Induction of biotransformation enzymes by polyhalogenated aromatic hydrocarbons (PHAHs): potential impact on animal physiology and health[1]

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ABSTRACT

Biotransformation and its role in the elimination of polyhalogenated aromatic hydrocarbons (PHAHs) has been the subject of many studies from the late seventies onwards. The notion of specific, high affinity interactions of phenolic PHAH metabolites with the plasma transport proteins of thyroid hormone and vitamin A, both *in vitro* and *in vivo*, stimulated further research into the possible role of biotransformation in the toxicity of certain PHAHs such as PCBs. Currently, phenolic metabolites of PCBs and related compounds have been identified as major metabolites in blood plasma of e.g. grey seals (*Halichoerus grypus*) and humans with background environmental exposure to these chemicals. The concentrations of the hydroxy-PCBs were in the same range as the most persistent parent congeners, such as PCB 153, 138 and 180. These phenolic metabolites were found to possess a specific range of biological activities, which differed from the parent compounds. Another potential adverse effect associated with persistent induction of biotransformation enzymes, like UDP-glucuronyl transferases (UGTs) by PHAHs, is a long-term enhanced elimination of several important endogenous ligands such as vitamin A and thyroid hormones. Reduced levels of vitamin A and thyroid hormones have been reported in most experimental animal and wildlife species exposed to PHAHs. The recent observation of the accumulation of high levels of phenolic PCB metabolites in blood and brain of late gestational rat foetuses, in parallel with reductions in both vitamin A and thyroid hormone levels, suggests that these metabolites may play an important role in the observed developmental toxicity of PHAHs.

KEYWORDS: BIOMARKERS; DISEASE; HAZARD ASSESSMENT; PHYSIOLOGY; POLLUTION-HAHs; POLLUTION-ORGANOCHLORINES; REPRODUCTION

INTRODUCTION

Exposure of laboratory animals and wildlife species to various xenobiotic compounds usually causes an enhanced biotransformation response, in most cases resulting in an accelerated catalytic breakdown and elimination of the xenobiotics. Polycyclic aromatic hydrocarbons (PAHs) are an example of this class of xenobiotics. However, due to the highly reactive nature of some of the metabolic PAH intermediates formed, a certain fraction of the PAH metabolites will not be eliminated but rather will give rise to DNA and protein adducts and associated toxicity. On the other hand, there are several classes of very persistent polyhalogenated aromatic hydrocarbon (PHAH) pollutants, such as polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) (Fig. 1), that give rise to a high induction of biotransformation enzymes, but are themselves hardly

degraded and eliminated by the enzymes they induce. Consequences of a persistent induction of certain biotransformation enzymes, such as cytochrome P450 isozymes, may involve: increased oxidative stress; increased formation of reactive intermediates from more readily degradable xenobiotic compounds present in the same exposure matrix; and enhanced elimination of physiologically important endogenous ligands (hormones, vitamins). Therefore, these classes of persistent PHAH pollutants may put the exposed laboratory and wildlife species at risk from adverse health effects. In this paper several aspects of the metabolism and physiological/toxicological consequences of exposure to PCBs and related compounds are discussed.
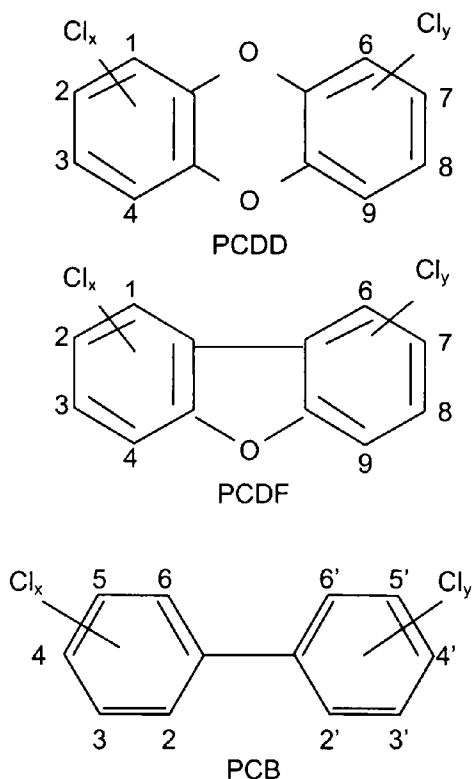

Fig. 1. General structures of PCDD, PCDF and PCB.

POLYCHLORINATED BIPHENYLS, DIBENZO-P-DIOXINS AND RELATED COMPOUNDS

PCBs are a class of ubiquitous and persistent environmental pollutants that accumulate extensively in food chains. All biota and species analysed so far have been found to contain a certain level of PCBs and related contaminants. Relatively high body burdens of PCBs and related compounds are found in species at higher trophic levels, especially those of the aquatic and marine environment. PCBs and related compounds are even detectable in marine species found in the Arctic and Antarctic, far from their source (e.g. harp seals, *Phoca groenlandica*, Antarctic fur seals, *Arctocephalus gazella*, ringed seals, *Phoca hispida* and

white whales, *Delphinapterus leucas*) due to the global transport of these persistent lipophilic compounds from warmer areas to colder areas (Norstrom and Muir, 1994; Oehme *et al.*, 1994a). Body burdens of PCBs and related compounds have been found to be dependent on a variety of factors including species, age, gender, season, diet and location. Therefore, it is difficult to discern temporal trends. However, overall there was a decline in PCB levels in most species in the early eighties, which now seems to have levelled off, at least in some areas (Norstrom *et al.*, 1988; Bignert *et al.*, 1993; De Boer and Hagel, 1994).

PHAH congener patterns
PHAH congener patterns present in biota of various trophic levels differ considerably. These differences are mainly due to variations in exposure and/or biotransformation capacity of the various species. In general, only 2,3,7,8-substituted polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) congeners are found in most biota, indicating a rapid degradation of the non-2,3,7,8-substituted PCDD/Fs by most species (Van den Berg *et al.*, 1994b). The relative concentrations of the various 2,3,7,8-PCDD/Fs increase with the number of chlorine substituents. Therefore, the highest concentrations are observed for octaCDD/F congeners. However, for PCB congeners the picture is more complex. In fact, PCB congeners have been divided into six structurally different groups of congeners that differ in their biostability. Some congeners appear to be very persistent, like the CB congeners 153, 138 and 180. However, the CB congener pattern differs between different species dependent on, for instance their biotransformation capacity. This is well illustrated by the variation of the ratio of the different non-*ortho* substituted, co-planar CBs, 3,3',4,4'-TeCB (CB 77), 3,3',4,4',5-PeCB (CB 126) and 3,3',4,4',5,5'-HxCB (CB 169) in various species (Kannan *et al.*, 1988; 1989a; b). In fish, crabs and blue mussels, CB 77 represents >90% of the non-*ortho* CBs in areas around Japan (Miyata *et al.*, 1994) and Taiwan (Lu *et al.*, 1994) and actually reflects the ratio of non-*ortho* CBs present in river sediments (Ohsaki *et al.*, 1994). In marine mammals, like Antarctic fur seals and harp seals, CB 77 and CB 126 are present in blubber in almost equal amounts, while CB 169 is present at a ratio of 0.1–0.2 towards CB 126 (Oehme *et al.*, 1994a; b). However, in human milk samples CB 77 is only a small proportion (<10%) of the non-*ortho* CB congeners (Koopman-Esseboom *et al.*, 1994).

PHAHs and toxicity
PCBs have been found to cause a wide spectrum of species-specific and congener-specific toxic and biochemical effects in experimental animals, including hepato-, immuno-, reproductive-, and dermal-toxicity, teratogenicity (congenital malformations), developmental neurotoxicity and carcinogenicity (McConnell, 1989; Safe, 1994). In general, early exposure during embryonal and neonatal development is a much more sensitive (more than 100-fold) time period than adult exposure for induction of adverse effects in laboratory animals and probably also in human infants (Brouwer *et al.*, 1995). Moreover, the effects induced early on in life have a much greater chance of being permanent without possibilities for compensation. In addition, PCBs and related compounds have been suggested to be involved in many epizootic diseases in fish, birds and marine mammals, such as 'blue sac like disease' in fish (Peterson *et al.*, 1993), skin lesions and liver tumors in flounder, *Platichthys flesus*, (Vethaak, 1993); reduced immune function in relation to outbreaks of lethal morbillivirus infections in seals in the North Sea and Baltic (Brouwer *et al.*, 1989b; De Swart *et al.*, 1994; Ross *et al.*, 1995, although see Kennedy, 1999); and reduced population size due to reproduction problems in ringed, *pusa hispida*, harbour seals, *Phoca vitulina*, (Helle *et al.*, 1976; Reijnders, 1986) and fish-eating birds, such as cormorants,

Phalacrolorax carbo, (Van den Berg *et al.*, 1994a). Early life stages in wildlife species also appear to be much more sensitive than adults for the adverse health effects of PCBs and related compounds (Colborn and Clement, 1992).

PHAHs and Ah-receptor

Most of the toxic and biochemical effects induced by planar, non-*ortho* substituted PCBs and 2,3,7,8-substituted PCDD and PCDF congeners are mediated through high affinity binding to a cytosolic protein, the aryl hydrocarbon-receptor (AhR; Poland and Knutson, 1982). After ligand binding the AhR is converted into an activated, DNA-binding form, subsequently transformed by the release of two heat shock proteins (HSP90) and translocated to the nucleus and complex formation to a second protein, the Ah-receptor nuclear translocator (ARNT; Whitlock, 1993; Hankinson, 1995). This heterodimer complex is bound to specific sequences, the dioxin responsive enhancer (DRE) sequences located in the promotor-region upstream of dioxin-responsive genes (Denison *et al.*, 1988a; b).

Nowadays a number of genes have been identified that contain one, or several DREs, and therefore their expression may be regulated via the dioxin-AhR pathway. A number of the DRE-containing genes encode cytochrome P450 isozymes, such as CYP1A1, CYP1A2, CYP1B1. In addition, several conjugation enzymes are identified with DREs in their promotor region, such as aldehyde dehydrogenase (Ahd4) gene, NAD(P)H-menadione oxidoreductase (Nmol) gene, UDP-glucuronyl transferase (UGT01*6) gene and the Glutathion-S-transferase (GST-pi) gene (Nebert *et al.*, 1993).

Induction of biotransformation enzymes

In laboratory rodents (mice, rats, guinea pigs) planar, non-*ortho* substituted PCBs cause a 50-fold to several hundred fold persistent induction of CYP1A1/2 activity, similar to 3-methylcholanthrene (3-MC), which therefore is also called 3-MC type of induction. However, in contrast to 3-MC, the CYP1A1/2 induction caused by the planar PCBs is persistent, reflecting the long half-life of PCB congeners in most species. A frequently used model substrate for CYP1A1/2 is ethoxyresorufin, which is deethylated during the enzymatic reaction. This activity is called ethoxyresorufin-O-deethylase (EROD). Another frequently used model substrate is benzo(a)pyrene, and in this case the corresponding enzymatic activity is called arylhydrocarbon hydroxylase (AHH). These activities are extensively used as markers for CYP1A1/2 induction by PHAHs (Safe, 1994). Several of the other AhR-pathway controlled gene products have also been found to be induced, such as increased UGT1 with associated increases in hepatic thyroxine (T_4) and 1-naphtol glucuronidation, following exposure of laboratory animals to PCBs and related compounds (Visser *et al.*, 1993; Van Birgelen *et al.*, 1995).

However, non-planar PCB congeners, such as diortho-substituted PCBs, do not cause induction of CYP1A1/2 genes, but instead induce CYP2B1/2 isozymes, similarly to phenobarbitone (PB). Because of this, such activity is also called PB-type of induction. A model reaction for CYP2B is the depentylation of pentoxyresorufin (PROD). The mechanism of CYP2B gene induction is less clear than that of CYP1A1/2. Both a receptor-mediated enhanced transcription and a post-transcriptional stabilisation of mRNAs have been suggested. In addition to CYP2B isozymes, PB-type inducers also enhance transcription of members of the CYP2C and CYP3A classes. Mono-*ortho* and some di-*ortho* substituted PCB congeners are so-called mixed type inducers' in that, they induce both CYP1A and CYP2B classes of isozymes.

In general, induction of orthologous CYP450 isozymes by PHAHs is also observed in wildlife species, like fish, fish-eating birds and marine mammals. In fish, CYP1A1 and the associated EROD activity is induced by PHAHs via an AhR mediated pathway (Hahn and

Stegeman, 1994). In the case of fish caught in the wild with different levels of environmental exposure to PHAHs, the correlation between exposure and EROD-induction is often less clear. This may be due partly to the problems associated with exposure of species in the wild to complex mixtures of PCBs and related compounds. Several PCB congeners, such as CB 77, appear to be able to competitively inhibit the EROD activity (Hahn and Stegeman, 1994; Morse *et al.*, 1995). In addition, several publications indicate the presence of Ah-receptor antagonists in PCB mixtures that partly abolish the EROD induction by 2,3,7,8-TCDD (Davis and Safe, 1990; Aarts *et al.*, 1993). Fish-eating birds, like the common tern (*Sterna hirundo*) and cormorants, as well as marine mammals, like harp seals and harbour porpoises (*Phocoena phocoena*) also show a good CYP1A1/2 inducibility following exposure to PHAHs (Goksøyr *et al.*, 1992; Van den Berg *et al.*, 1994a; Bosveld *et al.*, 1995). A major distinction between laboratory animals and fish and most fish-eating birds, is the absence of CYP2B isozymes in the latter species. However, there is limited information on the presence and inducibility of CYP2B isozymes in marine mammals (Goksøyr *et al.*, 1992; White *et al.*, 1994; Kannan *et al.*, 1995).

CONGENER PATTERNS IN DIFFERENT SPECIES

Comparison of congener patterns between sediments and fish, fish and marine mammals, fish and fish-eating birds, may provide valuable information on the metabolism of certain PHAH congeners. By comparing ratios of a non-metabolisable PHAH congener, like CB153 with the other CB congeners in different matrices one can deduce the relative impact of metabolism on PHAH congener patterns (Boon *et al.*, 1992). Only 2,3,7,8-substituted PCDD/Fs are found in biota and species at higher trophic levels, such as marine mammals and man, indicating that the non-2,3,7,8-PCDD/Fs are rapidly metabolised in the environment (Van den Berg *et al.*, 1994a). However, 2,3,7,8-TCDF which is present in biota is still rapidly metabolised in laboratory rats with a half-life of about two days. This 2,3,7,8-PCDF congener is probably quite extensively metabolised in marine mammals and human individuals as well. Metabolism of PCBs is more complex. PCB congeners have been divided into different structural groups with regards to cytochrome P450-dependent metabolism, based on experimental and field studies on seals and cetaceans (Boon *et al.*, 1994). Congeners that contribute to dioxin-type toxicity in laboratory animals are given in bold in this list.

I: Congeners without any vicinal hydrogen (H) atoms (e.g. CB153, **CB169**, CB180, CB183, CB187, CB194).

II: Congeners with vicinal H atoms only in the *ortho-* and *meta*-positions in combination with ≥ 2 *ortho*-Cl substituents (e.g. CB99, **CB128, CB138, CB170**).

III: Congeners with vicinal H atoms in the *ortho-* and *meta*-positions in combination with ≤ 1 *ortho* - CL (e.g., CB-28, **CB77, CB105, CB118, CB126, CB156**).

IV: Congeners with vicinal H atoms in the *meta-* and para-positions in combination with ≤ 2 *ortho*-Cl (e.g. CB44, CB49, CB52, CB101, CB110).

V: Congeners with vicinal H atoms in the *meta-* and para-positions in combination with ≥ 3 *ortho*-CL (e.g. CB136, CB149, CB151)

VI: Congeners with vicinal H atoms both in the *ortho-* and *meta*-positions and *meta-* and para-positions in combination with ≤ 1 *ortho*-Cl (e.g. CB31)

In seals, cetaceans and seabirds, congeners belonging to Groups I and II are persistent to biotransformation but, in polar bears, even some of these congeners appear to be metabolisable, especially when at least one unsubstituted para-position is available (Norstrom et al., 1988). Congeners of Group III are metabolisable (by CYP1A) in seals, harbour porpoises and dolphins. This is important because Group III contains most of the dioxin-type congeners. The capacity to metabolise congeners belonging to Groups IV and V seems to be more developed in seals than in cetaceans (Tanabe et al., 1988), although there is evidence for biotransformation of congeners of Group IV (Duinker et al., 1989; Boon et al., 1994) and even Group V congeners (Reijnders, 1994; Bruhn et al., 1995; Reijnders and de Ruiter-Dijkman, 1995) by the harbour porpoise too. The substitution pattern at the four available ortho-positions has two possible consequences: it strongly influences the ability of the molecule to reach a planar configuration (Cullen and Kaiser, 1984) which is very important for expressing dioxin-like toxicity, or the bulky chlorine atoms may simply prevent enzymatic attack.

Role of CYP1A1/2 in PHAH metabolism

In vitro incubations of a radiolabelled model compound (^{14}C-CB 77) in hepatic microsomal preparations from rats pretreated with different inducers of P450 isozymes, clearly indicated the importance of CYP1A1/2 in the phenolic CB metabolite formation (Ishida et al., 1991; Morse et al., 1995). The mechanism of CYP1A1/2-dependent formation of phenolic CB metabolites is thought to involve the formation of highly reactive arene oxide intermediates, which may spontaneously rearrange to phenols with a concomittant shift of substituents from the site of hydroxylation to the next neighbour carbon atom in the aromatic ring. Phenolic CB metabolites have been identified as major metabolites of both planar and non-planar CBs in in vitro and in vivo studies (Klasson-Wehler, 1989; Safe, 1989).

The rate of formation and the nature of the phenolic CB metabolites formed are both species and congener dependent (Murk et al., 1994b). In general, hydroxylation is favoured in the para-position of the least chlorinated ring. However, a phenolic derivative of 2,3,3',4',5-PeCB with the hydroxy-group in the most chlorinated ring was recently identified as the major metabolite present in blood plasma of mice (Bergman et al., 1994). Phenolic products are the major PCB metabolites formed in in vitro incubations with liver microsomes and in vivo by mammalian and avian species (Murk et al., 1994b). However, in liver microsomes of fish minimal formation of phenolic-PCB metabolites was observed, even when exposed experimentally to cytochrome P450-1A1/2 inducers.

TOXICOLOGICAL CONSEQUENCES OF PERSISTENT CYP1A1/2 INDUCTION BY PHAHs

(a) Phenolic PCB related effects

Metabolism and metabolite formation of most halogenated aromatic compounds, including PCBs have been regarded mainly as an elimination and detoxification route. Recent studies have indicated that phenolic and 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 blood plasma of several species (e.g. rats, mice, marine mammals and humans) either experimentally or environmentally exposed to PCBs (Klasson-Wehler et al., 1992; Bergman et al., 1994). Moreover, a considerable accumulation of phenolic PCB metabolites was observed in late gestational foetuses when their mothers were exposed to Aroclor 1254 from days 10 to 16 of gestation (Morse, 1995; Morse et al., 1995).

Effects on thyroid hormone metabolism and transport
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. For example, phenolic PCBs competitively inhibit thyroxine (T_4) binding to transthyretin (TTR; Lans *et al.*, 1993). TTR is 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 (Adams *et al.*, 1990), a pivotal enzyme in the activation/deactivation of thyroid hormone. Moreover, phenolic metabolites, but not their corresponding parent compounds are potent uncouplers of oxidative phosphorylation in rat liver mitochondria (Lans *et al.*, 1990; Narasimhan *et al.*, 1991). 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 (Brouwer and Van den Berg, 1986; Adams *et al.*, 1990).

Table 1

Observed *in vitro* biological effects of phenolic PCB metabolites and relative potency towards parent compounds. TTR: transthyretin; IC: intercellular communication; > or <: order of magnitude.

Biological effects of phenolic PCBs	Potency relative to parent compound
T_4- binding competition on TTR	OH-PCBs >>> PCBs
Inhibition of type 1- deiodinase	OH-PCBs >>> PCBs
Uncoupling mitochondrial respiration	OH-PCBs >> PCBs
Oestrogen receptor binding	OH-PCBs > PCBs
Ah-receptor binding (rat liver cytosol)	OH-PCBs < PCBs
IC inhibition (Hepalc1c7)	OH-PCBs < PCBs
Chick embryo mortality	OH-PCBs << PCBs
EROD induction (Hepalc1c7)	OH-PCBs <<< PCBs

Effects on the oestrogen system
Another interesting finding is that phenolic PCB metabolites, especially lower chlorinated ones, such as 4-hydroxy-2',4',6'-trichlorobiphenyl, show a substantial oestrogen receptor binding affinity, with a relative potency of 0.05 towards oestradiol (Korach *et al.*, 1987). Interestingly, Aroclor 1221 has been shown to increase uterine weight (an oestrogenic activity) *in vivo* in female rats (Gellert, 1978). 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-trichlorobiphenyl (Jansen *et al.*, 1993). It has been suggested that phenolic PCB metabolites may be more potent with respect to oestrogenic activity than their respective parent compounds, but the experimental evidence is scarce.

Effects on the Ah-receptor pathway
Ah-receptor binding has also been observed for some phenolic metabolites of CB77 (Klasson-Wehler *et al.*, 1990). The Ah-receptor binding affinities of 2-OH, 4-OH and 5-OH metabolites of CB77 were quite remarkable, only about two to three times lower than those for the parent compound CB77 itself, using rat liver cytosols. However, the potency to induce the Ah-receptor mediated EROD activity was three orders of magnitude lower for the

phenolic metabolites than for the parent CB77 compound itself, when using mouse Hepa1c1c7 cells (De Haan *et al.*, 1994). This striking difference may indicate that substantial metabolism of phenolic PCBs to more polar derivatives may take place in Hepa1c1c7 cells.

Effects on intercellular communication
Intercellular communication (IC) is an important regulatory mechanism for cell proliferation and differentiation. Recently, we have obtained evidence that phenolic metabolites of CB77 are also able to inhibit gap junctional IC in mouse Hepa1c1c7 cells (De Haan *et al.*, 1994). IC inhibition is believed to be an *in vitro* indicator of the tumor promotion potential of chemicals. The potency to inhibit IC for the phenolic CB77 metabolites was equal or higher than for the parent CB77. At the present time it is unclear as to whether the intrinsic potency of phenolic PCBs to inhibit IC may be even higher, due to the possibility of substantial secondary metabolism of these metabolites in mouse Hepa1c1c7 cells.

In vivo toxicity of phenolic metabolites
The *in vivo* toxic potency of phenolic metabolites relative to the parent compound CB77 is at least two orders of magnitude lower when tested in the chick embryo assay (Klasson-Wehler *et al.*, 1990). In addition, acute exposure of rats to phenolic metabolites of PCBs resulted in much less toxicity compared to their corresponding parent compounds (Yoshimura *et al.*, 1987; Koga *et al.*, 1990). However, it may not be possible to delineate the intrinsic toxic potency of phenolic PCBs from these *in vivo* studies. A large proportion of the phenolic PCBs, which were given as a bolus *ip* injection, may never have reached their target sites, due to secondary metabolism. Phenolic metabolites formed *in vivo* are extensively packaged into specific binding proteins, such as TTR. This may affect both the rate of secondary metabolism and the ligand concentration at the target sites, due to a TTR-specific delivery route, such as to the foetal compartment (Morse, 1995). Therefore, exposure studies with phenolic PCBs bound to their specific binding proteins, such as TTR, will be performed in our laboratory to investigate their toxic potency.

(b) Cyp1a1/2-induction and co-carcinogenesis
Induction of CYP1A1/2 and other isozymes also increases the metabolic capacity of the liver towards other xenobiotic compounds that may be present in the same exposure matrix, such as the polycyclic aromatic hydrocarbons (PAHs). Numerous studies have shown the cytochrome P450-dependent accelerated rate of formation of mutagenic, reactive intermediates of PAHs (Dipple *et al.*, 1984; Yang *et al.*, 1984). The well investigated bay-region diol-epoxides, such as benzo(a)pyrene-7,8-diol,9,10-epoxide, are highly mutagenic and readily form DNA-adducts. The formation of carcinogenic metabolites from PAHs by P450 isozymes is highly regio- and stereoselective. For example, the isozyme CYP1A2 is highly selective in the formation of 3,4-dihydrodiol metabolites from 7-methylbenz(c)acridine, while CYP3A4 is mainly involved in the proximate carcinogenic metabolite formation from dibenz(a,j)acridine (Roberts-Thomson *et al.*, 1995). There appears to be a good relationship between cytochrome P450 isozyme induction potency and the mutagenicity of the PAH-metabolites formed. Therefore, it is anticipated that a persistent exposure to PCBs and related P450 isozyme inducers may enhance the risk for co-carcinogenic events considerably when individuals are exposed to complex mixtures of PHAHs and PAHs present in the environment.

(c) Enhanced metabolism of endogenous substrates
Another observed consequence of long-term induction of biotransformation enzymes by PCB exposure is an accelerated metabolism and elimination of endogenously important components, such as vitamins and hormones. In this case, the enhanced elimination of endogenous compounds may not be due to CYP1A1/2, but to concomitantly induced enzymes such as UDP-glucuronyl transferases. Many studies have found an enhanced hepatic glucuronidation of thyroxine (T_4) and a concommittantly reduced level of T_4 in plasma of experimental animals exposed to PCBs and related compounds (Bastomsky, 1977; Collins and Capen, 1980; Visser *et al.*, 1993; Van Birgelen *et al.*, 1995). Both 3-MC and PB-type inducers can cause an accelerated hepatic glucuronidation of T_4, though through different isozymes, UGT1 and UGT2 (Visser *et al.*, 1993). Complex PCB mixtures may therefore possess a high potential to induce T_4-glucuronidation, since both planar and non-planar PCBs may contribute to this effect. Enhanced glucuronidation of certain vitamin A (retinoid) derivatives, such as retinoic acid, was also observed following incubation with hepatic microsomes of TCDD-pretreated rats (Bank *et al.*, 1989; Zile *et al.*, 1989). This enhanced conjugation of retinoid derivatives may also be involved in the dramatic depletion of hepatic and extrahepatic retinoid concentrations in laboratory animals exposed to PHAHs (Brouwer *et al.*, 1988; 1989a; Håkansson *et al.*, 1991; Chen *et al.*, 1992).

Reductions in both vitamin A and thyroid hormone levels in relation to PHAH exposure have been observed in several fish-eating bird species and in marine mammals (Brouwer *et al.*, 1989b; De Swart *et al.*, 1994; Murk *et al.*, 1994a; c; Van den Berg *et al.*, 1994a). In addition, Murk *et al.* (1994a; c) recently found a significant correlation between T_4-glucuronidation and PCB body burdens in common terns.

CONCLUSION

In conclusion, persistent induction of P450 isozymes by environmental pollutants, such as CYP1A1/2 by PHAHs, may put individuals (laboratory animals, wildlife species and man) at risk from adverse health effects, such as developmental toxicity and carcinogenesis. Therefore, given the long-lasting and relatively high levels of exposure of marine mammals to a variety of persistent chemical pollutants, it is of utmost importance to develop and use mechanistically-based biomarkers for PHAHs and other pollutants to monitor exposure levels and subtle indications of effects.

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