1 • **Manuscript title**

2 Disruption of thyroid hormone (TH) levels and TH-regulated gene expression by
3 polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and
4 hydroxylated PCBs in e-waste recycling workers

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Abstract

Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) are the primary toxicants released by electronic waste (e-waste) recycling, but their adverse effects on people working in e-waste recycling or living near e-waste sites have not been studied well. In the present study, the serum concentrations of PBDEs, PCBs, and hydroxylated PCBs, the circulating levels of thyroid hormone (TH), and the mRNA levels of seven TH-regulated genes in peripheral blood leukocytes of e-waste recycling workers were analyzed. The associations of the hormone levels and gene expression with the exposure to these contaminants were examined using multiple linear regression models. There were nearly no associations of the TH levels with PCBs and hydroxylated PCBs, whereas elevated hormone (T_4 and T_3) levels were associated with certain lower-brominated BDEs. While not statistically significant, we did observe a negative association between highly brominated PBDE congeners and thyroid-stimulating hormone (TSH) levels in the e-waste workers. The TH-regulated gene expression was more significantly associated with the organohalogen compounds (OHCs) than the TH levels in these workers. The TH-regulated gene expression was significantly associated with certain PCB and hydroxylated PCB congeners. However, the expression of most target genes was suppressed by PBDEs (mostly highly brominated congeners). This is the first evidence of alterations in TH-regulated gene expression in humans exposed to OHCs. Our findings indicated that OHCs may interfere with TH signaling and/or exert TH-like effects, leading to alterations in related gene expression in humans. Further

43 research is needed to investigate the mechanisms of action and associated biological
44 consequences of the gene expression disruption by OHCs.

45 **Keywords:** Thyroid hormone; Polybrominated diphenyl ethers; Polychlorinated
46 biphenyls; Gene expression; Serum; E-waste

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48 **1. Introduction**

49 Rapidly growing electronic waste (e-waste) has become a global environmental health
50 issue because of the expansion of the market for electronic products (Ogunseitan et al.,
51 2009). A substantial amount of e-waste generated worldwide was moved to several
52 developing countries, where the e-waste is dismantled using primitive techniques. These
53 uncontrolled activities release a wide range of hazardous substances, including flame
54 retardants, polychlorinated biphenyls (PCBs), and heavy metals, into the environment
55 (Breivik et al., 2011). PCBs and some of polybrominated diphenyl ethers (PBDEs),
56 which are an important class of brominated flame retardants, have been listed by the
57 Stockholm Convention on Persistent Organic Pollutants because of their ubiquitous
58 presence in the environment and adverse health effects (Miyazaki et al., 2008;
59 Abdelouahab et al., 2013). Thus, although declining levels of these organohalogen
60 compounds (OHCs) have been observed in developed countries after their phase-out
61 (Breivik et al., 2011; Sutton et al., 2015), continuous human exposure to these chemicals,
62 especially at the hotspots (e.g., e-waste site), remains a concern in some developing
63 countries, where the levels remain high due to insufficient control or treatment.

64 Thyroid hormones (THs) are essential for the normal development and maintenance of
65 normal physiological functions and play a critical role in fetal brain development
66 (Abdelouahab et al., 2013). Evidence from experimental and human studies has shown a
67 significant association between thyroid disruption and exposure to OHCs because of their

68 structural similarity to THs (Salay and Garabrant, 2009; Ibhazehiebo et al., 2011; Makey
69 et al., 2016). Potential mechanisms underlying the TH homeostasis disruption by these
70 contaminants include competition for binding to the thyroid transport proteins
71 (Alvarez-Pedrerol et al., 2009; Chevrier et al., 2010), increased TH metabolism in the
72 liver and brain (Meerts et al., 2002; Lema et al., 2008), and altered TH receptor (TR)
73 activity (Zoeller, 2005). However, the associations between OHC exposure and
74 circulating TH levels in human studies have not been consistent (Turyk et al., 2008;
75 Salay and Garabrant, 2009; Stapleton et al., 2011; Makey et al., 2016). Furthermore,
76 most studies were conducted in populations with background exposure levels, and
77 research on occupational exposure is limited (Julander et al., 2005; Zhang et al., 2010).

78 Gene expression endpoints mediate the majority of the biological actions of thyroid
79 hormones and are considered more promising approaches for the characterization of the
80 thyroid-toxic potential of anthropogenic contaminants (Buckman et al., 2011;
81 Ibhazehiebo et al., 2011). Moreover, gene expression analysis identifies the early
82 detection symptoms of contaminant exposure before the manifestation of higher-level
83 health effects (Tabuchi et al., 2006). Studies have linked changes in TR gene expression
84 to PCB exposure in wildlife species (Tabuchi et al., 2006; Buckman et al., 2011).
85 Wadzinski et al. (2014) recently found that the expression of dioxin-inducible enzyme
86 (CYP1A1) is strongly associated with that of TH-regulated target genes in human
87 placenta. However, to the best of our knowledge, the alterations in TH-regulated gene
88 expression in humans associated with environmental OHC exposure have not been

reported.

In the present study, the concentrations of PCBs, their hydroxylated metabolites, and PBDEs, which are typical organic pollutants released from e-waste recycling, and the circulating levels of THs, including total thyroxine (TT4), free T₄ (FT₄), total triiodothyronine (TT3), FT₃, and thyroid-stimulating hormone (TSH), were analyzed in the serum of e-waste recycling workers from an e-waste site in South China. In addition, the mRNA levels of seven TH-regulated genes, including the TR isoforms (TR α and TR β), forkhead box E1 (FOXO1, thyroid transcription factor 2), transcription factor Kruppel-like factor 9 (KLF9, also known as basic transcription element-binding protein), TSH receptor (TSHR), and plasma membrane integrin α v and β 3, in peripheral blood leukocytes (the cells of the immune system) of the e-waste workers were measured. These genes encode nuclear receptors, transcription factors, or integrins that are responsive to THs. It is widely accepted that THs act as modulators of the immune response, and some immune functions are altered under hypo- and hyper-thyroid conditions (De Vito et al., 2012). The main objective is to explore the relationships between these biomarkers and the exposure to OHCs in this cohort of workers who are prone to be exposed to high levels of a complex contaminant mixture.

2. Materials and methods

2.1 Sample collection

Blood samples were obtained from 79 fasting occupational e-waste recycling workers recruited from an e-waste site in South China in 2011. This site is one of the largest

e-waste sites in China, where the PCB and PBDE concentrations in the ambient air are markedly high (Tian et al., 2011; Chen et al., 2014). This study was launched with the authorization of the Ethics Committee of the School of Life Science, Sun Yat-sen University. The samples were collected by medical staff at a local hospital with consent from all participants after they were clearly informed of the study objectives. Duplicate venous blood samples were collected from each subject. The first portion (8–10 mL) was collected with an anticoagulant-free tube and processed within 3 h after collection to isolate serum by centrifugation at 3000 rpm for 5 min. Approximately 1-2 mL serum samples were stored at 4 °C and were used to measure the circulating thyroid hormone levels within two days after collection. The remaining serum samples were kept at -80 °C in the laboratory until the chemical analysis. The second portion (3-4 mL heparin-anticoagulated venous fresh blood) was used to isolate peripheral blood lymphocytes immediately after transportation. A short questionnaire and general physical examination, concerning the participants' age, gender, weight, height, smoking, and occupational history, were conducted.

2.2 Chemical analysis

The extraction and purification methods for PBDEs, PCBs, and hydroxylated PCBs in this study were similar to those used in our previous study (Yan et al., 2012) and modified according to the method described by (Park et al., 2009a). Briefly, the serum samples were denaturation with hydrochloric acid (6 M) and 2-propanol and were extracted with a mixture of hexane/methyl-tert-butyl ether (MTBE) (1:1, v/v). KCl (1%)

was added to the extracts, and the extracts were centrifuged. The upper organic layer containing the PBDEs and PCBs was purified by a multi-layer silica/alumina column after lipids were removed with concentrated sulfuric acid. The lower phenolic fraction (containing hydroxylated PCBs) was acidified with HCl, followed by derivatization with the excess of the freshly prepared diazomethane. The hydroxylated PCB derivatives were further cleaned by a multi-layer silica/alumina column. The eluent was finally condensed to 100 μ L under a gentle stream of nitrogen, and PCB24, PCB82, PCB198, BDE118, and BDE128 were added as internal standards for the quantification.

The recoveries of the surrogate standards BDE77, BDE181, ^{13}C -BDE209, CB65, and 204 were in the range of 69%–121%, 58%–105%, 63%–131%, 69–108%, and 66–108%, respectively, in the serum samples. The final results were not recovery-corrected. Serum procedural blanks (Milli-Q water) were run with each sample batch. Only trace amounts (< 5% of those in the corresponding serum extracts) were detected in the blanks, and these amounts were subtracted from the sample extracts. The mean recoveries in the spiked blanks ranged from 65% to 103% for nine individual PBDE congeners (BDE28, 47, 66, 99, 100, 153, 154, 183, and 209) and 62% to 108% for 21 PCBs.

The PCB and OH-PCB congeners were analyzed by an Agilent 6890 gas chromatograph equipped with a 5975B mass spectrometer (GC-MS) in electron impact (EI) ionization and electron capture negative ionization (ECNI) modes, respectively. These compounds were separated on a DB-5MS capillary column (60 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness) and a DB-XLB capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m

film thickness), respectively. PBDEs were analyzed by a Shimadzu 2010 GC-MS in ECNI mode, which was equipped with a DB-XLB (30 m × 0.25 mm × 0.25 μm) capillary column for the determination of tetra through hepta-BDEs and a DB-5HT (15 m × 0.25 × 0.1 μm) column for octa through deca-BDEs.

2.3 Thyroid hormone analysis

The analysis of TSH, T₃, T₄, FT₃ and FT₄ was conducted at the Guangdong Prevention and Treatment Center for Occupational Diseases. The hormones were analyzed by the ADVIA Centaur CP Immunoassay system (Siemens Healthcare Diagnostics Inc.) with accessory materials, including reagent standards and quality controls, following the standard methodologies. The analysis was operated automatically after loading the reagents and testing samples. The recoveries of the THs in dilutions (1:2, 1:4 and 1:8, v/v) from five serum samples ranged from 88.8% to 104.9%, and the recoveries ranged from 92.0%–112.5% in the spiked matrices. Standard reference materials (SRMs) for T₃, T₄, FT₃, FT₄ and TSH were tested three times before measuring the serum samples. The coefficients of variations were less than 6.3%, and the deviations of the SRMs were less than 13%.

2.4 Peripheral blood lymphocyte RNA extraction and cDNA synthesis.

Peripheral blood lymphocytes were isolated using a lymphocyte separation medium (LSM) (Haoyang Biological Manufacture Inc. Tianjin, China) designed for humans according to the manufacturer's protocol. Briefly, venous blood was diluted with PBS (1:1, v/v), which was then carefully overlaid on the same volume of LSM in a tube. The

tube was centrifuged at 2,000 rpm for 15 min, and lymphocytes on the upper layer were collected. The collected lymphocytes were rinsed with 5 mL PBS, which was then removed by gentle centrifugation. RNA was isolated from the lymphocytes using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Paisley, UK). After extraction, the purified RNA was subject to DNase I treatment to remove residual DNA. Then, the DNase I-treated RNA was reverse transcribed into complementary DNA (cDNA) using the first strand cDNA synthesis kit (Promega, Madison, USA) with random hexamer primers.

2.5 Quantitative Real-time PCR Assay

Several TH-regulated gene transcripts in the leukocytes of the e-waste recycling workers were selected to explore their responses to contaminant exposure. The mRNA levels of the genes were determined using real-time PCR in the LightCycler 480 Real-Time PCR System (Roche, German) with SYBR Premix Ex Taq II (Takara BIO, Inc., Dalian, China). All primers and their specificities are listed in Table S1 in the Supplemental Material. Negative control was included in each run with the same reaction reagent except for the cDNA template to confirm the reaction performance. Standard curves were created to calibrate the amplification efficiency by the target fragments for the target genes. All samples were run in triplicate and averaged before performing further calculations. 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.

2.6 Data analysis

A multiple linear regression was used to evaluate the associations of the circulating TH and gene expression mRNA levels with the serum OHC concentrations. Only OHC congeners with detection frequencies greater than 60% were included in the model, and for measurements below the limit of detection (LOD), a value of LOD/2 was assigned. The multiple linear regression was performed for both total and congener-specific concentrations. For PBDEs, the sub-totals of the pentaBDEs, octaBDEs, decaBDEs were also modeled, given their origins and toxic effects. The measurements in the model were all $\log_{10}$ -transformed to follow or closely approximate normality. The following covariates were considered in the regression models: sex (male/female), age (yr), occupational exposure duration (yr), body mass index (BMI), and smoking status (yes/no). The covariates were selected *a priori* by bivariate analyses, and those predicted at a p-value < 0.1 in their relationship with one or more OHCs or blood biomarker measurements were included in the adjusted model. The models were adjusted for the same covariates (sex, age, occupational exposure duration, and BMI) to maintain consistency. The Bonferroni-adjusted significance levels for the multiple regression were $\alpha = 0.005$ for TH variables (10 tests) and $\alpha = 0.0024$ for gene expression variables (21 tests). However, because the Bonferroni correction is considered to be overly conservative, and to be consistent with previous studies, we used uncorrected results and considered $p < 0.05$ to be statistically significant and $p < 0.1$ to be marginally significant

in this exploratory study. The gender differences in the OHC concentrations were compared using the Mann-Whitney Rank Sum test. The correlations between the different OHC concentrations and between the OHCs and occupational exposure durations were examined using Spearman correlation analysis. The statistical analyses were performed using SPSS software version 19 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1 OC Exposure

The sociodemographic characteristics of the participants are shown in Table 1. The enrolled e-waste recycling workers were aged 17–59 years (average of 43 years) and had engaged in e-waste recycling activities at this site for 1–20 years (average of 7.4 years). The participants all lived in this e-waste area (in several villages) and had a low school education level.

The OC concentrations are summarized in Table 2, and the descriptive statistics of the congener-specific values are shown in Table S2. Nineteen PCB congeners were detected in more than 60% of the serum samples. Their total concentrations ranged from 136 to 11,846 ng/g lipid, with a median of 2251 ng/g lipid. PCB118, 28, 153, and 138 collectively contributed 54.1% of the total PCB concentrations. The concentrations of the 19 hydroxylated PCBs ranged from 4.24 to 5233 ng/g lipid, with a median of 418 ng/g lipid. The hydroxylated PCBs were dominated by 4-OH-CB107 (average of 51.7%), followed by 4-OH-CB101, 4'-OH-CB79, 4-OH-CB146, 4-OH-CB187, 3-OH-CB138, and 3-OH-CB118, which together accounted for 32.3% of the total hydroxylated PCB

concentrations. The serum concentrations of PBDEs were between 105 and 35,902 ng/g lipid, with a median of 724 ng/g lipid. The maximum concentrations of PBDEs were observed in a female worker who had an e-waste recycling experience of eight years. BDE209, 207, 47, 206, 197, 183 were the primary congeners in the serum. Because of its lower bioavailability, the measurement of serum BDE209 was rarely included in previous studies (Sjodin et al., 2008; Brasseur et al., 2014; Haraguchi et al., 2016). However, the dominance of highly brominated congeners in the serum of the e-waste workers is likely due to the wide application of deca-BDE mixtures in obsolete electronics. Similar result was also found in serum of the general population in northern China (Zhu et al., 2009).

Our results indicated significant exposure of e-waste recycling workers to OHCs at this site. The PCB serum concentrations in the present study were much higher than those found in e-waste workers (median = 52 ng/g lipid) (Bi et al., 2007) and residents (118 ng/g lipid) (Zhao et al., 2010) at other e-waste sites in China. The PBDE concentrations in the studied cohort were also higher than those found in other e-waste sites, where medians of 81–600 ng/g lipid were reported (Yuan et al., 2008; Zhao et al., 2010). The concentrations in the present study were approximately five times higher than the values reported for the general population in other countries for both PCBs and PBDEs (with comparable congeners) (Sjodin et al., 2008; Park et al., 2009b; Dirtu et al., 2010; Stapleton et al., 2011).

There were no significant gender differences in the serum concentrations of these

contaminants ($p > 0.1$) except for the decaBDEs ($p = 0.015$). The serum OC concentrations were not significantly correlated with the workers' age (Table S3). Instead, we observed significant correlations between the workers' occupational exposure durations and PCBs ($r = 0.40$, $p = 0.003$), hydroxylated PCBs ($r = 0.42$, $p = 0.002$), and octaBDEs ($r = 0.32$, $p = 0.017$). The correlation analysis results for these OHCs indicated that the PCB and OH-PCB concentrations were highly correlated, and the octaBDE and decaBDE concentrations were significantly correlated but had no significant correlations with the pentaBDE concentrations.

3.2 Association between THs and OHCs

The levels of circulating THs in the e-waste workers (Table 2) were generally within the population reference ranges (Quinn et al., 2009). Seven participants had FT₄ levels above the reference range, and two and three participants had TSH levels below and above the reference range, respectively. There were no significant gender differences in the TH levels, except for FT₃. It should be noted that the TH changes within the normal population range may be associated with adverse effects for individuals because the normal range of fluctuations for an individual is narrower than that for a population (Andersen et al., 2002).

The results of the multiple linear regression regarding the associations between the circulating TH levels and OC exposure are shown in Table 3 (for congener-specific values with significant or marginal associations) and Table S4 (for total or sub-total concentrations). Generally, the levels of THs were positively associated with certain

lower-brominated congeners from the pentaBDE mixture. For instance, TT₃ was significantly and positively correlated with BDE47, 66, and 85, and a 10-fold increase in the serum BDE47 concentration was associated with 1.5-fold higher TT₃ levels in the serum [coefficient $\beta = 0.16$, 95% confidence interval (CI): 0.05–0.27]. The associations between TT₄ and PBDEs were significantly or marginally positive for congeners such as BDE66, 85, 153 and 154. However, a marginally inverse association was found between TT₄ and BDE209 ($\beta = -0.036$, 95% CI: -0.079–0.006). The circulating TSH levels also showed inverse associations with some highly brominated BDEs (202, 203, and 208), but there were no associations with the lower-brominated BDEs in the workers. In contrast, there were nearly no apparent associations between the THs and the PCBs or hydroxylated PCBs in the e-waste workers.

Changes in human serum TH levels associated with exposure to PBDEs have been reported in numerous epidemiologic studies, and the results are inconsistent (Table S5) (Julander et al., 2005; Alvarez-Pedrerol et al., 2009; Chevrier et al., 2010; Zhang et al., 2010; Stapleton et al., 2011; Makey et al., 2016). Studies conducted in the general population generally reported increased TT₄ or FT₄ levels in relation to PBDE concentrations (Bloom et al., 2008; Turyk et al., 2008; Dallaire et al., 2009; Stapleton et al., 2011), which was generally in agreement with the observations in the present study. However, Makey et al. (2016) found an inverse association between TT₄ and PBDEs and no consistent associations with FT₄ in North American adults. In maternal and cord blood, PBDE exposure in pregnant women has been found to be negatively associated with

changes in the T_4 levels, as summarized by Abdelouahab et al. (2013). The association between TT_3 or FT_3 and PBDE exposure is relatively less reported, and both non-significant associations (Turyk et al., 2008; Stapleton et al., 2011; Miranda et al., 2015; Makey et al., 2016) and positive associations (Dallaire et al., 2009; Vuong et al., 2015) have been found in a number of studies, similar to our results. A negative association with PBDE exposure was recently reported in Canadian and Korean pregnant women (Abdelouahab et al., 2013; Kim et al., 2013). Recent work by Leonetti et al. (2016) suggest that the correlations between placenta THs and PBDEs are sex-dependent; the authors found that BDE99 was positively associated with T_3 in female infants but that BDE99 and BDE209 were negatively associated with rT_3 in male infants.

In a longitudinal study of e-waste recycling workers, Julander et al. (2005) found significant positive associations between a few PBDEs and THs, such as T_3 and BDE183 and T_4 and BDE28 and 100. Xu et al. (2015) reported no correlations between FT_4 or FT_3 and the total PBDE concentrations in residents from another e-waste site in China. The inconsistent results (also see Table S5) observed at e-waste sites may be partly due to the heterogeneity of the subjects (involving e-waste recycling workers, residents, and pregnant women) and pollutant mixtures (dependent on the e-waste recycled) at these sites.

The positive associations between the THs and PBDEs in the present and many previous studies are not consistent with the results of laboratory animal studies, in which PBDEs typically lead to decreased levels of T_4 and T_3 (but to a lesser extent than T_4)

(Zhou et al., 2001; Tomy et al., 2004). A possible explanation is the difference in the thyroid functions by species. The competition between PBDEs and T₄ for binding sites on the principal binding protein in rodents (Marchesini et al., 2008) might not occur as robustly as that in humans. It was also hypothesized that PBDEs may inhibit the activities of deiodinases, which convert T₄ in peripheral tissues to T₃, leading to an increase in the circulating T₄ levels and a decrease in the T₃ levels (Alvarez-Pedrerol et al., 2009; Stapleton et al., 2011). Nevertheless, the strong positive association between TT₄ and TT₃ ($r = 0.620$, $p < 0.001$) observed in the present study did not support this hypothesis.

Most studies found that PBDE concentrations are not significantly associated with human TSH levels (Table S5). A number of studies reported inverse associations between lower-brominated PBDEs and TSH (Turyk et al., 2008; Chevrier et al., 2010; Stapleton et al., 2011), also inconsistent with the positive associations of laboratory animal studies. In the present study, the TSH showed no significant associations with the lower-brominated BDEs but did show likely inverse associations with several highly brominated congeners (Table 3). Scarce information is available regarding thyroid function with highly brominated PBDEs. In pregnant women in California, reduced TSH levels associated with BDE207 were also reported (Zota et al., 2011). For people living near e-waste site, reduced TSH levels in pregnant women and elevated levels in e-waste workers compared to unexposed cohorts have been reported (Yuan et al., 2008; Zhang et al., 2010). The inverse relationship with TSH has been considered a feedback response to

increased T₄ levels that are regulated by the pituitary (Stapleton et al., 2011). However, the circulating TSH levels were not strongly correlated with the TT₄ levels in e-waste recycling workers ($r = -0.240$, $p = 0.109$), suggesting an inhibited feedback of T₄ on the hypothalamic-pituitary-thyroid (HPT) axis.

For PCBs and hydroxylated PCBs, their associations with THs in the e-waste workers were weak. Most results reported previously demonstrated either nonsignificant or inverse associations (Salay and Garabrant, 2009). A previous study suggested that PCBs may be eliciting more potent endocrine effects on neonatal TH levels via prenatal exposure than PBDEs (Herbstman et al., 2008). However, our findings implied that thyroid homeostasis was affected more by PBDEs than PCBs and hydroxylate PCBs in the e-waste workers who had higher body burdens of these complex pollutant mixtures than the general population. To date, few studies have estimated the association between THs and highly brominated BDEs. Our results indicated potentially diverse effects of these congeners on human circulating TH levels from lower brominated PBDEs. The mechanisms of action of these OHCs underlying the TH disruption in humans are not well understood (Pearce and Braverman, 2009; Stapleton et al., 2011). Apparently, the varied actions of these chemicals on the THs may interfere with the associations between them.

3.3 TH-regulated gene transcripts

Despite the lack of associations between the serum PCB and hydroxylated PCB concentrations and the TH circulating levels, some congeners (mostly PCB28, PCB101,

4'-OH-CB79, and 3-OH-CB118) significantly activated TH-regulated gene transcripts in the leukocytes from the e-waste recycling workers (Table 4). PCB101, a nonplanar ortho-substituted congener that contains a single meta-substitution in both rings, exerted the most potent TH-like effect on gene expression. An association was not observed for the sum concentrations of PCBs or hydroxylated PCBs, except for an association between KLF9 expression and PCBs ($\beta = 0.76$, 95% CI: 0.07–1.46) (Table S6).

Field evidence of changes in TH-regulated gene expression related to OHC exposure is very rare. The increase in the mRNA expression of TR α associated with certain PCBs in the present study was in agreement with the results of the PCB-associated gene expression activation in harbor seals (Tabuchi et al., 2006) and killer whales (Buckman et al., 2011). One difference is that the TR β expression was more susceptible to the PCB exposure than TR α in the e-waste workers; however, in those marine animals, PCBs exerted no significant disruption effect on the TR β transcript levels. Nevertheless, it is noteworthy that THs exert divergent regulatory effects on genes from different tissues to fulfill their function, which may also account for the discrepancy. The activation effect of PCBs shown here was also similar to the results of the TR-regulated genes reported in previous *in vivo* and *in vitro* studies (Gauger et al., 2004; Zoeller, 2005; Ibhazehiebo et al., 2011).

The TH-regulated gene expression in the e-waste workers was also affected by certain PBDE congeners. However, in contrast to the consistently positive associations with PCB and hydroxylated PCB congeners, the associations with PBDEs varied. The gene

expression of TR α and KLF9, a small GC box-binding protein, was significantly upregulated by lower-brominated BDE28, which was similar to the actions of PCBs and hydroxylated PCBs. Cvorovic et al. (2015) proposed the hypothesis that TRs activate the transcription of KLF9 and that both transcription factors modulate each other's activity via multiple pathways. TR α and KLF9 were also found to be co-activated in intestinal stem cells, suggesting that KLF9 was induced by TR α (Kress et al., 2010). The finding in the present study also suggests the possible co-activation of KLF9 and TR α transcription in humans.

However, most of the gene transcripts (TR β , FOXE1, β 3, α v, and TSHR) were suppressed by the highly brominated BDEs (e.g., BDE153, 183, 196, 197, and 207). Only the transcript levels of α v were significantly inhibited by the total PBDE concentrations (β = -1.27, 95% CI: -2.350--0.201). THs play an important role in regulating physiological processes such as growth, development, and metabolism, largely through binding to the nuclear TR α and TR β (Tabuchi et al., 2006). Unlike TR α , which is activated by BDE28, TR β gene expression was suppressed by several highly brominated BDEs. This result was similar to the results of previous reporter gene assays showing that highly brominated BDEs are likely to reduce TR (especially TR β) transcription (Schriks et al., 2007; Ibhazehiebo et al., 2011). FOXE1 plays a crucial role in thyroid morphogenesis and has been reported as a promising biomarker for the detection of colorectal cancer (Szczepanek et al., 2011; Melotte et al., 2015). Montesinos et al. (2016) found that the expression of FOXE1 is stimulated by TSH at the

transcriptional level. Interestingly, we found that both the inhibited expression of FOXE1 and the reduced TSH circulating levels were associated with certain PBDEs in the e-waste workers. Thus, it is not clear whether FOXE1 expression was inhibited directly by these PBDEs or the reduced TSH levels.

The plasma membrane integrin $\alpha v\beta 3$ is a potential receptor for the activation of the nongenomic response by TH and the TH analogs (Barreiro Arcos et al., 2011). The TSHR is also a receptor that largely lies on the outer surface of the cell through which TSH affects various aspects of immunological development and function (Bagriacik and Klein, 2000). The multiple linear regression showed that the TSHR and integrin $\beta 3$ transcripts were less influenced by the OHCs than the other genes in the e-waste workers. In contrast, their expression showed the strongest correlations ($r = -0.365$, $p = 0.067$ for TSHR and $r = -0.294$, $p = 0.066$ for $\beta 3$) (although not significant) with T_3 compared to that of the other genes. This result suggests that in the leukocytes of e-waste workers, the expression of these two genes was, to a large extent, regulated by T_3 .

The circulating TH levels in humans can be influenced by a variety of factors, and TH levels alone may not be a robust indicator of thyroid status. Therefore, we used a number of TH-regulated genes as biomarkers to assess the TH disruption in e-waste recycling workers by these OHCs. Generally, the gene expression was more significantly associated with these OHCs than the hormone levels in the e-waste workers. The results indicated that exposure to these OHCs may interfere with TH signaling, leading to alterations in the TH-regulated gene expression in humans. To the best of our knowledge,

no additional information regarding the association between OC exposure and TH-regulated gene expression in humans is available. In a field study in harbor seals (Tabuchi et al., 2006) and a few laboratory studies in fetal rats (Zoeller, 2005), activated TH-regulated genes (such as TR α and TR β) and reduced circulating TH levels associated with PCB exposure have been found. In e-waste workers, we found that PCB28 was associated with reduced T₃ levels and increased TR β gene expression. A possible explanation is that the activated TR target gene expression enhanced the negative feedback effect of TH on TSH, which is mediated, at least partially, by TR β , as suggested by Zoeller et al. (2007).

The reasons for the difference in the disruption of gene expression between PBDEs and PCBs are not clear. It was suggested in two reporter gene assay studies that PBDEs and PCBs inhibited TH-regulated gene expression in monkey fibroblast-derived CV-1 and human medulloblastoma-derived TE671 cells via a common mechanism of dissociation of TR from TH response element (TRE) (Miyazaki et al., 2008; Ibhazehiebo et al., 2011). In an *in vitro* study, Ren et al. (2013) found that OH-PBDEs bind to TR with different binding affinity and exert agonistic or antagonistic activities depending on their degree of bromination (with highly brominated compounds being the antagonists). The authors attributed this result to the different binding geometries within the ligand binding pocket of the TR. This could also be a possible explanation for the different disruption effects of PCBs and PBDEs on gene expression.

We revealed significantly suppressed gene expression by the highly brominated

PBDEs that were mainly derived from the technical decaBDE mixture. This PBDE product is still used widely in many countries and has been found at high concentrations in indoor dust (Besis and Samara, 2012). Indoor dust ingestion constitutes a major portion of exposure to PBDEs in average people. Further research focused on the general population, particularly on children who have a higher dust intake than adults, are therefore needed. The health consequences of the gene expression alterations in humans are currently unknown. However, it is reasonable to speculate that the TH-regulated gene expression alterations associated with OHC exposure can occur in the brain because OHCs are able to cross the blood–brain barrier and accumulate in the brain (Miyazaki et al., 2008; Ibhazehiebo et al., 2011) and may disrupt normal brain development. There is a growing body of evidence indicating that these chemicals are developmental neurotoxicants.

4. Conclusion

This study is the first effort to explore the associations of circulating TH levels and TH-regulated gene expression with exposure to PBDEs, PCBs, and hydroxylated PCBs in humans. This study has limitations due to its small sample size and the failure to measure the serum hydroxylated PBDEs, which may interfere with thyroid homeostasis. Nevertheless, our results revealed that the TH-regulated gene transcription in leukocytes was disrupted by many of the contaminants released by e-waste recycling. Relatively, the TH circulating levels were associated with only a few PBDEs. Although it is unknown whether the gene expression alterations in peripheral leukocytes will affect

immunological development and function, the findings of our study provide implications for the potentially adverse effects of these chemicals on human health using novel gene expression endpoints. The present study also highlights the important potential health impact of highly brominated PBDEs, which are still widely used. Additional research is needed to investigate the mechanisms of action by which OHCs disrupt gene expression and the associated biological consequences.

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Tables

Table 1. Basic cohort characteristics of the e-waste recycling workers.^a

Characteristic	Range (mean $\pm$ SD) or percentage	
	Male (n = 33)	Female (n = 36)
Age (yr)	22-57 (42 $\pm$ 8)	22-59 (44 $\pm$ 8)
OED (yr)	1-20 (7 $\pm$ 4)	2-20 (8 $\pm$ 2)
BMI (kg/m ²)	18.3-28.7 (23.7 $\pm$ 2.6)	17.5-30.8 (23.5 $\pm$ 3.4)
Serum lipids (%)	0.28-1.03 (0.49 $\pm$ 0.16)	0.29-0.95 (0.45 $\pm$ 0.11)
Smoke	64%	2.8%

^a OED, occupational exposure duration; BMI, body mass index.

Table 2. Serum concentrations of PCBs, hydroxylated PCBs, and PBDEs (ng/g lipid) and the levels of thyroid hormones in e-waste recycling workers.^{a,b}

Analytes	Male			Female		
	Range	mean	Median	Range	mean	Median
PCBs	204-11846	3173	2603	136-11117	3423	2447
OH-PCBs	40.2-5233	1239	520	4.24-4161	923	452
Penta-BDEs	17.1-242	104	92.7	44.6-853	125	100
Octa-BDEs	8.02-283	77.3	62.1	4.58-2667	287	84.2
Deca-BDEs*	64.5-1494	509	409	100-34482	1896	743
PBDEs*	105-1806	690	572	206-35902	2309	1092
TT ₃ (nmol/L)	1.08-2.57	1.86	1.86	1.11-3.61	1.73	1.68
TT ₄ (nmol/L)	58.7-136	99.2	98.7	53.4-173	103	98.6
FT ₃ (pmol/L)**	3.76-5.47	4.73	4.79	3.12-8.34	4.45	4.30
FT ₄ (pmol/L)	12.2-22.0	16.67	16.8	9.03-33.3	16.2	15.7
TSH (mIU/L)	0.30-3.20	1.56	1.40	0.30-9.40	2.01	1.40

^a PCBs include PCB28, 74, 66/95, 99, 110, 146, 153, 138, 187, 177, 180, 164, 190, 101, 118, 105, 128, and 209. OH-PCBs include 4'-OH-CB79, 4-OH-CB101, 4'-OH-CB120, 3-OH-CB118, 4-OH-CB107, 3-OH-CB153, 4'-OH-CB146, 3-OH-CB138, 4-OH-CB130, 3'-OH-CB182, 4-OH-CB178, 3-OH-CB183, 4-OH-CB163, 4-OH-CB187, 4-OH-CB202, 4'-OH-CB172, 4-OH-CB199, and 4'-OH-CB208. Penta-BDEs include BDE28, 47, 66, 85, 99, and 100. Octa-BDEs include BDE153, 154, and 183. Deca-BDEs include BDE196, 197, 202, 203, 206, 207, 208, and 209. PBDEs include all these congeners.

^b *, gender difference is significant at $p < 0.05$; **, gender difference is significant at $p < 0.01$.

Table 3. Multiple linear regression coefficients (95% CI) for significant ($p < 0.05$) or marginal ($p < 0.1$) associations between specific PBDE, PCB, and hydroxylated PCB congeners and circulating TH levels.^a

TH	β	95% CI	p-value	OC
TT3	0.161	(0.051, 0.271)	0.005	BDE47
TT3	0.117	(0.029, 0.204)	0.010	BDE66
TT3	0.172	(0.056, 0.288)	0.004	BDE85
TT4	0.154	(0.033, 0.276)	0.014	BDE85
TT4	0.044	(-0.005, 0.093)	0.078	BDE153
TT4	0.161	(0.035, 0.286)	0.013	BDE66
TT4	0.045	(-0.006, 0.096)	0.085	BDE154
TT4	-0.036	(-0.079, 0.006)	0.088	BDE209
TSH	-0.207	(-0.444, 0.030)	0.086	BDE202
TSH	-0.209	(-0.434, 0.015)	0.067	BDE203
TSH	-0.172	(-0.373, 0.030)	0.094	BDE208
FT3	-0.035	(-0.061, -0.009)	0.010	PCB28
FT3	0.070	(0.006, 0.135)	0.033	BDE66
FT3	0.115	(0.028, 0.203)	0.011	BDE85
FT4	0.106	(0.021, 0.190)	0.016	BDE66
FT4	0.139	(0.021, 0.258)	0.022	BDE85
FT4	0.060	(-0.011, 0.130)	0.093	BDE153

^a Adjusted for sex, age (yr), occupational exposure duration of e-waste recycling (yr), and body mass index (BMI). The OC and gene expression values were log-transformed.

Table 4. Multiple linear regression coefficients (95% CI) for significant ($p < 0.05$) or marginal ($p < 0.1$) associations between specific PBDE, PCB, and hydroxylated PCB congeners and TH-regulated gene expression.^a

TH	β	95% CI	p-value	OC	TH	β	95% CI	p-value	OC
TR α	0.66	(0.213, 1.106)	0.005	PCB101	FOXE1	0.671	(-0.024, 1.367)	0.058	PCB28
TR α	0.738	(0.167, 1.310)	0.013	4'-OH-CB79	FOXE1	1.032	(0.494, 1.571)	0.000	PCB101
TR α	0.788	(0.130, 1.446)	0.020	3-OH-CB118	FOXE1	0.99	(0.291, 1.689)	0.007	4'-OH-CB79
TR α	0.957	(0.065, 1.849)	0.036	BDE28	FOXE1	-1.875	(-3.964, 0.215)	0.077	BDE66
					FOXE1	-2.045	(-3.969, -0.122)	0.038	BDE85
TR β	0.791	(0.095, 1.488)	0.027	PCB28	FOXE1	-0.755	(-1.478, -0.032)	0.041	BDE153
TR β	0.934	(0.373, 1.494)	0.002	PCB101	FOXE1	-0.836	(-1.520, -0.152)	0.018	BDE183
TR β	1.042	(0.269, 1.814)	0.010	4'-OH-CB79	FOXE1	-0.611	(-1.215, -0.007)	0.048	BDE196
TR β	0.814	(-0.107, 1.736)	0.081	3-OH-CB118					
TR β	-0.878	(-1.734, -0.021)	0.045	BDE153	KLF9	0.657	(0.141, 1.173)	0.014	PCB28
TR β	-0.861	(-1.642, -0.079)	0.032	BDE183	KLF9	0.434	(0.065, 0.803)	0.023	PCB66
TR β	-0.659	(-1.285, -0.032)	0.040	BDE196	KLF9	0.527	(0.032, 1.021)	0.038	PCB110
					KLF9	0.868	(0.478, 1.258)	0.000	PCB101
α v	0.726	(0.314, 1.139)	0.001	PCB101	KLF9	1.024	(0.344, 1.704)	0.005	PCB118
α v	0.762	(0.180, 1.343)	0.012	4'-OH-CB79	KLF9	0.688	(0.106, 1.271)	0.022	4'-OH-CB79
α v	-0.811	(-1.451, -0.170)	0.015	BDE153	KLF9	0.584	(-0.062, 1.230)	0.075	4'-OH-CB120
α v	-0.609	(-1.224, 0.007)	0.052	BDE183	KLF9	0.826	(0.219, 1.433)	0.009	3-OH-CB118
α v	-0.736	(-1.392, -0.079)	0.030	BDE197	KLF9	1.712	(0.437, 2.987)	0.010	BDE28
α v	-1.111	(-2.081, -0.142)	0.026	BDE202	KLF9	0.601	(-0.114, 1.316)	0.096	BDE100
α v	-1.161	(-2.130, -0.191)	0.021	BDE207					

αv	-0.82 5	(-1.660, 0.011)	0.053	BDE208	$\beta 3$	0.506	(0.058, 0.953)	0.02 9	PCB101
					$\beta 3$	-0.705	(-1.332, -0.077)	0.02 9	BDE183
TSH R	0.536	(0.013, 1.059)	0.045	PCB101	$\beta 3$	-0.732	(-1.463, -0.002)	0.04 9	BDE197
TSH R	-0.57 2	(-1.221, 0.076)	0.081	BDE197	$\beta 3$	-1.112	(-2.330, 0.106)	0.07 2	BDE207

^a Adjusted for sex, age (yr), occupational exposure duration of e-waste recycling (yr), and body mass index (BMI). The OC and gene expression values were log-transformed.