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Peroxisome Proliferator-activated Receptors as Potential Targets for Carcinogenic Activity of Polychlorinated Biphenyls: A Computational Perspective

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Abstract. *Background: Polychlorinated biphenyls (PCBs) are ubiquitous environment-contaminating synthetic chemicals that have been associated with increased risk of hepatic cancer, melanoma, non-Hodgkin lymphoma and cancer of many other body organs. Structural binding analyses of PCB 77 and PCB 118 with peroxisome proliferator-activated receptors (PPARα, PPARβ/δ and PPARγ) was performed to predict the association of PCBs with potential disruption of PPAR signaling pathways. Materials and Methods: The crystal structures of human PPARα, PPARβ/δ and PPARγ were obtained from the Protein Data Bank. Structures of PCB 77 and PCB 118 were obtained from PubChem database. Docking was performed using glide (Schrodinger) induced fit docking (IFD) module. Results: The PCB 77 and PCB 118 interacted with PPARα, PPARβ/δ and PPARγ showing an overlapping of 40-58% interacting amino acid residues with synthetic co-complex agonists of the three PPARs. The binding affinity was higher for PCB 118 than for PCB 77 during docking interactions with each of the three PPARs. Conclusion: The consistent commonality of interacting residues for PCB 77 and PCB 118 with co-complex synthetic agonists of the PPARs together with good binding affinity suggested that the PPAR signaling pathway is a potential target for toxicologic activity of PCBs.*

Environmental contamination, resulting as a consequence of global chemical industry, has become a major concern for the human and animal health throughout the world. Among the hundreds of thousands of the man-made chemicals manufactured for commercial purpose in the world, about a thousand chemicals are suspected to predispose the human population to the abnormal reproductive function, increased incidence of cancer, neurodevelopmental problems and impaired immune function (1-3).

Polychlorinated biphenyls (PCBs) are a class of hazardous man-made aromatic chemicals that are ubiquitous environmental pollutants due to their persistence in the environment (4, 5). PCBs have a biphenyl structure with two linked benzene rings where the hydrogen atom is substituted by chlorine (6, 7). The degree and position of chlorination can result in 209 different molecules also called as congeners and affects the physical nature of PCBs, which can be either oily liquid or solid. Commercial PCB products generally contain a mixture of different PCB congeners (8). On account of the low water solubility, low flammability with low electrical conductivity and lipophilic properties, PCBs were widely used since 1930s as coolants and lubricants in capacitors, transformers, cooling liquids, hydraulic fluid, pesticides and copy paper (9). The estimated total global production of PCBs until 1977 was about 1.5 billion pounds (8, 10). The PCBs were banned in the USA in 1977 but about 2/3rds of PCB products, such as insulation fluids, plastics, adhesives, paper, inks, *etc*., manufactured before the ban, are still used today and are a source of exposure together with landfills and waste dumps that contain the disposed PCB products (11). The United States Environmental Protection Agency has identified PCBs in 500 of the 1,598 most serious hazardous waste sites listed in its national priority list and these sites were targeted for long-term federal cleanup activities (8).

Human exposure of PCBs results from ingesting PCB contaminated food, water or polluted air and can also occur during repair and maintenance of PCB transformers or due to accidents, fires and spills involving PCB transformers and older computers and instruments (12). PCBs are bioaccumulative and may remain stored in the body organs for years. A recent study estimated the half-lives of some of the PCB congeners in the ranges of 21 to

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133 years (13). Based on carcinogenicity evidence in humans and experimental animals, PCBs were classified as probable carcinogens (group 1) by the International Agency for Research on Cancer (IARC) in 2013 (14). The PCBs-induced carcinogenesis is not due to genotoxicity but is a result of several non-genotoxic mechanisms, such as cancer promotion, peroxisome proliferation, hormone imbalance and cytotoxicity resulting in progressive cell division (15, 16).

Epidemiological and animal experimental studies have consistently shown association of PCBs with carcinogenicity (8, 17). An increase in the incidence of liver, gallbladder and biliary tract cancer was found on retrospective analyses in workers at two capacitor manufacturing plants in the United States (18). Several other studies (19–22) reported that exposure to insulating fluids containing PCBs was associated with an increase in incidence of malignant melanoma and brain cancer. Further, an increased risk for cancers of liver, stomach, intestines and thyroid was found in association of PCBs during mortality studies in workers at a capacitor manufacturing plant from 1944-1977 (23). Even without known occupational exposure, higher risk of non-Hodgkin lymphoma was found in patients with increased PCB levels in the adipose tissue and serum (24-26). In a recent study on a large cohort of 24,865 workers (27), PCB exposure was related with total cancer and intestinal cancer mortality of females and myeloma in males. A number of experimental studies in laboratory animals have also shown that all PCB mixtures caused gastrointestinal tract tumors, hepatocarcinomas, leukemia, lymphomas and pituitary tumors (8, 28, 29). There is considerable variability in toxicity of PCB congeners and, in general, coplanar PCBs are considered more toxic PCBs. The congeners 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118) are two examples of coplanar PCBs. PCB 77 is one of the three most toxic PCBs known. PCB 118 is one of the most frequently and consistently detected congener in the United States biomonitoring studies (30) and one of the two PCBs that share a toxicity equivalent of 25% for United States population (31).

Environmental contaminants are commonly thought to induce the adverse effects in human systems by disrupting nuclear receptor signaling pathways (1). One such signaling pathway involves peroxisome proliferator-activated receptors (PPARs) that belong to the ligand-activated nuclear receptor superfamilies and include the steroid, thyroid and retinoid hormone receptors (32, 33). There are three different PPAR isoforms, PPAR-α, PPAR-β/δ and PPAR-γ, with significant homology playing specific functional roles in the human body (34, 35). The PPARα plays a major role in regulating fatty acid homeostasis (36, 37) and regulates the peroxisomal and mitochondrial β-oxidation pathways, which are involved in the pathogenesis of various liver complications, such as hepatocarcinogenesis in a rodent model and drug-induced liver injury (38). The PPARβ/δ is associated with an increase in lipid catabolism in adipose tissue, skeletal muscle and the heart, as well as induction of cell proliferation and differentiation (39, 40). The PPARγ plays a major role in glucose metabolism and in differentiation of adipocytes and also functions as a transcriptional regulator in pathways that are required for these metabolic processes (37, 41, 42). Following binding with their corresponding ligands, PPARs undergo conformational changes that displace the co-repressors and recruit certain co-activator complexes that regulate several normal physiological processes and, thus, disruption of PPARs pathways contributes to disease progression in obesity, diabetes and cancers (34, 43).

In the present study, the potential molecular mechanisms for PCBs-mediated cancer formation were elucidated by investigating the structural binding characteristics of PCBs with PPARs using *in silico* predictive approaches. Docking studies involved the study of binding mechanisms, distinctive binding pattern and interacting residues of PPARα, PPARβ/δ and PPARγ with PCB 77 and PCB 118. It was expected that the approach via computational systems would help in predicting potential carcinogenic risks of the PCBs, which are ubiquitously contaminating the environment.

Materials and Methods

Data retrieval. The molecular structures of PCB 77 and PCB 118 were obtained from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/). The two dimensional structures of the PCB ligands are illustrated in Figure 1 and their abbreviations and PubChem compound identities (CIDs) are presented in Table I. Schrodinger 2015 suite with Maestro 10.3 (a graphical user interface) software (Schrodinger, LLC, New York, NY, USA) was used for docking studies of PCB 77 and PCB 118 with PPARα, PPARβ/δ and PPARγ (44).

Protein and ligand preparation. The Protein Data Bank ((PDB) http://www.rcsb.org/) was scanned for the crystal structure of human PPARα (PDB code: 3VI8), PPARβ/δ (PDB code: 3TKM) and PPARγ (PDB code: 3NOA). The crystal structures of the three PPARs were co-complexes with synthetic agonist ligands CHEMBL1956149, GW0742 and 5BC, respectively (see Table I for full IUPAC names), and the crystal structure of PPARα is shown in Figure 1 as an example. The details for the preparation of the receptors and the ligands for docking analysis using Schrodinger Glide (Schrodinger suite 2015-3; Schrodinger, LLC) were described previously (44).

Induced fit docking. Schrodinger's induced fit docking (IFD) module was used for docking analyses of the PCB 77 and PCB 118 with PPARα, PPARβ/δ and PPARγ as already described (44, 45). A softened-potential docking is performed in the first IFD

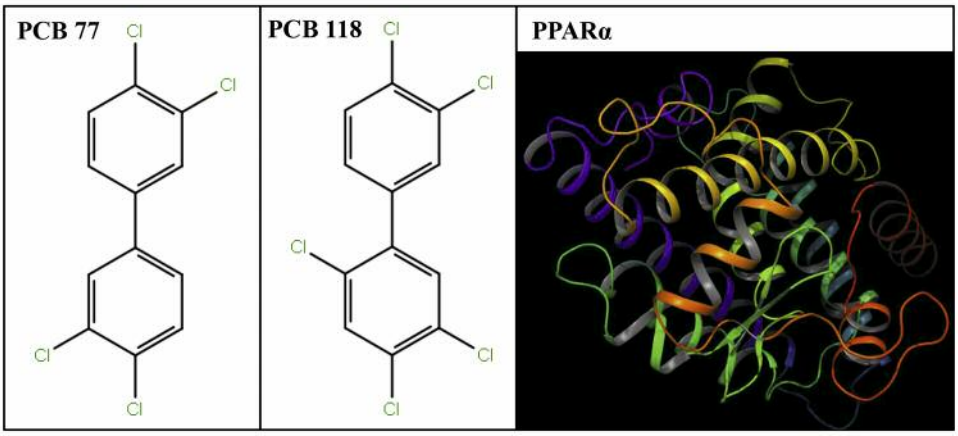

Figure 1. *Two-dimensional representation of the two PCBs, 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118), and ribbon form representation of crystal structure of peroxisome proliferator-activated receptor α (PPARα).*

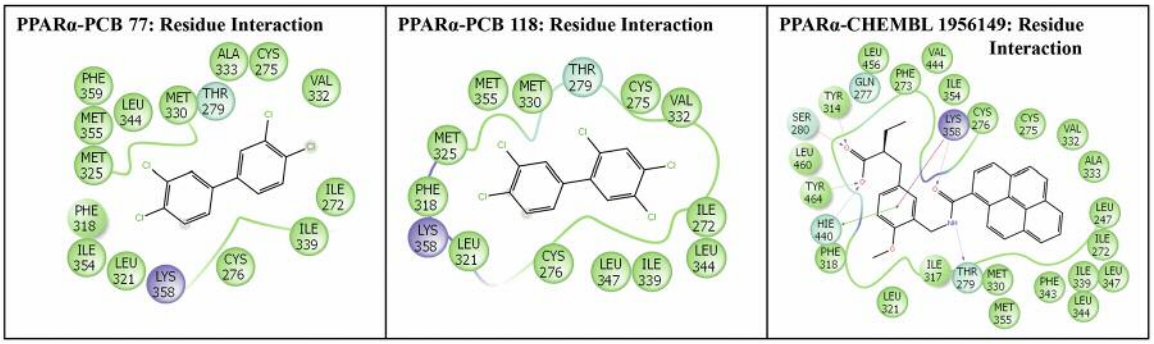

Figure 2. *Amino-acid residue interaction display in the binding pocket of peroxisome proliferator-activated receptor α (PPARα) for interactions with 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3',4,4',5-pentachlorobiphenyl (PCB 118) and PPARα co-complex synthetic agonist, CHEMBL19561.*

Table I. *Nomenclature, commonly used abbreviations and PubChem IDs of the two PCBs and the three co-complex bound ligands of peroxisome proliferator-activated receptors (PPARα, PPARβ/δ and PPARγ).*

S.No.	Name	Abbreviation	PubChem ID
1	3,3',4,4'-Tetrachlorobiphenyl	PCB 77	36187
2	2,3',4,4',5-Pentachlorobiphenyl	PCB 118	35823
3	(2S)-2-[[4-methoxy-3-[(pyrene-1-carbonylamino)methyl]phenyl]methyl]butanoic acid (co-complex ligand for PPARα)	CHEMBL1956149	25112371
4	2-[4-[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-1,3-thiazol-5-yl]methylsulfanyl]-2-methylphenoxy]acetic acid (co-complex ligand for PPARβ/δ) 9934458	GW0742	
5	2-[5-[3-[4-(4-phenylbenzoyl)-2-propylphenoxy]propoxy]indol-1-yl]acetic acid (co-complex ligand for PPARγ)	5BC	49850232

stage where docking of the ligand occurs into an ensemble of the binding protein conformations. Subsequently, complex minimization for highest ranked pose is performed where ligand, as well as the binding sites, are free to move. Prime module of Schrodinger 2015 with molecular mechanics generalized Born-surface area (MMGB-SA) function was used for the ligand binding affinity calculations against the PPARα, PPARβ/δ and PPARγ crystal complexes.

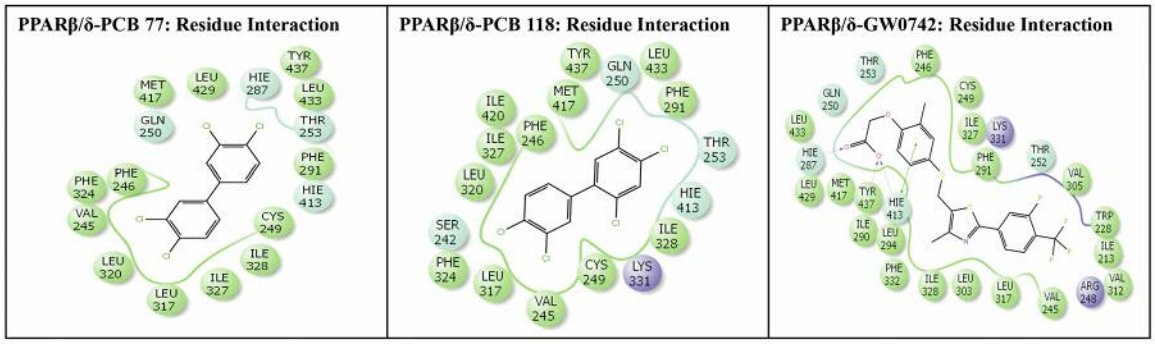

Figure 3. *Amino-acid residue interaction display in the binding pocket of peroxisome proliferator-activated receptor β/δ (PPARβ/δ) for interactions with 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3',4,4',5-pentachlorobiphenyl (PCB 118) and PPARβ/δ co-complex synthetic agonist, GW0742.*

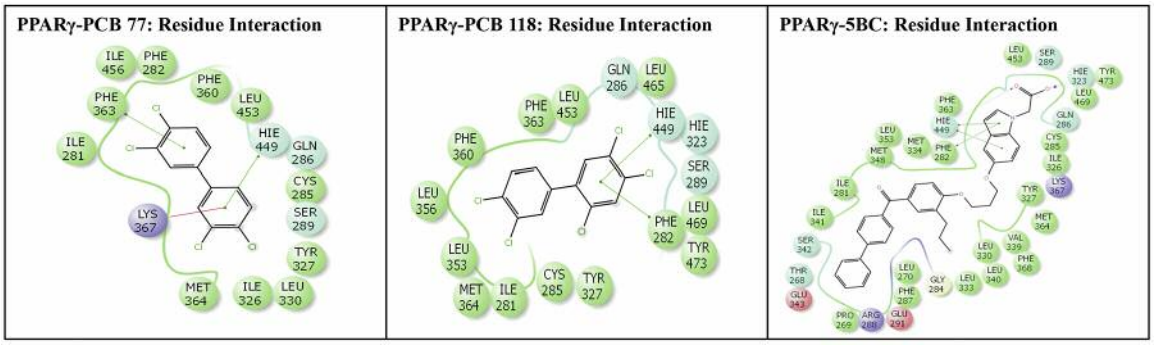

Figure 4. *Amino-acid residue interaction display in the binding pocket of peroxisome proliferator-activated receptor γ (PPARγ) for interactions with 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3',4,4',5-pentachlorobiphenyl (PCB 118) and PPARγ co-complex synthetic agonist, 5BC.*

Results

Docking simulation of PCB 77 and PCB 118 was successfully executed by IFD against PPARα, PPARβ/δ and PPARγ resulting in multiple docking poses for each ligand-receptor interaction. The best pose for each of the ligands with each of the three receptors was identified and used for computational analyses of ligand-receptor structural binding characteristics. Similarly, the best docking pose for bound co-complex ligands of each of the receptors were utilized for analyses.

Molecular docking studies of PCB 77 and PCB 118 with PPARα. The docking complexes of the two PCB ligands, PCB 77 and PCB 118, and co-complex ligand, CHEMBL 1956149, with PPARα showed interactions with 16, 14 and 27 amino-acid residues, respectively (Figure 2; Table II). Of these interacting residues, 14 residues for PCB 77 and 13 residues for PCB 118 overlapped with interacting residues for co-complex bound ligand CHEMBL 1956149 (commonality of 52% and 48%; Table II). Twelve residues of PPARα (Ile-272, Cys-275, Cys-276, Thr-279, Phe-318, Leu-321, Met-330, Val-332, Ile-339, Leu-344, Met-355 and Lys-358) were common among both the PCBs and the bound ligand. Further, PPARα interacting residues Ala-333 and Ile-354 were also common between PCB 77 and bound ligand but not for PCB 118. Conversely, residue Leu-347 was common for PCB 118 and the bound ligand but not for PCB 77. The bound ligand, CHEMBL 1956149, formed 6 hydrogen bonding interactions with interacting residues Thr-279, Ser-280, Tyr-314, Lys-358 and Tyr-464 of PPARα. Additionally, 1 pi-pi interaction displayed by His-440 and 1 cation-pi interaction is also observed between Lys-358 and CHEMBL. However, no hydrogen bonding interactions were present for both PCB docking complexes. The dock score, glide score and binding affinity were highest in the bound co-complex ligand followed by PCB 118 and PCB 77 (Table II).

Molecular docking studies of PCB 77 and PCB 118 with PPARβ/δ. The docking displays of the two PCB ligands,

Table II. *Number of interacting residues, number and percentage of residues common with co-complex bound ligands, induced fit docking (IFD) score, dock score, glide score and binding affinity values (molecular mechanics generalized Born-surface area (MMGB-SA) values) of 3,3',4,4'-tetrachlorobiphenyl (PCB 77) and 2,3',4,4',5-pentachlorobiphenyl (PCB 118) after induced fit docking (IFD) with human peroxisome proliferator-activated receptors (PPARα, PPARβ/δ and PPARγ).*

Target	Ligand	Number of interacting residues	Number of interacting residues common with native (%)	IFD score	Docking score (Kcal/mol)	Glide score (Kcal/mol)	MMGB-SA (Kcal/mol)
PPARα	PCB 77	16	14 (52%)	–594.81	–7.97	–7.97	–87.09
	PCB 118	14	13 (48%)	–595.63	–8.43	–8.43	–104.07
	Native	27	27 (100%)	–601.86	–14.21	–14.21	–130.11
PPARβ/δ	PCB 77	17	15 (58%)	–602.77	–7.81	–7.81	–84.61
	PCB 118	18	14 (54%)	–603.79	–8.43	–8.43	–96.94
	Native	26	26 (100%)	–610.49	–12.93	–12.93	–115.89
PPARγ	PCB 77	15	13 (39%)	–601.87	–8.36	–8.36	–92.85
	PCB 118	17	14 (42%)	–602.91	–8.78	–8.78	–107.74
	Native	33	33 (100%)	–612.12	–13.98	–13.98	–134.55

PCB 77 and PCB 118, and co-complex ligand, GW0742, with PPARβ/δ showed interactions with 17, 18 and 26 amino-acid residues, respectively (Figure 3; Table II). Of these interacting residues, 15 residues for PCB 77 and 14 residues for PCB 118 overlapped with interacting residues for co-complex bound ligand GW0742 (commonality of 58% and 54%; Table II). Thirteen residues of PPARβ/δ (Val-245, Phe-246, Cys-249, Gln-250, Thr-253, Phe-291, Leu-317, Ile-327, Ile-328, His-413, Met-417, Leu-433 and Tyr-437) were common among both the PCBs and the bound ligand. Further, PPARβ/δ interacting residues His-287 and Leu-429 were also common between PCB 77 and bound ligand but not for PCB 118. Conversely, residue Lys-331 was common for PCB 118 and the bound ligand but not for PCB 77. In addition, residues Leu-320 and Phe-324 were common for both PCBs but not for the bound ligand. The bound ligand, GW0742, formed 3 hydrogen bonding interactions with interacting residues His-287, His-413 and Tyr-437 of PPARβ/δ. In addition, 1 pi-pi interaction is also observed between GW0742 and His-413 but no hydrogen bonding interactions were present for both PCB docking complexes (Figure 3). The dock score, glide score and binding affinity were highest for the bound co-complex ligand, GW0742, followed by PCB 118 and PCB 77 (Table II).

Molecular docking studies of PCB 77 and PCB 118 with PPARγ. The docking displays of the two PCB ligands, PCB 77 and PCB 118, and co-complex ligand, 5BC, with PPARγ showed interactions with 15, 17 and 33 amino-acid residues, respectively (Figure 4; Table II). Of these interacting residues, 13 residues for PCB 77 and 14 residues for PCB 118 overlapped with interacting residues for co-complex bound ligand 5BC (commonality of 39% and 42%; Table II). Ten residues of PPARγ (Ile-281, Phe-282, Cys-285, Gln-286, Ser-289, Tyr-327, Phe-363, Met-364, His-449 and Leu-453) were common among both the PCBs and the bound ligand. Further, PPARγ interacting residues Ile-326, Leu-330 and Lys-367 were also common between PCB 77 and bound ligand but not for PCB 118. Conversely, residues, His-323, Leu-353, Leu-469 and Tyr-473 were common for PCB 118 and the bound ligand but not for PCB 77. In addition, PCB 77 formed 2 pi-pi interactions with residues Phe-363, His-449 and 1 cation-pi interaction with Lys-367 of PPARγ. PCB 118 formed 2 pi-pi interactions with residues Phe-282 and His-449 of PPARγ. The bound ligand, 5BC, formed 2 hydrogen bonding interactions with interacting residues His-323 and His-449 and 3 pi-pi interactions with residues Phe-282 and His-449 of PPARγ. However, no hydrogen bonding interactions were present for PCB 118 and PCB 77 docking complex (Figure 4). The dock score, glide score and binding affinity were highest for the bound co-complex ligand, 5BC, followed by PCB 118 and PCB 77 (Table II).

Discussion

The present computational study was undertaken to clarify the molecular interactions of PCB 77 and PCB 118 with PPARα, PPARβ/δ and PPARγ in order to predict the association of known carcinogenic activity of PCBs with potential disruption of PPAR signaling pathways. PCB 77 and PCB 118 are two dioxin-like congeners of coplanar PCBs present in the mixtures of commercially available synthetic PCBs that are used as coolants and lubricants in capacitors, transformers and other electrical equipment because of their good insulating and non-inflammable properties (9). Based on epidemiological evidence in humans and experimental results in animals, IARC classified PCBs as probable carcinogens (group 1) in 2013 (14). Despite the

ban on PCBs since 1977 and extensive and strict regulations, old PCB-containing products continue to be used commercially and, together with earlier persisting environmental contamination, remain a focus of constant attention and cause for concern. Recent biomonitoring data show that PCBs continue to be detected in the body fluids of the general population in the United States (30).

Structural binding analyses of the docking complexes of PCB 77 and PCB 118 with each of the PPARα, PPARβ/δ and PPARγ revealed that a number of important amino acid residues of each receptor were involved in interactions with both PCBs. Good dock scores and high binding affinity indicated that the docking complexes were in their favorable conformation resulting in good quality docking. The interacting amino acid residues exerted hydrophobic and hydrophilic interactions contributing to the stability of the docking complex. During docking of both the PCBs, PCB 77 and PCB 118, with each of the three PPARs there was an overlapping of 39-58% amino acid residues with interacting residues of highly specific synthetic co-complex ligands for each receptor indicating similarity in the interaction pattern of the PCBs with native co-complex ligand at the common ligand binding domain of each receptor. This consistent commonality of interacting residues between the two PCBs and the co-complex ligand for each PPAR suggested that the PCBs could potentially interfere in the normal receptor function of the PPARs and, thus, cause signaling dysfunction. The PCB 118 interacted with a higher binding affinity with the PPARs than PCB 77 indicating tighter interactions. Also, among the PPARα, PPARβ/δ and PPARγ, docking simulation was stronger for both PCBs with PPARα, PPARγ than with PPARβ/δ on the basis of dock score, glide score and binding affinity. Thus, on a preliminary basis, PCB 118 seemed to be a more potent PCB than the PCB 77 and the toxic effects of these congers could preferentially involve PPARα and PPARγ pathways.

Apparently, *in silico* studies of PCBs, including PCB 77 and PCB 118 with PPARs, have not been reported. However, studies on docking simulations of several coplanar PCBs with other nuclear receptors, such as aryl hydrocarbon receptor, are available (46, 47). *In vitro* competitive binding studies of PCB 77 and PCB 118 with PPARs are also not apparently available. In a recent study (48), a luciferase reporter assay did not show activation of PPARα and PPARγ; however, the tests were performed with Aroclor 1260, which is mixture of PCB congeners. At higher concentrations, Aroclor 1260 had an inhibitory effect on PPARα but no effect on PPARγ.

Several experimental studies have shown the toxic effects of PCBs, including PCB 77 and PCB 118, in animals and tissue culture (17, 29, 49). Although not related to the binding of PCB 77 and PCB 118 to the PPARS, some of these studies suggested, indirectly, an interaction of the two PCBs with PPARs. PCB 77 increased cellular damage and stimulated the activation of oxidative stress-sensitive and proinflammatory transcription factors (50). The inflammatory processes, thus, activated are critical for atherosclerogenic pathology in vascular endothelial cells. However, the PCB 77-induced inflammation was reduced by prior treatment of vascular endothelial cells with PPARα agonists (51). PCB 77 also decreased the *PPARα* and *PPARγ* mRNA and PPARα and PPARγ protein expression in a dose-dependent manner suggesting the relationship of PCB toxicity, in part, to inhibition of PPARs' function. Further, PCB 77-induced inhibition of PPARS also led to dysregulation of fatty acid uptake and metabolism, which may accelerate the adverse effects associated with inflammatory disease. Another study (52) also reported that coplanar PCB 126, which is structurally similar to PCB 77, acted as an inverse agonist and reduced peroxisomal activity in rats. In contrast to these studies, expression of PPARγ was shown to be increased by PCB 77 in another study (53). PCB 118 was also shown to perturb the steroidogenic activity and protein expression *in vitro* in human adrenocortical carcinoma cell cultures suggesting adverse effects on normal cellular processes, *e.g.*, protein synthesis, stress response and apoptosis (54). In another study (55), subchronic dietary exposure of PCB 77 and PCB 118 in rats induced moderate changes in the liver and thyroid and increased liver weight and hepatic ethoxyresorufin O-deethylase activity. In an exhaustive two-year study in rats (56), PCB 118 was shown to induce dose-dependent hepatic toxicity characterized by increased incidences of hepatocyte hypertrophy, inflammation, oval cell hyperplasia, pigmentation, eosinophilic and mixed cell foci, bile duct hyperplasia and neoplasms of liver, lung, pancreas and uterus.

In conclusion, the docking simulation study of PCB 77 and PCB 118 with PPARα, PPARβ/δ and PPARγ indicated that both PCBS were involved in interactions with important amino acid residues of each of the three PPARs. There was about 39-58% commonality of the interacting residues between the two PCBs and the co-complex synthetic agonists of each of three PPARs. PCB 118 formed tighter interactions with the PPARs than PCB 77. The interactions of both PCBs with PPARα and PPARγ were stronger than with PPARβ/δ. This study suggested that, on a preliminary basis, PPARα, PPARβ/δ and PPARγ are potential targets for the toxic carcinogenic effects of PCBs in the human body.

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Conflicts of Interest

The Authors have declared that no competing interests exist.

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