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Metabolism by Rat Hepatic Microsomes of Individual Isomers and Congeners in Aroclor 1016

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Polychlorinated biphenyls (PCBs) are abundant and persistent pollutants in the ecosystem. Commercial mixtures of PCBs (e.g. Aroclors) can contain up to 80 different isomers and congeners, many of which accumulate in the fatty tissues of species at the apex of food chains by the ingestion of PCB-contaminated lipid components of foods. These commercial mixtures of PCBs induce in hepatic microsomal membranes in vivo a variety of isoforms of the cytochrome P-450 components of monooxygenases involved in the metabolism of drugs and other xenobiotics including PCBs.

A number of studies have compared the accumulation of individual PCBs in biological tissues with their abundance in commercial mixtures of PCBs to which the animals had been exposed in the wild or in the laboratory (see Safe, 1984 for review). The results showed that for a wide range of species, many highly chlorinated PCBs are enriched in tissues whereas many less chlorinated PCBs are eliminated by metabolism and excretion.

Our studies in vivo on the accumulation of individual isomers and congeners of commercial mixtures of PCBs in the adipose and other tissues of fish-eating seabirds, pigeons and rats (Borlakoglu, 1989; Borlakoglu JT, Wilkins JPG, Walker CH, Dils RR, unpublished work) have shown that individual PCBs that lack adjacent H atoms in at least one of the rings are enriched in tissues, indicating that their accumulation exceeds their elimination by metabolism and excretion. Some of the PCBs which accumulated were non-ortho-substituted congeners (i.e. an approximately coplanar configuration) and mono-ortho-substituted congeners which are highly toxic when tested on laboratory animals. By contrast, individual PCBs that possessed meta-para-unsubstituted carbon atoms in at least one of the rings were not enriched in tissues, indicating that their metabolism in vivo by isoforms of the cytochrome P-450 components of hepatic microsomal monooxygenases exceeded their accumulation in tissues.

We report here experiments designed to test whether these structural rules governing the accumulation or metabolism of individual PCBs in vivo are supported by measurements in vitro of the metabolism of individual PCBs in a commercial mixtures of PCBs by preparations of rat hepatic microsomes.

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MATERIALS AND METHODS

Aroclor 1016 was obtained from the U.S. Environmental Protection Agency, Washington DC. This commercial mixtures of PCBs was found to contain at least 28 congeners, 12 of which were identified (see Table 1) by high performance capilliary g.c. using > 99% pure synthetic PCB isomers as standards (Borlakoglu, 1989). The IUPAC system of numbering individual PCB isomers and congeners (Ballschmitter & Zell, 1980) has been used.

Mature female Sprague-Dawley rats of average weight 175-200g were used. The hepatic microsomal fraction was prepared as described by Borlakoglu et al. (1990) and resuspended in 0.25M-sucrose, 5 mM-EDTA and 25 mM-Tris buffer, pH 7.4 (microsomal buffer solution) at a concentration of approximately 20 mg protein/ml. Portions were stored at -20°C, thawed once and diluted as necessary with this buffer.

The microsomal suspension was preincubated at 37°C with shaking for 1.5 min with a solution containing 55 mM-sodium phosphate buffer, pH 7.4, 12 mM-KCl, 4.8 mM-nicotinamide (final volume 4.8 ml) and 0.2 ml of a NADPH-generating system added that contained 21.4 mole glucose-6-phosphate, 4.6 mole $NADP^+$ and 1.6 units of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in the microsomal buffer solution. The reaction was started by adding 0.5-1.0 mmole of a solution of Aroclor 1016 in ethanol, the final concentration of ethanol per incubation being less than 4% (v/v). Incubation was then continued at 37°C with shaking for 2 h. Control incubations with no added Aroclor 1016 and with microsomal suspension that had been heated at 100°C for 20 min were used.

The reaction was stopped with 10 ml chloroform and the pH brought to > 9 with 5M-KOH. Phenolic metabolites partitioned into the aqueous phase and were separated from the non-metabolized substrates which remained in the chloroform layer as follows. The mixtures was centrifuged at 3000 rpm for 5 min and the aqueous phase reextracted three times with 10 ml portions of chloroform to remove residual non-metabolized substrates. The aqueous phase was brought to pH < 3 with 18M-sulphuric acid and again extracted three times with 10 ml portions of chloroform. The aqueous phases from five incubations were pooled, as were the chloroform extracts. The amounts of non-metabolized individual PCB congeners remaining and of the phenolic metabolites as their trimethylsily derivatives were measured by high resolution capilliary g.c. (Borlakoglu, JT, Wilkins JPG, Walker CH, Dils RR, unpublished work). The metabolism of individual PCBs in Aroclor 1016 was therefore measured as the percentage of the parent congener remaining at the end of the incubation and by the metabolites formed. The molecular formula of the metabolites was obtained by high resolution capilliary g.c.-m.s. (Borlakoglu JT, Wilkins JPG, Walker CH, Dils RR, unpublished work).

RESULTS AND DISCUSSION

Fig. 1 shows a typical result obtained when the pattern of PCBs in Aroclor 1016 is compared with the pattern of congeners remaining after incubating for 2 h at 37°C with rat hepatic microsomes as described above. Analysis of these results showed that 1-mono-,3-

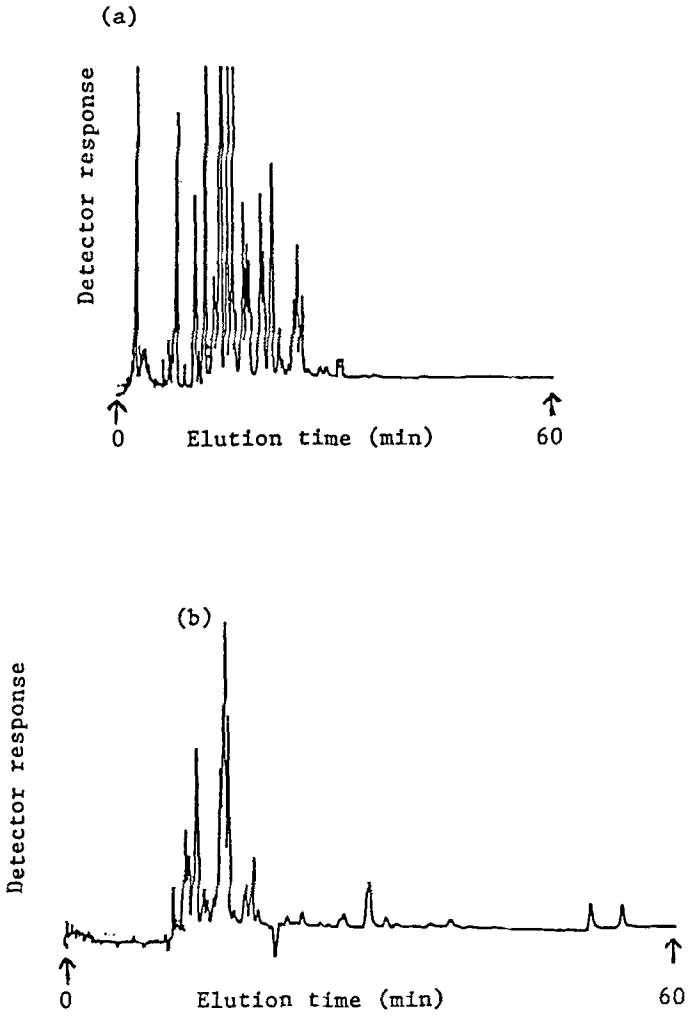

Figure 1 Metabolism of Aroclor 1016. Analysis by capilliary g.c. of (a) Aroclor 1016, (b) congeners remaining after incubation for 2h at 37°C with rat hepatic microsomes.

Table 1 Metabolism of Aroclor 1016 by rat hepatic microsomes : individual PCB isomers and congeners metabolized to mono-hydroxy-metabolites

Parent PCB isomer or congener						
IUPAC number	Position of Cl atoms	Pairs of H atoms at positions ortho-meta	meta-para	% (w/w) in Aroclor 1016	nmol Metabolite formed/2h per mg protein[a]	Molecular formula of mono-hydroxy metabolite formed
1	2	3	4	1.6	5	$C_{12} H_9 Cl 0$
6	2,3	3	3	1.0	5	$C_{12} H_8 Cl_2 0$
8	2,4'	3	2	14.8	74	$C_{12} H_8 Cl_2 0$
15	4,4'	4	0	3.2	12	$C_{12} H_8 Cl_2 0$
18	2,2',5	1	3	10.6	53	$C_{12} H_7 Cl_3 0$
21	2,3,4	3	2	0.05	0.5	$C_{12} H_7 Cl_3 0$
26	2,3',5	1	2	2.8	44	$C_{12} H_7 Cl_3 0$
28	2,4,4'	3	0	16.1	0	-
31	2,4',5	2	1	7.9	40	$C_{12} H_7 Cl_3 0$
44	2,2',3,5'	1	2	3.7	18	$C_{12} H_6 Cl_4 0$
47	2,2',4,4'	2	0	0.5	0	-
49	2,2',4,5'	1	1	2.3	4	$C_{12} H_6 Cl_4 0$
52	2,2',5,5'	0	2	7.7	31	$C_{12} H_6 Cl_4 0$
67	2,3',4,5	1	1	1.2	1	$C_{12} H_6 Cl_4 0$

[a] Average value of 4 independent experiments

di-, 5-tri- and 3-tetrachlorobiphenyls had been subjected to metabolism. Determination by g.c.-m.s. of the molecular formula of the metabolites formed identified twelve mono-hydroxylated metabolites (Table 1), which indicates the extensive metabolism of several PCB congeners in the Aroclor 1016. There was no evidence for the formation of dihydroxylated metabolites. Although this analysis by g.c.-m.s. confirmed the presence of phenolic metabolites, their mass fragmentation pattern in the electron impact mode used does not permit the precise position of the hydroxyl group on the carbon atoms to be assigned.

The relative rates of metabolism of individual PCBs was calculated as the percentage of the parent congener remaining at the end of the incubation. Table 1 shows that, with the exception of congener 15, the metabolism of isomers and congeners that lack H atoms in the meta-para position (e.g. congeners 28 and 47) were not metabolized at detectable rates. By contrast, congeners that possess meta-para-unsubstituted carbon atoms in at least one of the rings (congeners 1, 8, 18, 21, 26, 30, 31, 44, 49, 52 and 67) had been subjected to metabolism during the incubation. This confirms our proposed molecular rule based on studies in vivo (Borlakoglu, 1989; Borlakoglu JT, Wilkins JPG, Walker CH, Dils RR, unpublished work)

that this molecular feature is favourable for the interaction of the congener with isoforms of the cytochrome P-450 components of hepatic microsomal monooxygenases involved in the metabolism of drugs and other xenobiotics leading to the formation of oxygenated substrates and phenolic metabolites and their excretion in vivo.
The mechanisms involved in this metabolism of PCBs are unclear, but may involve isoforms of cytochrome P-450 abstracting a H atom from the substrate to permit oxidation ('abstraction theory') or a direct insertion of oxygen into the substrate ('direct insertion theory') (for reviews see White & Coon (1980) and Dawson & Eble (1986)). In addition, the metabolism of an individual PCB by this system might be affected by the presence of non-metabolizable PCB congeners. This possibility arises from the suggestion of Mills et al. (1985) that the metabolism of individual polybrominated biphenyls by '3-methylcholanthrene-type' induced isoforms of cytochrome P-450 can be inhibited by non-metabolizable congeners interacting with the active site of the cytochrome P-450. Hence results obtained for the metabolism of individual congeners in mixtures of PCBs need to be interpreted with some caution.
We had shown previously (Borlakoglu, 1989) that the induction in vivo of hepatic microsomal cytochrome P-450-dependent monooxygenases by PCBs results in up to 20-fold increases in the rate of metabolism of PCBs leading to significant increases in the formation of polyhydroxylated metabolites. It has been emphasised (see Birnbaum, 1985) that the metabolism of PCBs is a prerequisite for their excretion and that the study of the metabolism of individual PCB isomers and congeners both in vivo and in vitro is essential to predict their metabolic disposal. Such information about the molecular rules governing the enrichment of individual PCBs in tissues versus their metabolism and excretion should enable similar chemicals of commercial importance to be designed so as to minimize their accumulation in the tissues of humans and other animals and their toxic effects.
Although the metabolic activation of particular classes of polycyclic aromatic hydrocarbons (PAHs) leads to toxic effects in vivo (e.g. the metabolism of the pre-carcinogen benzo-(a)-pyrene to the stereo isome (R,S)-diol-(S,R) epoxide, the active carcinogen), the hydroxylation of very toxic PCBs such as 3,3',4,4'-tetrachlorobiphenyl (congener 77) significantly reduces their toxicity (Yoshimura et al., 1987). That particular features of the toxicity of PCB congeners can be explained by their ability to associate with a cytoplasmic receptor protein present in the tissues of a number of mammalian species (see Nebert & Eisen (1984) and Nebert & Gonzalez (1987) leads us to suggest that the hydroxylation of toxic PCBs reduces their ability to associate with this protein, thereby reducing their toxicity.
In conclusion, the results presented show that our proposed structural rules governing the accumulation or metabolism of individual PCBs in vivo are supported by measurements in vitro of the metabolism of individual PCBs in a commercial mixture of PCBs by rat hepatic microsomes. The work also shows that the separation and identification of individual PCBs and their metabolites by capilliary g.c.-m.s. provides a powerful and convenient method to assess the metabolism of PCBs and hence the potential toxic and

carcinogenic hazards that they present to humans and other animals.

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