Multi-class method for biomonitoring of hair samples using gas chromatography - mass spectrometry

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Abstract

Currently, non-invasive biomonitoring of human exposure to organic pollutants bases upon the analysis mainly of urine and human breast milk. While mostly persistent organic pollutants are the center of interest, the aim of our study was to develop a method for the determination of different chemical classes of emerging pollutants (organophosphorus flame retardants, plastic additives such as phthalates, bisphenol A, insecticides, antimicrobials, preservatives and musk fragrances) in hair by gas chromatography-mass spectrometry. The preferred sample preparation included hydrolysis of the hair with trifluoroacetic acid in methanol followed by a liquid–liquid extraction using hexane/ethyl acetate. The validated method is characterized by recoveries higher than 77 % for most analytes, relative standard deviations below 16 % and limits of detection between 2 pg mg^{-1} (HHCB) and 292 pg mg^{-1} (propylparaben) using 50 mg of dry hair. After respective blank corrections, bis-(2.ethylhexyl)phthalate (DEHP) and the musk fragrance HHCB were the predominant compounds determined in all hair samples at concentrations between 32 and 59 ng mg^{-1} and 0.8 – 13 ng mg^{-1}, respectively. The bactericide triclosan and the insect repellent N,N-diethyl-3-methylbenzamide (DEET) were detected in selected hair samples at 2 and 0.8 ng mg^{-1}, respectively.

Keywords: *Emerging pollutants; Hair analysis; Gas chromatography-mass spectrometry; Human biomonitoring*

1 Introduction

Humans are exposed permanently to a large variety of chemicals present in their indoor and outdoor environment, in food or beverages, or in clothes, personal care products etc. Numerous organic pollutants are known to impose potential health risks on human and the ecosystem due to their bioaccumulative and toxic character such as polychlorinated dioxins and –furanes (PCDD/F), -biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). Other wide spread applied chemicals such as UV filters, preservatives and antimicrobials, musk fragrances, and insect repellents used in personal care products as well as industrial chemicals (e.g. perfluorinated compounds (PFCs), phthalate esters, flame retardants (FRs) or alkyl phenols) evolved as potential hazards due to their biological effects found in laboratory experiments, environmental and epidemiological studies [1-3]. Along different routes, these pollutants can be incorporated in human tissue and may increase potential health risks.

Human biomonitoring allows assessing human exposure to chemicals via working environment or daily uptake from environment and diet. For epidemiological studies, non-invasive samples such as urine, breast milk, saliva and hair are favoured over blood and plasma because their sampling procedures are often connected with ethical and practical problems [4-8]. Particularly, hair analysis attained a suitable biomonitoring tool due to the easiness of sampling and sample storage that do not require any restricted measures as the presence of medical staff, adapted settings, or refrigerated conditions (9, 10). Furthermore, hair analysis enables a retrospective estimation of chronic and past exposure which is required in drug, doping and forensic studies.

In the context of assessing environmental exposure of humans to chemicals, hair analysis has been less frequently considered as biomonitoring tool so far because the substances determined in hair may reflect other metabolic pathways than those detected in urine, human milk or saliva.

Analytical methods for hair analysis have been developed with focus on defined pollutant classes such as organochlorine pesticides, brominated flame retardants (BFRs), PFCs, PAHs, PCBs, PCDD/Fs and illicit drugs [9,14]. Recent reports on the analysis of five metabolites of bis-(2-ethylhexyl) phthalate (DEHP) [15] and of bisphenol A [16] in human hair indicated the suitability of hair samples for monitoring the exposure of humans towards the ubiquitously present plastic additives.

Common sample preparation in hair analysis starts with washing and cutting or pulverizing the hair specimen. The next step is an acidic or alkaline treatment at

elevated temperature (40 °C-80 °C) that destroys the keratin structure of the hair. Subsequently the reaction mixture is extracted by liquid-liquid extraction using hexane, dichloromethane or ethyl acetate as reported for the determination of BFRs, heterocyclic aromatic amines and illicit drugs in hair [17-19]. Other extraction procedures preferred Soxhlet extraction [20] or hollow fiber solid phase microextraction (HF-SPME) [21]. In another study, ultrasonic extraction with methanol was chosen for hair extraction with the aim to assess the human exposure to organophosphorous pesticides [22]. In case of multicomponent analysis of pesticides, SPE was favoured over liquid-liquid extraction whereas different sorbent types were required to obtain best recoveries for the pesticides with different properties [23].

Depending on the polarity of the target analytes, their analysis has been performed either by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS-MS) [15].

An overview on established methodologies for hair analysis is given in the electronic supplement material as Table S1.

Although suspected of interfering with human health, pollutants such as musk fragrances, preservatives or antimicrobials have been less considered in hair analysis. While analysis of blood or urine samples utilizes commonly multi-class methods, appropriate approaches for biomonitoring by hair are missed. In order to complement data on pollutants in different biological materials, also for hair analysis highly sensitive multi-class methods are required [9, 24].

The aim of our investigations was to develop a multi-class method for the determination of fourteen emerging pollutants in hair by gas chromatography-mass spectrometry. The target compounds belonging to several chemical classes (phthalates, phenolic compounds, polycyclic musks, organophosphates, aromatic amide) are applied commonly as insecticides, antimicrobials, preservatives, flame retardants and fragrances. The new established protocol can be included in e.g. epidemiological investigations, workplace monitoring or health care studies.

2 Experimental

2.1 Chemicals

The substances investigated are listed in Table 1. BPA, ethyl (EtP) and n-propyl (n-PrP) esters of 4-hydroxybenzoic acid, 4-n-nonyphenol (4n-NP) as well as triclosan (TCS) were obtained from Sigma-Aldrich (Steinheim, Germany); Galaxolide (HHCB) and Tonalide (AHTN) were purchased from Promochem (Wesel, Germany); N,N-diethyl-3-methyl-benzamide (DEET), tri-n-butyl phosphate (TBP), triphenyl phosphate (TPP), tris(2-chloro-propyl) phosphate (TCPP), dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DBP) and DEHP were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany); and BPA-d_{16} (BPA-d_{16}) and di-n-butyl phthalate-d_4 (DBP-d_4) were supplied by Cambridge Isotope Laboratories,Inc. (Andover, MA, USA). All reference compounds were of purity >99 %. Acetone, ethyl acetate, n-hexane, methanol and trifluoroacetic acid (TFA) purchased from Merck (Darmstadt, Germany) were of chromatographic analysis grade. The shampoo “herbal essences-fresh balance” (Clairol) used for comparative experiments was bought in the supermarket.

Individual stock solutions were prepared at a concentration of 1 mg mL^{-1} in methanol. Mix standard solutions at different concentrations were prepared in methanol, when used to fortify hair samples, and in ethyl acetate, when considered to evaluate the performance of the GC-MS system, respectively.

2.2 Sample collection

Hair samples were collected from four female volunteers of general population and different age from Leipzig, Germany. All persons permitted the hair sampling and its use for scientific purposes. Their hair was not chemically treated (colored, curled) but the use of hairstyling products was reported within a little survey on the use of cosmetic products and perfumed household products such as fabric softener.

Hair specimens were cut from the posterior vertex region of the head, as close as possible to the scalp with a length of roughly 3-5 cm. All hair samples were stored in aluminum foil, at ambient temperature until further processing and analysis. Hair samples were washed twice with MilliQ water (ultrasonicated for 5 min) and isopropanol (5 min). The successive washing process removes endogenous substances as well as adsorbed chemicals from the surface of the hair [24]. After that, hair samples were cut in small pieces (2-3 mm), further dried at room temperature and wrapped in aluminum foil until the analysis. All wrapped samples were stored in the dark at room

158 temperature until analysis. Thus, all pollutant concentrations determined are related to
159 the weight of the dry hair.
160
161 *2.3 Hair analysis*
162 Fifty mg of hair were incubated with 1 mL of methanol/TFA (8.5:1.5, v/v) or for
163 comparative purposes, 1 mL 2M NaOH solution at 38 °C overnight. In each case, 20 µL
164 of the internal standard mixture at 0.4 ng mg^{-1} were added prior heating. After cooling
165 to room temperature, 4 mL hexane/ethyl acetate (1:1, v/v) were used for liquid-liquid
166 extraction (LLE) of the hair decomposed with methanol/TFA.
167 The samples incubated with NaOH were adjusted to pH 3 with acetic acid prior to LLE
168 with hexane/ethyl acetate. For LLE, the vial was shaken for 15 min and centrifuged at
169 4500 rpm for 10 min. The supernatant formed from the miscible solvents was separated
170 and evaporated to dryness at 40 °C under a stream of nitrogen. Finally, the dry residue
171 was reconstituted in 200 µL of ethyl acetate and 1 µL of this was injected for GC-MS
172 analysis.
173
174 *2.4 Gas chromatography-mass spectrometry*
175 Analyses were performed at a GC-MSD instrument (Agilent Technologies, San Jose,
176 CA, U.S.A.). Analytes were separated on a HP-5MS (30m×0.25mm, 0.25 µm, Agilent
177 Technologies) column. The oven temperature was programmed as follows: start at 50
178 °C for 1 min, increase at 10 K min^{-1} to 280 °C and held at 280 °C for 10 min to achieve
179 a running time of 34 min. The transfer line, ion source and quadrupole analyzer
180 temperatures were maintained at 280, 230 and 150 °C, respectively.
181 Helium was used as carrier gas at constant flow conditions of 1mL min^{-1}. The directly
182 coupled mass spectrometer determined the substances after electron impact ionization in
183 selected ion monitoring (SIM) mode. The target ions of the analytes are listed in Table
184 1. The extract of hair sample 4 and the shampoo extract were also analyzed at full scan
185 mode (mass range 50-400 u). Instrument blank was checked injecting 1 µL ethyl acetate
186 every four analysis and a standard mixture was multiply analyzed within the batch of
187 analyses.
188
189 *2.5 Method validation*
190 The washed and cut hair sample was spiked with the standard mixture in ethyl acetate.
191 The solvent was evaporated completely before the hair was incubated with

methanol/TFA. Method accuracy (expressed as recovery percentage) and precision (in terms of relative standard deviation (RSD)) were determined from recovery experiments in triplicate at high and low concentration levels in the range from 0.2-2.8 ng mg^{-1} depending on the pollutant. Recoveries were calculated by comparing the peak areas of hair samples spiked before extraction and a standard solution in ethyl acetate at the same concentration level. The peak areas of analytes found in the non-spiked hair were used to correct the signal areas of the spiked samples.

The linearity of the analysis method was studied by multi-level calibration curves built from the analysis of standard solutions in ethyl acetate in triplicate at 6 different concentration levels. Thus, the instrumental limits of detection (LOD) and quantification (LOQ) were estimated as the concentrations of the analyte corresponding to a signal-to-noise ratio of 3:1 and 10:1. In cases of DEHP, DBP, TCPP and BPA, the blank signals were considered for corrections. In order to calculate the method limits of detection (MDL) and quantification (LOQ), the recovery data (obtained with the low spiking concentration) were taken into account.

For quantification, the internal standards 4-n-NP, BPA-d_{16}, DBP-d_4 were used (Table 1). Intra-day precision was determined using 2 hair samples spiked at 2 ng mg^{-1} each analyte and measured in triplicate. For the determination of the inter-day precision, the same procedure was repeated on 3 different days.Matrix effects (ME) on the response of analytes were evaluated relating the signal areas of a spiked hair extract to a standard solution at the same concentration level (100 ng mL^{-1} for each substance in ethyl acetate, corresponds to 0.4 ng mg^{-1} hair). The signal areas of the analyte in the non-spiked extract were considered for correction of the spiked sample (Equ. 1).

$$ME = \frac{(\text{signal area hair extract spiked}) - (\text{signal area hair extract not spiked})}{\text{signal area STD}} \qquad \textit{Equation 1}$$

2.6 Blank analysis and instrument performance

Blanks of the individual solvents (1 mL of methanol/TFA and of 4 mL hexane/ethyl acetate) and of both mixtures together were measured following the sample preparation protocol. Results of the blank analyses are included in Table S2 (Supplement material) The blank signals e.g. of DEHP, DBP, TCPP, and BPA were subtracted in terms of the ion area (in counts per second, cps) from the respective target ion signals. The signal areas of the real sample extracts were corrected in the same way by the blank values.

The performance of the instrument was checked regularly by an external standard contained each target analytes at 100 ng mL^{-1}.

3 Results and discussion

3.1 Method optimization

One difficulty in hair analysis is to distinguish between the amount of pollutants adsorbed to the outer surface of the hair and the pollutants inside the hair which were incorporated from the human body. Thus commonly, the first step in hair analysis is a cleaning procedure to remove contaminants from the surface together with biogenic compounds, such as fatty acids, sphingolipids and steroids suspected to affect analysis. (Table S1).

3.1.1 Washing process

In a preliminary experiment, the efficiency of the cleaning procedure was evaluated using hair from a volunteer who has freshly washed the hair with a defined amount of commercial shampoo (2 g wet weight, "herbal essences-fresh balance", Clairol, UK). After air drying, 32 mg hair were taken from the volunteer and subjected to the cleaning procedure as described in 2.2. Another portion of 37 mg underwent the same cleaning procedure twice.

In each case the target substances remained on the hair surface were extracted with 4 mL hexane/ethyl acetate (1:1, v/v). After evaporation and reconstitution in ethyl acetate (200 µL), the target substances were analyzed. Most of the target substances were detected at trace concentration below 1 ng mg^{-1} hair except DEHP, DBP and AHTN which were detected in the range of 1 - 3 ng mg^{-1}(Fig. 1). However, these concentrations are significantly below the pollutant concentration detected later in the real hair samples. This suggests that the washing procedure was strong enough to remove contaminants from the hair's surface. Furthermore, the substance pattern of the shampoo extract was not reflected in the extract of the cleaned hair underlining the efficient removal of e.g. shampoo components by the washing procedure. Particularly the presence of AHTN has to be pointed up because this polycyclic musk compound was not determined in the shampoo extract. An extraction from inner hair parts cannot be excluded although for these cleaning experiments, the hair was not cut in small pieces. Thus, twice cleaning can reduce the substances on the hair surface (Fig. 1) but on the other site, multiple cleaning could extract already substances incorporated in the

hair. For this reason, in the protocol for the analysis of flame retardants Kucharska et al. abstained completely from a cleaning step of the hair [25b].
During our method development, one cleaning step consisting of twice washing with water and once with isopropanol was applied.

3.1.2 Denaturation of the hair sample

After the cleaning step, the denaturation of the hair by acidic, alkaline or enzymatic treatment [17, 18, 24] facilitates the release of the incorporated contaminants. In this study, acidic hydrolysis using MeOH/TFA and basic hydrolysis by aqueous NaOH were compared. Finally, the LLE extracts obtained from acidic hydrolysis were cleaner and exhibited lower noise in the GC-MS analysis compared to those of the alkaline hydrolysis that yielded turbid and dark extracts (Figure S1 in supplement material). These findings are in agreement with literature reports [17, 26].
Therefore, the hair treatment with TFA/methanol was selected for further optimization. The acidic hydrolysis is also advantageous with respect to the stability of esters such as phthalates and parabens. At acidic conditions, the hydrolysis half-life times of DBP and DEHP are > 500 d and > 800 d, respectively [27], and also parabens are known to resist acidic hydrolysis [28].

3.1.3 Liquid-liquid extraction

The LLE of a reaction mixture from 50 mg hair spiked with 2 ng mg^{-1} of each analyte was optimized. Hexane/ethyl acetate (1:1, v/v) and alternatively, dichloromethane were considered as extraction solvents because their suitability to extract substances of a broad range of polarity has been reported previously (e.g. cannabinol derivatives [19] and PCBs [17]). Similar recoveries were obtained for most analytes, except for TBP, HHCB and TCS (Fig. 2). For these three analytes, the recoveries were significantly higher using hexane/ethyl acetate (82 %, 103 %, 99 %) compared to dichloromethane (33 %, 68 %, 41%). Correspondingly, hexane/ethyl acetate was preferred for LLE.

3.2 Method validation

The method performance for each target substance was characterized in accordance to the FDA Guideline [29] determining the linearity of calibration of the instrumental method, the limits of detection (MDL) and quantification (LOQ) of the entire method using hair as well as its accuracy and precision . The calibration curves covered

293 different analyte-dependent concentration ranges (0.002 to -0.8 ng mg^{-1} (hair) for DMP,
294 DEET, TBP, TPP and AHTN; from 0.04 to 4 ng mg^{-1} for EtP, PrP, DEP, DBP, TCPP,
295 HHCB, BPA and TCS and from 0.4 to 80 ng mg^{-1} for DEHP. The linearity of the
296 calibration curves ranges over one to two orders of magnitude, with correlation
297 coefficients (r) from 0.9940 to 0.9998 for all compounds analyzed (Tab. 2).
298 Recoveries were determined from a selected hair sample which was spiked before
299 digestion. The blank analysis of this hair indicated traces of DBP, DEHP, TCPP, and
300 BPA (Tab. S2) which were subtracted from the respective target signals as blank
301 correction. Two concentration levels (0.2 to 2.8 ng mg^{-1}) were examined accounting
302 recoveries from 80% to 120% for most of the analytes (Tab. 2). For BPA, EtP and DBP,
303 the recoveries were found to be suboptimal requiring quantification with labeled
304 internal standards. The low recovery of BPA is probably caused by the extraction with
305 ethyl acetate/hexane. This solvent mixture extracts BPA less efficiently than methanol
306 which was preferred in the protocol described by Tzatzarakis et al. [16]. This study
307 focused on the determination of only BPA in hair samples and reported recoveries
308 between 88 % and 94 %.
309 With 50 mg of the selected hair sample used in our study, the limits of detection of the
310 method (MDL) ranged from 0.001 ng mg^{-1} (DMP) to about 0.3 ng mg^{-1} (PrP) and the
311 respective LOQs ranged from 0.006 ng mg^{-1} (DMP) to 1 ng mg^{-1} (PrP) (Table 2).
312 Probably, derivatization may improve the GC-MS selectivity and sensitivity of the
313 phenolic analytes (BPA, parabens, triclosan) [30] but in order to keep the method as
314 simple as possible for high sample throughput, no derivatization step was included.
315 The precision of the method was determined at a spiked concentration of 2 ng mg^{-1} each
316 analyte. The respective relative standard deviations (% RSD) for intra-day
317 measurements ranged between 2 – 10% and the inter-day precision was detected
318 between 5 – 16% (Table 2).
319 The influence of the hair matrix to GC-MS analysis was determined by comparing the
320 signal response of the analytes spiked at 100 ng mL^{-1} (would correspond to 0.4 ng mg^{-1}
321 hair) into a hair extract as well as in pure ethyl acetate solution. For most of the
322 compounds, matrix effects are weak and tolerable for quantification, except for TPP and
323 DEHP (>44% and 32 % signal enhancement, respectively; Table 2). For TPP, the signal
324 enhancement of 44% corresponds with the elevated recovery of 134 – 154% (Table 2).
325 Here another internal standard than DBP may be required for a better compensation of
326 matrix effects. In general, the use of isotope labeled internal standards for all the target

analytes would improve their quantification but the sensitivity of the instrumental method could suffer due to the extra target ions needed additionally for analysis.

The LOQ values (Table 2) span a wide range from 0.005 ng mg^{-1} for DMP to 0.97 ng mg^{-1} for PrP but allow a reliable detection of the selected pollutants in hair samples. In case of phosphorus flame retardant analysis, LC-MS/MS would be the more sensitive detection method with LOQs reported from 0.001 ng mg^{-1} to 0.033 ng mg^{-1} [25].

The method presented here manages on a small amount of sample and provides good sensitivity and precision without applying any special techniques such as negative chemical ionization or ECD which has been preferred for e.g. polyhalogenated pollutants.

While methods used for hair biomonitoring are often optimized for one substance such as BPA [16] or for a selected class of pollutants such as polybrominated diphenylethers (PBDEs) [31] or DEHP metabolites [15], our multi-class method allows to determine analytes at a broad range of properties (polarity range $=1.6 < \log K_{ow} < 7.6$) at concentrations relevant for biomonitoring. Extra cleanup and concentration steps as described for the analysis of other lipophilic pollutants in hair (examples in Tab. S1) are not required for our set of substances. Methods used to determine illicit drugs in hair are often comparably simple in sample preparation [19] but LC-MS/MS has been favored for the analysis of these semi polar and polar drugs. For instance, limits of quantification at about 0.2 pg mg^{-1} were reported allowing the evaluation of cannabis consumption.

3.3 Analysis of hair samples

To verify the suitability of our method, four hair samples of female adults were analyzed in duplicate. The two parallel analyses are in very good agreement with differences < 10% for most data (Table 3). Typical SIM chromatograms of a real hair sample are shown in Figure 3.

In total 12 of the 14 analytes were determined in at least one samples; BPA and TBP were not found considering the blank signals (Table 3). Highest concentrations were consistently found for DEHP (30 – 60 ng mg^{-1}), HHCB (1 – 12 ng mg^{-1}) and DBP (1 – 7 ng mg^{-1}).

The origin of both phthalates, DBP and DEHP, in the hair samples is not known, yet. They may be taken up from air or from phthalate-bearing dust particles [32]. Until now, only the DEHP metabolites mono (2-ethylhexyl) phthalate has been determined in hair

361 [15]. In case of the phthalates, hair may serve as an integral indicator for endogenous as
362 well as atmospheric exposure but data on the presence of phthalates in hair could not be
363 found in the literature.
364 Significant differences between the four hair samples were observed for the insect
365 repellent DEET, for the fragrance HHCB, the antibacterial agent triclosan (TCS), and
366 for DEP often used as ingredient in personal care products [33]. Sample 4 showed
367 significantly higher concentrations of TCS, DEET, DMP and DEP than the other three
368 samples while the concentration of HHCB was highest in sample 3 (Table 3). It can be
369 assumed that these differences are influenced by lifestyle (e.g. eating habits, personal
370 care) and the household environment [34-36], but the data set is not large enough to
371 elaborate on this.
372 Polycyclic musk compounds have been found in human milk, adipose tissue, blood and
373 urine [37, 38] but data on the occurrence in hair could not be found in literature. The
374 sorption of these volatile musk compounds from atmospheric environment is very
375 likely. Additionally, inhalation of HHCB and its dermal uptake may contribute to an
376 internal exposure and partition into hair. This assumption was supported by the
377 detection of HHCB-lactone a transformation product of HHCB in the extract of sample
378 4. The intensity of the GC-MS signal for the HHCB-lactone (ion trace of m/z 257)
379 exceeded that of HHCB (ion trace of m/z 243) by factor 4.7 but quantification was not
380 possible due to missing the proper reference. In a previous study, the HHCB-lactone has
381 been identified as a metabolite of HHCB measured in human milk samples [37] but its
382 formation by abiotic oxidative processes is possible, too. At least, the analysed
383 shampoo was free of the HHCB-lactone although HHCB was present at remarkable
384 amounts (ca. 500 µg g^{-1} shampoo). DEP, a phthalate often used in cosmetic products
385 might be introduced via inhalation from spray aerosols or contaminated dust particles of
386 indoor environment.
387 Information on the selected substances in hair samples is rare and comparative
388 conclusions difficult to draw.
389 As example, in our hair samples, TBP was not detected above its MDL (0.1 ng mg^{-1})
390 and concentrations of TPP were found in the range of 0.1 ng mg^{-1}– 1 ng mg^{-1}. These
391 concentrations were significantly lower than the mean concentrations reported by
392 Kucharska et al. [25a] with concentrations for TBP at 437 ng mg^{-1} and 82 ng mg^{-1} for
393 TPP whereat a cleaning of the hair prior to denaturation and extraction was not
394 performed [25b]. Thus, the different analytical protocols applied make the results not

fully comparable. This outlines the need to establish commonly accepted analytical protocols for hair analysis to produce reliable and comparable data bases for biomonitoring and related risk assessment.

4 Conclusions

The method developed in this study allows the determination of 14 contaminants, among them organophosphorus flame retardants, plasticizers, insecticides, antimicrobials, preservatives and musk fragrances. The analytes with a wide polarity range (logKow 1.6 – 7.6) can be determined simultaneously with sufficient sensitivity in hair samples using one protocol. A first application of the developed method led to the positive detection of 12 analytes in hair samples. BPA and TBP could not be determined above their LOQs indicating that although some of the contaminants are omnipresent, their concentration in hair can widely differ between individuals.

The use of hair analysis as an approach in biomonitoring of human exposure requires answers to a number of open questions: (a) how the pollutants sorbed from atmospheric environment can be distinguished from those incorporated by ingestion via inhalation, diet or dermal uptake? (b) What is the long term behavior of the contaminants in the hair?, (c) Is there a relationship between the exposure of a person and the amount of a contaminant found in its hair? (d) Is there a relationship between the concentrations found in hair and those found in commonly used body fluids such as breast milk, urine or blood? Further investigations are needed to answer these questions.

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Electronic Supplement Material is available.

References

1. Dann AB, Hontela A (2011) Triclosan: environmental exposure, toxicity and mechanisms of action. J App Tocicol 31: 285-311

2. Golden R, Gandy J, Vollmer G (2005) A review of the endocrine activity of parabens and implications for potential risks to human health. Crit Rev Toxicol 35/5:435–458

3. Dodson RE, Nishioka M, Standley LJ, Perovich LJ, Green Brody J, Rudel RA (2012) Endocrine disruptors and asthma-associated chemicals in consumer products. Environ Health Perspec 120: 935-943

4. Smolders R, Schramm KW, Nickmilder M, Schoeters G (2009) Applicability of non-invasively collected matrices for human biomonitoring. Environ Health 8:8. (open access)

5. Michalke B, Rossbach B, Göen T, Schäferhenrich A, Scherer G (2015) Saliva as a matrix for human biomonitoring in occupational and environmental medicine. Int Arch Occup Environ Health 88:1-44

6. Esteban M, Castaño A (2009) Non-invasive matrices in human biomonitoring: a review. Environ Int 35(2):438-49

7. Malarvannan G, Isobe T, Covaci A, Prudente M, Tanabe S (2013) 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 442:366–379

8. Högberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, Filipsson AF, Jansson B, Johansson N, Appelgren M, Håkansson H (2008) Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. Environ Health Perspect 116:334–339

9. Appenzeller BMR, Tsatsakis AM (2012) Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: State of the art, critical review and future needs.Toxicol Letters 210:119–140

452 10. Król S, Zabiegała B, Namiésnik J (2013) Human hair as a biomarker of human
453 exposure to persistent organic pollutants (POPs). Trends Anal Chem 47:84–98

454 11. Yusa V, Ye X, Calafat AM (2012) Methods for the determination of biomarkers of
455 exposure to emerging pollutants in human specimens. Trends Anal Chem 38:130–142

456 12. Rivier L (2000) Is there a place for hair analysis in doping controls? Forensic Sci Int
457 107(1-3):309-23.

458 13. Cooper GAA, Kronstrand R, Kintz P (2012) Society of Hair Testing guidelines for
459 drug testing in hair. Forensic Sci Inter 218:20-24.

460 14. Barbosa J, Faria J , Carvalho F, Pedro M , Queiros O , Moreira R , Dinis-Oliveira RJ
461 (2013) Hair as an alternative matrix in bioanalysis. Bioanalysis 5: 895-914

462 15. Chang Y-J, Lin K-L, Chang Y-Z (2013) Determination of Di-(2-
463 ethylhexyl)phthalate (DEHP) metabolites in human hair using liquid chromatography-
464 tandem mass spectrometry. Clin Chim Acta 420:155–159

465 16. Tzatzarakis MN, Vakonaki E, Kavvalakis MP, Barmpas M, Kokkinakis EN, Xenos
466 K, Tsatsakis AM (2015) Biomonitoring of bisphenol A in hair of Greek population.
467 Chemosphere 118: 336-341

468 17. Aleksa K, Liesivuori J, Koren G (2012) Hair as a biomarker of polybriminated
469 diethylethers´exposure in infants, children and adults. Toxicol Letters 210:198–202

470 18. Bessette EE, Yasa I, Dunbar D, Wilkens LR, Le Marchand L, Turesky RJ (2009)
471 Biomonitoring of carcinogenic heterocyclic aromatic amines in hair: a validation study.
472 Chem Res Toxicol 22:1454–1463

473 19. Dulaurent S, Gaulier JM, Imbert L, Morla A, Lachatre G (2014) Simultaneous
474 determination of Δ9-tetrahydrocannabinol, cannabidiol, cannabinol and 11-nor- Δ9-
475 tetrahydrocannabinol-9-carboxylic acid in hair using liquid chromatography-tandem
476 mass spectrometry. Forensic Sci Int 236:151–156

477 20. Goodpaster JV, Drumheller BC, Benner BA (2003) Evaluation of Extraction
478 Techniques for the Forensic Analysis of Human Scalp Hair Using Gas
479 Chromatography/Mass Spectrometry (GC/MS) J Forensic Sci 48: 1-8

480 21. Ebrahimi M, Eshaghi Z, Samadi F, Hosseini M-S (2011) Ionic liquid mediated sol-
481 gel sorbents for hollow fibre solid-phase microextraction of pesticide residues in water
482 and hair samples. J Chromatogr A 1218:8313– 8321

483 22. Tsatsakis AM, Barbounis MG, Kavalakis M, Kokkinakis M, Terzi I, Tzatzarakis
484 MN (2010) Determination of dialkyl phosphates in human hair for the biomonitoring of
485 exposure to organophosphate pesticides. J Chromatogr B 878:1246–1252

486 23. Duca RC, Salquebre G, Hardy, E, Appenzeller BMR (2014) Comparison of solid
487 phase- and liquid/liquid-extraction for the purification of hair extract prior to multiclass
488 pesticides analysis. J Chromatogr B 955-956:98-107

489 24. Schramm KW (2008) Hair biomonitoring of organic pollutants. Chemosphere
490 72:1103–1111

491 25.a) Kucharska A, Covaci A, Vanermen G, Voorspoels S (2014) Development of a
492 broad spectrum method for measuring flame retardants- Overcoming the challenges of
493 non-invasive human biomonitoring studies. Anal Bioanal Chem 406:6665-6675

494 25.b) Kucharska A, Covaci A, Vanermen G, Voorspoels S (2015) Non-invasive
495 biomonitoring for PFRs and PBDEs: new insights in analysis of human hair externally
496 exposed to selected flame retardants. Sci Total Environ 505:1062-1071

497 26. Tadeo JL, Sánchez-Brunete C, Miguel E (2009) Determination of polybrominated
498 diphenyl ethers in human hair by gas chromatography–mass spectrometry.Talanta
499 78:138–143

500 27. Lertsirisopon R, Soda S, Sei K, Ike M (2009) Abiotic degradation of four phthalic
501 acid esters in aqueous phase under natural sunlight irradiation. J Environ Sci 21: 285–
502 290

503 28. Alves A, Kucharska A, Erratico C, Xu F, Den Hond E, Koppen G, Vanermen G,
504 Covaci A, Voorspoels S (2014) Human biomonitoring of emerging pollutants through
505 non-invasive matrices: state of the art and future potential.Anal Bioanal Chem
506 406(17):4063-88.

507 29. U.S. Department of Health and Human Services, Food and Drug Administration
508 (2015) “Analytical Procedures and Methods Validation for Drugs and Biologics”,
509 Guidance for Industry, pp.3

510 30. Shanmugam G, Ramawamy BR, RadhakrishnanV, Tao H (2010) GC–MS method
511 for the determination of paraben preservatives in the human breast cancerous tissue.
512 Microchem J 96: 391-396

513 31. Król S, Namieśnik J, Zabiegała B (2014) Occurrence and levels of polybrominated
514 diphenyl ethers (PBDEs) in house dust and hair samples from Northern Poland; an
515 assessment of human exposure. Chemosphere 110:91–96

516 32. Cousins AP, Holmgren T, Remberger M (2014) Emissions of two phthalate esters
517 and BDE 209 to indoor air and their impact on urban air quality. Sci Total Environ 470-
518 471: 527-535

519 33. Api AM (2001) Toxicological profile of diethyl phthalate: a vehicle for fragrance
520 and cosmetic ingredients. Food Chem Toxicol. 39(2):97-108.

521 34. Altshul L, Covaci A, Hauser R (2004) The relationship between levels of PCBs and
522 pesticides in human hair and blood: preliminary results. Environ Health Perspect
523 112:1193–1199

524 35. Covaci A, Tutudaki M, Tsatsakis AM, Schepens P (2002) Hair analysis: another
525 approach for the assessment of human exposure to selected persistent organochlorine
526 pollutants. Chemosphere 46, 413–418

527 36. Chojnacka K, Górecka H, Górecki H (2006) The effect of age,sex, smoking habit
528 and hair color on the composition of hair.Environ Toxicol Pharmacol 22:52–57

529 37. Reiner JL, Wong CM, Arcaro KF, Kannan K (2007) Synthetic musk fragrances in
530 human milk from the United States. Environ Sci Technol 41:3815–20

531 38. Hond ED, Paulussen M, Geens T, Bruckers L, Baeyens W, David F, Dumont E,
532 Loots I, Morrens B, de Bellevaux BN, Nelen V, Schoeters G, Van Larebeke N, Covaci
533 A (2013) Biomarkers of human exposure to personal care products: results from the
534 Flemish Environment and Health Study (FLEHS 2007-2011). Sci Total Environ 463-
535 464:102–110

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Legend of figures

Figure 1. *above:* The pattern of the target substances in a commercial hair shampoo extract (2 g extracted with ethyl acetate), *below:* Efficiency of the number of cleaning processes using the procedure included in the protocol

Figure 2. Influence of the solvent extraction on the recoveries of the procedure (n = 3), 2 ng mg^{-1} of each analyte was used for spiking the hair

Figure 3. SIM chromatogram of a hair sample superimposed to a standard solution (500 ng mL^{-1}) and to a blank of ethyl acetate

Tables

Table 1. Studied compounds, their log K_{ow} values and target ions used for MS detection (SIM)

Table 2. Performance parameters of the method, R_{low}: Recovery of low concentration (2 ng mg^{-1} for TCS, BPA, DEHP, EtP and PrP; and 0.2 ng mg^{-1} for the rest of analytes), R_{high}: Recovery of high concentration (2.8 ng mg^{-1} for TCS, BPA, DEHP, EtP and PrP; and 2 ng mg^{-1} for the rest of analytes).*fortification with 100 -ng mL^{-1}-(0.4 ng mg^{-1} hair) each component, negative value = signal suppression, positive = signal enhancement

Table 3. Concentration of selected pollutants in four hair specimens (n=2)

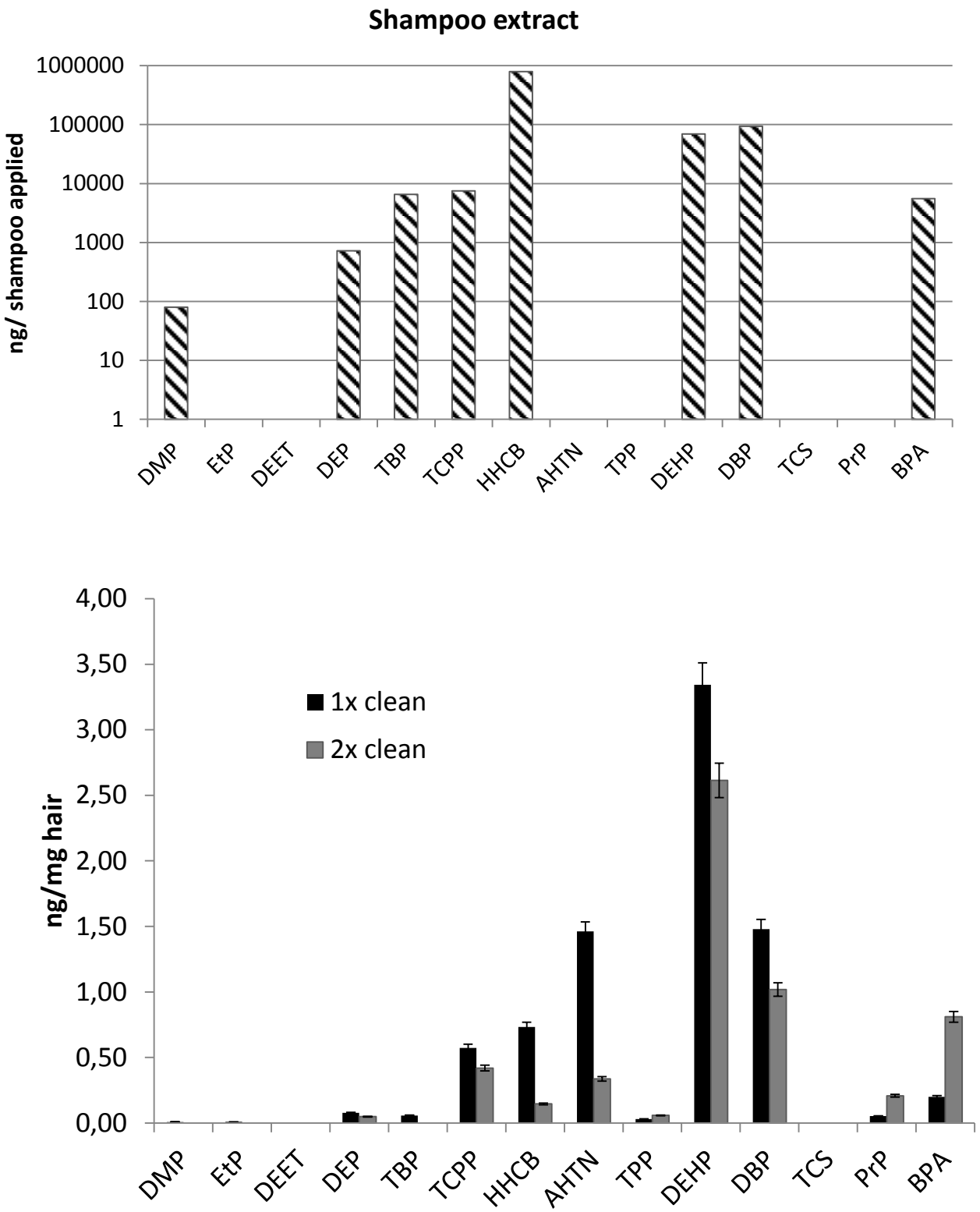
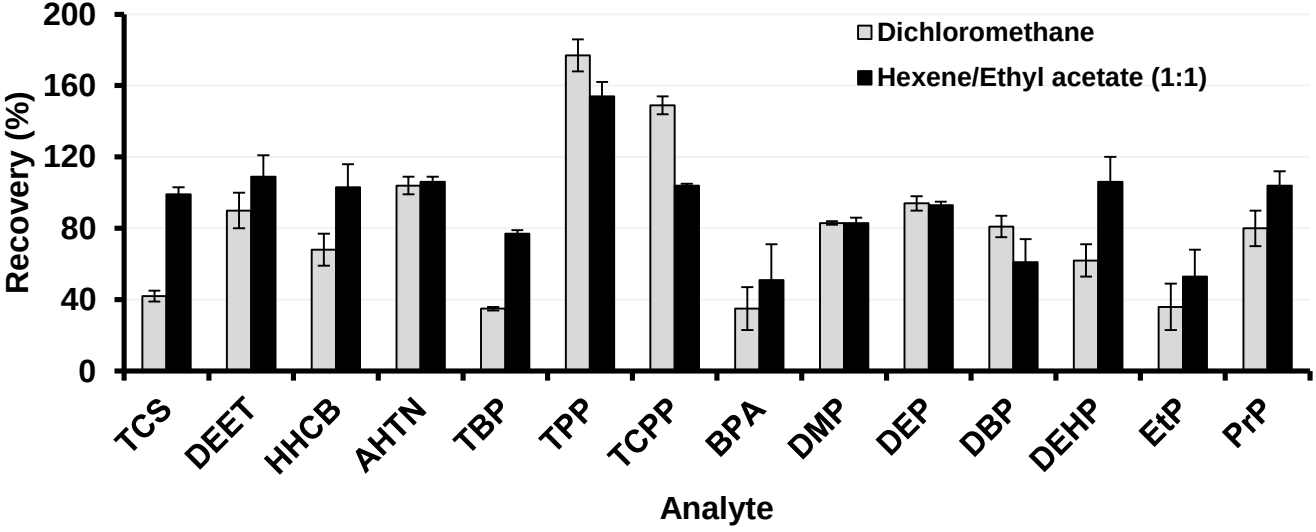

Shampoo extract
ng/ shampoo applied
1000000
100000
10000
1000
100
10
1
DMP
EtP
DEET
DEP
TBP
TCPP
HHCB
AHTN
TPP
DEHP
DBP
TCS
PrP
BPA
ng/mg hair
4,00
3,50
3,00
2,50
2,00
1,50
1,00
0,50
0,00
1x clean
2x clean

Figure 1


Dichloromethane
Hexene/Ethyl acetate (1:1)
Recovery (%)
200
160
120
80
40
0
TCS
DEET
HHCB
AHTN
TBP
TPP
TCPP
BPA
DMP
DEP
DBP
DEHP
EtP
PrP
Analyte

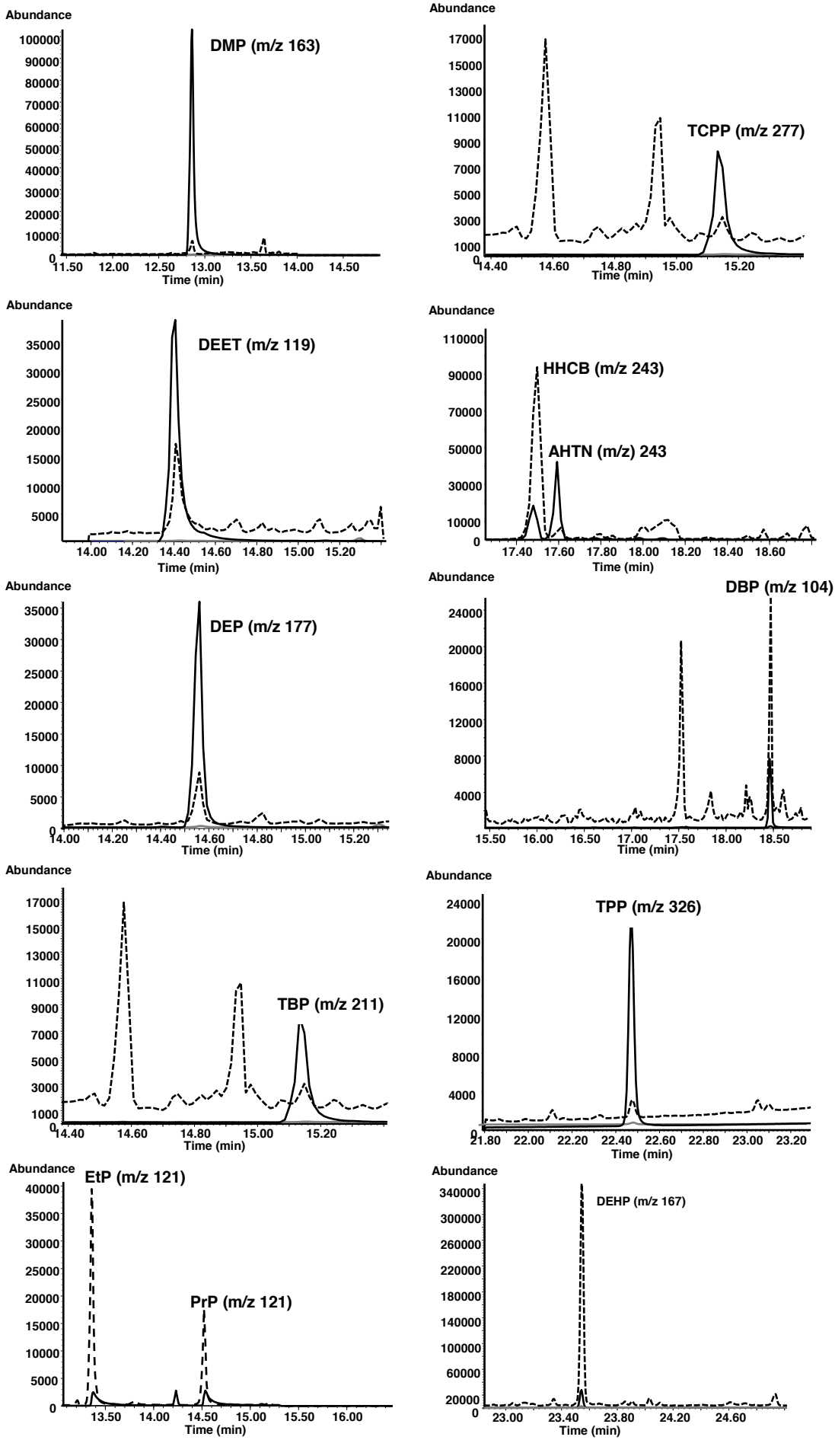

Abundance
DMP (m/z 163)
Time (min)
Abundance
TCPP (m/z 277)
Time (min)
Abundance
DEET (m/z 119)
Time (min)
Abundance
HHCB (m/z 243)
AHTN (m/z) 243
Time (min)
Abundance
DEP (m/z 177)
Time (min)
Abundance
DBP (m/z 104)
Time (min)
Abundance
TBP (m/z 211)
Time (min)
Abundance
TPP (m/z 326)
Time (min)
Abundance
EtP (m/z 121)
PrP (m/z 121)
Time (min)
Abundance
DEHP (m/z 167)
Time (min)
Blank ethyl acetate
Mixture standard solution (500 ng/ml)
Hair sample

Table 1. Studied compounds, their log K_{ow} values and target ions used for MS detection (SIM) and gas chromatographic retention times R_t

Target compound (abbreviation)	CAS number	Chemical class/ usage	Log K_{ow}[a]	Target ions for SIM mode (m/z)[b]	R_t (min)	Internal Standard for quantitation
Triclosan (TCS)	3380-34-5	chlorinated phenoxy phenol/ antimicrobial	4.76	**218**, 288, 290	21.3	4n-NP
N,N-diethyl-meta-toluamide (DEET)	134-62-3	phenyl amide/ insect repellent	2.18	**91**, 119, 190	15.3	4n-NP
Galaxolide (HHCB)	1222-05-5	polycyclic musk/ fragrance	5.9	**243**, 258, 213	18.7	4n-NP
Tonalide (AHTN)	1506-02-1		5.2	**243**, 258	18.9	4n-NP
Tri-n-butyl phosphate (TBP)	126-73-8	organophosphorus compound/ flame retardant	4	**211**, 155	16.2	DBP-d4
Triphenyl phosphate (TPP)	115-86-6		4.59	**325**, 326	24.0	DBP-d4
Tris(chloro-2-propyl) phosphate (TCPP)	13674-84-5		2.89	**277**, 279	18.1	DBP-d4
Bisphenol A (BPA)	80-05-7	phenolic compounds/ antioxidant	3.32	**213**, 228	21.8	BPA-d16
Dimethyl phthalate (DMP)	131-11-3	phthalates/ plasticizer	1.6	**163**, 77	13.7	DBP-d4
Diethyl phthalate (DEP)	84-66-2		2.42	**149**, 177	15.5	DBP-d4
Di-n-butyl phthalate (DBP)	84-74-2		4.5	**104**, 149	19.7	DBP-d4
Di-2-ethylhexyl phthalate (DEHP)	117-81-7		7.6	**149**, 167	25.2	DBP-d4
Ethylparaben (EtP)	120-47-8	hydroxyl-benzoic acid ester/ preservative	2.47	**121**, 138, 166	14.5	4n-NP
n-Propylparaben (PrP)	94-13-3		3.04	**121**, 138, 180	15.8	4n-NP

Internal standards				
4n-Nonylphenol (n-NP)[a]	25154-52-3	5.71	**107**, 220	18.7
Bisphenol A d16 (BPA-d16)[a]	96210-87-6		**224**, 242	21.9
Di-n-butyl phthalate-d4 (DBP-d4)a	93952-11-5		**153**, 223	19.6

[a]: Experimental values from Database ChemSpider; [b]: quantifier ion bold

Table 2. Performance parameters of the method, R_{low}: Recovery of low concentration (2 ng mg^{-1} for TCS, BPA, DEHP, EtP and PrP; and 0.2 ng mg^{-1} for the rest of analytes), R_{high}: Recovery of high concentration (2.8 ng mg^{-1} for TCS, BPA, DEHP, EtP and PrP; and 2 ng mg^{-1} for the rest of analytes).*fortification with 100 -ng mL^{-1}- (0.4 ng mg^{-1} hair) each component, negative value = signal suppression, positive = signal enhancement

Compound	**r^2**	**Linear range (MDL to…) (ng mg^{-1})**	**Method precision (RSD %) (n=3) (2 ng mg^{-1} each)**		**MDL (ng mg^{-1})**	**LOQ (ng mg^{-1})**	**Recovery R in % (± % RSD, n=3)**		**Matrix effect* (%)**
			Intra-day	**Inter-day**			**R_{low}**	**R_{high}**	
TCS	0.9948	4	2	5	0.276	0.920	99 (4)	80 (6)	-5

DEET	0.9957	0.8	10	12	0.022	0.072	109 (12)	111 (14)	12
HHCB	0.9992	4	2	10	0.002	0.007	103 (13)	90 (13)	5
AHTN	0.9998	0.8	9	9	0.002	0.007	106 (3)	104 (13)	13
TBP	0.9966	0.8	2	5	0.031	0.102	77 (10)	82 (9)	-5
TPP	0.9986	0.8	6	8	0.031	0.102	154 (8)	134 (2)	44
TCPP	0.9995	4	5	8	0.021	0.069	104 (1)	93 (10)	6
BPA	0.9988	4	9	13	0.041	0.137	51 (20)	72 (14)	-10
DMP	0.9974	0.8	9	7	0.001	0.005	83 (3)	105 (10)	-3
DEP	0.9991	4	2	6	0.019	0.062	93 (9)	110 (13)	-4
DBP	0.9985	4	9	10	0.020	0.065	61 (13)	87 (13)	-15
DEHP	0.9940	80	4	8	0.025	0.080	106 (14)	120 (9)	32
EtP	0.9940	4	8	12	0.149	0.498	53 (15)	32 (21)	15
PrP	0.9971	4	7	16	0.292	0.972	104 (8)	110 (12)	19

Table 3. Concentration of selected pollutants in four hair specimens (n=2)

Compound	Concentration (ng mg^{-1})							
	Sample 1		Sample 2		Sample 3		Sample 4	
TCS	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.84	1.87
DEET	0.14	0.13	0.07*	0.07*	0.19	0.22	0.79	0.73
HHCB	4.00	4.20	0.82	0.84	11.95	12.7	2.68	2.67
AHTN	0.23	0.29	0.12	0.12	1.16	1.26	0.45	0.51

TBP	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
TPP	0.78	0.91	0.14	0.10	0.21	0.20	0.78	0.67
TCPP	1.00	1.09	0.19	0.18	0.48	0.57	1.70	1.55
BPA	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
DMP	0.13	0.13	0.06	0.07	0.02	0.02	0.36	0.36
DEP	2.41	2.40	0.49	0.44	0.06*	0.06*	15.31	15.42
DBP	6.25	6.48	5.23	5.16	1.04	1.49	7.08	6.74
DEHP	42.91	46.31	34.96	32.52	43.21	58.78	55.04	46.33
EtP	1.83	1.98	0.81	0.84	<MDL	<MDL	<MDL	<MDL
PrP	1.42	1.52	0.42*	0.40*	<MDL	<MDL	<MDL	<MDL

<MDL: below method limit of detection; * below LOQ (see Tab. 2)

Electronic supplement material

Title: Multi-class method for biomonitoring of hair samples using gas chromatography - mass spectrometry

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Content:

Abbreviations:

ACN: Acetonitrile; BPA: Bisphenol A; DCM: Dichloromethane; DEHP: Di-(2-ethylhexyl)phthalate; SPME: Solid phase microextraction; ECD: Electron capture detector; EtOH: Ethanol;GPC: Gel permeation chromatography; HBCDs: Hexabromocyclododecanes; HF-SPME: Hollow fiber-solid phase microextraction; HS-SPME: Head space-solid phase microextraction; FID: Flame ionization detector; Fl: Fluorescence detector; HAAs: heterocyclic aromatic amines; PBDEs: polybrominated diphenyl ethers; NBFRs: novel brominated flame retardants; MeOH: Methanol; BPs: bromophenols; PCBs: polychlorinated biphenyls; OCPs: organochlorine pesticides; OPPs: organophosphate pesticides; PFCs: Perfluoroalkyl compounds; PAHs: polycyclic aromatic hydrocarbons; PCDDs: polychlorinated dibenzo-p-dioxins; PCDFs: polychlorinated dibenzofurans; SDS: Sodium dodecyl sulphate; SFE: Supercritical fluid extraction; TFA. Trifluoroacetic acid.

Table S1. Overview of procedures reported for the determination of different groups of organic pollutants in human hair

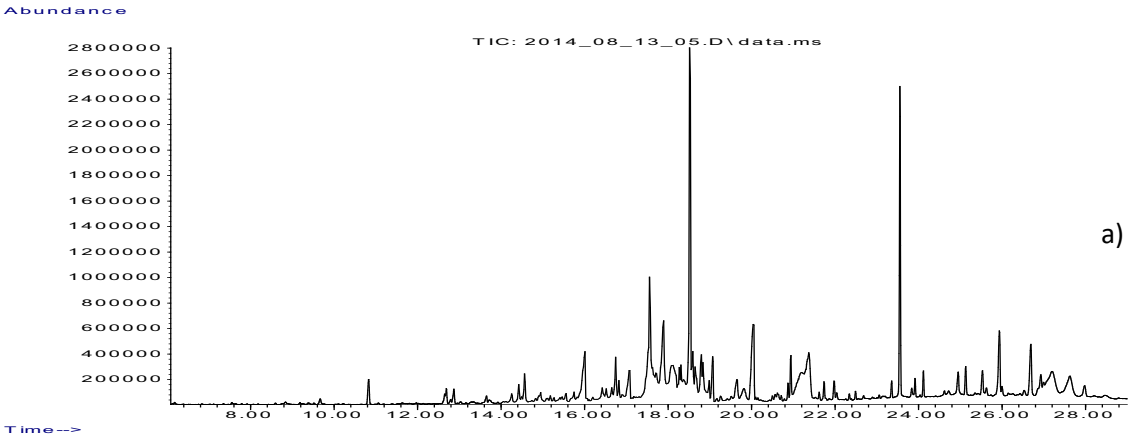
Pollutants	Sample-precleaning	Sample amount	Sample preparation	Extraction	Clean-up	Analysis method	Reference
8 PBDEs		10-40 mg	2mL hexane:DCM (4:1), 1.5mL 4N HCl (14h, 40ºC)		Na_2SO_4/Florisil SPE cartridges	GC-MS	[17]
PBDEs, NBFRs, BPs, PCBs, OCPs and their metabolites	2xH2O (24h)	80 mg	5mL 3N HCl, 5mL hexane/DCM (4:1) (12h, 45ºC)	3x5mL hexane/DCM (4:1)	Silica-Bond Elut cartridges	GC-MS	Ali et al., 2013
57 PCBs and 9 OCPs	35mL hot H2O, 35mL H2O with shampoo, 5x30mL H2O (30min)		3N HCl (12h, 40ºC)	3xhexane/DCM (4:1, v/v)	SPE cartridge: deactivated alumina, acidified silica and anhydrous Na_2SO_4	GC-ECD	[36]
9 OCPs, 7 PCBs	3xdestilled H2O	500 mg	4mL 4M HCl and 3mL hexane/DCM (4:1, v:v) (40ºC)	2X4mL hexane/DCM (4:1, v:v)	SPE cartridge: acidified silica and anhydrous Na_2SO_4	GC-MS/MS	Behrooz et al., 2012
3 HAAs	3x1mL 0.1N HCl, 3x1mLMeOH	0.25-2 g	1mL 1N NaOH (80ºC, 1h)	2X5mL ethyl acetate	SPE Oasis MCX	LC-MS/MS	[18]
5 DEHP metabolites	2mL DCM	25 mg	0.5mLMeOH/TFA (8.5:1.5, v/v) (45 °C, overnight). Adjusted ph 3 (glacial acid)	2mL ethyl acetate		LC-MS/MS	[15]
Antipsychotic (Clozapine)	2x2mL deionized H2O, 2x2mL ethyl acetate (3min)	5 mg	USE 900 ul mobile phase (1h). Centrifuged			LC-MS/MS	Chen et al., 2014
10 OCPs and 5 PCBs	5mL H2O (5min)	200 mg	2mL 3N HCl (12h, 40ºC)	2 X 3mL hexane/DCM (4:1)	SPE cartridge: deactivated alumina, acidified silica and anhydrous Na_2SO_4	GC/ECD	[37]
5 OCPs, 3 OPPs	H2O (SDS)	200 mg		SFE: CO2 extraction		GC-MS	Cuong et al., 2012
4 Illicit drugs	H2O (2min), 2xDCM	20 mg	1mL 1M NaOH (10 min, 100ºC). 1mL of acetic	7mL of a hexane/ethyl		LC-MS/MS	[19]

	(1min)		acid	acetate (90/10, v/v)			
6 pesticides	20mL DCM, 20mL acetoneand 15mLMeOH	50 mg	2mLMeOH, phosphate buffer (55ºC, 5h)	Multi-walled carbon nanotubes (MWCNTs). HF-SPME using hollow fiber-supported ionic liquid mediated sol–gel sorbent		HPLC-DAD	[21]
4 monocyclic aromatic compounds	20mL DCM, 15mL acetone, 15mLMeOH and 10mLMeOH (5min)	50 mg	MeOH (2mL, 50ºC, 5h, ph 7.4 phosphate buffer). Filtered and rinsed with 2mLEtOH	HF-SPME (containing carbon nanotube reinforced sol–gel)		GC-FID	Es'haghi et al., 2011
7 opiate						GC/MS/MS	Gambelunghe et al., 2005
52 monohydroxylated metabolites of PAHs	10mL H2O (2 min)	50 mg	1mL 1N NaOH (overnight, 40 °C). 500µL 2N HCl and 1mLammonium acetate buffer	2x2mL DCM. Dried, 2mL cyclohexane and 2mLMeOH/H2O (80:20; v/v)	Envi-Chrom P SPE column	GC-MS/MS	Grova et al., 2013
Antiepilptic, abuse drug (Clonazepan)	2mL H2O (0.1% SDS), 2mL H2O, 2mL MeOH (3 min)	50 mg	1mL of phosphate buffer (50mM, 4h, ultrasonicated)	Vortexed with a 1mL diethyl ether:chloroform (70:30, v:v)		LC-MS/MS	John et al., 2014
14 HAAs	3x1mLMeOH (3 min)	10 mg	0.2mL 1M NaOH (60 min, 100ºC)	2M HCl; 0.2mL 0.1M phosphate buffer; H2O to1mL. Centrifugated. In tube-SPME		LC-MS/MS	Kataoka et al., 2013
2 insecticides	5mL H2O (10min) and 2x5mLMeOH (1min)			2x2mLMeOH (3h)		LC-MS/MS	Kavalakis et al., 2013
18 opioids and metabolites	2x2mL MeOH, 2mL H2O, 2x2mL MeOH	10 mg	2mLMeOH (16 h)			LC-MS/MS	Kim J et al., 2014
8 PBDEs	H2O, 2xshampoo, H2O (10min)	0.5-2.5 g		hexane/acetone (3:1, v/v)	SPE glass columns: activated alumina and acidified silica gel	GC-MS/MS	[30]
2 PFCs	20mL H2O (10min), 2xacetone	100 mg		3x10mLAcN (2h, 55ºC)	SPE Oasis WAX cartridges	LC-MS/MS	Li et al., 2013

29 PCBs	H2O (1%SDS), H2O	500 mg	4mL 4M HCl (12h, 40ºC) 3mL of hexane/DCM (4:1, v/v)	2x4mL hexane/DCM (4:1, v/v). Concentrated to 2-3mL; dehydrated with Na2SO4	SPE cartridge: deactivated alumina, acidified silica and anhydrous Na2SO4	GC-MS	Liang et al., 2014
8 OCPs, 4 PBDEs and 12 PCBs	3x1mL Acetone (1min)	1 g	MAE: 3mL acetone/formic acid (4:1, v/v) (110ºC, 15min)	3X3mL hexane/DCM (4:1, v/v)	GPC	GC-MS/MS	Lu et al., 2014
5 PCDDs, 5PCDFs and 10 PBDEs	3xH2O (10min)	3-10 g		Soxhlet: 400mL, DCM/hexane (3:1) (24h)	SPE cartridge: silica gel, acidified silica and anhydrous Na_2SO_4	GC/ECD	Ma et al., 2011
PCBs and PBDEs, HBCDs	Milli-Q H2O (1h, 40°C) with shampoo,tap H2O, distilled H2O and 10x Milli-Q H2O	3-10 g	20mL 4M HCl (overnight 40°C) and 20mL of hexane/DCM (4:1, v/v)	2x4mL hexane/DCM (4:1, v/v)	GPC and activated silica gel	GC-MS	[7]
Pesticides (dichloro-diphenyl-trichloroethanes (DDTs))	H2O (1% SDS) (5min), H2O	2 g		Soxhlet: 150mL of hexane/DCM (18h)	GPC: acidified silica gel and florisil	GC-MS	Man et al., 2014
Herbicides: terbuthylazine and desethylterbuthylazine	2.5mL H2O (15s)	50 mg		2.5mLMeOH (5h 55°C, sonicated)		LC-MS/MS	Mercadante et al., 2012
96 drugs: opiates, amphetamines, hallucinogens, benzodiazepines, antihistamines, antidepressants, antipsychotics, barbiturates, musclerelaxants	isopropanol, 2xH2O	10 mg		MeOH/ACN/NH_4OOCH (37ºC, 18h), homogenized and centrifugated,filtrated, diluted		UPLC-MS/MS	Montesano et al., 2014
21 PFCs	H2O (15 min), 2x acetone	250 mg	0.5mL Acetone (1h)	5 mL ACN (15min)		LC-MS/MS	Pérez et al., 2012
Pesticides: carbamates, OPPs, OCPs, pyrethroids, and a chloroacetanilideherbicide		50 mg		2x2mL hexane (6h)		GC-MS	Posecion et al., 2006
22 Pesticides: OCPs, OPPs, dinitroanilin, nicotianilin, phenol, azole and pyrethroids	H2O (2min) and AcN (2min) (40 ºC)	50 mg	1mL ACN (40ºC, 12 h); centrifugate	800 uL of supernatant and 7mL phosphate buffer) DI-SPME (30 and 90ºC)		GC-MS/MS	Salquebre et al., 2012
13 metabolites of PAHs		50 mg	1 M NaOH (60ºC, 30 min). Centrifuged. 1mL of	2x2mL DCM		GC-MS	Schummer et

			acetate buffer and 800 uL 2M HCl to adjust pH 5				al., 2009
50 Pesticides: OCPs, OPPs, pyrethroids,acetanilides and carbamates	H2O(1min), AcN (1min)	50 mg	1mL ACN (12 h, 40ºC) Centrifuged. 700 uL were mixed with 7.3mL phosphate buffer (pH 7)	SPME ((poly)dimethylsiloxane divinylbenzenefiber (60min)		GC-MS/MS	Schummer et al., 2012
15 Basic drugs	H2O (5min), acetone (5 min)	10 mg	0.5g Na_2SO_4, 1mL 1M NaOH and phosphate buffer	HS-SPME (30min, 90ºC or 15 min 60ºC)		GC-MS	Sporkert et al., 2000
18 PBDE	H2O and shampoo	200 mg	3mL 3N HCl (overnight, 40ºC)	4X2mL hexane	Glass chromatographic column	GC-MS	[26]
10 PAHs	hexane	50 mg	2.5M NaOH	Hexane		HPLC-Fl	Toriba et al., 2003
4 OPP	2xH2O and MeOH (3min)	500 mg	2mLMeOH (12h, 37ºC).	2mL H2O, 3mL ethylacetate		GC-ECD	Tsatsakis et al., 2008a
9 OCP	2xH2O and MeOH (3min)	200 mg	2mL 3M HCl (40ºC, 12h)	2x3mL hexane/DCM (4:1)	SPE cartridge: deactivated alumina, acidified silica and anhydrous Na_2SO_4	GC-MS	Tsatsakis et al., 2008b
4 OPP	5mL H2O (10min) and 5mLMeOH (1min)	100 mg		2mLMeOH (30min, 40ºC). Centrifugated		GC-MS	[22]
6 OCPs and 6 PCBs	2xH2O (5min, 40ºC)	100 mg		4mL 4M HCl and 4mL hexane/DCM (4:1, v/v) (40ºC, 12h)		GC-MS/MS	Wielgomas et al., 2012
23 PBBs, 12 PBDEs, and 27 PCBs	20mL H2O (10min), 2xshampoo and H2O	2-5 g		Soxhlet:hexane/acetone (3:1, v/v) (24 h)	Multilayer silica gel column	GC-MS	Zhao et al., 2008
23 PBDEs	2x H2O (1h, 40ºC)	2 g	40mL 4M, HCl (12h, 40ºC) and 40mL of hexane/DCM (4:1, v/v)	3x 40mL of hexane/DCM (4:1, v/v)	Multi-layersilica/aluminacolumn	GC-MS	Zheng et al., 2011
BPA	2x5 mL H_2O ultrasonication (5 min),	100 mg	Cut, +internal standard	2x2mL MeOH, 50°C, ultrasonication for 2x 2 h		LC-(APCI)MS	[16]

2mL MeOH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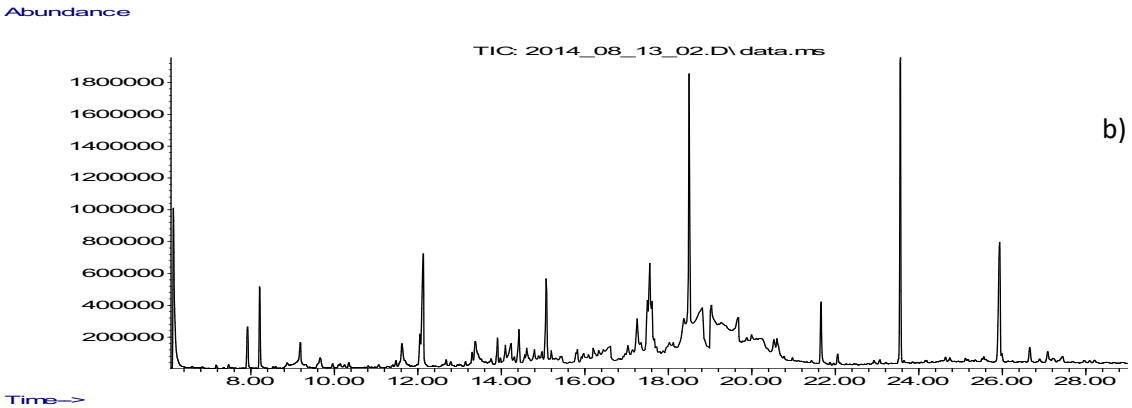
a)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b)

Figure S1. Total ion chromatograms of GC-MS(SIM) analysis of hair extracts (non-spiked) a) treated with MeOH/TFA and extracted with ethyl acetate and b) treated with NaOH solution, then adjusted to pH 3 with acetic acid and extracted with ethyl acetate.

Table S2. Blanks of the solvents used for the individual sample preparation steps and of the complete method without hair (n=2). Values are given as amount per injection. (n.d. = not detected)

	MeOH/TFA pg/µL (±%RSD)	hexane/ethylacetate pg/µL (±%RSD)	Complete method pg/µL (±%RSD)
TCS	n.d.	1 (6)	n.d.
DEET	n.d.	n.d.	n.d.
HHCB	1 (8)	1 (5)	1(6)
AHTN	1 (6)	n.d.	n.d.
TBP	n.d.	n.d.	n.d.
TPP	n.d.	1(1)	1(3)
TCPP	6 (2)	6 (1)	7(2)
BPA	n.d.	2 (10)	2(8)
DMP	n.d.	n.d.	n.d.
DEP	1 (4)	1 (4)	2 (4)
DBP	11 (5)	8 (6)	13(6)
DEHP	14 (5)	13 (5)	16(3)
EtP	n.d.	n.d.	n.d.
PrP	n.d.	n.d.	n.d.

References for SI

Behrooz RD, Barghi M, Bahramifar N, Esmaili-Sari A (2012) Chemosphere 86:235–241

Chen H, Xiang P, Shen M (2014) J Forensic Leg Med 22:62–67

Cuong LP, Evgen'ev MI, Gumerov FM (2012) J Supercritical Fluids 61:86–91

Eshaghi Z, Ebrahimi M, Hosseini M-S (2011) J Chromatogr A 1218:3400–3406

Grova N, Salquèbre G, Appenzeller BMR (2013) Anal Bioanal Chem 405:8897–8911

John C, Ghosh P, Varshney KM, Kaur S, Shukla SK, Satyanarayana S (2014) J Liquid Chromatogr Related Technol 37:1917–1928

Kataoka H, Inoue T, Saito K, Kato H, Masuda K (2013) Anal Chim Acta 786:54–60

Kavalakis MP, Tzatzarakis MN, Theodoropoulou EP, Barbounis EG, Tsakalof AK, Tsatsakis AM (2013) Chemosphere 93:2612-2620

Kim J, Ji D, Kang S, Park M, Yang W, Kim E, Choi H, Lee S (2014b) J Pharm Biomed Anal 89:99– 105

Li J, Guo F, Wang Y, Zhang J, Zhong Y, Zhao Y, Wu Y (2013) Environ Int 53:47–52

Liang B, Liu X, Hou J, Liang G, Gong W, Xu D, Zhang L (2014) Environ Pollut 185:10–15

Lu D, Feng C, Lin Y, Wang D, Ip HSS, Qiu X, Wang G, She J (2014) Chemosphere 114:327–336

Ma J, Cheng J, Wang W, Kunisue T, Wu M, Kannan K (2011) J Haz Mat 186:1966–1971

Man YB, Chan JKY, ShengWang H, Wu SC, HungWong M (2014) Environ Int 65:73–82

Mercadante R, Polledri E, Giavini E, Menegola E, Bertazzi PA, Fustinoni S (2012) Toxicol Letters 210:169–173

Montesano C, Johansen SS, Nielsen MKK (2014) J Pharm Biomed Anal 88:295–306

Perez F, Llorca M, Farré M, Barceló D (2012) Anal Bioanal Chem 402:2369–2378

Posecion Jr N, Ostrea Jr E, Bielawski D, Corrion M, Seagraves J, Jin Y (2006) Chromatographia 64:681–687

Salquèbre G, Schummer C, Millet M, Briand O, Appenzeller BMR (2012) Anal Chim Acta 710:65–74

Schummer C, Appenzeller BMR, Millet M, Wennig R (2009)
J Chromatogr A 1216: 6012–6019

Schummer C, Salquèbre G, Briand O, Millet M, Appenzeller BMR (2012) Toxicol Letters 210:203–210

Sporkert F, Pragst F (2000) J Chromatogr B 746:255–264

Toriba A, Kuramae Y, Chetiyanukornkul T, Kizu R, Makino T, Nakazawa H, Hayakawa K (2003) Biomed Chromatogr 17:126–132

Tsatsakis AM, Tzatzarakis MN, Tutudaki M (2008a) Forensic Sci Int 176:67–71

Tsatsakis AM, Tzatzarakis MN, Tutudaki M, Babatsikou F, Alegakis AK, Koutis C (2008b) Hum Exp Toxicol 27:933–940

Wielgomas B, Czarnowski W, Jansen EHJM (2012) Chemosphere 89: 975–981

Zhao G, Wang Z, Dong MH, Rao K, Luo J, Wang D, Zha J, Huang S, Xu Y, Ma M (2008) Sci Total Environ 397:46–57

Zheng J, Luo X-J, Yuan J-G, Wang J, Wang Y-T, Chen S-J, Mai B-X, Yang Z-Y (2011) Environ Pollut 159:3706–3713