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Dioxin-related compounds in breast milk of women from Vietnamese e-waste recycling sites: Levels, toxic equivalents and relevance of non-dietary exposure

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ABSTRACT

Although informal e-waste recycling sites (EWRSs) are hotspots of both polychlorinated and polybrominated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs and PBDD/Fs), human exposure to the latter has not been studied in details. This study investigated the accumulation levels and profiles of dioxin-related compounds (DRCs) in breast milk samples from women living in two Vietnamese EWRSs and estimated the intake contribution from e-waste-related exposure. Screening results using Dioxin-Responsive Chemically Activated LUciferase gene eXpression assay (DR-CALUX) showed higher dioxin-like (DL) activities in samples from the EWRS Bui Dau than in those from the EWRS Trang Minh and a reference site (2.3–10 vs 1.7–4.8 and 0.60–5.7 pg CALUX-TEQ/g lipid, $n=10$, 6 and 9, respectively). Chemical analysis results of selected samples show that the WHO-TEQ levels of PCDD/Fs, DL-PCBs and PBDD/Fs in EWRS samples were not significantly higher than in those from the reference site (0.22–7.4 vs 1.1–3.0 pg/g lipid) and within the Vietnamese background range, but women involved in recycling accumulated higher concentrations of PCDFs (13–15 vs 2.3–8.8 pg/g lipid) and PBDFs (1.1–1.5 vs <1.1 pg/g lipid). By comparing the DRC profile in milk of these women with the reported profile in house dust from the same site, dust ingestion was estimated to contribute most of the intake for tetraBDF, 37 per cent to 55 per cent for penta–octaCDFs, but less than twenty per cent for PCDDs and DL-PCBs, and 26 per cent for total WHO-TEQs. The DL activities in some EWRS milk samples were not fully explained by chemical data, suggesting contribution from unidentified compounds. The estimated WHO-TEQ intake doses for breastfed infants (1.3–33 pg/kg/d) mostly exceeded the tolerable value, especially for those living in the EWRSs; and unidentified DRCs might increase further the dioxin-related health risk.

1. Introduction

Rapid growth in the amount of waste electrical and electronic equipment (WEEE), also known as e-waste, and continuing informal recycling activities of these materials pose serious risks of environmental pollution and human health impacts (Zhang et al., 2012). Primitive e-waste processing methods such as acid-stripping and open burning of wires for metal retrieval, heating circuit boards for dismantling, and chipping and melting of plastics not only facilitate the release of hazardous chemicals contained in e-waste (e.g. toxic metals, brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs), etc.), but also generate dioxin-related compounds (DRCs)—including polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDD/Fs), their brominated (PBDD/Fs) and mixed brominated/chlorinated homologues (PXDD/Fs)—as secondary pollutants (Tue et al., 2013b).

The occurrence of PBDD/Fs in EWRSs has been associated with high content of BFRs, especially polybrominated diphenyl ethers (PBDEs), in e-waste plastics (Ma et al., 2009; Tue et al., 2010). Technical PBDE formulations have been found to contain PBDFs as impurities (Hanari et al., 2006). PBDD/Fs can be generated from these precursors not only during waste incineration (Duan et al., 2011; Weber and Kuch, 2003), as is the case with the more well known PCDD/Fs, but also through degradation at <300 °C (Weber and Kuch, 2003) or under natural light (Kajiwara et al., 2008).

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PBDD/Fs have been found in soil, dust and air of Asian e-waste recycling sites (EWRSs) at concentrations greater than those of PCDD/Fs (Tue et al., 2013b). Considering that PBDD/Fs can produce dioxin-like (DL) toxic effects by activating the aryl hydrocarbon receptor (AhR) signalling pathway with similar relative effect potencies (REPs) to those of PCDD/Fs, and have recently been recommended for inclusion in the World Health Organisation Toxicity Equivalency Factor (WHO-TEF) concept (van den Berg et al., 2013), it is important to include PBDD/Fs in the assessment of human exposure and potential risks related to DRCs released from informal e-waste recycling.

Environmental contamination with PCDD/Fs in EWRSs resulted in high accumulation in human via soil/dust ingestion, inhalation and consumption of contaminated local food, as reported in various studies in China (reviewed by Chan and Wong, 2013). For PBDD/Fs, although high exposure levels from non-dietary sources have been estimated (Tue et al., 2013b), information on the levels of these DRCs in EWRS residents and their diet is notably lacking. Such information will be a useful addition to the scarce data on PBDD/Fs in human, currently available only for small numbers of adipose tissue and breast milk samples (in the range of a few picogram per gram lipid; Choi et al., 2003; Ericson Jogsten et al., 2010; Croes et al., 2013). The present study investigated breast milk samples as bioindicators for human exposure to DRCs in two Vietnamese EWRSs, where our previous survey found relatively high PBDF concentrations in house dust (median 23–49 ng/g; Tue et al., 2010). We aimed to elucidate the accumulation levels and profiles of DRCs including PBDD/Fs as well as the relevance of non-dietary exposure for human intake, to evaluate the contribution of various DRCs to the total DL toxicity levels in breast milk and to assess the risk for breastfeeding infants. Using a combined *in vitro* bioassay/instrumental analysis approach, we used the Dioxin-Responsive Chemically Activated LUciferase gene eXpression (DR-CALUX) assay as a tool for screening and measuring the total DL toxicity of all potential DRCs present in the breast milk samples.

2. Material and Methods

2.1. Sample collection

This sampling was conducted in three locations in northern Vietnam: two e-waste recycling sites Trang Minh (TM, Kien An district, Hai Phong city) and Bui Dau (BD, My Hao district, Hung Yen province), and a reference site Thach Hoa (TH, Thach That district, suburban Hanoi). Information on the populations and recycling activities of the EWRSs has been provided in our previous reports (Tue et al., 2010, 2013a). Breast milk was chosen as the bioindicator because the large collectable volume and relatively high lipid content are suitable for studying the human accumulation of lipophilic contaminants such as DRCs from e-waste recycling. Milk samples ($n=6$, 9, and 10 for TH, TM and BD, respectively) were collected in September 2008 with informed consent of the donors. All donors were healthy and non-smokers (see supplementary Table S1 for further details). Milk was expressed by the donor or with the help of a midwife into a solvent-precleaned glass bottle with Teflon-lined screw caps. All samples were kept with gel ice immediately and then sent within eight hours to our laboratory at Hanoi University of Science and frozen at −20 °C. The frozen samples were later air-transported with gel ice to Ehime University and stored at −25 °C until analysis.

2.2. Sample extraction

Approximately 50 g of milk sample was freeze-dried and extracted with an acetone/hexane mixture (1:1 v/v) using a rapid solvent extractor (SE100, Mitsubishi Chemical Analytech, Japan) for 30 min at 35 °C and a flow rate of 10 ml/min. Five per cent of the extract was used for gravimetric determination of the lipid content, and the remaining extract was subjected to gel permeation chromatography for lipid removal. Two portions equivalent to 13.5 and 25 g wet weight were used for DR-CALUX and chemical analysis, respectively. A procedural blank was analysed with every set of seven samples.

2.3. DR-CALUX assay

To remove non-persistent compounds such as polyaromatic hydrocarbons (PAHs) which are not considered as DRCs, the extract was treated with sulphuric acid-impregnated silica gel column according to a previously reported method (Tue et al., 2010), then concentrated and solvent-exchanged into 50 μL biochemical-grade dimethyl sulphoxide (DMSO) for bioassay. Dioxin-like activity was measured using DR-CALUX assay with a rat hepatoma cell line having an AhR-regulated luciferase gene construct (H4IIE-luc, BioDetection Systems b.v., The Netherlands). The culture conditions, assay procedures and data analysis followed those used in our previous reports (Suzuki et al., 2010; Tue et al., 2010). All measurements satisfied the criteria of the standard operating procedure regarding the calculated EC_{50} of 2,3,7,8-CDD (TCDD) (11.3 ± 1.1 pM, $n=25$) and the maximum induction relative to DMSO control (8.6 ± 1.6). Results were expressed in picogram CALUX TCDD-equivalent (CALUX-TEQ) per gram milk lipid.

2.4. Chemical analyses of DRCs

Fourteen selected milk extracts containing more than 0.03 pg CALUX-TEQ/g wet weight were spiked with $^{13}C_{12}$-labelled surrogates (2,3,7,8-substituted tetra–octaCDD/Fs, tetra–hexaBDD/Fs and DL-PCBs) and then subjected to additional clean-up with multilayer silica gel column (silica, ten per cent $AgNO_3$-impregnated silica, silica, 22 per cent H_2SO_4-impregnated silica, 44 per cent H_2SO_4-impregnated silica and silica in bottom-up order) and separation over activated carbon-impregnated silica gel column. $^{13}C_{12}$-labelled 1,2,3,4-TeCDD, 1,2,3,4,6,9-HxCDF, 1,2,3,4,6,8,9-HpCDF were added to the PCDD/F-PBDD/F fraction and $^{13}C_{12}$-CB-111 to the DL-PCB fraction as internal standards. 2,3,7,8-substituted PCDD/Fs, PBDD/Fs and DL-PCBs were quantified using a gas chromatograph (HP-6890, Agilent) and a high resolution mass spectrometer (AutoSpec Ultima, Waters). PCDD/Fs and DL-PCBs were separated using a BPX-DXN column (60 m × 0.25 mm, SGE, Australia) with a temperature programme of 20 °C/min from 150 to 220 °C, 2 °C/min to 260 °C, 5 °C/min to 320 °C, hold 8 min, and a RH-12ms column (60 m × 0.25 mm, Inventx, USA) with a programme of 20 °C/min from 150 to 210 °C, 3 °C/min to 280 °C, 10 °C/min to 320 °C, hold 13 min. PBDD/Fs were separated using a DB-5MS column (15 m × 0.25 mm, Agilent) and a programme of 120 °C for one minute, 20 °C/min to 240 °C, 10 °C/min to 300 °C and hold thirteen minutes. All analyses were carried out by an accredited laboratory (Shimadzu Techno-Research Inc., Japan) which frequently participates in worldwide intercalibration studies for PCDD/Fs, DL-PCBs (UNEP, 2005) and PBDD/Fs (Takahashi et al., 2006). The average recoveries of the ^{13}C-labelled surrogates were 80–97 per cent, 66–114 per cent and 95–110 per cent for PCDD/Fs, DL-PCBs and PBDD/Fs, respectively. Results were expressed in picogram per gram milk lipid. WHO-TEQs were calculated for PCDD/Fs and DL-PCBs using the World Health Organisation toxic equivalency factors (TEFs) (van den Berg et al., 2006) and for PBDD/Fs using TEFs of similarly substituted PCDD/Fs (van den Berg et al., 2013).

3. Results and discussion

3.1. Dioxin-like activities and accumulation levels of DRCs in human milk

All sulphuric acid-treated human milk extracts exhibited DL activities as evidenced by the dose-dependent luciferase induction in the DR-CALUX assay (supplementary Fig. S1). The CALUX-TEQ levels were 0.60–10 pg/g lipid in the whole sample set (Table 1). The levels in TM and TH were not different but those from BD were significantly higher (Wilcoxon rank sum test $p < 0.05$), showing 3.2 and 1.7-fold difference in terms of medians compared with TH and TM, respectively. The minimum CALUX-TEQ levels in the EWRSs were still the same or higher than the median in TH.

PCDD/Fs and DL-PCBs were detected in all fourteen samples selected for chemical analysis. PBDFs were found in only two samples whereas PBDDs were not detected (Table 1). In general, there was no significant difference in DRCs concentrations for the three study locations. However, the two samples from BD recyclers had considerably higher concentrations of PCDFs (13–15 pg/g lipid) and DL-PCBs (6600–7600 pg/g lipid) as compared with TH and TM samples (2.3–8.8 and 430–5000 pg/g lipid, respectively). These were also the only samples with detectable levels of PBDFs (1.1–1.5 pg/g lipid). In general, the most abundant among PCDD/Fs were OCDD and 1,2,3,4,6,7,8-HpCDD (42–56 per cent and 10–13 per cent of total concentrations), and among DL-PCBs were