Intestinal Absorption of Polychlorinated Biphenyls in Rats

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The polychlorinated biphenyls (PCB's) are becoming of increasing concern as environmental contaminants (1,2,3). Dangers associated with the widespread use and toxicity of the PCB's have been discussed in some detail by Risebrough and Bodine (4) and by Sax (5). Various modes of entry of PCB's into the ecosystem have been suggested (1), including inhalation of vapors from burning trash containing PCB's, and absorption through the skin. It is the purpose of the present report to provide some quantitative data on the extent to which the PCB's can be absorbed from the diet.

MATERIALS AND METHODS

2-Chlorobiphenyl and 3-chlorobiphenyl were from K & K Laboratories, Inc., Plainview, N. Y. Biphenyl, 4-chlorobiphenyl and 4,4'-dichlorobiphenyl were from Aldrich Chemical Co., Inc., Milwaukee, Wis. 2,2'-Dichlorobiphenyl was from Chemicals Procurement Laboratories, Inc., College Point, N. Y. 2,5,2',5'- and 3,4,3',4'-tetrachlorobiphenyls, 2,4,5,2',5'-pentachlorobiphenyl and 2,4,5,2',4',5'-hexachlorobiphenyl were kindly provided by Dr. Ronald G. Webb, Southeast Water Laboratory, Athens, Ga. The other PCB's used in this study were synthesized here by methods identical or analogous to those described by Hutzinger et al. (6).

GLC packings were obtained from Applied Science Laboratories or Supelco, Inc. Male CD strain rats were supplied by Charles River Breeding Labs and equilibrated on D & G laboratory diet obtained from Price-Wilhoite Co., Frederick, Md. Florisil was obtained fully activated from the Floridin Co., and hydrated by equilibration for one week with 5% of its weight of water.

The procedure used to assay uptake of PCB's by rats was a modification of the squalane-based system previously described as applicable to the study of hydrocarbon absorption (7). Briefly, the procedure consists of (a) feeding a mixture of PCB's and squalane to rats, (b) collecting feces until no further excretion of intact PCB's or squalane occurs (about 4 days), (c) extracting lipids from the feces with a chloroform:methanol mixture, (d) purifying the extract to remove ester lipids and sterols, and (e) comparing the molar ratios (PCB:squalane) in

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the feces to those in the mixture fed. Details of the procedure may be found in the original reference (7).

Excreted PCB's were isolated along with the squalane marker as follows: the fecal extract was taken to dryness *in vacuo* and redissolved in hexane:diethyl ether 98:2 (v/v). Five grams of Florisil (5% hydrated) were added to 15 ml of solution and the mixture was shaken for 5 minutes. The mixture was then filtered by suction through a scintered glass funnel and the solvent evaporated from the filtrate *in vacuo*. The residue was chromatographed on a 1 cm diameter column packed with 5g of Florisil, eluting squalane and PCB's together with 15 ml of hexane:benzene 4:1 (v/v). Control experiments showed that all of the PCB's used in this study were quantitatively eluted under these conditions, while lipids of polarities equal to or greater than that of methyl palmitate were retained on the column. The preliminary batch treatment with Florisil removed enough lipid to prevent overloading the column. The described process did not change the composition of the "fed" PCB:squalane mixtures when they were added to control feces, re-isolated and analyzed by gas chromatography.

Gas-lipid chromatography (GLC) of the hexane:benzene column eluate was performed on Hewlett-Packard Model 5750 and Varian Aerograph Model 1200 gas chromatographs equipped with hydrogen flame ionization detectors and 1 mV recorders. Injection ports and detector blocks were maintained at 260° and 300°, respectively. Helium carrier gas flow rates, measured at the column outlets were: (a) for OV-101, 30 ml/min., (b) for cyclohexanedimethanol succinate (CHDMS), 35 ml/min., (c) for OV-225, 40 ml/min. Columns and column temperature conditions were as follows: (a) 3m x 2mm (ID) column packed with 10% OV-101 on 80-100 mesh HP chromosorb W. This column was operated isothermally at 200° for qualitative analysis and linearly programmed from 150 to 280° at 8°/min. for quantitative analysis. (b) 3m x 2mm (ID) column packed with 10% OV-225 on 100-120 mesh Supelcoport. Column temperature was 190° for qualitative and 140-240° at 2°/min. for quantitative analysis. (c) 4m x 2mm (ID) column packed with 3% CHDMS on 100-120 mesh Gas Chrom Q. Column temperature was 200° for qualitative and 140-225° at 2°/min. for quantitative analysis.

Hydrocarbon mixtures were run along with the PCB's under isothermal conditions to obtain apparent retention indices (8), which were determined graphically as 100 times the alkane-equivalent chain lengths (9). Relative molar responses of the hydrogen flame ionization detector were determined in terms of peak areas (height times width at half-height) for the PCB's used in this study. Although these parameters were not needed for determination of percentage excretion since changes in peak area ratios were being measured, knowledge of relative molar responses were useful in checking the chromatograms for signs of decomposition or adsorption of the PCB's. No such losses were detected in the present study, but PCB's have been found to interact destructively with the detector elements of thermal conductivity cells (communication from

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Dr. Webb).

RESULTS AND DISCUSSION

GLC parameters for the compounds used in this study are listed in Table 1 for isothermal conditions. The use of three columns differing in selectivity toward the various PCB's permitted confirmation of the identifications of the peaks being measured from the feces extracts.

The retentions of the various PCB's (fed minus excreted) are summarized in Table 2. All of the PCB's were retained to an extent greater than 90% of the amount fed. For those tested at several dose levels, percentage retention was apparently independent of the dose. Two factors contributed to the higher standard deviations in Table 2: "noise" from instrumental and background contaminants when the very small amounts of PCB in feces from the lowest dose were analyzed, and occurrence of fairly non-polar fecal material giving rise to peaks close to those of a few of the PCB's in the other cases. In every case, however, one or another of the three GLC columns was able to "isolate" each PCB adequately for quantitation.

The very low levels of PCB excreted tended to obscure any relationships between substitution pattern and absorptivity that may have existed. When similar tests were performed using aliphatic hydrocarbons (7), a clear relationship between percentage excreted in the feces and molecular weight was observed; this relationship was thought to reflect a diffusion rate-limited step in intestinal absorption of aliphatic hydrocarbons.

In the case of PCB's, no significant difference in excretion rates was observed over a molecular weight range of 116.5 to 289, suggesting that diffusion may not be rate-limiting for uptake of these compounds. The presence of a cyclic structure probably does not account for this difference between PCB's and aliphatic hydrocarbons, since pentadecylcyclohexane and heneicosane were absorbed at the same rate (7). Unfortunately, no information is presently available on absorptivity of aromatic hydrocarbons as a function of molecular weight.

In this initial study we have not investigated the possibility of intestinal metabolism of PCB's, possible recirculation of PCB's in the bile, or effects of dietary status on retention of PCB's, all of which would be required for a full elucidation of the PCB absorption process. We have shown, however, that PCB's having from one to six chlorine atoms per molecule are very well absorbed and/or metabolized by rats when the PCB's are fed in a single dose of between 5 and 100 mg per kg body weight. This observation further emphasizes our need to be alert for possible contamination of the environment and food in particular by these compounds, and to ascertain whether there would be detrimental effects of such contamination.

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TABLE 1

GLC Parameters for PCB Isomers.

Isomer[a]	Retention Index[b] OV-101	OV-225	CHDMS	Rel. Resp.[c]
Biphenyl	1396	1831	2001	1.000
2-Cl	1509	1969	2120	1.549
3-Cl	1575	2060	2247	1.072
4-Cl	1584	2080	2267	0.998
2,6-Cl_2	1620	2105	2248	1.201
2,2'-Cl_2	1620	2123	2264	1.218
2,4-Cl_2	1667	2140	2318	1.580
2,3'-Cl_2	1688	2195	2376	1.640
2,4'-Cl_2	1695	2203	2382	1.565
3,4'-Cl_2	1763	2318	2520	1.089
4,4'-Cl_2	1774	2342	2557	1.019
2,5,2'-Cl_3	1772	2278	2448	1.301
2,4,2'-Cl_3	1779	2280	2457	1.209
2,4,4'-Cl_3	1855	2390	2597	1.605
2,3',5'-Cl_3	1863	2434	2642	1.690
3,4,3'-Cl_3	1956	2550	2772	1.090
2,5,2',5'-Cl_4	1930	2462	2640	1.460
2,3,4,5-Cl_4	2039	2570	2750	1.500
3,4,3',4'-Cl_4	2161	2802	3055	0.961
2,4,5,2',5'-Cl_5	2095	2618	2830	1.208
2,4,5,2',4',5'-Cl_6	2262	2799	3025	1.277
Squalane	2645	2600	2585	4.69

[a]PCB's described in terms of positions substituted by chlorine and total number of chlorine atoms.

[b]Retention index taken as 100 x ECL relative to straight chain, saturated hydrocarbons. 200° for OV-101 and CHDMS, 190° for OV-225.

[c]Peak area response per mole relative to biphenyl.

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TABLE 2

Uptake of PCB's by Rats.

Compound[a]	Percentage Retained[b] 5mg/kg	±S.D.	50mg/kg	±S.D.	100mg/kg	±S.D.
Biphenyl	99.5	0.11	98.8	0.25	98.2	0.30
2-Cl	98.9	0.45	98.0	0.20	98.4	0.15
3-Cl	98.0	1.01	96.6	0.10	97.3	0.40
4-Cl	97.3	1.73	96.3	0.40	96.4	0.42
2,6-Cl_2			95.0	0.96	94.9	0.75
2,2'-Cl_2	95.0	2.42	97.4	0.20	98.2	2.10
2,4-Cl_2			95.7	0.40	97.1	0.35
2,3'-Cl_2			96.8	0.51	97.9	0.28
2,4'-Cl_2	97.2	3.38	95.5	1.20	97.6	0.78
3,4'-Cl_2	95.7	3.45	94.3	0.86	97.1	0.63
4,4'-Cl_2	95.4	3.05	95.6	1.12	97.4	1.10
2,5,2'-Cl_3			95.1	3.00	94.3	3.18
2,3',5'-Cl_3			95.6	1.05	95.0	1.12
3,4,3'-Cl_3			93.5	2.85	90.3	3.58
2,4,4'-Cl_3			92.6	0.86	94.0	1.50
2,5,2',5'-Cl_4	96.0	1.75				
2,3,4,5-Cl_4	97.3	0.86				
3,4,3',4'-Cl_4	91.8	0.57				
2,4,5,2',5'-Cl_5	95.1	0.57				
2,4,5,2',4',5'-Cl_6	95.3	1.11				

[a] See corresponding footnote, Table 1.

[b] Each value is the average of three determinations on each of two rats.

$$\text{Percentage Retained} = \frac{\text{(amount fed)} - \text{(amount excreted)}}{\text{amount fed}} \times 100\%$$

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SUMMARY

Chlorinated biphenyls having from one to six chlorine atoms per molecule were fed to rats at doses between 5 and 100 mg/kg body weight. Less than 10% of the amounts fed were excreted in the feces, indicating a high degree of absorption and/or metabolism of these compounds.

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g ±S.D.

0.30
0.15
0.40
0.42
0.75
2.10
0.35
0.28
0.78
0.63
1.10
3.18
1.12
3.58
1.50

of two

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