Considerations for estimating daily intake values of nonpersistent environmental endocrine disruptors based on urinary biomonitoring data

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Abstract

Human exposure to chemicals may be estimated by back-calculating urinary concentrations resulting from biomonitoring studies if knowledge of the chemical's toxicokinetic properties is available. In this paper, available toxicokinetic data for back-calculating urinary concentrations into daily intake values for bisphenol A (BPA), phthalates, parabens, and triclosan (TCS) are reviewed and knowledge gaps are identified. Human data is evaluated and presented with relevant animal data. Focus is on the recovery of the administered dose, the route of administration, and differences between humans and animals. Two human toxicokinetic studies are currently used to conclude that an oral dose of BPA is recoverable in urine and that no free BPA is present in plasma in spite of several contradicting biomonitoring studies. Urinary recovery of an oral dose of phthalates in humans is complicated to assess due to extensive metabolism. In animals using ^{14}C -marked phthalates, near-complete recovery is observed. An oral dose of ^{14}C -marked parabens is also almost completely recovered in animals. In both humans and animals, however, two unspecific metabolites are formed, which complicates the back-calculation of parabens in humans. The recovery of both oral and dermal TCS in humans has been studied, but due to background levels of TCS, the back-calculation is difficult to perform. In conclusion, due to limited data, reasonable estimates of daily intake values based on urinary data are often not possible to obtain. Several knowledge gaps are identified and new studies are suggested. The route of administration used in toxicokinetic studies often does not match realistic scenarios.

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Introduction

Urinary concentrations of environmental chemicals are often used as a measure of exposure in biomonitoring studies. To be used for regulatory purposes, these urinary concentrations should be converted into daily intake values of the chemicals in question. In order to perform this conversion, knowledge about the toxicokinetic

properties of the compounds needs to be available. That is administration, distribution, metabolism and excretion (the ADME properties). Different chemicals possess different challenges regarding these toxicokinetic properties. There might be exposure via many routes (oral, dermal and by inhalation like for some of the phthalates (Koch *et al.* 2013)). Some may be distributed to many different tissues in the body like some UV-filters (Søbørg *et al.* 2006). Some may be converted into biologically active metabolites like paracetamol, as reviewed by Graham *et al.* (2013) or be metabolised so fast, that only a fraction of the compound is bioavailable as may be the case for bisphenol A (BPA) under certain circumstances (Völkel *et al.* 2002). Chemicals may also be metabolised locally in the skin like triclosan (TCS) (Moss *et al.* 2000) and finally, some may bioaccumulate and show limited excretion like some perfluorinated compounds (Olsen *et al.* 2007) or be excreted through sweat like some of the phthalates (Genuis *et al.* 2012). A general overview of these processes is presented in Fig. 1.

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Figure 1 From exposure to excretion, the administration/exposure, distribution, metabolism and excretion (ADME) properties are shown.

In this study, the challenge of converting urinary concentrations of non-persistent environmental endocrine disruptors into daily intake values will be discussed with focus on recovery of the administered dose, the relevance of the route of administration for individual compounds and differences between metabolism in humans and animals. In order to do this, data from human studies will be supplied with relevant data from animal studies. BPA, di-*n*-butyl phthalate (DnBP), di-ethylhexyl phthalate (DEHP), methyl paraben (MeP), *n*-propyl paraben (nPrP) and TCS will be used as model compounds representing different physicochemical properties and different patterns of usage. Furthermore, the need for further research will be identified in order to estimate daily intake values in humans for these compounds based on measured urinary concentrations.

Bisphenol A

Humans are exposed to BPA from a number of sources, including polycarbonate plastics and epoxy resins, both of which may be used in food contact materials. Furthermore, BPA may be found in thermal and recycled paper and in polyvinyl chloride and various other polymers, as e.g. reviewed by EFSA (2013). As reviewed by Vandenberg *et al.* (2013), orally administered BPA is rapidly absorbed from the gastrointestinal tract and via first-pass metabolism in the liver converted to BPA-glucuronide and to a minor extent BPA-sulfate. Unlike free BPA, both conjugates do not bind to the estrogen receptor (Matthews *et al.* 2001; Shimizu *et al.* 2002). They are excreted in urine and can be quantified as BPA following deconjugation in almost every urine sample from different parts of the world (Calafat *et al.* 2008b; Kim *et al.* 2011; Frederiksen *et al.* 2013b).

In order to estimate the recovery of an oral dose of BPA, Völkel *et al.* (2002) administered 5 mg BPA- d_{16} to healthy volunteers in hard gelatine capsules and quantified the free and the glucuronidated substance in blood and urine samples (see Table 1). In this study, no free BPA was detected in either blood or urine (LODs of 10 nM (2.3 ng/ml) and 6 nM (1.4 ng/ml), respectively), but the oral dose was completely recovered as BPA-glucuronide in urine within 24–34 h. In a later study by Völkel *et al.* (2008), BPA- d_{16} was administered in drinking water and the compound was subsequently quantified in urine samples. Again, no free BPA- d_{16} was detected in urine, whereas the glucuronidated compound could be quantified in amounts corresponding to near-complete recovery of the oral dose (see Table 1). No blood samples were analysed in this later study.

By using the results from Völkel *et al.* (2002, 2008), it may be concluded that quantification of total BPA in urine samples following deconjugation reflects the total exposure to BPA. However, these two studies by Völkel and colleagues are of limited value for evaluating bioavailable BPA, i.e. free BPA that may reach and interact with susceptible receptors in the body. This information is crucial for risk assessment. In the one study where serum samples were analysed (Völkel *et al.* 2002),

Table 1 Recovery of the administered dose/exposure in urine (human data).

Compound	Administration			References and comments
	Oral	Dermal	Inhalation	
BPA	~100%	No data	No data	Völkel <i>et al.</i> (2002, 2008). After 24–34 h
DEHP	31.0 ± 5.0%	No data	No data	Kessler <i>et al.</i> (2012). After 46 h
	47.1 ± 8.5%			Anderson <i>et al.</i> (2011). After 48 h. MECPP in urine accounted for 13.93% of the dose
	74.3%			Koch <i>et al.</i> (2005). After 44 h. MCMHP in urine accounted for 5.4% of the dose
DnBP	No data	1.82%	No data	Janjua <i>et al.</i> (2008). After 24 h
MeP	No data	No data	No data	
nPrP	No data	No data	No data	
TCS	54%	5.9 ± 2.1%	No data	Oral: Sandborgh-Englund <i>et al.</i> (2006). After 96 h. Dermal: Queckenberg <i>et al.</i> (2010). After 168 h

no free BPA was detected. This may be explained by the relative insensitive analytical method applied and the use of hard gelatine capsules for administering BPA, which does not reflect BPA exposure via food. Thus, possible uptake of BPA by absorption through the buccal mucosa cannot be studied using this design. In dogs, the oral (gavage) bioavailability of BPA (20 mg/kg) has recently been found to be 0.72%, whereas the bioavailability of sublingually administered BPA (5 mg/kg) has been found to be 70% (Gayraud *et al.* 2013). In another study in mice, it was shown that BPA-d₆ exposure via the diet resulted in higher serum BPA-d₆ levels than exposure via oral bolus and thus higher levels of bioavailable BPA (Sieli *et al.* 2011). Indeed, as reviewed by Vandenberg *et al.* (2007, 2010), several studies have reported levels of free BPA in serum in low nanogram per milliliter range. These reported serum levels of BPA do not seem to correlate with the estimated total exposure of 0.5–0.6 µg/kg per day (with 78–99% of BPA from dietary sources) recently published by the European Food Safety Agency (EFSA 2013) or the 0.1–0.2 µg/kg per day from dietary sources as estimated by the US Food and Drug Administration (U.S. Food and Drug Administration. Bisphenol A (BPA): Use in Food Contact Application. 6-4-2013. Accessed 14-8-2013, <http://www.fda.gov/newsevents/publichealthfocus/ucm064437.htm>). On the contrary, Taylor *et al.* (2011) had to administer 400 µg deuterated BPA/kg in feed to adult rhesus monkeys to obtain serum levels of (deuterated) BPA similar to those reported in humans. In the same study, the toxicokinetics of the adult rhesus monkeys, CD-1 mice and the women from the Völkel study (Völkel *et al.* 2002) were found to be very similar. Thus, exposure to BPA may be via other nondietary routes that to a lesser extent lead to extensive first-pass metabolism, which was also suggested by Stahlhut *et al.* (2009) after failing to observe decreasing urinary BPA concentrations after 8.5–24 h of fasting (following an initial decrease in BPA concentrations from 4.5 to 8 h). Several potentially relevant nondietary sources of BPA exposure have been identified (e.g., thermal and recycled paper, cigarette filters, and contaminated dust), as reviewed by EFSA (2013) and Vandenberg *et al.* (2013). The above studies highlight the importance of the route of administration. The vast majority of animal studies for risk assessment are conducted using oral gavage, which does not mimic the way humans are exposed, and thus may underestimate the level of bioavailable BPA in humans.

To the best of our knowledge, no studies exposing healthy volunteers to deuterated BPA have been conducted since the Völkel *et al.* (2002, 2008), which were hampered by relatively insensitive analytical methods, non-realistic routes of exposure and few study participants. However, in support of the results by Völkel *et al.* (2002, 2008), a recent comprehensive review using four different methods for estimating human serum concentrations of BPA based on several pharmacokinetic/toxicokinetic- and biomonitoring studies concluded that

serum BPA concentrations should be orders of magnitude lower than levels reported (Teeguarden *et al.* 2013). Based on their results, the authors in general question reports of measurable BPA in human serum and suggest contamination with BPA during handling and analysis of the samples as a possible explanation of detectable BPA. As evident, results are contradicting. A human study using deuterated BPA spiked in different types of food (acidic, high in fat etc.) and with dermal exposure (e.g. in semi-solid topical dosage forms) with subsequent chemical analysis of free and conjugated BPA in urine and serum samples by state-of-the art methods would – in our opinion – add considerably to the understanding of BPA toxicokinetics and thus, aid in the risk assessment conducted by relevant authorities.

Phthalates

Phthalates are present in a number of products including food packaging materials, toys, cosmetics, medical devices, some types of medicines, paints and building products, as e.g. reviewed by the National Research Council of the National Academies (2008). Phthalates may be classified into two groups based on molecular weight. Low-molecular-weight (LMW) phthalates including DnBP are often used in cosmetics, whereas high-molecular-weight (HMW) phthalates including DEHP can be found in PVC polymer and plastisol applications, as reviewed by Wittassek *et al.* (2011).

Following oral and topical administration, phthalates are quickly metabolised and excreted in humans (Koch *et al.* 2005, Koch & Angerer 2007, Janjua *et al.* 2008) and because of the ubiquitous distribution in the environment, phthalate metabolites can be quantified in the vast majority of urine samples from many parts of the world (Silva *et al.* 2004, Irvin *et al.* 2010, Frederiksen *et al.* 2011a, Kim *et al.* 2013).

In a recent study, exposure to DEHP and other HMW phthalates was concluded to be mainly from dietary sources, whereas exposure to DnBP and other LMW phthalates appeared to be from non-dietary sources like personal care products, contaminated dust and indoor air (Koch *et al.* 2013). This is in accordance with higher amounts of monoethyl phthalate (MEP) (a metabolite of the LMW diethyl phthalate (DEP)) being excreted in adolescents – who supposedly are more exposed to cosmetics and personal care products – than in young children, as reported by Frederiksen *et al.* (2011a).

For DEP, almost complete recovery of an i.v. or oral dose (160 µg/kg) of ¹⁴C-DEP in urine and feces was observed in juvenile beagle dogs, with recovery in feces accounting for 3% of the total dose. After 24 h, MEP accounted for 72–74% of the recovered dose in urine and three unidentified metabolites were found to account for 11%, ≤1%, and ≤1% of the dose, respectively (Kao *et al.* 2012). An oral dose of 0.01 mg ¹⁴C-DEHP/g feed was completely recovered in the feces

(4%) and urine (96%) of rats (Williams & Blanchfield 1974), and similar results were obtained for an oral dose of 0.06 mg ^{14}C -DnBP/g feed in rats (Tanaka *et al.* 1978). Thus in animal studies using ^{14}C -marked phthalates (DEP, DEHP, and DnBP), complete or near-complete recovery of oral doses was observed predominantly in urine within 48 h of exposure (Williams & Blanchfield 1974, Tanaka *et al.* 1978, Kao *et al.* 2012).

Oral administration of a single dose of DEHP- d_4 in a saccharose solution to four healthy male volunteers with subsequent analysis of the compound and its metabolites in serum and urine resulted in $31.0 \pm 5.0\%$ of the administered dose being recovered in urine within 46 h (with $29.1 \pm 4.8\%$ being accounted for after 22 h) (see Table 1). After 24 h, the deuterated compounds were all below or close to the LODs of the analytical method (9 nM) (Kessler *et al.* 2012). In this study, the metabolite mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) was not analyzed. However, Anderson *et al.* (2011) carried out a similar study in 20 healthy volunteers and recovered $47.1 \pm 8.5\%$ of the administered oral dose of DEHP- d_4 in olive oil (as metabolites) in urine after 48 h including the recovery of 13.9% of the administered dose as MECPP (see Table 1). Finally, Koch *et al.* (2005) studied the metabolism and excretion of orally administered DEHP- d_4 in butter served on bread at three dose levels in a single healthy volunteer and recovered 74.3% in urine with no observed differences in metabolism and excretion with dose within 44 h. In this study, five major metabolites of DEHP including mono-(2-carboxymethylhexyl) phthalate (MCMHP) (accounting for 5.4% of the dose) were quantified in urine.

One important question seems to be why near-complete recovery of an oral dose of a phthalate may be obtained in animals using ^{14}C -marked substances, while only approximately ~50–75% can be accounted for in humans orally exposed to deuterated phthalates (Koch *et al.* 2005, Anderson *et al.* 2011, Kessler *et al.* 2012)? The obvious answer could be that not all metabolites in urine are detected and quantified, e.g. phthalic acid and hippuric acid, which have been identified in the urine from animals exposed to phthalates (Tanaka *et al.* 1978, Albro *et al.* 1982, Nativelle *et al.* 1999, Silva *et al.* 2011). Both are nonspecific metabolites of phthalates and other compounds and thus not suited for biomonitoring purposes. Hence, only when using radioactive substances such as ^{14}C -labeled phthalates, phthalic acid, and hippuric acid originating from the administered phthalate may be quantified. Another possible explanation could be alternative routes of elimination and/or bioaccumulation. Both LMW and HMW phthalates are lipophilic with estimated log P values for DnBP and DEHP at 4.7 and 7.4 (Pubchem. <http://pubchem.ncbi.nlm.nih.gov/>. Accessed 9-10-2013.), respectively, suggesting their potential for accumulation in adipose tissue. In humans, bioaccumulation of phthalates was indeed suggested, as increasing

concentrations of several phthalate metabolites in urine were observed during weight loss in obese, although with no clear distinction between LMW and HMW phthalates (Dirtu *et al.* 2013). Excretion in other sources than urine was also reported when DEHP and DnBP were quantified in sweat samples more frequently than in serum samples. Metabolites of the two compounds were quantified in sweat, serum, and urine samples, with sweat being an important contributor to the excretion of MEHP and mono-iso-butyl phthalate (MiBP) with 4.6 and 1.4 times higher concentration in sweat than in urine (Genius *et al.* 2012). The two DEHP metabolites mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) were detected in urine but not in sweat and serum.

In both rats and humans, the major metabolite in urine following oral exposure to DnBP was found to be mono-butyl phthalate (MnBP), indicating similar metabolism and excretion patterns in the two species (Silva *et al.* 2007). Likewise, the same metabolites seemed to be present in urine of rats and humans following exposure to DEHP (Silva *et al.* 2006). Analyzing urine samples from NHANES, Silva *et al.* (2003) found that MEP accounted for 71% of the total urinary amount of free and conjugated MEP, whereas MnBP and MEHP, the monoester of DEHP, were found to be excreted in urine predominantly as glucuronidated substances. Thus, different physicochemical properties of phthalates result in differences in metabolism and excretion.

Dermal uptake and subsequent excretion of phthalates in humans was studied by Janjua *et al.* (2007; 2008). In these studies, whole-body application of semi-solid topical dosage forms containing DEP, DnBP and butyl paraben (BuP) was performed on 26 healthy male volunteers. Following application (2 mg/cm^2), MEP and MnBP (and BuP) were quantified in serum and in urine following deconjugation. The amounts recovered in urine within 24 h after exposure corresponded to 5.79, 1.82 and 0.32 % of the applied dose of DEP, DnBP and BuP, respectively.

Conducting a study in a suitable animal model exposed to similar doses of both ^{14}C -marked and deuterated phthalate(s) with subsequent analysis of radioactivity and quantification of known metabolites in urine using state-of-the-art analytical methods (e.g. isotope diluted LC-MS/MS) would enable not only an evaluation of the amount of metabolites missing in present analytical methods, but also an evaluation of possible bioaccumulation of phthalates and of alternative routes of elimination.

Parabens

Parabens have been quantified in the vast majority of human urine samples in biomonitoring studies from different parts of the world (Calafat *et al.* 2010, Frederiksen *et al.* 2011b, Ma *et al.* 2013). Humans are exposed to parabens mainly via personal care products and cosmetics, as reviewed e.g. by Darbre & Harvey (2008), but parabens

may also be present in food (Liao *et al.* 2013) and in medicinal products (Ursino *et al.* 2011).

By oral gavage, Mathews *et al.* (2012) exposed rats to ^{14}C -BuP at three dose levels and recovered 83–85% of the radioactive dose in urine and 3–6% in feces within 72 h. Faster excretion but similar levels of recovery in urine and feces were observed 72 h after i.v. exposure to 10 mg/kg. Following dermal exposure (72 h) to 10 and 100 mg/kg, 21 and 39% of the dose were recovered in skin (unabsorbed dose), respectively, and 16 and 43% of the dose were recovered in the dose-site skin, respectively. At the dermal doses of 10 and 100 mg/kg 46 and 7%, respectively, was recovered in urine. These results indicate that the absorption capacity through the skin becomes saturated. The authors furthermore studied BuP metabolism *in vitro* using rat and human hepatocytes. In both species, an unspecific compound, *p*-hydroxybenzoic acid (HBA), was identified as the main metabolite after 5 h of incubation with 92–100 and 78–84% of the dose being converted to HBA in rats and humans, respectively. In human hepatocytes, another unspecific metabolite, *p*-hydroxyhippuric acid (HHA), accounted for 16–22%. Finally, BuP metabolites in rat urine were identified using LC–MS/MS. The major metabolites were HBA and HHA following i.v. and oral exposure to 10 mg ^{14}C -BuP/kg. However, different amounts were observed depending on the route of exposure, with HHA being the major metabolite following i.v. administration and HBA and butyl-containing metabolites being the major metabolites following oral exposure. Apart from HBA and HHA, several other urinary metabolites were identified, but the amounts of these metabolites including free, sulfated, and glucuronidated BuP were not quantified (Mathews *et al.* 2012). A similar study in rats using ^{14}C -MeP, -nPrP, and -BuP has recently been conducted (Aubert *et al.* 2012): following oral exposure by gavage, recovery of the administered dose was found to be similar for the three compounds and the results was comparable to the findings of Mathews *et al.* (2012) with complete recovery (urine, feces, and cage wash) after 168 h. Following dermal administration, systemic exposure was found to be highest for the short-chain-length MeP and lowest for the long-chain-length BuP. Urinary excretion of radioactivity following dermal application of 100 mg/kg was around 20% for MeP and nPrP and around 26% for BuP after 168 h.

Also in human, parabens are readily hydrolyzed to HBA and HHA and conjugated with glucuronic and sulfuric acid before excretion, as reviewed by Soni *et al.* (2005). In a biomonitoring study, Ye *et al.* (2006) quantified free, glucuronidated and sulfated MeP (but not HBA and HHA) in urine samples from 100 volunteers and found the three substances to account for 5, 28 and 67% of the total amount of excreted MeP and MEP conjugates, respectively. Likewise, free, glucuronidated and sulfated nPrP accounted for 2, 43 and 55% of the total amount of excreted nPrP and nPrP conjugates, respectively.

Following five daily whole-body applications (2 mg/cm^2) of a semi-solid topical dosage form containing 2% w/w BuP (and DnBP and DEP) corresponding to an average daily dose of 10 mg/kg BuP to male healthy volunteers, free and glucuronidated BuP was quantified in urine corresponding to 0.32% of the applied dose (Janjua *et al.* 2008).

As described earlier, regardless of the route of exposure (oral, topical, or i.v.) a dose of one or more parabens seems to be almost completely recoverable in urine and feces (and in the skin after dermal application). The problem is, however, that HBA and HHA, which are the major urinary metabolites of parabens in rats and after metabolism by rat and human hepatocytes *in vitro*, are not specific for parabens and thus are not useful for biomonitoring purposes. However, following exposure to parabens, small amounts of the dose can be detected as the parent compound in urine in almost every sample analyzed, and thus, quantification of free and conjugated parabens in urine samples could provide a basis for estimating the daily intake in humans. Unfortunately, it is presently not known at which ratios HBA, HHA, free, and conjugated parabens are excreted in urine following oral and dermal exposure, which makes this kind of back-calculation impossible. To the best of our knowledge, the study by Janjua *et al.* (2008) is the only study where recovery of a dose of a paraben in humans has been estimated based on the quantification of the free and conjugated compound in urine. In this study, only 0.32% of the dermally applied dose was recovered in urine as the free and glucuronidated compound. However, as shown by Mathews *et al.* (2012) in rats, a large fraction of a topically applied dose corresponding to the dose used by Janjua *et al.* (2008) was recovered in skin as unabsorbed dose (21%), and thus, this needs to be taken into account when trying to back-calculate the concentration of paraben found in urine into an estimated intake/uptake. To obtain valuable knowledge for doing this back-calculation, a study using realistic concentrations of parabens in various topical dosage forms applied to healthy volunteers could be carried out. Subsequently, quantification of free and conjugated parabens in urine should be done, as well as quantification of unabsorbed parabens at the dose-site (appropriate use of an animal or an *ex vivo* model could prove useful for this part of the study). To obtain information about the ratios of metabolites in urine, studies such as the ones performed by Aubert *et al.* (2012) and Mathews *et al.* (2012) but with detailed quantification of the excreted metabolites would be very useful.

Triclosan

TCS is extensively used as an antimicrobial agent in a variety of products such as toothpaste, disinfectants, detergents, soaps, fabrics, mouthwash, and veterinary, industrial, other household and personal care products,

as reviewed e.g. by Dann & Hontela (2011). Urinary concentrations of TCS have been measured in samples from many parts of the world, and detectable amounts of TCS were found in 74.6% of samples from the USA (Calafat *et al.* 2008a), in 80% of samples from Denmark (Frederiksen *et al.* 2013b) and in 92.6% of samples from Korea (Kim *et al.* 2011).

Topically applied TCS permeates human skin with some metabolism taking place locally. Free TCS and the glucuronic- and sulfuric acid-conjugated TCS were found following permeation through human skin *in vitro* (Moss *et al.* 2000). In the same study, more than 50% of a topically applied dose of ^3H -TCS to rats was recovered in the *stratum corneum* and in the rinse-off of the skin after 24 h of exposure. Following topical application of approximately 20 mg TCS/kg to the skin of healthy volunteers for 12 h (ended by washing off excess cream), 5.9% of the dose was recovered in urine within 168 h (4.9% recovered within 48 h) (Queckenberg *et al.* 2010) (see Table 1). This is in accordance with previous findings reported by Moss *et al.* (2000), and with the view of the Scientific Committee on Consumer Products, which states that the amount of the dose absorbed under normal use conditions in humans (topically or when using toothpaste) is assumed to be 5–10% (European Commission Health and Consumer Protection Directorate-General 2009). This is also in line with the results of Lin (2000), who found that after the use of TCS-containing mouthwash, 7.33% of the dose was absorbed through the buccal mucosa.

Following a single oral dose of 4 mg TCS (in mouthwash) in humans, 54% was recovered as free and glucuronidated TCS in urine within four days (with the vast majority of the dose being excreted within 24 h) (Sandborgh-Englund *et al.* 2006; see Table 1). Calculations in the study were difficult due to baseline levels of TCS being present in all participants in spite of a 6-day wash-out period before the exposure. Unidentified sources of TCS exposure most likely explain the TCS background levels. In rats *in vivo*, TCS is metabolized predominantly by glucuronidation, sulfation, and hydroxylation, with the end-products of these three processes being the only metabolites detected in urine following oral exposure to 5 mg/kg (Wu *et al.* 2010).

A daily intake of the total amount of TCS (free and conjugated) that has entered the body based on the quantification of free and conjugated TCS in urine can be estimated with the data currently available (described above). With humans being exposed to TCS from a variety of products and via different routes of exposure (oral and dermal and possibly also through dust (Fan *et al.* 2010)) with different grades of absorption, the bioavailable dose (free TCS in plasma), however, is hard to estimate based on urinary measurements due to likely differences in metabolism and avoidance of first-pass metabolism dependent of the routes of exposure.

Discussion and conclusions

In this paper, the ADME properties of selected non-persistent environmental endocrine disruptors with different physicochemical properties and different use patterns have been reviewed with the purpose of converting urinary concentrations into estimated daily intake values. As can be seen from the above, this is in many cases not an easy task and in the following, a few general issues will be discussed.

Conducting studies using oral gavage as the route of administration is an example of a design that may not reflect the way humans are exposed to chemicals (with the notable exception of phthalate exposure via enteric coated medicinal capsules (Hauser *et al.* 2004)). In these studies, absorption of the chemical is exclusively taking place from the gastrointestinal tract, where the chemical via *vena portae* is transported to the liver and undergoes first-pass metabolism, which normally greatly reduces the toxicity of a chemical and increases the rate of excretion. Parenterally administered chemicals avoid first-pass metabolism and may thus enter the systemic circulation and relevant tissues in the body as the parent compound. However, in the vast majority of animal studies, the concentration of the administered chemical and/or its metabolites in serum or urine following exposure to a specific dose is not quantified. If a toxicological effect is observed (or is not observed) no connection can be made from the dose given to the animal.

BPA may be used as an example to highlight the importance of including routes of administration other than oral for assessing human exposure to nonpersistent environmental endocrine disruptors. According to relevant authorities, human exposure to BPA occurs mainly via the diet (EFSA 2013, U.S. Food and Drug Administration. Bisphenol A (BPA): Use in Food Contact Application. 6-4-2013. Accessed 14-8-2013, <http://www.fda.gov/newsevents/publichealthfocus/ucm064437.htm>) with estimated total daily intakes of 0.1–0.6 µg/kg per day. These estimates do not seem to correlate with the observations made by Stahlhut *et al.* (2009) of stabilizing levels of urinary excreted BPA after fasting, and with the results reported by Taylor *et al.* (2011), who fed rhesus monkeys 400 µg BPA/kg per day to obtain serum levels of free BPA comparable with reported concentrations of free BPA in human serum (Vandenberg *et al.* 2010). It should be noted that the recent EFSA draft scientific opinion on BPA (EFSA 2013) does not estimate free and thus bioavailable BPA and states that this conversion step needs to be considered in the subsequent step of risk characterization of BPA. The above findings thus indicate non-identified, significant alternative routes of exposure and/or limited non-dietary exposure leading to very high bioavailability of BPA. Likewise, following a six-day wash-out period where all identifiable sources of TCS were avoided, TCS was

still present in urine and plasma of healthy volunteers (Sandborgh-Englund *et al.* 2006).

The available human data on the metabolism of chemicals is to a large extent based on studies on healthy adult volunteers, which may not reflect the situation in special and/or vulnerable populations. Indeed, it has recently been shown that the metabolism of DEHP and di-iso-nonyl phthalate (DiNP) changed dramatically with postnatal age in newborns during the first year of life (unpublished results). In the same study, it was also shown that preterm babies may be highly exposed to DEHP, DiNP, and butylbenzyl phthalate via medical devices in hospital settings. Persons with filaggrin gene loss-of-function mutations (7.5% of the study population), which is associated with facilitated transfer of allergens across the epidermis, constitute another special but widespread population. Male carriers of the filaggrin-mutation have been shown to excrete up to 33% higher amounts of 14 phthalate metabolites in their urine compared to men with a normal barrier function of the skin indicating high exposure to phthalates via the dermal route of this population (Joensen *et al.* 2013). Both these studies investigated phthalates, but the observed special metabolism in babies and presumed increased uptake through the skin of persons with filaggrin gene loss-of-function mutations are likely also to apply to other chemicals such as the one reviewed in this paper. These results therefore highlight the need for special attention toward studies designed to take into account special populations and special circumstances. In risk assessment, safety factors are applied to cover inter- and intra-species differences, e.g. between rat and human and between different sub-populations, respectively. Thus, newborns and carriers of the filaggrin-mutation should in theory already be taken into consideration when risks are assessed. However, data from well-conducted studies in relevant, vulnerable sub-populations such the one described above would always add to the quality of the risk assessment and thus should be performed when possible.

Biomonitoring studies to a large extent rely on quantitative analyses of urine samples in order to estimate human exposure to various chemicals. As evident from Table 1, not much information is available on the urinary recovery of an administered dose of the nonpersistent environmental endocrine disruptors discussed in this paper and more dedicated studies are needed to fill these identified knowledge gaps. The results of these studies will enable us to provide better exposure estimates from biomonitoring data for use in human risk assessment.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Albro PW, Jordan ST, Schroeder JL & Corbett JT 1982 Chromatographic separation and quantitative determination of the metabolites of di-(2-ethylhexyl) phthalate from urine of laboratory animals. *Journal of Chromatography. A* **244** 65–79. (doi:10.1016/S0021-9673(00)80123-5)
- Anderson WA, Castle L, Hird S, Jeffery J & Scotter MJ 2011 A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. *Food and Chemical Toxicology* **49** 2022–2029. (doi:10.1016/j.fct.2011.05.013)
- Aubert N, Ameller T & Legrand JJ 2012 Systemic exposure to parabens: pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [¹⁴C]-methyl-, propyl- and butylparaben in rats after oral, topical or subcutaneous administration. *Food and Chemical Toxicology* **50** 445–454. (doi:10.1016/j.fct.2011.12.045)
- Calafat AM, Xiaoyun Y, Lee-Yang W, Reidy JA & Needham LL 2008a Urinary concentrations of triclosan in the U.S. population: 2003–2004. *Environmental Health Perspectives* **116** 303–307. (doi:10.1289/ehp.10768)
- Calafat AM, Ye X, Wong L-Y, Reidy JA & Needham LL 2008b Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. *Environmental Health Perspectives* **116** 39–44. (doi:10.1289/ehp.10753)
- Calafat AM, Xiaoyun Y, Lee-Yang W, Bishop AM & Needham LL 2010 Urinary concentrations of four parabens in the U.S. population: NHANES 2005–2006. *Environmental Health Perspectives* **118** 679–685. (doi:10.1289/ehp.0901560)
- Dann A & Hontela A 2011 Triclosan: environmental exposure, toxicity and mechanisms of action. *Journal of Applied Toxicology* **31** 285–311. (doi:10.1002/jat.1660)
- Darbre PD & Harvey PW 2008 Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. *Journal of Applied Toxicology* **28** 561–578. (doi:10.1002/jat.1358)
- Dirtu AC, Geens T, Dirinck E, Malarvannan G, Neels H, Van Gaal L, Jorens PG & Covaci A 2013 Phthalate metabolites in obese individuals undergoing weight loss: urinary levels and estimation of the phthalates daily intake. *Environment International* **59** 344–353. (doi:10.1016/j.envint.2013.06.023)
- EFSA 2013 Draft scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs – Part: exposure assessment.
- European Commission Health and Consumer Protection Directorate-General 2009 SCCP (Scientific Committee on Consumer Products), Opinion on triclosan, 21 January 2009.
- Fan X, Kubwabo C, Rasmussen P & Jones-Otazo H 2010 Simultaneous quantitation of parabens, triclosan, and methyl triclosan in indoor house dust using solid phase extraction and gas chromatography–mass spectrometry. *Journal of Environmental Monitoring* **12** 1891–1897. (doi:10.1039/c0em00189a)

- Frederiksen H, Aksglaede L, Sorensen K, Skakkebaek NE, Juul A & Andersson AM 2011a Urinary excretion of phthalate metabolites in 129 healthy Danish children and adolescents: estimation of daily phthalate intake. *Environmental Research* **111** 656–663. (doi:10.1016/j.envres.2011.03.005)
- Frederiksen H, Jørgensen N & Andersson AM 2011b Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS). *Journal of Exposure Science and Environmental Epidemiology* **21** 262–271. (doi:10.1038/jes.2010.6)
- Frederiksen H, Aksglaede L, Sorensen K, Nielsen O, Main KM, Skakkebaek NE, Juul A & Andersson AM 2013a Bisphenol A and other phenols in urine from Danish children and adolescents analyzed by isotope diluted TurboFlow–LC–MS/MS. *International Journal of Hygiene and Environmental Health* **216** 710–720. (doi:10.1016/j.ijheh.2013.01.007)
- Frederiksen H, Nielsen JK, Mørck TA, Hansen PW, Jensen JF, Nielsen O, Andersson AM & Knudsen LE 2013b Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother–child pairs. *International Journal of Hygiene and Environmental Health* **216** 772–783. (doi:10.1016/j.ijheh.2013.02.006)
- Gayraud V, Lacroix M, Collet S, Bousquet-Melou A, Toutain P-L & Picard-Hagen N 2013 High bioavailability of bisphenol a from sublingual exposure. *Environmental Health Perspectives* **121** 951–956. (doi:10.1289/ehp.1307424R)
- Genuis S, Beeson S, Lobo R & Birkholz D 2012 Human elimination of phthalate compounds: Blood, Urine, and Sweat (BUS) Study. *Scientific World Journal* **2012** Article ID 615068. (doi:10.1100/2012/615068)
- Graham G, Davies M, Day R, Mohamudally A & Scott K 2013 The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. *Inflammopharmacology* **21** 201–232. (doi:10.1007/s10787-013-0172-x)
- Hauser R, Duty S, Godfrey-Bailey L & Calafat AM 2004 Medications as a source of human exposure to phthalates. *Environmental Health Perspectives* **112** 751–753. (doi:10.1289/ehp.6804)
- Irvine EA, Calafat AM, Silva MJ, Aguilar-Villalobos M, Needham LL, Hall DB, Cassidy B & Naeher LP 2010 An estimate of phthalate exposure among pregnant women living in Trujillo, Peru. *Chemosphere* **80** 1301–1307. (doi:10.1016/j.chemosphere.2010.06.048)
- Janjua NR, Mortensen GK, Andersson AM, Kongshoj B & Wulf HC 2007 Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. *Environmental Science & Technology* **41** 5564–5570. (doi:10.1021/es0628755)
- Janjua NR, Frederiksen H, Skakkebaek NE, Wulf HC & Andersson AM 2008 Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. *International Journal of Andrology* **31** 118–130. (doi:10.1111/j.1365-2605.2007.00841.x)
- Joensen UN, Jørgensen N, Medgaard M, Frederiksen H, Andersson AM, Menné T, Johansen JD, Carlsen BC, Stender S & Szecsi PB 2013 Increased urinary levels of phthalate metabolites in carriers of the filaggrin gene null mutation. *Environmental Health Perspectives*. (doi:10.1289/ehp.1306720)
- Kao M, Ruoff B, Bower N, Aoki T, Smart C & Mannens G 2012 Pharmacokinetics, metabolism and excretion of ¹⁴C-monoethyl phthalate (MEP) and ¹⁴C-diethyl phthalate (DEP) after single oral and IV administration in the juvenile dog. *Xenobiotica* **42** 389–397. (doi:10.3109/00498254.2011.627478)
- Kessler W, Numtip W, Völkel W, Seckin E, Csanády G, Pütz C, Klein D, Fromme H & Filser JG 2012 Kinetics of di(2-ethylhexyl) phthalate (DEHP) and mono(2-ethylhexyl) phthalate in blood and of DEHP metabolites in urine of male volunteers after single ingestion of ring-deuterated DEHP. *Toxicology and Applied Pharmacology* **264** 284–291. (doi:10.1016/j.taap.2012.08.009)
- Kim K, Park H, Yang W & Lee JH 2011 Urinary concentrations of bisphenol A and triclosan and associations with demographic factors in the Korean population. *Environmental Research* **111** 1280–1285. (doi:10.1016/j.envres.2011.09.003)
- Kim M, Song NR, Choi JH, Lee J & Pyo H 2014 Simultaneous analysis of urinary phthalate metabolites of residents in Korea using isotope dilution gas chromatography–mass spectrometry. *Science of the Total Environment* **470–471** 1408–1413. (doi:10.1016/j.scitotenv.2013.07.037)
- Koch HM & Angerer J 2007 Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. *International Journal of Hygiene and Environmental Health* **210** 9–19. (doi:10.1016/j.ijheh.2006.11.008)
- Koch HM, Bolt HM, Preuss R & Angerer J 2005 New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Archives of Toxicology* **79** 367–376. (doi:10.1007/s00204-004-0642-4)
- Koch HM, Lorber M, Christensen KLY, Pålme C, Koslitz S & Brüning T 2013 Identifying sources of phthalate exposure with human biomonitoring: results of a 48h fasting study with urine collection and personal activity patterns. *International Journal of Hygiene and Environmental Health* **216** 672–681. (doi:10.1016/j.ijheh.2012.12.002)
- Liao C, Liu F & Kannan K 2013 Occurrence of and dietary exposure to parabens in foodstuffs from the United States. *Environmental Science & Technology* **47** 3918–3925. (doi:10.1021/es400724s)
- Lin YJ 2000 Buccal absorption of triclosan following topical mouthrinse application. *American Journal of Dentistry* **13** 215–217.
- Ma WL, Wang L, Guo Y, Liu LY, Qi H, Zhu NZ, Gao CJ, Li YF & Kannan K 2013 Urinary concentrations of parabens in Chinese young adults: implications for human exposure. *Archives of Environmental Contamination and Toxicology* **65** 611–618. (doi:10.1007/s00244-013-9924-2)
- Mathews J, Brown S, Patel P, Black S, Banks T, Etheridge A, Fennell T, Snyder R, Blystone CR & Waidyanatha S 2012 Metabolism and disposition of [¹⁴C]n-butyl-p-hydroxybenzoate in male and female Harlan Sprague Dawley rats following oral administration and dermal application. *Xenobiotica* **43** 169–181. (doi:10.3109/00498254.2012.702935)
- Matthews JB, Twomey K & Zacharewski TR 2001 *In vitro* and *in vivo* interactions of bisphenol a and its metabolite, bisphenol a glucuronide, with estrogen receptors α and β . *Chemical Research in Toxicology* **14** 149–157. (doi:10.1021/tx0001833)
- Moss T, Howes D & Williams FM 2000 Percutaneous penetration and dermal metabolism of triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether). *Food and Chemical Toxicology* **38** 361–370. (doi:10.1016/S0278-6915(99)00164-7)
- National Research Council 2008 Phthalates and cumulative risk assessment. The tasks ahead. Washington, DC, USA: The National Academies Press.
- Nativelle C, Picard K, Valentin I, Lhuguenot JC & Chagnon MC 1999 Metabolism of n-butyl benzyl phthalate in the female wistar rat. Identification of new metabolites. *Food and Chemical Toxicology* **37** 905–917. (doi:10.1016/S0278-6915(99)00071-X)
- Olsen GW, Burris JM, Ehresman DJ, Froelich JW, Seacat AM, Butenhoff JL & Zobel LR 2007 Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorocarbon production workers. *Environmental Health Perspectives* **115** 1298–1305. (doi:10.1289/ehp.10009)
- Queckenberg C, Meins J, Wachall B, Doroshyenko O, Tomalik-Scharte D, Bastian B, Abdel-Tawab M & Fuhr U 2010 Absorption, pharmacokinetics, and safety of triclosan after dermal administration. *Antimicrobial Agents and Chemotherapy* **54** 570–572. (doi:10.1128/AAC.00615-09)
- Sandborgh-Englund G, Adolfsson-Erici M, Odham G & Ekstrand J 2006 Pharmacokinetics of triclosan following oral ingestion in humans. *Journal of Toxicology and Environmental Health. Part A* **69** 1861–1873. (doi:10.1080/15287390600631706)
- Shimizu M, Ohta K, Matsumoto Y, Fukuoka M, Ohno Y & Ozawa S 2002 Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells. *Toxicology in Vitro* **16** 549–556. (doi:10.1016/S0887-2333(02)00055-3)
- Sieli P, Jašarevic E, Warsak D, Mao J, Eilersieck M, Liao C, Kannan K, Collet S, Toutain P-L, vom Saal FS *et al.* 2011 Comparison of serum bisphenol A concentrations in mice exposed to bisphenol A through the diet versus oral bolus exposure. *Environmental Health Perspectives* **119** 1260–1265. (doi:10.1289/ehp.1003385)
- Silva M, Barr D, Reidy J, Kato K, Malek N, Hodge C, Hurtz D III, Calafat A, Needham LL & Brock J 2003 Glucuronidation patterns of common urinary and serum monoester phthalate metabolites. *Archives of Toxicology* **77** 561–567. (doi:10.1007/s00204-003-0486-3)
- Silva MJ, Barr D, Reidy JA, Malek N, Hodge C, Caudill S, Brock J, Needham LL & Calafat AM 2004 Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000. *Environmental Health Perspectives* **112** 331–338. (doi:10.1289/ehp.6723)

- Silva MJ, Samandar E, Preau J, Needham LL & Calafat AM 2006 Urinary oxidative metabolites of di(2-ethylhexyl) phthalate in humans. *Toxicology* **219** 22–32. (doi:10.1016/j.tox.2005.10.018)
- Silva MJ, Samandar E, Reidy JA, Hauser R, Needham LL & Calafat AM 2007 Metabolite profiles of di-*n*-butyl phthalate in humans and rats. *Environmental Science & Technology* **41** 7576–7580. (doi:10.1021/es071142x)
- Silva MJ, Furr J, Samandar E, Preau J, Gray LE, Needham LL & Calafat AM 2011 Urinary and serum metabolites of di-*n*-pentyl phthalate in rats. *Chemosphere* **82** 431–436. (doi:10.1016/j.chemosphere.2010.09.052)
- Søborg T, Ganderup NC, Kristensen JH, Bjerregaard P, Pedersen KL, Bollen P, Hansen SH & Halling-Sørensen B 2006 Distribution of the UV filter 3-benzylidene camphor in rat following topical application. *Journal of Chromatography. B* **834** 117–121. (doi:10.1016/j.jchromb.2006.02.026)
- Soni MG, Carabin IG & Burdock GA 2005 Safety assessment of esters of *p*-hydroxybenzoic acid (parabens). *Food and Chemical Toxicology* **43** 985–1015. (doi:10.1016/j.fct.2005.01.020)
- Stahlhut R, Welshons WV & Swan S 2009 Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environmental Health Perspectives* **117** 784–789. (doi:10.1289/ehp.0800376)
- Tanaka A, Matsumoto A & Yamaha T 1978 Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. *Toxicology* **9** 109–123. (doi:10.1016/0300-483X(78)90036-7)
- Taylor J, vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain P-L, Laffont C & VandeVoort C 2011 Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: relevance for human exposure. *Environmental Health Perspectives* **119** 422–430. (doi:10.1289/ehp.1002514)
- Teeguarden J, Hanson-Drury S, Fisher JW & Doerge DR 2013 Are typical human serum BPA concentrations measurable and sufficient to be estrogenic in the general population? *Food and Chemical Toxicology* **62** 949–963. (doi:10.1016/j.fct.2013.08.001)
- Ursino MG, Poluzzi E, Caramella C & De Ponti F 2011 Excipients in medicinal products used in gastroenterology as a possible cause of side effects. *Regulatory Toxicology and Pharmacology* **60** 93–105. (doi:10.1016/j.yrtph.2011.02.010)
- Vandenberg LN, Hauser R, Marcus M, Olea N & Welshons WV 2007 Human exposure to bisphenol A (BPA). *Reproductive Toxicology* **24** 139–177. (doi:10.1016/j.reprotox.2007.07.010)
- Vandenberg LN, Chahoud I, Heindel J, Padmanabhan V, Paumgarten F & Schoenfelder G 2010 Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. *Environmental Health Perspectives* **118** 1055–1070. (doi:10.1289/ehp.0901716)
- Vandenberg LN, Hunt PA, Myers JP & vom Saal FS 2013 Human exposures to bisphenol A: mismatches between data and assumptions. *Reviews on Environmental Health* **28** 37–58. (doi:10.1515/reveh-2012-0034)
- Völkel W, Colnot T, Csanády G, Filser JG & Dekant W 2002 Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chemical Research in Toxicology* **15** 1281–1287. (doi:10.1021/tx025548t)
- Völkel W, Kiranoglu M & Fromme H 2008 Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. *Toxicology Letters* **179** 155–162. (doi:10.1016/j.toxlet.2008.05.002)
- Williams D & Blanchfield B 1974 Retention, excretion and metabolism of di-(2-ethylhexyl) phthalate administered orally to the rat. *Bulletin of Environmental Contamination and Toxicology* **11** 371–378. (doi:10.1007/BF01684945)
- Wittassek M, Koch HM, Angerer J & Brüning T 2011 Assessing exposure to phthalates – the human biomonitoring approach. *Molecular Nutrition & Food Research* **55** 7–31. (doi:10.1002/mnfr.201000121)
- Wu JL, Liu J & Cai Z 2010 Determination of triclosan metabolites by using in-source fragmentation from high-performance liquid chromatography/negative atmospheric pressure chemical ionization ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry* **24** 1828–1834. (doi:10.1002/rcm.4558)
- Ye X, Bishop AM, Reidy JA, Needham LL & Calafat AM 2006 Parabens as urinary biomarkers of exposure in humans. *Environmental Health Perspectives* **114** 1843–1846. (doi:10.1289/ehp.9413)

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