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Endocrine-disrupting mechanisms of polychlorinated biphenyls

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Abstract

The extent of human exposure to chemicals, their endocrine-disrupting mechanisms of action, and the relationship between exposure and various human diseases raise significant scientific and public health concerns. Polychlorinated biphenyls (PCBs) belong to the group of organic compounds known as persistent organic pollutants, characterized by long-range transport, persistence, bioaccumulation, and high toxicity. They have been identified as endocrine-disrupting chemicals. *In vivo* and *in vitro* studies have shown that endocrine-disrupting effects of PCBs mainly involve thyroid and reproductive function. In the review presented, these effects were placed in the context of the most recent findings on PCB-induced endocrine-disrupting mechanisms and modes of action.

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Keywords

Polychlorinated biphenyls, Thyroid disruption, Reproductive endocrine disruption, Mechanisms and modes of actions.

Abbreviations

PCBs, Polychlorinated biphenyls; EDCs, endocrine-disrupting chemicals; NMDR, nonmonotonic dose–response; POPs, persistent organic pollutants; MoAs, mode of actions; TH, thyroid hormone; TSH, thyroid-stimulating hormone; HPT, hypothalamic–pituitary–thyroid; OH-PCBs, hydroxylated PCBs; TR, TH receptors; TTR, transthyretin receptor; T4, thyroxine; TBG, thyroxin-binding globulin; UDPGTs, glucuronyl transferases; AhR, aryl hydrocarbon receptor; RyRs, ryanodine-sensitive Ca^{2+} release channels; Dio, deiodinase; T3, triiodothyronine; rT3, reverse T3; IL-6, interleukin-6; TNF-α, tumor necrosis factor α; ICAM-1, intercellular adhesion molecule-1; NIS, natrium-iodide symporter; HPA, hypothalamic–pituitary–adrenal axis.

Introduction

Endocrine-disrupting chemicals (EDCs) are defined as 'an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations' [1]. WHO/IPCS 2002 definition of EDCs claims the existence of reasonable evidence of a biologically plausible causal relationship between the endocrine activity and the induced adverse effect(s) seen in an intact organism, or a (sub)population is a prerogative for identification of the substance as EDC. This definition of EDCs was later endorsed by the Scientific Committee of the European Food Safety Authority [2]. It was concluded that the three criteria define the presence of (i) an adverse effect in an intact organism or a (sub)population; (ii) an endocrine activity; and (iii) a plausible causal relationship between the two. The list of the substances characterized as EDCs is continually increasing, and although substantial scientific progress has been made in the field, public concern remains high. There are two main reasons for this concern, first is the extent of the consumer exposure to EDCs and the second one is that the evidence linking EDC exposure and various human diseases has become stronger in the past decades [3–10]. Furthermore, an increasing body of evidence shows that EDCs can produce additive or even synergistic mixture or 'cocktail' effects suggesting that adverse effects on endocrine system can occur even at doses that *per se* have not been observed [11–14]. Another point that needs to be addressed in EDC toxicity is that the applicability of the 'safe threshold' concept used in assessing their safety has been a significant source of controversy [8,15]. The understanding of the basic endocrine principles by which EDCs act including nonmonotonic dose–response (NMDR), low-dose effects, and developmental vulnerability is essential for translating findings on EDCs to human health [16].

Among the chemicals, known to be EDCs are the chemicals known as persistent organic pollutants (POPs), organic compounds characterized by long-range transport, persistence, bioaccumulation, and high toxicity. The results of the Stockholm Convention on POPs in 2001 give a list of the top 12 chemicals for regulating, nicknamed the 'dirty dozen' [17]. Among

these chemicals, polychlorinated biphenyls (PCBs) have been identified as EDCs. The aim of this review is to give a concise view of state-of-the-art knowledge in this field regarding the potential of PCBs to exert this effect. We will accomplish this by focusing on the identification of mechanisms of endocrine disruption, and modes of these actions (MoAs) are key means by which toxicity test data are translated to evidence-based human health risk assessment.

PCBs as endocrine disruptors

Exposure to PCBs is related to several disorders in humans, predominantly developmental toxicity [18,19], immunotoxicity [20], metabolic diseases such as type II diabetes [21], thyroid disorders [22], and disruption of female and male reproductive health [23–25]. All these effects might be linked with their interactions with the endocrine system. Generally, from the mechanistic point of view, depending on chemical structure PCBs may exert either dioxin-like effects (coplanar PCBs) mediated through interaction with aryl hydrocarbon receptor (AhR) or nondioxin-like effects shown with other groups of PCB congeners. PCB-induced effects that may involve both Ah-receptor-dependent and -independent mechanisms include liver hypertrophy, neurodevelopmental effects or reproduction effects involving changes in steroid hormone homeostasis and/or thyroid hormone disruption, immunological effects, and cancer through nongenotoxic mechanisms.

Animal and *in vitro* studies have shown that endocrine-disrupting effects of PCBs mainly involve thyroid and reproductive function, and all mentioned disorders that are related to PCB exposure can be explained at least partly by PCB-induced disruption of these endocrine functions. We will discuss the thyroid and reproductive disrupting effects of PCBs in the context of their mechanisms and modes of actions.

Thyroid disruption

The results of the studies examining the relationship between PCBs body burden and thyroid hormone levels in humans are controversial. As reviewed by Zoeller [26], PCB levels were shown to be connected with either elevated or decreased thyroid hormone (TH) levels, with no effect on the levels of thyroid-stimulating hormone (TSH), or the thyroid gland itself. There is a vast number of biological sources of variation to affect the relationship between PCB and TH levels. Namely, the relationship between prenatal exposure to PCBs and neonatal TH levels was shown to be affected by the birth delivery method [27].

On the other hand, animal studies are rather compliant and nearly uniformly confirm the inverse association between PCB levels and TH circulating levels. The thyroid function is regulated by sensitive feedback mechanisms of circulating thyroid hormones at the hypothalamic and pituitary levels [28]. The appropriate response of the feedback loop would result in elevated TSH levels which might result in compensatory hyperplasia of thyroid tissue and histopathological changes as observed in some animal studies [29,30].

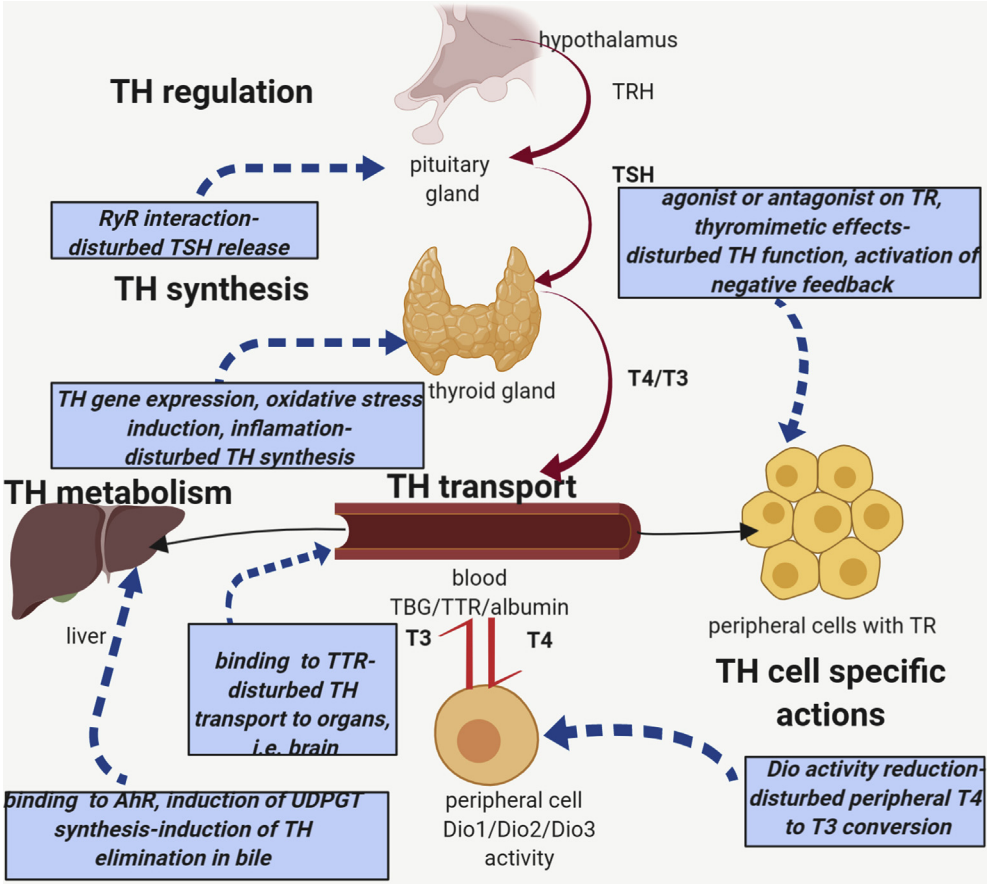
Despite the evident advances in analytical methods to study these chemicals in biological tissue, many difficulties remain in identifying reliable markers of the effects of PCBs on thyroid function, precise mode of actions (MOAs), and/or mechanisms of action. However, some of them are well known and studied by human, animal, *in vitro* and computational studies.

PCB congeners are metabolized into hydroxylated metabolites (OH-PCBs). The OH-PCBs are similar in structure to TH, resulting in interaction with TH receptors (TR) leading to disruption of normal thyroid homeostasis [31]. PCBs might disturb TRs acting like agonist or antagonist [26]. In a recent study utilizing the combination of experimental data and computational modeling, it was revealed that higher chlorinated PCBs tend to have TR-agonistic activities while lower ones were agonist [32]. Both *in vitro* and *in vivo* animal studies showed that PCBs and OH-PCBs are able to competitively bind to thyroxine (T4) carrier, transthyretin receptor (TTR) [33,34]. Although in humans, most T4 transport happens through thyroxin-binding globulin (TBG), binding of T4 to TTR might be important for blood–brain barrier transport, altering brain levels of TH in humans. Another possible MOA of PCBs' thyroid-disrupting effects is altered gene transcription as shown in a study which correlated the circulating levels of PCBs, OH-PCBs, and TSH with the mRNA levels of seven TH-regulated genes in the serum of e-waste recycling workers [35]. The study revealed that TH-regulated gene expression was significantly associated with certain PCBs and OH-PCBs. Another suggested mechanism is the induction of hepatic uridine diphosphate glucuronyl transferases (UDPGTs) by the AhR activation by coplanar PCB 126 congener [36] as observed in the rodent study. On the other hand, nonplanar, that is, *ortho*-substituted PCB congeners produce AhR-independent effects and produce their effects through CYP enzyme induction. In a study examining acute ED effects of two different PCB congeners in weanling female Sprague–Dawley rats exposure to *ortho*-PCB congeners caused a reduction in T4 levels without T3 nor TSH levels being changed suggesting possible effects of *ortho*-congeners on feedback mechanisms of hypothalamic–pituitary–thyroid (HPT) axis [37]. The mechanism of this interception would include their resemblance to TH in the amount that they interact with the TR and cause thyromimetic effects preventing the body from perceiving the state of thyroxinemia. Interference with Ca^{2+} homeostasis is also of importance, having in mind

that intracellular Ca^{2+} regulates hormone secretion, among many other functions. *Ortho*-substituted PCBs were found to have direct activity towards the ryanodine-sensitive Ca^{2+} release channels (RyRs) in the skeletal and muscles [38] suggesting the potential role of PCBs and RyRs interactions in disturbed TSH release from the pituitary gland.

The results of a Dutch cohort of 100 mother–infant pairs, exposed to background PCB levels, suggested that PCBs have a negative effect on deiodinase (Dio) type 3 activity, as reflected by a positive correlation with the triiodothyronine (T3)/reverse T3 (rT3) ratio [39]. Another important mechanism is certainly oxidative stress induction [40]; however, little data exist on the role of this mechanism in the EDC activity of these chemicals. A recent study in rats suggested that NADPH oxidase inhibition attenuates PCB-induced thyroid dysfunction, presumably due to its role in reactive oxygen species (ROS) generation prevention and inhibition of NF-κB pathways [41]. The effects of PCB 126 in pregnant albino rats revealed the potential for maternofetal hypothyroid effect, shown by negative effects on fetal pituitary–thyroid axis and cytokine levels which may contribute to thyroid hypofunctioning [42]. Other studies buttressed the role of inflammation in PCB-induced thyroid disruption, showing an increased generation of interleukin-6 (IL-6), tumor necrosis factor α (TNF-α) and intercellular adhesion molecule-1 as a consequence of PCB 118 congener which resulted in inhibition of natrium-iodide symporter (NIS) [43]. The effects of PCBs on NIS were also investigated in *in vitro* studies dealing with molecular mechanisms by which PCBs induce thyroid disruption, revealing a decrease in NIS mRNA expression levels [44,45]. The potential mechanisms and modes of actions of PCBs' effects on HPT axis regulation are illustrated in Figure 1.

Figure 1



Some of the suggested mechanisms and modes of PCBs action on HPT axis regulation. The HPT axis can be disturbed at several points of regulation including hypothalamus and pituitary gland control of TH synthesis, TH synthesis in thyroid gland, TH transport through blood on proteins (TBG, TTR, and albumin), selective uptake into tissues and cells, and metabolism at the site of action and in liver through enzymes glucuronidases or sulfotransferases. The circulated blue dotted arrows indicate the potential organs/tissues targeted by PCBs. Square blue shape annotates possible PCBs mechanisms and MOAs with resulting effects. PCB, polychlorinated biphenyls; MOAs, mode of actions; TH, thyroid hormone; TSH, thyroid-stimulating hormone; HPT, hypothalamic–pituitary–thyroid axis; TR, TH receptors; TTR, transthyretin receptor; T4, thyroxine; TBG, thyroxin-binding globulin; UDPGTs, glucuronyl transferases; AhR, aryl hydrocarbon receptor; RyRs, ryanodine-sensitive Ca^{2+} release channels; Dio, deiodinase; T3, triiodothyronine. Created with BioRender.com.

Questions regarding the level of protection that the current toxicological threshold for PCBs provides were addressed in a study in ovariectomized rats [46]. The study showed that PCBs have a strong potential to cause thyroid disruption as shown by a low reference point using the Benchmark dose method with tT4 levels as the most sensitive endpoint while tT3 serum levels proved to be a less sensitive endpoint. The presence of the gap between T3 and T4 levels was also noted on the male rat model [13,29] suggesting that PCBs affect mainly T4 production in the thyroid gland and its metabolism in the liver. It should be noted that the EDCs interact with hormone action a manner that is quite specific and that traditional toxicological endpoints are not sufficient enough to predict adverse outcomes [8]. These chemicals produce nonlinear dose responses both *in vitro* and *in vivo*, including NMDR, as well as suggesting that for chemicals such as PCBs no threshold can be assumed. A recent paper that reviewed epidemiological studies of prenatal exposure to mixtures of EDCs concluded that many studies make simplified assumptions on EDCs' additivity, linearity, relative potency, etc. [47].

Table 1 Molecular mechanisms/modes of actions of PCB-induced reproductive endocrine disruption identified by the most recent studies (sorted by publication year).

PCB mixture/ congener	Type of study	Cell culture/species	Exposure regime	Mechanism/mode of action	Reference
Aroclor 1242	*in vitro*	Mouse Leydig cell line TM3	Five concentrations ranging from 10 to 16 to 10-6 M; 24 h	↓ Cell viability at 10-6 M and 10-8 M ↑ Lipid peroxidation and reactive oxygen species relative to the concentration ↓ Antioxidant systems relative to the concentration ↓ 3-Hydroxysteroid dehydrogenase [HSD] and 17-HSD relative to the concentration	[44]
Delor 103/106/103+106	*in vitro*	Mouse Leydig cell line MA-10	0.2 and 2 ng/mL; 24 h	↑ Estrogen-related receptors expression (dependent by dose- and type regime) ↑ Calcium (Ca^{2+}) concentration (independently of dose and type regime) ↑ Sex steroid secretion, both androgens and estrogens (independently of dose and type regime) Altered mitochondria ultrastructure without membrane potential affected (independently of dose and type regime)	[45]
Aroclor 1254	*in vivo*	Male rats (F1 offspring)	Lactationally exposed (dams were orally treated with 1, 2 and 5 mg/kg bw/day from postpartum day 1–20)	Altered testicular architecture ↓ Testosterone, estradiol and androgen-binding protein levels in serum and testicular interstitial fluid (dose-dependently) ↓ Gene expression level of follicle-stimulating hormone receptor, androgen-binding protein, estrogen receptor β and androgen receptor (dose-dependently) ↑ Inhibin β gene expression level	[46]
(±)-PCB 136	*in vitro*	Human breast carcinoma MCF-7 cells; human prostate carcinoma LNCaP; human hepatic HepaRG® cells	Six concentrations ranging from 0.01 to 25 μ M; 24 h	Estrogenic activity by (+)-PCB 136 and antiestrogenic activity by (–)-PCB 136 Antiandrogenic activity by both PCB 136 stereoisomers ↑ Constitutive androgen receptor (CAR)-dependent gene expression by both PCB 136 stereoisomers ↑ Pregnane X receptor (PXR)-dependent gene expression with (–)-PCB 136 being more potent	[47]
Aroclor 1254	*in vitro*	Sheep embryonic fibroblasts (SEF) and amniocytes (SA)	1 μg/mL; 120 days	↓ Proliferation ↑ Permanent DNA hypermethylation in SEF ↑ Sister chromatid exchange rate ↑ Histone γ-H2A.X phosphorylation of fibroblasts ↑ Chromosomal abnormalities in SEF	[51]

Another concern regarding thyroid-disrupting effects of PCBs is that recent studies examining mixtures of PCBs with other EDCs suggested synergism between chemicals on thyroid function implicating potential health effects of low-dose EDC mixtures present in the environment [13,48,49]. This is of great importance having in mind the fact that exposure to low-dose mixtures of EDCs characterizes the real-life human scenarios [50].

Reproductive endocrine disruption

Numerous human and animal studies link exposure to PCBs to a variety of toxic reproductive outcomes in both males and females. However, relevant underlying mechanistic data are still needed to support the observed effects. PCBs have been shown to exert estrogenic and antiestrogenic activity, impact on various receptor levels, and modulate steroidogenesis. The most recent studies have focused on the detrimental effects of PCBs on Leydig cell homeostasis *in vitro*. These studies were aimed to expand molecular targets considering the dose dependence of the effects and to contribute to the weight of evidence for already identified molecular mechanisms. Lipid peroxidation along with increased ROS and decreased antioxidant enzyme system and cell viability were found in mouse Leydig cells, *in vitro* exposed to Aroclor 1242. This was followed by decreased 3β- and 17β-hydroxysteroid dehydrogenase activity, indicating impaired testosterone synthesis [51]. In another study, mouse Leydig cell lines were treated with PCB 103 or 106. The study revealed dose- and type-specific increase in estrogen-related receptor type α expression increased calcium concentration and sex steroid secretion, as well as altered mitochondria ultrastructure but without a change in mitochondrial membrane potential [52]. The study on male rats exposed to Aroclor 1254 during lactation revealed altered testicular architecture, altered testosterone, estradiol and androgen-binding protein levels in both serum and testicular interstitial fluid. Furthermore, the authors also detected a dose-dependent decrease in gene expression levels of follicle-stimulating hormone, androgen-binding protein, estrogen receptor β, and androgen receptor [53]. These findings have provided mechanistic support of Sertoli cell dysfunction as a consequence of PCBs' exposure.

Recent *in vitro* findings on enantioselective action of single congener towards nuclear receptors important for endocrine regulation or metabolism further increase the evidence of the complexity of PCBs' reproductive toxicity [54]. More precisely, (+) and (−)-PCB 136 have shown opposite effects on estrogen receptor activation, and different potency to induce pregnane X receptor-mediated gene expression. On the other hand, they did show similarity in the induction of constitutive androgen receptor-dependent gene expression and the suppression of androgen receptor-mediated gene expression.

Importantly, impaired reproductive physiology has been identified as an inter- and transgenerational effect of PCBs in rodents [55,56]. Although epigenetic mechanisms have been postulated to mediate transmission effects in multiple generations, they have yet to be clarified/studied in connection to PCBs [57]. In this regard, altered DNA methylation and genomic integrity of fetal sheep cells in a simplified *in vitro* model of pregnancy exposure to Aroclor1254 have been recently reported [58].

Molecular mechanisms and MOAs of PCB-induced reproductive endocrine disruption identified by the most recent studies are annotated in Table 1.

Conclusions and perspectives

PCBs are complex mixtures of various congeners, each with its own unique molecular structure and, hence potentially different toxicity mechanisms and toxic outcomes. Although methods and outcomes differ between studies, it can be postulated that most animal data confirm PCBs' ability to act as EDCs. However, certain studies in humans did not succeed to unambiguously confirm their role in diabetes mellitus, thyroid disorders, and metabolic syndrome [59–61]. Furthermore, a recent study designed to test the activity of PCB mixture identified in pregnant women with increased risk for neurodevelopmental disorders implicated ryanodine receptors as targets in environmentally triggered neurodevelopmental disorders suggesting that thyroid disruption is not a predominant mechanism driving PCBs' neurodevelopmental toxicity [62]. Hence, results obtained in human studies certainly indicate that PCB-induced alterations in endocrine function go beyond structures and circuits discussed in this paper. Additionally, PCBs were shown to have an impact on cortisol level as well with cortisol synthesis being identified as a sensitive target for PCBs [63]. To this end, their ability to affect not only HPT but hypothalamic–pituitary–adrenal axis was proved as well. It holds that the actual role of PCBs in endocrine-related diseases and general health is yet to be determined, as well as the complexity of PCBs' ED properties. Identifying new sensitive and reliable approaches and models to help further clarification of complex molecular mechanisms of PCBs' endocrine disruption along with the systematic monitoring of endocrine function with regards to these chemicals' levels are of utmost importance from the clinical, scientific, and regulatory point of view.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** of outstanding interest

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