chlorine substitution. In the case of 2,6,2',6'-TCB, no detectable residues (0.001 ppm≤) were found in any of the samples, possibly because of the extremely high metabolic lability of this isomer. Even among the remaining isomers more than 130-fold difference in residues was observed at 1 day after the end of exposure.

TABLE 4

Regression lines for relationship between concentrations of TCB isomers and time after end of exposure

Compound	Regression line[a] $\log Y = -kX + \log Y_0$
Experiment A	
2,3,2',3'-TCB	
Carcass	$\log Y = -0.3490X + \bar{1}.1067$
2,4,2',4'-TCB	
Carcass	$\log Y = -0.0329X + 0.9970$
Liver	$\log Y = -0.0384X + 0.6937$
2,5,2',5'-TCB	
Carcass	$\log Y = -0.0898X + 0.6395$
Liver	$\log Y = -0.1162X + \bar{1}.9246$
3,4,3',4'-TCB	
Carcass	$\log Y = -0.3311X + \bar{1}.3977$
3,5,3',5'-TCB	
Carcass	$\log Y = -0.1408X + 0.6137$
Liver	$\log Y = -0.1346X + \bar{1}.7239$
Experiment B	
2,4,2',4'-TCB + PB	
Carcass	$\log Y = -0.0619X + 0.3579$
2,5,2',5'-TCB + PB	
Carcass	$\log Y = -0.1164X + \bar{1}.4426$
3,5,3',5'-TCB + PB	
Carcass	$\log Y = -0.2008X + 0.4106$

[a] Y = concentration in μg/g of TCB, X = time in days after end of exposure

The concentration of each isomer in liver was less than that in carcass by a factor of 5 or more with the exception of 2,4,2',4'-TCB. The relatively high liver residue of 2,4,2',4'-TCB may be explained by a certain special affinity of this isomer for liver tissue.

When the concentration (Y) of each isomer were plotted against time (X) after the end of exposure on semilogalithmic paper, approximately linear relationship was observed. Therefore, the results were fitted to the following general equation by the method of least squeares.

$$\log Y = -kX + \log Y_0 \qquad (1)$$

TABLE 5

Biological half-lives and initial concentrations of TCB isomers

Compound	$t_{1/2}$ (days)	Confidence limits P=0.95	Initial concn. (μg/g)
Experiment A			
2,3,2',3'-TCB			
Carcass	0.9	(0.7 - 12)	0.13
2,4,2',4'-TCB			
Carcass	9.2	(6.4 - 16)	9.93
Liver	7.8	(6.4 - 10)	4.94
2,5,2',5'-TCB			
Carcass	3.4	(2.2 - 7.3)	4.36
Liver	2.6	(1.8 - 4.5)	0.84
3,4,3',4'-TCB			
Carcass	0.9	(0.7 - 1.5)	0.25
3,5,3',5'-TCB			
Carcass	2.1	(1.5 - 3.7)	4.11
Liver	2.2	(1.4 - 5.2)	0.53
Experiment B			
2,4,2',4'-TCB + PB			
Carcass	4.9	(3.7 - 7.0)	2.28
2,5,2',5'-TCB + PB			
Carcass	2.6	(1.6 - 6.2)	0.28
3,5,3',5'-TCB + PB			
Carcass	1.5	(1.2 - 2.1)	2.57

where k is the rate constant of decline and Y_0 is the initial concentration. The results are shown in Table 4. Analysis of variance showed that there exists no significant deviation from linearity in each case ($P>0.01$). The biological half-lives and the estimates of initial concentration were calculated from the slopes and intercepts of regression lines, respectively. The results are shown in Table 5.

There were clean differences in the biological half-lives of the isomers, but no apparent relationship between substitution pattern and biological half-life could be observed.

In the case of any isomers, there were no significant difference between the values of the rate constants for carcass (excluded with liver) and liver. It is indicated, therefore, that a dynamic equilibrium exists between the concentrations in these two parts of body.

Dosing with PB, a potent inducer of hepatic drug-metabolizing enzymes, throughout experiment B has not significantly increased the rates of decline of 2,4,2',4'-, 2,5,2',5'-, and 3,5,3',5'-TCB's ($P>0.01$). These results suggest that the rate limiting step in the declines of these isomers is the elimination process from a certain storage site, possibly body fat mass, rather than the metabolic one in liver. In contrast, the initial residue levels of 2,4,2',4'- and 2,5,2',5'-TCB's were markedly reduced by the treatment of mice with PB. These observations could be atributed to a reduced intake of the isomers into the storage site. The relative constancy of the initial residue levels of 3,5,3',5'-TCB between experiment A and B seems to reflect the lower susceptibility of this isomer to hepatic metabolism, probably because this isomer contains only isolated, unsubstituted carbon atoms in the biphenyl ring.

If we assume that the constant amount (I_s) of an orally ingested compound daily entered the storage site during the dosing period, whereas the elimination of the compound from the storage site occured even during the dosing period according to the first order equation with the rate constant k, the body burden (A_t) at time t would be given by the equation:

$$A_t = (I_s/2.303k)(1 - e^{-2.303kt}) \qquad (2)$$

where t is time after the beginning of exposure. The value of body burden (A_{20}) at t = 20 (i.e. at the end of

exposure) can be estimated by the equation:

$$A_{20} = w\ Y_0 \qquad (3)$$

where w is the average body weight of mice at the end of exposure. By use of the numerical values of k shown in Table 4 and A_{20} calculated by equation (3), the estimate of the daily amount (I_s) which entered the storage site was calculated from equation (2). To assess the degree of accumulation we introduced an index, storage ratio, defined as

$$\text{storage ratio} = I_s/I_o \qquad (4)$$

where I_o is the measured average daily amount of oral ingestion. The storage ratios and the values of the quantities A_{20}, I_o, and I_s used in the calculation of the storage ratios are shown in Table 6.

Storage ratio may be correlated to the extents to which a given compound is absorbed and metabolized prior to deposition in the storage site. For example,

TABLE 6

Body burdens (A_{20}) at end of exposure, daily intakes into storage site (I_s), daily oral intakes (I_o), and storage ratios (I_s/I_o) of TCB isomers

Compound	A_{20} (μg)	I_s (μg/day)	I_o (μg/day)	I_s/I_o
Experiment A				
2,3,2',3'-TCB	3.4	2.8	49	0.06
2,4,2',4'-TCB	230.4	22.4	40	0.6
2,5,2',5'-TCB	105.1	21.2	39	0.5
3,4,3',4'-TCB	6.2	4.7	44	0.1
3,5,3',5'-TCB	93.1	30.2	45	0.7
Experiment B				
2,4,2',4'-TCB + PB	56.8	8.6	39	0.2
2,5,2',5'-TCB + PB	6.8	1.8	47	0.04
3,5,3',5'-TCB + PB	67.0	31.0	47	0.7

when a compound can be completely absorbed and hardly metabolized, its storage ratio will be close to unity. ALBRO and FISHBEIN (1972) has indicated that various chlorobiphenyl isomers show a high degree of absorption and varying degrees and patterns of substitution cause no appreciable difference in absorption rate. Therefore, storage ratio can be expected to reflect predominantly the rate of metabolism.

The lower values of storage ratio for 2,3,2',3'- and 3,4,3',4'-TCB's, hence, imply that these orientations of chlorine substituents, as well as that of 2,6,2',6'-TCB, are favoured for ready metabolic degradation. The storage ratios for 2,4,2',4'-, 2,5,2',5'-, and 3,5,3',5'-TCB's in experiment A were very similar, but the induction of microsomal enzyme activity caused by PB-treatment in experiment B resulted in clear difference in the value of storage ratio among these isomers. The increasing order of the storage ratios in experiment B, 2,5,2',5'-TCB < 2,4,2',4'-TCB < 3,5,3',5'-TCB, would correspond to the increasing inherent recalcitrancy to metabolic degradation.

The actual magnitude of accumulation during the course of a prolonged daily exposure depends upon both the biological half-life and the storage ratio of each compound. For example, the biological half-life of 3,5,3',5'-TCB was at most 2 times that of 2,3,2',3'-TCB, whereas the former exhibited more than 40-fold accumulation at the end of exposure. This discrepancy can be accounted for by the large difference in storage ratio between these two isomers. Thus, the significance of the storage ratio, hereinabove defined as a measure of bioaccumulability, would be obviously understood as well as of biological half-life.

Further work is in progress on the more highly chlorinated biphenyl isomers.

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