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

Jing Zheng [a], Chun-Tao He [c], She-Jun Chen [b,*], Xiao Yan [a], Mi-Na Guo [d], Mei-Huan Wang [a], Yun-Jiang Yu [a], Zhong-Yi Yang [c], Bi-Xian Mai [b,*]

[a] Center for Environmental Health Research, South China Institute of Environmental Sciences, Ministry of Environmental Protection, Guangzhou, China
[b] State Key Laboratory of Organic Geochemistry, Guangdong Provincial Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China
[c] State Key Laboratory for Biocontrol, School of Life Sciences, Sun Yat-Sen University, Guangzhou, China
[d] College of Natural Resources, University of California-Berkeley, Berkeley, CA, USA

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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 hormones (THs), 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 research is needed to investigate the mechanisms of action and associated biological consequences of the gene expression disruption by OHCs.

1. Introduction

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

Thyroid hormones (THs) are essential for the normal development and maintenance of normal physiological functions and play a critical role in fetal brain development (Abdelouahab et al., 2013). Evidence from experimental and human studies has shown a significant

* Corresponding authors at: Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China.
E-mail addresses: chenshejun@gig.ac.cn (S.-J. Chen), nancymai@gig.ac.cn (B.-X. Mai).

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

Gene expression endpoints mediate the majority of the biological actions of thyroid hormones and are considered more promising approaches for the characterization of the thyroid-toxic potential of anthropogenic contaminants (Buckman et al., 2011; Ibhazehiebo et al., 2011). Moreover, gene expression analysis identifies the early detection symptoms of contaminant exposure before the manifestation of higher-level health effects (Tabuchi et al., 2006). Studies have linked changes in TR gene expression to PCB exposure in wildlife species (Tabuchi et al., 2006; Buckman et al., 2011). Wadzinski et al. (2014) recently found that the expression of dioxin-inducible enzyme (CYP1A1) is strongly associated with that of TH-regulated target genes in human placenta. However, to the best of our knowledge, the alterations in TH-regulated gene 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 (TT_4), free T_4 (FT_4), total triiodothyronine (TT_3), FT_3, 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 (TRα and TRβ), forkhead box E1 (FOXE1, thyroid transcription factor 2), transcript factor Kruppel-like factor 9 (KLF9, also known as basic transcription element-binding protein), TSH receptor (TSHR), and plasma membrane integrin αv and β3, 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 μL 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 × 0.25 mm × 0.25 μm film thickness) and a DB-XLB capillary column (30 m × 0.25 mm × 0.25 μm 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, T_3, T_4, FT_3 and FT_4 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, FT_3, FT_4 and TSH were tested three times before measuring the serum samples. The coefficients of variations were <6.3%, and the deviations of the SRMs were <13%.

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