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In Vitro Metabolism of Polychlorinated Biphenyls-^{14}C

by W. GREB[1], W. KLEIN,[2] F. COULSTON, L. GOLBERG, and F. KORTE[2]

Institute of Experimental Pathology and Toxicology
Albany Medical College, Albany, N.Y. 12208
[1]Chemisches Institut der Universität Bonn
53 Bonn, W. Germany
[2]Institut für ökologische Chemie der Gesellschaft für
Strahlen- und Umweltforschung mbH, München
D-5205 St. Augustin 1, W. Germany

INTRODUCTION

Commercial polychlorinated biphenyls (PCBs) are unsuitable for metabolic studies as they are mixtures consisting of many individual ingredients resulting from the method of manufacture. Toxicological testing of all the various chlorinated biphenyls is a formidable task, and initially we can only investigate certain representative compounds. At naturally occurring levels of exposure, the toxicity of PCBs to mammals is generally very low. Lower chlorinated biphenyls, however, are of special interest because their biological degradability might lead to metabolites of increased toxicity. Lower chlorinated biphenyls are excreted very rapidly by the Rhesus monkey (GREB et al. 1973) and are converted to hydroxylated products (BLOCK and CORNISH 1959, HUTZINGER et al. 1972, YOSHIMURA and YAMAMOTO 1973) by animals. In chronic feeding studies the main changes were found in the livers: hepatic porphyria in chickens and rabbits (VOS 1972) and proliferation of hepatic smooth endoplasmic reticulum in rats (NORBACK and ALLEN 1970), mice and monkeys (NISHIZUMI 1970, ALLEN and NORBACK 1973). Enzyme induction has been reported for PCBs with high and low chlorine contents (LITTERST et al. 1972, BENTHE et al. 1972, BICKERS et al. 1972). As the hydroxylating enzymes are located in the smooth endoplasmic reticulum their metabolic activity might be indirectly responsible for the gross pathological changes observed. Therefore, we investigated the effect of these enzymes on the PCBs by incubating lower chlorinated biphenyls with the microsomal fraction of rat liver homogenate. Identification of metabolites is an essential step in the evaluation of safety of these compounds.

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EXPERIMENTAL

The compounds studied were 2,2'-dichlorobiphenyl, 2,4'-dichlorobiphenyl, and 2,5,2'-trichlorobiphenyl. They were labelled with ^{14}C and synthesized in the German laboratory, except the last one, which was bought from Mallinckrodt Chem. Corp. (Lot Nr. 4449a). Trying to get a maximum conversion rate, adult female rats (Swiss Webster strain) weighing 200 - 300 g were treated with phenobarbital daily for 3 days (50 mg/kg i.p.) to increase mixed function oxidase activity. The animals were killed on the fourth day, the livers were removed, washed and homogenized, and centrifuged at 10,000 g. The supernatant was centrifuged at 100,000 g for 1 hour and the pellet resuspended in buffer (0.25 M sucrose in 0.01 M Tris-HCl, pH 7.4). The NADPH regenerating system and incubation medium consisted of 30 mM $MgCl_2$, 0.1 ml 1N nicotinamide, 0.05 ml 30 mM TPN, 150 mM glucose-6-phosphate, 100 units glucose-6-phosphatedehydrogenase, and 1 ml 50 mM Tris-HCl (pH = 8.6) per ml microsomes. Biphenyl hydroxylase activity was measured by the method of CRAVEN et al. (1965) and protein content determined using bovine serum albumin as a standard (SUTHERLAND et al. 1949).

The low degree of aqueous solubility of the PCBs presented a problem since many organic solvents are known to decrease microsomal enzyme activity. After testing the inhibitory effect of a series of solvents, dimethylsulfoxide (DMSO) was selected together with an emulsifier as vehicle. Although DMSO stimulates aniline metabolism in rats in vivo (STOCK et al. 1969), it partly inhibits enzyme activity in vitro. The PCBs were administered in 0.1 ml DMSO, 20 mg Tween 80, and 0.2 ml H_2O and incubated for 1 hour at 37°C. The reaction was stopped by adding 2 N HCl. The acidified mixture was extracted with 2 x 20 ml hexane and 3 x 10 ml chloroform/methanol (3:1) so that 99 % of the radioactivity could be recovered. After concentration, the compounds were purified by preparative layer chromatography on silica gel (Merck Nr. 5765, 5766, solvent: dichloromethane). Radioactivity was determined by the "scraping technique" and liquid scintillation counting (Packard Model 3380) or autoradiography (Kodak X-ray film) (CECIL et al. 1966). Compounds were methylated by diazomethane in ether overnight, GC carried out on Packard model 873 (EC-detector, 2 m, 1% OV-1) and final analysis performed with a GC-MS- combination (LKB 9000 A).

Experimental details are provided in Table 1.

TABLE 1

Experimental Data on PCB Application to Rat Liver Homogenate

	2,2'-Dichlorobiphenyl	2,4'-Dichlorobiphenyl	2,5,2'-Trichlorobiphenyl
dose (μmoles)	3.97	1.51	1.43
dose (μCi)	1.02	7.72	14.25
g liver/ml homogenate	1.25	1.12	1.12
mg protein/ml	14.8	13.4	13.4
enzyme activity (% 4-OH-BP formed from 1.2 μ moles biphenyl/ml)	16.3	12.6	12.6
vol. of microsome suspension (ml)	6.0	12.0	6.0
level of PCB used, dose (μmoles/ml)	0.66	0.12	0.23
conversion in %	10.0	35.8	31.0

RESULTS AND DISCUSSION

The rate of conversion of the PCBs varied between 10 and 35.8 % on the basis of their concentrations in μmoles per ml incubation medium. Smaller concentrations increased the yield of metabolites. The difference in yield between doses of 0.23 and 0.12 μmoles/ml, however, was very small (31 % and 35.8 %, respectively). Determining the enzyme activity, 16.3 % 4-hydroxybiphenyl were formed from 1.2 μmoles/ml biphenyl, while only 10 % of 0.66 μmoles/ml 2, 2'-dichlorobiphenyl were converted. Although less concentrated, the PCB was metabolized more slowly than the biphenyl. Radioactive extracts of the incubation mixtures showed thin layer chromatograms similar to each other: a main peak corresponding to unchanged PCB, two smaller peaks corresponding to mono- and dihydroxymetabolites, and a small fraction at the origin which was not identified.

Table 2 shows GLC - and MS-data of 2,2'-dichlorobiphenyl and its methylated metabolites. Four monohydroxy derivatives (R_1-R_4) were detected and characterized by GLC/MS. Although four metabolites were detected on TLC, matching dihydroxy derivatives (R_5-R_8), only one of them (R_5) occurred in amounts sufficient for methylation, GLC and MS.

Table 3 gives GLC - and MS-data of 2,4'-dichlorobiphenyl and its metabolites. Two monohydroxy derivatives (U_1 - U_2) and two dihydroxy derivatives (U_3 - U_4) were isolated and characterized by GLC/MS.

In Table 4, figures are listed related to GLC and MS of 2,5,2'-trichlorobiphenyl and its metabolites, three monohydroxy derivatives (L_1 - L_3) and two dihydroxy derivatives (L_4 - L_5).

All identified metabolites contained one or two hydroxyl groups in the PCB-molecule. Primarily monohydroxy products were formed. While we found all possible monohydroxy-dichloroisomers of the 2,2'-dichlorobiphenyl, only two of the maximum six 2,4'-dichlorobiphenyl-monohydroxy-metabolites and three of seven of the 2,5,2'-trichlorobiphenyl-monohydroxy-metabolites were formed. Although the first experiment showed that all different positions in the biphenyl-molecule could be hydroxylated, the chlorine substituent might have a directing influence on the position hydroxylated by the enzymes. Some isomers were more likely to be formed than others. Biotransformation of the PCBs in vitro proceeded to metabolites containing up to two hydroxyl groups per molecule.

Fig. 1 gives a survey of all metabolites identified in this study.

The high yields and variety of products formed in these experiments demonstrate that lower chlorinated biphenyls are readily metabolized by hepatic enzymes, probably mixed function oxidases, of rats.

This investigation of the metabolism of three individual PCBs also provides some indication of their general biodegradability. Thus it is likely that at least one commercial mixture of lower chlorinated biphenyls, Aroclor 1221, contains mostly ingredients of low persistence. On the basis of the composition of Aroclor 1221 reported by WILLIS and ADDISON 1972, a flowsheet of metabolic conversions (Fig. 2) has been drawn up to summarize our present information.

TABLE 2

GLC - and MS-Data of 2,2'-Dichlorobiphenyl and its Methylated Metabolites

Data		2,2'-DCB	OH-Metabolite R_1	OH-Metabolite R_2	OH-Metabolite R_3	OH-Metabolite R_4	$(OH)_2$-Metabolite R_5
GLC R_t (min.)		4.0/185°C	7.3/185°C	8.8/185°C	10.8/185°C	7.3/188°C for 8 min. then temp. raised 5°C/min.	13.5/200°C for 8 min., then temp. raised 5°C/min.
MS-fragment	M^+	222 s	252 s	252 s	252 s	252 s	282 s
"	M^+-CH_3	-	-	237 l	-	237 l	267 l
"	M^+-Cl	187 s	217 l	217 m	217 l	217 l	247 l
"	metastable	158*	-	222*-5	-	222*-5	-
"	M^+-CH_3-CO	-	209 m	209 s	209 m	209 s	239 m
"	M^+-CH_3-Cl	-	-	202 s	202 l	202 m	-
"	M^+-Cl_2	152 l	-	182 l	-	182 m	-
"	$M^+-Cl-HCl$	151 l	-	-	-	-	-
"	$M^+-CH_3-CO-HCl$	-	173 m	173 m	173 l	173 m	

Peak-Intensities: s = strong, m = medium, l = low

TABLE 3

GLC - and MS-Data of 2,4'-Dichlorobiphenyl and its Metabolites

Data	2,4'-DCB	OH-Metabolite U_1 (not methylated)	OH-Metabolite U_2 (methylated)	$(OH)_2$-Metabolite U_3 (methylated)	$(OH)_2$-Metabolite U_4 (methylated)
GLC R_t (min.)	3.6/185°C	5.1/185°C	9.5/188°C for 8 min., then temp. raised 5°C/min.	13.0/200°C for 8 min., then temp. raised 5°C/min.	11.5/200°C for 8 min., then temp. raised 5°C/min.
MS-fragment M^+	222 s	238 s	252 s	282 s	282 s
" M^+-CH_3	-	-	-	267 l	-
" M^+-CO-H	-	209 m	-	247 l	247 l
" M^+-Cl	203 m	203 m	217 l	-	-
" M^+-HCl	202 s	202 s	-	-	-
" $M^+-CO-CH_3$	-	-	209 m	239 m	209 m
" M^+-Cl_2	152 s	168 s	-	-	-
" $M^+-Cl-HCl$	151 m	167 m	-	-	-
" $M^+-Cl_2-H_2O$	-	149 s	-	-	-
" $M^+-CO-CH_3-HCl$	-	-	173 l	-	-

Peak-Intensities: s = strong, m = medium, l = low

TABLE 4

GLC - and MS-Data of 2,5,2'-Trichlorobiphenyl and its Metabolites

Data	2,5,2'-TCB	OH-Metabolite L_1 (not methylated)	OH-Metabolite L_2 (methylated)	OH-Metabolite L_3 (methylated)	$(OH)_2$-Metabolite L_4 (methylated)	$(OH)_2$-Metabol.L_5 (methyl.)
GLC R_t (min.)	5.1/185°C	8.9/185°C	11.9/182°C for 9 min., then temp. raised 8°C/min.	13.9/185°C for 8 min., then temp. raised 5°C/min.	16.2/200°C for 8 min., then temp. raised 5°C/min.	14.0/200°C for 8 min., then temp. raised 5°C/min.
MS-fragment M^+	256 s	272 s	286 s	286 s	316 s	316 s
" M^+-CH_3	-	-	-	271 m	301 l	-
" M^+-Cl	221 m	237 l	251 l	251 l	281 m	-
" $M^+-CO-CH_3$	-	-	243 m	243 s	273 m	273 m
" $M^+-CO-Cl$	-	209 m	-	-	-	-
" M^+-CH_3-Cl	-	-	236	236 l	-	-
" M^+-Cl_2	186 s	202	216 l	216 l	-	-
" $M^+-CO-CH_3$	-	-	173 l	173 m	-	-
" M^+-Cl_3	151 m	-	-	-	-	-

Peak-Intensities: s= strong, m = medium, l = low

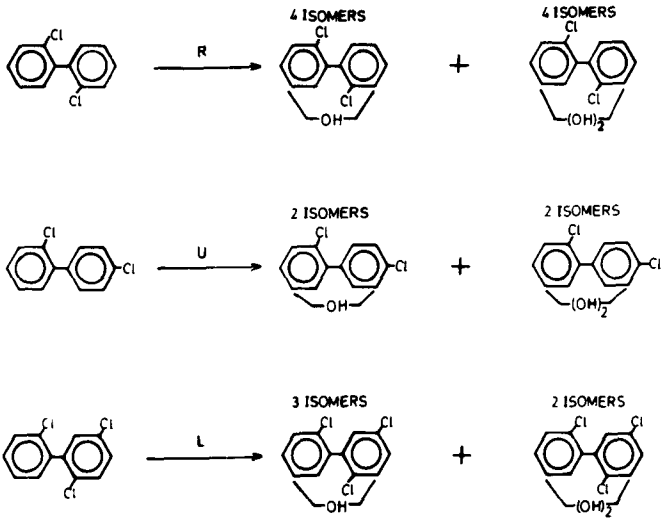

Fig.1. Metabolism of PCB by Rat Liver Microsomes

COMPONENT OF AROCLOR 1221 METABOLISM

RAIG and AMMON 1970

12.7% Biphenyl (BP) → 4-OH-BP → 4,4'-$(OH)_2$-BP
12.7% Biphenyl (BP) → 4-OH-BP → 3,4-$(OH)_2$-BP
12.7% Biphenyl (BP) → 2-OH-BP

28.4% 2-Cl-BP

BLOCK and CORNISH 1959
HUTZINGER et al. 1972

18.7% 4-Cl-BP → 4-Cl-OH-BP → 4-Cl-$(OH)_2$-BP

13.6% 2,4'-Cl_2-BP → 2,4'-Cl_2-OH-BP (two isomers) → 2,4'-Cl_2-$(OH)_2$-BP (two isomers)

9.2% 2,2'-Cl_2-BP → 2,2'-Cl_2-OH-BP (four isomers) → 2,2'-Cl_2-$(OH)_2$-BP (four isomers)

HUTZINGER et al. 1972

6.2% 4,4'-Cl_2-BP → 4,4'-Cl_2-OH-BP

3.5% 2,4-Cl_2-BP

92.3%

Fig. 2. Components of Aroclor 1221 (in % weight) and their known Metabolites

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