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Featured structure-activity relationships for some tri- and tetrachlorobiphenyls in human CYP2E1-activated mutagenicity — Impact of the extent of *ortho*-chlorination

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HIGHLIGHTS

- Activation of tetra-Cl-PCBs by CYP2E1 is limited by the increase in *ortho* chlorines.
- Distinct structure-mutagenicity relationships exist between tri- and tetra-Cl-PCBs.
- Human CYP2E1 can activate some noncoplanar PCBs for aneugenic potentials.

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ABSTRACT

Polychlorinated biphenyls (PCBs) as a group of persistent organic pollutants are confirmed human carcinogens; however, their mutagenicity remains mostly unknown. We have reported the mutagenicity of some PCBs with one to four chlorines in mammalian cells expressing human CYP2E1. To further explore the structural requirements for the mutagenicity of PCBs, eight tri- and tetrachlorobiphenyls untested before were investigated for the induction of gene mutations and micronuclei in a V79-derived cell line expressing both human CYP2E1 and sulfotransferase (SULT) 1A1 (V79-hCYP2E1-hSULT1A1), with SULT1A1 activity inhibited by pentachlorophenol, a potent SULT1 inhibitor. 2,2′,6-Tri-, 2,3′,6-tri, 2,4′,6-tri-, and 2,2′,5-trichlorobiphenyls (PCBs 19, 27, 32, and 18, respectively) induced micronuclei and gene mutations in V79-hCYP2E1-hSULT1A1 cells, at potencies slightly higher than 2,6-dichlorobiphenyl, but one order of magnitude below that by 2,3,3′- and 2,3,4′-trichlorobiphenyls as reported recently; in the parental V79-Mz cells, they were nonmutagenic and weak in micronuclei induction. Among the four tetrachlorobiphenyls with varying number of *ortho* chlorines, 2,3,3′,4′-tetrachlorobiphenyl (PCB 56) induced both micronuclei and gene mutations in V79-hCYP2E1-hSULT1A1 cells with a potency greater than the above compounds; however, 2,2′,3,3′-tetrachlorobiphenyl was equivocal and 2,2′,3,6′-tetra- and 2,2′,6,6′-tetrachlorobiphenyls inactive in V79-hCYP2E1-hSULT1A1 cells. Immunofluorescent staining of micronuclei formed by PCBs 32 and 56 in V79-hCYP2E1-hSULT1A1 cells with centromere protein B antibodies indicated that they were predominantly whole chromosomes, implying aneugenic potentials. This study suggests that tri- and tetrachlorobiphenyls with a single *ortho* chlorine can be most mutagenic under activation by human CYP2E1, and greater numbers of *ortho* chlorines may cause a drastic decline in the activity, especially for tetrachlorobiphenyls.

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of organic compounds consisting of 209 congeners, with varying number and distribution of chlorine substituents on the biphenyl rings. PCBs had been produced and discharged to the environment from 1929 to late in 1970s, when the banning of these compounds began in various countries and regions. Nevertheless, human exposure to PCBs has persisted to the present, mostly due to their stability in the environment, bio-accumulation, bio-magnification through food chains, and ever-lasting production in some processes such as pigment production and burning of chlorine-containing organic materials. They have also been present in human tissues, as

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Abbreviations

PCB	Polychlorinated biphenyl
CENP-B	Centromere protein B
AhR	Aryl hydrocarbon receptor
TCDD	2,3,7,8-Tetrachlorodibenzo-p-dioxin
DMSO	Dimethyl sulfoxide
UGTs	UDP-glucuronosyltransferases
SULTs	Sulfotransferases
EMS	Ethyl methanesulfonate
NDMA	N-Nitrosodimethylamine
PCP	Pentachlorophenol
VCR	Vincristine

reflected by their occurrence in breast milk (Haque et al., 2017). PCBs are associated with a broad range of adverse effects such as endocrine disruption (Miyabara and Yonemoto, 2000), neurotoxicity (Boix et al., 2011), and carcinogenicity (Lauby-Secretan et al., 2013). In addition, quinoid metabolites of PCBs, rather than the prototype compounds, may induce gene mutations (Zettner et al., 2007) and polyploidy in mammalian cells (Flor and Ludewig, 2010). In 2013, PCBs have been classified by the International Agency for Research on Cancer (IARC) as human (group 1) carcinogens (Lauby-Secretan et al., 2013).

According to the degree of chlorination and patterns of chlorine distribution, PCB congeners can be categorized into coplanar and noncoplanar PCBs (Safe, 1994). PCBs with at least four chlorines all at non-*ortho*-positions or in some cases with a single *ortho* chlorine, thus possessing a coplanar configuration, are defined as coplanar PCBs; they include 12 congeners. Coplanar PCBs are potent ligands for the aryl hydrocarbon receptor (AhR), similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a representative AhR activator; for this reason, coplanar PCBs are also termed dioxin-like PCBs. In contrast, PCBs with varying number of *ortho* chlorines are noncoplanar PCBs; they are ligands for the constitutive androstane receptor (CAR) and pregnane X receptor (PXR) (Al-Salman and Plant, 2012), without significant affinity for AhR, and they are also called non-dioxin-like PCBs.

Cytochrome P450 (CYP) enzymes catalyze the metabolism of a wide range of xenobiotics, which may result in either the detoxification or activation of xenobiotics. PCBs with varied configurations may be metabolized by different CYP enzymes. Some coplanar PCBs can be catalyzed by CYP1A enzymes into their hydroxylated metabolites (Safe, 1994; Shimada et al., 2003; Yamazaki et al., 2011; Grimm et al., 2015), while noncoplanar PCBs are substrates for CYP2B or CYP3A enzymes (Kania-Korwel et al., 2008; Grimm et al., 2015). Recently, we have reported that a series of lower chlorinated and noncoplanar PCBs are mutagenic (inducing micronuclei and gene mutations) in mammalian cells with human CYP2E1 being the major activating enzyme (Zhang et al., 2016; Liu et al., 2017). We have observed that 2,3- and 2,6-dicholobiphenyls (PCBs 5 and 10, respectively) are the most mutagenic dichlorobiphenyls in V79-hCYP2E1-hSULT1A1 cells (Zhang et al., 2016). Addition of an extra chlorine to the nonsubstituted ring of PCB 5 at the *para*- or *meta*-position leads to about ten-fold increase in the potency of mutagenicity, as demonstrated by the effects of 2,3,3′-tri- and 2,3,4′-trichlorobiphenyls (PCBs 20 and 22, respectively) in V79-hCYP2E1-hSULT1A1 cells (Liu et al., 2017). We were interested in the effect of a tetrachlorobiphenyl structurally relevant to PCBs 20 and 22, i.e., 2,3,3′,4′-tetrachlorobiphenyl (PCB 56), as compared with its chemical isomers (other tetrachlorobiphenyls) with more *ortho* chlorines, which are conferred with increasing noncoplanarity. We were also curious about the mutagenicity of trichlorobiphenyls with an additional chlorine on the nonsubstituted ring of PCB 10 at varying positions, i.e., 2,2′,6-, 2,3′,6-, and 2,4′,6-trichlorobiphenyls (PCBs 19, 27, and 32, respectively) in human CYP2E1-expressing cells, as compared with the single *ortho*-chlorine-containing PCBs 20 and 22, the most mutagenic PCBs activated by human CYP2E1 (Liu et al., 2017). Experimental results with these compounds might reveal an impact of the number of *ortho* chlorines on the mutagenicity of tri- and tetrachlorobiphenyls.

Two end points of mutagenicity, i.e., gene mutations at the *Hprt* locus and micronuclei formation in V79-hCYP2E1-hSULT1A1 and the parental V79-Mz cell line, were employed in this study to explore the mutagenicity of PCB compounds with differently positioned chlorines as described above. The *Hprt* mutagenicity assay detects a wide range of mutational events, including base pair substitutions, frameshift mutations, and large segment deletions (Stone-Wolff et al., 1985; Cariello and Skopek, 1993). The micronucleus test detects both breaks and loss of chromosomes, termed clastogenic and aneugenic effects, respectively. Yet, a simple micronucleus test cannot differentiate a clastogenic from aneugenic effect, unless it is combined with an immunofluorescence assay of centromere (kinetochore) in the formed micronuclei, or use of a centromeric DNA probe in the fluorescent in situ hybridization (FISH) with the micronuclei (Renzi et al., 1996). Traditionally, CREST antibodies (serum obtained from patients with CREST syndrome) were used for the detection of centromere-containing micronuclei (Earnshaw and Rothfield, 1985). Centromere proteins (CENP) include components as many as at least 16 members, of which CENP-A-specific antibodies have been used in the detection of centromere-containing micronuclei (Benameur et al., 2011). CENP-B (Bejarano and Valdivia, 1996), another CEBP component, is present in the serum of most (97.1%) patients with CREST syndrome (Song et al., 2013). Antibodies raised in animals against CENP-B have been commercially available; therefore, in this study the sources of test PCB-induced micronuclei were differentiated by immunofluorescent staining of CENP-B.

2. Materials and methods

2.1. Chemicals

Cell counting kit (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Ethyl methanesulfonate (EMS, purity of 98.5%) was purchased from J & K Chemical Ltd (Suzhou, China). 2,6-Dichlorobiphenyl (PCB 10), 2,2′,5-, 2,2′,6-, 2,3′,6-, and 2,4′,6-trichlorobiphenyls (PCBs 18, 19, 27, and 32, respectively) and 2,2′,3,3'-, 2,2′,3,6'-, 2,2′,6,6'-, and 2,3,3′,4′-tetrachlorobiphenyls (PCBs 40, 46, 54, and 56, respectively) were purchased from Accustandard Inc. (New Haven, CT, USA). Pentachlorophenol (PCP) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Dimethyl sulfoxide (DMSO), 6-thioguanine, and *N*-nitrosodimethylamine (NDMA) were all from Sigma-Aldrich (St. Louis, MO, USA). 6-Diamidino-2-phenyl-indole (DAPI) was from Yeasen Biotech Co., Ltd (Shanghai, China) and vincristine (VCR) from Selleck (Houston TX). Each test PCB was dissolved in DMSO before exposing cell cultures, with the concentration of DMSO being controlled at or lower than 0.8‰ (v:v). Each of EMS, NDMA, and VCR was dissolved in pure water before being used to expose cell cultures.

2.2. Cell lines

Both V79-Mz and V79-hCYP2E1-hSULT1A1 cell lines were generous gifts from Dr. Hansruedi Glatt (Nuthetal, Germany). The V79-Mz cell line, which was used as the control cells in this study,