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ABSTRACT

Phosphate flame retardants (PFRs) and novel brominated flame retardants (NBFRs) (2-ethylhexyl-2,3,4,5-tetrabromo-benzoate (EH-TBB) and bis-(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (BEH-TEBP)) were measured in free-range chicken eggs from three e-waste recycling sites and a negative control site located in Guangdong province, Southern China. BEH-TEBP, tris-(chloroethyl)-phosphate (TCEP), tris-(chloropropyl)-phosphate (∑TCPP, two isomers) and tris-(1,3-dichloroisopropyl)-phosphate (TDCIPP) were detected in more than 50% of eggs samples with low concentrations. The median values of BEH-TEBP and total PFRs were 0.17-0.46 ng/g ww (wet weight) and 1.62-2.59 ng/g ww in eggs from the e-waste sites, respectively. The results indicate that EH-TBB, BEH-TEBP and PFRs are less persistent and bioaccumulative than polybrominated diphenyl ethers (PBDEs) in chicken eggs, and possibly also in other bio-matrices. Triphenyl phosphate (TPHP) were identified in albumen with higher frequencies, but at similar concentrations compared to yolk, while BEH-TEBP was mainly detected in yolk. The estimated daily intake (EDI) of BEH-TEBP and total PFRs from consumption of chicken eggs ranged from 0.03-0.09 and 0.32-0.52 ng/kg bw/day for adults, and 0.20-0.54 and 1.89-3.02 ng/kg bw/day for children in e-waste sites, respectively. Indoor dust ingestion seems to be a more important pathway for the intake of these FRs, while egg consumption is probably a more important exposure pathway for PBDEs.

Keywords: Flame retardants; dietary exposure; home-produced eggs; e-waste recycling region

1. Introduction

With the ban of polybrominated diphenyl ethers (PBDEs) in Europe and the United States (European Court of Justice, 2008; UNEP, 2009), the usage of alternative flame retardants (FRs), like 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), bis-(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (BEH-TEBP) and phosphate flame retardants (PFRs), has significantly increased in recent years (Covaci et al, 2011; van der Veen and de Boer, 2012; Wei et al, 2015). As novel brominated flame retardants (NBFRs), EH-TBB and BEH-TEBP have been used as alternative of Penta-BDE (Covaci et al, 2011; Roberts et al, 2012). They have been reported as major components of Firemaster 550, a commercial fire safety additive, with a ratio of 4:1 (Stapleton et al, 2008), while another additive FR product, BZ-45, was also found to contain them (Davis and Stapleton, 2009). PFRs, such as tris-(1-chloro-isopropyl)-phosphate (TCIPP), triphenyl phosphate (TPHP) and tris-(2-butoxyethyl)-phosphate (TBOEP), have been widely used in commercial products such in textures, cable coating, paints, foams and electronic products, and also being applied as plasticizers or additives in lubricant (van der Veen and de Boer, 2012). EH-TBB, BEH-TEBP and PFRs contain ester bonds in their chemical structures, given them less persistent properties and different bioaccumulation characters comparing with persistent FRs, such as PBDEs, dechlorane plus (DPs), decabromodiphenyl ethane (DBDPE), or 1,2-bis-(2,4,6-tribromophenoxy) ethane (BTBPE).

The occurrence of NBFRs and PFRs has been frequently reported in environment, including air, indoor dust, soil, and sediment (Brandsma et al, 2015; Cequier et al, 2014; Kim et al, 2014; Liu et al, 2014), but limited studies investigated their levels in biota, such as fish,

wild birds, mammals or humans (van der Veen and de Boer, 2012; Wei et al, 2015). Sundkvist et al. (2010) detected TCIPP (0.4-16 ng/g ww, wet weight), TPHP (0.04-2.3 ng/g ww) and TBOEP (0.86-4.2 ng/g ww) in fish tissues, which had similar PFR levels with those in fish from Netherland (Brandsma et al, 2015). Tri-ethylhexyl phosphate (TEHP) (6 ng/g ww) and 2-ethylhexyl diphenyl phosphate (EHDPHP) (3.5 ng/g ww) were reported as main PFRs in fish from Philippines (Kim et al, 2011a, b). PFR values in fish tissues from the Dutch North Sea were not correlated with lipid percentage (Brandsma et al, 2015), suggesting that PFRs do not follow the same distribution as lipids like other PBDEs (Brandsma et al, 2015, Malarvannan et al, 2015). Based on the limited studies on PFRs in creatures, the bioaccumulation of PFRs seemed to be different from PBDEs (Brandsma et al, 2015, Kim et al, 2011a, b; Malarvannan et al, 2015; Sundkvist et al, 2010). EH-TBB and BEH-TEBP might undergo hydrolysis in gastrointestinal tract, with 70% degradation of BEH-TEBP being reported in a gastrointestinal absorption model (Fang and Stapleton, 2014). The rapid hepatic elimination of tris-(1,3-dichloroisopropyl)-phosphate (TDCIPP) was reported in an *in vitro* study using chicken hepatocytes, where TDCIPP was all transformed into bis-(1,3-dichloroisopropyl) phosphate (BDCIPP) in 36 h (Farhat et al, 2014).

Qingyuan is one of the largest e-waste recycling areas in China. However, the improper handling process during e-waste recycling leads to terrible pollution to regional environment and seriously threatens the health of on-site workers and local residents. The environmental contamination in e-waste recycling regions could further pass down to local agriculture and eventually threaten food safety (Song and Li, 2014).

Dietary intake has been considered as an important human exposure pathway for

persistent FRs, but data about less persistent FRs in food is still limited (Domingo, 2014; Xu et al, 2015). Eggs from free-range chicken were recognized as to have higher level of organic pollutants than eggs from caged chicken and other food, due to the (more) intensive contact of hens with the environment (Covaci et al, 2009; Domingo, 2014). In our previous studies, intakes of persistent FRs (including PBDE, DPs and DBDPE) via local home-produced free-range egg consumption were proved to be a great threat to health of the locals in e-waste recycling area (Zheng et al, 2012). As a follow-up study, we aimed (1) to investigate the extent of contamination of EH-TBB, BEH-TEBP and PFRs in free-range chicken eggs from e-waste recycling region; and (2) to assess the human exposure risks of these FRs via egg consumption for local residents. To the best of our knowledge, this is the first study on human dietary exposure of PFRs in e-waste recycling sites.

2. Methods and materials

2.1. Sampling

Free-range chicken eggs (N = 45) were collected from three villages that rely on e-waste recycling business (Site 1, N 23°32′ E 113°03′; Site 2, N 23°36′ E 113°04′; and Site 3, N 23°34′ E 113°02′) and a negative control site (N 23°34′ E 113°03′) in Qingyuan (Guangdong Province, China) in July, 2010. The free-ranged hens were raised on the recycling sites where the e-waste was primitively dismantled and extracted. The control site is about 5 km away from other three recycling sites and free of e-waste recycling activities. More details about sampling areas were provided in our previous study (Zheng et al, 2012). The collected egg samples were transported to our laboratory within 12 h. The albumen of eight eggs was

separated from yolk, and analyzed individually, while the remains of each egg were homogenized. All samples were then lyophilized individually, and the water content of each sample was gravimetrically determined. Dry samples were packed with aluminum foil, sealed in zip bags and stored at -20 °C until analysis.

2.2. Chemicals and materials

Standards of EH-TBB, BEH-TEBP and their isotope labeled internal standards (IS) $^{13}C_6$-BEH-TEBP-D_{34} (MBEH-TEBP) and $^{13}C_6$-EH-TBB-D_{17} (MEH-TBB) were purchased from Wellington Laboratories (Guelph, ON, Canada). Standards of tricresyl phosphate (TMPP, mixtures of 4 isomers), TEHP, EHDPHP, tri-n-propyl phosphate (TNPP), tri-n-butyl phosphate (TNBP), TPHP, tris(2-chloroethyl) phosphate (TCEP) and TDCIPP were purchased from Chiron AS (Trondheim, Norway). TCPP mixture (2 isomers, tris (1-chloroisopropyl) phosphate (TCIPP) is the major component) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Triamyl phosphate (TAP, IS) was purchased from TCI Europe (Zwijndrecht, Belgium). Isotope labeled IS of PFRs, TCEP-D_{12}, TDCIPP-D_{15}, TPHP-D_{15}, and TBOEP-D_6 were synthesized by Dr. Vladimir Belov (Max Plank, Germany) and had a purity of >98%. TBOEP standard was purchased from Acros (Belgium) and had a purity of 94%. DSC-18 sorbent, Z-SEP sorbent and Supelclean™ ENVI™-Florisil® cartridges (500 mg, 3 mL) were purchased from Supelco (Bellefonte, PA, USA). Aminopropyl silica (APS) cartridges (500 mg, 3 mL) were purchased from Agilent (Santa Clara, CA, USA). Silica gel, anhydrous magnesium sulfate ($MgSO_4$), concentrated sulfuric acid (H_2SO_4, 98%) and all solvent in used (chromatography grade) were purchased from Merck (Darmstadt, Germany).

2.3. Sample preparation and analysis

The sample preparation and analysis method were described in details by Xu et al, (2015) and are given in the Supplementary Information (SI). Briefly, about 2 g freeze-dried yolk or whole egg sample (for albumen only 1 g was used) was spiked with IS (MEH-TBB, MBEH-TEBP, TAP, TPHP-D_{15}, TDCIPP-D_{15} and TCEP-D_{12}), then extracted with ultrasonication and vortexation in 5 mL acetonitrile:toluene (9:1, v/v). After solvent exchange to hexane, the extract was performed with multi-step clean-up to further remove lipid and pigment. First, the extract was fractionated on a Florisil cartridge: the first fraction (F1) was eluted with 8 mL hexane and the second fraction (F2) was eluted with 5 mL acetonitrile. After concentrated under a gentle nitrogen flow, the F1 was further cleaned-up on 2 g acid silica (10%, pre-cleaned with 6 mL hexane) with 10 mL hexane:dichloromethane (1:1, v/v, F3). F2 was concentrated to 2.5 mL, adding with 200 mg Z-SEP/DSC18 mixture sorbent to perform dispersive SPE for the removal of interference. After centrifugation, the supernatant of F2 was combined with F3, then, solvent-exchange to 2 mL of hexane, which was further fractionated on an APS cartridge. Elution was performed with 10 mL hexane (F4) and 12 mL hexane:dichloromethane (1:1, v/v, F5). Both fractions were evaporated to nearly dryness, and then resolubilized in 100 μL of iso-octane prior to GC-MS analysis.

EH-TBB and BEH-TEBP (in F4) were analyzed by 6890 Agilent (Palo Alto, CA, USA) gas chromatography (GC) coupled to a 5973 mass spectrometer (MS) operated in electron capture negative ionization (ECNI). A DB-5 column (15 m × 0.25 mm × 0.10 μm) was used for separation and the MS was deployed in selected ion monitoring (SIM) mode. The analysis

of PFRs (in F5) was performed by GC-MS in electron ionization (EI) mode. A HT-8 column (25 m × 0.22 mm × 0.25 μm) was used and the MS was operated in SIM mode with two characteristic ions acquired for each compound. Detailed information about analytical parameters was provided by Xu et al. (2015).

2.4. Quantification and quality assurance

For quality control, procedural blanks (N = 9 in total) and spiked egg samples (N = 6 in total) were added in the each batch of sample extraction. All target analytes were blank-corrected. The recoveries of target compounds in spiked egg samples ranged from 98 ± 12% to 134 ± 38%. The limits of quantification (LOQs, table 1) were set as the mean values plus three times standard deviations of blanks. For the undetected compounds in blanks, the LOQs were estimated as a signal to noise ratio of 10.

2.5. Statistical analysis

Statistical analysis was performed with SPSS 16 for Windows (SPSS, Inc., Chicago, IL). Data below LOQs were replaced with ½*LOQ in statistical analysis when the chemical have over 50% detection frequency (DF). After log-transformation, data was performed with one-way analysis of variance (ANOVA) to compare the differences of TCEP, ∑TCPP, and total PFRs levels in eggs from different sites.

3. Results and discussion

3.1. Levels of target FRs in home-produced eggs

EH-TBB was detected in 30% of eggs from e-waste sites, but not in eggs from the

control site. BEH-TEBP could be quantified in two thirds of eggs from e-waste sites, ranging from nd-1.82, 0.077-0.96 and nd-0.31 ng/g ww in Sites 1-3, respectively (Table 1), but it was also found in two out of eight eggs from the control site. A weak relationship could link e-waste recycling activities with EH-TBB/BEH-TEBP contamination in free-range chicken eggs. By contrast, if considering approximately 10% lipid content in eggs, Labunska et al. (2015) reported higher levels of EH-TBB (average: 4.3 ng/g lw) and similar levels of BEH-TEBP (average: 1.1 ng/g lw) in poultry eggs from another e-waste recycling area in southern China. Similar to our findings, the concentrations of EH-TBB and BEH-TEBP in their egg samples were only 2-5 times higher than the levels (or LOQ) for control site, while distinctive differences of persistent contaminants, such as PBDEs, hexabromocyclododecane (HBCD) and polychlorinated biphenyls (PCBs) could be seen in eggs from e-waste sites and control sites (1-3 orders of magnitude higher in eggs from e-waste sites than control sites) (Labunska et al, 2013, 2015; Zheng et al 2012).

Among the PFRs measured in the present study, TPHP, EHDPHP, TCEP, ∑TCPP and TDCIPP were detected in eggs, but only TCEP, ∑TCPP and TDCIPP have over 50% DF in eggs from e-waste recycling sites. TEHP, TNPP, TNBP, TBOEP and TMPP were not detected in all samples. Total PFRs levels were 0.92-7.61, 0.48-15.8, 1.02-3.62 and 0.62-3.03 ng/g ww in eggs from three e-waste sites and the control site, respectively (Table 1). All three chlorinated PFRs, TCEP, ∑TCPP and TDCIPP, could be found in eggs, while only EHDPHP and TPHP, out of five non-chlorinated PFRs were detected, probably implying that chlorinated PFRs might be more persistent or bioaccumulative in eggs than non-chlorinated PFRs. No significant differences were found between levels of TCEP, ∑TCPP and total PFRs

in eggs from e-waste sites and the control site (one-way ANOVA, $p > 0.05$). Accordingly, e-waste recycling activities seem to have little influence on the PFR levels in eggs.

Only limited studies reported the PFR levels in food or biota. Chen et al. (2012) reported low levels of EHDPHP (<0.09-0.17 ng/g ww), TBOEP (0.16-2.2 ng/g ww), TCEP (<0.10-0.55 ng/g ww) and TCPP (<0.20-4.1 ng/g ww) in herring gull eggs from the Channel-Shelter Island (Lake Huron, US) in 2010, while TEHP and TPHP were not detected. In another study about herring gull eggs from the Great Lakes basin during 2013, TCEP was detected in 58% of samples (max 13 ng/g ww) (Chu and Letcher, 2015). In free-range chicken eggs from the Belgium market, only TPHP (0.27 ng/g ww) could be found (Xu et al, 2015). These studies about PFRs in eggs showed similar range of PFR levels with our results. Moreover, Malarvannan et al. (2015) reported a median of 8.4 ng/g ww (range 3.4-44 ng/g ww) of total PFR levels in eel muscle from catchments in Belgium. In Western Scheldt catchment (Netherlands and Belgium), the median levels of total PFRs in different aquatic species ranged from 3 to 22 ng/g ww (Brandsma et al, 2015). So far, there are no reports on correlations between the bioaccumulation of PFRs in biota and the contamination of ambient environment. Kim et al (2011a) observed no accumulation for nine PFRs, except TPHP, in demersal (aquatic) species. Trophic magnification was tentatively observed for TCEP, TCIPP and TBOEP in the benthic aquatic food web, but PFRs showed trophic dilution in the pelagic food web (Brandsma et al, 2015).

Although it is not possible to estimate the emission of PBDEs and PFRs from e-waste recycling to local environment, we found the ratio of total PFRs (2200-6800 ng/g) and PBDEs (680-24000 ng/g) were range from 0.3-4 in indoor dust from the same area with this study

(Zheng et al, 2015). In another study, PBDE levels were 10-50 times higher in eggs from e-waste sites (2600-14000 ng/g lw) than from the control site (300 ng/g lw) (Zheng et al, 2012). However, little differences of PFR profiles could be observed between e-waste sites and control site in the same egg samples. In terms of contamination levels in eggs from e-waste area, PFRs, as well as EH-TBB and BEH-TEBP, were hundreds folds lower than PBDE concentrations (Zheng et al, 2012), implying their weaker bioaccumulation and persistency potential in eggs compared to PBDEs. This could possibly due to several factors. First, the bioaccumulation of organic chemicals was influenced by hydrophobicity (or K_{OW}), and chemicals with log K_{OW} around 6-8 tend to be more bioaccumulative (Gobas et al, 2003; Kelly et al, 2007). Most PFRs have lower log K_{OW} 1.4-5.1), except TEHP (log K_{OW} = 9.5), than PBDEs (log K_{OW} 6-10) (Bergman et al, 2012; CEPA, 1999; van der Veen and de Boer, 2013). Although EH-TBB and BEH-TEBP have log K_{OW} of 7.7 and 9.3, respectively, their bioaccumulation might be also influenced by other factors. Second, the ester bonds of EH-TBB, BEH-TEBP and PFRs could be broken during metabolism processes, such as in gastrointestinal tract or liver (Roberts et al, 2012; Van den Eede et al, 2013). TDCIPP can be completely metabolized to BDCIPP by chicken embryo hepatocytes in 36 h (Farhat et al, 2014). BEH-TEBP and EH-TBB were found to degrade by almost 70% in digestive fluids in an *in vitro* study (Fang and Stapleton, 2014). Many PFR metabolites have been identified in *in vitro* microsomal studies and in human urine samples (Ballesteros-Gómez et al, 2015; Van den Eede et al, 2013, 2014, 2015). As a result, metabolism might be an important reason explaining why only low levels of our target compounds present in the eggs. Further studies are necessary to elucidate the absorption, metabolism and elimination of PFRs, EH-TBB and

BEH-TEBP in organisms.

3.2 Distribution of FRs between albumen and yolk

Eight eggs were randomly selected to investigate the distribution of FRs between albumen and yolk. TPHP and ∑TCPP were detected in albumen and BEH-TEBP and TDCIPP were detected in yolk, all with values slightly higher than LOQs. BEH-TEBP levels ranged from nd-0.22 ng/g ww (median = 0.15 ng/g ww) in yolk, while only one albumen sample was detected with BEH-TEBP above LOQ (0.26 ng/g ww). TPHP had a higher detection frequency in albumen (62% DF, nd-0.92 ng/g ww) than in yolk (38% DF, 0.28-0.50 ng/g ww). ∑TCPP (2.06 ng/g ww) was detected in one albumen sample but not in yolk, while TDCIPP (4.74 ng/g ww) was detected in one yolk sample but not in albumen. PBDEs, BTBPE and DBDPE were found in yolk, not in albumen (Zheng et al 2012), while same phenomenon was reported by Rawn et al (2011). Trace amount of DPs were detected in albumen (median: 0.06 ng/g ww), which were much lower than those in yolk (median: 2.78 ng/g ww). It seems that PBDEs, DPs, BEH-TEBP and other NBFRs tend to accumulate in yolk, and TPHP tend to accumulate in albumen. The distribution of other PFRs between albumen and yolk was still not clear, because the lower available amount of albumen samples used in analysis enhance their LOQ in albumen.

Albumen is mainly composed of water and albumin, while yolk contains higher proportion of lipids, including fat and cholesterol derivatives. The distribution patterns of NBFRs and PFRs in eggs yolk and albumen could probably link to the K_{OW} of each compound. It may relate to the different affinity for albumin and lipid compounds. Compared

with our results, Greaves and Letcher (2014) reported higher levels of PFRs in both albumen (14.8 ± 5.9 ng/g ww) and yolk (14.8 ± 2.4 ng/g ww) of herring gull eggs with different profiles. TBOEP accounted for 66% of the PFR concentrations in their albumen samples, but only 13% in yolk, while TNBP accounted for 25% of the PFR concentrations in yolk with no detection in albumen (Greaves and Letcher, 2014). Whereas the herring gulls are predators and their oviposition period/amount is also different compared to hens, it is reasonable to observe a different (and more contaminated) PFR profile in gull eggs.

3.3 Human exposure of EH-TBB, BEH-TEBP and PFRs: comparison with indoor dust

Based on a diet consumption survey in Guangdong Province conducted in 2002, the average egg consumption was 14.0 g/day/person in the rural regions (Ma et al, 2005). Applying an average body weight (bw) of 70 kg for adults and 12 kg for children (Zheng et al, 2015), the median exposure our target contaminants via egg consumption were estimated (Table 2). The estimated dietary exposure of BEH-TEBP ranged from 0.03-0.09 and 0.20-0.54 ng/kg bw/day for adults and children, respectively, which were similar with assessment for another e-waste recycling region (0.07 and 0.28 ng/kg bw/day for adults and children) (Labunska, 2015). PFR intakes were 0.32-0.52 and 1.89-3.02 ng/kg bw/day for adults and children in e-waste recycling sites, which was slightly higher than PFR intake values in the control site (0.28 and 1.62 ng/kg bw/day for adults and children, respectively).

The human exposure risks of PBDEs, NBFRs and PFRs via dust ingestion have been reported in the same sampling region (Zheng et al, 2015). Figure 1 compared the human exposure via dust ingestion and eggs consumption to PBDEs, NBFRs and PFRs. PBDEs,

BTBPE and DPs exposure via eggs were at least 5 times higher than exposure via indoor dust. Meanwhile, with exception of site 1, DBDPE, BEH-TEBP, and PFRs exposure via dust ingestion was over four times higher than via egg consumption. Egg consumption seems to be the main exposure pathway for PBDEs, BTBPE and DPs, while indoor dust ingestion is probably a more important pathway for DBDPE, BEH-TEBP, and PFRs. However, people usually have at least one egg (about 40-70 g) each time that leads to a pulse exposure of 3-5 times of proposed average daily exposure for only one egg consumption. Furthermore, if considering consumption of other food, the FR exposure risk via diet might be higher. As a result, EH-TBB, BEH-TEBP and PFRs exposure via other food product consumption should also be investigated in the future for more comprehensive risk assessment.

4 Conclusions

EH-TBB, BEH-TEBP and eight PFRs were measured in free-range chicken eggs from three e-waste recycling sites and a control site. BEH-TEBP, TCEP, ∑TCPP and TDCIPP were detected in more than 50% of eggs. BEH-TEBP had a higher detection frequency and higher concentrations in yolk than albumen, while TPHP was more detected in albumen, likely due to its different physical-chemical properties (e.g., K_{OW}) and to its affinity for albumin or lipids. Human exposure risks to BEH-TEBP and PFRs via egg consumption were lower than exposure via indoor dust in e-waste recycling region. However, as the food intake is thousands times higher than the dust intake amount, and egg is only a small part of our daily diet, total dietary exposure to these FRs might be higher. Therefore, more studies about EH-TBB, BEH-TEBP and PFRs in food products are required in order to achieve better

assessment of their exposure risk via diet.

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References

Ballesteros-Gómez A, Van den Eede N, Covaci A. *In vitro* human metabolism of the flame retardant Resorcinol Bis(diphenylphosphate) (RDP). Environ Sci Technol, 2015, 49:3897-3904.

Bergman Å, Rydén A, Law RJ, de Boer J, Covaci A, Alaee M, Birnbaum L, Petreas M, Rose M, Sakai S, Van den Eede N, van der Veen I. A novel abbreviation standard for organobromine, organochlorine and organophosphorus flame retardants and some characteristics of the chemicals. Environ Int, 2012, 49:57-82.

Brandsma SH, Leonards PE, Leslie HA, de Boer J. Tracing organophosphorus and brominated flame retardants and plasticizers in an estuarine food web. Sci Total Environ, 2015, 505:22-31.

Canadian Environmental Protection Act (CEPA), 1999. Environmental Screening Assessment

328 Report on Polybrominated Diphenyl Ethers (PBDEs).
329 Cequier E, Ionas AC, Covaci A, Marcé RM, Becher G, Thomsen C. Occurrence of a broad
330 range of legacy and emerging flame retardants in indoor environments in Norway. Environ
331 Sci Technol, 2014, 48:6827-6835.
332 Chen D, Letcher RJ, Chu S. Determination of non-halogenated, chlorinated and brominated
333 organophosphate flame retardants in herring gull eggs based on liquid
334 chromatography-tandem quadrupole mass spectrometry. J Chromatogr A, 2012,
335 1220:169-174.
336 Chu S and Letcher RJ. Determination of organophosphate flame retardants and plasticizers in
337 lipid-rich matrices using dispersive solid-phase extraction as a sample cleanup step and
338 ultra-high performance liquid chromatography with atmospheric pressure chemical
339 ionization mass spectrometry. Anal Chim Acta, 2015, doi:10.1016/j.aca.2015.05.024.
340 Covaci A, Harrad S, Abdallah M A E, Ali N, Law R J, Herzke D, de Wit C A. Novel
341 brominated flame retardants: A review of their analysis, environmental fate and behaviour.
342 Environ Int, 2011, 37:532-556.
343 Covaci A, Roosens L, Dirtu AC, Waegeneers N, Van Overmeire I, Neels H, Goeyens L.
344 Brominated flame retardants in Belgian home-produced eggs: levels and contamination
345 sources. Sci Total Environ, 2009, 407:4387-4396.
346 Davis EF and Stapleton HM. Photodegradation pathways of nonabrominated diphenyl ethers,
347 2-Ethylhexyltetrabromobenzoate and di(2-ethylhexyl)tetrabromophthalate: identifying
348 potential markers of photodegradation. Environ Sci Technol, 2009, 43:5739-5746.
349 Domingo JL. Health risks of human exposure to chemical contaminants through egg
350 consumption: A review. Food Res Int, 2014, 56:159-165.
351 European Court of Justice 2008–04–01, Case C–14/06, 2008.
352 Fang M and Stapleton HM. Evaluating the bioaccessibility of flame retardants in house dust
353 using an *in vitro* Tenax bead-assisted sorptive physiologically based method. Environ Sci
354 Technol, 2014, 48:13323-13330.
355 Farhat A, Crump D, Porter E, Chiu S, Letcher RJ, Su G, Kennedy SW. Time-dependent
356 effects of the flame retardant tris(1,3-dichloro-2-propyl) phosphate (TDCPP) on mrna
357 expression, in vitro and in ovo, reveal optimal sampling times for rapidly metabolized

compounds. Environ Toxicol Chem, 2014, 33:2842-2849.

Greaves AK and Letcher RJ. Comparative body compartment composition and in ovo transfer of organophosphate flame retardants in North American Great Lakes herring gulls. Environ Sci Technol, 2014, 48:7942-7950.

Gobas F, Kelly BC, Arnot JA. Quantitative structure activity relationships for predicting the bioaccumulation of POPs in terrestrial food-webs. Qsar Comb Sci, 2003, 22:329-336.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas F. Food web-specific biomagnification of persistent organic pollutants. Science, 2007, 317:236-239.

Kim JW, Isobe T, Chang KH, Amano A, Maneja RH, Zamora PB, Siringan FP, Tanabe S. Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ Pollut, 2011a, 159:3653-3659.

Kim JW, Isobe T, Muto M, Tue NM, Katsura K, Malarvannan G, Sudaryanto A, Chang KH, Prudente M, Viet PH, Takahashi S, Tanabe S. Organophosphorus flame retardants (PFRs) in human breast milk from several Asian countries. Chemosphere, 2014, 116:91-97.

Kim JW, Ramaswamy BR, Chang KH, Isobe T, Tanabe S. Multi residue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry. J Chromatogr A, 2011b, 1218:3511-3520.

Labunska I, Harrad S, Santillo D, Johnston P, Yun L. Domestic duck eggs: an important pathway of human exposure to PBDEs around E-Waste and scrap metal processing areas in Eastern China. Environ Sci Technol, 2013, 47:9258-9266.

Labunska I, Abdallah MAE, Eulaers I, Covaci A, Tao F, Wang M, Santillo D, Johnston P, Harrad S. Human dietary intake of organohalogen contaminants at e-waste recycling sites in Eastern China. Environ Int, 2015, 74:209-220.

Liu H, Hu Y, Luo P, Bao L, Qiu J, Leung LMY, Zeng EY. Occurrence of halogenated flame retardants in sediment off an urbanized coastal zone: association with urbanization and industrialization. Environ Sci Technol, 2014, 48:8465-8473.

Ma W, Deng F, Xu Y, Xu H, Nie S, Li J, Deng H, Li H. The study on dietary intake and nutritional status of residents in Guangdong Province, China. South China J Prev Med, 2005, 31:1-5, (in Chinese).

388 Malarvannan G, Belpaire C, Geeraerts C, Eulaers I, Neels H, Covaci A. Organophosphorus
389 flame retardants in the European eel in Flanders, Belgium: Occurrence, fate and human
390 health risk. Environ Res, 2015, 140:604-610.
391 Rawn DFK, Sadler A, Quade SC, Sun WF, Lau BPY, Kosarac I, Hayward S, Ryan JJ.
392 Brominated flame retardants in Canadian chicken egg yolks. Food Addit Contam A, 2011,
393 28:807-815.
394 Roberts SC, Macaulay LJ, Stapleton HM. In vitro metabolism of the brominated flame
395 retardants 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl)
396 2,3,4,5-tetrabromophthalate (BEH-TEBP) in human and rat tissues. Chem Res Toxicol,
397 2012, 25:1435-1441.
398 Song Q and Li J. A systematic review of the human body burden of e-waste exposure in China.
399 Environ Int, 2014, 68: 82-93.
400 Stapleton HM, Allen JG, Kelly SM, Konstantinov A, Klosterhaus S, Watkins D, Mcclean MD,
401 Webster TF. Alternate and new brominated flame retardants detected in U.S. house dust.
402 Environ Sci Technol, 2008, 42:6910-6916.
403 Sundkvist AM, Olofsson U, Haglund P. Organophosphorus flame retardants and plasticizers
404 in marine and fresh water biota and in human milk. J Environ Monitor, 2010, 12: 943-951.
405 United Nations Environment Programme (UNEP). Stockholm Convention text and annexes as
406 amended in 2009. Available at:
407 http://chm.pops.int/Convention/tabid/54/language/en-US/Default.aspx#convtext (accessed
408 May 2015).
409 Van den Eede N, Maho W, Erratico C, Neels H, Covaci A. First insights in the metabolism of
410 phosphate flame retardants and plasticizers using human liver fractions. Toxicol Lett, 2013,
411 223:9-15.
412 Van den Eede N, Heffernan AL, Aylward LL, Hobson P, Neels H, Mueller JF. Age as a
413 determinant of phosphate flame retardant exposure of the Australian population and
414 identification of novel urinary PFR metabolites. Environ Int, 2014, 74:1-8.
415 Covaci, A.Van den Eede N, Erratico C, Exarchou V, Maho W, Neels H, Covaci A. In vitro
416 biotransformation of tris(2-butoxyethyl) phosphate (TBOEP) in human liver and serum.
417 Toxicol Appl Pharm, 2015, 284:246-253.

van der Veen I and de Boer J. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere, 2012, 88:1119-1153.

Wei GL, Li DQ, Zhuo MN, Liao YS, Xie ZY, Guo TL, Li JJ, Zhang SY, Liang ZQ. Organophosphorus flame retardants and plasticizers: Sources, occurrence, toxicity and human exposure. Environ Pollut, 2015, 196:29-46.

Xu F, García-Bermejo Á, Malarvannan, Gómara B, Neels H, Covaci A. Multi-contaminant analysis of organophosphate and halogenated flame retardants in food matrices using ultrasonication and vacuum assisted extraction, multi-stage cleanup and gas chromatography–mass spectrometry. J Chromatogr A, 2015, 1404:33-41.

Zheng XB, Wu JP, Luo XJ, Zeng YH, She YZ, Mai BX. Halogenated flame retardants in home-produced eggs from an electronic waste recycling region in South China: Levels, composition profiles, and human dietary exposure assessment. Environ Int, 2012, 45:122-128.

Zheng XB, Xu FC, Chen KH, Zeng YH, Luo XJ, Chen SJ, Mai BX, Covaci A. Flame retardants and organochlorines in indoor dust from several e-waste recycling sites in South China: Composition variations and implications for human exposure. Environ Int, 2015, 78:1-7.

435 Table 1. Medians (range) in ng/g ww (detection frequency) of PFRs, EH-TBB, and
436 BEH-TEBP in home-produced eggs.

437

	LOQs	E-waste Sites			Control Site
		Site 1 (n=12)	Site 2 (n=7)	Site 3 (n=10)	(n=8)
TPHP	0.23	nd[a]-0.69 (50%)	nd-0.36 (43%)	nd-0.43 (40%)	nd-0.29 (25%)
EHDPHP	0.30	nd-0.40 (17%)	nd-0.38 (28%)	nd-0.68 (40%)	nd-0.83 (50%)
TCEP	0.11	1.08 (0.44-2.38) (100%)	0.67 (0.32-1.07) (100%)	0.72 (0.32-0.94) (100%)	0.65 (0.62-1.29) (100%)
TDCIPP	0.60	nd-5.84 (42%)	nd-13.1 (43%)	0.49 (nd-1.95) (70%)	0.67[b]
∑TCPP	0.15	0.56 (nd-3.49) (92%)	0.37 (0.16-1.53) (100%)	0.33 (0.19-0.94) (100%)	0.17 (nd-1.26) (75%)
∑PFRs	-	2.59 (0.92-7.61)	1.62 (0.48-15.8)	1.95 (1.02-3.62)	1.39 (0.62-3.03)
EH-TBB	0.005	0.005	0.058	0.062 (nd-0.10) (70%)	nd
BEH-TEBP	0.05	nd-1.82 (42%)	0.46 (0.077-0.96) (100%)	0.17 (nd-0.31) (80%)	nd-0.54 (25%)

438 [a] not detected

439 [b] only found in one sample

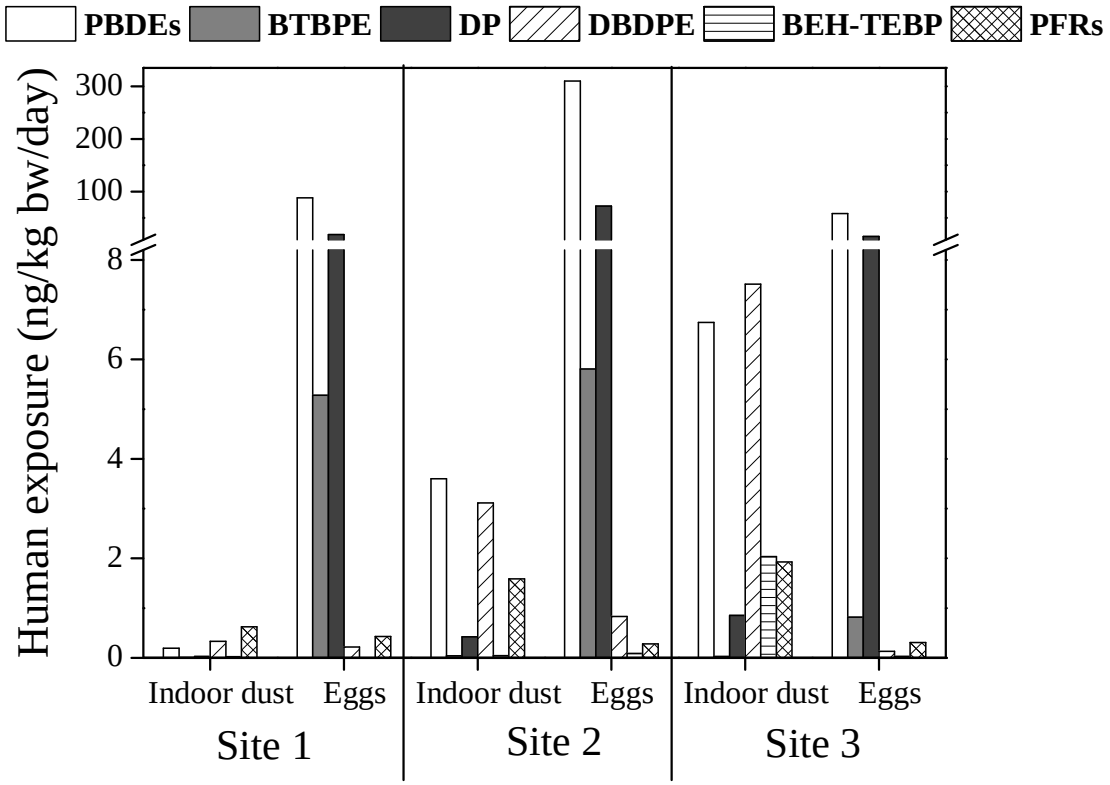
440

441 Table 2. Human exposure assessment of BEH-TEBP and PFRs via egg consumption (ng/kg
442 bw/day).
443

		E-waste sites			Control site
		Site 1	**Site 2**	**Site 3**	
BEH-TEBP	Adult	NA	0.09	0.03	NA
(ng/kg bw /day)	Children	NA	0.54	0.20	NA
PFRs	Adult	0.52	0.32	0.39	0.28
(ng/kg bw /day)	Children	3.02	1.89	2.28	1.62

444 NA – Not available

445 Figure 1. Human exposure of FRs via dust ingestion and egg consumption in e-waste sites.
446



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