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In vitro profiling of toxic effects of prominent environmental lower-chlorinated PCB congeners linked with endocrine disruption and tumor promotion☆

Kateřina Pěnčíková [a], Lucie Svržková [a], Simona Strapáčová [a], Jiří Neča [a], Iveta Bartoňková [b], Zdeněk Dvořák [b], Martina Hýžďalová [a, c], Jakub Pivnička [c], Lenka Pálková [a], Hans-Joachim Lehmler [d], Xueshu Li [d], Jan Vondráček [c, *], Miroslav Machala [a, **]

[a] Department of Chemistry and Toxicology, Veterinary Research Institute, Hudcova 70, 62100 Brno, Czech Republic
[b] Department of Cell Biology and Genetics, Faculty of Science, Šlechtitelů 11, Palacký University, 78371 Olomouc, Czech Republic
[c] Department of Cytokinetics, Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, 61265 Brno, Czech Republic
[d] Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, 52242, IA, USA

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ABSTRACT

The mechanisms contributing to toxic effects of airborne lower-chlorinated PCB congeners (LC-PCBs) remain poorly characterized. We evaluated *in vitro* toxicities of environmental LC-PCBs found in both indoor and outdoor air (PCB 4, 8, 11, 18, 28 and 31), and selected hydroxylated metabolites of PCB 8, 11 and 18, using reporter gene assays, as well as other functional cellular bioassays. We focused on processes linked with endocrine disruption, tumor promotion and/or regulation of transcription factors controlling metabolism of both endogenous compounds and xenobiotics. The tested LC-PCBs were found to be mostly efficient anti-androgenic (within nanomolar – micromolar range) and estrogenic (at micromolar concentrations) compounds, as well as inhibitors of gap junctional intercellular communication (GJIC) at micromolar concentrations. PCB 8, 28 and 31 were found to partially inhibit the aryl hydrocarbon receptor (AhR)-mediated activity. The tested LC-PCBs were also partial constitutive androstane receptor (CAR) and pregnane X receptor (PXR) agonists, with PCB 4, 8 and 18 being the most active compounds. They were inactive towards other nuclear receptors, such as vitamin D receptor, thyroid receptor α, glucocorticoid receptor or peroxisome proliferator-activated receptor γ. We found that only PCB 8 contributed to generation of oxidative stress, while all tested LC-PCBs induced arachidonic acid release (albeit without further modulations of arachidonic acid metabolism) in human lung epithelial cells. Importantly, estrogenic effects of hydroxylated (OH-PCB) metabolites of LC-PCBs (4-OH-PCB 8, 4-OH-PCB 11 and 4′-OH-PCB 18) were higher than those of the parent PCBs, while their other toxic effects were only slightly altered or suppressed. This suggested that metabolism may alter toxicity profiles of LC-PCBs in a receptor-specific manner. In summary, anti-androgenic and estrogenic activities, acute inhibition of GJIC and suppression of the AhR-mediated activity were found to be the most relevant modes of action of airborne LC-PCBs, although they partially affected also additional cellular targets.

1. Introduction

Polychlorinated biphenyls (PCBs) are widely distributed organic pollutants, which have been used as electrical insulating fluids, in building construction materials or added as plasticizers and flame retardants to a variety of consumer products. Despite the ban on their production and their declining levels in the environment, PCB contamination remains a major environmental problem in many

☆ This paper has been recommended for acceptance by David Carpenter.
* Corresponding author.
** Corresponding author.
E-mail addresses: vondracek@ibp.cz (J. Vondráček), machala@vri.cz (M. Machala).

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parts of the world, as they continue to be released into the environment through the use or disposal of various PCB-containing products (Lehmann et al., 2015). In particular, the volatile PCB congeners continue to present a threat to human health, since significant levels of the airborne lower-chlorinated PCB congeners (LC-PCBs) are found in PCB-contaminated buildings or within industrial and densely populated urban areas (Herrick et al., 2004; Lehmann et al., 2015). Their indoor concentrations are often higher than in the respective outdoor areas (Jamshidi et al., 2007). A recent study comparing indoor versus outdoor levels of PCBs in PCB-contaminated U.S. schools revealed median indoor air levels of Σ PCBs ranging from 2.05 to 111.83 ng/m^3 (95th percentile 5.14–187.92 ng/m^3) (Marek et al., 2017). In contrast, outdoor air levels in the same study were much lower and ranged from 0.16 to 0.58 ng/m^3 (95th percentile: 0.50–2.38 ng/m^3). LC-PCBs have been also identified in blood samples of exposed human populations (Fitzgerald et al., 2011; Gabrio et al., 2000). During recent years, LC-PCBs have become a point of a renewed interest, as several PCBs, such as PCB 11, have been discovered to be byproducts of pigment production (Hu and Hornbuckle, 2010; Anezaki et al., 2015). These so-called non-legacy or unintentional PCBs are found, together with other LC-PCB congeners, in consumer products, as well as in the environment (Grimm et al., 2015).

Based on their toxicological mechanism of action, PCBs can be divided into two groups. The dioxin-like coplanar PCBs (DL-PCBs) activate the aryl hydrocarbon receptor (AhR) and this is considered their principle mode of action (Van den Berg et al., 2006). In contrast, the most abundant environmental non-dioxin-like PCBs (NDL-PCBs) do not activate the AhR, and there is no generally accepted risk concept for these compounds (Hamers et al., 2011), although some NDL-PCBs have been shown to inhibit the AhR transcriptional activity (Brenerová et al., 2016; Ovesen et al., 2011). Nevertheless, NDL-PCBs exert numerous specific modes of action linked with carcinogenicity, neurotoxicity or endocrine disruption (Gore et al., 2015; Grandjean and Landrigan, 2006; Lauby-Secretan et al., 2013). They have been shown: i) to interact with nuclear receptors controlling metabolism of both endogenous and exogenous compounds (including hormones), such as constitutive androstane receptor (CAR) or pregnane X receptor (PXR); ii) to directly modulate gene expression dependent on activation of estrogen (ER), androgen (AR) or thyroid receptors; iii) to exert effects linked with carcinogenesis/tumor promotion, such as generation of reactive oxygen species (ROS) or inhibition of gap junctional intercellular communication (GJIC); or to produce neurotoxic effects (Gore et al., 2015; Grimm et al., 2015; Hamers et al., 2011; Knerr and Schrenk, 2006; Machala et al., 2003; Pessah et al., 2006; Simon et al., 2007). Effects of NDL-PCBs can also be associated with further alterations of physiological processes within the body, as illustrated e.g. in case of disruption of CAR/PXR signaling by PCBs, linked with deregulation of energy metabolism, inflammation and liver diseases (Wahlang et al., 2016).

Therefore, both DL- and NDL-PCBs may act through multiple mechanisms contributing to their toxic action, which can be used to derive their respective relative effective potency values. Such data may aid the risk assessment of both individual PCB congeners and their mixtures (Hamers et al., 2011). However, the available information about specific toxicities of LC-PCBs is very limited. Just recently, a study comparing activities of LC-PCBs towards several hormonal receptors and AhR has suggested that LC-PCBs may act as agonists or antagonists of ER, AR, glucocorticoid receptor (GR) or AhR (Takeuchi et al., 2017). However, this study used ectopic expression of specific nuclear receptors together with the respective reporter genes in rodent, not human, cells. Importantly, it also did not investigate some LC-PCBs found at significant levels in both indoor and outdoor air, such as PCB 18, 28 and 31 (Carpenter, 2015; Marek et al., 2017). Therefore, we still have only a limited information about the activity of environmental LC-PCBs within the context of endogenous nuclear receptor expression in human cell models. Furthermore, little is known about their further impact on cells, including their potential effects linked with tumor promotion. The rapid metabolism of abundant LC-PCB congeners may lead to formation of hydroxylated metabolites with specific toxicity profiles (Grimm et al., 2015); however, the toxicities of hydroxylated LC-PCBs are less known than those of their parent compounds, although hydroxylation significantly alters the activities of a number of PCB congeners (Dhakal et al., 2018; Machala et al., 2004), and some of them could be highly active towards ER or AR (Takeuchi et al., 2011).

Therefore, we employed a series of *in vitro* assays to determine toxicity profiles of six abundant LC-PCBs that are present at high levels in Aroclor 1260 vapor, and/or that are among the most abundant LC-PCB congeners found in contaminated school buildings – PCB 4, 8, 11, 18, 28 and 31 (Carpenter, 2015; Marek et al., 2017). Next to these LC-PCBs, we determined *in vitro* toxicities of major hydroxylated metabolites of PCB 8, 11 and 18: 4-OH-PCB 8, 4-OH-PCB 11, 5-OH-PCB 11 and 4′-OH-PCB 18. As impurities may alter cellular responses to NDL-PCBs, the purity of all test compounds was rigorously tested by gas chromatography-mass spectrometry (GC-MS). We tested the potencies of selected LC-PCBs and OH-LC-PCBs to interact with AhR, ER, AR, GR, thyroid receptor (TR), vitamin D receptor (VDR), peroxisomal-proliferator activated receptor γ (PPARγ), using human cell lines endogenously expressing the receptors and stably transfected with the respective reporter vectors. Activation of CAR/PXR was estimated using determination of expression of their respective endogenous gene targets - cytochrome P450 2B6 (CYP2B6) and CYP3A4 in differentiated HepaRG cells (Anthérieu et al., 2010). The estimation of receptor activities was further complemented with functional cell bioassays, focusing on the effects leading to generation of oxidative stress and oxidative DNA damage, deregulation of ER-dependent cell proliferation, stimulation of arachidonic acid (AA) release and disruption of GJIC.

2. Materials and methods

2.1. Reagents

2,2′-Dichlorobiphenyl (PCB 4), 2,4′-dichlorobiphenyl (PCB 8), 3,3′-dichlorobiphenyl (PCB 11), 2,2′,5-trichlorobiphenyl (PCB 18), 2,4′,5 -trichlorobiphenyl (PCB 31) and 2,2′,5-trichlorobiphenyl-4′-ol (4′-OH-PCB 18) were purchased from AccuStandard (New Haven, USA). 2,4,4′-Trichlorobiphenyl (PCB 28) was from Dr. Ehrenstorfer (LGC Standards, Teddington, UK). 2,4′-Dichlorobiphenyl-4-ol (4-OH-PCB 8), 3,3′-dichlorobiphenyl-4-ol (4-OH-PCB 11) and 3,3′-dichlorobiphenyl-5-ol (5-OH-PCB 11) were synthesized as described in Supplementary Experimental Procedures. The purities of all LC-PCB congeners and trace impurities are reported in Supplementary Tables 1 and 2. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 99% purity, was from Cambridge Isotope Laboratories (Andover, USA). ICI 182,780 was from Tocris Bioscience (Bristol, UK). Mifepristone was purchased from (Cayman Chemical). Cyproterone acetate (CPTA) was from Santa Cruz Biotechnology (Santa Cruz, USA). 17β-Estradiol (E2), 5α-dihydrotestosterone (DHT), triiodothyronine (T3), rosiglitazone (RGS), phenobarbital (PB) and rifampicin (RIF) were from Sigma-Aldrich (Prague, Czech