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Dermal uptake of benzophenone-3 from clothing

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Key words

benzophenone-3, clothing, dermal uptake, exposure, biomonitoring

Abstract

Benzophenone-3 (aka BP-3, oxybenzone) is added to sunscreens, plastics and some coatings to filter UV radiation. The suspected endocrine disruptor BP-3 has been detected in the air and settled dust of homes and is expected to redistribute from its original sources to other indoor compartments, including clothing. Given its physical-chemical properties, we hypothesized that dermal uptake from clothing could contribute to the body burden of this compound. First, cotton shirts were exposed to air at an elevated concentration of BP-3 for 32 days; the final air concentration was 4.4 μg/m^3. Then three participants wore the exposed shirts for 3 hours. After this 3-h exposure, participants wore their usual clothing while collecting urine samples for the next 48 hours. Urine was analyzed for BP-3 and a metabolite, BP-1, and six other UV filters. The rate of urinary excretion of the sum of BP-1 and BP-3 increased for all participants during and following the 3-hour exposure. The summed mass of BP-1 and BP-3 excreted during the first 24 hours attributable to wearing exposed t-shirts were 12, 9.9 and 82 μg for participants 1, 2 and 3 respectively. Analysis of these results coupled with predictions of steady-state models suggest that dermal uptake of BP-3 from clothing could meaningfully contribute to overall body burden.

Introduction

2-Hydroxy-4-methoxybenzophenone commonly known as benzophenone-3 (BP-3), is an ultraviolet (UV) light filter used in sunscreen, cosmetics and other personal care products. It is also added to plastics and coatings to reduce UV damage in industrial and consumer products[1]. BP-3 and its metabolites have been found in blood and urine of people in the US, Europe and China[2–5]. Based on its weak estrogenic activity as shown in several in vitro and in vivo studies,

BP-3 has been flagged as a suspected endocrine disruptor and is on the EU-commission priority list of potential endocrine disrupting chemicals[6]. The endocrine disrupting ability of BP-3 was recently confirmed in a study showing a skewed sex ratio favoring females in zebrafish following developmental exposure[7]. In addition, recent in vitro studies have shown that BP-3 and some other chemical UV filters mimic the effect of progesterone on the CatSper Ca^{2+} channel in human sperm cell, triggering multiple sperm cell functions essential for fertilization of the egg and exposure to BP-3 is therefore suspected to impact on male fertility[8]. Furthermore, the major metabolite of BP-3, 2,4-dihydroxybenzophenone or benzophenone-1 (BP-1), is a suspected endocrine disruptor and is included on the Substitute it Now (SIN) list[6]. In vitro studies have identified both estrogenic and anti-androgenic activity for BP-1[9], and urinary BP-1 has been shown to be associated with endometriosis in US women[10].

Dermal uptake from personal care products and cosmetic products is believed to constitute the major exposure pathway for BP-3. A number of studies have documented dermal uptake of BP-3 following its topical application[11–16] In a human subject trial, Janjua et al.[16] observed a rapid rise in the BP-3 concentration in plasma and urine after applying a BP-3 containing cream to 2 m^2 of body area. Median urine concentrations after 24 h (12 ng/ml for females; 81 ng/ml for males) were within the 25-75th percentile range observed in the US population (5.8-94 ng/ml)[17], but somewhat higher than the 25-75th percentile range observed in Danish pregnant women (1.1-14 ng/ml) and young Danish men from the general population (1.3-7.8 ng/ml)[18]. Zamoiski et al.[19] observed a positive correlation between self-reported sunscreen use and urinary BP-3. In an intervention study, Harley et al.[20] observed an average 36% decrease in urinary BP-3 concentrations in adolescent girls after being encouraged to use BP-3-free personal care products

provided by the researchers. BP-3 was found in most personal care products tested by Liao et al.[21] and they estimated that 80% of dermal exposure is due to skin lotions and face creams.

Dermal uptake of BP-3 may also occur from contaminated air and clothing, although uptake from either has not been directly observed. Weschler and Nazaroff[22] predicted that dermal uptake to bare skin from indoor air could contribute substantially to the body-burden of many semi-volatile organic compounds (SVOCs). Weschler et al.[23] supported these predictions by demonstrating the dermal uptake of two phthalates from air for six bare-skinned subjects. Applying the Weschler and Nazaroff model, Morrison et al.[24] predicted that dermal uptake of BP-3 from the gas phase is probable, if it is present in indoor air. Wan et al.[25] observed BP-3 concentrations in the air of US homes ranging from 0.19 to 72 ng/m^3. Although they estimated that inhalation would not be an important pathway, they did not consider the potential for dermal uptake from air or clothing.

Dermal uptake from air can also be influenced or even enhanced by wearing clothing that had been exposed to, or impregnated with, skin-permeable chemicals[26–32]. Xue et al[33] detected BP-3 in 70% of newly purchased infant clothing and estimated a mean dermal dose of about 7 ng/kg body weight /day. Clothing can also have a high sorptive capacity for SVOCs present in homes and other buildings[34–39], resulting in high-intensity sources of exposure close to the skin. Therefore, BP-3 adsorbed to clothing from indoor air may meaningfully contribute to overall body-burden of BP-3. While cosmetics and other personal care products can contribute significantly to exposure[19,20], we are concerned about the persistent, all-year background exposure which is seemingly independent of sunscreen or personal care product application[40]. Efforts to reduce BP-3 in these products[41] may have a limited effect if substantial amounts are also absorbed from indoor air and clothing.

The objective of this research is to investigate the hypothesis that dermal uptake can occur for individuals wearing clothing that has had the opportunity to sorb BP-3 from air.

Methods.

Shirt dosing and participant exposure experiments took place at the Technical University of Denmark (DTU), Lyngby, Denmark. Plasma and blood analysis took place at the Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark. BP-3 air samples were collected at DTU and analyzed at Fraunhofer, WKI Braunschweig, Germany. The research protocol was approved by the Capital Region of Denmark Committee for Research Ethics (case no. H-16018670).

Shirts. Five new, dark blue, long-sleeve t-shirts were purchased on May 4, 2016 from a local department store in Lyngby, Denmark. They were made of 100% cotton according to the attached tag. They were identical except for size (one small, one medium, two large). The measured density and thickness were 0.28 g/cm^3 and 0.062 cm, respectively. They were washed in a mechanical washer with hot water and a fragrance-free detergent. They were dried with an electric hot air dryer. Four of the t-shirts were then transferred to the dosing chamber, while the fifth was kept as an unexposed control.

BP-3. BP-3 of 98+ % purity was purchased from Alfa Aesar, Thermo Fisher GmbH, Karslruhe, Germany. The properties of BP-3 can be found in Supporting Information, Table S1.

Shirt dosing chamber and shirt preparation. A small closet-sized, sealed dosing chamber was constructed to expose shirts to an elevated air concentration of BP-3. The chamber was constructed of foam-board covered in aluminum foil with a metal internal support frame. Approximately 20 g of BP-3, a solid at room temperature, was heated until melted and brushed

116 onto three clean 0.15 m^2 aluminum sheet pans to increase the exposed surface area. These pans
117 were placed on the floor of the chamber and a small muffin fan was installed to enhance air
118 movement. Four shirts were hung inside-out on plastic hangers in the sealed chamber for 32
119 days. On day 27, the pans coated with BP-3 were removed from the chamber, but the shirts were
120 not removed, and the chamber was re-sealed. Removing the pans for several days allows shirts to
121 equilibrate with the surrounding air without an emission source driving sorption. Chamber
122 concentrations were measured (see *Air concentrations* section) on day 31 and shirts were worn
123 by participants immediately after being removed from the dosing chamber on day 32.

124 **Participant exposure chamber**. The participant exposure chamber has been described in detail
125 elsewhere[23]. Briefly, the 55 m^3 chamber is a converted room with a controllable ventilation
126 system operated at an air exchange rate of 0.7/h. Unlike prior dermal uptake experiments[23,28], the
127 concentration of the target analyte was not intentionally elevated in the chamber and participants
128 did not wear breathing hoods during the three-hour exposure period. Even though the
129 participants were wearing shirts with sorbed BP-3, we anticipated that the breathing zone
130 concentration would be low because the chamber was well ventilated and well mixed with fans.
131 As a check of this assumption, the air concentration near the breathing zone was measured to
132 determine if inhalation contributed significantly to total dose (see *Air concentrations* section).
133 The temperature was between 20-23ºC during the exposure period.

134 **Participants**. Prior to the exposure, the 3 male participants, all with normal characteristics
135 (Table 1) were asked not to apply any sunscreen or any other personal care products that may
136 have contained UV filters two days before and two days after the three-hour exposure period.
137 The participants were also asked not to shower after exposure until the following morning. Each
138 participant chose a shirt that they felt fit them best. The shirts were normal-to-close fitting, but

neither skin-tight nor very loose. See Supporting Information Figure S1 for images of participants wearing shirts.

Table 1. Personal characteristics.

	Participant 1	Participant 2	Participant 3
Age (y)	27	36	51
Weight (kg)	73	71	84
Height (m)	1.80	1.80	1.84
BMI (kg/m^2)	22.5	21.9	24.8
BSA (m^2)	1.92	1.89	2.08

BMI; body mass index is calculated as (body weight (kg))/(height (m))^2

BSA; body surface area (based on method of DuBois and DuBois[42]

Urine sample collection. The participants collected the total volume of three consecutive first-morning urine voids, the first one on the morning prior to exposure (day 1). During the period between the first-morning urination on the exposure day and the first-morning urination the following day (day 2), they also collected the total volume of each spot urine voids separately. Finally, during the period between the first- morning urination on day 2 and the first-morning urination on day 3 they collected all spot urine voids as one pool (Figure 1). All urine samples were collected in 1 L or 2.5 L polyethylene bottles.

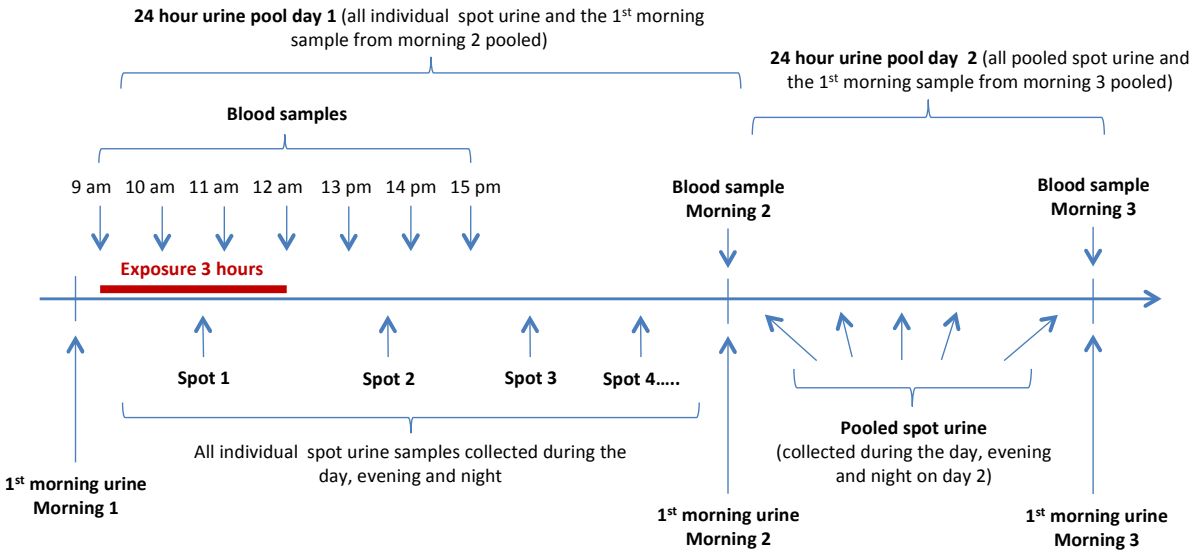


Figure 1. Schedule of urine and blood sample collection

In the chemical laboratory, urine samples were handled immediately after being received. All urine samples were weighed and 5 ml aliquots of all samples were stored. Subsequently a 24-hour urine pool for day 1 was prepared by pooling the remaining volume of all spot urine samples of day 1 together with the first-morning urine of day 2. For a 24 hour urine pool of day 2 the remaining volume of the spot urine pool from day 2 was combined with the first-morning urine of day 3. Aliquots of all urine samples were stored at -20°C until chemical analysis.

Shortly before exposure began, a peripheral venous catheter (venflon) was placed in the antecubital vein of each participant. Through this catheter a blood sample was collected immediately before the start of the exposure period and five additional blood samples were collected one hour apart over the next five hours after which the catheter was removed. Additionally, blood samples were taken in the morning of day 2 and 3 by venipuncture of the cubital vein (Figure 1). Blood samples were allowed to clot and serum was obtained by

centrifugation for 10 min at 2000g and subsequently aliquoted and stored at -20°C until chemical analysis.

Chemical analyses. All urine and serum samples were analyzed for the free and total (sum of free and conjugated) content of eight different UV filters (Supporting Information, Table S2) by a recently developed method for UV filters analyzed in urine using isotope dilution TurboFlow-liquid chromatography – tandem mass spectrometry (LC-MS/MS) with prior enzymatic deconjugation.[43] In addition, the method was optimized and validated for analysis of UV filters in serum. The limit of detections (LOD) for BP-1 and BP-3 were respectively 0.25 and 0.28 ng/ml in urine and 0.13 and 0.12 ng/ml in serum. Other LODs are shown in Supporting Information, Table S2.

In short, the samples were analyzed in four batches for, respectively, urine and serum samples with and without enzymatic deconjugation. Each batch included standards for calibration curves, 24 urine or 27 serum samples, three blanks, and control material of respectively urine or serum containing three samples pooled unspiked, three samples pooled spiked with native UV filter standards at a low level and three samples pooled spiked at a high level.

The recovery for all analytes spiked in urine or serum in both spike levels was ≥90%, except for 5-chloro-2-hydroxybenzophenone (BP-7) at low (70%) and high (75%) spike levels in serum and at both spike levels (>79%) in urine.

Air concentrations. Air samples were collected by drawing 10-20 L of air through sorbent tubes containing Tenax-TA (Buchem, BV) with a calibrated sampling pump set at 0.1-0.2 L/min. Three samples were taken from the shirt dosing chamber one day before the participant exposure experiments, and a field blank was set aside for later analysis. The field blank was treated exactly

as other tubes except that no air sample was collected. Air samples were also collected from the breathing zone of two participants during the 3 h exposure period. The sample tube was attached to the participant's work desk, with the inlet approximately 20-30 cm from the nose and mouth region. The tubes were analyzed for BP-3 via thermal desorption (TD-100, Markes Int.) and gas chromatography (7890B GC, Agilent Technologies) coupled with mass spectrometry (5977A MSD, Agilent Technologies). Separation was performed on a DB-5MS column (60 m x 0.25 mm x 0.25 μm). The mass spectrometer was operated in selected ion monitoring mode using m/z 227 as quantifier and m/z 151 as qualifier mass. Calibration information and determination of LOD is shown in Supplementary Information (Section S1 and Figures S2 and S3). We did not quantify airborne BP-1 in the shirt dosing chamber or in breathing zone.

Extraction from fabrics. After exposure, each shirt was placed into separate, cleaned glass jars and shipped to Missouri S&T for analysis. Four square pieces, approximately 10 cm^2 each, were cut from four quadrants of the back of each shirt. Each piece was extracted in 5 ml of acetonitrile, sonicated for 30 min and filtered. The extract was analyzed by injecting 15 μl into a Phenomenex Syrengi 4u Hydro-RP 80A column on a LabTech UV-600 HPLC using 10% water and 90% acetonitrile in gradient mode with detection at 325 nm. A seven point calibration was performed between 0.5 μg/ml and 25 μg/ml. The limit of detection was estimated to be 0.06 μg/ml, which corresponded to approximately 1.7 μg/g of shirt material.

Calculation of excretion mass and rates. Excreted mass was calculated by multiplying the urine concentration by the urine mass and density (assumed to be 1 g/cm^3). The excretion mass rate for each interval was calculated by dividing the excreted mass for each urination by the elapsed time since the previous urination. The time of urination on the evening prior to the first morning urination was not recorded. We assume that this time is identical to the time interval

between second morning urination and the last urination of the previous evening. To estimate the mass excreted that is attributable to wearing the exposed t-shirt, the excretion mass rate was integrated over the first 24 hours (up to and including the 2nd morning urination) after subtracting out the background rate associated with the first morning urination. For results in which BP-1 and BP-3 are combined, such as summed concentration, excreted mass and excretion rate, the BP-1 result is converted to BP-3 equivalents by multiplying it by the ratio of the molecular weights: (228.2 g BP-3/mol)/(214.2 g BP-1/mol).

Results and discussion

Air concentrations in the dosing chamber and breathing zone

Based on three replicate samples, the BP-3 air concentration in the shirt dosing chamber on day 31 was 4.4±0.5 μg/m^3. At 25°C, based on vapor pressure estimates from SPARC, the saturation air concentration over pure BP-3 is 9.3 μg/m^3; based on estimates from EPI Suite, 81 μg/m^3 (Table S1). In either case, the shirt dosing chamber concentration was a significant fraction of the saturation concentration under these conditions. During the human subject exposure experiments, the air concentration in the breathing zone was below the limit of detection (0.3 μg/m^3).

Clothing extraction

The BP-3 concentrations in clothing are shown in Table 2 for an unworn shirt and for shirts after they were worn by subjects. In all dosed shirt samples the concentrations were well above the limit of detection, ranging from 61 to 132 μg/g. Participant 1 wore the shirt with the highest average concentration and lowest relative standard deviation (RSD). The shirt concentrations were about 40% lower for participants 2 and 3 but the RSD was much higher indicating that the

sorption from the dosing chamber air was not as complete or as uniform for their shirts. The dosed but unworn shirt absorbed less than half of BP-3 compared with the shirt worn by participant 1. Non-uniformities in mixing within the chamber or spacing between the shirts may have accounted for the observed variation among and within shirt samples. Xue et al.[33] observed a mean BP-3 concentration in fabrics of 12.6 ng/g, which is about 10^4 times lower than measured here. The shirts in the current study were exposed to BP-3 at a concentration far greater than those reported by Wan et al.[25] for air in buildings. To provide an approximate (order-of-magnitude) estimate of the anticipated concentrations in the shirts exposed to BP-3 in a residence we first assume linear sorption, then we multiply the observed range in the current study (61 to 132 µg/g) by the ratio of the mean value reported by Wan et al. (1.18 ng/m^3 for public places)[25] and the air concentration in the exposure chamber (4400 ng/m^3). The resulting range, 16-35 ng/m^3, is centered in the range reported by Xue et al. (<2.2 – 41.8 ng/m^3)[33]. This suggests that the BP-3 identified by Xue et al. may have derived from building air instead of from the manufacturing process. This is supported by the observation of Xue et al. that there was no significant difference between raw fabrics and purchased clothing, or among fabric materials (e.g. cotton vs. polyester) in their study[33]. In other words, the BP-3 concentration in clothing may be more dependent on its most recent environmental conditions, such as room air, than its composition or manufacturing process.

Table 2. Concentration of BP-3 in t-shirts, averages of 4 samples of each shirt. Limits of detection (LOD) were 1.7 µg/g, 0.03 µg/cm^2, and 0.48 µg/cm^3.

	mass BP-3/ mass cloth (µg/g)	mass BP-3/ area shirt (µg/cm2)	mass BP-3/ volume shirt (µg/cm3)	RSD*
Participant 1	132	2.3	37	0.04
Participant 2	81	1.4	23	0.26
Participant 3	80	1.4	22	0.30

Exposed but not worn	61	1.1	17	0.13
Unexposed (blank)	<LOD	<LOD	<LOD	

* RSD = relative standard deviation for 4 samples of same shirt

Urine

Of the eight target analytes, only BP-1 and BP-3 were detected consistently in both first-morning and spot urine. Major results for these two analytes are shown in Table 3. The concentration of 3-benzylidene camphor (3-BC) was above detection limit in all samples from subject 1, suggesting a unique source for that subject. All other analytes were below detection limits in most urine samples (Supplementary Information, Table S3).

Table 3. Urinary concentrations, mass and excretion rates

	Participant 1			Participant 2			Participant 3	
	BP-1	**BP-3**		**BP-1**	**BP-3**		**BP-1**	**BP-3**
First morning urination (ng/ml)								
Morning 1	7.07	29.6		2.23	7.38		3.39	16.7
Morning 2	7.39	22.4		1.65	3.53		14.5	79.5
Morning 3	4.58	16.5		0.71	2.14		3.81	31.7
24 h urine (ng/kg bw/24h)								
Pool 1	108	346		64.6	202		178	1153
Pool 2	68.1	168		24.8	54.7		94.9	678
First morning excretion rate (μg/h)								
	0.18	0.74		0.09	0.29		0.22	1.1
Total excreted after exposure up to and including second morning urination (μg)								
Individual	7.9	25		4.6	14		15	97
Sum of BP-1 and BP-3 (BP-3 equiv.)	34			19			110	
Mass excreted* (μg)								
Sum of BP-1 and BP-3 (BP-3 equiv.)	12			9.9			82	
Normalized mass excreted (μg/(m^2)/(μg/m^3)**								
Sum of BP-1 and BP-3 (BP-3 equiv.)	2.7			2.4			18	
Average ratio of the concentrations of BP-1 and BP-3								
BP-1/BP-3	0.31			0.35			0.15	

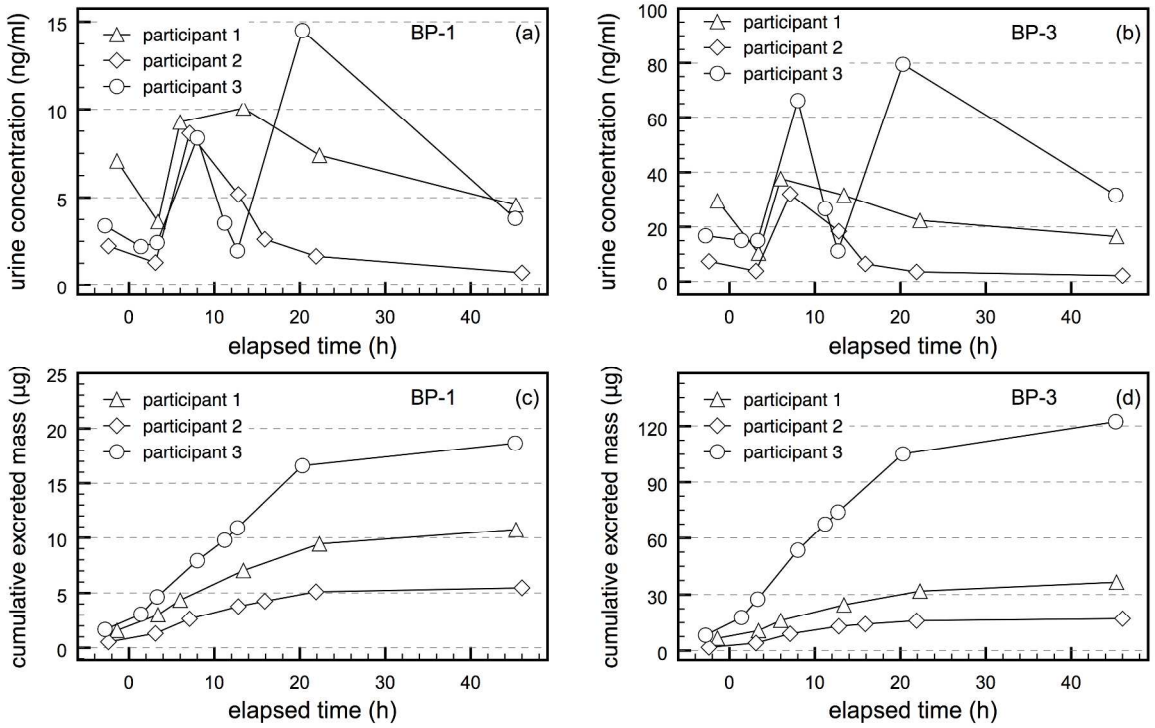
*** Mass excreted, corrected for background excretion rate during first 24 h up to and including second morning urination, (μg)**

**** Mass excreted during first 24 h, normalized by shirt-covered surface area (0.5*BSA) and dosing BP-3 air concentration, (μg/(m^2)/(μg/m^3))**

Concentrations and accumulated urinary excretion of BP-1 and BP-3 are shown in Figure 2. Pre-exposure (background) BP-3 first-morning urine concentration from participants 1, 2 and 3 were 30, 7 and 17 ng/ml, respectively. Their individual peak concentrations in subsequent spot urines were 38, 32 and 79 ng/ml, respectively. These values are comparable to values within the 25-

75th percentile of the US population (5.8-94 ng/ml)[17], mostly lower than the mean for an Australian population (61.5 ng/ml)[44], but much higher than values reported for Danish young men (25-75th percentile = 1.3-7.8 ng/ml) and other Danish population groups such as kindergarten children, school children and adolescents, pregnant women and children and their mothers[18,40,43]. Chinese young adults (0.55 ng/ml median; 0.26 ng/ml geometric mean)[4,45] or Belgian adults (1.3 ng/ml mean)[46]. The median peak urine concentration of BP-3 after whole-body application of a BP-3 containing lotion was 44 and 81 ng/ml for female and male subjects, respectively[16].

There are fewer published measurements of urinary BP-1 available for comparison with subjects in this study. Background BP-1 urine concentration from participants 1, 2 and 3 in the current study were 7.1, 2.2 and 3.4 ng/ml, respectively. Their peak concentrations were 10.1, 8.6 and 14.5 ng/ml respectively. In Chinese adults, Zhang et al.[4] reported a geometric mean of 0.28 ng/ml. Frederiksen et al.[43] observed a median concentration in Danish children and adolescents of 0.54 ng/ml in 24 h urine.



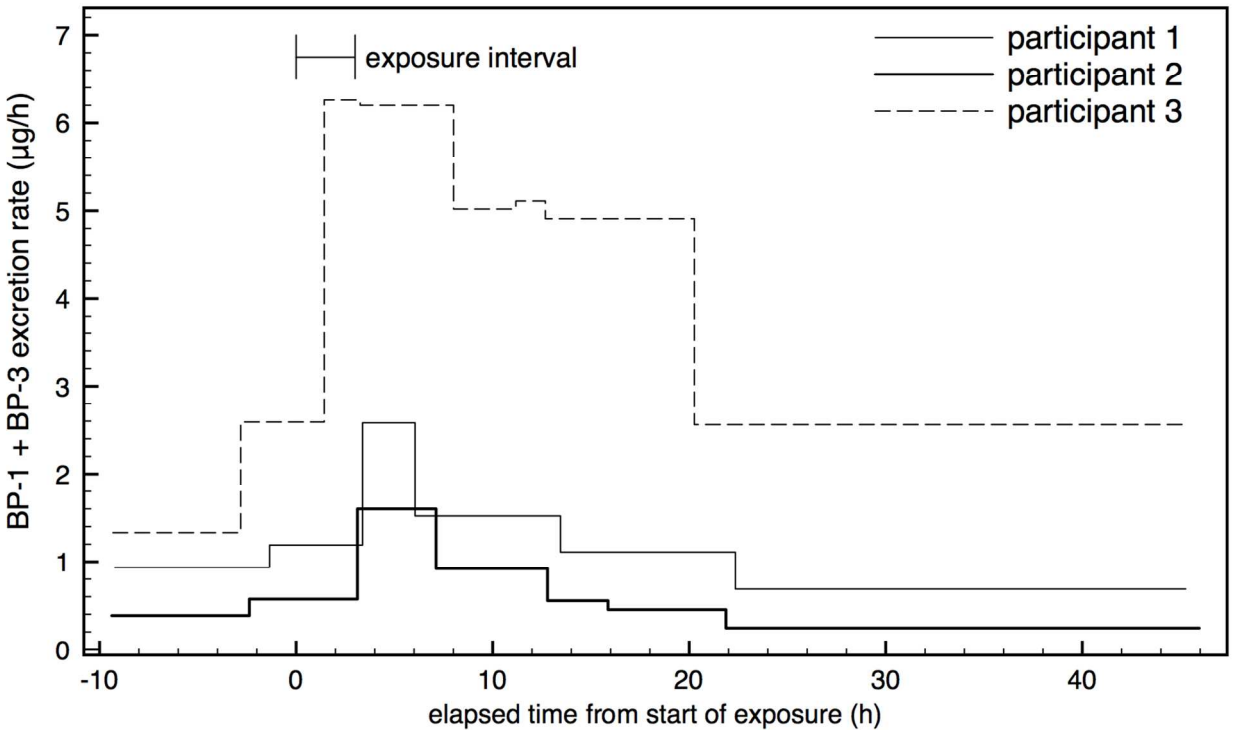
284

285 **Figure 2**. Urine concentration of BP-1 (a) and BP3 (b), and accumulated urinary excretion of
286 BP-1 (c) and BP-3 (d). The x-axis shows the elapsed time from when the participant began
287 wearing the exposed t-shirt, i.e. start of exposure.

288 Urine concentrations of, and mass excreted per urination for, BP-1 and BP-3 were highly
289 correlated (see Figures S4a and S4b of Supplementary Information). For all participants R^2
290 values were 0.8 or greater. Although BP-1 has other possible sources and is also per se used as a
291 UV filter in consumer products, the correlation suggests that the measured BP-1 here is mainly
292 present as a metabolite of BP-3, similar to the metabolism of BP-3 in rats shown in
293 pharmacokinetic studies[47,48]. Both compounds exhibit peak concentrations within 4-8 hours of
294 the start of the exposure for subjects 1 and 2. A second peak is observed for subject 3 which is
295 due to a larger void volume of the previous sample (i.e. dilution) and accumulation overnight. It

is also possible that there was some secondary exposure to BP-3 in the evening of the intervention exposure. In all three subjects, the accumulated urinary excretion of BP-1 (Figure 2c) and BP-3 (Figure 2d) occurs faster during the 24h period after exposure.

Shown in Figure 3 are excretion rates for the sum of BP-1 and BP-3. This figure shows more clearly than Figure 2 the effect of wearing dosed shirts. Excretion rates peak 4-8 hours after donning the dosed shirts, then decay. Although Participant 3 has a much higher excretion rate than Participants 1 or 2, the dose pattern and the decay in the excretion rate is similar. While it is possible that Participant 3 encountered a source of BP-3 prior to or after the experimental exposure, the similarity in the pattern of the excretion rate suggests that this individual absorbed most of the excreted BP-3 during the experiment. Excretion continues for 20+ hours after exposure, suggesting that skin acts as a reservoir for BP-3 that accumulated during the 3 h that BP-3 exposed shirts were worn. This is consistent with a recent study contrasting oral and dermal uptake of bisphenol-A (BPA). After dermal exposure, Liu and Martin observed that cumulative excretion increased linearly for 2 days, and half the participants still had detectable urinary total BPA-*d16* after 1 week.[49] The rates shown in Figure 3 are based on the urination that takes place at the end of an averaging period. Therefore, the first urination after exposure is averaged over the entire period since the previous urination, which took place prior to exposure. This makes it appear that elevated excretion occurs prior to wearing shirts; this is instead an artifact of the method.



315

316 **Figure 3**. Urinary excretion rates (µg/h) of the sum of BP-1 and BP-3 for each participant. The
317 x-axis shows the elapsed time, with "0" indicating when the participant dons the exposed t-shirt,
318 i.e. start of exposure.

319 The summed masses of BP-1 and BP-3 (BP-3 equivalent) excreted during the first 24 hours
320 attributable to wearing exposed t-shirts were 11, 9.9 and 82 µg for participants 1, 2 and 3
321 respectively. We believe these values represent a lower-bound on the amount absorbed during
322 the experiment. This value only accounts for the first 24 h, whereas there is evidence that
323 excretion of BP-3 can occur for several days after exposure[11,16]. Additionally, other metabolites
324 of BP-3 may not be accounted for with this method. Uptake by inhalation during the exposure is
325 estimated to be negligible. The air concentrations in the breathing zone were below detection
326 limits. Using the detection limit as the upper limit on the air concentration in the exposure

chamber, and an average inhalation rate of 0.7 m^3/h, uptake of BP-3 is less than 0.6 μg by inhalation.

It is apparent from the results shown in Table 3 and Figures 2-3 that participant 3 had substantially larger dermal uptake of BP-3 than the other two participants. When corrected for the pre-exposure excretion rates, participant 3's uptake is about 7-9 times greater than that of participants 1 and 2. Gonzalez et al.[11] observed a wide range of excretion rates due to whole-body application of sunscreen containing BP-3 for 25 subjects (male and female); the highest percent excretion was 7.3 times greater than the lowest. In our study, this difference is not due to differences in dosing of BP-3 to the shirts since the concentration in the shirt worn by participant 3 was lower than that worn by participant 1 and similar to that worn by participant 2. Participant 3 was 24 and 15 years older than participants 1 and 2, respectively. In a previous study examining dermal uptake of diethylphthalate and di(n-butyl)phthalate directly from air[23], older subjects had substantially greater uptake than younger subjects. The authors speculated that this may have been due to the thinner stratum corneum and reduced lipid content of older skin compared to younger skin[50]. The reduced lipid content may be especially important for lipophilic compounds such as phthalate esters and BP-3. In support of this, BP-3 has been shown to accumulate in adipose (lipid) tissue[51].Additionally, the shirt worn by participant 3 had noticeable amounts of dry skin flakes, whereas the other shirts did not. Participant 3 may suffer from dry skin, which is known to compromise the skin's barrier function[52].

The higher transdermal uptake of subject 3 may also indicate that he had a different skin type than the other two participants. Filaggrin is an epidermal protein that is crucial for skin barrier function. We have recently shown that carriers of a filaggrin gene (*FLG*) loss-of function mutation have a significantly higher urinary excretion of several of the most common phthalate

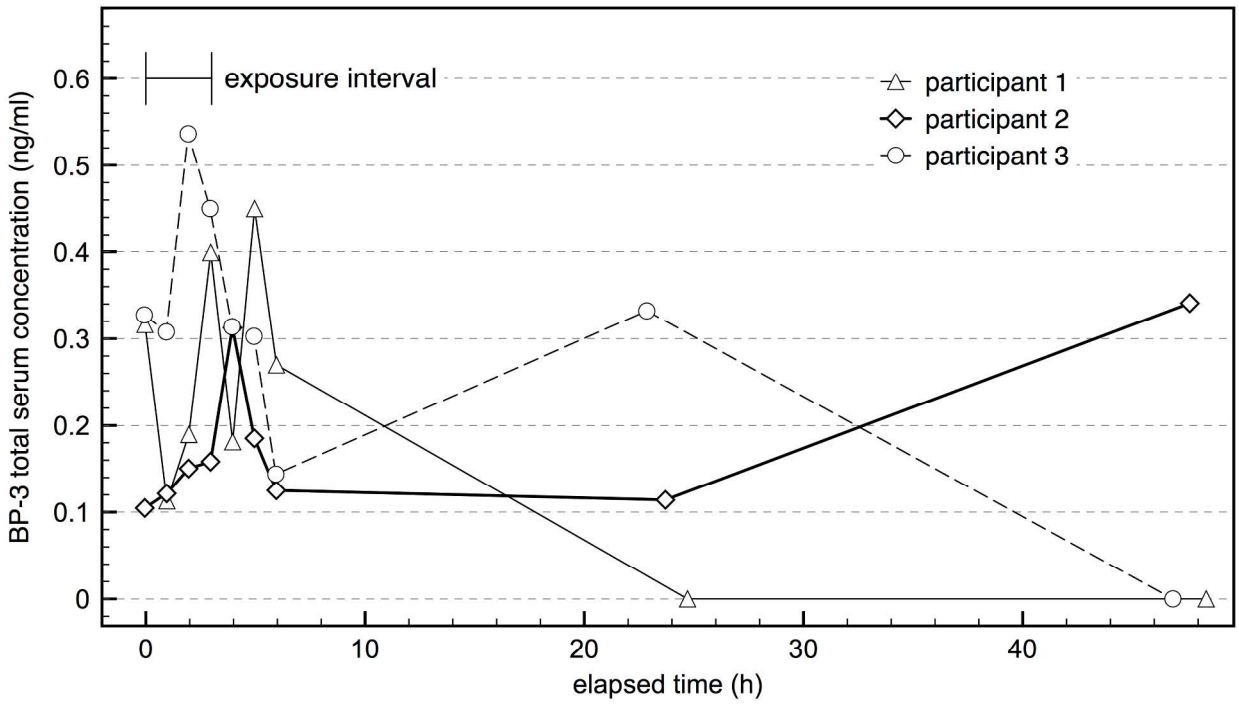
metabolites and parabens and a tendency to increased urinary excretion of both BP-3 and BP-1 compared to controls with no mutations[5,53]. Up to 10% of Europeans and Asians are heterozygotes for *FLG*-loss-of function mutations, which causes dry skin, and are likely to experience facilitated transfer of allergens such as nickel and chromium across the epidermis.

Table 3 also shows the average ratio of the urine concentrations of BP-1 and BP-3 for the participants. Participant 3 had a lower value for BP-1/BP-3 (0.15) than participant 1 (0.31) and participant 2 (0.35). This indicates less metabolism of the BP-3 absorbed through the skin of participant 3 compared to the younger participants. As adults age, a decrease in the rate of metabolism for chemicals such as BP-3 is not unusual and for BP-3 this is supported by higher ratios reported in the urine of children: the value of BP-1/BP-3 was 0.75 and 0.55 for Danish kindergarten children and adolescents respectively[40,43]. These results may also reflect differences among the subjects in the amount of metabolism occurring in the skin compared to metabolism that occurs after BP-3 enters the bloodstream[54].

Serum

Serum concentrations of total BP-3 (sum of free and conjugated forms) are shown in Figure 4. We found conjugated BP-3 in almost all samples and free BP-3 in about half of the samples (Supplementary Table S3); the correlation between free and conjugated BP-3 was poor. BP-1 concentrations in the serum were below detection limits in all three participants. 4-HBP, a possible metabolite of BP-3[55,56], was observed in all serum samples and a weak correlation between the free and conjugated form was observed. There was no correlation between total BP-3 and 4-HBP in serum, suggesting that the source for 4-HBP exposure may be different from that of BP-3. Free and total 4-MBP was measured in some of the serum samples, but again there was

373 no correlation with BP-3, and it may derive from a different source. Total BP-3 was elevated
374 soon after initiating exposure, with concentrations peaking 2 – 5 hours after putting on the shirts.
375 However, the impact of wearing BP-3 exposed shirts is not as obvious in blood samples as in the
376 urine samples. Considering all participants, the average concentration of the samples taken each
377 hour during and 2 hours after wearing the shirt was 0.26±0.13 ng/ml, while the average over the
378 samples taken at 24 and 48 hours after exposure was 0.13±0.16 ng/ml. As was the case in the
379 urine samples, participant 3 had higher total BP-3 serum concentrations than participants 1 and 2.



380

381 **Figure 4**. Serum concentrations of BP-3. The x-axis shows the elapsed time from when the
382 participant dons the exposed t-shirt, i.e. start of exposure.

383 ## Implications for population exposure

384 Dermal uptake is a dynamic and complex process. An airborne chemical first transfers to the skin
385 surface, then accumulates and transports through the skin layers to dermal capillaries. The

compound or its metabolites are eventually excreted. Because of these dynamics, it is challenging to extrapolate the results of this 3h exposure to predict uptake associated with daily residential exposure to BP-3 that has sorbed to clothing from indoor air. Since uptake had not reached equilibrium, our observed excretion may be considered a lower-bound estimate of the steady-state uptake under these conditions. The background-corrected excreted mass of the sum of BP-3 and BP-1 during the first 24 h, normalized by shirt-covered surface area (0.5*BSA) and dosing BP-3 air concentration (4.4 $\mu g/m^3$) ranges from 2.7 to 18 $\mu g/(m^2)/(\mu g/m^3)$ (Table 3). This corresponds to a mass-normalized excretion rate of 52 to 330 ng/kg/hr. The steady-state uptake from a shirt equilibrated with air in the dosing chamber, at a BP-3 concentration of 4.4 $\mu g/m^3$, can be estimated using the model described in Morrison et al.[57]. If the gap between skin and shirt is 1 mm, the model predicts a steady-state BP-3 uptake of about 1000 ng/kg/h. Therefore, in only a 3 h exposure, the mass (summed BP-1 and BP-3) excreted is 5-30% of the steady-state estimate of BP-3 uptake; as noted earlier, the excreted mass is an underestimate of the mass absorbed.

The steady-state uptake model can be used to predict uptake of BP-3 from clothing that has equilibrated with air in residences. Wan et al.[25] reported residential airborne concentrations that ranged between 0.07 and 18 ng/m^3. Here, we assume that a reasonable airborne concentration range is ½ to twice the median reported concentration of 1.64 ng/m^3 (0.82 to 3.28 ng/m^3), which is consistent with the range of exposure estimates provided in Wan et al.[25] Using the same method as above[57] and an adult surface area to mass ratio of 0.025 m^2/kg[58], steady-state uptake from equilibrated clothing is estimated to range between 8.8 to 35 ng/kg/day. This is similar to the 6-7 ng/kg/day estimated by Xue et al.[33], using a US EPA exposure estimation method[58], for dermal uptake of BP-3 for infants wearing newly purchased clothing. (Although BP-1 has been

408 measured in house dust[59], we were unable to find measured indoor airborne concentrations of
409 BP-1. Therefore, we did not estimate dermal uptake of BP-1 by the airborne route.)

410 The range of 8.8 to 35 ng/kg/day is consistent with the lower daily dose estimates in various
411 populations. Based on concentrations in urine, Gao et al.[45] estimated the mean daily excretion
412 rate in a Chinese population to be 27 ng/kg/day of BP-3. In Denmark, the median daily excretion
413 rate in kindergarten children was estimated to be 136 ng/kg/day at summer time and 32
414 ng/kg/day at winter[40], while the median daily excretion rate in Danish adolescents (17-21 year
415 old, boys and girls) was estimated to be 33 ng/kg/day in samples collected in winter time[43].
416 Based on a survey of U.S. residents, Calafat et al.[17] reported geometric mean urine
417 concentrations for BP-3 of 30.7 ng/ml for females (n = 1288) and 16.8 ng/ml for males (n =
418 1229). Daily excretion rates can be estimated assuming an average urine volume of 1.3 L/day[60]
419 and an average body weight of 75 kg for females and 89 kg for males[61]. The resulting estimated
420 excretion rates are 532 ng/kg/day for females and 245 ng/kg/day for males. Dewalque et al.[46]
421 reported a mean urine concentration of 1.3 ng/ml in a Belgian population, which corresponds to
422 22 ng/kg/day using the same method. Overall, the observed uptake rates and model predictions
423 suggest that the clothing-enhanced dermal uptake route is competitive with other exposure
424 routes, especially for individuals that have intentionally avoided personal care products
425 containing UV filters.

426 For three male volunteers, wearing cotton shirts that had been exposed to airborne BP-3 resulted
427 in dermal uptake of this chemical as evidenced by BP-3 in their blood as well as both BP-3 and
428 BP-1 (a metabolite) in their urine. In an average adult population, direct applications of
429 sunscreen, cosmetics and other personal care products are anticipated to be the dominant
430 contributors to BP-3 exposure. However, given that BP-3 is commonly present in indoor air[25],

this unintentional exposure pathway (i.e., dermal uptake from air and clothing) is likely to contribute meaningfully to the overall body-burden of BP-3. For infants and children not using BP-3 containing lotions, inadvertent exposure from air and clothing may be the major source of BP-3 in their bodies.

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References

(1) MST. *Survey of chemical substances in consumer products*; 142; Ministry of Environment and Food of Denmark. Environmental Protection Agency., 2015.

(2) Louis, G. M. B.; Kannan, K.; Sapra, K. J.; Maisog, J.; Sundaram, R. Urinary concentrations of benzophenone-type ultraviolet radiation filters and couples' fecundity. *Am. J. Epidemiol.* **2014**, *180* (12), 1168–1175.

(3) CDC. *Fourth National report on Human Exposure to Environmental Chemicals (2009) with updated tables, January 2017*; Centers for Disease Control and Prevention, 2017.

(4) Zhang, T.; Sun, H.; Qin, X.; Wu, Q.; Zhang, Y.; Ma, J.; Kannan, K. Benzophenone-type UV filters in urine and blood from children, adults, and pregnant women in China: partitioning between blood and urine as well as maternal and fetal cord blood. *Sci. Total Environ.* **2013**, *461*, 49–55.

(5) Joensen, U. N.; Jørgensen, N.; Thyssen, J.; Petersen, J. H.; Szecsi, P. B.; Stender, S.; Andersson, A.-M.; Skakkebaek, N. E.; Frederiksen, H. Exposure to phenols, parabens and UV filters: Associations with loss-of-function mutations in the filaggrin gene in men from the general population. *Environ. Int.* **2017**, *In Press*.

(6) Hass, U.; Christiansen, S.; Axelstad, A.; Boberg, J.; Andersson, A. M.; Skakkebaek, N. E.; Bay, K.; Holbech, H.; Kinnberg, K. L.; Bjerregaard, P. *Evaluation of 22 SIN List 2.0 substances according to the Danish proposal on criteria for endocrine disruptors, Danish Centre on Endocrine Disrupters*; SIN67169 and Annex; Danish Centre on Endocrine Disrupters, 2012.

(7) Kinnberg, K. L.; Petersen, G. I.; Albrektsen, M.; Minghlani, M.; Awad, S. M.; Holbech, B. F.; Green, J. W.; Bjerregaard, P.; Holbech, H. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, Danio rerio. *Environ. Toxicol. Chem.* **2015**, *34* (12), 2833–2840.

(8) Rehfeld, A.; Dissing, S.; Skakkebaek, N. E. Chemical UV filters mimic the effect of progesterone on Ca(2+) signaling in human sperm cells. *Endocrinology* **2016**, *157* (11), 4297–4308.

(9) Suzuki, T.; Kitamura, S.; Khota, R.; Sugihara, K.; Fujimoto, N.; Ohta, S. Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. *Toxicol. Appl. Pharmacol.* **2005**, *203* (1), 9–17.

(10) Kunisue, T.; Chen, Z.; Louis, G. M. B.; Sundaram, R.; Hediger, M. L.; Sun, L.; Kannan, K. Urinary concentrations of benzophenone-type UV filters in US women and their association with endometriosis. *Environ. Sci. Technol.* **2012**, *46* (8), 4624.

(11) Gonzalez, H.; Farbrot, A.; Larkö, O.; Wennberg, A. Percutaneous absorption of the sunscreen benzophenone-3 after repeated whole-body applications, with and without ultraviolet irradiation. *Br. J. Dermatol.* **2006**, *154* (2), 337–340.

(12) Gonzalez, H.; Farbrot, A.; Larkö, O. Percutaneous absorption of benzophenone-3, a common component of topical sunscreens. *Clin. Exp. Dermatol.* **2002**, *27* (8), 691–694.

(13) Schauer, U. M.; Völkel, W.; Heusener, A.; Colnot, T.; Broschard, T. H.; von Landenberg, F.; Dekant, W. Kinetics of 3-(4-methylbenzylidene) camphor in rats and humans after dermal application. *Toxicol. Appl. Pharmacol.* **2006**, *216* (2), 339–346.

(14) Sarveiya, V.; Risk, S.; Benson, H. A. Liquid chromatographic assay for common sunscreen agents: application to in vivo assessment of skin penetration and systemic absorption in human volunteers. *J. Chromatogr. B* **2004**, *803* (2), 225–231.

(15) Janjua, N. R.; Mogensen, B.; Andersson, A.-M.; Petersen, J. H.; Henriksen, M.; Skakkebæk, N. E.; Wulf, H. C. Systemic absorption of the sunscreens benzophenone-3, octyl-methoxycinnamate, and 3-(4-methyl-benzylidene) camphor after whole-body topical application and reproductive hormone levels in humans. *J. Invest. Dermatol.* **2004**, *123* (1), 57–61.

(16) Janjua, N.; Kongshoj, B.; Andersson, A.; Wulf, H. C. Sunscreens in human plasma and urine after repeated whole-body topical application. *J. Eur. Acad. Dermatol. Venereol.* **2008**, *22* (4), 456–461.

(17) Calafat, A. M.; Wong, L.-Y.; Ye, X.; Reidy, J. A.; Needham, L. L. Concentrations of the sunscreen agent benzophenone-3 in residents of the United States: National Health and Nutrition Examination Survey 2003-2004. *Environ. Health Perspect.* **2008**, *116* (7), 893.

(18) Frederiksen, H.; Jensen, T. K.; Jørgensen, N.; Kyhl, H. B.; Husby, S.; Skakkebæk, N. E.; Main, K. M.; Juul, A.; Andersson, A.-M. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. *Reproduction* **2014**, *147* (4), 555–565.

(19) Zamoiski, R. D.; Cahoon, E. K.; Freedman, D. M.; Linet, M. S. Self-reported sunscreen use and urinary benzophenone-3 concentrations in the United States: NHANES 2003–2006 and 2009–2012. *Environ. Res.* **2015**, *142*, 563–567.

(20) Harley, K. G.; Kogut, K.; Madrigal, D. S.; Cardenas, M.; Vera, I. A.; Meza-Alfaro, G.; She, J.; Qi, G.; Zahedi, R.; Bradman, A. Reducing phthalate, paraben, and phenol exposure from personal care products in adolescent girls: findings from the HERMOSA Intervention Study. *Environ. Health Perspect. Online* **2016**, *124* (10), 1600.

(21) Liao, C.; Kannan, K. Widespread occurrence of benzophenone-type UV light filters in personal care products from China and the United States: an assessment of human exposure. *Environ. Sci. Technol.* **2014**, *48* (7), 4103–4109.

(22) Weschler, C. J.; Nazaroff, W. W. SVOC exposure indoors: Fresh look at dermal pathways. *Indoor Air* **2012**, *22* (5), 356–377.

(23) Weschler, C. J.; Beko, G.; Koch, H. M.; Salthammer, T.; Schripp, T.; Toftum, J.; Clausen, G. Transdermal uptake of diethyl- and di(n-butyl) phthalate directly from air: experimental verification. *Environ. Health Perspect.* **2015**, *123* (10), 928–934.

(24) Morrison, G. C.; Weschler, C. J.; Bekö, G. Dermal uptake directly from air under transient conditions: advances in modeling and comparisons with experimental results for human subjects. *Indoor Air* **2016**, *26* (6), 913–924.

(25) Wan, Y.; Xue, J.; Kannan, K. Occurrence of benzophenone-3 in indoor air from Albany, New York, USA, and its implications for inhalation exposure. *Sci. Total Environ.* **2015**, *537*, 304–308.

(26) Piotrowski, J. Further investigations on the evaluation of exposure to nitrobenzene. *Br. J. Ind. Med.* **1967**, *24*, 60–65.

(27) Morrison, G. C.; Weschler, C. J.; Bekö, G.; Koch, H. M.; Salthammer, T.; Schripp, T.; Toftum, J.; Clausen, G. Role of clothing in both accelerating and impeding dermal absorption of airborne SVOCs. *J. Expo. Sci. Environ. Epidemiol.* **2016**, *26* (1), 113–118.

(28) Bekö, G.; Morrison, G.; Weschler, C. J.; Koch, H. M.; Pälmke, C.; Salthammer, T.; Schripp, T.; Toftum, J.; Clausen, G. Measurements of dermal uptake of nicotine directly from air and clothing. *Indoor Air* **2017**, *27* (2), 427–433.

(29) Blum, A.; Gold, M. D.; Ames, B. N.; Jones, F. R.; Hett, E. A.; Dougherty, R. C.; Horning, E. C.; Dzidic, I.; Carroll, D. I.; Stillwell, R. N.; et al. Children absorb tris-BP flame retardant from sleepwear: urine contains the mutagenic metabolite, 2,3-dibromopropanol. *Science* **1978**, *201* (4360), 1020–1023.

(30) Appel, K. E.; Gundert-Remy, U.; Fischer, H.; Faulde, M.; Mross, K. G.; Letzel, S.; Rossbach, B. Risk assessment of Bundeswehr (German Federal Armed Forces) permethrin-impregnated battle dress uniforms (BDU). *Int. J. Hyg. Environ. Health* **2008**, *211* (1-2), 88–104.

(31) Kegel, P.; Letzel, S.; Rossbach, B. Biomonitoring in wearers of permethrin impregnated battle dress uniforms in Afghanistan and Germany. *Occup. Environ. Med.* **2014**, *71* (2), 112–117.

(32) Rossbach, B.; Appel, K. E.; Mross, K. G.; Letzel, S. Uptake of permethrin from impregnated clothing. *Toxicol. Lett.* **2010**, *192* (1), 50–55.

(33) Xue, J.; Liu, W.; Kannan, K. Bisphenols, benzophenones, and bisphenol A diglycidyl ethers in textiles and infant clothing. *Environ. Sci. Technol.* **2017**.

(34) Saini, A.; Rauert, C.; Simpson, M. J.; Harrad, S.; Diamond, M. L. Characterizing the sorption of polybrominated diphenyl ethers (PBDEs) to cotton and polyester fabrics under controlled conditions. *Sci. Total Environ.* **2016**, *563–564*, 99–107.

(35) Saini, A.; Okeme, J.; Mark Parnis, J.; McQueen, R.; Diamond, M. From air to clothing: characterizing the accumulation of semi-volatile organic compounds to fabrics in indoor environments. *Indoor Air* **2016**, *27* (3), 631–641.

(36) Piadé, J. J.; D'Andrés, S.; Sanders, E. B. Sorption phenomena of nicotine and ethenylpyridine vapors on different materials in a test chamber. *Environ. Sci. Technol.* **1999**, *33* (12), 2046–2052.

(37) Morrison, G.; Li, H.; Mishra, S.; Buechlein, M. Airborne phthalate partitioning to cotton clothing. *Atmos. Environ.* **2015**, *115*, 149–152.

(38) Morrison, G.; Shakila, N. V.; Parker, K. Accumulation of gas-phase methamphetamine on clothing, toy fabrics, and skin oil. *Indoor Air* **2015**, *25* (4), 405–414.

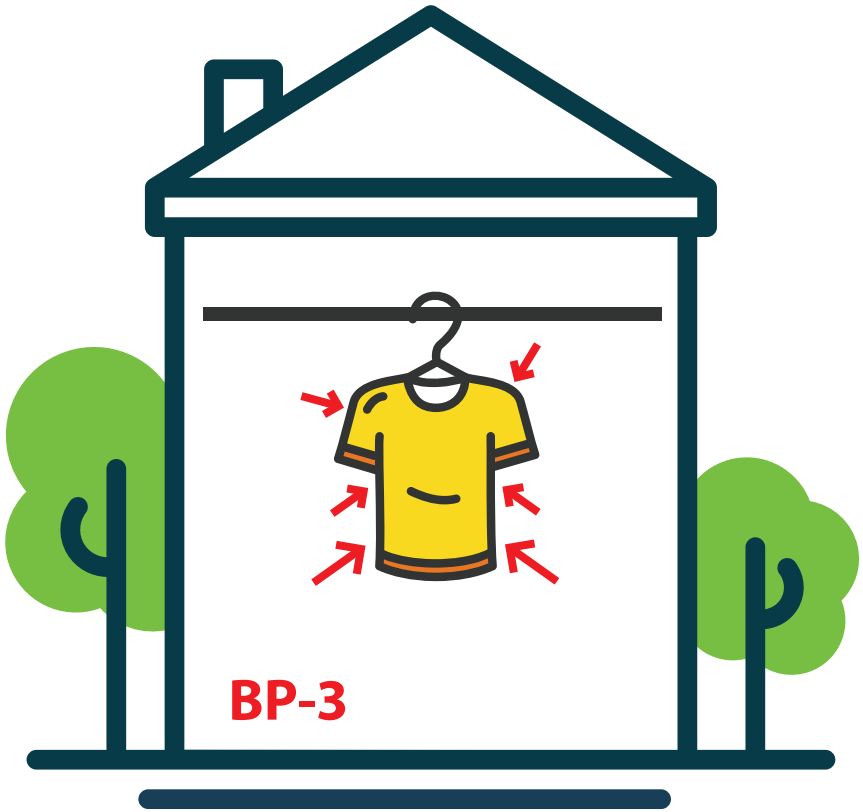
(39) Cao, J.; Weschler, C. J.; Luo, J.; Zhang, Y. Cm-History Method, a Novel Approach to Simultaneously Measure Source and Sink Parameters Important for Estimating Indoor Exposures to Phthalates. *Env. Sci Technol* **2016**, *50*, 825–834.

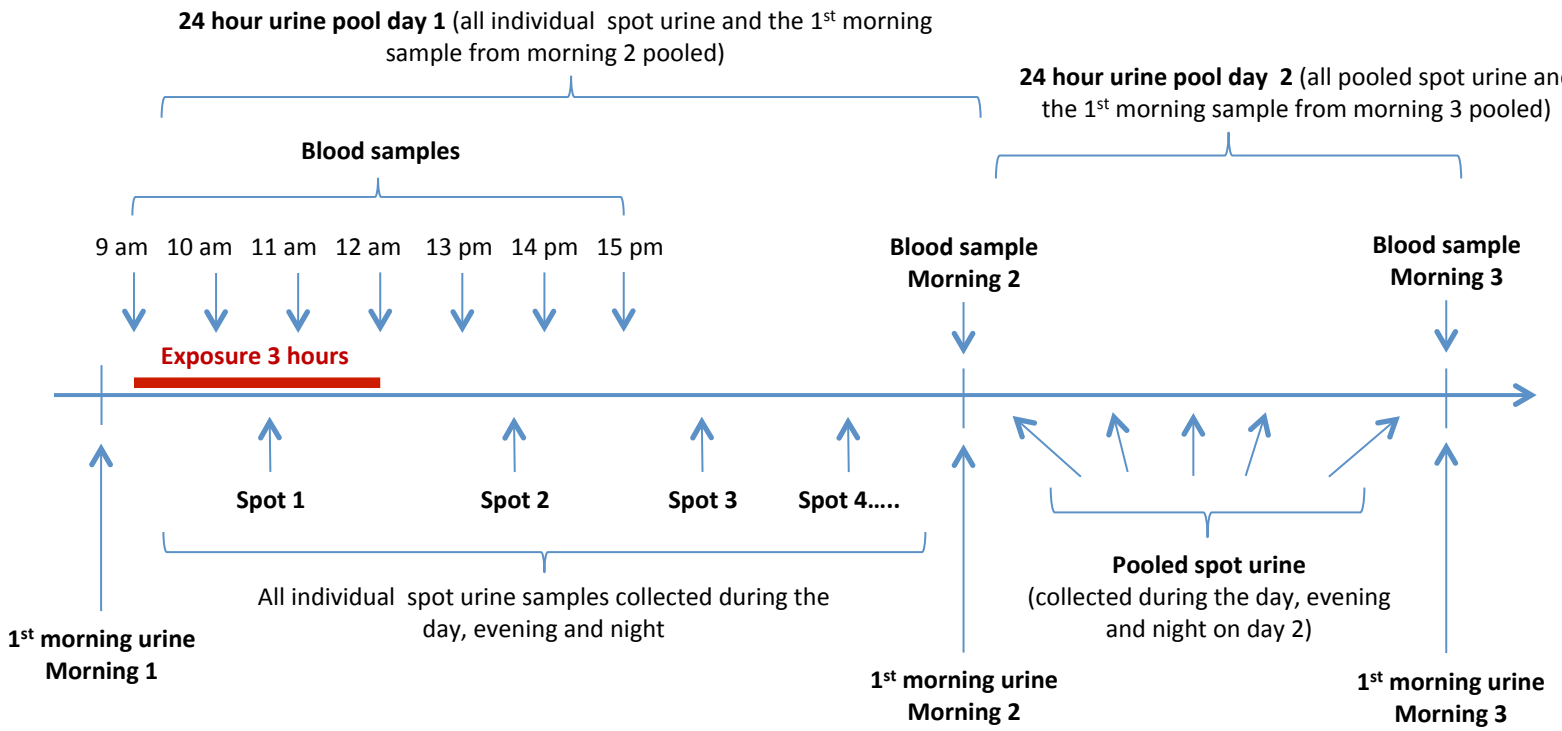
(40) Krause, M.; Andersson, A.-M.; Skakkebaek, N. E.; Frederiksen, H. Exposure to UV filters during summer and winter in Danish kindergarten children. *Environ. Int.* **2017**, *99*, 177–184.

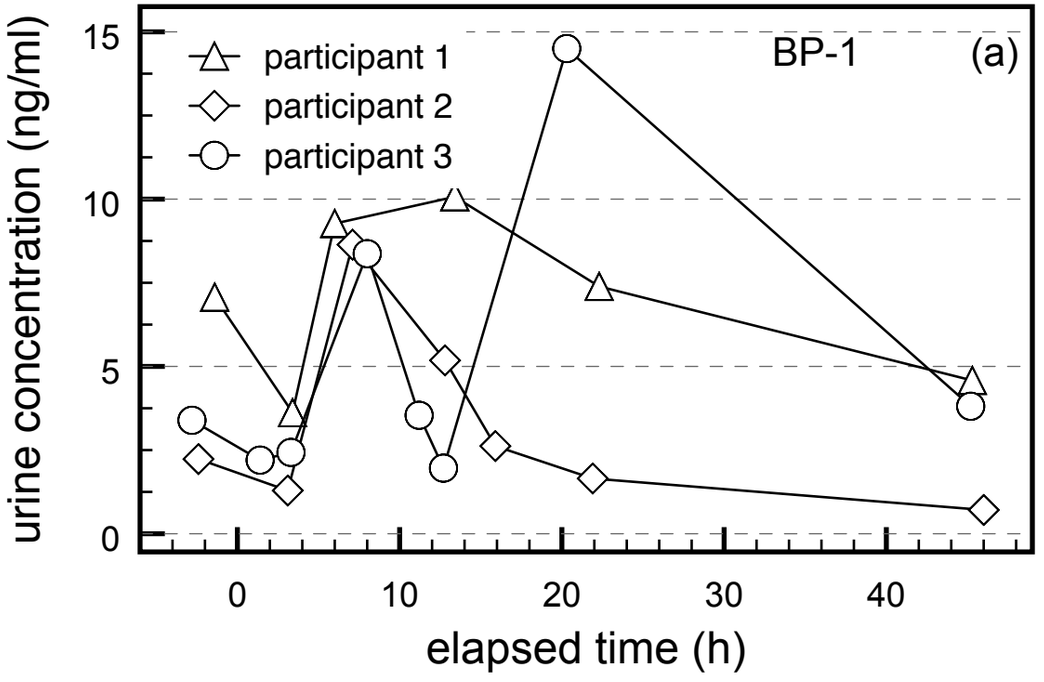
(41) European Union. *Commission Regulation (EU) 2017/238 of 10 February 2017 amending Annex VI to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products*; 2017; Vol. (EU) 2017/238.
(42) DuBois, D.; DuBois, D. A formula to estimate the approximate surface area if height and weight be known. *Arch. Intern. Med.* **1916**, *17*, 863–871.
(43) Frederiksen, H.; Nielsen, O.; Skakkebaek, N. E.; Juul, A.; Andersson, A.-M. UV filters analyzed by isotope diluted TurboFlow-LC–MS/MS in urine from Danish children and adolescents. *Int. J. Hyg. Environ. Health* **2016**.
(44) Heffernan, A.; Baduel, C.; Toms, L.; Calafat, A.; Ye, X.; Hobson, P.; Broomhall, S.; Mueller, J. Use of pooled samples to assess human exposure to parabens, benzophenone-3 and triclosan in Queensland, Australia. *Environ. Int.* **2015**, *85*, 77–83.
(45) Gao, C.; Liu, L.; Ma, W.; Zhu, N.; Jiang, L.; Li, Y.-F.; Kannan, K. Benzonphenone-type UV filters in urine of Chinese young adults: concentration, source and exposure. *Environ. Pollut.* **2015**, *203*, 1–6.
(46) Dewalque, L.; Pirard, C.; Charlier, C. Measurement of urinary biomarkers of parabens, benzophenone-3, and phthalates in a Belgian population. *BioMed Res. Int.* **2014**, *2014*.
(47) Jeon, H.-K.; Sarma, S. N.; Kim, Y.-J.; Ryu, J.-C. Toxicokinetics and metabolisms of benzophenone-type UV filters in rats. *Toxicology* **2008**, *248* (2), 89–95.
(48) Okereke, C. S.; Abdel-Rhaman, M. S.; Friedman, M. A. Disposition of benzophenone-3 after dermal administration in male rats. *Toxicol. Lett.* **1994**, *73* (2), 113–122.
(49) Liu, J.; Martin, J. W. Prolonged exposure to bisphenol A from single dermal contact events. *Environ. Sci. Technol.* **2017**, *In press*.
(50) Harvell, J.; Maibach, H. I. Percutaneous-absorption and inflammation in aged skin - a review. *J. Am. Acad. Dermatol.* **1994**, *31* (6), 1015–1021.
(51) Wang, L.; Asimakopoulos, A. G.; Kannan, K. Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue. *Environ. Int.* **2015**, *78*, 45–50.
(52) Halling-Overgaard, A.; Kezic, S.; Jakasa, I.; Engebretsen, K.; Maibach, H.; Thyssen, J. Skin absorption through atopic dermatitis skin: a systematic review. *Br. J. Dermatol.* **2016**.
(53) Joensen, U. N.; Jørgensen, N.; Meldgaard, M.; Frederiksen, H.; Andersson, A.-M.; Menné, T.; Johansen, J. D.; Carlsen, B. C.; Stender, S.; Szecsi, P. B. Associations of filaggrin gene loss-of-function variants with urinary phthalate metabolites and testicular function in young Danish men. *Environ. Health Perspect. Online* **2014**, *122* (4), 345.
(54) Hopf, N.; Berthet, A.; Vernez, D.; Langard, E.; Spring, P.; Gaudin, R. Skin permeation and metabolism of di (2-ethylhexyl) phthalate (DEHP). *Toxicol. Lett.* **2014**, *224* (1), 47–53.
(55) Bae, J.; Kim, S.; Kannan, K.; Louis, G. M. B. Couples' urinary concentrations of benzophenone-type ultraviolet filters and the secondary sex ratio. *Sci. Total Environ.* **2016**, *543*, 28–36.
(56) Kang, H.-S.; Ko, A.; Kwon, J.-E.; Kyung, M.-S.; Im Moon, G.; Park, J.-H.; Lee, H.-S.; Suh, J.-H.; Lee, J.-M.; Hwang, M.-S. Urinary benzophenone concentrations and their association with demographic factors in a South Korean population. *Environ. Res.* **2016**, *149*, 1–7.
(57) Morrison, G. C.; Weschler, C. J.; Bekö, G. Dermal uptake of phthalates from clothing: Comparison of model to human participant results. *Indoor Air* **2017**, *27* (3), 642–649.
(58) USEPA. *Exposure Factors Handbook: 2011 Edition*; EPA/600/R-090/052F; US Environmental Protection Agency: Washington D.C, 2011.
(59) Wang, L.; Asimakopoulos, A. G.; Moon, H.-B.; Nakata, H.; Kannan, K. Benzotriazole, benzothiazole, and benzophenone compounds in indoor dust from the United States and East Asian countries. *Environ. Sci. Technol.* **2013**, *47* (9), 4752–4759.
(60) Hays, S. M.; Aylward, L. L.; Blount, B. C. Variation in Urinary Flow Rates According to Demographic Characteristics and Body Mass Index in NHANES: Potential Confounding of

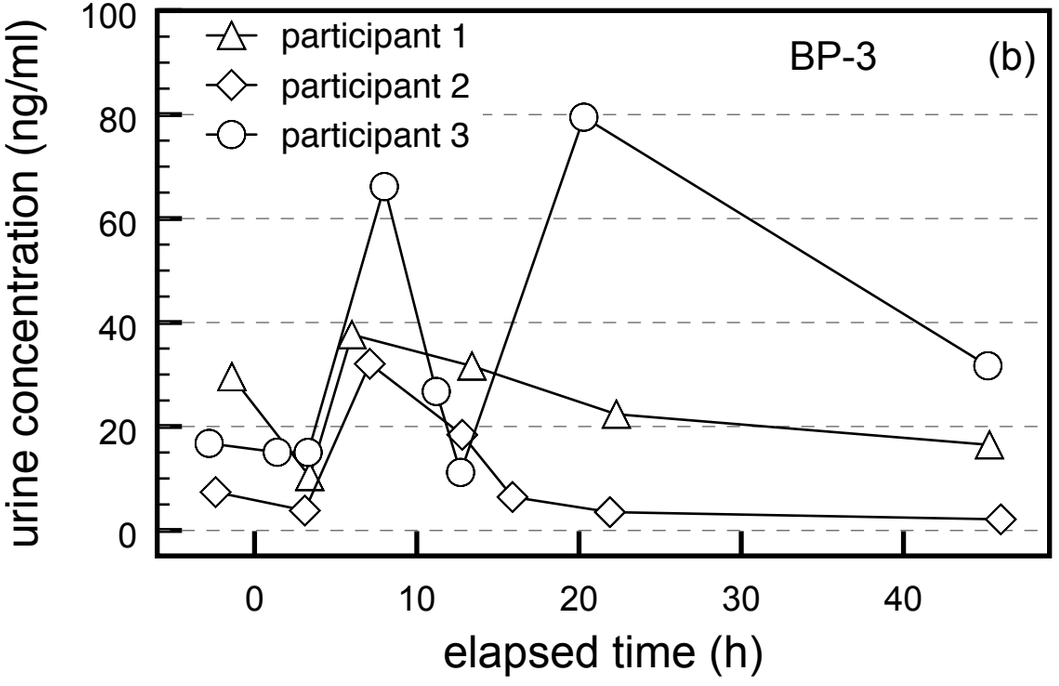
Associations between Health Outcomes and Urinary Biomarker Concentrations. *Environ. Health Perspect.* **2015**, *123* (4), 293–400.

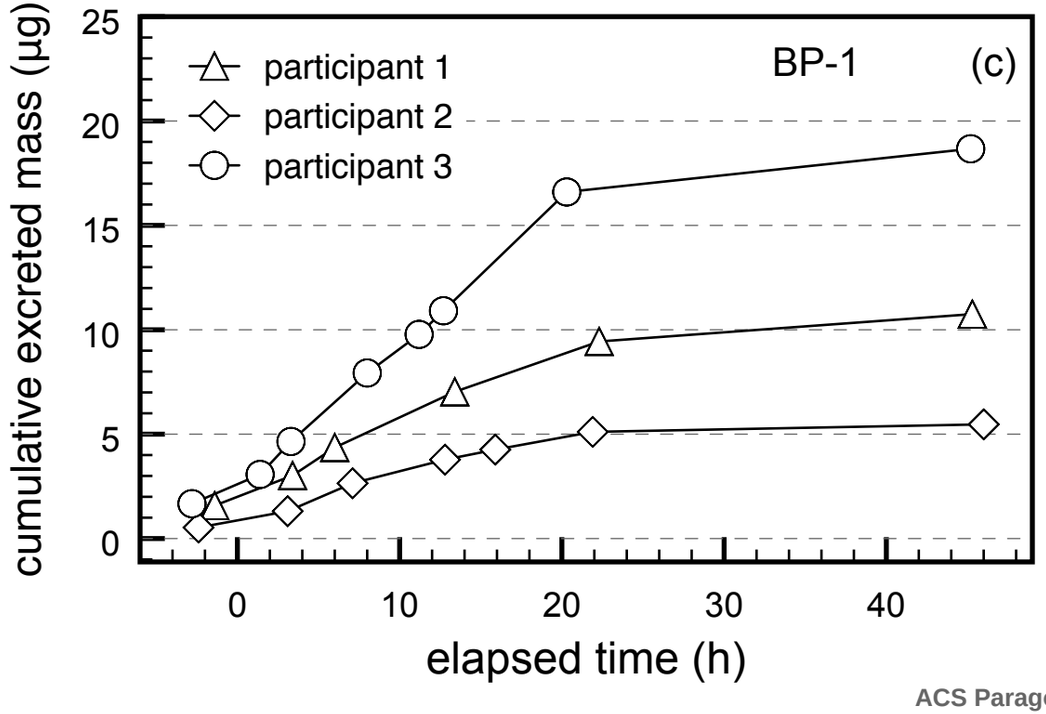
(61) CDC. *Anthropometric Reference Data for Children and Adults: United States, 2007–2010*; Vital and health statistics. Series 11, Data from the National Health Examination Survey, the National Health and Nutrition Examination Surveys, and the Hispanic Health and Nutrition Examination Survey ; no. 252; HHS publication ; no. (PHS) 2012–1602; Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES), 2012.

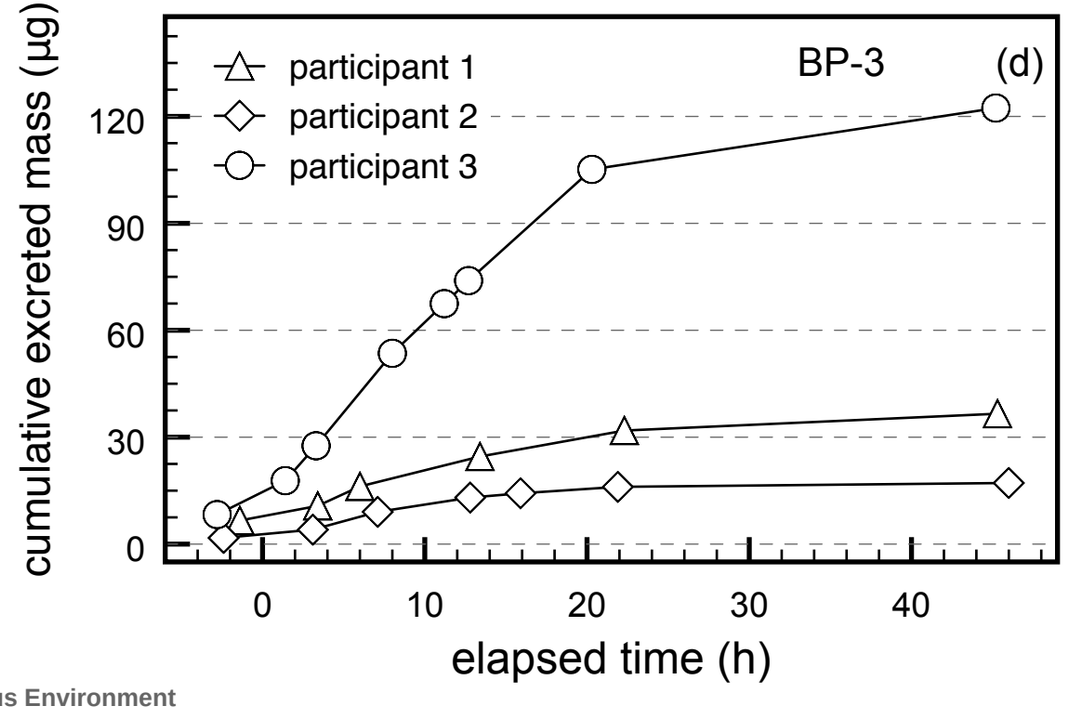

BP-3

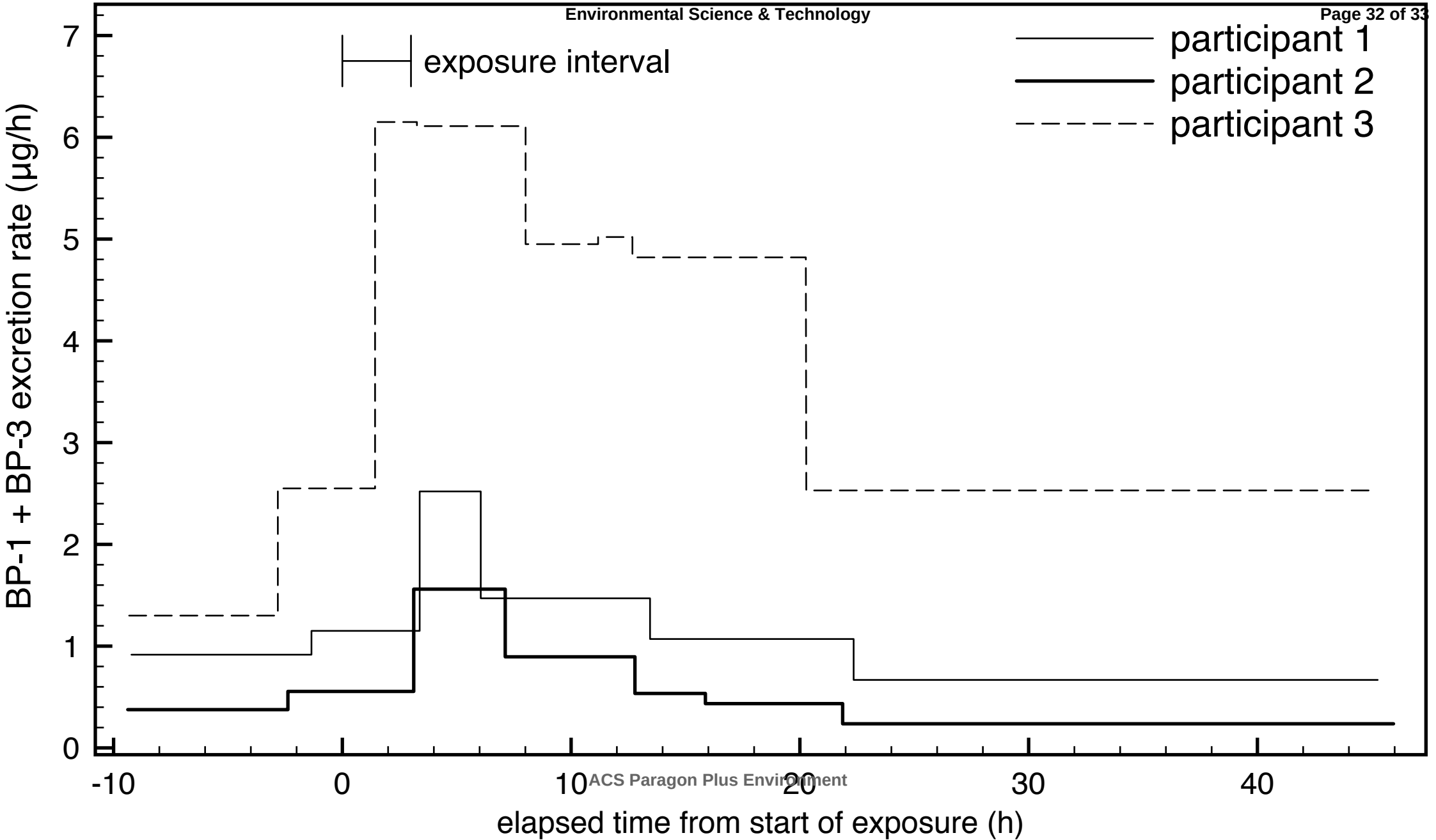

24 hour urine pool day 1 (all individual spot urine and the 1st morning sample from morning 2 pooled)
24 hour urine pool day 2 (all pooled spot urine and the 1st morning sample from morning 3 pooled)
Blood samples
9 am
10 am
11 am
12 am
13 pm
14 pm
15 pm
Blood sample Morning 2
Blood sample Morning 3
Exposure 3 hours
Spot 1
Spot 2
Spot 3
Spot 4…..
All individual spot urine samples collected during the day, evening and night
Pooled spot urine (collected during the day, evening and night on day 2)
1st morning urine Morning 1
1st morning urine Morning 2
1st morning urine Morning 3


urine concentration (ng/ml)
participant 1
participant 2
participant 3
BP-1
(a)
elapsed time (h)


urine concentration (ng/ml)
participant 1
participant 2
participant 3
BP-3
(b)
elapsed time (h)


cumulative excreted mass (µg)
participant 1
participant 2
participant 3
BP-1
(c)
elapsed time (h)


cumulative excreted mass (µg)
participant 1
participant 2
participant 3
BP-3
(d)
elapsed time (h)


exposure interval
participant 1
participant 2
participant 3
BP-1 + BP-3 excretion rate (µg/h)
0
1
2
3
4
5
6
7
-10
0
10
20
30
40
elapsed time from start of exposure (h)