Non-invasive biomonitoring for PFRs and PSDEs: New insights in analysis of human hair externally exposed to selected flame retardants

Non-invasive biomonitoring for PFRs and PBDEs: new insights in analysis of human hair externally exposed to selected flame retardants

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Abstract

In this study, we investigated the hypothesis whether externally adsorbed and internally deposited flame retardants (FRs) in hair could be distinguished. To this extent, hair samples collected from one volunteer were exposed under controlled conditions to phosphate FR (PFR) and polybrominated diphenyl ether (PBDE) standards to mimic external contamination. Afterwards, suitable washing procedures to selectively remove contaminants from the hair surface were investigated. The samples were measured by GC-(ECNI)-MS for PBDEs and LC-(ESI+)-MS/MS for PFRs. All investigated compounds were transferred onto the hair surface. One of the most important finding was that dust particles are not mandatory to transfer compounds on the hair surface and to be able to measure high levels of compounds in human hair. To assess different protocols to selectively remove external contamination, the exposed hair samples were washed in different media before analysis: water, methanol, hexane:dichloromethane (1:1, v:v), acetone and shampoo. Results indicated that there is no washing medium able to entirely and exclusively remove external contamination. Among investigated media, methanol removed a meaningful part of the external contamination (42-105 %), but the removal efficiencies differed among compounds. We therefore concluded that hair should not be washed prior to analysis or in case of visible contamination (e.g. with cosmetic products), water would be the recommended agent. Organic solvents should not be used for the washing step. Although it is impossible to distinguish external from internal exposure, hair samples may be used as valuable biomarker of human exposure, providing a measure of integral exposure. To the best of our knowledge, this is the first study which has used externally exposed hair samples to PBDEs and PFRs.

1. Introduction

Human biomonitoring is a useful tool for the assessment of a population's exposure to environmental contaminants by directly measuring substances or their metabolites (CDC 2008). Blood is commonly

considered as an ideal matrix but several limitations apply: (i) it is invasive matrix that has a significant impact on the response of study participants, (ii) ethical issues, especially when sampling children, (iii) sample amount is limited (need for very powerful analytical tools). Therefore, current developments in the field of human biomonitoring are discussing on the utility of non-invasive matrices such as hair, nails, urine, saliva, etc. (Alves et al., 2014). The main advantages of non-invasive methods are ease and low cost of sampling procedures, simplicity of the ethical dossier and recruitment. Among the possible non-invasive matrices, hair seems to be one of the most promising matrices. It has many advantages, such as sample stability, large window of detection, information on short to long term exposure (from weeks to months or even years, depending on hair length), etc. Its high lipid content (2-4 %) also makes this sample useful in measurement of lipophilic chemicals (such as persistent organic pollutants - POPs).

Two routes of exposure are relevant for hair sample analysis, namely external (deposition from air and dust) and internal exposure (through contact with blood at the hair follicle/root) (Covaci et al., 2002). Hair was already used in 1960s and 1970s in the analysis of toxic metals, such as arsenic, lead or mercury (Kintz, 2004) and more recently for the analysis of pharmaceuticals and drugs of abuse (Boumba et al., 2006; Kintz, 2007; Mieczkowski et al., 2001; Nakahara, 1999; Pragst and Balikova, 2006; Psillakis et al., 1999; Tsatsakis et al., 2000; Tsatsakis et al., 1997; Wang et al., 2009). Compared to the application in forensics, there is less knowledge about analysis of POPs in human hair samples. There are some data about analysis of pesticides and selected POPs (Schramm et al., 1992; Schramm, 1997; Tsatsakis and Tutudaki, 2004). Much less information is available about brominated flame retardants - BFRs (e.g. polybrominated diphenyl ethers (PBDEs): Leung et al., 2011, Tadeo et al., 2009, Aleksa et al., 2012). Levels of PBDEs and dioxins in hair from an occupationally-exposed population from e-waste, China were meaningfully elevated when compared to the levels in a control group (Ma et al., 2011). Further, very few publications deal with establishing relations between the levels of POPs in paired human hair and blood samples. Varying correlations between the hair and blood levels were reported only for *p,p'*-DDT, *p,p'*-DDE, PCB 28, and PCB 74 (Alsthul et al., 2004). Similar correlations were found for PCDD/Fs and coplanar PCBs (Nakao et al., 2002). Apart from meaningful relationship between levels of PBDEs in hair and internal organs reported by D'Havé et al. (2005) the lack of clear correlations hampers interpretation of POPs' results in hair, which might be explained by confounding internal and external exposure. Selective removal of external contamination by means of washing seems to be appropriate in hair sample analysis especially when the distinction between internal and external exposure is required. There is no generally accepted approach among laboratories regarding this washing step and water, shampoo and/or organic solvents were mainly used to decontaminate hair samples (Barbounis et al., 2012; Behrooz et al., 2012; Covaci et al., 2008; Zupancik-Kralj et al., 1992; Malarvannan et al., 2013; Nakao et al., 2005; Nakao et al., 2002, Schramm, 2008; Tsatsakis et al., 2008; Zhao et al., 2008). Further, there is no information available on the efficacy of the various washing procedures used.

FRs are widely used as additives in e.g. furniture, plastics, electronics, and textiles to enhance fire safety (American Chemistry Council, 2014). While there are no recent figures on FR consumption available in the public domain, the use of these chemicals is significant. Only in Europe, the total consumption of FRs was 465,000 tons in 2006, of which 10 % were BFRs (CEFIC, 2007). PBDEs were the most frequently used BFRs until recently. The rising concerns about adverse effects on human health have led to worldwide restrictions of some FRs, such as PBDEs (European Court of Justice 2008). Upcoming legislation has however resulted in increased production of alternative FRs, such as phosphate flame retardants, PFRs (US EPA, 2005; Stapleton et al., 2009). The most frequently used PFRs are tris(2-chloroethyl)phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP), tris(1,3-dichloro-*iso*-propyl) phosphate (TDCIPP) and also non-halogenated PFRs such as tri-n-butyl phosphate (TnBP), tri-phenyl phosphate (TPhP), tris(2-ethylhexyl) phosphate (TEHP), tris(2-butoxyethyl) phosphate (TBEP) and tri-cresyl phosphate (TCP) (Van der Veen and de Boer, 2012). Similar to PBDEs, PFRs can be easily released into the environment either by volatilization, leaching or abrasion (Marklund et al., 2005). Because of their wide use, PFRs are constantly released into

the environment and hence were already detected in indoor air (Otake et al., 2001; Sjödin et al., 2001; Hartmann et al., 2004), house dust (Marklund et al., 2003; Stapleton et al., 2009; Van den Eede et al., 2011), drinking water (Stackelberg et al., 2007), sediment, wildlife and human breast milk (Sundkvist et al., 2010; Kim et al., 2011; Van der Veen and de Boer, 2012). The knowledge about PFR toxicity is limited and there are few publications on adverse effects following PFR exposure. Apart from neurotoxic properties after chronic exposure (WHO 1998, 1991a, 1991b), it is known that PFRs can be adsorbed through the human skin, can act as skin irritants (WHO 2000, 1998, 1991a, 1991b) and can cause hemolysis (Sato et al., 1997). Chlorinated PFRs, such as TDCIPP and TCEP also showed carcinogenic effects (WHO, 1998). Monitoring of environmental behavior of chemicals displaying such properties is therefore justifiable.

Taking into account the above we focused our present study on investigation of the deposition of selected FRs onto the surface of hair. The main aim of this part was to check whether contribution of dust particles is mandatory in order to transfer the compounds on the surface of hair and if dust might be the main reason of measurable levels of FRs in hair. In the second part several and the commonly used washing media were tested to evaluate their effectiveness of removal of external exposure from the hair and whether distinction between external and internal exposure is possible. We believe that our findings and conclusions can significantly contribute to the knowledge about analysis of FRs in human hair and to better understanding how the exposure data should be interpreted. To the best of our knowledge, this is the first study which tests externally exposed human hair samples to these FRs and in such way.

2. Experimental

2.1 Materials (chemicals and reagents)

All solvents used were of analytical grade. Hexane (Hex), dichloromethane (DCM), toluene, methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Ethyl acetate (EtAc), water (H_2O) and formic acid were purchased from Biosolve (Valkenswaard, the Netherlands). Florisil, anhydrous sodium sulphate (Na_2SO_4), nitric acid (HNO_3, 65 %), hydrochloric acid (HCl, 37 %) and concentrated sulphuric acid (H_2SO_4, 98 %) were purchased from Merck (Darmstadt, Germany). Silica gel was purchased from J.T. Baker chemicals (Deventer, Holland). Standards of PBDE congeners (BDE-28, 47, 100, 99, 154, 153, 183) were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE-77 was used as internal standard (IS) for PBDEs and was purchased from AccuStandard INC (New Haven, CT, USA). All neat PFR standards were purchased from Sigma-Aldrich: TEHP (with purity 98 %), TBEP (94 %), TPhP (99 %), TDCIPP (96 %), TCPP (68 %), TnBP (98 %), TCEP (97 %), TCP (technical mixture, 90 %). Deuterated PFR were used as IS: TPP-d_{15} was purchased from Sigma-Aldrich, TnBP-d_{27} from Chiron, TBEP-d_6, TDCPP-d_{15}, TCEP-d_{12} were synthesized by Dr. Vladimir Belov (Max Planck Institute for Biophysical Chemistry, Goettingen, Germany). Standard solutions were prepared in toluene (for PBDEs) and in methanol (for PFRs). All standard concentrations were calculated using standard purity.

Empty polypropylene SPE cartridges (Chromabond, 6 mL) were purchased from Macherey-Nagel (Germany). Before use, silica gel, anhydrous Na_2SO_4 and Florisil were washed with Hex under ultrasonication. After washing the reagents were activated at 160-200 °C overnight. Acidified silica gel (44 %, *w/w*) was prepared as described by Kucharska et al. (2014). To ensure low laboratory background levels, all glassware was heated at 450 °C overnight. Just before use, all glass tubes were rinsed with Hex.

2.2 Sample collection

All experiments were performed on one batch of human hair sample collected from the same individual. The hair donor never used any bleaching or coloring agents. The hair was washed the day before sampling. In total 18 g was collected, cut into small pieces using stainless steel scissors (1-2 mm) and homogenized by

shaking in a closed container and afterwards split into 5 equal parts. No additional pretreatment such as washing was done before the experiments.

2.3 Method description

2.3.1 Exposure experiment

In order to assess the efficiency of different washing procedures, externally contaminated hair samples were prepared. To ensure a sufficient contaminant load onto the hair surface, different exposure times were used. The hair sample was split into 5 equal parts (3 g) and placed into 5 plastic boxes (of which 4 were used for exposure and 1 as a reference blank). Standard solutions of PBDEs and PFRs were spiked onto two watch glasses (resulting in 8 µg and 4 µg, respectively) placed inside the boxes containing the hair sample. The sample arrangement can be visualized in the supporting information (Figure S1). After evaporation of the solvent, the boxes were closed with a lid, sealed with parafilm and wrapped in aluminum foil in order to protect the hair and the standards from light. Exposure experiments ran for 24 h (box 1), 48 h (box 2), 72 h (box 3) and 10 days (box 4) at room temperature. Hair samples in box 5 were not exposed and were used as a control sample (further called blank hair sample). After each exposure period hair samples were mixed by shaking and stored until analysis in aluminum foil and paper envelopes at -18 °C. No additional homogenization such as grinding was applied to avoid potential contamination of tools and areas in the laboratory.

2.3.2 Washing procedures

To assess different washing procedures, hair samples with a sufficient exposure time were used to enable measurement of sufficiently high levels in both hair and in washing media. Therefore, samples after 72 h of exposure (for PFR measurement) and 10 days of exposure (for PBDEs) were used for this purpose. Five hair samples after exposure (200 mg each) were washed in 5 different washing media: water, methanol, Hex:DCM (1:1, *v/v*), acetone, and shampoo. The selection was based on what was primarily used in other hair studies for similar compounds. The washing procedure included 10 min sonication in 10 mL of each relevant medium followed by vortexing and centrifugation over 6 min at 3600 rpm. This step was repeated 3 times. All fractions of each washing medium were collected in glass containers, spiked with IS, evaporated (except water and shampoo) and fractionated on Florisil as described below and shown on Figure 1. Also hair samples after washing in each medium were analyzed according to the method described below. Shampoo was not analyzed due to difficulties of operation. Water had to be additionally extracted 3 times by liquid-liquid extraction with hex:DCM (4:1, *v/v*) and then the organic fractions were treated as the other media-spiked with IS, evaporated, reconstituted in 1 mL of Hex and fractionated on Florisil.

2.3.3 Sample analysis

To calculate mass balances following the exposure experiments, both hair samples and washing liquids needed to be analyzed. To this extent, a solid-liquid and a liquid-liquid extraction protocol was used. After completion of the exposure experiments all samples were analyzed under repeatability conditions. The analytical method is described elsewhere (Kucharska et al, 2014) and is briefly summarized below. Around 200 mg of hair were accurately weighed, spiked with IS (BDE-77 and TPP-d_{15}, TCEP-d_{12}, TBEP-d_6, TDCPP-d_{15}, TnBP-d_{27}), incubated with 3 mL of 10 % HNO_3 under ultrasonication at 25 °C for 25 min and consecutively extracted with 2 x 3 mL of Hex:DCM (4:1, *v/v*) under vortex agitation. After each extraction cycle, hair extracts were centrifuged at 3500 rpm for 6 min. The supernatants were collected into clean glass tubes. The combined fractions of organic solvent were evaporated to near dryness under a gentle nitrogen stream and reconstituted in 1 mL of Hex. The extracts were further fractionated to allow for both GC (PBDEs) and LC (PFRs) analysis. Empty cartridges were filled up from the bottom with 1 g of Florisil and 250 mg of

anhydrous Na_2SO_4 and were further conditioned with 10 mL of EtAc and 6 mL of Hex. The extracts were quantitatively transferred and eluted with 10 mL of Hex and 6 mL of Hex:DCM (6:1, *v/v*) (Fraction A) and 10 mL of EtAc (Fraction B). Fraction A was evaporated to near dryness and reconstituted in 1 mL of Hex. The extract was further cleaned on a cartridge containing 44 % acidified silica and anhydrous Na_2SO_4. PBDEs were eluted with 9 mL of Hex and 3 mL of DCM. The cleaned extract was evaporated until dryness under a gentle nitrogen stream, reconstituted in 100 µL of toluene and analyzed by GC-ECNI-MS. The second fraction (Fraction B), was evaporated to dryness, resolubilized in 500 µL of MeOH and measured by LC-(ESI +)-MS/MS. A scheme of the sample preparation is shown in Figure 1. All the fractions of each relevant medium after hair washing were collected, spiked with the IS, evaporated and fractionated on Florisil according to the procedure described above and shown in Figure 1.

2.3.4 GC-MS analysis

Analysis of Fraction A containing PBDEs was performed with a Hewlett Packard HP 6890 Series GC system coupled to an Agilent 5975C MS operated in electron captured negative ionization (ECNI) mode. One µL of the cleaned extract was injected on a DB-5MS column (20 m × 0.18 mm × 0.18 µm) using pulsed splitless injection. The injection temperature was set at 300 °C and in pulsed pressure of 10 psi until 1.25 min and purge flow to split vent 70 mL/min after 1.25 min. The GC temperature program was set 110 °C held 1.25 min, ramp 30 °C/min to 230 °C, held 4 min, ramp 40 °C/min to 300 °C and held 10 min. Helium was used as carrier gas with a constant flow rate of 1.0 mL/min. The mass spectrometer was operated in selected ion monitoring (SIM) mode and *m/z* 79 and 81 were monitored. Dwell times were set on 40 ms. The ion source, quadrupole and interface temperatures were set at 230 °C, 150 °C and 300 °C, respectively, and the electron multiplier voltage was at 2271 V. Methane was used as moderating gas. In the GC-MS measurement of PBDEs, BDE-77 was used as IS (Table S1 of supporting information).

2.3.5 LC-MS analysis

Fraction B containing PFRs was analyzed on Waters Acquity UPLC system (Waters, Milford, MA, USA). The PFRs were separated at 40 °C on an Acquity UPLC BEH C18 column (2.1 mm x 100 mm, 1.7 µm) with a Van Guard Acquity UPLC BEH C18 precolumn (2.1 mm x 5 mm, 1.7 µm). Optimum separation was obtained with a binary mobile phase consisting of ultrapure water (solvent A) and MeOH (solvent B), both solvents acidified with 0.1 % formic acid. The mobile phase flow rate was set 0.4 mL/min and the following gradient was employed: 0-0.25 min 95 % A, 0.25-0.75 min 30 % A, 0.75-5.50 0 % A held 2 min, 7.50 – 7.60 min 95 % A (return to initial conditions) and held 2.40 min. Five µL of extract was injected into the LC system. The UPLC system was coupled to a Waters Quattro Premier XE Micromass tandem mass spectrometer that was operated in the positive electrospray ionization mode (ESI+). The parameters of the mass spectrometer were as follows: electrospray source block and desolvation temperature 120 °C and 350 °C, respectively; argon collision gas flow 0.22 mL/min; cone and desolvation nitrogen gas flow 50 L/h and 800 L/h, respectively. A capillary voltage of 3.20 kV was used for all compounds. The cone voltage and collision energy were compound-dependent. Parent and daughter ions were selected in the multiple reaction monitoring (MRM) mode (Table S1 of supporting information). For the quantification of PFRs, deuterated PFRs were used as internal standards, namely: TnBP-d_{27} (for TnBP), TPhP-d_{15} (for TPhP, TCP, TCPP, TEHP), TCEP-d_{12} (for TCEP), TBEP-d_6 (for TBEP), and TDCPP-d_{15} (for TDCIPP) (Table S1 of supporting information).

2.3.6 QA/QC

Hair samples were analyzed according to the method that was fully validated and the results are shown elsewhere (Kucharska et al, 2014). Briefly, procedural blank samples were run in parallel with each batch of samples (usually 3 procedural blanks per 10 human hair samples). As a blank procedural sample, 50 mg of

non-contaminated human hair pre-washed in methanol was used and no meaningful contamination during sample preparation was observed. Instrumental QC also included injection of solvent blanks (methanol) every five samples and after standard solutions and quality control samples (spiked human hair samples, both with native and ISs). The recoveries of standards (native and ISs) for the quality control samples were always in agreement to the recoveries found during method validation. The limit of detection (LOQ - defined as 3 times the standards deviation (SD) of the mean of the blank measurements or when non detects in blanks 3 times SD of the instrumental limit of detection) were in the range between 1-9 ng/g (33 ng/g for TCEP) for PFRs and 0.8 ng/g (for all the investigated PBDE congeners).

3. Results and discussion

3.1 Exposure experiments

An important objective of these experiments was to produce externally contaminated hair samples. Although the set-up of the exposure chamber was rather basic and had its limitations, it allowed us to gain insight on the atmospheric transfer of the compounds under study. The hair samples were analyzed before and after exposure and transfer efficiencies were based on the amount of chemical found on hair and the total amount of chemical that was added to the exposure chamber. The absorption capacity of the inner walls of the boxes and the watch glasses was not taken into account. Further, these exposed samples were used to assess different washing media for the efficiencies to remove external contamination.

Except for BDE-153, BDE-183, levels of all compounds were found to be increased in the hair samples after the maximum exposure time of 10 days. Transfer efficiency varied among FRs for intermediate exposure times (24h, 48h, 72h and 10 days). All results of hair analyses for PBDEs and PFRs are given in table S2 in the supporting information and shown graphically in figure 2 (for PBDEs) and figure 3 (for PFRs). The highest values are observed for BDE-28 and they are positively correlated with the exposure time. Relatively high RSDs in table S2 are supposedly caused by inefficient hair sample homogenization. As already mentioned, no grinding, nor additional cutting was used in order to avoid contamination of tools and working place by the exposed hair samples. The calculated transfer efficiencies and selected data of physico-chemical properties of FRs are given in table 1. The compound transfer of FRs within the closed boxes is assumed to be driven by their physico-chemical properties of the chemicals (vapor pressure, boiling point, volatility, etc.) and by the atmospheric conditions during the experiments (temperature, humidity, etc). PFRs under study reached the steady-state on the hair surface after 48 h. TDCPP and TnBP were transferred relatively quickly and reached steady-state already after 24 h of exposure (or even earlier, but this was not investigated in this study). In contrast to transfer speed (related to the steady state), the transfer efficiency (related to how likely the FRs are to accumulate onto hair) varied considerably among the different PFRs. The highest transfer efficiencies were calculated for TCEP, TCPP and TnBP and were 21, 38 and 54 %, respectively. These high values can be most likely explained by the relatively high vapor pressure values of these PFRs; indeed TnBP is the most volatile compound in the group of PFRs. Estimated transfer efficiencies for the other PFRs were around 2 %. Figure 4 depicts the correlation between calculated transfer efficiencies and log vapor pressure. We have not observed meaningful correlations between the calculated transfer efficiencies and the compound solubility in water or log K_{ow}. As mentioned earlier, the potential loss and/or adsorption of FRs onto the inner wall of the exposure boxes and the watch glasses was not considered.

Although hair samples in the present study were directly exposed to FRs (by the use of standards) and in relatively small area boxes, this simple test might mimic/reflect the situation that happens when FRs are released from products and materials where they are used. Both PBDEs and PFRs are categorized as semivolatile organic compounds (SVOCs) – compounds with vapor pressures in the range of 7.5×10^{-11} - 7.5×10^{-3} (mmHg) (Weschler and Nazaroff, 2008; WHO, 1997) and with boiling points 240 – 400 °C. PFRs are

not chemically bound to the original materials, therefore they are gradually and easily released to the environment mainly by abrasion and volatilization. When these FRs are released into the air (by volatilization), they are further distributed between the gas phase and particles (Weschler and Nazaroff, 2008) with exposure to humans mainly through ingestion, inhalation of dust particles and dermal sorption (WHO, 1990; Hughes et al., 2001). Weschler and Nazaroff (2008) have suggested that SVOCs have the potential to be much faster transferred via the air–to-skin (or clothing and hair) route than via inhalation. The vapor pressure for SVOCs in normal indoor conditions is low and consequently, their partition to particles is higher than to the air (Weschler, 2003). Moreover, the indoor air concentrations of SVOCs are usually low, because there are easily absorbed to surfaces and dust particles. But in general, the probable exposure routes in the indoor environment are therefore skin contact with surfaces, aerosol deposition on skin and inhalation (Järnström et al., 2009). In our study, the boxes were well closed and sealed and therefore dust particles were not present in the small air area. Nevertheless, we still observed that FRs were easily transferred onto the hair surface. The differences observed in the transfer efficiencies were caused by different physical-chemical properties of the investigated compounds.

Further, no steady-state was reached during timeframe of the exposure experiments for PBDEs: the longer the hair was exposed to PBDEs, the higher levels were detected afterwards (Figure 2, Table S2). In this group, the highest transfer efficiency was calculated for BDE-28, BDE-47 and BDE-100 (Table 1). Again, the higher transfer efficiency value was obtained for the more volatile compounds or the one with the highest vapor pressure. Within the timeframe of this experiment, several PBDE congeners were transferred onto the hair. However, levels of BDE-99, BDE-154, BDE-153 and BDE-183 transferred to the hair surface were too low to determine quantitatively. Thus, we have not considered these latter compounds for further calculations and for the discussion on the washing efficiency. PBDEs have been found to be ubiquitous in the environment and evidence for bioaccumulation exists (Wang et al., 2007). PBDEs toxicity decrease as the number of bromines increases (Birnbaum and Staskal, 2004). The lower PBDE congeners are predominant in air samples (Birnbaum and Staskal, 2004) and in the present study they were much easier distributed and absorbed by hair than higher PBDE congeners. Recent studies indicated that the indoor environment is a major exposure route to PBDEs for young children (Harrad et al., 2010). PBDEs, like other SVOCs, are partitioned between the gaseous and particulate phases in the air and are likely to undergo air surface exchange and long-range atmospheric transport (Wania and Dugani, 2003).

The aspects briefly discussed above raise concerns about interpretation of results of hair analysis as it is impossible to assess the contribution of external and internal exposure. After the exposure experiment, the hair was sufficiently exposed to the 2 FR groups. Due to the nature of exposure, all contamination could be considered external. The 10 days (in case of PBDEs) and 72 h (in case of PFRs) exposed samples were used therefore to assess the efficiency of the washing procedures.

3.2 Washing efficiency (PFRs and PBDEs)

The main goal of this experiment was to find the most efficient washing medium and to develop the most effective washing procedure that can preferably completely remove external contamination, leaving the internal contaminant load untouched. Currently, the interpretation of results of hair analysis is under discussion since external contamination can have a severe impact on the total contaminant load, which might not be interrelated with levels measured in other tissues. In order to remove potentially confounding external contamination, the washing step prior to hair analysis is considered crucial. It is generally accepted that by removing the external contamination, hair analysis would lead to more reliable information about internal exposure. The use of water and/or shampoo and also sporadically organic solvents was the most common approach in the analysis of POPs (Altshul et al., 2004; Malarvannan et al., 2013; Nakao et al., 2005; Nakao et al., 2002; Schramm, 2008; Zhao et al., 2008). In the present study, we compared several washing media, such as water, methanol, a mixture of Hex:DCM (1:1, *v/v*), acetone and shampoo, as the most

suitable for their ability to remove external contamination. Levels of FRs in the exposed hair samples (H_{exp}), in the washing media ($WM\_{name\ of\ medium}$), hair after washing ($H\_{name\ of\ medium}$), calculated mass balances (% Mass balance) and percentage of removal of external contamination (% Removal) are given in Table 2 (for PBDEs) and Table 3 (for PFRs) and in Figures S2 and Figure S3 in the supporting information. The calculation of the values is explained at the bottom of each table. The following approach showed that even when hair is washed many compounds were still present in the hair samples.

Due to analytical limitations/difficulties (agent requiring larger dilution), shampoo was not analyzed. Therefore, mass balance was not calculated in this particular case and also % removal of external contamination was estimated by the use of concentration of compounds in hair after washing in shampoo as given in Tables 2 and 3. Mass balances were acceptable, keeping in mind the large number of sample preparation steps, the insufficient homogenization of hair sample and the watch glasses from which any potential residues were not analyzed as it was not the case of the study. On the other hand, mass balances are not matching perfectly only in case of 3 compounds, namely TCP, TBEP, TPhP independently and always after each type of washing (Table 3). This might indicate that hair samples are well homogenized and the differences are caused only by the nature of certain compounds. Removal efficiencies of PBDE congeners investigated here were more similar after application of each medium and range between 60 – 77 % except water which remove only 10 % of the external contamination with BDE-28. This might be explained by the higher solubility of BDE-28 in water (171 µg/L at 25 °C, table 1), compared to the other investigated PBDEs. BDE-47 and BDE-100 were not detected in the washing medium (water), but they were still present at relatively high levels in the hair after washing with water (51 and 15 ng/g, respectively), and indeed also their water solubility is lower (54 and 48 µg/L, respectively). Although the removal efficiencies between solvents (except water) used as washing media are not significantly different, Hex:DCM (1:1, *v/v*) and MeOH were the most efficient media for the removal of PBDE contamination (Table 2). On the other hand, significant amounts (around 23-34 %) of PBDEs were still present in the hair after washings what might lead to conclusion that these media are not perfect. Further, removal efficiencies differ within the PFR group, most probably due to differences in polarity, molecular size, etc. Again, water was the least efficient medium and removal efficiencies ranged between 4 % (for TCP and TEHP, which have a very low solubility 0.36 and 0.6 mg/L, respectively) and 80 % (for TCEP, the most water soluble PFR at 7300 mg/L at 25 °C). Hex:DCM (1:1, v/v) was not as efficient as for PBDE congeners and removed entirely only two compounds - 109 % of TnBP and 101% TCEP, the other removal efficiencies for this medium ranged between 18 % (TDCIPP) and 48 % (TEHP). Acetone, considered also as a "good cleaning" agent, gave a very widely range of removal efficiencies, between 15 % (for TDCIPP) and 94 % (for TnBP). Shampoo gave relatively constant removal efficiencies between 49 % (TDCIPP) and 97 % (for TCEP), but still too low in order to select it as the best removal agent. As we noticed several times, TDCIPP was one of the most difficult compounds in terms of removal. Within the investigated media, only methanol removed 84 % of TDCIPP and also levels of other PFRs were still present in hair after methanol wash (Figure S3). Methanol therefore should be considered as potentially the best washing medium with removal efficiencies relatively constant and ranged between 42 % (for TCP) and 97 % (for TnBP).

In general, even after application of ultrasounds and vortexing repeatedly for 3 times, relatively high levels could be found in the hair after washing, both for PBDEs and PFRs (Figures S2 and S3). The main issue is related to discrimination in removal of the investigated compounds – procedure with the use of a certain washing media might be efficient for one compound, but not for the others. In practice, it is difficult to find a compromise, since usually several compounds are being measured from one hair sample intake.

Water and shampoo, which are the most often used washing agents, did not sufficiently remove the external contamination. Levels of some FRs were still high when the water or shampoo was used for washing. Within the tested media, the most efficient was methanol as it might penetrate to the inner part of the hair and can extract compounds of the interest from the inner structure. There are some studies

where washing with *n*-hexane, methanol and dichloromethane was tested in order to remove external contamination with Polycyclic Aromatic Hydrocarbons (PAHs), but hair samples were not exposed externally to these compounds (Toriba et al, 2013). Toriba et al. (2013) indicated *n*-hexane as the most appropriate medium for external hair decontamination. Methanol, due to higher levels of PAHs in the washing solvent after hair washing, was also considered as a medium that can extract PAHs from the inner part of the hair.

For the investigated FRs, we could not find the most appropriate washing medium to remove only the external contamination. Although methanol removed an important part of FRs, it is not possible to assess how much was extracted from the inner part of hair and in consequence how much this would contribute to the internal exposure. Further, there are some studies where direct extraction in methanol combined with incubation is successfully being used in analysis of cortisol and cortisone (Xing et al., 2013) and steroid hormones (Gao et al., 2013) in human hair. That might also confirm our concerns, that direct extraction with the use of appropriate solvents might be sufficient for hair sample analysis. Based on the outcome of our experiments, we suggest that we should not wash hair samples prior to analysis and rather avoid any potential contamination during sample collection. Hair samples may be used as such and any external contamination that is present on the hair is supposedly deposited from within the personal cloud of the sampled individual. This would enable the use of untreated hair as a suitable proxy for integral (internal and external) exposure monitoring.

4. Implications

To the best of our knowledge, this is the first study performed using hair samples that were externally exposed to BFRs and PFRs. In our study we conclude that dust might be not the only one, potential source of exposure. Also, the presence of dust particles in the air is not mandatory in order to transfer FRs onto the hair surface. Therefore, it is not known yet what is the contribution of each exposure (namely external or internal) and based on our findings we suggest that distinction between these two exposure pathways is practically impossible. Moreover, the profile of compounds in hair and dust is not always similar; levels in hair are usually lower than in dust (Kucharska et al., 2014). During the day, people spend time at many places other than at home (work, school, cars, etc.), therefore human exposure assessment based on the house dust levels might not give a complete picture. Profiles and levels in hair are actually more relevant, as not everything present in dust can be transferred to/onto the human body. Thus, an exposure assessment based on levels in dust might lead to misinterpretations. Further, the experiments indicated that there is no efficient medium that could entirely and exclusively remove external contamination. We therefore suggest that hair samples should not be washed before analysis, but rather all necessary precautions during hair sampling should be ensured (clean scissors, one-use gloves, sample storage, etc.). Water might be an ideal medium only in order to remove larger particles such as sand, dust, and when cosmetics (hair sprays, gel, etc.) were used. Moreover, organic solvents such as methanol should not be used for washing as they might also remove a part of compounds from internal exposure, what might lead to further underestimations in the exposure assessment. Regarding the interpretations of results of hair analysis, the integral exposure should be indicated as the more reliable. We suggest that hair can be used as a passive sampler which is attached to our body 24h/day (in close vicinity of the head and mouth) and integrating exposure from multiple environments, giving an insight in our personal “cloud of exposure”. We conclude that levels detected in hair (independently whether they come from internal or external exposure) will significantly contribute to the exposure assessment and in the future hair might be used as a biomarker of human exposure.

Acknowledgements

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Appendix A. Supporting information

Supporting information and data associated with this article can be found in the online version.

References:

Aleksa K, Carnevale A, Goodyer C, Koren G. Detection of Polybrominated biphenyl ethers (PBDEs) in pediatric hair as a tool for determining in utero exposure. Forensic Sci Intern 2012; 218: 37-43

Altshul L, Covaci A, Hauser R. The relationship between levels of PCBs and pesticides in human hair and blood: preliminary results. Environ Health Perspect 2004; 112: 1193-1199

Alves A, Kucharska A, Erratico C, Xu F, Den Hond E, Koppen G, Vanermen G, Covaci A, Voorspoels S. Human biomonitoring of emerging pollutants through non-invasive matrices: state of the art and future potential. Anal Bioanal Chem 2014; 17: 4063-4088

American Chemistry Council (2014) http://flameretardants.americanchemistry.com/FR-Basics

Barbounis E, Tzatzarakis M, Alegakis A, Kokkinaki A, Karamanos N, Tsakalof A, Tsatsakis A. Assessment of PCBs exposure in human hair using double focusing high resolution mass spectrometry and single quadrupole mass spectrometry. Toxicol Lett. 2012; 210(2):225-31

Behrooz R, Barghi M, Bahramifar N, Esmaili-Sari A, Organochlorine contaminants in the hair of Iranian pregnant women. Chemosphere 2012; 86: 235-241

Birnbaum L, Staskal D. Brominated flame retardants: cause of concern? Environ Health Perspect 2004; 112: 9–17

Boumba V, Ziavrou K, Vougiouklakis T. Hair as a biological indicator of drug use, drug abuse or chronic exposure to environmental toxicants. Int J Toxicol 2006; 25: 143-163

CDC (Centres for Disease Control and Prevention). National biomonitoring program; 2008. http://www.cdc.gov/biomonitoring Accessed: 2008

CEFIC European Flame Retardants Association (CEFIC), What are FRs? Flame Retardant Market Statistics, CEFIC, Brussels, Belgium. www.cefic-efra.eu 2011 (accessed 27.11.2011)

Covaci A, Hura C, Gheorghe A, Neels H, Dirtu A. Organochlorine contaminants in hair of adolescents from Iassy, Romania. Chemosphere 2008; 72: 16-20

Covaci A, Tutudaki M, Tsatsakis A M, Schepens P. Hair analysis: another approach for the assessment of human exposure to selected persistent organochlorine pollutants. Chemosphere 2002; 46: 413–418

D'Havé H, Covaci A, Scheirs J, Schepens P, Verhagen R, de Coen W. Hair as an indicator of endogenous tissue levels of brominated flame retardants in mammals. Environ Sci Technol 2005; 39: 6016-6020

European Court of Justice 2008-04-01. Case C-14/06 (2008)

Gao W, Stalder T, Foley P, Rauh M, Deng H, Kirschbaum C. Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay. J Chromatogr B Anal Technol Biomed Life Sci. 2013; 928: 1-8

Harrad S, de Wit C, Abdallah M, Bergh C, Björklund J, Covaci A, Darnerud P, de Boer J, Diamond M, Huber S, Leonards P, Mandalakis M, Östman C, Småstuen Haug L, Thomsen C, Webster T. Indoor contamination with hexabromocyclododecanes, polybrominated diphenyl ethers and perfluoroalkyl compounds: An important exposure pathway for people? Env Sci Technol 2010; 44: 3221–3231

Hartmann P, Burgi D, Giger W. Organophosphate flame retardants and plasticizers in indoor air. Chemosphere 2004; 57: 781-787

Hughes M, Edwards B, Mitchell C, Bhooshan B. In vitro dermal absorption of flame retardant chemicals. Food Chem Toxicol 2001; 39: 1263–1270

Järnström H, Vares S, Airaksinen M. Semi volatile organic compouns and flame retardants. Occurrence in indoor environment for indoor exposure; VTT TIEDOTTEITA RESEARCH NOTES 2009: 2486

Kim J, Isobe T, Chang K-H, Amano A, Maneja R, Zamora P, Siringan F, Tanabe S. Levels and distribution of organophosphorus flame retardants and plasticizers in fishes from Manila Bay, the Philippines. Environ Poll 2011; 159: 3653-3659

Kintz P. Value of hair analysis in postmortem toxicology. Forensic Sci Intern 2004, 142: 127-134

Kintz P. Analytical and practical aspects of drug testing in hair. CRC Press; Taylor & Francis 2007

Kucharska A, Covaci A, Vanermen G, Voorspoels S. Development of a broad spectrum method for measuring flame retardants - Overcoming the challenges of non-invasive human biomonitoring studies. Anal Bioanal Chem (2014); DOI: 10.1007/s00216-014-8106-z

Leung A, Chan J, Xing G, Xu Y, Wu S, Wong C, Leung C, Wong M. Body burden of Polybrominated diphenyl ethers in childbearing-aged women at an intensive electronic-waste recycling site in China. Environ Sci Pollut Res 2011; 17: 1300-1313

Ma J, Cheng J, Wang W, Kunisue T, Wu M, Kannan K. Elevated concentrations of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans and polybrominated diphenyl ethers in hair from workers at an electronic waste recycling facility in Eastern China. J Hazard Mater 2011; 186: 1966-1971

Malarvannan G, Isobe T, Covaci A, Prudente M, Tanabe S. Accumulation of brominated flame retardants and polychlorinated biphenyls in human breast milk and scalp hair from the Philippines: Levels, distribution and profiles. Sci Total Environ 2013; 442: 366-379

Marklund A, Anderson B, Haglund, P. Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere 2003; 53: 1137-1146

473 Marklund A, Andersson B, Haglund P. Organophosphorus flame retardants and plasticizers in Swedish
474 sewage treatment plants. Env Sci Technol 2005; 39: 7423–7429

475 Mieczkowski T, Tsatsakis A, Kruger, Psillakis T. The concentration of three anti-seizure medications in hair:
476 the effects of hair color, controlling for dose and age. BMC Clin Pharmacol 2001; 1: 2

477 Nakahara Y. Hair analysis for abused and therapeutic drugs. J Chromatogr B 1999; 733: 161-180

478 Nakao T, Aozasa O, Ohta S, Miyata H. Assessment of human exposure to PCDDs, PCDFs and Co-PCBs using
479 hair as a human pollution indicator sample 1: development of analytical method for human hair and
480 evaluation for exposure assessment. Chemosphere 2002; 48: 885–895

481 Nakao T, Aozasa O, Ohta S, Miyata H. Survey of human exposure to PCDDs, PCDFs, and coplanar PCBs using
482 hair as an indicator. Arch Environ Contam Toxicol 2005; 49: 124-130

483 Otake T, Yoshinaga J, Yanagisava Y. Analysis of organic esters of plasticizer in indoor air by GC-MS and GC-
484 FPD. Environ Sci Technol 2001; 35: 3099-3102

485 Pragst F, Balikova M. State of the art in hair analysis for detection of drug and alcohol abuse. Clin Chim Acta
486 2006; 370: 17-49

487 Psillakis T, Tsatsakis A, Christodoulou P, Michalodimitrakis M, Paritsis N, Helidonis E. Carbamazepine levels
488 in head hair of patients under long-term treatment: a method to evaluate the history of drug use. J Clin
489 Pharmacol 1999; 39: 55-67

490 Sato T, Watanabe K, Nagase H, Kito H, Niikawa M, Yoshioka Y. Investigation of the hemolytic effects of
491 various organophosphoric acid triesters (OPEs) and their structure-activity relationship. Toxicol Environ
492 Chem. 1997; 59: 305–313

493 Schramm K. Hair: A Matric for Non-invasive Biomonitoring of Organic Chemicals in Mammals Bull. Environ.
494 Contam. Toxicol 1997; 59: 396-402

495 Schramm K. Hair-biomonitoring of organic pollutants. Review. Chemosphere 2008; 72: 1103-1111

496 Schramm K, Kuettner T, Weber S., Lutzke K., Dioxin hair analysis as monitoring tool. Chemosphere 1992; 24:
497 351-358

498 Sjödin A, Carlsson H, Thuresson K, Sjölin S, Bergman A, Östman, C. Flame retardants in indoor air at an
499 electronics recycling plant and at other work environments. Environ Sci Technol 2001; 35: 448-454

500 Spearman RIC 1977. Biochemistry of hair formation. In: Jarrett, A. (Ed.), The Physiology and
501 Pathophysiology of the Skin. Academic Press, London, United Kingdom. pp. 1302–1305.

502 Stackelberg P, Gibs J, Furlong E, Meyer M, Zaugg S, Lippincott R. Efficiency of conventional drinking-water
503 treatment processes in removal of pharmaceuticals and other organic compounds. Sci Total Environ 2007;
504 377: 255-272

505 Stapleton H, Klosterhaus, Eagle S, Fuh J, Meeker J, Blum A, Webster T. Detection of organophosphate flame
506 retardants in furniture foam and US house dust. Environ Sci Technol 2009; 43: 7490-7495

Sundkvist A, Olofsson U, Haglund P. Organophosphorus flame retardants and plasticizers in marine and fresh water biota and in human milk. J Environ Monit 2010, 12, 4, 943-951

Tadeo J, Sanchez-Brunete C, Miguel E. Determination of polybrominated diphenyl ethers in human hair by gas chromatography-mass spectrometry. Talanta 2009; 78: 138-143

Tsatsakis A, Psillakis T, Paritsis N. Phenytoin concentration in head hair sections: a method to evaluate the history of drug use. J Clin Psychopharmacol 2000; 20: 560-573

Tsatsakis A, Psillakis T, Tzatzarakis M, Kourtopoulos H, Paritsis N. Carbamazepine levels in the hair of patients under long-term treatment: a preliminary study. Clin Chim Acta 1997; 263: 187-195

Tsatsakis A, Tzatzarakis M, M. Pesticide levels in head hair samples of Cretan population as an indicator of present and past exposure. Forensic Sci Int 2008; 176: 67-71

Tsatsakis A, Tutudaki M. Progress in pesticide and POPs hair analysis for the assessment of exposure. Forensic Sci Int 2004; 145:195–199.

Toriba A, Kuramae Y, Chetiyanukornkul T, Kizu R, Makino T, Nakazawa H, Hayakawa K.Quantification of polycyclic aromatic hydrocarbons (PAHs) in human hair by HPLC with fluorescence detection: a biological monitoring method to evaluate the exposure to PAHs. Biomed Chromatogr 2003; 17:126-132.

ToxNet Data Network http://toxnet.nlm.nih.gov/

US Agency for Toxic Substances and Disease Registry (US ATSDR). Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers (PBBs and PBDEs), Atlanta, GA (2004) ATSDR: http://www.atsdr.cdc.gov/toxprofiles/tp68-c6.pdf

US EPA 2005. Furniture Flame Retardancy Partnership: Environmental Profiles of Chemical Flame Retardant Alternatives. 742-R-05-002A

Van den Eede N, Dirtu A, Neels H, Covaci A. Analytical developments and primary assessment of human exposure to organophosphate flame retardants from indoor dust. Environ Int 2011; 37: 454-461

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

Wang T, Fu J, Wang Y, Liao C, Tao Y, Jiang G. Use of scalp hair as indicator of human exposure to heavy metals in an electronic waste recycling area. Environ Pollut 2009; 157: 2445-2451

Wang Y, Jiang G, Lam P, Li A. Polybrominated diphenyl ether in the East Asian environment: a critical review. Env Int 2007; 33: 963-973

Wania F, Dugani C. Assessing the long-range transport potential of polybrominated diphenyl ethers: a comparison on four multimedia models. Env Toxicol and Chem 2003; 22: 1252-1261

Weschler C. Indoor/outdoor connections exemplified by processes that depend on an organic compound's saturation vapor pressure. Atm Env 2003; 37: 5455–5465

Weschler C, Nazaroff W. Semivolatile organic compounds in indoor environments. Atm Environ 2008; 42: 9018-9040

World Health Organization. Flame retardants: tris(2-butoxyethyl) phosphate, tris(2-ethylhexyl) phosphare and tetrakis(hydroxymethyl) phosphonium salts, Environmental Health Criteria 218. Geneva, Switzerland, 2000

World Health Organization. Flame retardants: tris(chloropropyl) phosphate and tris(2-chloroethyl) phosphate, Environmental Health Criteria 209. Geneva, Switzerland, 1998

World Health Organization. International programme on chemical safety. Environmental Health Criteria 192. 81 p. Geneva, Switzerland, 1997

World Health Organization. Triphenyl phosphate. Environmental Health Criteria 111. Geneva, Switzerland, 1991a

World Health Organization. Tri-n-butyl-phosphate. Environmental Health Criteria 112. Geneva, Switzerland, 1991b

World Health Organization. Tricresyl phosphate. Environmental Health Criteria 110. Geneva, Switzerland, 1990

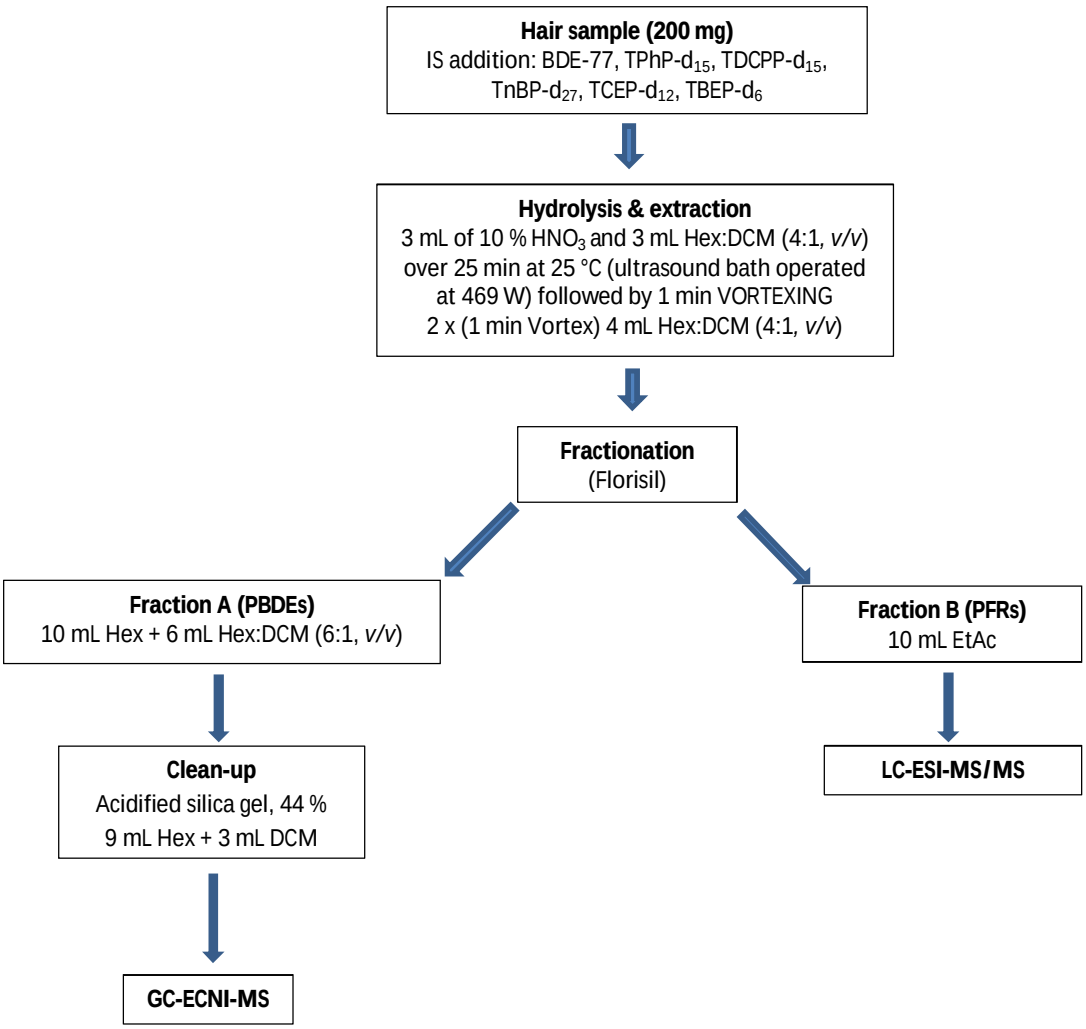
Xing X, Chen Z, Li J, Zhang J, Deng H, Lu Z. Study on dissolution mechanism of cortisol and cortisone from hair matrix with liquid chromatography–tandem mass spectrometry Clin Chim Acta 2013; 421:62-72

Yue C, Li L. Filling the gap: Estimating physicochemical properties of the full array of polybrominated diphenyl ethers (PBDEs). Env Poll 2013; 312-323

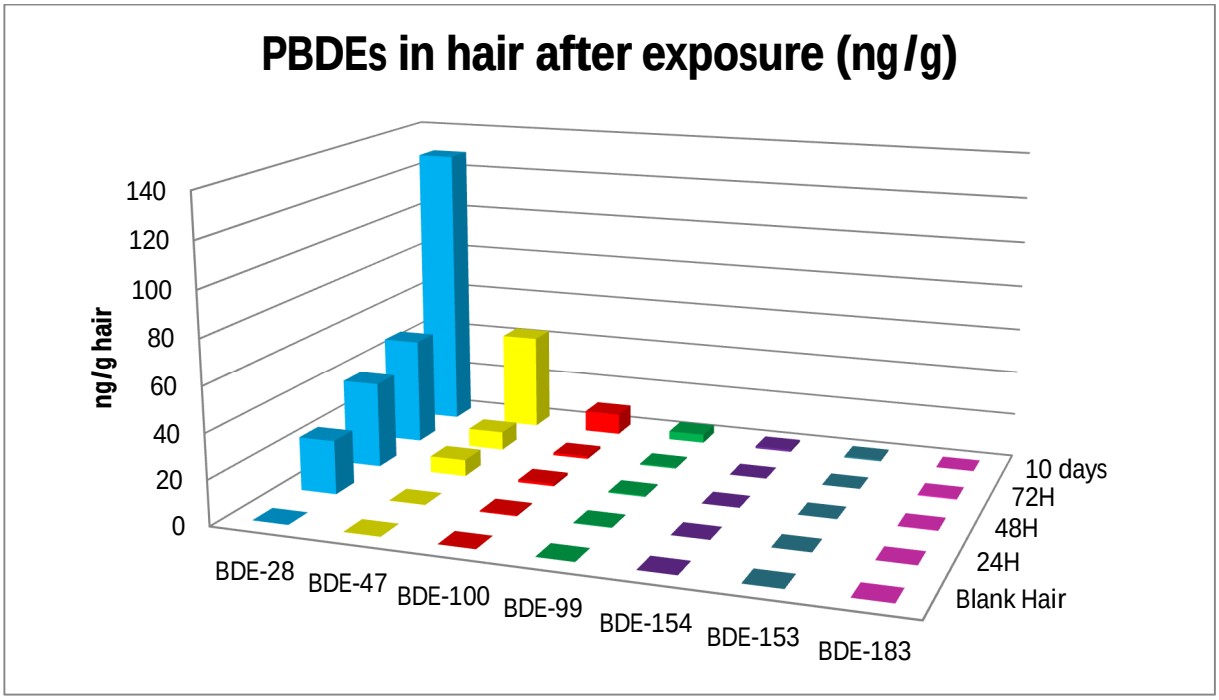
Zhao GF, Wang ZJ, Dong MH, Rao KF, Luo JP, Wang DH, et al. PBBs, PBDEs, and PCBs levels in hair of residents around e-waste disassembly sites in Zhejiang Province, China, and their potential sources. Sci Total Environ 2008; 397: 46-57

Zupancik-Kralj L, Jan J, Marsel J. Assessment of polychlorobiphenyls in human/poultry fat and hair/plumage from a contaminated area. Chemosphere 1992; 25: 1861-1867

Figure 1. Scheme of the analytical procedure



585 Figure 2. Levels of PBDEs after 24h, 48h, 72h and 10 days of exposure (ng/g hair)



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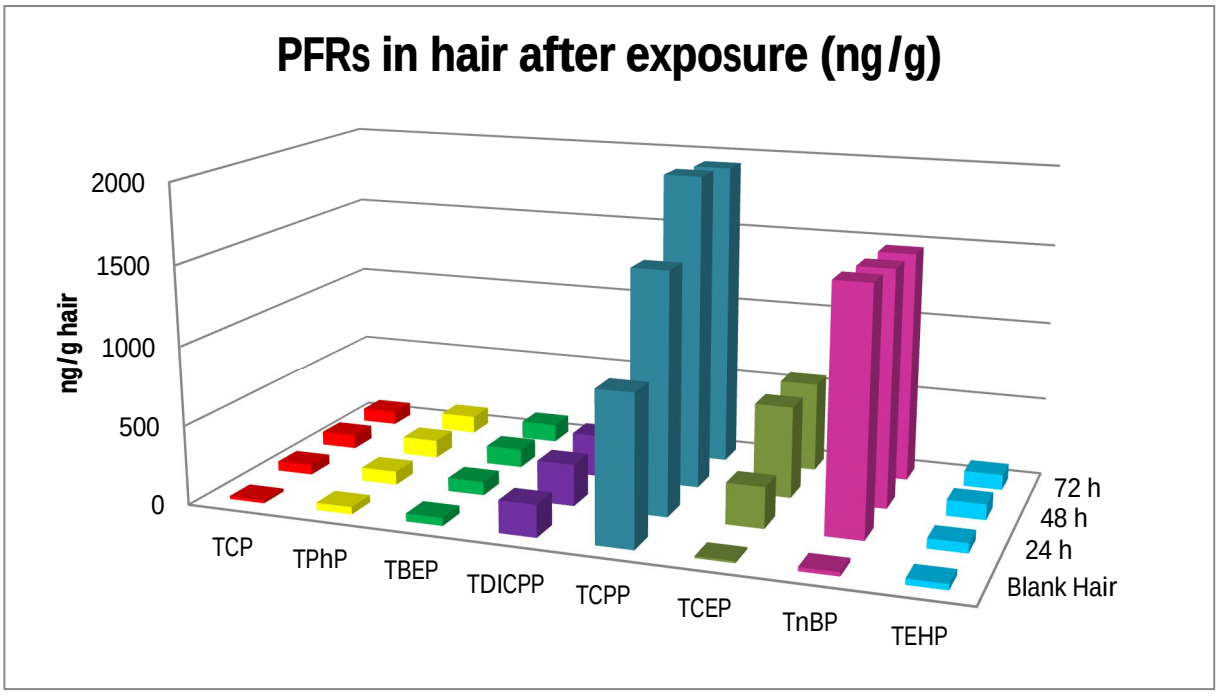
587 Figure 3. Levels of PFRs in hair after 24h, 48h and 72h of exposure (ng/g hair)



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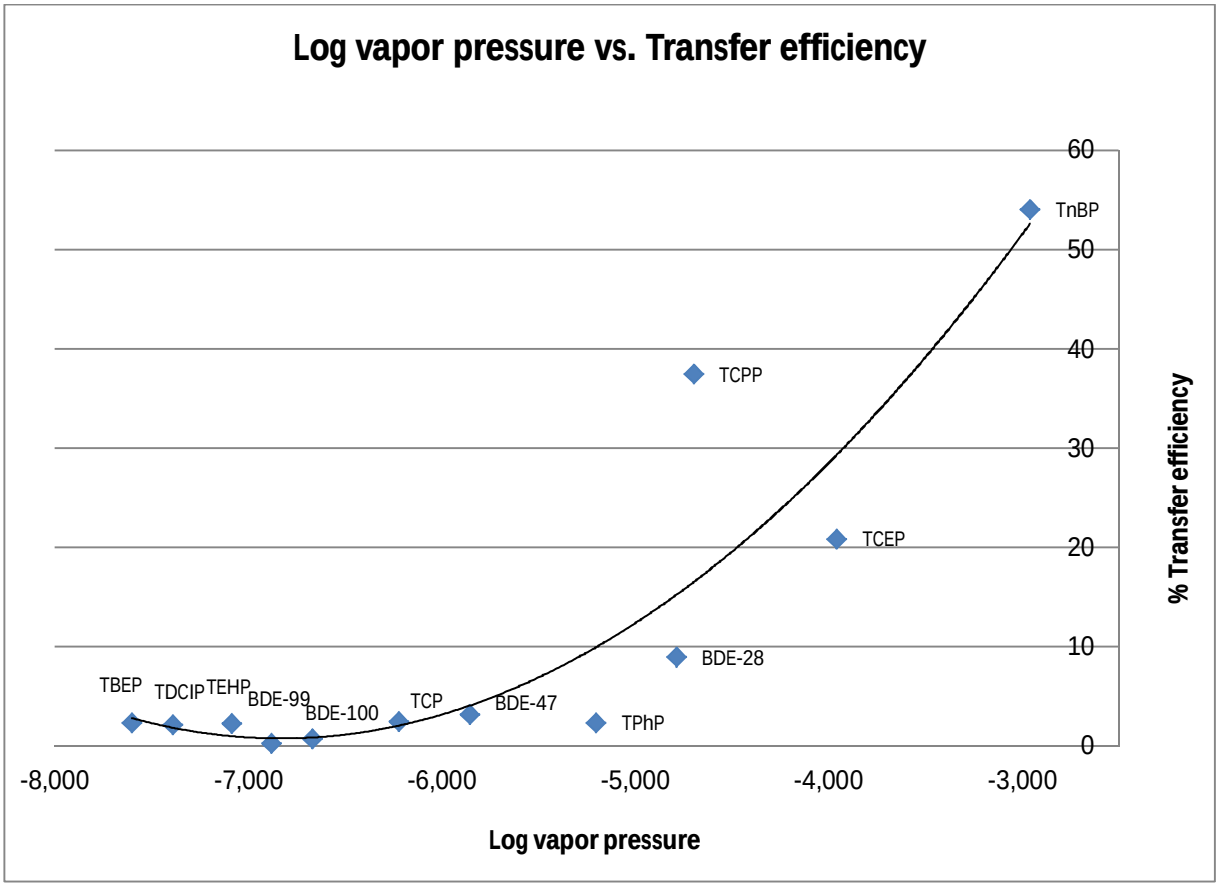
590 Figure 4. Log Vapor pressure vs. transfer efficiency (PBDEs and PFRs)



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596 Table 1. Transfer efficiency vs. physical-chemical properties of PFRs

Compound	% Transfer efficiency (for PFRs after 72H of exposure; for PBDEs after 10* days of exposure) *%T=(Amount in 3g/Amount spiked on watch glasses)100 %)*	Vapor Pressure (mmHg) [a, b]	$logK_{ow}$ [b]	Solubility in water (mg/L for PFRs and µg/L for PBDEs) at 25 °C [a, c, d]
BDE-28	9.0	$1.64x10^{-5}$	5.94	171
BDE-47	3.2	$1.40x10^{-6}$	6.81	54
BDE-100	0.8	$2.15x10^{-7}$	7.24	48
BDE-99	0.3	$1.32x10^{-7}$	7.32	43
BDE-154	-**	$2.85x10^{-8}$	7.82	1
BDE-153	-**	$1.57x10^{-8}$	7.90	1
BDE-183	-**	$3.51x10^{-9}$	8.27	2
TCP	2.5	$6.00x10^{-7}$	5.11	0.36
TPhP	2.4	$6.28x10^{-6}$	4.59	1.9
TBEP	2.4	$2.5x10^{-8}$	3.75	1.1
TDCPP	2.2	$4.07x10^{-8}$	3.27	7.0
TCPP	37.5	$2.01x10^{-5}$	2.59	$1.60x10^{3}$
TCEP	20.9	$1.1x10^{-4}$	1.47	$7x10^{3}$
TnBP	54.1	$1.1x10^{-3}$	4.0	280
TEHP	2.3	8.2510^{-8}	9.49	0.6

597 *- PBDEs were not transferred onto the surface of hair significantly after 72 h of exposure and quantitative determination was not
598 possible. The exposure test therefore in case of PBDEs only was extended towards 10 days
599 **- these congeners were not detected thus transfer efficiency could not be calculated.
600 [a] – US ATSDR 2004 (for PBDEs)
601 [b] - Bergman (2012), units converted from Pascal to mm Hg (for PFRs)
602 [c] - TOXNET-Toxicology Data Network
603 [d] – Yue and Li (2013), units converted from mmol/m^3 to µg/L

604

Table 2. Levels of PBDEs in hair (ng/g) after 72h of exposure, in hair after washing and in washing media after washing. Mass balance and % removal of external contamination

H_2O					
Compound	Hair after exposure (10 days); H_{exp} [1]	H_H_2O [2]	WM_H_2O [3]	% Mass balance [4]	% Removal [5]
BDE-28	129	128	12.6	109	10
BDE-47	43	51	not detected	117	0
BDE-100	10	15	not detected	157	0
MeOH					
	Hair after exposure (10 days); H_{exp}	H_MeOH	WM_MeOH	% Mass balance	% Removal
BDE-28	129	4	85	69	66
BDE-47	43	0.1	31	72	72
BDE-100	10	not detected	7	73	73
Hex:DCM					
Compound	Hair after exposure (10 days); H_{exp}	H_Hex:DCM	WM_Hex:DCM	% Mass balance	% Removal
BDE-28	129	14	89	79	69
BDE-47	43	not detected	34	78	77
BDE-100	10	0.2	7	78	76
Acetone					
Compound	Hair after exposure (10 days); H_{exp}	H_Acetone	WM_Acetone	% Mass balance	% Removal
BDE-28	129	17	77	73	60
BDE-47	43	4	30	78	68
BDE-100	10	0.2	7	70	68
Shampoo [6]					
Compound	Hair after exposure (10 days); H_{exp}	H_Shampoo	WM_Shampoo	% Mass balance	% Removal
BDE-28	129	47	not analyzed	not calculated	63
BDE-47	43	17	not analyzed	not calculated	61
BDE-100	10	3	not analyzed	not calculated	65

[1] H_{exp} – levels/concentration of compounds in hair after exposure
[2] H_H_2O - levels/concentration of compounds in hair after washing in H_2O
[3] WM_H_2O - levels/concentration of comounds in washing medium (H_2O) after washing
[4] % Mass balance = $(H\_H_2O + WM\_H_2O/H_{exp})*100$ %
[5] % Removal = $(WM\_H_2O/H_{exp})*100$ %
[6] % Removal calculated as follows % Removal = (100 % – $(H_{\_Shampoo})/H_{exp})*100$ %); $H_{\_Shampoo}$ – concentration of compounds in hair after washing in Shampoo; C_{expH} – concentration in hair after exposure. Mass balance was not calculated due to the lack of concentration data for Shampoo
Note: The same approach as explained above was used for other washing media

618 Table 3. Levels of PFRs in hair (ng/g) after 72h of exposure, in hair after washing and in washing media after
619 washing. Mass balance and % removal of external contamination

H_2O					
Compound	Hair after exposure (72h); H_{exp}[1]	H_H_2O[2]	WM_H_2O[3]	% Mass balance[4]	% Removal[5]
TCP	87	32	3	41	4
TPhP	111	63	18	73	16
TBEP	114	30	29	52	26
TDCIPP	262	181	26	79	10
TCPP	1937	927	673	83	35
TCEP	571	65	464	93	81
TnBP	1468	829	745	107	51
TEHP	97	55	4	60	4
MeOH					
	Hair after exposure (72h); H_{exp}	H_MeOH	WM_MeOH	% Mass balance	% Removal
TCP	87	0	37	43	42
TPhP	111	2	73	67	66
TBEP	114	0.00	62	55	55
TDCIPP	262	42	220	100	84
TCPP	1937	140	1598	90	82
TCEP	571	7	454	81	80
TnBP	1468	9	1549	106	105
TEHP	97	14	65	81	67
Hex:DCM					
	Hair after exposure (72h); H_{exp}	H_Hex:DCM	WM_Hex:DCM	% Mass balance	% Removal
TCP	87	14	29	49	34
TPhP	111	44	49	83	44
TBEP	114	30	40	61	35
TDCIPP	262	174	46	84	18
TCPP	1937	822	850	86	44
TCEP	571	63	620	120	109
TnBP	1468	241	1485	118	101
TEHP	97	27	47	76	48
Acetone					
	Hair after exposure (72h); H_{exp}	H_Acetone	WM_Acetone	% Mass balance	% Removal
TCP	87	10	14	28	16
TPhP	111	30	30	54	27
TBEP	114	27	19	40	16
TDCIPP	262	149	39	72	15
TCPP	1937	719	770	77	40
TCEP	571	17	386	70	68
TnBP	1468	178	1374	106	94
TEHP	97	33	34	69	35
Shampoo[6]					
	Hair after exposure (72h); H_{exp}	H_Shampoo	WM_Shampoo	% Mass balance	% Removal
TCP	87	12	Not analyzed	Not calculated	87
TPhP	111	24	Not analyzed	Not calculated	79

TBEP	114	18	Not analyzed	Not calculated	85
TDCIPP	262	133	Not analyzed	Not calculated	49
TCPP	1937	638	Not analyzed	Not calculated	67
TCEP	571	18	Not analyzed	Not calculated	97
TnBP	1468	550	Not analyzed	Not calculated	63
TEHP	97	42	Not analyzed	Not calculated	57

[1] H_{exp} – levels/concentration of compounds in hair after exposure

[2] H_H_2O – levels/concentration of compounds in hair after washing in H_2O

[3] WM_H_2O – levels/concentration of compounds in washing medium (H_2O) after washing

[4] % Mass balance = $(H\_H_2O + WM\_H_2O/H_{exp})*100$ %

[5] % Removal = (WM_H_2O/H_{exp}) *100 %

[6] % Removal calculated as follows % Removal =$(100\% – (H_{\_Shampoo)}/H_{exp})*100$ %); $H_{\_Shampoo}$ – concentration of compounds in hair after washing in Shampoo; C_{expH} – concentration in hair after exposure. Mass balance was not calculated due to the lack of concentration data for Shampoo

Note: The same approach in calulations as explained above was used for other washing media