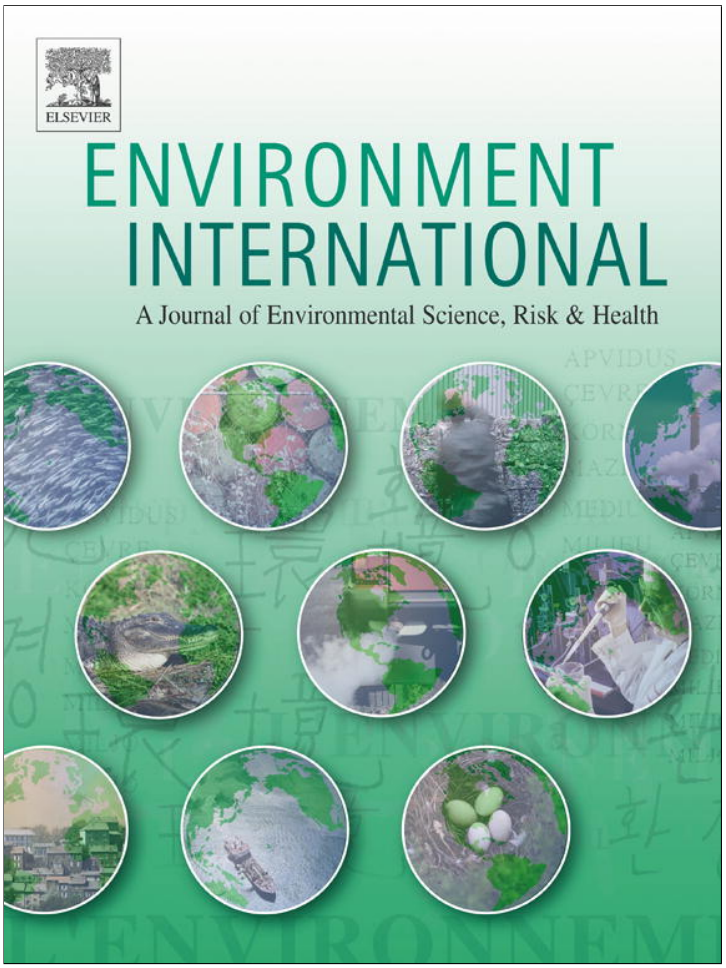

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Urinary levels of bisphenol A, triclosan and 4-nonylphenol in a general Belgian population

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ABSTRACT

Bisphenol A, triclosan and 4-nonylphenol are among the endocrine disruptors which are widely used in daily products. In this study, we reported total urinary levels of bisphenol A, triclosan and 4-nonylphenol, in order to evaluate the baseline contamination of a general population in Belgium. Bisphenol A and triclosan were detected in respectively 97.7% and 74.6% of the samples examined demonstrating that the general Belgian population is extensively exposed to both chemicals. On the other hand, 4-nonylphenol was not detected in any urine samples analyzed, suggesting either low exposure, inadequate biomarker, or that urine is an inappropriate biological matrix for assessing exposure to nonylphenol commercial mixtures. Geometric mean concentration was determined for bisphenol A at 2.55 μg/l and for triclosan at 2.70 μg/l. No significant difference was observed between levels and gender for both bisphenol A and triclosan. When classified by age, the 20–39 year group showed the highest triclosan levels, while all age groups seemed to be similarly exposed to bisphenol A. Both bisphenol A and triclosan urinary levels were not correlated with creatinine excretion in our healthy population, questioning the relevance of the creatinine adjustment in reporting these chemical levels. Bisphenol A levels in urine of people living in the same home and collected on the same time were fairly correlated, confirming the assumption that dietary intake would be the primary route of exposure. Triclosan urinary levels were not correlated with bisphenol A levels.

1. Introduction

This past decade bipshenol A (2,2′-bis-[4-hydroxyphenyl]propane), triclosan (2,4,4′-trichloro-2′-hydroxy-diphenyl ether) and 4-nonylphenol are of growing concerns for scientific and politic communities. Although their adverse health effects still remain controversial (Laws et al., 2000; Vandenberg et al., 2009), they are suspected to have estrogenic properties and are commonly classified as endocrine disruptors.

Worldwide production of bisphenol A (BPA) has drastically increased since 1980, reaching in 2008 the amount of 5.2 million tons per year (The Dow Chemical Company, 2012). BPA is mainly used as monomer to manufacture polycarbonate plastics and epoxy resins. Polycarbonates are used to manufacture a wide variety of products such as digital media (CDs, DVDs, etc.), electrical and electronic parts, safety equipment, etc., and to a less extent for food packaging such as drinking bottles, baby bottles, food and beverage containers (Vandenberg et al., 2007; Von Goetz et al., 2010). Epoxy resins are used as coating on food and drink cans, but also in electrical and electronic parts, in adhesive or in dental cements (Geens et al., 2011; Vandenberg et al., 2007). BPA has been demonstrated to leach out of the food container into the food itself (Cao and Corriveau, 2008; Geens et al., 2010; Munguia-Lopez et al., 2005), making dietary ingestion the primary source of exposure for the general population (Geens et al., 2009a; Morgan et al., 2011; Vandenberg et al., 2007; Wilson et al., 2007). Infants would be particularly exposed to BPA through its migration from polycarbonate baby bottles (Von Goetz et al., 2010), leading the European Commission to restrict the use of BPA in baby bottle fabrication (Off. J. Eur. Union, 2011). BPA has been detected ubiquitously in our environment (indoor and outdoor air, house dust) (Wilson et al., 2007), nevertheless air and dust inhalation as well as dermal absorption are thought to be minor exposure pathway (Dekant and Vöelkel, 2008; Geens et al., 2009a; Vandenberg et al., 2007).

Triclosan (TCS) is a broad spectrum antimicrobial and antifungal agent added in a wide variety of personal care products such as soaps, toothpastes, mouthwashes, acne medications, deodorants, disinfection solutions, shampoos and others, but also in industrial or household products including detergents, dishwashing liquids, etc. Its worldwide manufacture exceeds 1500 tons per year, from which almost 25% are produced in Europe. Although TCS is thought to have low acute toxicity, its concentration in personal care is regulated by the European Community Cosmetic Directive (Sci. Comm. Consum. Saf., 2010) and the United States Food and Drug Agency (Dann and

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Hontela, 2011), at concentrations of 0.3% and 0.1% respectively. In view of its commercial uses, major human exposure pathways are thought to be ingestion and dermal absorption (Calafat et al., 2008a).

Four-nonylphenol (NP) is an alkyphenol primarily involved in the production of alkylphenol ethoxylates (APEs), which are surfactants mainly used for their wetting, dispersing and emulsifying properties. They are particularly found in domestic products like detergents, cosmetic products, in pesticide formulation or in paint, plastic and paper products. Other NP industrial applications include the manufacturing of antioxidants and lubricating oil additives. Annual worldwide NP production in the early 2000s approached 250,000 tons, with the United States of America (USA) and Europe principal producers (Soares et al., 2008). The main source of NP in the environment is the degradation of APEs in sewage treatment plants (Langford and Lester, 2002), leading to its widespread presence in the aquatic environment where it exhibits high toxicity. While NP is still currently used and produced in Asia and South America, Europe initiated the phasing out of this chemical in the mid-nineties, following by the USA more than 10 years later (U.S. E.P.A., 2010). Drinking water and foodstuffs are thought to be the main route of exposure for humans, as food contamination was demonstrated to be ubiquitous (Guenther et al., 2002).

Focusing on BPA and TCS, urinary excretion of free and conjugated compounds was demonstrated to be their major elimination route (Dekant and Vöelkel, 2008; Sandborgh-Englund et al., 2006; Teeguarden et al., 2011; Vandenberg et al., 2007). The metabolic pathway of NP seems to be more complex and is still not fully understood (Calafat et al., 2005; Müller et al., 1998; Ye et al., 2007). Urine should therefore be relevant biological fluid in biomonitoring for at least BPA and TCS human exposure assessment.

Whereas BPA urinary levels have become frequently reported in North America and in Asia, European data is limited. On the other hand, studies on TCS and NP concentrations in urine still remain scarce. The aim of this work is to estimate the levels of BPA, TCS and NP in 131 urine samples collected from a non-occupationally exposed population aged from 1 to 75 years living in Liege and the surrounding areas, to estimate baseline values concentration in Belgium. To our knowledge these are the first peer-reviewed published data related to a Belgian general population including all age ranges, although the present cohort should not be considered strictly speaking as a large-scale and fully representative population regarding socio-economical ranges and spatial distribution of living areas in Belgium.

2. Materials and methods

2.1. Sample collection

This study was approved by the Hospital Faculty Ethics Committee of the University (Liege, Belgium). One hundred and thirty-one healthy males and females from the general population, living in Liege and surrounding areas, and aged from 1 to 75 years agreed to participate in this study and gave informed consent. For infants, informed consent was obtained from parents or guardians. Because subjects did not include neonates, no specific sampling devices were required. Participants were asked to provide first morning urine void in a 100 ml polypropylene container, and fill in a brief questionnaire including data on age, size and weight to obtain BMI status (weight divided by height squared), smoking habits, current residence, and certain eating habits. Some characteristics of the studied population are summarized in Table 1. Samples were collected from May to July 2011, and immediately frozen at −20 °C since Calafat et al. (2005) demonstrated that urinary BPA is stable at −20 °C for one year. Before analysis, the samples were left to thaw for 24 h at 4 °C. Ten polypropylene containers used for the samples' collection were randomly selected and screened for the potential contaminations of BPA, TCS and NP, and no target analytes were detected in these containers.

Table 1
Demographic details on the studied population.

	Men	Women
N	65 (49.6%)	66 (50.4%)
Average age (years)	29.2	29.9
BMI (%)		
BMI<18.5	3.1	5.7
18.5<BMI<24.9	56.2	74.3
BMI≥25	28.1	11.4
BMI≥30	12.5	8.6
Residence		
Rural	60.9	53.0
Urban	39.1	47.0
Smoker habit		
Never	75.4	76.9
Former	12.3	9.2
Current	7.7	4.6
Passive exposition	4.6	9.2

2.2. Chemicals and materials

Native BPA, NP, and labeled BPA-d_{14}, TCS-d_3 and NP-d_8 were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), while native TCS was bought from Accustandard (New Haven, USA). Acetonitrile, methanol and toluene were HPLC grade (Labscan, Gliwice, Poland), dichloromethane (HPLC grade), n-hexane (95% ultra resi-analyzed) and isooctane (UV-spectrum quality) were purchased from J.T. Baker (Pennsylvania, USA), acetone and potassium hydroxide (p.a.) were purchased from Merck Group (Darmstadt, Germany), formic acid + 99% p.a. was obtained from Accros Organics (Geel, Belgium), sodium acetate was Normapur grade (Prolabo, VWR International, Pennsylvania, USA), beta-glucuronidase and sulfatase both from Helix Pomatia, and 2,3,4,5,6-pentafluorobenzoyl chloride were bought from Sigma-Aldrich (St Louis, USA), and finally Oasis HLB cartridge (3 cm^3, 60 mg) were purchased from Waters (Milford, USA).

2.3. Extraction and derivatization

Extraction and derivatization were adapted from the method developed by Geens et al. (2009b). Briefly, internal standard (BPA-d_{14}, TCS-d_3, nNP-d_8) was added to 3 ml of urine prior to hydrolysis with β-glucuronidase and sulfatase in sodium acetate buffer (pH = 4.5) and formic acid for 30 min at 40 °C. The hydrolyzate was then loaded on Oasis HLB cartridges (3cm^3, 60 mg, Waters) previously prewashed with dichloromethane, methanol and bi-distilled water. After a short drying step using a vacuum manifold, targets were eluted with a 50/50 (v/v) dichloromethane–methanol mixture and evaporated to dryness under a gentle nitrogen stream at 30 °C. Derivatization was performed with pentafluorobenzoylchloride (PFBCl) in toluene, followed by double liquid–liquid extraction with hexane and aqueous potassium hydroxide. The combined organic phases were then evaporated until dryness and reconstituted in isooctane (50 μl) prior to analysis by gas chromatography coupled to mass spectrometry (GC–MS/MS).

2.4. GC–MS/MS analysis

GC–MS/MS analyses were carried out using an Agilent 7890A GC/7000A GC Triple Quad mass spectrometer (Agilent Technologies, California, USA) equipped with an Agilent HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). One microliter was injected in pulsed splitless mode (15 psi for 1.25 min) at 90 °C held for 1.15 min and then increased at 700 °C/min until 290 °C. Helium (He N60, Air Liquide, France) was used as carrier gas at a constant flow of 1.23 ml/min.

The GC run was optimized as follows: initial oven temperature of 90 °C for 1.25 min, then increased at 25 °C/min to 300 °C and held for

15 min. The mass spectrometer operated in Multiple Reaction Monitoring (MRM), with Negative Chemical Ionisation source (NCI) using methane (CH_4 N55, Air Liquide, France) as reagent gas at a flow of 40 ml/min. The source and quadrupoles were set at 150 °C while the transfer line temperature was fixed at 230 °C. The following transitions of PFB derivatives were monitored for quantification: 485>289.9 and 481.8>286.9 for TCS-d3 and TCS respectively, 421.9>356.7 and 413.9>350.1 for NP-d8 and NP, 630>372 for BPA-d14, 616>358.1 and 616>242.6 for BPA. Masses 485, 481.8, 421.9, 413.9, 630 and 616 were also traced for respectively TCS-d3, TCS, NP-d8, NP BPA-d14, and BPA to ensure specificity. These unfragmented masses were monitored instead of second transitions to obtain high signal and produce fairly accurate ratio.

2.5. Urinary creatinine adjustment

Creatinine determinations were performed using the automate ARCHITECT ci 4100 (ABBOTT, Illinois, USA) according to the manufacturer's instructions, and using Abbott reagent and calibration kits. This analysis method was based on enzymatic chain reactions resulting in a change of absorbance proportional to the creatinine (creat) concentration.

2.6. Quality assurance/quality control

The analytical method was validated according to ISO17025 and the guidelines of the French Society of Pharmaceutical Sciences and Techniques (SFSTP), and based on a total error approach using the ENOVAL software (Arlenda, Liege, Belgium). The total error evaluates the ability of the method to produce accurate results. According to this validation method, linearity, inter and intra assay precision (repeatability and reproducibility) and trueness were evaluated by measuring in triplicate fortified urine samples at different levels (from 0.5 to 30 μg/l for BPA and NP, and from 0.5 to 15 μg/l for TCS) in a single day, and replicating these determinations on two consecutive days. The concentration of these validation standards covered the dosing range of unknown samples. The limit of quantification (LOQ) was the smallest concentration determined in fortified urine samples measurable with a total error not exceeding 50%. Limit of detection (LOD) is one-third of the LOQ. The determination of LOD, LOQ and the other validation parameters are thoroughly described elsewhere (Dubois et al., 2012). Table 2 gathers values obtained for these parameters.

Table 2
Validation parameters.

	Target concentrations (μg/l)	Trueness relative bias (%)	Repeatability (RSD%)	Reproducibility (RSD%)	LOD (μg/l)	LOQ (μg/l)
BPA					0.16	0.50
	0.5	8.3	20.9	21.8		
	1	−1.1	4.1	8.5		
	2	−0.3	3.2	3.2		
	4	0.4	4.6	6.0		
	8	−1.5	4.0	4.5		
	15	1.4	4.5	4.6		
	30	0.3	6.1	6.1		
NP					0.23	0.68
	0.5	12.5	8.2	24.7		
	1	6.9	5.8	7.4		
	2	9.6	4.8	7.4		
	4	5.9	4.2	9.9		
	15	3.1	5.6	8.2		
	30		3.2	4.6		
TCS					0.33	1.00
	1	−25.7	9.3	10.7		
	2	−11.1	5.2	5.2		
	4	−15.7	10.0	10.0		
	8	−6.1	6.7	6.7		
	15	2.1	10.5	10.5		

2.7. Determination of unknown samples

For each compound (BPA, TCS and NP) the more pertinent calibration curve determined during the validation and ranging from 2.5 to 20 μg/l, was performed on fortified "blank" urine (urine of anonymous donors). As these urine samples contained target chemical background levels (except for NP), analyses were performed using the method of standard addition. These calibration standards were used to define the response function to quantify the validation standards during the validation process, and each unknown samples. Therefore unknown sample sequences included the four level calibration curve, a "blank" urine, and two home-made quality controls (5 and 15 μg/l). An internal quality control (from INSPQ, Quebec, Canada) containing BPA only was also added in each sequence. When the level determined was above the validated concentration range (from 0.5 to 15 μg/l), the analysis was carried out again on the urine sample diluted with water.

2.8. Statistical analysis

Measurements below limits of quantification (LOQs) were treated as half the LOQ value of the compound considered (Hornung and Reed, 1990). Analysis of variance (one-way Anova) was calculated using Statistica 10 software (StatSoft, Inc., Oklahoma, USA) on log transformed concentrations. Geometric mean (GM) and percentiles were determined by statistical functions in Microsoft Office Excel 2007 (Microsoft Corporation, Washington, USA).

3. Results

BPA and TCS were detected in 97.7% and 74.6% of the urine samples respectively, while none showed a NP urinary level higher than the LOD (0.23 μg/l). The GM concentration was determined for BPA at 2.55 μg/l (2.54 μg/g creat), ranging from not detected (ND) to 29.36 μg/l (ND to 27.44 μg/g creat), and for TCS at 2.70 μg/l (2.68 μg/g creat), with a range of ND to 598.95 μg/l (ND to 476.11 μg/g creat). The 2.5, 50 and 97.5 percentiles were respectively 0.57 μg/l (0.58 μg/g creat), 2.46 μg/l (2.25 μg/g creat) and 12.29 μg/l (16.68 μg/g creat) for BPA and 0.5 μg/l (0.30 μg/g creat), 2.24 μg/l (2.31 μg/g creat) and 72.96 μg/l (52.82 μg/g creat) for TCS. All participants were categorized in six age groups (0–6, 7–11, 12–19, 20–39, 40–59, >60), with a minimum of 10 men and 10 women per group. Frequency of detection, GM and percentile for each age group, and for women and men separately are detailed in Table 3. One-way ANOVA analyses did not show any significant difference between both BPA and TCS mean concentrations and gender. Comparison of BPA levels among age groups did not show any statistical difference. On the other hand, TCS urinary level was significantly higher ($p<0.001$) for participants included in the age group of 20–39 years old. Correlation between urinary BPA concentrations among people living in the same home (collected on the same day) is shown in Fig. 1. These relatives were ranked by pair, with BPA levels (in μg/l) of one member of the pairs reported on the X-axis, and BPA levels of the other member assigned to the Y-axes. Relatives included husband and wife but also brothers, sisters, parents, etc. Therefore males and females were randomly assigned to the axes since no significant differences were observed between BPA urinary levels in male and females. Comparison between urinary chemical levels and smoking habits was not conducted due to the very small fraction of smokers participating in this study. Urinary levels (logarithm transformed values) of both BPA and TCS were quite fairly correlated with their respective creatinine adjusted levels ($R=0.777$ and 0.927 for BPA and TCS respectively), as shown in Fig. 2. Conversely we found no correlation between BPA and TCS urinary levels.

Table 3
Number of individuals (N), frequency of detection, geometric mean, range levels and percentile for BPA and TCS.

Age (year)	N	Positive samples (%)	Geometric mean μg/l (μg/g creat)	Range μg/l (μg/g creat)	2.5th μg/l (μg/g creat)	50th μg/l (μg/g creat)	97.5th μg/l (μg/g creat)
BPA							
All	131	74.6	2.55 (2.54)	ND–29.36 (ND–27.44)	0.57 (0.58)	2.46 (2.25)	12.29 (16.68)
0 to 6	21	100.0	2.71 (3.63)	0.56–22.96 (0.50–16.34)	0.50 (0.50)	2.65 (3.70)	8.97 (15.53)
7 to11	21	100.0	3.27 (3.04)	1.04–29.36 (0.79–27.44)	1.05 (0.80)	2.82 (2.90)	20.23 (24.32)
12 to 19	22	100.0	2.61 (2.96)	0.67–10.27 (0.52–7.83)	0.71 (0.55)	2.86 (1.73)	8.70 (7.21)
20 to 39	22	95.5	2.52 (1.93)	ND–10.58 (ND–16.69)	0.57 (0.57)	2.54 (1.87)	9.19 (11.02)
40 to 59	23	95.5	2.82 (2.81)	ND–12.64(ND–18.43)	0.50 (0.76)	2.52 (2.61)	17.75 (14.02)
>60	22	95.8	2.21 (2.83)	ND–7.63(ND–16.67)	0.45 (1.01)	1.97 (2.73)	7.60 (14.08)
Male	65	98.5	2.70 (2.23)	ND–29.36 (ND–27.44)	0.73 (0.69)	2.66 (1.99)	16.25 (15.29)
Female	66	96.9	2.40 (2.90)	ND–23.40 (ND–18.43)	0.44 (0.55)	2.35 (2.53)	10.79 (17.38)
TCS							
All	131	74.6	2.70 (2.68)	ND–598.95 (ND–476.11)	<LOQ (<LOQ)	2.24 (2.31)	72.96 (52.82)
0 to 6	21	70.0	1.70 (2.32)	ND–10.96 (ND–14.48)	<LOQ (<LOQ)	1.48 (2.29)	8.91 (12.27)
7 to11	21	71.4	1.37 (1.21)	ND–9.76 (ND–4.82)	<LOQ (<LOQ)	1.51 (1.27)	7.03 (4.67)
12 to 19	22	86.4	3.35 (2.41)	ND–403.06 (ND–296.59)	<LOQ (<LOQ)	3.16 (2.22)	208.40 (155.20)
20 to 39	22	100.0	11.17* (9.26)	1.86–598.95 (0.75–476.11)	1.57 (1.03)	8.77 (5.53)	590.16 (438.27)
40 to 59	23	61.9	2.40 (2.47)	ND–34.60 (ND–45.97)	<LOQ (<LOQ)	2.24 (2.14)	32.92 (38.24)
>60	22	56.5	1.65 (2.30)	ND–63.38 (ND–101.08)	<LOQ (<LOQ)	1.26 (1.49)	50.29 (77.19)
Male	65	70.8	2.84 (2.35)	ND–598.95 (ND–476.11)	<LOQ (<LOQ)	2.49 (2.28)	465.76 (334.19)
Female	66	78.5	2.57 (3.06)	ND–220.19 (ND–164.94)	<LOQ (<LOQ)	1.89 (2.35)	57.36 (59.05)

* Statistically different ($p<0.001$) compared to levels detected in the other age groups.

4. Discussion

Literature data on NP levels in exposed or non-exposed populations are scarce. Chen et al. (2009) found urinary NP in 30% of Taiwanese pubertal students, Xiao et al. (2011) observed the presence of NP in nearly 50% of their 60 Chinese volunteers and Calafat et al. (2005) positively detected NP in urine of 51% of the US general population but with a GM below their LOD (0.1 μg/l). As an explanation to quite low level and detection frequency of NP, Calafat et al. (2005) suggested that the prevalent metabolism pathway which occurred would lead to oxidative metabolites, and that NP would not be representative for nonylphenol commercial mixtures. This assumption was based on a pharmacokinetic study carried out by Müller et al. (1998) demonstrating that only 10% of the applied dose (intravenous and per os administration) was found as free or conjugated parent compound in urine and feces. In our study, NP was not detected in any sample of analyzed urine. In the light of the pharmacokinetic behavior of NP and a previous American study (Calafat et al., 2005), it is not so surprising because our limit of detection was twice as high as the American one. Since NP is massively produced and used worldwide, the population should be unequivocally exposed and therefore NP or NP metabolites should be found in human biological media. Our results confirm that NP (free or conjugated) should not be an adequate biomarker for NP exposure, or perhaps that urine may not be the ideal biological media for NP biomonitoring. Other human compartments such as serum or adipose tissue should be investigated as NP is known to be more lipophilic than BPA or TCS.

Frequencies of detection of BPA and TCS, 97.7 and 74.6% respectively were consistent with those reported in North America, in Asia or in Europe ranging from 90 to 95% for BPA, and from 68 to 75% for TCS, depending on the target population and the limits of detection of the analytical method (Calafat et al., 2005, 2008a; Casas et al., 2011; Kim et al., 2011; Mendiola et al., 2010; Yamano et al., 2008; Ye et al., 2005; Zhang et al., 2011).

Fig. 3 compares BPA urinary concentrations in Belgium (present study) and levels in worldwide studies conducted in healthy and non-occupationally exposed mixed populations. They were selected because of the similarity of age averages and ranges (from 0 to up to 65 years old, with an average of around 30 years old), and similar gender ratio. BPA levels found in Belgians seemed to be comparable to those found in the general US population (Calafat et al., 2008b; Carwile and Michels, 2011; Mendiola et al., 2010) except for those reported by Calafat et al. (2005) and Mahalingaiah et al. (2008). Concentrations measured in China or Korea were likewise in the same

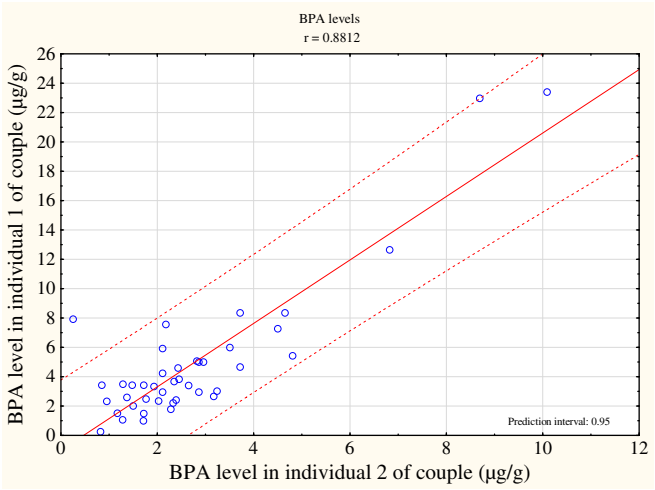

Fig. 1. BPA levels (μg/l) of people living in the same home. 0.8812 was extracted as Pearson coefficient (r). Dotted line represents the prediction interval for 95%.

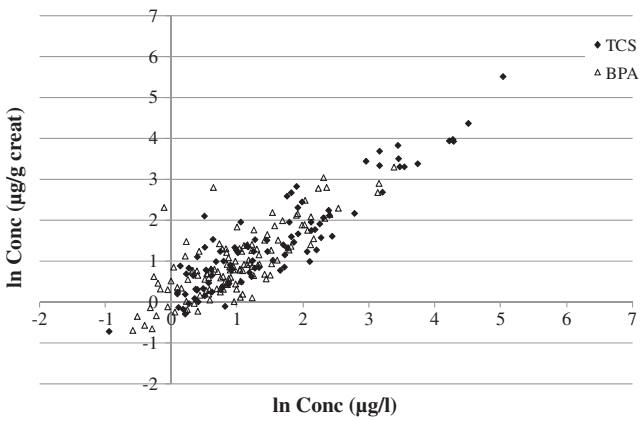

Fig. 2. Correlation between logarithm of raw (μg/l) and creatinine (μg/g creat) adjusted levels of BPA and TCS (R = 0.777 and 0.927 for BPA and TCS respectively).

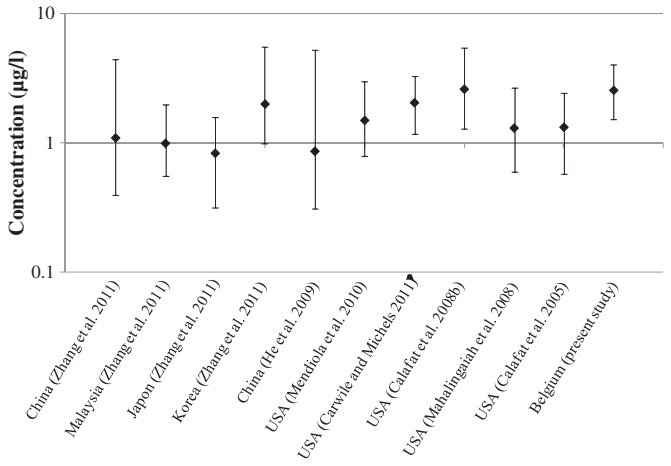

Fig. 3. Urinary BPA levels in different countries. The lower and upper boundaries represent the 25th and 75th percentiles.

range, contrary to what was observed in Japan and Malaysia where BPA exposure seemed to be lower. Very few studies have evaluated the BPA body burden in European populations. The present Belgian GM (2.55 µg/l) was in the same magnitude order that the ones detected in Spanish (2.2 µg/l) or Norwegian (2.8 µg/l) pregnant women (Casas et al., 2011; Ye et al., 2009), but were slightly higher than levels reported in Dutch pregnant women (Ye et al., 2008) and in a general German population (Völkel et al., 2008), with GM and median of 1.1 µg/l and 1.2 µg/l respectively. To our knowledge, the only study on BPA carried out in Belgium (Geens et al., 2009a) concerned 20 adolescents. The mean level found in this small-size and specific population was 1.25 µg/l, which was lower than what we found in this present study.

Nevertheless care must be taken when comparing different studies not only because of the different characteristics of the population studied, but also because of the difference in the sampling approach. It has been demonstrated that huge between-individual but also within-person variability used to occur in BPA level measured within a day (Mahalingaiah et al., 2008; Teeguarden et al., 2011; Teitelbaum et al., 2008; Ye et al., 2011). In order to assess as rigorously as possible BPA exposure, the best solution would be to collect 24 hour urine samples but even then this would represent daily and not long term exposure. However one-spot urine samples have become more and more well-accepted as reflecting exposure over weeks to months, ideally timed-randomly collected from a large population (Mahalingaiah et al., 2008; Teitelbaum et al., 2008; Ye et al., 2011). Almost all studies used in this discussion for BPA level comparison had analyzed one spot urine samples collected randomly at different times throughout the day. In the present work, first morning voids were collected from all the participants to obtain samples with the highest concentration as possible (Barr et al., 2005) and therefore allow the chemicals to be positively detected in as many samples as possible. Unlike what was observed by Wolff et al. (2007) for BPA, TCS and phthalates, we did not find any correlation between target chemical levels and creatinine in our healthy population. This suggests that in our case BPA or TCS did not show similar renal behavior as creatinine, questioning the creatinine adjustment in reporting urinary chemical levels. On the other hand we highlighted strong correlation between BPA as well as TCS levels and their respective levels adjusted for creatinine content (Fig. 2). This correlation reinforces our choice of sample collect principle because in our specific healthy population, first morning voids would not be much affected by dilution and therefore would not need adjustment.

BPA levels become more and more reported in the literature mainly in the USA or in Asia. This is not the same for TCS for which few data is currently available. Calafat et al. (2008a) found 13 µg/l as GM in the US general population, while in Spanish pregnant women Casas et al. (2011) reported a median of 6.1 µg/l. These results are 2 to 4 fold higher than what we observed in our Belgian population. This is not surprising since the US population is well-known for frequently using personal care products containing TCS (Calafat et al., 2008a). On the other hand, our results are very close to levels reported in Korea (Kim et al., 2011), and slightly higher than concentrations detected by Geens et al. (2009a) in Belgian adolescents. Again different cosmetic usage patterns could partly explain those minimal differences. One can reasonably think that teenagers normally use less often personal care product than older population and be therefore less exposed to TCS.

Differences in urinary BPA and TCS levels related to age, gender, household income, urban or rural residence, are still controversial. Some studies demonstrated that BPA and TCS urinary concentration were higher with increasing age (Calafat et al., 2008a; Kim et al., 2011), while other studies observed the opposite trend (He et al., 2009; Zhang et al., 2011). Concerning gender effect, some surveys did not show a significant difference between male and females for both TCS or BPA (Kim et al., 2011; Yamano et al., 2008; Yang et al., 2006; Zhang et al., 2011), while others observed higher BPA levels for men (Carwile and Michels, 2011; He et al., 2009; Lakind and Naiman, 2008; Mahalingaiah et al., 2008). In the present population, no statistical difference was found neither for BPA and TCS levels between male and females. One must keep in mind that the small size of our population could lead to missing a significant trend. While no statistical difference was observed for BPA levels between age groups, TCS concentrations were significantly higher for people between 20 and 39 years old and comparable for all other age groups. As previously observed in a US population (Mahalingaiah et al., 2008), we found correlation for BPA levels in people living in the same home for which samples were collected on the same day. This correlation was not true for TCS. Considering that these individuals had the same diet, and adding to the fact that we did not find any correlation between BPA and TCS levels in urine samples, these results seemed to show that BPA and TCS should have a different primary route of exposure, with respectively dietary intake and personal care products (by oral or dermal absorption) as major sources for BPA and TCS human exposure. This confirms the assumption already made by studies on BPA and TCS intake assessment (Braun et al., 2011; Geens et al., 2009a; Li et al., 2010; Morgan et al., 2011; Teeguarden et al., 2011; Wilson et al., 2007). Again comparisons should be interpreted with caution since our population does not cover all socio-economical classes and this could bias our results. Some studies have already demonstrated the influence of income on both BPA and TCS body burden (Calafat et al., 2008a; Clayton et al., 2011), increasing income resulting most of the time in an increase of TCS levels and a decrease of BPA levels.

5. Conclusion

Even if literature on BPA and TCS body burden has increased over the past five years, European data still remains limited. This study conducted in Belgium (in Liege and the surrounding area) on a non-occupationally exposed population aged from 1 to 75 years provides, to our knowledge, the first available data related to BPA and TCS levels in a Belgian general population. As already described worldwide, BPA and TCS were positively detected in almost all samples (97.7% and 74.6% respectively). On the other hand NP was not detected in any samples of urine analyzed, confirming that urinary NP (free or conjugated) may not adequately assess nonylphenol exposure. Compared to other countries, BPA urinary levels were comparable to what was observed in the USA but higher than levels reported in other European countries or in Asia, while conversely TCS levels measured were similar than in the Korean and lower than in the American population. In the present population, no statistical difference was

found neither for BPA and TCS levels between male and female, nor according to the age for BPA body burden. TCS concentrations were significantly higher for people aged between 20 and 39 years old, and comparable for all other age groups.

Creatinine adjustment should be used with caution for reporting BPA and TCS urinary levels in a healthy population because both chemicals would not show similar renal behavior than creatinine. BPA levels in urine of people living in the same home and collected on the same time were fairly correlated, confirming the assumption that dietary intake would be the primary route of exposure for BPA.

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