Beiträge zur Ökologischen Chemie LXXXIV[+]
Metabolism of Lower Polychlorinated Biphenyls-^{14}C in the Rhesus Monkey

by W. GREB, W. KLEIN, F. COULSTON*, L. GOLBERG*, and F. KORTE

Institut für ökologische Chemie der Gesellschaft für Strahlen- und Umweltforschung mbH, München D-5205 St. Augustin 1, W. Germany

**Institute of Experimental Pathology and Toxicology Albany Medical College, Albany, N.Y. 12208*

INTRODUCTION

Metabolism studies on PCB are important for estimating the toxicity of metabolites after knowing their identity and biological pathway. Some pure PCB-isomers were shown to be converted to hydroxylated products by rat liver microsomes (GREB et al. 1974), rabbits (BLOCK and CORNISH 1959), rats (HUTZINGER et al. 1972, YOSHIMURA and YAMAMOTO 1973) and pigeons (YOSHIMURA and YAMAMOTO 1973). The purpose of this study was to identify all major metabolites after PCB-administration to Rhesus monkeys and to measure the amounts of different excreted metabolites. Correlation of conversions and elimination pattern of PCBs with different chlorine content might lead to conclusions about the behavior of other PCBs.

The excretion rates of 2,4'-dichlorobiphenyl and 2,5,2'-trichlorobiphenyl have been described elsewhere (GREB et al. 1973); we now wish to report the identification of their metabolites.

EXPERIMENTAL

The PCBs investigated were 2,4'-dichlorobiphenyl and 2,5,2'-trichlorobiphenyl, both major components of the lower AROCLOR series. They were labelled with ^{14}C. Female Rhesus monkeys were housed in metabolic cages and the PCB was injected once in the left forearm vein. Three animals received 2,4'-dichlorobiphenyl in doses of 16.8, 77.6 and 566 µg/kg, while 82.6 µg/kg of the 2,5,2'-trichlorobiphenyl were administered to one monkey. Urine and feces were collected daily. Conjugates were hydrolyzed by refluxing urine with an equal volume of 8 N H_2SO_4 for one hour and extracted with ether. Feces were mixed with anhydrous sodium-sulfate and extracted for two days in a Soxhlet apparatus with CH_2Cl_2 and then with methanol. The combined extracts were concen-

[+]LXXXIII. Communication: GREB, W., W. Klein, F.COULSTON, L.GOLBERG,and F.KORTE: Bull.Environ.Contam. Toxicol., in press.

Bulletin of Environmental Contamination & Toxicology, Vol. 13, No. 4 ©1975 by Springer-Verlag New York Inc.

trated under a nitrogen stream and purified by preparative layer chromatography on silica gel Merck Nr.5765, 5766(solvent: CH_2Cl_2). After methylation by diazomethane (DE BOER and BACKER 1963) the metabolites were characterized by GLC (Packard 873, EC-detector, 1% OV-1, 2m) and identified by GLC-MS (LKB 9000 A).

RESULTS AND DISCUSSION

After 14 days about 77% of the administered radioactivity were recovered,and reaction of urine with Glusulase (Endo Laboratories) according to K.D. VOIGT (1965) showed that about 17% of metabolites were conjugated with sulfuric or glucuronic acid. Incubation of urine and feces in vitro with the original PCBs revealed no formation of metabolites due to reaction with bacteria from the intestines.

In vivo urinary and fecal metabolites were identical and the metabolic pattern in excreta was constant between the first and fifth day after application. Only metabolites, no parent compound, were detected in the excreta.

Fig. 1 gives a survey of the metabolites formed from both PCBs.

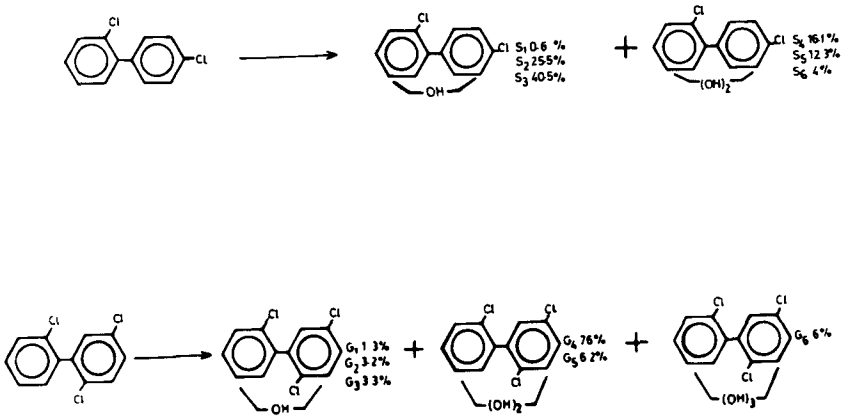

Fig.1. Metabolism of Polychlorinated Biphenyls by Rhesus Monkeys

TABLE 1

TLC -, GLC- and MS-Data of 2,4'-Dichlorobiphenyl Metabolites in Monkeys

Data	OH-Metabolite S_1	OH-Metabolite S_2	OH-Metabolite S_3	$(OH)_2$-Metabolite S_4	$(OH)_2$-Metabol. S_5	$(OH)_2$-Metabol. S_6
R_f before methylation (CH_2Cl_2)	0.66	0.50	0.25	0.13	< 0.1	< 0.1
R_f after methylation (CH_2Cl_2)	0.82	0.70	0.66	0.59	0.64	0.52
GLC after methylation R_t (min.)	10.2/179°C	12.1/185°C for 9 min. then temp. raised 5°C min.	11.4/185°C for 9 min. then temp. raised 5°C min.	14.8/200°C for 8 min., then temp. raised 5°C/ min.	13.1/200°C for 8 min. then temp. raised 5°C/ min.	11.6/200°C for 8 min., then temp. raised 5°C/ min.
MS-fragment after methylation M^+	252 s	252 s	252 s	282 s	282 s	282 s
" M^+-CH_3	-	237 l	237 m	267 l	267 m	267 m
" metastable	-	-	223* m	-	-	-
" M^+-Cl	217 l	217 m	-	247 m	247 m	-
" M^+-HCl	-	-	-	246 m	246 m	-
" M^+-CH_3-CO	209 m	209 s	209 m	239 m	239 l	239 l
" M^+-CH_3-Cl	-	-	202 l	-	-	-
" $M^+-CH_3-CO-HCl$	173 l	-	173 m	-	-	-
" $M^+-CH_3-CO-Cl_2$	-	-	139 m	204 l	-	-

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

TABLE 2

TLC-, GLC - and MS-Data of 2,5,2'-Trichlorobiphenyl Metabolites in Monkeys

Data	OH-Metabolite G_1	OH-Metabolite G_2	OH-Metabolite G_3	$(OH)_2$-Metabolite G_4	$(OH)_2$-Metabolite G_5	$(OH)_3$-Metabol. G_6
R_f before methylation (CH_2Cl_2)	0.49	0.25	0.25	0.11	< 0.1	< 0.1
R_f after methylation (CH_2Cl_2)	0.76	0.72	0.72	0.64	0.53	0.32
GLC after methylation R_t (min.)	13.8/180°C	15.4/185°C	16.2/185°C	17.0/200°C for 8 min., then temp. raised 5°C/min.	13.7/200°C for 8 min., then temp. raised 5°C/min.	17.8/200°C for 8 min., then temp. raised 5°C/min.
MS-fragment after methylation M^+	286 s	286 s	286 s	316 s	316 s	346 s
" M^+-CH_3	271 l	271 l	-	301 l	301 l	-
" M^+-Cl	251 m	-	251 l	281 l	-	-
" M^+-CH_3-CO	243 m	243 l	243 l	273 m	273 l	-
" M^+-CH_3-Cl	-	-	-	266 l	-	-
" M^+-Cl_2	186 l	-	-	246 l	246 s	276 l
" $M^+-CH_3-CO-Cl_2$	173 l	173	-	203 l	-	-

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

After methylation all purified metabolites showed in the mass spectra -OCH_3-groups indicating that the original metabolites were phenolic derivatives. In both cases a number of isomers had been formed. Isomers only differed in peak-intensities. All isolated compounds showed the typical isotopic distribution pattern corresponding to the number of chlorine atoms in the parent PCB. For 2,4'-dichlorobiphenyl there was no conversion exceeding the introduction of 2 OH-groups per molecule. For the 2,5,2'-trichlorobiphenyl we observed a metabolite with three OH-groups. Corresponding to its formation the monohydroxy-metabolite with the lowest concentration should be the precursor of the highest concentrated dihydroxy-compound. Only three monohydroxy-derivatives were formed although six (resp. seven for the trichlorobiphenyl) structures are possible. For each PCB a highly polar zone of 1 and 4% resp. of radioactivity could not be identified. Data of the metabolites are shown in Table 1 - 2.

Comparing the excreted amounts (in % of totally excreted radioactivity) of metabolites formed, the dichlorobiphenyl was mainly excreted as monohydroxy-derivatives, while the trichlorobiphenyl was mainly eliminated as dihydroxy-compounds (Table 3).

TABLE 3

Comparison of Metabolites formed of PCBs by Rhesus Monkeys

Metabolites	Dichloro-biphenyl	Trichloro-biphenyl
Monohydroxy-metabolites	66.6%	7.8%
Dihydroxy-metabolites	32.4%	82.2%
Trihydroxy-metabolites	-	6 %
Polar metabolites, unidentified	1 %	4 %

The following might explain this different behavior. Due to the additional chlorine atom, the trichlorobiphenyl is more lipophilic than the dichlorobiphenyl. The monohydroxy-metabolites react in the same way: the monohydroxy-dichlorobiphenyl is excreted rapidly, while the water solubility of the monohydroxy-trichlorobiphenyl is still low. After further hydroxylation the trichlorobiphenyl-metabolites reach a polarity to be easier eliminated. A dihydroxy-trichlorobiphenyl thus should correspond to a monohydroxy-dichlorobiphenyl as regards speed of elimination. For higher chlorinated BPs, a higher degree of hydroxylation is necessary and will cause a longer retention time of the compound in the body, that is, a slow excretion rate. This might explain their accumulation.

CONCLUSION

Metabolism of PCB with high chlorine content will be difficult and proceed slowly. Concerning lower chlorinated biphenyls, however, this study reveals that total degradation to hydroxylated metabolites and the rapid excretion of the PCBs investigated in primates indicate that accumulation of lower chlorinated biphenyls at low doses will not occur.

REFERENCES

BLOCK, W.D., and H.H. CORNISH: J. Biol. Chem. 234, 3301 (1959).

DE BOER, Th. J., and H.J.BACKER: Org. Syn. Coll. 4, 943 (1963).

GREB, W., W. KLEIN, F. COULSTON, L. GOLBERG, and F. KORTE: Chemosphere 2, 143 (1973).

GREB, W., W. KLEIN, F. COULSTON, L. GOLBERG, and F. KORTE: Bull. Environ. Contam. Toxicol., in press (1974).

HUTZINGER, O. et al.: Science 178, 312 (1972).

VOIGT, K.D. in H.D. BERGMEIER: Methods of Enzymatic Analysis. Acad. Press 1965, p. 462.

YOSHIMURA, H., and H. YAMAMOTO: Chem. Pharm. Bull.21, 1168 (1973).