Formation and Toxicological Aspects of Phenolic Metabolites of Polychlorobiphenyls (PCBs), and Related Compounds.

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1. Abstract

Phenolic metabolites of PCBs and related compounds are observed at relatively high levels in bloodplasma of several species, including man. These phenolic metabolites have specific range of biological activities which differs from the parent compounds. In addition, they may add to the effects of parent compounds. The observation that relatively high levels of phenolic PCBs may accumulate in late gestational fetusses, suggests that these metabolites may play an important role in the observed developmental toxicity by PCBs and related chemicals.

2. Introduction

Biotransformation and its role in elimination of PCBs and related compounds has been a subject of many studies from the late seventies onward. The notion of specific, high affinity interactions of phenolic PCB metabolites with the plasma transport proteins of thyroid hormone and vitamin A, both *in vitro*[1] and *in vivo*[2], in the mid eighties, further stimulated research into biotransformation of PCBs and its possible role in toxicity of these compounds. Since then much additional *in vitro* and *in vivo* information on the formation, occurence in organs and body fluids, and biochemical/toxic effects of hydroxylated PCBs and related compounds has been gathered, some of which will be addressed in this paper.

3. Formation of phenolic metabolites of PCBs and related compounds

Phenolic products are the major PCB metabolites formed in *in vitro* incubations with liver microsomes and *in vivo* by mammalian and avian species[3]. The mechanism of formation of phenolic PCB metabolites is suggested to involve a cytochroom P450 mediated production of arene oxide intermediates, which spontaneously rearrange to phenols with a concomittant shift (NIH-shift) of substituents from the site of hydroxylation to the next neighbour carbon atom in the aromatic ring. NIH-shift phenolic PCB metabolites have been identified as major metabolites of both planar and non-planar PCBs in *in vitro* and *in vivo* studies[3,4].
The rate of formation and the structure of phenolic metabolites formed may be both species and congener dependent. An example of congener dependency is that hydroxylation is favored at the para position in the least chlorinated ring. However, recent studies indicate the presence of phenolic metabolites of 2,3,3',4',5-pentachlorobiphenyl in bloodplasma of mice with the

hydroxy-group substituted on the most chlorinated ring[5]. In addition, the availability of two vicinal unsubstituted carbon atoms facilitates oxidative metabolism. Increased chlorination on both phenyl rings will reduce the rate of metabolism drastically. Also species differences in type and rate of phenolic PCB metabolites formed have been observed[6]. *In vitro* metabolism of 3,3',4,4'-tetrachlorobiphenyl (PCB 77), using liver microsomes of different species, indicated that the rate of phenolic metabolite formation correlated well with the microsomal ethoxyresorufin-O-deethylase (EROD) activity, indicating the involvement of cytochrome P450-1A1/2 activity. However, in liver microsomes of fish hardly any formation of phenolic-PCB metabolites was observed, even when exposed experimentally to cytochrome P450-1A1/2 inducers. On the other hand, liver microsomes of mammalian and avian species sensitive to cytochrome P450-1A1/2 inducers were able to substantially metabolise PCB 77 with the formation of phenolic compounds with the hydroxy-group on the 4 (NIH-shift), 5 and 6 position. Relatively high levels of NIH-shift phenolic PCB metabolites are also observed in *in vivo* exposure studies in rats[7] and mice[4].

3) Biological activities of phenolic PCB metabolites

Metabolism and metabolite formation of most halogenated aromatic compounds, including PCBs have been regarded mainly as an elimination route. Recent studies have indicated that considerable amounts of phenolic and also methylsulphone metabolites of PCBs and related compounds are not readily excreted, but instead may be present in relatively large quantities in organs and body fluids for a prolonged time period. For example, relatively large amounts of phenolic PCB metabolites have been identified in bloodplasma of several species, either experimentally, or environmentally exposed to PCBs, such as rats, mice, marine mammals and humans[7]. Moreover, a considerable accumulation of phenolic PCB metabolites was observed in late gestational fetuses, when their mothers were exposed to Aroclor 1254 from day 10 to 16 of gestation (Morse, personal communication).
In addition, several studies have indicated that phenolic metabolites of PCBs and related compounds do have their own metabolite-specific range of biological activities and may also add to some parent compound-specific biological effects (Table 1).

Phenolic PCB metabolites are much more potent than their respective parent compounds in terms of interference with thyroid hormones for their key proteins in transport and metabolism. This is observed for e.g., competitive inhibition of thyroxine (T_4) binding to transthyretin (TTR)[9], the major plasma transport protein of T_4 in most species, except in larger mammals including humans. Phenolic metabolites of PCBs are also much more potent than their parent compounds with respect to competitive inhibition of hepatic type 1 deiodinase[10], a pivotal enzyme in the activation/deactivation of thyroid hormone, and for uncoupling of oxidative phosphorylation in rat liver mitochondria[11,12]. Next to these *in vitro* observations there are also *in vivo* observations indicating that both TTR and type 1-deiodinase activities are affected by phenolic metabolites of PCBs[2,10].

Another interesting finding is that phenolic PCB metabolites, especially lower chlorinated ones, such as 4-hydroxy-2',4',6'-trichlorobiphenyl do show a substantial estrogen receptor binding affinity, with a relative potency of 0.05 towards estradiol[13]. Interestingly Aroclor 1221 has been shown to increase uterine weight (an estrogenic activity) *in vivo* in female rats[14]. Increases in uterine weight have also been observed in immature rats, following exposure to 2,2',5,5'-tetrachlorobiphenyl, the Aroclor 1242 mixture and the phenolic metabolite 4'-OH-2,4,6-trichlorobiphenylol[15] It is suggested that phenolic PCB metabolites may be more potent than their respective parent compounds, but the experimental evidence is scarce.

Ah receptor binding has also been observed for some phenolic metabolites of PCB 77[14]. The Ah receptor binding affinities of 2-OH, 4-OH and 5-OH metabolites of PCB 77 were quite remarkable, only about two to three times lower than those for the parent compound PCB 77 itself, using rat liver cytosols. However, the potency to induce the Ah receptor mediated EROD activity was much smaller by the phenolic metabolites than the parent PCB 77 compound, i.e., three orders of magnitude lower, when using mouse Hepa1c1c7 cells[17]. This striking difference may indicate that substantial metabolism of phenolic PCBs to more polar derivatives may take place in Hepa1c1c7 cells.

Table 1. Observed *in vitro* biological effects of phenolic PCB metabolites and their relative potency towards parent compounds.

Biological effects of phenolic PCBs	Potency relative to parent compound	Reference
T_4-binding competition on TTR	OH-PCBs > > > PCBs	1,9
Inhibition of type 1-deiodinase	OH-PCBs > > > PCBs	10
Uncoupling mitochondrial respiration	OH-PCBs > > PCBs	11,12
Estrogen receptor binding	OH-PCBs > PCBs	13
Ah-receptor binding (rat liver cytosol)	OH-PCBs < PCBs	15
IC inhibition (Hepa1c1c7)	OH-PCBs < PCBs	17
Chick embryo mortality	OH-PCBs << PCBs	16
EROD induction (Hepa1c1c7)	OH-PCBs < < < PCBs	17

Note: TTR: transthyretin; IC: intercellular communication; > or <: order of magnitude

Recently, we have obtained evidence that phenolic metabolites of PCB 77 are also able to inhibit gap junctional intercellular communication (IC) in mouse Hepa1c1c7 cells[17]. IC inhibition is believed to be an *in vitro* indicator of tumor promotion potential of chemicals. However, the potency to inhibit IC was about 10 to 100 times lower as compared to the parent compound PCB 77. At this moment it is unclear, whether the intrinsic potency of phenolic PCBs is lower, or whether substantial metabolism of phenolic PCBs in mouse Hepa1c1c7 may be the cause of these differences in IC inhibition between PCB 77 and its phenolic metabolites.

The toxic potency of phenolic metabolites relative to the parent compound PCB 77 is considerably lower (at least two orders of magnitude) when tested in the chick embryo assay[16]. In addition, exposure of rats to phenolic metabolites of PCBs generally resulted in much less toxicity compared to their corresponding parent compounds[18,19]. Also in these *in vivo* studies, it may not be possible to delineate the intrinsic toxic potency of phenolic PCBs, since a large proportion of the phenolic PCBs, when given as a bolus ip injection, may never reach its target sites, due to extensive secondary metabolism. Phenolic metabolites formed *in vivo* are to a considerable extent packaged into specific binding proteins, such as TTR, and therefore are less prone to secondary metabolism. In addition, these binding proteins may be involved in transport and disposition of phenolic compounds. Therefore, exposure studies with phenolic PCBs bound to their specific binding proteins, e.g., TTR will be performed in our laboratory to investigate their toxic potency.

5. Discussion and conclusions

From *in vitro* studies several biological effects were reported by phenolic PCB metabolites. Their relative potency compared to parent compounds was high when considering interactions with key proteins in thyroid hormone metabolism and in hepatic mitochondria, moderate with respect to Ah-receptor binding, estrogen receptor binding and IC inhibition potencies and low for EROD induction and chick embryo mortality.
Therefore, it is anticipated that phenolic metabolites of PCBs and related chemicals may predominantly contribute to disturbances in thyroid hormone levels and metabolism and to a lesser extent to alterations in mitochondrial function, estrogen binding and function and possibly tumor promotion effects observed following exposure of experimental animals to these chemicals. The extent to which phenolic metabolites may play a role in these effects in the *in vivo* situation may depend both on the biotransformation potential of the species exposed and the metabolisability of the congener, or mixture exposed to. The recently observed drastic accumulation of phenolic PCB metabolites in late gestational fetuses, following Aroclor 1254 exposure of the dams from day 10 to 16 of gestation, suggests that the developing fetus may be particularly at risk for toxic effects associated with phenolic PCB metabolites (Morse, personal communication).

6. References

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