Disruption of thyroid hormone regulated proteins and gene expression by polychlorinated biphenyls, polybrominated diphenyl ethers and new flame retardants in residents of an e-waste region

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ABSTRACT:

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and new flame retardants (NFRs) are known thyroid hormone (TH) disruptors, but their disrupting mechanisms in humans are not completely understood. In this study, we aimed to explore the disrupting mechanisms of the aforementioned chemicals via

examining TH-regulated proteins and gene expression in human serum. Adult participants from an e-waste dismantling (exposed group) and a control region (control group) in South China provided blood samples for the research. Some compounds of PCBs, PBDEs, and NFRs showed strong binding affinity to the thyroid-stimulating hormone (TSH), thyroglobulin, thyroxine-binding globulin (TBG), gene expression of TH receptor α (TR α) and β , and iodothyronine deiodinase I (ID1). The highly exposed individuals had lower levels of TBG, TSH, and expression of TR α , but higher expression of ID1 than those of the control group. The disruption of TH-regulated proteins and gene expression suggested the exertion of different and, at times, even contradictory effects on TH disruption. However, no statistically significant difference was found in the TH levels between the exposed and the control group, implying that the TH disruption induced by these chemicals depends on the combined influence of multiple mechanisms. Gene expression appears to be an effective approach for investigations of TH disruption and the potential health effects.

Keywords: Polychlorinated biphenyl; Polybrominated diphenyl ether; New flame retardant; Thyroid hormone; Gene expression

Capsule: High exposure levels of PCBs, PBDEs, and NFRs decrease the levels of TBG, TSH, expression of TR α and increase expression of ID1.

1. Introduction

Once used as additives in electronic products, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have gradually accumulated in the environment and have been found in humans (Covaci et al., 2003; Sjödin et al., 2003; Hagmar, 2004; Beyer and Biziuk, 2009). As substitutes for PBDEs (Eljarrat and Barceló, 2010; Covaci et al., 2011), new flame retardants (NFRs) have recently been identified in the environment and in humans (Covaci et al., 2011; He et al., 2013; Liu et al., 2014b; Kuang et al., 2016). In China, e-waste dismantling releases PCBs, PBDEs, and NFRs to the surrounding environment (Chen et al., 2009; Robinson, 2009; Gao et al., 2011), potentially exposing dismantling workers and local residents to large quantities of these chemicals (Qu et al., 2007; Zhang et al., 2010; Zheng et al., 2011; Xu et al., 2014; Xu et al., 2015).

PCBs, PBDEs, and NFRs have been revealed to interrupt the equilibrium of thyroid hormone (TH) levels (Giera and Zoeller, 2013). Since TH is crucial for normal growth, development and metabolism (Turakulov et al., 1975), the disruption of TH leads to damage in the normal physiological functions and the overall human health (Miller et al., 2009). Generally, TH levels are disrupted via the response of the hypothalamus–pituitary–thyroid (HPT) axis (Liu et al., 2014a; Thornton et al., 2016). The known mechanisms identified through in-vitro and animal experiments include the prevention of thyroxine carrier proteins from binding TH (Meerts et al., 2000; Hamers et al., 2006; Marchesini et al., 2008; Cao et al., 2010; Audet-Delage et al., 2013), damage to the iodothyronine deiodinases (Gereben et al., 2008; Butt et al.,

2011; Dong et al., 2014; Roberts et al., 2015), and competitive binding to the TH receptor (TR) (Kitamura et al., 2008; Miyazaki et al., 2008; Cai et al., 2011). Whether these mechanisms are relevant to interpreting TH disruption from PCBs, PBDEs, and NFRs in humans is still unknown (Thornton et al., 2016). Without knowing these mechanisms, we cannot interpret the inconsistent results in TH disruption reported in previous studies after exposure of humans to these chemicals (Guo et al., 2018b).

Changes in TH-regulated proteins, e.g., upstream and downstream hormones, carrier proteins and receptor proteins, are indicators of TH disrupting mechanisms (Sinha and Yen, 2016). However, as some of them are not distributed in blood but in other organs (Sinha and Yen, 2016), detection of TH-regulated proteins cannot be performed by the use of standard blood sampling methods. To overcome this issue, TH-regulated gene expression in human blood cells was developed (Zheng et al., 2017). Gene expression endpoints have proven to be an effective approach to investigate TH disruption of PBDEs (Fritsche et al., 2005; Ren et al., 2015; Roberts et al., 2015). The understanding of the change in TH-regulated proteins and gene expression in the human body can facilitate the elucidation of the TH disrupting mechanisms, and further interpret more comprehensively the changes in TH levels and the inconsistent results reported in previous studies (Hagmar, 2004).

To better understand the impacts of existing and emerging chemical groups on humans, we selected local residents from an e-waste dismantling site. In addition to PCBs, PBDEs, NFRs, and TH (including triiodothyronine (T_3), thyroxine (T_4), free T_3

(FT₃), and free T₄ (FT₄)), TH-regulated proteins and gene expression were analyzed. These TH-regulated proteins and gene expression, e.g., thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), thyroglobulin (TG), thyroxine-binding globulin (TBG), TH receptor α (TR α), TH receptor β (TR β), iodothyronine deiodinase I (ID1), and iodothyronine deiodinase II (ID2), are closely associated with the secretion, synthesis, transport, function and degradation of TH, respectively (Sinha and Yen, 2016). The associations between the levels of PCBs, PBDEs, NFRs and TH-regulated proteins and gene expression were investigated. The findings obtained are important for extending our understanding of TH disrupting mechanisms and focusing our attention on the human health effects exerted by these chemicals.

2. Materials and methods

2.1. Sample collection

Two towns in Q city, South China were chosen for the study. One of them had e-waste dismantling activities (exposed region) while the other did not include any industrial or e-waste pollution source (control region). The distance between the two towns is approximately 50 kilometers. The participants with no past history of critical illness were randomly selected from local residents of the two towns (n = 54 for the exposed group and n = 58 for the control group). Details of the sample chosen were presented in our previous study (Guo et al., 2018a).

Prior to blood sampling, participants signed an informed consent form and completed a questionnaire. The questionnaires included questions related to the

participants' age, sex, incidence of neurological illness, respiratory illness, and smoking, which were all binary variables ("Yes" or "No"). Blood samples were collected from September 20 to 28, 2016. Blood samples were analysed for PCBs, PBDEs, and NFRs (10 mL), serum lipids and TH-regulated proteins (3.0 mL), and TH-regulated gene expression (1.0 mL). Samples for gene expression analysis were stored at -80°C until analysis, the rest of the samples were stored at -20°C until analysis.

The health survey and blood sampling process was approved by the Ethics and Human Subject Committee of Guangdong Provincial Center for Disease Control and Prevention.

2.2. Laboratory analysis

The analysis of serum PCBs, PBDEs, NFRs, and serum lipids were described in our previous study (Guo et al., 2018a). Serum T₃, T₄, FT₃, FT₄, TSH, TG, and TBG were determined by chemiluminescent immunoassay (Beckman Coulter, Inc., Brea, CA). The detection limits of the assays were 0.20 nmol L⁻¹ for T₃, 6.4 nmol L⁻¹ for T₄, 1.4 pmol L⁻¹ for FT₃, 3.2 pmol L⁻¹ for FT₄, 0.0010 MIU L⁻¹ for TSH, 0.055 µmol L⁻¹ for TBG, and 0.15 pmol L⁻¹ for TG.

RNA was extracted from whole blood using PureLink RNA Mini Kit (Thermo Fisher Scientific, Carlsbad, CA), then reverse transferred into complementary DNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Carlsbad, CA). Five TH-regulated gene transcripts (TRH, TR α , TR β , ID1, and ID2)

in blood of the participants were chosen for gene expression analysis (Table S1, "S" is used in name of figures and tables in the Supplementary Material). The mRNA levels of the genes were determined using real-time polymerase chain reaction in the 7900 HT Fast Real Time PCR system (Applied Biosystems, Waltham, MA). All samples were run in triplicate and averaged before performing further calculations. To obtain reliable expression values, gene transcript was abandoned if the cycle threshold (Ct) of amplification reaction was more than 40. Based on this criterion, subsequent analyses were performed only of the gene expression of TR α , TR β , and ID1. The relative expression values were obtained by normalizing the mRNA expression levels (fold change) to the β -actin abundance level using the comparative ($2^{-\Delta\Delta C_t}$) method.

2.3. Data analysis

Summation of PCB-28, -52, -101, -138, -153, -180, and -209 is referred to as Σ PCB. Summation of BDE-28, -47, -85, -99, -100, -153, -154, -183, -196, -204, -206, -207, and -209 is referred to as Σ PBDE. Summation of tetrabromoethylcyclohexane (TBECH), hexachlorocyclopentadienyldibromocyclooctane (HCDBCO), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 1,2-bis(trbromophenoxy)-ethane (BTBPE), bis(2-ethylhexyl)-tetrabromophthalate (TBPH), dechlorane plus syn (DPs), dechlorane plus anti (DPa), and 1,2-bis(2,3,4,5,6-pentabromophenyl)ethane (DBDPE) is referred to as Σ NFR.

Associations between PCBs, PBDEs, NFRs, and TH-regulated proteins, gene expression were modeled by both linear regression analyses and generalized additive models (GAM). The results of the two methods were partially identical, whereas others complemented each other (Table S2, S3 and Figure S1). Significant associations fitted by GAM and associations that were statistically significantly fitted only by linear regression methods but not GAM were chosen for subsequent analyses (Table 1). Details of the statistical analysis are shown in the Supplementary Material. All the statistical analysis was conducted using R software, and $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Demographic information and exposure levels

The characteristics of participants are listed in Table 2. Most participants were aged from 46 to 65, about half of them were women. No statistically significant difference was identified for sex, age, incidence of neurological illness and respiratory illness, smoking, serum lipid level, and body mass index (BMI) between the two groups ($p > 0.05$, Table 2 and 3). Compared to the participants in the exposed group, more participants in the control group lived far from e-waste factories (Table 2).

Σ PCB, Σ PBDE and Σ NFR levels were significantly greater in the exposed group (median level, 310, 190 and 350 ng g⁻¹ lipid, respectively) than in the control group (median level, 42, 74 and 110 ng g⁻¹ lipid, respectively) ($p < 0.05$, Table 4). The levels of Σ PCB and Σ PBDE in the exposed group were also greater than the most

previous results, which indicated that the e-waste dismantling activities had greatly increased environmental exposure of local residents (Guo et al., 2018a). The levels of individual congeners are provided in Table S4.

3.2. Disruption to TH-regulated proteins

PCBs congeners were nonmonotonically associated with TBG and TSH (Table 1 and Figure S1 d and e), and the curves fluctuated with the PCBs levels, e.g., PCB-138, -180 with TBG, PCB-209 with TSH. Our results were different from Japanese pregnant women where no association was found between the levels of PCB-138, -180 and TBG, PCB-209 and TSH (Hisada et al., 2014). The various exposure levels in the different studies may be the reason for that discrepancy (Guo et al., 2018b), e.g., the levels of PCBs in this study (25, 19, and 10 ng g⁻¹ lipid for PCB-138, -180, and -209, respectively, the median level of the exposed group and control group) were much greater than those (8.9, 8.8, and 0.33 ng g⁻¹ lipid for PCB-138, -180, and -209, respectively) in the Japanese pregnant women (Hisada et al., 2014).

More PBDEs congeners (e.g., BDE-153, -154, -183, and -209) and Σ PBDE were negatively associated with TBG, while less congeners were positively (e.g., BDE-99) or nonmonotonically associated (e.g., BDE-85) with TBG (Table 1 and Figure S1 e). These results were different from a previous study which found no association between PBDEs and TBG (Turyk et al., 2008) and different exposure levels might be one of the reasons, e.g., the level of Σ PBDE in this study (140 ng g lipid⁻¹, median value for the exposed group and the control group) were greater than that in men of

Great Lakes (38 ng g lipid⁻¹, median value)(Turyk et al., 2008). Another reason is these previous results were based on congeners with less bromine atoms (e.g., BDE-47, -99, -100, and -153), while results in this study were based on congeners with more bromine atoms (e.g., BDE-209). Compared to the above mentioned congeners, congeners with less bromine atoms were not associated with the TBG levels (e.g., BDE-28 and -47), which supported previous laboratory findings that binding affinity of PBDEs to TBG increased with the rise in the number of bromine atoms and their substituent positions (Ren and Guo, 2012; Liang et al., 2017).

A few PBDEs congeners (e.g., BDE-47, -100) were negatively associated with TSH (Table 1 and Figure S1 d). Most previous studies found no association between them (Stapleton et al., 2011; Kim et al., 2013; Huang et al., 2014; Xu et al., 2015; Makey et al., 2016), whereas a few studies detected statistically significant associations between them (Turyk et al., 2008; Chevrier et al., 2010; Zota et al., 2011). Possibly because TSH is controlled by the negative feedback of TH, and disruptors (e.g., PBDEs, PCBs) may slightly disturb the circulation of TSH via disrupting TH indirectly (Yu et al., 2010; Yu et al., 2013; Liu et al., 2014a; Zhu et al., 2018). Only BDE-138 and -153 were negatively associated with TG (Table 1), which was consistent with a previous study that exposure to PBDEs inhibits the release of TG in human thyroid cell (Kronborg et al., 2017).

Similar to PBDEs, NFRs had similar disrupting effects on TBG and TSH. Three species of NFRs (e.g., TBB, DP₁ and DP₂) and Σ NFR were negatively associated with TBG, while a few of them were positively (e.g., BTBPE) or nonmonotonically

associated (e.g., TBECH) with TBG (Table 1 and Figure S1 e). Σ NFR was negatively associated with TSH. Though three species of NFRs (e.g., TBECH, DPs, DPa) were nonmonotonically associated with TSH (Table 1 and Figure S1 d), monotonic decreasing trends between them were observed in most ranges of NFRs concentrations. A plausible explanation is NFRs and PBDEs share similar molecular structures, e.g., BTBPE and BDE-99 (Covaci et al., 2011), which enable them to exert similar endocrine disrupting effects (Ezechiáš et al., 2012; Kim and Oh, 2014).

3.3. Disruption to gene expression of ID1

PCBs (e.g., PCB-180 and Σ PCB) were nonmonotonically associated with expression of ID1. PBDEs (e.g., BDE-153, -183, -196, and -204) were positively associated with expression of ID1. For NFRs, DPs, DPa, and Σ NFR were positively associated with expression of ID1, whereas TBB and TBPH were nonmonotonically associated with expression of ID1 (Table 1 and Figure S1 i). Some studies found exposure to these chemicals up-regulated expression of ID1 in fish and polar bears (Yu et al., 2010; Noyes et al., 2013; Dong et al., 2014; Gabrielsen et al., 2015), while others found no significant associations (Liu et al., 2014a; Thornton et al., 2016). One reason for these diverse results is that these chemicals disrupt ID1 indirectly, i.e., IDs are sensitive to the HPT axis and are prone to compensate for any change in TH levels (Jarque and Piña, 2014), and hence these chemicals can disrupt IDs through a change in TH levels. Additionally, the gene of ID1 was extracted from peripheral blood in this study, whereas they were extracted from hepatocytes or brain tissues in

animal studies, respectively (Dong et al., 2013; Thornton et al., 2016). Hence, ID1 from different organs may respond differently under exposure to these chemicals (Dong et al., 2013; Li et al., 2014). In particular, PBDE congeners with more bromine atoms were more likely to alter expression of ID1 than congeners with less bromine atoms, and similar results were obtained in animal studies (François et al., 2016; François and Verreault, 2018). This is because the bromine atom can prevent the IDs from binding to TH, and as such the inhibition increases with more bromine atoms (Marsan and Bayse, 2017).

Moreover, pairwise interactions of Σ PCB and Σ PBDE, Σ PCB and Σ NFR tended to increase expression of ID1 ($p < 0.05$, Table S5, Figure S2 e and f).

3.4. Disruption to gene expression of $TR\alpha$ and $TR\beta$

Many compounds of PCBs, PBDEs, and NFRs were negatively associated with expression of $TR\alpha$, including PCB-138, -180, BDE-47, -153, -183, -196, -204, -206, TBB, TBPH, DPs, DPa, Σ PBDE, and Σ NFR. However, a few of them were nonmonotonically associated with expression of $TR\alpha$ (e.g., PCB-28, -153, BDE-28, -154, -207, TBECH, DBDPE, and Σ PCB), and these curves were widely different from one another (Table 1 and Figure S1 g). Obviously, these disrupting effects did not depend on the chemicals' molecular structures and the number of substitute atoms, which were different from OH-PCBs and OH-PBDEs (Kitamura et al., 2005; Ren et al., 2013). Binding affinities of TRs for OH-PCBs, OH-PBDEs are enhanced by hydroxy substitution (Kitamura et al., 2005), whereas those for PCBs and PBDEs

were not. Previous studies obtained similar results, i.e., PCBs and PBDEs may prevent or stimulate the function of TR (Miyazaki et al., 2008; Ibhazehiebo et al., 2011), and the reason for contradictory disrupting effects calls for further research.

Moreover, pairwise interactions of Σ PCB with Σ PBDE, Σ PCB with Σ NFR tended to increase expression of TR α ($p < 0.05$, Table S5, Figure S2 c and d). The results indicated Σ PCB and Σ PBDE have synergistic effects on TR α , possibly because they disrupt TR via similar pathways, i.e., directly altering TR actions (Gauger et al., 2004; Miyazaki et al., 2008; Ibhazehiebo et al., 2011) or indirectly affecting TRs via TH levels (Yu et al., 2013; Thornton et al., 2016; Chen et al., 2017). NFRs may have synergistic effects with PCBs, and more research is needed to support this assertion.

Few compounds of these chemicals were statistically significantly associated with gene expression of TR β (Table 1 and Figure S1 h), e.g., DBDPE was positively associated with expression of TR β , PCB-52 and BDE-153 were negatively associated with expression of TR β , PCB-153, BDE-207, and TBECH were nonmonotonically associated with expression of TR β . The number of the significant associations for TR β was much less than those of TR α , indicating the selectivity of PCBs, PBDEs, and NFRs on TR isoform disruption (Zoeller, 2005; Tabuchi et al., 2006; Schriks et al., 2007). In fact, TR α and TR β serve different functions which lead to different responses to the chemical exposure (Kim et al., 2015a; Cao et al., 2016; Du et al., 2016).

3.5. Disruption to TH

Few statistically significant associations were observed between levels of these chemicals and TH (Table 1 and Figure S1 a, b and c). PCB congeners (e.g., PCB-28, -52, -101, -138 and -153) were negatively associated with FT₄, whereas Σ PCB was nonmonotonically associated with FT₄. Some PBDE congeners were positively associated with T₃ (e.g., BDE-85 and -99) and FT₃ (e.g., BDE-47), while some others were nonmonotonically associated with T₃ (e.g., BDE-47, -204 and -207) and FT₃ (e.g., BDE-204 and -207). Also, PBDEs were negatively associated with T₄ (e.g., BDE-153, -183 and Σ PBDE) and FT₄ (e.g., BDE-153, -183, -204 and -207).

The statistically significant associations in this study and previous reports published over the last decade were compared (Table 5). The results of this study were partly similar to some of previous ones (Chevrier et al., 2008; Abdelouahab et al., 2011; Huang et al., 2014; Wang et al., 2014), while they were different from most of others (Turyk et al., 2008; Bloom et al., 2009; Abdelouahab et al., 2011; Stapleton et al., 2011; Kim et al., 2013; Bloom et al., 2014; Huang et al., 2014; Xu et al., 2014; Miranda et al., 2015; Vuong et al., 2015; Makey et al., 2016; Liu et al., 2017). One reason for these discrepancies was the different exposure levels, i.e., the participants in some studies were exposed to much higher levels of PCBs and PBDEs than in others. For example, PCB-153 in this study (12 ng g⁻¹ lipid, median level for the exposed group and control group) was much lower than that in adults from New York (74 ng g⁻¹ lipid, median level), and PCB-153 was negatively associated with FT₄ in this study, whereas it was positively associated with FT₄ in adults from New York (Bloom et al., 2009). The second reason may lie in the different TH levels, e.g., the

TH levels of pregnant women were widely different from those of adult men (Vuong et al., 2015). The usage of different control variables among the studies may be another reason - BMI, sex, chest tightness, dyspnea, and smoking were used in this study, while they were not necessarily used in other studies (Kim et al., 2013; Huang et al., 2014). For NFRs, TBECH was nonmonotonically associated with T_3 , which was similar to the findings of a previous study on fish (Park et al., 2011). Other species of NFRs were not statistically significantly associated with TH; a finding similar to that of our previous research on children, indicating that NFRs have weaker TH disrupting effects than PBDEs (Guo et al., 2018b).

3.6. Levels of TH-regulated proteins, gene expression and TH between two groups

The levels of some TH-regulated proteins and gene expression were statistically significantly different between the two groups, while others were not (Table 3 and Graphical Abstract). These differences may be attributed to the statistically significant associations between these chemicals and TH-regulated proteins and gene expression. First, the levels of TBG were lower in the exposed group than in the control group ($p < 0.05$), for large amount of negative associations ($n = 8$) promoted the decreases of TBG levels. Similarly, expression of ID1 was greater in the exposed group than in the control group ($p < 0.05$), for six positive associations promoted the increase in expression of ID1, and interactions of Σ PCB and Σ PBDE, Σ PCB and Σ NFR were also prone to increase expression of ID1. Second, the levels of TSH and expression of TR α were slightly lower in the exposed group than in the

control group ($p < 0.1$). For TSH, only three negative associations were found which may be the reason. For $TR\alpha$, though thirteen negative associations detected suggested decreases in expression of $TR\alpha$, opposite effects were found for the interactions of ΣPCB with $\Sigma PBDE$, ΣPCB with ΣNFR , which tended to increase expression of $TR\alpha$. In contrast, the levels of TG and expression of $TR\beta$ were not statistically significantly different between the two groups ($p > 0.05$), possibly because of the few significant associations ($n = 2$ for TG and $n = 3$ for $TR\beta$). These results suggest that these chemicals altered some TH-regulated proteins and gene expression, and disturbed the secretion, transport, degradation, and receptors of TH in human. Of note, the changes in TH-regulated proteins and gene expression may correspond to some potential illnesses, e.g., expression of ID1 and the level of TBG may be associated with nonthyroid illness syndrome (Jirasakuldech et al., 2000; Castro et al., 2013; Vidart et al., 2014) and expression of $TR\alpha$ may be associated with breast cancer (Silva et al., 2002). Thus, the health effects relevant to TH-regulated proteins and gene expression should be focused on in future research.

In contrast to that for TH-regulated proteins and gene expression, no TH levels were statistically significantly different between the two groups ($p > 0.05$, Table 3 and Graphical Abstract). Compared to the aforementioned proteins and gene expression, TH are less sensitive to these chemicals. Since TH homeostasis are controlled via the HPT axis (Liu et al., 2014a; Thornton et al., 2016), multiple disrupting mechanisms involved in the HPT axis may interrupt TH homeostasis. The above mentioned TH-regulated proteins and gene expression can partly explain these

mechanisms and the variation of TH levels. On one hand, the lower TBG levels in the exposed group suggest greater FT₃ and FT₄ levels than those in the control group, for TBG binds a large amount of serum TH (nearly 70%) and serves as an additional thyroidal reservoir of TH (Nilsson and Peterson, 1975). The lower TSH levels in the exposed group also imply greater TH levels than the ones in the control group, as the secretion of TSH is partly controlled by the negative feedback of TH (Yu et al., 2010; Kim et al., 2015a; Zhu et al., 2018). On the other hand, the greater gene expression of ID1 in the exposed group suggests lower TH levels compared to the control group, for ID1 controls the degradation of TH (Jarque and Piña, 2014) and plays a critical role in homeostasis of TH levels (Yu et al., 2010). The lower expression of TR α in the exposed group also reflects lower TH levels compared to those of the control group, as gene expression of TR α is regulated by TH levels (Tabuchi et al., 2006; Liu et al., 2014a; Chen et al., 2017; Chang et al., 2018). Besides, expression of TR β and the levels of TG were not statistically significantly different between the two groups, which suggest these chemicals may not change TH levels via disrupting TR β or TG.

These results clearly indicate the action of contradictory mechanisms, and the overall effects on TH of these chemicals may depend on combined actions of these mechanisms (Graphical Abstract)(Byrne et al., 2018). Nevertheless, the factors influencing the combination are still not clear. The exposure levels, physique and the health status of participants, which differ among various studies, may partially explain that discrepancies. Different combinations of these TH-regulated proteins and gene expression may be responsible for the inconsistent results in most previous

studies (Hagmar, 2004; Kim et al., 2013, 2015b). More comprehensive research on TH-regulated proteins and gene expression are needed to better understand the combined effects of these mechanisms and predict the changes in the TH levels.

This study has some limitations. First, we are not sure whether PCBs, PBDEs, and NFRs can disrupt other TH-regulated proteins and gene expression which also control the synthesis and secretion, metabolism and receptors of TH, e.g., TRH (Pekary, 2013), TSH receptor (Szkudlinski et al., 2002), sodium-iodide (Na^+/I^-) symporter (Hallinger et al., 2017), thyroid peroxidase (Song et al., 2012), ID2 (Dong et al., 2014), uridinediphosphate-glucuronosyltransferase and sulfotransferase (Tseng et al., 2008; Leonetti et al., 2018). Knowing their levels or gene expression could facilitate the exploration of the disrupting mechanisms more comprehensively. Second, this study did not consider the metabolisms of PCBs and PBDEs in serum, which may also interface with the homeostasis of TH. Third, the associations were mainly based on linear regression and GAM, which could not fully present the disrupting effects on TH. Other statistical methods, which promote a better understanding of the TH disruption, should also be employed. Further research is needed to address these limitations and broaden the knowledge of the health effects of these TH disrupting chemicals.

4. Conclusion

The results suggest that PCBs, PBDEs, and NFRs have strong binding affinity to TBG, TSH, ID1, and $\text{TR}\alpha$, and high exposures to these chemicals can decrease the

levels of TBG, TSH, expression of TR α , but increase expression of ID1. Moreover, our results indicate that a combination of TH disrupting mechanisms is crucial to TH disruption. The present findings improve our understanding of TH disruption, broaden the utilization of gene expression in interpreting disrupting mechanisms, and focus further research attention on the potential illnesses in which TH-regulated proteins and gene expression are involved.

Acknowledgements

This study was aided financially by the National Key R&D Program of China (2016YFC0207001), National Natural Science Foundation of China (21507013) and Science and Technology Program of Guangzhou, China (201707010476).

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Table 1 Statistically significant associations ($p < 0.05$) between serum PCBs, PBDEs, NFRs and TH regulated proteins and gene expression, adjusted for BMI, sex, dyspnea, chest tightness, and smoking.

		T ₃	T ₄	FT ₃	FT ₄	TG	TSH	TBG	ID1	TR α	TR β
PCB-28	-	-	-	-	↓	-	-	-	-	N	-
PCB-52	-	-	-	-	↓	-	-	-	-	-	↓
PCB-101	-	-	-	-	↓	-	-	-	-	-	-
PCB-138	-	-	-	-	↓	↓	-	N	-	↓	-
PCB-153	-	-	-	-	↓	↓	-	-	-	N	N
PCB-180	-	-	-	-	-	-	-	N	N	↓	-
PCB-209	-	-	-	-	-	-	N	-	-	-	-
BDE-28	-	-	-	-	-	-	-	-	-	N	-
BDE-47	N	-	-	↑	-	-	↓	-	-	↓	-
BDE-85	↑	-	-	-	-	-	-	N	-	-	-
BDE-99	↑	-	-	-	-	-	-	↑	-	-	-
BDE-100	-	-	-	-	-	-	↓	-	-	-	-
BDE-153	-	-	↓	-	↓	-	-	↓	↑	↓	↓
BDE-154	-	-	-	-	-	-	-	↓	-	N	-
BDE-183	-	-	↓	-	↓	-	-	↓	↑	↓	-
BDE-196	-	-	-	-	-	-	-	-	↑	↓	-

774	BDE-204	N	-	N	↓	-	-	-	↑	↓	-
775	BDE-206	-	-	-	-	-	-	-	-	↓	-
776	BDE-207	N	-	N	↓	-	-	-	-	N	N
777	BDE-209	-	-	-	-	-	-	↓	-	-	-
778	TBECH	N	-	-	-	-	N	N	-	N	N
779	HCDBCO	-	-	-	-	-	-	-	-	-	-
780	TBB	-	-	-	-	-	-	↓	N	↓	-
781	BTBPE	-	-	-	-	-	-	↑	-	-	-
782	TBPH	-	-	-	-	-	-	-	N	↓	-
783	DPs	-	-	-	-	-	N	↓	↑	↓	-
784	DPa	-	-	-	-	-	N	↓	↑	↓	-
785	DPDBE	-	-	-	-	N	-	-	-	N	↑
786	ΣPCB ^k	-	-	-	N	-	-	-	N	N	-
787	ΣPBDE ^l	-	↓	-	-	-	-	↓	-	↓	-
788	ΣNFR ^m	-	-	-	-	-	↓	↓	↑	↓	-

789 ^aTriiodothyronine. ^bThyroxine. ^cFree triiodothyronine. ^dFree thyroxine. ^eThyroglobulin. ^fThyroid stimulating hormone. ^gThyroxine-binding globulin.
790 ^hIodothyronine deiodinase I. ⁱThyroid hormone receptor α . ^jThyroid hormone receptor β . ^kSum of PCB-28, -52, -101, -138, -153, -180 and -209. ^lSum of
791 BDE-28, BDE-47, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-196, BDE-204, BDE-206, BDE-207 and BDE-209. ^mSum of TBECH,
792 HCDBCO, TBB, BTBPE, TBPH, DPs, DPa and DBDPE. ↑Positive association. ↓Negative association. N Nonmonotonic association.

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Table 2 Demographic characteristics of participants.

Total (n = 112)	Exposed group		Control group	
	Male ^a	Female	Male	Female
Number	26	28	25	33
Age (years)				
26-35	6	0	5	3
36-45	5	5	6	5
46-55	5	7	10	15
56-65	6	11	5	8
66-75	3	2	2	2
Personal symptoms of neurological illness				
Headache	3	2	3	4
Dizzy	0	1	0	2
Insomnia	1	0	0	0
Memory loss	1	1	0	0
Languor	0	1	0	0
Personal symptoms of respiratory illness				
Sore throat	2	3	1	0
Dyspnea	1	0	0	1
Chest tightness	0	1	1	1
Chest pain	0	0	0	0
Dry cough	1	1	3	2
Loose cough	0	0	1	1
Palpitation	0	0	0	2
Smoking	19	0	15	0
Annual household incomes (RMB of China)				
< 10000	3	3	2	2
10000-30000	1	1	6	6
30000-50000	6	5	6	7
>50000	10	5	7	8
Unclear	3	8	1	5
Not respond	3	6	3	5

The distances between participants' house and a nearest e-waste factory

827	< 0.1 km	6	4	0	0
828	0.1-0.5 km	3	1	0	0
829	0.5-1 km	9	9	1	0
830	1-2 km	3	0	0	0
831	2-3 km	1	2	0	0
832	> 3 km	1	3	11	14
833	Not respond	3	9	13	19

^aOne of the male participants in the exposed group did not reveal his age or personal symptoms.

Table 3 Levels of TH regulated proteins, gene expression, lipids and BMI in study participants.

		Exposed group			Control group		
		Median	Mean $\pm$ SD	Range	Median	Mean $\pm$ SD	Range
839	T ₃ ^a	1.7	1.7 $\pm$ 0.27	0.96–2.3	1.7	1.7 $\pm$ 0.25	1.2–2.5
840	T ₄ ^b	99	96 $\pm$ 15	67–120	99	98 $\pm$ 19	51–150
841	FT ₃ ^c	4.8	4.8 $\pm$ 0.57	3.3–6.1	4.8	4.9 $\pm$ 0.69	3.5–7.7
842	FT ₄ ^d	17	17 $\pm$ 2.2	13–22	17	17 $\pm$ 3.2	7.1–25
843	TG ^e	8.5	13 $\pm$ 13	0.10–71	9.7	14 $\pm$ 16	0.10–100
844	TSH ^f	1.6	1.7 $\pm$ 1.0	0.14–5.4	1.6	2.6 $\pm$ 3.9	0.34–27
845	TBG ^g	17	18 $\pm$ 4.7	9.0–28	20	20 $\pm$ 4.4	11–37
846	ID1 ^h	4.3 $\times 10^{-3}$	(4.2 $\pm$ 1.8) $\times 10^{-3}$	0.1 $\times 10^{-3}$ –9.6 $\times 10^{-3}$	3.3 $\times 10^{-3}$	(3.2 $\pm$ 1.8) $\times 10^{-3}$	0.031 $\times 10^{-3}$ –6.4 $\times 10^{-3}$
847	TR α ⁱ	3.6 $\times 10^{-3}$	(14 $\pm$ 33) $\times 10^{-3}$	0.093 $\times 10^{-3}$ –190 $\times 10^{-3}$	9.4 $\times 10^{-3}$	(29 $\pm$ 46) $\times 10^{-3}$	0.33 $\times 10^{-3}$ –220 $\times 10^{-3}$
848	TR β ^j	0.30 $\times 10^{-3}$	(0.47 $\pm$ 0.38) $\times 10^{-3}$	0.045 $\times 10^{-3}$ –1.6 $\times 10^{-3}$	0.32 $\times 10^{-3}$	(1.0 $\pm$ 2.6) $\times 10^{-3}$	0.11 $\times 10^{-3}$ –15 $\times 10^{-3}$
849	TL ^k	4.3	4.9 $\pm$ 2.7	2.9–23	4.6	4.7 $\pm$ 0.91	3.3–8.5
850	TC ^l	1.9	2.0 $\pm$ 0.67	1.0–5.2	2.0	2.0 $\pm$ 0.37	0.97–3.0
851	TG ^m	0.68	1.1 $\pm$ 1.6	0.36–12	0.79	0.92 $\pm$ 0.47	0.30–3.2
852	BMI	25	24 $\pm$ 2.9	19–31	25	25 $\pm$ 3.0	17–33

^aTriiodothyronine (nmol L⁻¹). ^bThyroxine (nmol L⁻¹). ^cFree triiodothyronine (pmol L⁻¹). ^dFree thyroxine (pmol L⁻¹). ^eThyroglobulin (pmol L⁻¹). ^fThyroid stimulating hormone (MIU L⁻¹). ^gThyroxine-binding globulin (μ mol L⁻¹). ^hIodothyronine deiodinase I. ⁱThyroid hormone receptor α . ^jThyroid hormone

855 receptor β . ^kTotal lipid (g L⁻¹). ^lTotal cholesterol (g L⁻¹). ^mTriglycerides (g L⁻¹).

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Table 4 Serum Levels of Σ PCB, Σ PBDE, and Σ NFR (ng g⁻¹ lipid) in participants.

	Exposed group (n = 54)			Control group (n = 58)		
	Median	Mean $\pm$ SD	Range	Median	Mean $\pm$ SD	Range
Σ PCB ^a	310	390 $\pm$ 260	86–1400	42	78 $\pm$ 61	12–350
Σ PBDE ^b	190	240 $\pm$ 120	87–620	74	93 $\pm$ 50	27–210
Σ NFR ^c	350	540 $\pm$ 420	160–2800	110	140 $\pm$ 84	40–420
^a Sum of PCB-28, -52, -101, -138, -153, -180 and -209. ^b Sum of BDE-28, -47, -85, -99, -100, -153, -154, -183, -196, -204, -206, -207 and -209. ^c Sum of TBECH, HCDBCO, TBB, BTBPE, TBPH, DPs, DPa and DBDPE.						

Table 5 Statistically significant associations between levels of PCBs, PBDEs and TH in the previous reports.

Chemical	Population	N	Associations between chemicals and TH	Reference
PCBs				
PCB-28, -52, -101, -138, -153	Pregnant Women	334	Negatively associated with FT ₄ ^e	Chevrier et al. (2008)
PCB-153, -180	Adults	38	Positively associated with FT ₄ ^e	Bloom et al. (2009)
ΣPCB ^a	Adults	55	Negatively associated with FT ₄ ^e	Xu et al. (2015)
PBDEs				
BDE-47	Men	51	Positively associated with FT ₄ ^e	Turyk et al. (2008)
BDE-47, -99, -100, ΣPBDE ^b	Pregnant Women	137	Positively associated with T ₄ ^f	Stapleton et al. (2011)
BDE-47, -99, ΣPBDE ^b	Pregnant Women	137	Positively associated with FT ₄ ^e	Stapleton et al. (2011)
BDE-47, -99, ΣPBDE ^b	Men	52	Negatively associated with T ₄ ^f	Abdelouahab et al. (2011)
BDE-47	Pregnant Women	138	Negatively associated with T ₃ ^g	Kim et al. (2013)
BDE-28, -47, -153, -183	Adults	124	Negatively associated with T ₃ ^g	Huang et al. (2014)
BDE-99, -209	Adults	124	Positively associated with T ₃ ^g	Huang et al. (2014)
ΣPBDE ^c	Men	127	Negatively associated with FT ₄ ^e	Bloom et al. (2014)
BDE-28, -47	Women	187	Positively associated with T ₄ ^f and FT ₃ ^h	Vuong et al. (2015)
BDE-47	Women	187	Positively associated with T ₃ ^g and FT ₄ ^e	Vuong et al. (2015)
BDE-99, -100	Pregnant Women	140	Negatively associated with T ₃ ^g	Miranda et al. (2015)
BDE-47, -99, -100	Adults	51	Negatively associated with T ₄ ^f	Makey et al. (2016)
BDE-153	Adults	51	Positively associated with FT ₄ ^e	Makey et al. (2016)

886	Σ PBDE ^d	Adults	33	Negatively associated with FT ₄ ^e	Liu et al. (2017)
887	^a Sum of PCB-28, -52, -101, -138, -153, and -180. ^b Sum of BDE -47, -99, -100, and -153. ^c Sum of BDE-28, -47, -66, -85, -99, -100, -138, -153, and -154. ^d Sum				
888	of BDE-28, -47, -99, -100, -153, -154, and -183. ^e Free triiodothyronine. ^f Thyroxine. ^g Triiodothyronine. ^h Free thyroxine.				
889					

PCBs, PBDEs and NFRs disrupted some TH-regulated proteins and gene expression.

Levels of TBG, TSH and expression of ID1, TR α were different between the two groups.

The disruption on TH-regulated proteins and gene expression may do harm to health.

The overall effects on TH depend on a combination of multiple disrupting mechanisms.

Conflict of interest statement

Title: Disruption of thyroid hormone regulated proteins and gene expression by polychlorinated biphenyls, polybrominated diphenyl ethers and new flame retardants in residents of an e-waste region.

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June 5th, 2019