EXPOSURE ASSESMENT TO BISPHENOLS: COMBINING BIOMONITORING AND DUPLICATE DIET STUDIES

Nieves González Paradell

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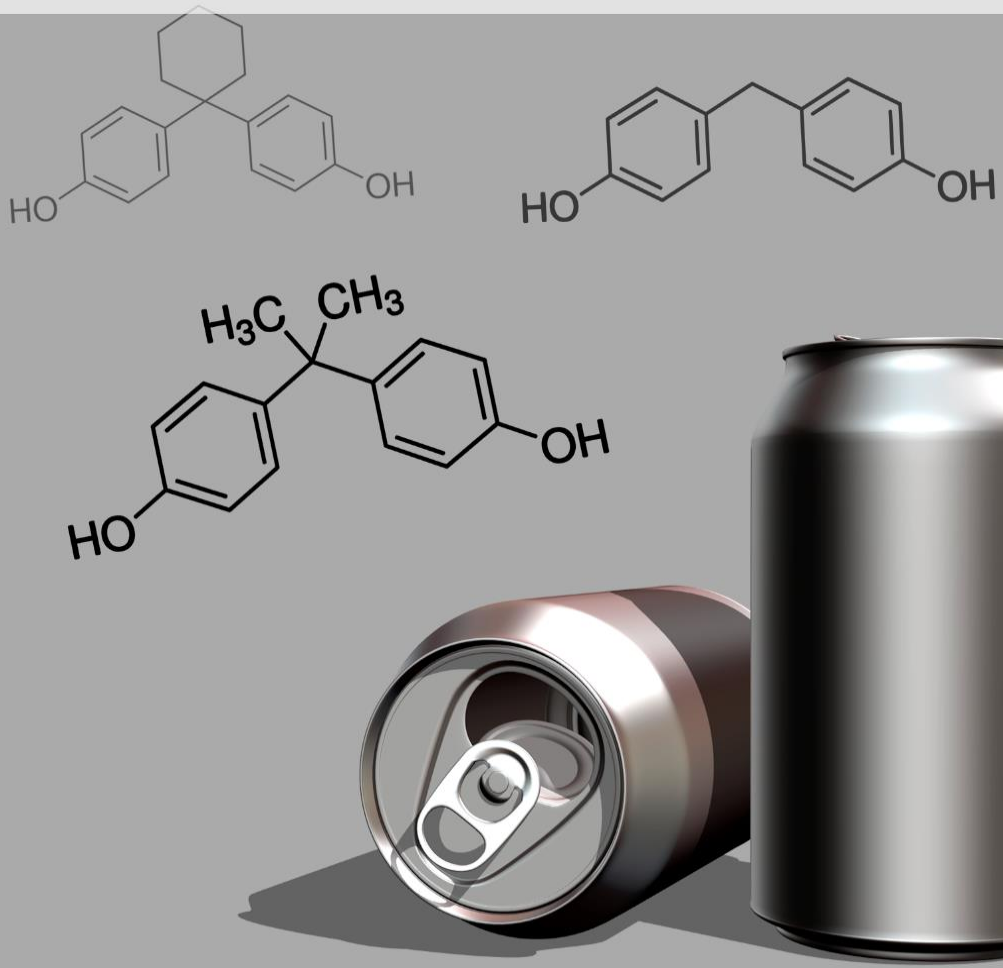
Nieves González Paradell - DOCTORAL THESIS 2020



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Exposure assesment to bisphenols: Combining biomonitoring and duplicate diet studies

DOCTORAL THESIS

Supervised by Dr. Montse Marquès Bueno, Dr. Martí Nadal Lomas and Prof. José Luis Domingo Roig

Department of Basic Medical Sciences

Reus

2020

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Dr. Montse Marquès, Dr. Martí Nadal and Prof. José Luis Domingo, researchers at the laboratory of Toxicology and Environmental Health, Universitat Rovira i Virgili

CERTIFY

That the present doctoral thesis, entitled "**Exposure assessment to bisphenols: Combining biomonitoring and duplicate diet studies**" presented by Nieves González Paradell for the award of the degree of Doctor, has been carried out under our supervision at the Department of Basic Medical Sciences at Universitat Rovira i Virgili, and it fulfills all the requirements to be eligible for the Distinction of International Doctor.

Reus, February 2020



Dr. Montse Marquès Bueno Dr. Martí Nadal Lomas Prof. José Luis Domingo Roig

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ACKNOWLEDGMENTS

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En primer lloc, donar gràcies a la Montse, al Martí i al Dr. Domingo per haver sigut els meus directors de tesi, per haver-me guiat en aquest camí i per haver-me dedicat tant de temps, inclús quan no el teníeu. A més a més, vull agrair especialment al Dr. Domingo per haver-me donat l'oportunitat de poder fer la tesi en aquest grup.

En segon lloc, gràcies a tots els companys i companyes de laboratori, sense vosaltres la meva estada en aquest laboratori no hagués sigut la mateixa. Gràcies per tots els moments viscuts durant aquest temps.

Per últim, però no menys important, agrair a la meva família tot el suport donat durant aquests anys. Sense vosaltres no hagués pogut arribar on sóc ara. Gràcies de tot cor.

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ABBREVIATIONS

UNIVERSITAT ROVIRA I VIRGILI
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A

AA: Anhydride acetic

ACSA: Agència Catalana de Seguretat Alimentària

ADME: Absortion, distribution, metabolism, excretion

As: Arsenic

B

BMDL: Benchmark dose level

BPA: Bisphenol A

BPA_{d16}: Deutered bisphenol A

BPA-G: Bisphenol A glucuronide

BPA-S: Bisphenol A sulfate

BPAF: Bisphenol AF

BPAP: Bisphenol AP

BPB: Bisphenol B

BPE: Bisphenol E

BPF: Bisphenol F

BPS: Bisphenol S

BPS-G: Bisphenol S glucuronide

BPS-S: Bisphenol S sulfate

BPs: Bisphenols

BPZ: Bisphenol Z

BSTFA: N,O-Bis(trimethylsilyl)trifluoroacetamide

BSTFA-TMC: BSTFA with trimethylchlorosilane

BZPs: Benzophenones

C

cAMP: Cyclic adenosine monophosphate

Cd: Cadmium

CV: Coefficient variation

D

DDS: Duplicate diet study

DDT: Dichlorodiphenyltrichloroethane

DES: Diethylstilboestrol

DLLME: Dispersive liquid-Liquid microextraction

DNA: deoxyribonucleic acid

E

EDC: Endocrine-disrupting chemical

EFSA: European Food Safety Agency

EI: Electron ionization

F

FAO/WHO: Food and Agriculture Organization/World Health Organization

FVU: First void urine

G

GC: Gas chromatography

GC-MS: Gas chromatography – mass spectrometry

GM: Geometrical mean

H

H295R: angiotensin-II-responsive steroid-producing adrenocortical cell line

HBM: Human biomonitoring

HCB: Hexachlorobenzene

Hg: Mercury

HUVEC: Human umbilical vein endothelial cell

HWI: Hazardous waste incinerator

I

IISPV: Institut d'investigació sanitaria Pere Virgili

IS: Internal standard

L

LOAEL: Lowest-observed-adverse-effect level

LOD: Limit of detection

LOQ: Limit of quantification

M

MCF7: Michigan Cancer Foundation-7 cell line

MeCN: Acetonitrile

MS: Mass spectrometry

N

ND: not detected

NQ: Not quantified

NOAEL: No-observed-adverse-effect level

P

Pb: Lead

PBPK model: Physiologically-based pharmacokinetic model

PCBs: Polychlorinated biphenyls

PCDD/Fs: polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans

PCP: Personal care products

PDE4: Phosphodiesterase-4

PFASs: Perfluoroalkyls

PhyEs: Phytoestrogens

POPs: Persistent organic pollutants

PVC: Polyvinyl chloride

Q

QuEChERS: Quick, easy, cheap, effective rugged and safe

R

RBC: Red blood cell

S

SIM: Selected ion monitoring

SML: Specific migration limit

S/N ratio: Signal to noise ratio

T

T4CE: Tetrachloroethylene

TFA: Trifluoroacetamide

TDI: Tolerable daily intake

TDS: Total diet study

TIC: Total ion chromatogram

t-TDI: Temporary tolerable daily intake

U

UGT: Uridine 5'-diphospho-glucuronosyltransferases

V

VEGF: Vascular endothelial growth factor

W

WHO: World Health Organization

Z

Z-sep: Zirconium oxide-based sorbent

SUMMARY

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Bisphenols (BPs) are a group of organic compounds that are used in many consumer products. The most important source of exposure in humans is through the consumption of foodstuffs, either canned or packed in polycarbonate plastic. Among all the BPs, bisphenol A (BPA) is the most used analogue in the industry, being also ubiquitously present in the environment. The endocrine disrupting potential of BPA has been largely demonstrated, while other adverse health effects, such as reproductive, developmental or fertility problems are linked to its exposure. Therefore, regulation against BPA is getting stricter throughout the world. In this sense, manufacturers started to replace BPA by other BP analogues. Nonetheless, similar or even greater endocrine-disrupting capacity has been reported for some alternative analogues.

Biomonitoring studies consist in analysing target compounds or metabolites in biological samples, being an effective strategy to assess the exposure to BPs. In addition, the analysis of BPs in food samples is suitable to accurately estimate the real exposure to BPs, considering that the diet is the main exposure pathway. This approach is frequently tackled through duplicate diet studies, which consist in collecting two food portions: one for chemical analysis and the other for consumption.

The present doctoral thesis was aimed at assessing the dietary exposure to BPs in a general population cohort by means of a duplicate diet study of canned food.

In Chapter 1, the optimization and validation of the analytical method was carried out. The procedure of extraction and derivatization of BP analogues in biological tissues (blood) was therefore optimized. Different parameters were tested to enhance the quantification of the chromatographic peaks of BPs including sample volumes, the addition of salts or derivatizing agents. Results showed that gas chromatography – mass spectrometry (GC-MS) is an appropriate analytical technique for most of BP analogues. However, for some analogues (namely BPS) the analysis through GC-MS might not be the most optimal procedure.

In Chapter 2, the dietary exposure to BPs was assessed by analysing their co-occurrence in food. A number of foodstuffs including canned food, fresh products or food packed in other BP-free materials, were included. Among 8 BPs analogues, only BPA, BPE and BPB were detected, being BPA the most abundant compound. Importantly, one sample of canned asparagus presented BPA levels above the specific migration limit (SML) established by the European Commission. Unexpectedly, BPs were also reported for non-canned food, being food processing a potential contamination source. Anyhow, the estimated dietary intake of BPA by an adult consuming a canned food diet was below the tolerable daily intake (TDI) established by the European Food Safety Authority (EFSA).

Finally, in Chapter 3, the content of BPs in biological samples of the volunteers who participated in the duplicate diet study was analysed by applying the method optimized and validated in Chapter 1. Biological samples were urine and blood (whole blood, plasma and red blood cell (RBC) fractions) collected for 2 days of study. As expected, urine was reported as the best matrix to carry out biomonitoring of BPs due to the fast metabolism and excretion of these compounds. BPA was detected in most urine samples, for both groups, either exposed and control. Anyhow, concentrations of BPA in urine of the exposed group were significantly higher than in the control group. Other BP analogues were only found at trace quantities in few random samples, being hardly correlated to the consumption of canned food and their use as BPA substitutes. These results confirm that diets based on canned food significantly increases the exposure to BPA, but not to other BP analogues. Since spot urine sample, instead of 24-h composites, were collected, it could be confirmed that the shorter intervals of urine sampling the better the exposure to BPA is predicted, being successfully correlated with the dietary ingestion of BPA. Thus, 4-h interval was found to be an optimal interval. Finally, the exposure to BPA was calculated considering the concentrations of BPA in urine. Results are similar to the dietary exposure predicted in Chapter 2, representing less than the 3% of the TDI. Consequently, the low exposure to BPA means that no harmful effects are likely to occur in humans, even when consuming a diet of canned food.

Summarizing, BPA is here reported to be ubiquitously present in foodstuffs, regardless the food packaging. However, BPA content is higher in canned foodstuffs than fresh or glass-packed food. Therefore, the consumption of canned food leads to a higher exposure to BPA, although the TDI is not exceeded, even when following a canned food diet. Interestingly, the occurrence of other analogues in food and urine is hardly linked to the intake of canned foodstuffs, and the use of personal care products and thermal paper could be other potential sources. Anyhow, the potential increasing occurrence of other unregulated BPs might mean a significant exposure to BPs in the future, considering the potential endocrine-disrupting effects of these chemicals, the regulation of not only BPA but also other analogues is mandatory.

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INTRODUCTION

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1. Endocrine system

The endocrine system is a group of glands and cells that forms the main regulatory mechanism and controls the major human body functions. In humans, the main glands are the pituitary gland and the hypothalamus, which constitute the hypothalamus-pituitary axis. The human body counts with other glands, such as pineal gland, thyroid, parathyroid, pancreas, adrenal glands, and ovaries or testes (Malespin & Nassri, 2019) (Figure 1). These glands are in charge of synthesizing and excreting hormones, which are the messengers of the endocrine system. In turn, hormones mediate many growths, homeostatic and developmental systems, such as cardiovascular system, central nervous system or reproductive system, among others (Lintelmann et al., 2003). Regarding the latter, the main sex hormones are estrogen and progesterone for females, and testosterone for males. These hormones are mainly synthesized by the ovaries and the testis, respectively. However, the liver and the adipose tissue, which are known as secondary endocrine organs, can also produce them locally (Wierman, 2007; Malespin & Nassri, 2019).

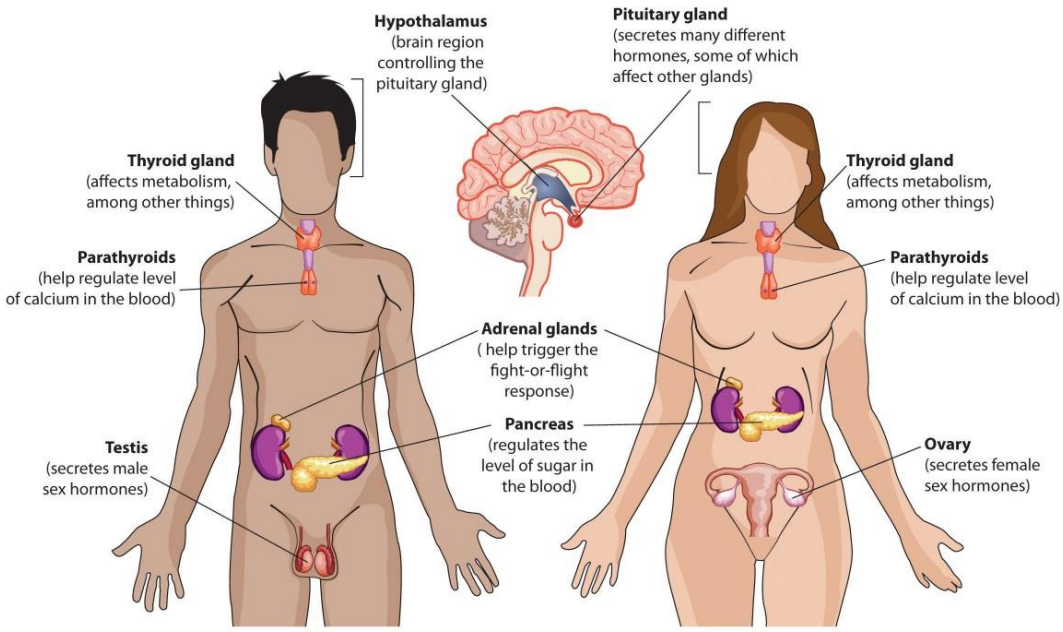

Figure 1. Main glands of the endocrine system. Source: Anatomy medicine, 2019

Estrogens are responsible for secondary sex characteristics and reproductive. In turn, progesterone has an important role on the menstruation cycle and on the development of foetal membranes and mammary glands during pregnancy. Regarding male sex steroids, testosterone helps on the determination of the secondary sex characteristics and sperm production. In addition, all of them interfere in other non-reproductive functions and systems, like bone growth and protein synthesis, and cardiovascular and immune systems (Lintelmann et al., 2003; Guerriero, 2009; Morales-Montor et al., 2011).

Secreted hormones travel through the bloodstream to target tissues and cells, where they initiate the cellular cascade binding to the protein receptor. Each class of sex steroid binds to different intracellular receptors, namely estrogen receptors, progesterone receptors or androgen receptors. Generally, they are ligand-dependent transcription factors, meaning that gene transcription is only possible when the hormone binds to the receptor (Ikeda et al., 2019).

However, not only reproductive hormones but also endocrine-disrupting chemicals (EDCs) are able to bind to sex steroid receptors.

2. Endocrine disruptors

According to the World Health Organization (WHO), EDCs are described as "*exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*" (Usman & Ahmad, 2016; WHO/IPCS, 2002). Generally, EDCs are lipophilic compounds, and hence, they can be accumulated in the adipose tissue. Consequently, some of them have a long half-life in the human body.

Thus, the exposure to EDCs is not limited to one single compound, but resulting in a co-exposure to a mixture of EDCs. Different EDCs may occur at low levels and have additive or synergistic effects because they interact with the same targets. For example, one EDC by itself might not have an effect on the human body, but if a combined exposure occurs, additive exposure of different compounds might trigger the molecular pathways and result in an adverse outcome for the human health. Therefore, the potential cumulative, additive or synergic effects, resulting from this co-exposure, need further attention (Lauretta et al., 2019; Mantovani, 2015).

In addition, it is also important to notice that there are multiple factors that modulates the risks associated to the exposure to EDCs. Life stage is the most important. Newborns and infants are more susceptible because an early burden may be derived from *in utero* exposure or from maternal breast milk or, in some cases, a limited variety of food. Additionally, pathways for the detoxification of EDCs are less developed than in adults. On the other hand, children and adolescents may be also higher exposed due to the fact that they ingest more food in relation to their body weight and their diet may be consisting of non-healthy dietary habits. Pregnant women are also a critical population regarding EDCs susceptibility since exposure to EDCs might lead to adverse birth outcomes (Mantovani, 2015).

Gender is also an important modulating factor, being male offspring especially susceptible to estrogenic EDCs (García et al., 2017; Schreiber et al., 2019). However, in general, both genders can be affected by EDCs because some of them can interact with estrogen and androgen receptors (Mantovani, 2015).

2.1. Mechanism of action

EDCs can act through several mechanisms, but the most important are agonistic and antagonistic (Figure 2) (Lintelmann, 2003; Combarnous and Nguyen, 2019):

1. *Agonistic action*: the xenoestrogen binds to the hormone receptors and triggers the transcription factors and leads to the same effects as the natural-occurring hormone. The potency of the agonist depends on its affinity for the receptor. Diethylstilbestrol (DES) is a well-known EDC with this mechanism of action.
2. *Antagonistic action*: An antagonist binds to the receptor and blocks or diminishes the effects caused by the activation of that receptor. The inhibition can be competitive, because the antagonist binds to the active binding site of the hormone, or non-competitive, because the antagonists binds to the hormone-receptor complex but not to the binding site. Competitive inhibition can lead to a completely deactivation and non-competitive inhibition can produce a diminished effect of the receptor. Herbicides, like linuron or vinclozolin, are examples of exogenous compounds with antagonistic action.
3. *Interaction with components of hormone signalling pathways*: some EDCs do not interact with the hormone receptor but with the signalling pathway components after receptor activation. In this mode of action, the exogenous compounds can disrupt different sites which can be difficult to describe. One example is atrazine, a widely-used herbicide. This compound facilitates cAMP accumulation by inhibiting PDE4 which is a cAMP-specific phosphodiesterase.
4. *Stimulation of an endogenous hormone biosynthesis*: some EDCs can influence the concentration of natural-occurring hormones by affecting their biosynthesis. These kind of compounds are structurally different from hormones, since they

do not bind to the hormone receptor. An example of this mechanism of action is present in triclosan which induces the secretion of Vascular Endothelial Growth Factor (VEGF) by human prostate cancer cells.

5. *Inhibition of an endogenous hormone biosynthesis*: It is the same mechanism of action than the above-mentioned but instead of promoting the biosynthesis it inhibits the formation of the hormones. One example are parabens, which inhibit the 17β-hydroxysteroid dehydrogenase enzyme and this leads to the inhibition of the oestrogen degradation and, consequently, increases their concentrations in blood.
6. *Binding to circulating hormone-binding protein*: hydrophobic EDCs can compete with the binding to transportation proteins. These proteins transport hydrophobic hormones, such as steroids and thyroid hormones. EDCs that act with this mechanism have structural similarities with natural-occurring hormones. Some EDCs have been shown to interact with α-fetoprotein or steroid hormone-binding protein.
7. *Stimulation or inhibition of hormone-binding protein synthesis or degradation*: similar to the above-mentioned, EDCs acting with this mechanism interfere with the synthesis of the hormone-binding proteins which leads to a variation of the total hormone concentration. These type of EDCs might not be structurally similar to endogenous hormones since they interfere with the protein biosynthesis.
8. *Stimulation of hormone receptor expression*: endocrine homeostasis can be disrupted by the stimulation of hormone receptors. For example, HUVEC cells exposed to cadmium has been shown to increase estradiol receptor β.
9. *Inhibition of hormone receptor expression*: In contrast to the aforementioned mechanism, there are some EDCs that can inhibit the expression of the hormone receptors. For example, BPA has been described to inhibit androgen receptors *in vivo*. Receptor synthesis and degradation is controlled by the natural-occurring

hormones so, EDCs might resemble the endogenous hormones to exert their effects.

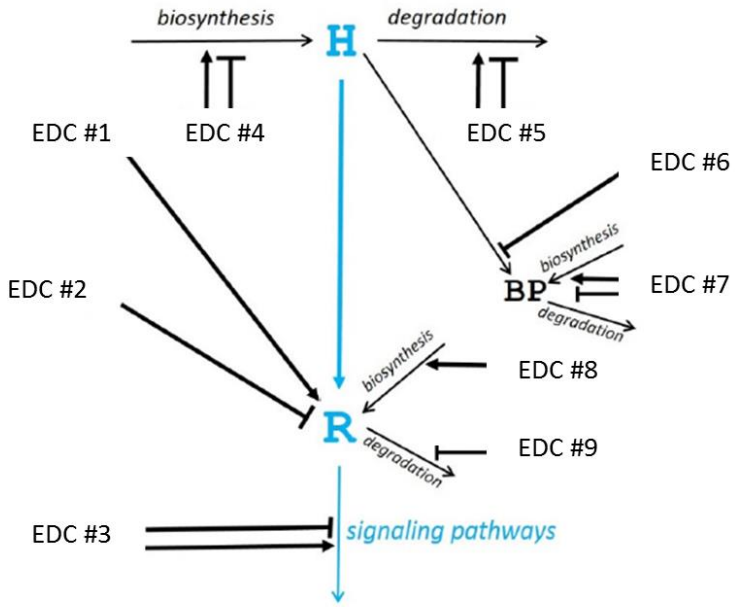

Figure 2. Scheme of different mechanisms of action of EDCs. H: hormone; R: receptor. Adapted from Combarnous & Nguyen, 2019

2.2. Types of EDCs

Generally, EDCs includes naturally occurring chemicals, known as phytoestrogens (PhyEs), and synthetic compounds, widely used in the industry (Grześkowiak et al., 2017; Hampl et al., 2016).

2.2.1. Phytoestrogens

PhyEs are plant-derived substances that are structurally similar to the human female estrogen 17-β estradiol (Figure 3), therefore, they can bind to estrogen receptors. PhyEs have shown both estrogenic and anti-estrogenic properties, thus, they can act as agonists or antagonists. According to their chemical structure, they are divided into three main classes: isoflavones, coumestants and lignans (Nie et al., 2015; Paterni et al., 2016). PhyEs have shown beneficial health effects, namely lowering of osteoporosis, breast cancer,

heart disease and menopausal symptoms (Nie et al., 2015). Nonetheless, their related side-effects should not be underestimated. Some authors suggest that almost all PhyEs derive into pro-apoptotic effects in some cells and they may cause potential harm to the human health due to their estrogenic disrupting activity (Nie et al., 2015; Paterni et al., 2016; Patisaul, 2016).

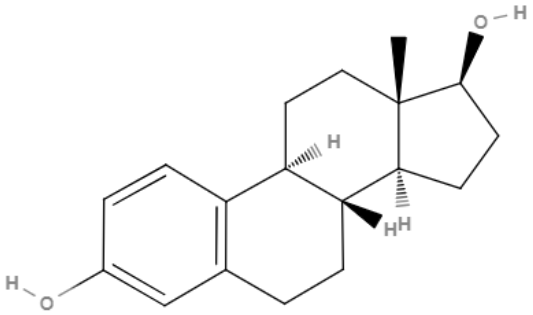

Figure 3. Molecular structure of 17-β estradiol

2.2.2. Synthetic compounds

Synthetic compounds are man-made chemicals that can be used with different purposes, such as industrial (polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs)), agricultural (pesticides), residential (bisphenol A (BPA)) and pharmaceutical (parabens) activities (Lauretta et al., 2019; Kabir et al., 2015). Synthetic compounds have been widely studied and found in several products, such as toys, personal care products, plastics, detergents, etc. Moreover, reported effects for the human health are of high concern because they can affect many functions of the human body. Exposure to these EDCs may lead to adverse health outcomes like obesity, diabetes, neurobehavioral disorders, autoimmune diseases, asthma, and even cancer (Nowak et al., 2019). Due to their harmful effects, some of them have been banned in some countries, for example, dichlorodiphenyltrichloroethane (DDT), DES or BPA (Kabir et al., 2015).

3. Bisphenols

Bisphenols (BPs) are a group of organic compounds with a hydroxyl residue bounded to an aromatic ring (Figure 4). They are obtained by condensing a phenol with a ketone or an aldehyde. This reaction occurs in the presence of an acidic ion-exchange resin (Corrales et al., 2015; Geens et al., 2012; Michałowicz, 2014). Nowadays, there are many analogues described in the literature (Pelch et al., 2017).

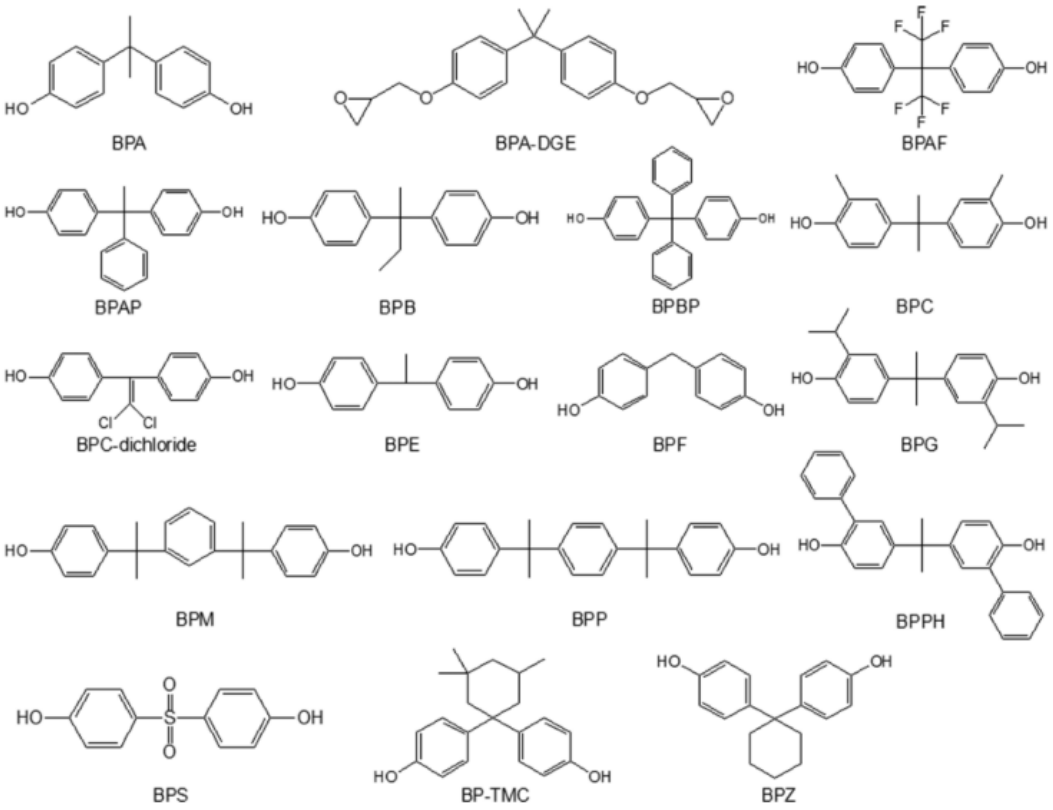

Figure 4. A number of bisphenol analogues described in the literature. Source: Chen et al., 2016

BPs are widely used in the industry. Due to its physical and chemical properties, such as high strength over a wide range of temperatures, lightweight, hardness, resistance to chemicals and to impact, transparency, colourability and low manufacturing costs, they are used in many everyday products (Mikołajewska, 2015; Eladak et al., 2015; Gostner et al., 2015). It is also important to remark that BPs have some undesirable properties that

make their application controversial, which are incomplete polymerization and seepage from the materials (Gostner et al., 2015). The most important analogue is BPA.

4. Bisphenol A

BPA was firstly synthetized in 1891 by Alexander Dianin (Tyl, 2014; Eladak et al., 2015). However, its estrogenic activity was not reported until 1936, when it was found out that a phenanthrene nucleus – like in estradiol – was not necessary to induce an estrogenic activity (Dodds & Lawson, 1936). Moreover, it was also reported that compounds containing two phenol rings bounded by a carbon chain were active, and their activity varied depending on this chain (Dodds & Lawson, 1938).

Even though the endocrine disruption potential of BPA was well known, this compound became relevant for the industry in the 1950s (Mikołajewska, 2015; Eladak et al., 2015). Since then its production has been increasing surprisingly every year. Nowadays, the global demand of BPA is projected by 10.6 million metric tons in 2022 (Lemhler et al., 2018). About 95% of the BPA production is aimed at the fabrication of plastics, in particular polycarbonate plastics (71%) and epoxy resins (29%) (Careghini et al., 2015). Additionally, BPA is also used for the production of thermal paper (Michałowicz, 2014). Its applications comprise coating for food packaging and beverage cans, lacquers, metal jar lids, medical equipment, dental sealants, toys, personal care products, adhesive plastics, automobile parts, etc. (Chen et al, 2016; Kang et al., 2006; EFSA, 2015; Liao & Kannan, 2014). Therefore, humans are largely exposed to BPA.

Consequently, traces of BPA occur in a wide range of biological and environmental matrices. One of the first studies to report the leaching of this xenoestrogen from coatings of food cans was carried out by Brotons et al, (1995). These authors tested if the liquid from the canned food could enhance the proliferation of MCF7 human breast cancer cells similar to 17β-estradiol. As expected, the proliferation effect was 58% of that obtained with 17β-estradiol. It meant an estrogenic effect, although lower than that obtained with the natural estrogen. This study was preceded by Kishnan et al, (1993),

who reported the release of BPA from polycarbonate plastics during the autoclaving process. It was found that an estrogenic compound occurring in the yeast-culture medium was not a yeast product – as firstly thought – but a component of the polycarbonate flask used for the cell growth. They observed its estrogenic effect due to the induction of progesterone receptors in MCF7 cells when these were grown using medium with autoclaved water (Kishnan et al., 1993). Other studies also reported the leaching of BPA from dental sealants and reusable polycarbonate plastics (Biles et al., 1997; Olea et al., 1996).

4.1. Exposure pathways

Although there are many exposure routes to BPA, they can be basically divided into dietary and non-dietary pathways (Figure 5).

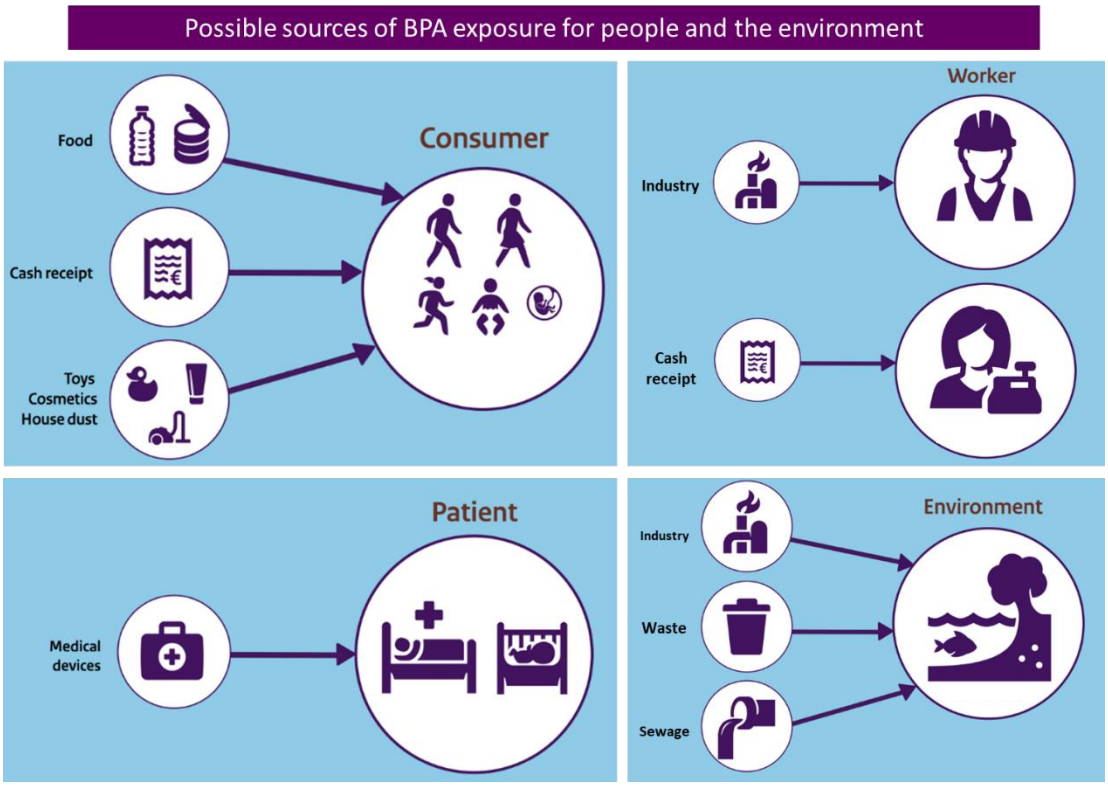

Figure 5. Sources of BPA exposure for people and the environment. Adapted from: RIVM, 2019

4.1.1. Dietary sources

Diet is the main route of exposure to BPA (Martínez et al., 2018). BPA contamination in food is usually caused by the contact to packaging materials that are made of polycarbonate or epoxy resins (Geens et al., 2012). However, some authors have reported that BP contamination could occur even during the primary production of the food (Mercogliano & Santonicola, 2018; Santonicola et al., 2018). One example is cow milk, which is a high-fat food and BPA residues are likely to accumulate in these matrix. BPA is able to go in via contaminated feed, soil or veterinary medicines. On the other hand, BPA can enter into milk chain through various pathways during milk production (polyvinyl chloride (PVC) tubes used for the milk process or transfer to storage tanks). Hence, BPA concentration can reach a high level in the final product, being a great concern for the public health because dairy products are widely consumed by the human population (Mercogliano & Santonicola, 2018; Santonicola et al., 2018).

Even though food packaging is made mainly to prevent deterioration and to lengthen the product life, food can also be contaminated by the presence of residual amounts of monomer that remain after the polymerization process of the jar lids of glass containers (García Ibarra et al., 2019; Noonan et al., 2011). Moreover, temperature and storage conditions have a direct impact on the migration of BPA. Regarding to this, Errico et al., (2014) exposed tomato cans to three different temperatures (25, 37 and 45°C) and storage conditions (undamaged and dented cans). Results showed that heating of canned tomatoes and/or can damage during transportation and storage were an aggravating factor for BPA migration to food products (Errico et al., 2014).

Many authors have determined BPA concentration in food samples, especially canned food. Many different types of food have been analysed in the literature. Some of the most analysed food groups are meat and meat products, fish and seafood, fruit, vegetables and beverages (Table 1). In general, fish and seafood is the type of food that contains higher levels of BPA. In contrast, fruit and beverages are the groups that present lower levels of BPA. According to the literature, contamination of the food is not

dependent on the country nor the continent, as BPA contamination is widely spread around the globe.

Table 1. BPA concentrations in different types of food and countries.

Country	Food type	Type of container	BPA concentration (µg/kg)	Reference
Norway	Meat and meat products	Non-canned	0.24	Sakhi et al., 2014
Belgium		Canned and non-canned	0.86 to 26.7	Geens et al., 2010
Korea		Canned	29.57	Choi et al., 2018
Korea		Canned	24.49	Lim et al., 2009
Japan		Canned	6.8	Kawamura et al., 2014
Canada		Canned	10.5	Cao et al., 2011
Egypt		Canned	231.77	Osman et al., 2018
Portugal	Fish and seafood	Canned	19.1	Cunha et al., 2012
Portugal		Canned	21	Cunha et al., 2017
Italy		Canned	19.1 to 187	Fattore et al., 2015
Belgium		Canned and non-canned	0.67 to 169.3	Geens et al., 2010
Norway		Canned and non-canned	1.2	Sakhi et al., 2014
Korea		Canned	22.6 to 174	Choi et al., 2018
Korea		Canned	39.78	Lim et al., 2009
Japan		Canned	7.9	Kawamura et al., 2014
Canada		Canned and non-canned	0.48 to 106	Cao et al., 2011
Egypt		Canned	117.34	Osman et al., 2018
Norway	Fruit	Non-canned	0.38	Sakhi et al., 2014
Belgium		Canned and non-canned	0.11 to 20	Geens et al., 2010
Korea		Canned	<1.87 to 60.6	Choi et al., 2018
Korea		Canned	8.6	Lim et al., 2009
Japan		Canned	<1	Kawamura et al., 2014
Canada		Canned	0.51 to 3.24	Cao et al., 2011
Egypt		Canned	51.53	Osman et al., 2018

Table 1. BPA concentrations in different types of food and countries *(continued)*

Country	Food type	Type of container	BPA concentration (µg/kg)	Reference
Italy	Vegetables	Canned	0.30 to 3.80	Errico et al., 2014
Norway		Non-canned	<0.1	Sakhi et al., 2014
Belgium		Canned and non-canned	0.10 to 116.3	Geens et al., 2010
Korea		Canned	<1.74 to 11.83	Choi et al., 2018
Korea		Canned	3.10	Lim et al., 2009
Japan		Canned	4.10	Kawamura et al., 2014
Canada		Canned and non-canned	0.41 to 83.7	Cao et al., 2011
Egypt		Canned	51.53	Osman et al., 2018
Norway	Beverages	Canned and non-canned	<0.02 to 0.37	Sakhi et al., 2014
Belgium		Canned	0.36	Geens et al., 2010
Korea		Canned	1.81 to 15.25	Choi et al., 2018
Korea		Canned	8.3 to 45.51	Lim et al., 2009
Japan		Canned	<1 to 1.1	Kawamura et al., 2014
Canada		Canned and non-canned	0.24 to 0.74	Cao et al., 2011
Egypt		Canned	45.81	Osman et al., 2018

4.1.2. Non-dietary

Although the major pathway of BPA intake is through the diet, there are more routes of exposure that should be considered to assess the exposure to BPs.

4.1.2.1. Dermal

Transfer of the exogenous compounds across the human skin is influenced by a number of factors, namely, molecular weight or lipophilicity. BPA is a small molecule (288 Da) and also lipophilic, hence, it is likely to cross the human skin and enter the blood stream with little difficulties (Healy et al., 2015). In fact, some skin models have proved that BPA can be absorbed when applied directly to the skin. Moreover, they have observed that 9.5-33% of the applied dose can pass through the epidermis and the dermis as free BPA (Demierre et al., 2012; Zalko et al., 2011).

The most relevant source of dermal exposure is thermal paper. BPA is used as an additive acting like a colour developer under heat or pressure in printing devices, like cash registers or credit card terminals (Geens et al., 2012). Although general population is daily in contact with thermal paper, cashiers are the population group that is exposed the most. Some authors have determined whether handling thermal receipts increase the urinary levels of BPA. However, results are contradictory. Porras et al, (2014) noted that any contact with thermal paper does not have an effect on the total BPA concentration in urine, while Lee et al, (2018) and Ndaw et al, (2016) concluded that handling of thermal paper increase urinary total BPA levels.

Even though thermal paper is not recyclable and should be placed in the non-recyclable bin, most people place it in the paper bin. Hence, it enters into the recycling system where some BPA is removed as paper sludge but some of it will remain and contaminate the recycled products. Therefore, its exposure potential is very high and not easy to control (Pivnenko et al., 2019).

Nowadays, there are a number of alternatives that are used as substitutes for BPA in thermal paper, such as phenol-based substitutes (i.e.: BPS) or phenol-free substitutes (i.e.: Pergafast®, urea urethane) (Pivnenko et al., 2019). The use of these alternatives is spread across the European market. Nowadays, 1 out of 3 thermal paper receipts contains an alternative developer (Vervliet et al., 2019), being BPA the colour developer most frequently used in the European receipts (Martin et al., 2017; Björnsdotter et al., 2017a; Vervliet et al., 2019). Regarding BPA alternatives, phenol-free compound Pergafast® is the most used in Germany and Norway (Martin et al., 2017; Björnsdotter et al., 2017b), while in the Netherlands, Spain and Sweden, BPS is the main substitute (Björnsdotter et al., 2017b).

Personal care products (PCP) are also a potential source of dermal exposure to BPA. Although BPA is not a product component itself, migration from containers might occur. Its presence has been documented in different types of PCP like toothpastes, hair products, body washes, hand soaps, sanitary products and make-up products (Liao and Kannan, 2014). Although these kind of products are used on a daily basis, the contribution only accounts with the 0.07-2.87% of the total exposure to BPA (Lu et al., 2018; Martínez et al., 2018). These results are in accordance with another study finding out a positive correlation between lip balm and urinary BPA (Husøy et al., 2019).

Recently, attention has also been paid on the occurrence of BPA in textiles and the exposure derived from their use. Xue et al, (2017a) and Freire et al, (2019) determined that BPA occurred in 82 to 91% of textile samples, being the dermal exposure estimated to be between 0.93 and 248 pg/kg bw/day.

4.1.2.2. Inhalation

Inhalation is another important non-dietary exposure pathway to BPA, being air and dust the most significant sources (Velázquez-Gómez & Lacorte, 2019). In fact, BPA is used in a wide range of indoor applications. As it occurs with all toxics, children are the most vulnerable population because they have higher oxygen requirements, higher respiration rate and higher hand-to-mouth contact than adults (Healy et al., 2015; Geens et al., 2012).

Thus, the concentrations of BPA in dust ranged between <0.5 and 10200 ng/g (Loganathan and Kannan, 2011), being the analogue most predominant in air (particulate phase, vapour phase and bulk air) among all bisphenols (Xue et al., 2017b).

4.2. Metabolism

BPA is rapidly conjugated in the gut or in the liver upon ingestion and only less than 1% of the free BPA remains unconjugated in blood (Thayer et al., 2015). Hepatic uridine 5'-diphospho-glucuronosyltransferase (UGT) is the main enzyme that catalyses BPA-glucuronide (BPA-G) formation, which is the main BPA metabolite. BPA-sulfate (BPA-S), catalysed by sulfotransferases, is also a relevant BPA metabolite (Gramec Skledar & Peterlin Mašič, 2016) (Figure 6). In fact, quantification of free BPA and its metabolites proved that BPA-G was the major metabolite (69.5%), followed by BPA-S (21%) and unconjugated BPA (9.5%) (Ye et al., 2005). These results are consistent with Gerona et al., 2016, where urine from pregnant women was analysed and it was reported that total BPA (free + conjugated) consisted of 71% of BPA-G, 15% of BPA-S and 14% of free BPA.

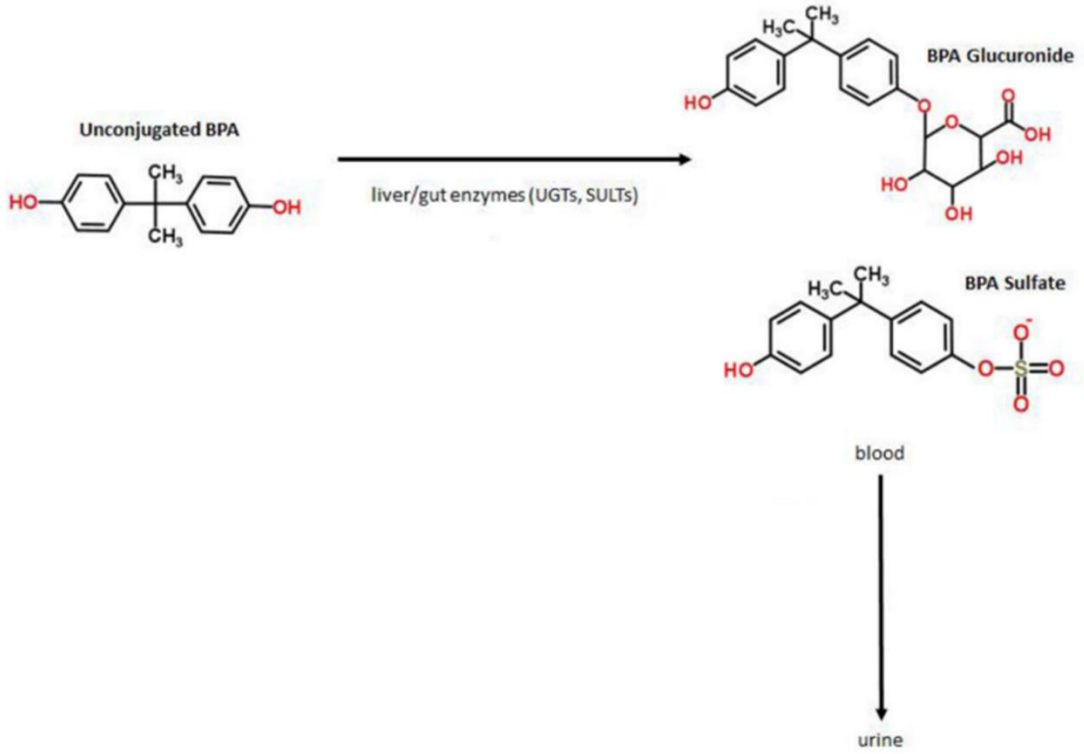

Figure 6. Scheme of the BPA metabolites formed *in vivo*. Adapted from: Thayer et al., 2015

Initially, it was believed that both of them (BPA-G and BPA-S) were inactive forms of BPA, but, recently, some authors suggested that BPA-G is biologically active and induces lipid accumulation and differentiation of preadypocites yet it does not have estrogenic activity like unconjugated BPA (Boucher et al., 2015). BPA-S activity remains unexplored.

Regarding BPA pharmacokinetics, the maximum concentration of total BPA is reached, approximately, 1 h to 1.6 h after its ingestion, while it can be detected in serum after 5 minutes. Moreover, its half-life in human body is determined between 5.5 h and 6 h, being more than 90%, excreted as metabolites (Thayer et al., 2015; Teeguarden et al., 2015). On the other hand, half-life of BPA-G was lower than free BPA, occurring at 0.29 h. Moreover, maximum BPA-G concentration occurred 24 min earlier than the corresponding to free BPA (Teeguarden et al., 2015). On the other hand, half-life of BPA-S was 0.62 h, two times slower than BPA-G. Peak concentration of BPA-S occurred at 2.2 h, 1 h later than the corresponding to BPA-G and 36 min after maximum concentration of free BPA (Teeguarden et al., 2015).

4.3. Health effects

4.3.1. BPA and reproduction

BPA is involved in different dysfunctionalities in the human health. Taking into account its endocrine disrupting potential, BPA hazard is mainly focused on the reproductive system. Animal studies show that BPA can affect female fertility, delays on the onset of female puberty and changes on the oestrous cycle. Negative impact on female fertility is derived from impaired oocyte cytoskeletal dynamics, DNA damage, oxidative stress and epigenetic modifications (Matuszczak et al., 2019).

4.3.2. BPA and cancer

The exposure to BPA is also associated with different types of cancer. In fact, there is an accumulated evidence suggesting that sex steroids are involved in ovarian cancer. It has been reported that approximately one-half of the ovarian epithelial cancer cells express higher levels of estrogen receptors than normal cells. Estrogens can provide a hormonal environment that promotes tumour progression (Gao et al., 2015). However, there is still a lack of evidence that relates BPA exposure to the development of the ovarian cancer.

Mammary gland development is connected to estrogen signalling pathways. BPA effects on mammary glands and breast carcinogenesis have been extensively studied *in vitro* and *in vivo.* Evidence suggests that low-dose exposure to BPA during the foetal development alters cell proliferation, apoptosis and development of mammary glands, which can influence mammary gland carcinogenesis in the future. Nevertheless, the exact mechanisms of action remain undisclosed (Gao et al., 2015). In addition, estrogens are involved in the etiology of prostate cancer, and uses of anti-estrogens have been recognized to have therapeutic effects against prostate cancer. BPA is believed to regulate the proliferation and migration of prostate cancer cells, but the main mechanism of action is still unknown (Gao et al., 2015).

4.3.3. BPA and fertility

Male fertility might also be affected by the exposure to BPA. Human studies reported a decrease in the sexual function related to BPA exposure in a dose-dependent way. In addition, Rochester et al., 2013 and Radwan et al., 2018 found that higher urinary BPA was significantly correlated with lower sperm quality measures (concentrations, count, vitality and mobility). Effects on the spermatogenesis are due to the disruption of the hypothalamus-pituitary axis (Rahman and Pang, 2019).

4.3.4. BPA and development

In turn, the exposure to BPA is able to cause miscarriages and premature deliveries in pregnant women (Hu et al., 2019; Huang et al., 2019a). Notwithstanding, data is very limited on these effects and further investigations should be carried out (Mikołajewska et al., 2015).

Among all the population groups, infants and children are the most susceptible populations, as they are not fully developed like adult population. According to some authors, BPA exposure could affect birth's weight, produce male genital abnormalities and even affect to their behaviour and neurodevelopment (Rochester, 2013; Mikołajewska et al., 2015; Almeida et al., 2018).

4.3.5. BPA and metabolism

BPA might also trigger metabolic disorders. Mainly, diabetes and obesity are linked to BPA exposure. Obesity is the result of many behavioural factors, such as excessive food intake and sedentary life, along with genetic predisposition, but these do not explain completely the increasing incidence. BPA is a compound called "obesogen", meaning that it can downregulate lipid metabolism and increase adipogenesis. It has been suspected that BPA may be modulated by methyl donors and this could cause permanent damage to adipogenesis, appetite and energy metabolism (Bertoli et al., 2015). In turn, diabetes has also been related to urinary BPA (Murphy et al., 2019; Duan et al., 2018). Finally, BPA exposure has also been linked to other metabolic diseases, like non-alcoholic fatty liver and

cardiovascular disease (Rochester, 2013; Bertoli et al., 2015; Rahmani et al., 2018; Kim et al., 2019).

4.4. Regulation of BPA

The rising strong evidence of the negative health outcomes provoked by the widespread exposure to BPA made necessary its regulation. Thus, a strong regulation against it entered into force since 2010s decade. The first country that banned the sale, the import and the advertisement of baby bottles containing BPA was Canada (Government of Canada, 2010). On 2011, the European Commission adopted a new regulation that established a specific migration limit (SML) of 0.6 mg/kg for the manufacture of polycarbonate feeding bottles for infants (2011/8/EU) (European Commission, 2011). Later on, a more restrictive SML was established being set at 0.05 mg/kg for all polycarbonate plastic. Moreover, no migration is permitted from varnishes or coatings applied to materials and products that are specifically intended to be used in food products for young children up to 3 years old (2018/213/EU) (European Commission, 2018).

Much strict is the regulation in France, where BPA has been banned in all the food contact materials that might be in contact with all the population, not only limited to toddlers and children (Journal Officiel de la République Française, 2015). This law caused great discomfort among the European plastic industry, complaining that this legislation would cause a loss of 1.5 billion €/year (DG GROW, 2015). Afterwards, France decided to partially lift the ban on the manufacture and export of BPA-containing food contact materials because it posed an “unjustifiable restriction of trade”. However, the ban remained valid at the national level (QPC, 2015).

Beyond the regulation of polycarbonate plastics used in food by the European Commission, toys and thermal paper have also been regulated. In this sence, BPA migration from toys has been lowered from 0.1 mg/L, which means that during this extraction a maximum of 0.01 mg BPA may migrate out of the toy material, to 0.04 mg/L (2017/898/EU) (European Commission, 2017). Regarding thermal paper, the use of BPA has been recently regulated.

Regulation 2016/2235/EU establishes that BPA shall not be used in thermal paper in a concentration equal or greater than 0.02% of its weight and this amend will enter into force on January 2020 (European Commission, 2016).

In parallel, the European Food Safety Authority (EFSA) delivered a scientific opinion on BPA and performed a risk characterization for this compound. The tolerable daily intake (TDI) derived from this opinion was established in 0.05 mg/kg bw/day, based on the no adverse effect level of 5 mg/kg body weight per day in rodent studies (EFSA, 2006). Almost ten years later, EFSA re-evaluated the hazard risk for this compound and lowered the former limit to a temporary tolerable daily intake (t-TDI) of 4 μg/kg bw/day, concluding that estimated dietary intake to BPA for the highest exposed groups (toddlers, infants, children and adolescents) is below the t-TDI. This result demonstrates that there is no health concern at this level of exposure even when taking aggregated exposure into account (EFSA, 2015). Nowadays, a new safety re-evaluation is being conducted by EFSA and their opinion is expected to be delivered in 2020.

4.5. BPA analogues

As a result of the increasing regulation against BPA, manufacturers started to replace it with other analogues. The most used and studied analogues are bisphenol S (BPS), bisphenol F (BPF), and bisphenol B (BPB) (Andra et al., 2015; Karrer et al., 2018).

4.5.1. BPS

BPS was first synthetized as a dye in 1869 and it was one of the first analogues to act as a substitute of BPA in the 2000s (Wu et al., 2018). Its main uses are for epoxy glues, coatings and, especially, thermal paper. It is also used as an additive in dyes and tanning agents (Chen et al., 2016). Its higher thermal stability makes it a good alternative for BPA (Gramec Skledar, 2016).

Regarding the metabolism, BPS is mainly glucuronated to form BPS glucuronide (BPS-G) in the liver, but a small portion can be metabolized in the intestine upon ingestion. BPS sulfate

(BPS-S) is also a common metabolite although in a much lower proportion than BPS-G (Oh et al., 2018). The pharmacokinetic model for BPS was also characterized. Similar to what happens to BPA, BPS is rapidly absorbed by the gastrointestinal tract 1 h after ingestion and the half-life is determined to be 6.93 hours (Oh et al., 2018).

Occurrence of BPS in the environment is ubiquitous. Several matrices were reported to present measurable levels of BPS as environmental compartments (indoor dust, fluvial and surface water, sewage sludge) (Wu et al., 2018; Xue et al., 2017; Qiu et al.,2019); thermal paper (Bjornsdotter et al., 2017; Vervliet et al., 2019; Qiu et al., 2019); food (Liao et al., 2014; Liao and Kannan, 2013) and, also, human urine and blood (Wu et al., 2018; Qiu et al., 2019). In general, the presence of BPS in all the aforementioned compartments is increasing since it is being used as a BPA alternative since 2012, although it is still lower than BPA (Qiu et al., 2019). However, the occurrence of BPS in the environment will most probably become similar or even greater than BPA, causing harmful effects to human health in the future (Qiu et al., 2019).

Chen et al., (2002) proved the weak estrogenic activity belonging to BPS (Chen et al., 2002). Since then many studies have reported adverse health effects caused by the exposure to this compound. BPS is also capable to cause reproductive disruption in males, as it can inhibit gonadotropin secretion, induce oxidative stress in the testicular tissues and affect spermatogenesis (Ullah et al., 2018). Zhang et al, (2016) reported time- and concentration-dependent cytotoxicity in various cell types, such as renal cells or hepatocytes. Regarding animal models, embryonic exposure to BPS was found to affect neural functionality in *C. elegans* (Mersha et al., 2015) and, in zebrafish, BPS induced precocious hypothalamic neurogenesis (Kinch et al., 2015). In general, BPS affects many body functions but it has lower estrogenic potential and lower acute toxicity than BPA (Chen et al., 2002; Qiu et al., 2019).

4.5.2. BPF

BPF is being used as a replacement of BPA due to its lower viscosity and better resistance to solvents (Lu et al., 2018). It is used in lacquers, varnishes, adhesives plastics, pipe linings, dental sealants and coatings for food packaging (Chen et al., 2016; Usman et al., 2019). Interestingly, BPF naturally occurs in mustard at a concentration of 8 mg/kg (Zoller et al., 2016). Some authors even reported the presence of this BPF in plants used in traditional medicine at higher concentrations than in mustard (Huang et al., 2019b).

Regarding the metabolism, BPF is absorbed and distributed to the whole body. As BPA, it is mainly excreted through the urine but it was found that 7-9% of the administered dose was still present in the tissues after 96 h upon ingestion. Moreover, an *in vitro* study suggest that BPF is metabolized by hepatic and intestinal cells (Usman et al., 2019). Biotransformation of BPF *in vivo* indicates that BPF sulfate is the major urinary metabolite (Usman and Ahmad, 2016).

BPF is found to be as hormonally active as BPA (Rochester and Bolden, 2015) and at 10 nmol/l is able to decrease the secretion of basal testosterone (Eladak et al., 2015). Meng et al., 2019 suggested that BPF can affect the liver function by disturbing the antioxidant defence system and leading to metabolic disorders because it affects to the levels of several key metabolites.

BPF has already been detected in human matrices like urine (Li et al., 2018) or serum (Song et al., 2019). In turn, its presence is extensive in the environment, being found in numerous matrices namely, sewage sludge, sediments, indoor dust, surface and ground water, soil, food products or personal care products (Usman et al., 2019; Choi et al., 2018; Jin & Zhu, 2016; Lu et al., 2018).

4.5.3. BPB

BPB is an analogue used as an epoxy resin in the manufacture industry (Cunha & Fernandes, 2010). It shows slower degradation under aerobic and anaerobic conditions when compared to BPA (Ike et al., 2006).

Occurrence of BPB is not as widespread as the above-mentioned BPs. This compound has been detected in several matrices like wastewaters (Česen et al., 2018), canned food (Cunha et al., 2012; Fattore et al., 2015), freshwater (Liu et al., 2017), sewage sludge (Yu et al., 2015) and urine (Yang et al., 2014; Cunha & Fernandes, 2010). However, its detection rates and concentration levels are much lower than BPA, BPS or BPF.

Data regarding the metabolism is very scarce and only studies with an S9 fraction have been published. The oxidative metabolites detected were *ortho*-hydroxylated BPB, *ortho*-quinone and two other BPB metabolites with lower polarity (Gramec Skledar and Peterlin Mašič, 2016). Anyways, Cunha and Fernandes, 2010, detected BPB after deconjugation with β-glucuronidase, so conjugation pathways of BPB could also be an important route of its metabolism.

Regarding the health effects, BPB is able to disrupt male reproductive function in rats by inducing oxidative stress, disrupting sperm production and damaging the DNA of the spermatozoa (Ullah et al., 2019). Also, BPB is capable to supress gonadotropin secretion, while presenting estrogenic and anti-androgenic properties (Ullah et al., 2018). Estrogenic potential of BPB has been predicted to be higher than BPA, while anti-androgenic activity is similar to that of BPA (Conroy-Ben et al., 2018).

4.5.4. Other analogues (BPAF, BPE, BPP, BPAP, BPZ)

This subsection includes other BPA analogues less used by the industry. Recently, these analogues have been included in environmental monitoring campaigns. They have been detected in wastewaters (Česen et al., 2018), water and sediments (Jin & Zhu, 2016),

freshwater (Liu et al., 2017), foodstuffs (Chen et al., 2016) but also in human matrices, like urine (Li et al., 2018) and blood (Jin et al., 2018).

Metabolism pathways have not been explored for these compounds yet and further studies should be performed in order to elucidate them.

Regarding health effects, this group of BPs analogues are expected to behave similar to BPA, as they have similar molecular structure. However, there is a strong data gap in literature. Zhu et al., 2020 suggested that bisphenol AF (BPAF) is more cytotoxic than BPA, while potential synergistic and additive effects of BPs were observed. Additionally, Feng et al., 2016 indicated that BPAF modulates steroidogenic gene expression and hormone synthesis in H295R cells.

5. Biomonitoring

One of the best strategy to assess the exposure to BPs is through biomonitoring studies. Human biomonitoring (HBM) is the method to quantify chemicals or toxic metabolites. Usually, blood or urine are the most used matrices, but hair, sweat or breast milk are also common matrices to perform the biomonitoring. The monitored substances should take into account different factors besides their potential toxicology, like their use in the society, the possible environmental contamination, the availability of proper analytical techniques and the cost, among others (Ibarzulea et al., 2016). Hence, human biomonitoring becomes useful to: i) quantify the distribution of the substances among the general population or in population subgroups; ii) Identify overexposed population groups; iii) establish reference values; iv) determine the temporal trend; v) compare the results between different subpopulations; vi) identify public health problems; vii) establish and assess legislative actions or environmental interventions; viii) promote the study of the health effects.

Biomonitoring programs are carried out in most of the developed countries. Classic pollutants, such as persistent organic pollutants (POPs), organochlorine pesticides (as DDT or hexachlorobenze (HCB)), PCDD/Fs, PCBs and heavy metals (like arsenic (As), mercury (Hg), lead (Pb), cadmium (Cd), among others) have been largely biomonitored during the

last decades. More recently, attention has been paid on emerging compounds, being EDCs of great interest because their potential harmful effects. Some examples of recent biomonitoring studies conducted in Spain are shown in Table 2.

Table 2. Summary of recent biomonitoring studies conducted in Spain

Human sample	EDCs	Reference
Urine Blood Human milk	Parabens Alkylphenols BPA Triclosan	Azzouz et al., 2016
Hair	BPA Parabens Perfluoroalkyl substances (PFASs) Brominated flame retardants	Martín et al., 2016
Hair	BPA Parabens Benzophenones (BZPs) PFASs	Rodríguez-Gómez et al., 2017
Human milk	Bisphenols Parabens BZPs	Vela-Soria et al., 2016
Nails	Bisphenols Parabens PFASs Triclocarban	Martín-Pozo et al., 2020
Nasal lavages	Organophosphorus flame retardants Phtalates Alkylphenols BPA	Velázquez-Gómez & Lacorte, 2019
Human milk	Bisphenols	Dualde et al., 2019
Adipose tissue	Phenols Parabens	Artacho-Cordón et al., 2018

The German Human Biomonitoring Commission derives health-related guidance values for the general population which can be grouped as: i) values that represent the concentration of a substance, at which and below of which, there is no risk of adverse effects observed (HBM I value), and ii) values that represent the concentration, at which or above which, adverse effects are possible (HBM II value). There are three methods to establish the new values (Apel et al., 2017):

1) Based on human data

It is based on epidemiological data on human toxicity. Values for some toxic metals and PCBs are derived through this concept. These values are the most reliable for the interpretation of the human internal exposure. However, only a few substances have enough epidemiological data available to derive HBM values.

2) Based on a tolerable daily intake

HBM values based on this concept are equivalent to the TDI values established by the European organisations – like EFSA – and is calculated by means of toxicokinetic extrapolation. Therefore, toxicokinetic information from humans in necessary to derive HBM values. In addition, there are many substances that have no TDI or any other reference values established.

3) Based on critical effects on animal models

HBM values are derived through the assessment factors (to consider differences and uncertainties) and toxicokinetic extrapolation of the critical effects observed in animal studies in substances that TDI is missing. To derive HBM values through this concept NOAEL, LOAEL of BMDL are selected. It is also important to take into account the availability of the data like the dose-effect curve, the toxicological mechanisms and the severity and the type of the effect.

6. Diet studies

Diet is the major route of exposure for most of the chemical substances that we are exposed to. Consequently, information about human exposure through dietary intake is necessary to perform human health risk assessments. Based on the Food and Agriculture Organization/World Health Organization (FAO/WHO) recommendations, two main approaches may be used to assess the levels and the intake of contaminants in the food supply (WHO, 1985).

6.1. Total diet studies

Total Diet Studies (TDS) select, collect and analyse the most commonly consumed food. Food is marketed in retail stores, supermarkets or grocery stores to represent the typical diet. Food is processed as for consumption, and then is pooled into representative food groups. Finally, analysis to determine harmful or beneficial chemical substances are carried out (EFSA, 2011). TDS are widely used to assess contamination that may be distributed across the entire food supply and that may occur at low levels, such as heavy metals or dioxins.

A TDS is suitable to (EFSA, 2011): i) identify food groups needing detailed monitoring or surveillance; ii) develop priorities for possible public intervention and provide advice; iii) identify possible trends in dietary exposure to chemical substance in the general population and in specific population groups; iv) serve as a cost-effective complement to other food safety activities; v) communicate the evaluation of the chemical safety of the food supply chain as part of the risk assessment process.

TDS are very popular among developed countries. In 2000, a surveillance program to determine the levels of different chemical substances, such as toxic metals, dioxins, PCBs, etc was launched in Catalonia (Spain). To date, 5 more TDS have been conducted in Catalonia (Llobet et al., 2003; Martí-Cid et al., 2008; Martorell et al., 2011; Perelló et al., 2014; González et al., 2019).

6.2. Duplicate diet studies

Duplicate diet studies (DDS) require to purchase and prepare twice the usual portions of food that would be consumed by an individual. Individual food of interest would have to be provided as a separate duplicate sample and stored as such. The collection of food can be done at any period of time, but the dietary pattern should be reflected (WHO, 1985).

This approach is useful for institutional groups and small surveys, rather than large-scale food consumption studies due to the cost and the time involved. Additionally, food is often collected and stored all together in food containers, being difficult the individual analysis. Finally, a food diary is needed to record the food item and quantity that was eaten or purchased (WHO, 1985).

Among all the chemical substances, the most popular determined through DDS are: toxic metals (Hayashi et al., 2019; Tinggi & Schoendorfer, 2018), minerals (Muros et al., 2019), persistent organic pollutants (Bramwell et al., 2017), perfluorinated substances (Papadopoulou et al., 2017; Fromme et al., 2007) and polycyclic aromatic hydrocarbons (Nie et al., 2014; Martorell et al., 2012).

7. Analytical techniques

7.1. QuEChERS

QuEChERS (quick, easy, cheap, effective rugged and safe) were developed by Anastassiades et al., (2003) as an alternative to determine the occurrence of pesticides in fruits and vegetables. However, due to its great versatility, this procedure has been applied for the extraction of different analytes in different matrices, obtaining excellent results (Santana-mayor et al., 2019). The original procedure involves a solid-liquid extraction following a liquid-liquid partitioning with a salting out process, using anhydrous $MgSO_4$ and NaCl. Afterwards, a clean-up is performed by a dispersive solid-phase extraction, adding anhydrous $MgSO_4$ and a primary secondary amine sorbent (Anastassiades et al., 2003). Recently, new sorbents have been developed to improve the clean-up. One example is the zirconium oxide-based (Z-sep) sorbent, enhancing the remove of fat (López-Blanco et al., 2016; Lozano et al., 2014)

QuEChERS provide satisfactory results in a cost-effective way and within a short time (less than 30 minutes) (Santana-Mayor et al., 2019; Anastassiades et al., 2003). This technique can be used in combination with other extraction methods, such as dispersive liquid-liquid microextraction (DLLME).

7.2. Dispersive liquid-liquid microextraction

DLLME method was first developed by Rezaee et al., (2006). It is a simple and high performance method with high enrichment, while providing high recovery results. Regarding the procedure, a cloudy state is formed due to the solvent droplets after the injection of the binary solvent mixture (extraction and disperser solvents) into an aqueous sample. The large surface area between the fine droplets and the aqueous phase facilitates the quick transfer of analytes from the sample solution into the extraction phase. Subsequent centrifugation of the mixture causes sedimentation of the fine droplets at the bottom of the conical tube. Finally, the sediment phase can be collected and analysed with the appropriate analytical equipment (Hashemi et al., 2017).

7.3. Gas Cromatography – Mass Spectometry

Gas chromatography (GC) is an analytical technique used to determine the concentration of an analyte in gas mixtures. The chromatographic equipment is constituted by a thermostable oven with the column inside. The column is a thin glass capillary tube. Inside the tube, a thin layer of the stationary phase is fixed in the internal walls. This substance should be affine with the gas mixture in order to separate the analytes before arriving to the sensor. There is also a mobile carrier – usually an inert gas like helium, hydrogen or nitrogen – that transports the mixture into the column (De Blasio, 2019).

The components in the gas mixture show certain affinity for the stationary phase of the column. Therefore, some analytes will travel faster than other inside the column and will be separated, reaching the sensor at different times. Subsequently, the detector can convert the difference in absorbance in diverse current signals and plot them against time which will generate a chromatogram (De Blasio, 2019).

Besides the GC equipment, there are different technologies that provide an improvement of the analytical information with highest level of accuracy. The mass spectrometer (MS) receives the stream of separated compound from the GC into the ion source, which is a metallic filament where voltage is applied. Analytes are ionized by this voltage and can be

further fragmented to predictable pattern. Finally, fragments enter to the detector and they are analysed (Sparkman et al., 2011).

HYPOTHESIS AND OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
EXPOSURE ASSESMENT TO BISPHENOLS: COMBINING BIOMONITORING AND DUPLICATE DIET STUDIES
Nieves González Paradell

EDCs have become a topic of concern due to their adverse health effects in humans. Among EDCs, BPA is considered of great interest because of its widespread use by the food industry, especially to provide resistance to polycarbonate plastic, or as can lining.

Dietary intake has been extensively identified as the most relevant pathway of exposure to environmental and processing pollutants, such as BPA. However, the dietary co-exposure to other bisphenol analogues, like BPS, BPF, BPB, BPAF, BPZ, BPE and BPAP, is still unknown. Data on the occurrence of BPA substitutes in food are scarce in the scientific literature.

It is here hypothesized that the dietary exposure to bisphenols – BPA and analogues – might be higher in a diet formed by canned foodstuffs and packed in polycarbonate plastics, than in a diet consisting of fresh and glass-packed food.

General objective

The present doctoral thesis was aimed at assessing the dietary co-exposure to bisphenol analogues in a general population cohort by means of a duplicate diet study.

Specific objectives

1) To optimize and validate an analytical method to identify and quantify bisphenol analogues (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE and BPAP) by means of GC-MS in samples of blood.
2) To analyse the concentrations of the eight bisphenol analogues in food samples marketed in Spain. Food samples were packed in cans and polycarbonate plastics, glass or other BPA-free materials.
3) To estimate the dietary co-exposure to BPA and some BPs analogues for an adult cohort following a canned food-based diet.
4) To analyse the concentrations of selected BP analogues in urine and blood samples obtained from a general population cohort.

5) To assess the real co-exposure to BPA and BPs analogues and to further compare such exposure to estimate values obtained from the dietary intake.
6) To model the BPA excretion through urine and to determine optimal time intervals of urine sampling for a more sensitive assessment of BPA exposure.
7) To assess whether the consumption of canned foodstuffs might pose a risk for human health, in terms of BPA exposure.

RESULTS

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EXPOSURE ASSESMENT TO BISPHENOLS: COMBINING BIOMONITORING AND DUPLICATE DIET STUDIES
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CHAPTER 1

Quantification of eight bisphenol analogues in blood and urine samples of workers in a hazardous waste incinerator

Neus González[a], Sara C. Cunha[b], Carolina Monteiro[b], José O. Fernandes[b], Montse Marquès[a], José L. Domingo[a], Martí Nadal [a]

Environmental research 176 (2019) 108576

[a] *Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain*

[b] *LAQV-REQUIMTE, Department of Bromatology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*

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ABSTRACT

Bisphenol A (BPA) has been widely used in the manufacture of polycarbonate plastic and epoxy resins. In recent years, producers have started replacing BPA by other chemical analogues, such as bisphenol -S (BPS) and -F (BPF), all of them under the label "BPA-free". However, despite bisphenol (BP) analogues have a very similar structure, their endocrine-disrupting properties could differ from those of BPA. Unfortunately, information regarding human exposure to BP analogues is very limited, not only as single substances, but also as chemical mixtures. The aim of this study was to determine the levels of 8 BP analogues (A, S, F, B, AF, Z, E, and AP) in biological samples from a controlled cohort of workers in a hazardous waste incinerator (HWI) located in Constantí (Catalonia, Spain). Firstly, a chemical method to analyze a mixture of those 8 analogues in total blood and urine was optimized, being samples quantified by means of gas chromatography coupled to mass spectrometry (GC-MS). Furthermore, a biomonitoring study was performed by collecting samples of total blood and urine of 29 people working in the HWI. Among the 8 BP analogues assessed, BPA presented the highest levels in both biological samples, with mean total (free + conjugated) BPA concentrations of 0.58 and 0.86 μg/L in blood and urine, respectively. Free vs. total BPA levels presented a mean percentage of 79% in blood and 19% in urine. Beyond BPA, traces of BPB were also found in a single sample of blood. Furthermore, none of the remaining BP analogues was detected in blood or urine. Despite BPA has been regulated, it is still very present in the environment, being human exposure to this chemical still an issue of concern for the public health.

Keywords: bisphenol A (BPA), analogues, chemical mixture, blood, urine, biomonitoring

INTRODUCTION

Bisphenol A (BPA) was firstly synthesized in 1891 (Tyl, 2014), but only became relevant in the plastic industry in the 1950s (Corrales et al., 2015; Eladak et al., 2015; Gao et al., 2015). Due to its physical properties (namely, heat and chemical resistance, transparency, and ductility), BPA is one of the preferred monomers for the manufacture of polycarbonate plastics and epoxy resins (Mikołajewska et al., 2015). Nowadays, a wide range of products such as plastic bottles, cans, dental fillings, medical devices, toys and also thermal paper, incorporate traces of BPA (EFSA, 2015; Liao and Kannan, 2014). Even before its first industrial uses, the estrogenic activity associated to BPA was already reported (Dodds and Lawson, 1936).

Due to the similar structure to 17β-estradiol, BPA can interact with the endocrine receptors and act as an agonist of estradiol receptors, or as an antagonist of androgenic receptors, causing a dysfunctionality of the endocrine system (Björnsdotter et al., 2017; Rochester, 2013; Usman and Ahmad, 2016). Exposure to BPA may lead to diverse health outcomes, such as obesity and diabetes , as well as infertility, precocious puberty, reduced sperm quality, polycystic ovary syndrome, and breast and prostate cancer, among others (Bertoli et al., 2015; Heindel et al., 2017; Diamanti-Kandarakis et al., 2009; Gao et al., 2015; Konieczna et al., 2015; Rochester, 2013).

In the early 1990s, it was found that BPA could leach from polycarbonate plastics into water content (Krishnan et al., 1993). Furthermore, the potential leaching of BPA from other products, like food cans, dental sealants and baby bottles, has been also reported (Biles et al., 1997; Brotons et al., 1995; Olea et al., 1996).

The main pathway of human exposure to BPA is the diet, representing more than the 99% of total exposure (LaKind et al., 2019; Martínez et al., 2018; Sarigiannis et al., 2019). Canned fruits, vegetables, and canned meat and meat products have been identified as the most important contributors to the dietary intake of BPA (Martínez et al., 2017).

Anyhow, other routes like dermal absorption and inhalation should not be ignored (Geens et al., 2012; Lu et al., 2018).

As a result of the increased social concern, the European Union banned the use of BPA in baby bottles (European Commission, 2011), setting specific migration limits for food contact materials (EFSA, 2015, 2006). Because of the strict laws, producers started to replace BPA with other analogues. Among the 24 bisphenol (BP) analogues described in the literature, bisphenol S (BPS) and bisphenol F (BPF) are the most frequently detected (Pelch et al., 2017). BPS is mostly used as thermal paper, but it has also applications as can coating, epoxy glue, tanning agent and as an additive in dyes. On the other hand, BPF is mostly used in painting applications, like liners, lacquers and varnishes, as well as in dental sealants and coatings for food packages (Chen et al., 2016). Since these analogues have a very similar structure to BPA, they could have similar or greater endocrine-disrupting properties than those of BPA (Eladak et al., 2015; Rochester and Bolden, 2015; Žalmanová et al., 2016).

The occurrence of bisphenol analogues in a number of environmental compartments, such as wastewater, sediments or air, has been largely described (Česen et al., 2018; Jin and Zhu, 2016; Liao et al., 2012; Liu et al., 2017; Xue et al., 2016; Yu et al., 2015). Moreover, traces in some consumer products, namely canned foods, have been also reported (Cunha et al., 2012; Geens et al., 2010; Lim et al., 2009; Mariscal-Arcas et al., 2009; Thomson and Grounds, 2005). Unfortunately, a comprehensive assessment of human exposure to BP analogues, other than BPA, from dietary and non-dietary sources is still lacking. Data on human biomonitoring of BP analogues are still very limited, being most studies focused only on 2 or 3 analogues (mainly BPA, BPB and BPS) (Cunha and Fernandes, 2010; Genuis et al., 2012; Jin et al., 2018; Martínez et al., 2019; Morgan et al., 2018; Owczarek et al., 2018; Philips et al., 2018; Porucznik et al., 2015; Yang et al., 2014; Ye et al., 2015; Žalmanová et al., 2016; Lehmler et al., 2018). Therefore, the development of reliable analytical methods for the simultaneous determination of BP analogues is essential in order to elucidate the joint exposure to the mixture of BPs.

This study was aimed at assessing the concentrations of 8 BP analogues (A, S, F, B, AF, Z, E, and AP) in samples of total blood and urine from workers in a hazardous waste incinerator located in Tarragona (Catalonia, Spain). The determination of BPs was based in a dispersive liquid-liquid microextraction (DLLME) with *in situ* derivatization with anhydride acetic followed by GC-MS (Cunha and Fernandes , 2010).

MATERIALS AND METHODS

Chemicals

BPA (99% purity), BPB (98% purity), BPF (98% purity), BPE (98% purity), BPAF (98% purity), BPZ (99% purity) and BPAP (99% purity) were purchased from Sigma-Aldrich (West Chester, PA, USA). $_{d16}$-bisphenol A (BPA_{d16}; 98 atom % D), used as internal standard (I.S.), was purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Individual solutions of the standards and internal standards were prepared in methanol (HPLC grade from Sigma-Aldrich) at a concentration of 2000 µg/L. Acetonitrile (MeCN, gradient grade for HPLC), acetic anhydride (AA, >99% purity), tetrachloroethylene (T4CE, >99% purity) N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), BSTFA with trimethylchlorosilane (BSTFA-TMC), trifluoroacetamide (TFA) and pyridine were acquired from Sigma-Aldrich. β-Glucuronidase (Type 1 from Helix pomatia, ≥3000,000 U/g solid glucuronidase and ≥10,000U/g solid sulfatase) was purchased from Sigma-Aldrich.

Equipment

A gas chromatograph 6890 (Agilent, Little Falls, DE, USA) equipped with a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) and an electronically controlled split/splitless injection port, was interfaced to a single quadrupole inert mass selective detector (5975B, Agilent) with electron ionization (EI) chamber. GC separation was performed on a DB-5MS column (30 m × 0.25 mm I.D. × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Helium was the carrier gas, with a constant flow of 1 mL/min. The injection was made in splitless mode (purge-off time, 60 s) at 280 °C. The oven

temperature program was as follows: 100 °C held for 1 min, ramped to 280 °C at 30 °C/min held for 8.0 min. Total run time was 15 min. The MS transfer line was held at 280 °C. Mass spectrometric parameters were the following: electron ionization with 70 eV energy; ion source temperature, 230 °C and MS quadrupole temperature, 150 °C. Agilent ChemStation (version D.0200SP1) was used for data collection/processing and GC–MS control. The MS was operated in selected ion monitoring (SIM) detecting at least three ions per analyte (Table 1).

Table 1. GC-MS acquisition parameters.

Analyte	tR (min)	Ions (*m/z*) Quantification	Qualification			
BPAF	7.38	267	420	336	359	197
BPF	8.12	200	284	242	152	107
BPE	8.31	199	256	298	214	241
$BPAd_{16}$ (IS)	8.46	224	242	284	266	326
BPA	8.52	213	228	255	270	312
BPB	8.98	213	255	297	281	326
BPS	10.32	250	334	292	141	186
BPZ	11.56	268	352	310	225	251
BPAP	12.69	275	317	312	359	374

Quality Control/Quality Assurance

Plastic material was avoided, while glassware was previously baked for 1 h at 300 ºC and then washed with acetone to avoid background contamination. Before the entire analytical procedure, samples were spiked with both standard and IS (BPA_{d16}) to evaluate analytical performances such as linearity, sensitivity, precision and accuracy (European Commission, 2017). Linearity was assessed through multilevel matrix-matched calibration with nine calibration levels. Calibration curves were built by the least squares linear regression model, plotting the peak area ratios of target compound and IS versus the concentration of each target substance. In each sample batch a blank extract (sample free of bisphenol) was injected and between samples solvent injections were performed in order guarantee no contamination.

Sampling

Samples of total blood and urine corresponding to 29 adults (11 women and 18 men), who work in a hazardous waste incinerator (HWI; Constantí, Tarragona, Catalonia, Spain) were collected. The procedure was executed in accordance with the guidelines of the IISPV Ethical Committee on Clinical Investigation (Tarragona, Catalonia, Spain). Samples were collected as part of the health surveillance plan that is periodically conducted in order to determine the occupational exposure to organic substances and heavy metals (Mari et al., 2007, 2009, 2013). Subjects were classified according to the specific workplace. Out of 29 participants, 7 were administrative assistants, 6 were laboratory workers, and the remaining 16 were plant operators. All samples were stored at -20ºC until analysis.

Analysis of creatinine in urine

Creatinine (QCA kits, Tarragona, Spain) was analyzed in urine samples. A Cobas Mira automatic analyzer (Roche Pharmaceuticals, Basel, Switzerland) was used according to the instructions provided by the manufacturer.

Total blood samples preparation

Free BP levels

Briefly, total blood samples were thawed at room temperature and homogenized by vortex. 1.5 mL of MeCN was then added to 500 µL of total blood sample. Each sample was firstly spiked with 20 µL of BPA_{d16}, kept at room temperature for 10 min, and afterwards centrifuged at 3500 rpm for 4 min. Subsequently, 1 mL of sample was transferred to a clean vial, adding 85 µL of T4CE and 100 µL of AA to the sample. In a glass tube with conical bottom, 3 mL of deionized water and 300 µL of K_2CO_3 (to achieve pH ≥ 10) were added. Rapidly, the sample was transferred to the glass tube and vortexed. Samples were then centrifuged at 2100 rpm for 4 min. Finally, 70 µL of the lower phase were transferred to a 100 µL insert, being 1 µL injected into the GC-MS system.

Total (conjugated + free) BP levels

Total blood samples were thawed at room temperature and mixed by vortex. Subsequently, 1.5 mL of sample was transferred to a clean vial and 12 µL of β-glucoronidase solution (20,000 U/mL in 1M ammonium acetate buffer pH 5.0) were added. Samples were incubated overnight at 37ºC to grant the hydrolysis. After cooling at room temperature, samples were extracted as previously described in section 2.6.1.

Urine samples preparation

Free BP levels

The method for BP determination in urine samples was previously optimized by Cunha and Fernandes (2010). Briefly, urine samples were thawed at room temperature and homogenized by vortex. Then, 5 mL of sample were transferred to a glass tube with conical bottom and spiked with 200 µL of $BPA_{d16.}$ Rapidly, 1325 µL of MeCN, 85 µL of T4CE and 125 µL of AA were added to the sample. Tubes were shaken and centrifuged for 20 minutes at 2500 rpm and 4ºC. Finally, 70 µL of the lower phase were transferred to a 100 µL insert, being 1 µL injected in GC-MS.

Total (conjugated plus free) BP levels

Urine samples were thawed at room temperature and homogenized by vortex. Afterwards, 5 mL of sample were transferred to a glass tube with conical bottom and spiked with 200 µL of BPA_{d16}. Then, 40 µL of β-glucoronidase solution (20,000 U/mL in 1M ammonium acetate buffer pH 5.0) were added. The mixture was incubated overnight at 37ºC in order to grant the hydrolysis. After cooling at room temperature, samples were extracted as above stated (section 2.7.1).

Statistics

Data treatment was performed by means of the statistical package SPSS 20.0. Statistically significant differences between groups, according to workplace and gender, were set at a level of significance of 0.05 ($p < 0.05$). The Levene test was used to compare the

homogeneity of the variances. Subsequently, the significance of the data was computed by an ANOVA or the Mann-Whitney U-test. For calculations, non-detected values were excluded from data treatment, while non-quantified samples were assumed to have a concentration equal to one-half of the limit of quantification (NQ = 1/2 LOQ).

RESULTS AND DISCUSSION

Method optimization

In order to improve the quantification method of BPs in total blood, extraction and derivatization of samples were optimized. Firstly, two different sample volumes (500 and 1000 µL) were tested. Results showed that 1000 µL did not provide a proportional increase of the peaks. Therefore, a volume of 500 µL was chosen (data not shown). MeCN was selected as a dispersive agent in the DLLME procedure, according to the results of Cunha and Fernandes (2010). The use of ultrasonic bath was tested in the dissolution step of the sample. The results showed that ultrasounds break down the molecules of BPs, and consequently, their determination could not be performed. Hence, ultrasonic bath was not used (data not shown). The addition of salts was tested in order to assess the improvement of the extraction process. Up to 400 mg of $MgSO_4$ and 100 mg of $C_2H_3NaO_2$ were added into the sample after MeCN addition, with no enhancement of the quantification peaks. Thus, the use of salts was discarded (data not shown). Finally, AA, BSTFA, TFA and BSTFA:TMCS-pyridine were tested as derivatizing agents for a mixture of standards at 1 mg/L. AA showed the best performance for all the compounds (Figure 1).

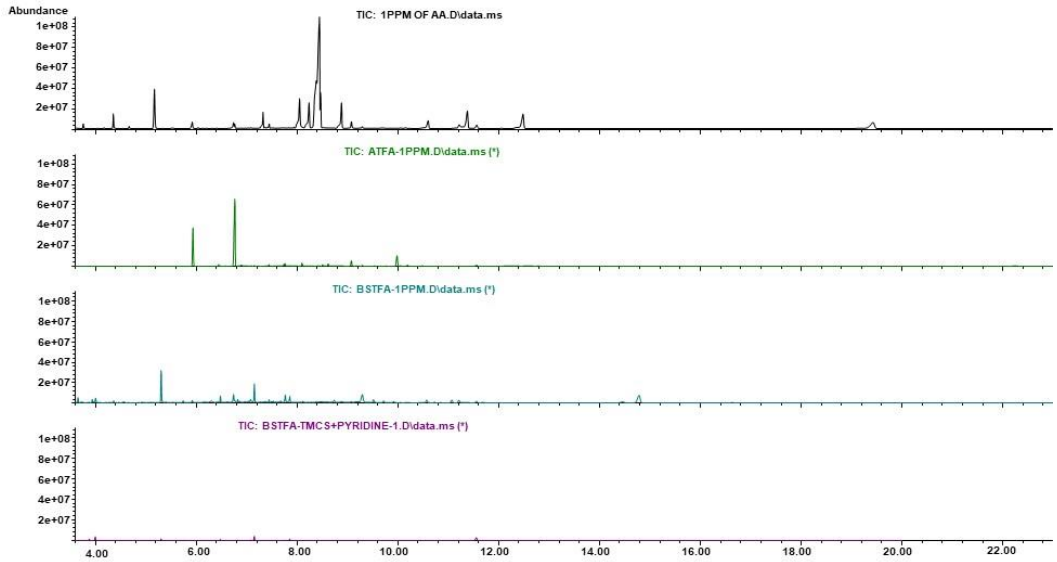

Figure 1. Total ion chromatogram (TIC) of 8 bisphenols at 1 mg/L using different derivitizing agents tested: a) AA; b) TFA; c) BSTFA; d) BSTFA-TMCS- pyridine. All in the same scale

Blank samples (bold and urine) were spiked with both analytes and internal standard to evaluate linearity, linear range, sensitivity, precision and accuracy, according to European Commission, 2017. The linearity of the method was evaluated by analyzing matrix-matched standards prepared at nine concentration levels, ranging from the limit of quantification (LOQ) to about 50 to 150 µg/L. Matrix-matched calibration standards were used to overcome the enhancement of analytical response verified when used solvent standards. Linearity with correlation coefficients equal or greater than 0.99 for all the studied analytes was obtained. Method detection and quantification limits (LOD and LOQ, respectively) were calculated using low level points for blood in order to achieve signal-to-noise ratios (S/N) of 3 and 10, respectively. The LOD and LOQ of urine were considered those reported by Cunha and Fernandes (2010). The recovery was determined by comparing the analytical response of the analytes in spiked samples, before and after the extraction step, for three concentration levels, being each level performed five times (in the same day). Precision (repeatability and intermediate

precision) was assessed at three different levels (five replicates per level per day), in two non-consecutive days (Table 2).

Table 2. Linearity range, average of repeatability (%RSD, n=5), extraction efficiency (%, n=5), LOD (µg/L) s and standard combined uncertainty (u´c tg)

Analytes	Total blood						Urine			
	Linearity range	5 µg/L % extraction efficiency (% RSD)	10 µg/L % extraction efficiency (% RSD)	20 µg/L % extraction efficiency (% RSD)	$U'_c{}^{tg}$	LOD	Linearity range	2.5 µg/L % extraction efficiency (% RSD)	10 µg/L % extraction efficiency (% RSD)	$U'_c{}^{tg}$
BPAF	0.1-50	96 (13)	75 (5)	74 (6)	13	0.03	0.2-50	70 (18)	101 (16)	16
BPF	0.1-50	97 (5)	95 (2)	97 (3)	11	0.03	0.5-50	66 (9)	74 (19)	22
BPE	0.2-50	102 (5)	92 (7)	92 (4)	11	0.06	0.2-50	75 (14)	87 (15)	24
BPA	0.1-50	116 (9)	88 (5)	92 (4)	12	0.03	0.1-50	73 (13)	101 (20)	23
BPB	2.5-50	95 (19)	88 (7)	85 (5)	15	0.76	0.2-50	77 (6)	96 (16)	21
BPS	15-50	103 (17)	116 (8)	83 (9)	15	4.55	2.5-50	62 (9)	84 (1)	11
BPZ	7.5-50	88 (19)	103 (2)	97 (5)	15	2.27	0.5-50	99 (5)	103 (2)	21
BPAP	15-50	95 (16)	102 (13)	101 (9)	16	4.55	0.5-50	93 (17)	89 (10)	24

Calculation of a reasonable estimate of the total uncertainty for a measurement result obtained with our method was accomplished considering the data from measurement performance characteristics. The target standard combined uncertainty ($u'_c{}^{tg}$), reflecting the combination of precision ($u'_{ra}{}^{tg}$) and uncertainty on bias ($u'_{sy}{}^{tg}$) was calculated with the showed formula (1), where $u_{ra}{}^{tg}$ was estimated from the defined target value for the coefficient of variation (CV), and $u_{sy}{}^{tg}$ was estimated from the maximum/minimum permissible mean error (of R max = 120% and R min = 70%) (Bettencourt da Silva et al., 2015; Solaesa et al., 2019).

(1) $$u'_c = \sqrt{(u'^{tg}_{ra})^2 + (u'_{sy}{}^{tg})^2}$$

$u'_{sy}{}^{tg} = (Rmax - Rmin)/2*6^{1/2}$

As can be observed in Table 2, measurement uncertainty value was below 24% which is lower than the target value of 25% setting from the evaluation criteria European Union proficiency tests.

Levels of BP analogues in total blood

The concentrations of the detected BP analogues in the 29 samples of total blood are summarized in Table 3. BPA and BPB were the only two BPs detected in at least one sample of blood. Moreover, all the samples presented concentrations of the remaining BP analogues (S, F, Z, AP, AF and E) under the LOD. Free BPA was detected in 65% of the samples, with levels ranging from undetected values to 1.65 µg/L and a mean concentration of 0.46 µg/L. As example, the chromatogram corresponding to the sample of total blood with the highest concentration is depicted in Figure 2. On the other hand, total (free + conjugated) BPA was detected in 23 out of the 29 samples (79%), with a mean concentration of 0.58 µg/L and levels ranging from undetected values to 1.60 µg/L. In contrast to BPA, only one sample had detectable levels of BPB, being the concentrations of free and total BPB 2.53 and 2.69 µg/L, respectively.

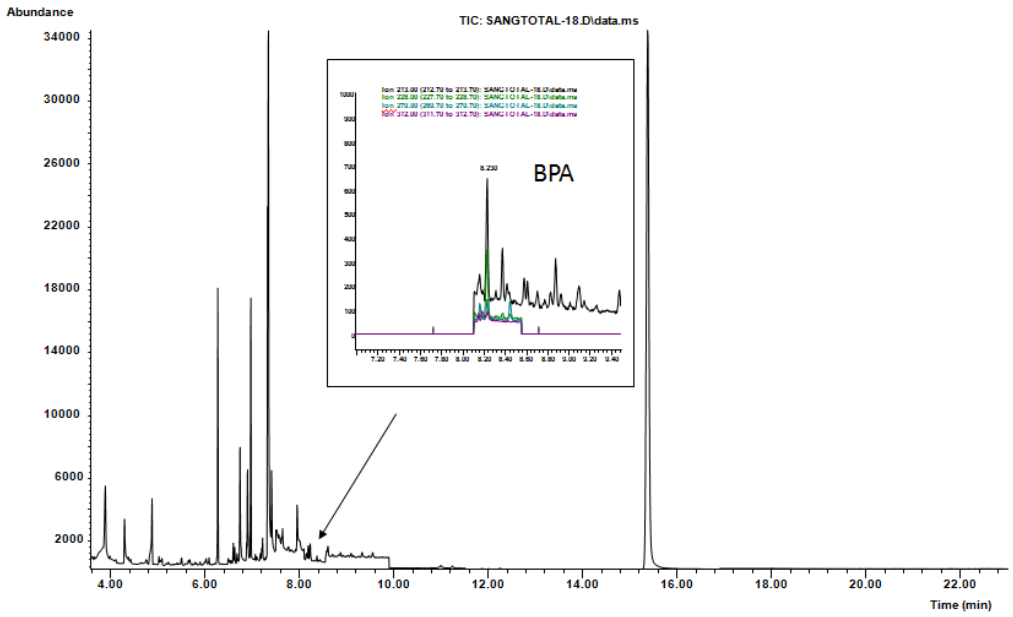

Figure 2. Total ion chromatogram (TIC) of a total blood sample (1.65 µg/L of free BPA) obtained by the DLLME—heart-cutting GC–MS method, together with the individual chromatograms in SIM mode.

A specific analysis of BPA levels in total blood, according to the gender and workplace, was conducted. Mean free BPA was higher in men than women (0.76 vs. 0.45 µg/L, respectively), but the difference was not statistically significant ($p>0.05$). In contrast, mean levels of total (free + conjugated) BPA was significantly higher in men (0.93 µg/L) than in women (0.37 µg/L). On the other hand, no significant differences were observed according to the workplace for both free and total BPA. The mean concentrations of free BPA in people working in the administration building, the laboratory and the plant were 0.50, 0.66 and 0.18 µg/L ($p>0.05$), respectively, while those of total BPA were 0.74, 0.62 and 0.25 µg/L ($p>0.05$), respectively.

Table 3. Free and total (free + conjugated) BPA and BPB concentrations in total blood samples. Data given as μg/L.

Sample	Gender	Workplace	Free		Total	
			BPA	BPB	BPA	BPB
1	M	Plant	<0.03	<0.76	<0.03	<0.76
2	F	Administration	<0.03	<0.76	<0.03	<0.76
3	M	Plant	<0.03	<0.76	<0.03	<0.76
4	M	Plant	<0.03	<0.76	<0.03	<0.76
5	M	Plant	<0.1	<0.76	1.40	<0.76
6	F	Administration	<0.03	<0.76	<0.03	<0.76
7	F	Administration	<0.03	<0.76	<0.1	<0.76
8	F	Laboratory	<0.03	<0.76	<0.1	<0.76
9	M	Plant	0.95	<0.76	1.10	<0.76
10	M	Plant	0.27	<0.76	0.62	<0.76
11	M	Plant	<0.1	<0.76	<0.1	<0.76
12	M	Plant	<0.1	<0.76	<0.1	<0.76
13	M	Laboratory	<0.1	<0.76	<0.1	<0.76
14	M	Plant	<0.03	<0.76	<0.03	<0.76
15	F	Laboratory	<0.1	<0.76	<0.1	<0.76
16	F	Administration	1.60	<0.76	1.60	<0.76
17	M	Plant	0.40	<0.76	0.47	<0.76
18	M	Plant	1.65	<0.76	1.65	<0.76
19	M	Plant	0.53	<0.76	1.01	<0.76
20	M	Administration	0.33	<0.76	1.19	<0.76
21	M	Plant	1.25	<0.76	1.52	<0.76
22	F	Laboratory	<0.03	<0.76	0.58	<0.76
23	F	Laboratory	0.52	<0.76	0.70	<0.76
24	M	Plant	<0.1	<0.76	<0.1	<0.76
25	F	Administration	<0.03	<0.76	0.20	<0.76
26	M	Plant	<0.1	<0.76	<0.1	<0.76
27	F	Administration	<0.1	2.53	<0.1	2.69
28	M	Plant	0.76	<0.76	0.93	<0.76
29	F	Laboratory	<0.1	<0.76	0.04	<0.76

F, female; M, male.
LOQ of BPA: 0.1 μg/L; LOQ of BPB: 2.5 μg/L.
LOD of BPA: 0.03 μg/L; LOD of BPB: 0.76 μg/L.

Information regarding the levels of BPs in total blood is still very limited. Zhang et al. (2013) analyzed the content of BPA in total blood samples from 50 adults in China, reporting a mean level of 0.20 μg/L, a value lower than that found in the current study. Most investigations reporting biomonitoring levels of BPA and BP analogues use plasma as matrix, instead of total blood. However, our results highlight the importance of considering all the

blood fractions in order to avoid underestimating the total BP exposure and to determine the real partitioning potential of each BP analogue.

Unlike total blood, the levels of BPA in plasma samples have been largely reported in the scientific literature. However, data differ substantially, as they depend on a number of social, dietary and lifestyle factors. In China, BPA mean concentrations of 0.40 µg/L (Jin et al., 2018) and 0.95 µg/L (Wan et al., 2013) were found, being similar to those here observed. Other authors reported notable higher values in India (7.43 µg/L) and the Czech Republic (range: <LOQ to 8.17 µg/L) (Kolatorova Sosvorova et al., 2017 Shekhar et al., 2017). Recently, Azzouz et al. (2016) analyzed the levels of BPA in samples of healthy volunteers in different hospitals in Jaén (Spain) and reported concentrations ranging from <LOD to 7.1 µg/L. It should be noted that in all these aforementioned studies only the concentration of free BPA was determined.

Levels of BP analogues in urine

The urinary concentrations of BPA, the only analogue detected, are shown in Table 4. Because of methodological limitations, four of the samples were missed and consequently were excluded from the subsequent analysis. Free BPA was detected in 52% of the samples, with levels ranging from undetected values to 0.59 µg/L, and a mean concentration of 0.16 µg/L. The total (free + conjugated) BPA mean concentration was 0.86 µg/L, being detected in 60% of the samples, with levels ranging from undetected values to 2.82 µg/L.

Table 4. Free and total (free + conjugated) BPA concentrations in urine samples. Data given as µg/L (µg/g creatinine).

Sample	Gender	Workplace	Free BPA	Total BPA
1	M	Plant	<0.1	0.40 (0.03)
2	F	Administration	<0.03	<0.03
3	M	Plant	<0.03	<0.03
4	M	Plant	<0.03	<0.03
5	M	Plant	<0.03	<0.03
7	F	Administration	<0.03	<0.03
8	F	Laboratory	<0.03	<0.03
9	M	Plant	<0.03	<0.03
10	M	Plant	<0.03	<0.03
11	M	Plant	0.14 (0.017)	0.09 (0.011)
13	M	Laboratory	0.26 (0.013)	0.39 (0.020)
14	M	Plant	<0.1	<0.1
15	F	Laboratory	<0.1	0.39 (0.032)
16	F	Administration	<0.03	<0.03
17	M	Plant	<0.03	0.81 (0.034)
18	M	Plant	0.59 (0.027)	1.18 (0.054)
19	M	Plant	<0.1	0.21 (0.015)
20	M	Administration	<0.1	1.38 (0.11)
22	F	Laboratory	0.41 (0.029)	2.82 (0.20)
23	F	Laboratory	<0.1	2.43 (0.29)
25	F	Administration	<0.03	0.33 (0.024)
26	M	Plant	0.22 (0.010)	0.28 (0.013)
27	F	Administration	<0.03	<0.03
28	M	Plant	<0.1	2.06 (0.13)
29	F	Laboratory	<0.1	<0.1

F, female; M, male.
LOQ: 0.1 µg/L; LOD: 0.03 µg/L.

Free BPA concentrations were very similar in men and women (0.16 vs. 0.14 µg/L). In contrast, the levels of total (free + conjugated) BPA were 2-fold higher in female workers (0.68 and 1.20 µg/L in men and women, respectively), but this difference did not reach the level of statistical significance ($p<0.05$). According to the workplace, individuals working in the plant and the laboratory building showed very similar concentrations of free BPA (0.16 µg/L in both cases), while most of the administration workers presented BPA levels under the limit of detection. With respect to total (free + conjugated) BPA, notable -but not significan- differences were found according to the workplace, which followed this pattern: laboratory > administration > plant (1.22, 0.85 and 0.63 µg/L, respectively). Anyhow, the

differences according to gender and workplace may be confusing, as most workers in the plant are men, while most of the laboratory workers are women.

The detection rate of BPA is in accordance to that found in Portugal (Cunha and Fernandes, 2010), where 45% of the samples were positive for BPA. However, the detection percentages here observed are lower than those frequently found in countries such as China (84%) (Zhang et al., 2013) or the United States (74-99%) (Ye et al., 2015). BPs are rapidly excreted after ingestion, being their half-life of 6 h approximately (Thayer et al., 2015). Hence, urine is considered as the preferred matrix to perform biomonitoring studies of BPs.

Table 5 summarizes the levels of free and total (free + conjugated) BPA found in a number of countries. The levels of free BPA observed in the present study would be in the lowest part of the range, while those of total (free + conjugated) BPA would be similar to those previously reported. Anyway, the current concentrations in workers of the HWI are far below the threshold value established by the German Human Biomonitoring Commission, which is 200 µg/L for adults (UBA, 2017).

Table 5. Concentrations of BPA in total blood and urine: a summary of recent data from various countries.

Country	Matrix	Free BPA (µg/L)	Total BPA (µg/L)	Reference
China	Total blood	0.20	-	Zhang et al., 2013
Spain	Total blood	0.43	0.56	This study
United States	Urine	0.36	-	Ye et al., 2015
China	Urine	0.39	0.89	Zhang et al., 2013
Spain	Urine	LOD – 8.9	-	Azzouz et al., 2016
Portugal	Urine	0.41 – 1.64	0.39 – 4.99	Cunha and Fernandes, 2010
Poland	Urine	1.84	-	Radwan et al., 2018
Saudi Arabia	Urine	1.3	-	Li et al., 2018
Norway	Urine	3.82	-	Sakhi et al., 2018
Slovenia	Urine	-	1.02 (mothers) 0.32 (fathers)	Snoj Tratnik et al., 2019
Spain	Urine	0.16	0.86	This study

CONCLUSIONS

To the best of our knowledge, this is the first study considering the simultaneous analysis of 8 different BP analogues in 2 biological matrices. BPA was the most detectable analogue

regardless the biological matrix (total blood or urine), while BPB was also identified, but in a much lower rate. Interestingly, no traces of other BP analogues were detected in any sample. Nonetheless, further efforts should be done to evaluate the human exposure to these endocrine disruptors, taking into account they are present as a chemical mixture and not as single substances. To date, data on the body burdens of BP analogues are really limited, enhancing the need to investigate and to pressure to obtain "safe" alternatives to BPA. In some recent studies, it has been confirmed that some BP analogues may affect in a similar way to that of BPA. They can play a role in some diseases like diabetes (Liu et al., 2017) and can also cause adverse effects on reproduction, affecting the following generations (Horan et al., 2018). Essentially, most studies have been only focused on BPS and BPF analogues, which neglects the potential harm of other analogues, as well as the effects of the BP mixtures.

The unexpected potential role of the workplace on the exposure to BPs was also highlighted. Despite dietary intake seems to be the predominant route of exposure to BPA and other BP analogues, occupational exposure to these chemicals should not be disregarded. Among the 16 plant workers, 12 had detectable levels of BPA in total blood samples (75%), compared to percentages of 43% and 67% of administration and laboratory workers, respectively. Regarding urine samples, 5 of the 6 laboratory workers showed detectable levels of BPA (free and total), while 7 and 8 out of the 13 plant operators had detectable levels of free and total BPA, respectively. In turn, only 1 and 2 out of 6 administration workers had detectable levels of free and total BPA, respectively. Although mean concentrations were not significant in most cases, these results would suggest that plant workers could be more exposed to BPs. However, the gender may be a critical confusing factor, as most of the plant operators are men. Anyway, further studies focused on determining the exact pathway of exposure to BPs in the HWI workers are clearly necessary. The manipulation of hazardous materials as a potential source of exposure to BPs should be also carefully assessed.

ACKNOWLEDGEMENTS

This study was financially supported by the Spanish Ministry of Economy and Competitiveness, through MODELBIS Project (AGL2016-78942-R). Sara C. Cunha, Carolina Monteiro and José O. Fernandes thanks to UID/QUI/50006/2019 with funding from FCT/MCTES. Sara C. Cunha also acknowledges FCT for the IF/01616/2015 contract.

REFERENCES

Azzouz, A., Rascón, A.J., Ballesteros, E., 2016. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. J. Pharm. Biomed. Anal. 119, 16–26.

Bertoli, S., Leone, A., Battezzati, A., 2015. Human Bisphenol A Exposure and the "'Diabesity Phenotype.'" Dose-Response 13, 1–12.

Bettencourt da Silva, R., Williams, A., 2015. Eurachem/CITAC Guide: Setting and Using Target Uncertainty in Chemical Measurement, (1st ed. 2015). Available from ww.eurachem.org

Biles, J.E., McNeal, T.P., Begley, T.H., Hollifield, H.C., 1997. Determination of bisphenol-A in reusable polycarbonate food-contact plastics and migration to to food-simulating liquids. J. Agric. Food Chem. 45, 3541–3544.

Björnsdotter, M.K., de Boer, J., Ballesteros-Gómez, A., 2017. Bisphenol A and replacements in thermal paper: A review. Chemosphere 182, 691–706.

Brotons, J.A., Olea-serrano, M.F., Villalobos, M., Pedraza, V., Olea, N., 1995. Xenoestrogens Released from Lacquer Coatings in Food Cans. Environ. Health Perspect. 103, 608–612.

Česen, M., Lenarčič, K., Mislej, V., Levstek, M., Kovačič, A., Cimrmančič, B., Uranjek, N., Kosjek, T., Heath, D., Dolenc, M.S., Heath, E., 2018. The occurrence and source identification of bisphenol compounds in wastewaters. Sci. Total Environ. 616–617, 744–752.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y. L., Wu, Y., Widelka, M. 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity - A review. Environ. Sci. Technol. 50, 5438-5453.

Corrales, J., Kristofco, L.A., Baylor Steele, W., Yates, B.S., Breed, C.S., Spencer Williams, E., Brooks, B.W., 2015. Global assessment of bisphenol a in the environment: Review and analysis of its occurrence and bioaccumulation. Dose-Response 13, 1–29.

Cunha, S.C., Cunha, C., Ferreira, A.R., Fernandes, J.O., 2012. Determination of bisphenol A and bisphenol B in canned seafood combining QuEChERS extraction with dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 404, 2453–2463.

Cunha, S.C., Fernandes, J.O., 2010. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta 83, 117–125.

Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting chemicals: An endocrine society scientific statement. Endocr. Rev. 30, 293–342.

Dodds, E.C., Lawson, W., 1936. Synthetic strogenic agents without the phenanthrene nucleus. Nature 137, 996.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA J. 13, 3978.

EFSA, 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A). EFSA J. 428, 1–75.

Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S., Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol A story: Bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil. Steril. 103, 11–21.

European Commission, 2011. Commission Directive 2011/8/EU. Off. J. Eur. Communities 29–32.

European Commission, 2017. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11813/2017.

Gao, H., Yang, B.-J., Li, N., Feng, L.-M., Shi, X.-Y., Zhao, W.-H., Liu, S.-J., 2015. Bisphenol A and hormone-associated cancers: current progress and perspectives. Medicine (Baltimore). 94, e211.

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. 50, 3725–3740.

Geens, T., Apelbaum, T.Z., Goeyens, L., Neels, H., Covaci, A., 2010. Intake of bisphenol A from canned beverages and foods on the Belgian market. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 27, 1627–1637.

Genuis, S.J., Beesoon, S., Birkholz, D., Lobo, R.A., 2012. Human excretion of bisphenol A: Blood, urine, and sweat (BUS) study. J. Environ. Public Health 2012. https://doi.org/10.1155/2012/185731

Heindel, J.J., Blumberg, B., Cave, M., Machtinger, R., Mantovani, A., Mendez, M.A., Nadal, A., Palanza, P., Panzica, G., Sargis, R., Vandenberg, L.N., Vom Saal, F., 2017. Metabolism disrupting chemicals and metabolic disorders. Reprod. Toxicol. 68, 3–33.

Horan, T.S., Pulcastro, H., Lawson, C., Gerona, R., Martin, S., Gieske, M.C., Sartain, C.V., Hunt, P.A. 2018. Replacement bisphenols adversely affect mouse gametogenesis with consequences for subsequent generations. Curr. Biol. 28: 2948-2954

Jin, H., Zhu, J., Chen, Z., Hong, Y., Cai, Z., 2018. Occurrence and partitioning of bisphenol analogues in adults' blood from China. Environ. Sci. Technol. 52, 812–820.

Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from Liaohe River Basin and Taihu Lake, China. Water Res. 103, 343–351.

Kolatorova Sosvorova, L., Chlupacova, T., Vitku, J., Vlk, M., Heracek, J., Starka, L., Saman, D., Simkova, M., Hampl, R., 2017. Determination of selected bisphenols, parabens and estrogens in human plasma using LC-MS/MS. Talanta 174, 21–28.

Konieczna, A., Rutkowska, A., Rachoń, D., 2015. Health risk of exposure to bisphenol A (BPA). Rocz. Panstw. Zakl. Hig. 66, 5–11.

Krishnan, A. V, Stathis, P., Permuth, S.F., Tokes, L., Feldman, D., 1993. Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132, 2279–2286.

LaKind, J.S., Pollock, T., Naiman, D.Q., Kim, S., Nagasawa, A., Clarke, J., 2019. Factors affecting interpretation of national biomonitoring data from multiple countries: BPA as a case study. Environ. Res. 173, 318-329.

Lehmler, H-S., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to Bisphenol A, Bisphenol F, and Bisphenol S in U.S. Adults and Children: The National Health and Nutrition Examination Survey 2013−2014. ACS Omega. 3, 6523-6532.

Li, A.J., Xue, J., Lin, S., Al-Malki, A.L., Al-Ghamdi, M.A., Kumosani, T.A., Kannan, K., 2018. Urinary concentrations of environmental phenols and their association with type 2 diabetes in a population in Jeddah, Saudi Arabia. Environ. Res. 166, 544–552.

Liao, C., Kannan, K., 2014. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 31, 319–329.

Liao, C., Liu, F., Moon, H.-B., Yamashita, N., Yun, S., Kannan, K., 2012. Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: apatial and temporal distributions. Environ. Sci. Technol. 46, 11558–11565.

Lim, D.S., Kwack, S.J., Kim, K.B., Kim, H.S., Lee, B.M., 2009. Risk assessment of bisphenol A migrated from canned foods in Korea. J. Toxicol. Environ. Heal. - Part A Curr. Issues 72, 1327–1335.

Liu, B., LehmLer, H.J., Sun, Y., Xu, G., Liu, Y., Zong, G., Sun, Q., Hu, F.B., Wallace, R.B., Bao, W. 2017. Bisphenol A substitutes and obesity in US adults: analysis of a population-based, cross-sectional study. Lancet Planet Health 1, e114-e122.

Liu, Y., Zhang, S., Song, N., Guo, R., Chen, M., Mai, D., Yan, Z., Han, Z., Chen, J., 2017. Occurrence, distribution and sources of bisphenol analogues in a shallow Chinese freshwater lake (Taihu Lake): Implications for ecological and human health risk. Sci. Total Environ. 599–600, 1090–1098.

Lu, S., Yu, Y., Ren, L., Zhang, X., Liu, G., Yu, Y., 2018. Estimation of intake and uptake of bisphenols and triclosan from personal care products by dermal contact. Sci. Total Environ. 621, 1389–1396.

Mari, M., Borrajo, M.A., Schuhmacher, M., Domingo, J.L., 2007. Monitoring PCDD/Fs and other organic substances in workers of a hazardous waste incinerator: A case study. Chemosphere 67, 574-581.

Mari, M., Nadal, M., Schuhmacher, M., Domingo, J.L., 2013. Body burden monitoring of dioxins and other organic substances in workers at a hazardous waste incinerator. Int. J. Hyg. Environ. Health 216, 728-734.

Mari, M., Schuhmacher, M., Domingo, J.L., 2009. Levels of metals and organic substances in workers at a hazardous waste incinerator: A follow-up study. Int. Arch. Occup. Environ. Health 82, 519-528.

Mariscal-Arcas, M., Rivas, A., Granada, A., Monteagudo, C., Murcia, M.A., Olea-Serrano, F., 2009. Dietary exposure assessment of pregnant women to bisphenol-A from cans and microwave containers in Southern Spain. Food Chem. Toxicol. 47, 506–510.

Martínez, M.Á., Castro, I., Rovira, J., Ares, S., Rodríguez, J.M., Cunha, S.C., Casal, S., Fernandes, J.O., Schuhmacher, M., Nadal, M., 2019. Early-life intake of major trace elements, bisphenol A,

tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas. Environ. Res. 169, 246–255.

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25–34.

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ. Res. 158, 566-575.

Mikołajewska, K., Stragierowicz, J., Gromadzińska, J., 2015. Bisphenol A – Application, sources of exposure and potential risks in infants, children and pregnant women. Int. J. Occup. Med. Environ. Health 28, 209–241.

Morgan, M.K., Nash, M., Barr, D.B., Starr, J.M., Clifton, M.S., Sobus, J.R., 2018. Distribution, variability, and predictors of urinary bisphenol A levels in 50 North Carolina adults over a six-week monitoring period. Environ. Int. 112, 85–99.

Olea, N., Pulgar, R., Pérez, P., Olea-Serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M., Sonnenschein, C., 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environ. Health Perspect. 104, 298–305.

Owczarek, K., Kubica, P., Kudłak, B., Rutkowska, A., Konieczna, A., Rachoń, D., Namieśnik, J., Wasik, A., 2018. Determination of trace levels of eleven bisphenol A analogues in human blood serum by high performance liquid chromatography–tandem mass spectrometry. Sci. Total Environ. 628–629, 1362–1368.

Pelch, K.E., Wignall, J.A., Goldstone, A.E., Ross, P.K., Blain, R.B., Shapiro, A.J., Holmgren, S.D., Hsieh, J-H., Svoboda, D., Auerbach, S.S., Parham, F.M., Masten, S.A., Thayer, K.A. 2017. NTP Research Report on Biological Activity of Bisphenol A (BPA) Structural Analogues and Functional Alternatives. NTP RR 4. Research Triangle Park, NC: National Toxicology Program. (4): 1-78.

Philips, E.M., Jaddoe, V.W. V, Asimakopoulos, A.G., Kannan, K., Steegers, E.A.P., Santos, S., Trasande, L., 2018. Bisphenol and phtalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ. Res. 161, 562–572.

Porucznik, C.A., Cox, K.J., Wilkins, D.G., Anderson, D.J., Bailey, N.M., Szczotka, K.M., Stanford, J.B., 2015. A preliminary study of biomonitoring for bisphenol-A in human sweat. J. Anal. Toxicol. 39, 562–566.

Radwan, M., Wielgomas, B., Dziewirska, E., Radwan, P., Kałużny, P., Klimowska, A., Hanke, W., Jurewicz, J., 2018. Urinary bisphenol A levels and male fertility. Am. J. Mens. Health 12, 2144–2151.

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–155.

Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ. Health Perspect. 123, 643–650.

Sakhi, A.K., Sabaredzovic, A., Papadopoulou, E., Cequier, E., Thomsen, C., 2018. Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children. Environ. Int. 114, 242–251.

Sarigiannis, D.A., Tratnik, J.S., Mazej, D., Kosjek, T., Heath, E., Horvat, M., Anesti, O., Karakitsios, S.P., 2019. Risk characterization of bisphenol-A in the Slovenian population starting from human biomonitoring data. Environ. Res., 170, 293-300.

Shekhar, S., Sood, S., Showkat, S., Lite, C., Chandrasekhar, A., Vairamani, M., Barathi, S., Santosh, W., 2017. Detection of phenolic endocrine disrupting chemicals (EDCs) from maternal blood plasma and amniotic fluid in Indian population. Gen. Comp. Endocrinol. 241, 100–107.

Snoj Tratnik, J., Kosjek, T., Heath, E., Mazej, D., Ćehić, S., Karakitsios, S.P., Sarigiannis, D.A., Horvat, M., 2019. Urinary bisphenol A in children, mothers and fathers from Slovenia: Overall results and determinants of exposure. Environ. Res. 168, 32-40.

Solaesa, A.G., Fernandes, J.O., Sanz, M.T., Benito-Román, Ó., Cunha, S.C., 2019. Green determination of brominated flame retardants and organochloride pollutants in fish oils by vortex assisted liquid-liquid microextraction and gas chromatography-tandem mass spectrometry. Talanta. 195, 251-257.

Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ. Int. 83, 107–15.

Thomson, B.M., Grounds, P.R., 2005. Bisphenol A in canned foods in New Zealand: an exposure assessment. Food Addit. Contam. 22, 65–72.

Tyl, R.W., 2014. Abbreviated assessment of bisphenol A toxicology literature. Semin. Fetal Neonatal Med. 19, 195–202. https://doi.org/10.1016/j.siny.2013.11.010

UBA, 2017. German Human Biomonitoring Commission. Available at https://www.umweltbundesamt.de/sites/default/files/medien/355/bilder/dateien/hbm-werte_engl_stand_2017_02_06_2.pdf

Usman, A., Ahmad, M., 2016. From BPA to its analogues: Is it a safe journey? Chemosphere 158, 131–142.

Wan, H.T., Leung, P.Y., Zhao, Y.G., Wei, X., Wong, M.H., Wong, C.K.C., 2013. Blood plasma concentrations of endocrine disrupting chemicals in Hong Kong populations. J. Hazard. Mater. 261, 763–769.

Xue, J., Wan, Y., Kannan, K., 2016. Occurrence of bisphenols, bisphenol A diglycidyl ethers (BADGEs), and novolac glycidyl ethers (NOGEs) in indoor air from Albany, New York, USA, and its implications for inhalation exposure. Chemosphere 151, 1–8.

Yang, Y., Guan, J., Yin, J., Shao, B., Li, H., 2014. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere 112, 481–486.

Ye, X., Wong, L.Y., Kramer, J., Zhou, X., Jia, T., Calafat, A.M., 2015. Urinary concentrations of bisphenol A and three Other bisphenols in convenience samples of U.S. sdults during 2000-2014. Environ. Sci. Technol. 49, 11834–11839.

Yu, X., Xue, J., Yao, H., Wu, Q., Venkatesan, A.K., Halden, R.U., Kannan, K., 2015. Occurrence and estrogenic potency of eight bisphenol analogs in sewage sludge from the U.S. EPA targeted national sewage sludge survey. J. Hazard. Mater. 299, 733–739.

Žalmanová, T., Hošková, K., Nevoral, J., Prokešová, Š., Zámostná, K., Kott, T., Petr, J., 2016. Bisphenol S instead of bisphenol A: A story of reproductive disruption by regretable substitution - A review. Czech J. Anim. Sci. 61, 433–449.

Zhang, T., Sun, H., Kannan, K., 2013. Blood and urinary bisphenol A concentrations in children, adults, and pregnant women from China: partitioning between blood and urine and maternal and fetal cord blood. Sci. Total Environ. 47, 4686–4694.

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EXPOSURE ASSESMENT TO BISPHENOLS: COMBINING BIOMONITORING AND DUPLICATE DIET STUDIES
Nieves González Paradell

CHAPTER 2

Concentrations of nine bisphenol analogues in food purchased from Catalonia (Spain): Comparison of canned and non-canned foodstuffs

Neus González[a], Sara C. Cunha[b], Ricardo Ferreira[b], José O. Fernandes[b], Montse Marquès[a], Martí Nadal[a,*], José L. Domingo[a]

Food and Chemical Toxicology 136 (2020) 110992

[a]*Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain*

[b]*LAQV-REQUIMTE, Department of Bromatology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*

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Nieves González Paradell

ABSTRACT

The present study was aimed at assessing the exposure of an adult population to nine BPs analogues (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE, BPAP and BPP) through a duplicate diet study. Up to 40 canned and non-canned food samples were purchased from Tarragona (Catalonia, Spain) and further analysed. Three of the nine BPs - BPA, BPB and BPE - were detected in the food samples. BPA was found in 93% and 36% of canned and non-canned samples, respectively, with a mean concentration of 22.49 and 4.73 µg/kg, respectively. Only one sample of canned asparagus (88.66 µg/kg) exceeded the new threshold set by the European Commission (50 µg/kg). BPB was found in canned and non-canned chicken and olive oil samples, with lower levels for canned chicken and non-canned olive oil. Finally, BPE was detected in non-canned mushrooms and nuts (2.40 and 12.35 µg/kg, respectively). Based on the current results, dietary intake for BPA was estimated to be 24.9 and 3.11 µg/day for canned and non-canned groups, respectively. The unexpected occurrence of BPs in non-canned products highlights the ubiquity of these compounds along the food production chain, beyond to the packaging.

Keywords: bisphenol A (BPA), bisphenol analogues, food, QuEChERS, dietary intake

INTRODUCTION

Food products are sensitive to contamination at any stage of the production chain, from farm-to-fork (Mancini et al., 2016). Food contaminants can have a wide range of sources, including the environment, processing, and packaging, among others (Rather et al., 2017). Regarding food packaging, in recent years bisphenols (BPs) have received a great attention. BPs are organic compounds containing two phenol rings, which are connected by a different binding bridge, usually a methyl bridge (Bisphenol A, BPA), a methylene bridge (Bisphenol F, BPF), or a sulphur dioxide group (Bisphenol S, BPS), depending on the analogue (Kang et al., 2006; Usman and Ahmad, 2016). It has been widely reported that BPs can play an important role in diseases like diabetes and obesity (Mirmira and Evans-Molina, 2014), as well as to cause harmful developmental and reproduction effects (Rochester, 2013).

BPA is the most used BP analogue in the food industry, with a projected consumption of 10.6 million metric tons in 2022 (Lemhler et al., 2018). It is used as a monomer for the manufacture of polycarbonate plastics and can linings. With respect to its chemical structure, there is a similarity to that of 17β-estradiol, a natural occurring hormone. Thus, BPA can bind to endocrine receptors causing a dysfunctionality of the endocrine system (Matuszczak et al., 2019; Rochester, 2013; Usman and Ahmad, 2016).

In 2011, the regulation 2011/8/EU banned the use of BPA in baby bottles and set a specific migration limit of 0.6 mg/kg of food from varnishes or coatings applied to materials (European Commission, 2011). Recently, a new regulation (2018/213/EU) was adopted setting a more restrictive migration limit (0.05 mg/kg), while no migration of BPA, from varnishes or coatings applied to materials and articles specifically intended to infants and young children up to 3 years old, is permitted (European Commission, 2018).

As a consequence of these restrictions on BPA, manufacturing companies are gradually replacing BPA by other BP analogues. Nowadays, there are 24 analogues described in the literature (Pelch et al., 2017). Hence, exposure to BPs persists, occurring through

different pathways, such as diet, inhalation and dermal contact. However, it has been reported that diet means up to the 99% of the exposure to BPA (Martínez et al., 2018). Therefore, an additional knowledge on the levels of BPs in foodstuffs, as well as risk assessment studies, are required to protect human health.

Even though BPs have gained attention in the last years, BPA is still the core research. Since their properties, structure and potential human health effects are very much alike, research on BP analogues – other than BPA – is needed. The present study was aimed at assessing the dietary exposure to nine bisphenol analogues (BPA, BPS, BPF, BPB, BPAF, BPZ, BPE, BPAP and BPP). The concentrations of these BPs analogues were determined in 40 canned and non-canned food samples consumed during a two days duplicate diet study. To the best of our knowledge, this is the very first study focused on assessing the dietary co-exposure to 9 BPs in Spain.

MATERIALS AND METHODS

Standards and chemicals

BPA (99% purity), BPB (98% purity), BPF (98% purity), BPE (98% purity), BPAF (98% purity), BPZ (99% purity) and BPAP (99% purity) were purchased from Sigma-Aldrich (West Chester, PA, USA). d16-bisphenol A ($BPAd_{16}$; 98 atom % D), used as internal standard (I.S.), was purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Individual standard solutions and internal standards were prepared in methanol (HPLC grade from Sigma-Aldrich) at concentrations of 2000 µg/L. Acetonitrile (MeCN, gradient grade for HPLC), acetic anhydride (AA; >99% purity) and tetrachloroethylene (T4CE, >99% purity) were acquired from Sigma-Aldrich. Sodium chloride and potassium carbonate (both analytical grade) were obtained from PanReac Quimica (Barcelona, Spain) and magnesium sulfate was acquired from Sigma-Aldrich. Supel™ QuE Z-Sep$^+$ was purchased from Supelco (Bellefont, PA, USA).

Instrument

BPs analyses were performed in a gas chromatograph 6890 (Agilent, Little Falls, DE, USA) equipped with a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) and a mass selective detector (5975B, Agilent), with an electron ionization (EI) chamber. The separation was performed on a DB-5MS column (30 m × 0.25 mm I.D. × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Chromatographic and detection specifications have already been reported (González et al., 2019).

Quality Control/Quality Assurance

Procedural blanks were measured each batch of 10 samples. Blank samples were spiked with both recovery and internal standards to evaluate linearity, linear range, sensitivity, precision and accuracy, according to EU guidelines (European Commission, 2017). A multilevel matrix-matched calibration -with nine calibration levels- was generated by the least squares' linear regression model. The peak area ratios of target analyte, and internal standard versus the concentration of each target compound, were plotted. Detection limits were calculated using low level points to achieve signal-to-noise ratios of 3. The quantification limits were established as the lowest concentration assayed with acceptable accuracy and precision, corresponding to the lowest calibration level of the calibration curve.

Food sampling

A total of 40 food samples were purchased in a big grocery store in Tarragona (Catalonia, Spain). Foodstuffs were divided into 2 food baskets: 1) canned food, and 2) non-canned food (including fresh food, packed in glass containers, or other BP-free materials). Canned food included tuna, pâté, nuts, mushrooms, artichokes, asparagus, corn, olive oil, green beans, red beans, peach in syrup, fruit salad in syrup, mackerel and squid. Non-canned food included the same foodstuffs than the canned group, but in glass containers, excepting mackerel and chicken. In addition, canned group included yogurt in plastic, and pre-cooked quinoa and rice, while non-canned group included yogurt in glass, dry quinoa

and rice, and fresh salmon – replacing canned mackerel – and chicken, packed in waxed paper. Both groups included fresh salad and banana, as well as toasts and cookies packed with plastic free of BPs.

Duplicate diet study

A duplicate diet study was performed to assess exposure to BPs of an adult. A cohort of 26 individuals was divided into two groups: 1) a potential high-BPA diet, consisting of the "canned food basket" above described, and 2) BPA-free diet, made of fresh food and food packed in glass containers and other BP-free materials, consisting of the "non-canned food basket" also above described. The cohort followed a two-days of balanced diet (Table 1), which was reviewed and approved by a nutritionist. Participants were able to drink as much water as they wished. However, the sources (tap, bottled, etc.) should be recorded. In parallel, each food item was homogenized using a domestic shredder and stored at -20ºC until further analysis. Only edible parts of each food item were used.

Table 3. Food consumption (g/day) for all analysed samples.

		DAY 1	
	Foodstuff	**Food weight (g)**	**Homemade measures**
Breakfast	Pâté	37.5	½ can
	Toasts	40	4 slices
Snack	Nuts	25	1 handful
Lunch	Quinoa	125	1 cup
	Mushrooms	115	1 can
	Chicken	42	1 can
	Yoghurt	115	1 unit
Snack	Fruit salad in syrup	140	½ can
Dinner	Salad	150	½ bag
	Asparagus	80	3 units
	Corn	55	½ can
	Stuffed squid	72	1 can
	Toasts	40	4 slices
	Fruit	125	1 piece
		DAY 2	
Breakfast	Tuna	52	1 can
	Toasts	40	
Snack	Nuts	25	1 handful

Lunch	Rice	125	1 cup
	Red beans	60	6 spoonful
	Artichokes	115	1 can
	Toasts	40	4 slices
	Peach in syrup	115	1 can
Snack	Yoghurt	115	1 unit
	Cookies	35	7 units
Dinner	Green beans	130	1 can
	Mackerel/salmon	85	1 can
	Toasts	40	4 slices
	Yoghurt	115	1 unit

Food samples treatment

Sample preparation is described elsewhere (Cunha et al., 2012). Briefly, each food item was blended separately with a domestic shredder before weighting 10 g of sample and adding 100 µl of BPA_{d16} and 10 ml of deionised water. For the fatty samples, 5 ml of n-heptane was added, vigorously shaked and centrifuged at 1690 g for 2 minutes. The upper-layer was discarded. Then, 10 ml of MeCN were added and samples were vortexed and agitated for 10 minutes. Afterwards, 4 g of $MgSO_4$ and 1.2 g of NaCl were added and agitated for 15 minutes. Finally, samples were centrifuged at 1690 g for 5 minutes. An additional clean-up was needed for fatty food samples, consisting of the inclusion of 1.2 g $MgSO_4$ and 50 mg of Z-SEP in the clean-up step.

A DLLME (Dispersive Liquid-Liquid MicroExtraction) procedure was subsequently performed: 85 µl of T4CE and 100 µl of AA were added to 1 ml of the MeCN extract. Rapidly, the mixture was transferred to a 25-ml screw cap glass tube, with conical bottom containing 3 ml of deionised water and 300 µl of 5% K_2CO_3 solution to ensure a pH ≥ 10. Samples were gently shaked by hand and centrifuged at 1690 g for 4 minutes. Finally, 70 µl of the lower phase were transferred to a vial with a 100-µl insert and 1 µl was injected to the GC system.

Calculation of the dietary exposure

Food consumption data are shown in Table 1. The dietary intake of each BP analogue was calculated by multiplying its concentration in each food item by the quantity of consumed food. Total dietary exposure to BPs was obtained by summing the respective intakes of all food items. Exposure was also calculated according to the average body weight of the study participants (mean: 68 kg) in order to compare the estimated exposure to the threshold limit. For calculations, when the concentration of a BP analogue was under the respective limit of detection (LOD), it was assumed to be one-half of that limit (ND=1/2LOD).

Statistics

Data treatment was performed by means of the statistical package SPSS 20.0. A Kolmogorov-Smirnov test was used to compare the homogeneity of the variances. Subsequently, the significance of the data was computed by an ANOVA or the Mann-Whitney U-test. For calculations, non-detected values were excluded from data treatment, while non-quantified samples were assumed to have a concentration equal to one-half of the limit of quantification (NQ = 1/2 LOQ).

RESULTS AND DISCUSSION

Levels of BPs in food

The concentrations of BPs in the 40 canned and non-canned food samples are summarized in Table 2. BPA, BPB and BPE were the three analogues with levels above the LOD. BPA was identified in 58% of the food samples, presenting a mean concentration of 15.54 µg/kg. Regarding canned food, BPA was detected in 14 of 15 food items. Levels of BPA ranged from <0.17 for the olive oil – the only canned food item below its LOD – to 88.66 µg/kg for the asparagus (mean concentration of BPA = 22.49 µg/kg). In turn, BPA was found in the 36% of the non-canned food samples, with a mean concentration of 4.73 µg/kg. Toasts, quinoa, yogurt, salad, asparagus, fresh squid, banana, nuts, rice,

artichokes, peach in syrup, cookies, green beans, salmon and olive oil were the food items with levels below the LOD. The highest concentration in non-canned food corresponded to mushrooms (9.56 µg/kg).

The levels of BPA in canned food were found to be higher than those observed in non-canned food. Pairs of foodstuffs with quantifiable concentrations of BPA were: pâté (13.39 vs 5.10 µg/kg), mushrooms (19.88 vs 9.56 µg/kg), chicken (20.91 vs 1.41 µg/kg), fruit salad in syrup (11.69 vs 3.85 µg/kg), corn (10.65 vs 4.21 µg/kg), tuna (32.22 vs 5.68 µg/kg) and red beans (26.16 vs 8.78 µg/kg). Also, pre-cooked quinoa and rice had detectable levels of BPA (2.93 and 1.04 µg/kg, respectively), while dry quinoa and rice were below the LOD.

Table 2. BPA, BPB and BPE concentrations (µg/kg) in canned and non-canned foods.

Food sample	Packaging	BPA	BPB	BPE
Pâté	Can	13.39	<0.33	<0.83
	Glass	5.10	<0.33	<0.83
Toasts	Plastic	<0.17	<0.17	<0.17
Quinoa	Plastic (pre-cooked)	2.93	<0.17	<0.17
	Plastic (dry)	<0.17	<0.17	<0.17
Mushrooms	Can	19.88	<0.17	<0.17
	Glass	9.56	<0.17	2.40
Chicken	Can	20.91	3.86	<0.83
	Fresh	1.41	4.19	<0.83
Yogurt	Plastic	<0.17	<0.17	<0.17
	Glass	<0.17	<0.17	<0.17
Fruit salad in syrup	Can	11.69	<0.17	<0.17
	Glass	3.85	<0.17	<0.17
Salad	Plastic	<0.17	<0.17	<0.17
Asparagus	Can	88.66	<0.17	<0.17
	Glass	<0.17	<0.17	<0.17
Corn	Can	10.65	<0.17	<0.17
	Glass	4.21	<0.17	<0.17
Squid	Can	30.85	<0.33	<0.83
	Fresh	<0.33	<0.33	<0.83
Banana	Fresh	<0.17	<0.17	<0.17
Tuna	Can	32.22	<0.33	<0.83
	Glass	5.68	<0.33	<0.83
Nuts	Can	3.45	<0.17	<0.17
	Plastic	<0.17	<0.17	12.35
Rice	Plastic (pre-cooked)	1.04	<0.17	<0.17

	Plastic (dry)	<0.17	<0.17	<0.17
Red beans	Can	26.16	<0.17	<0.17
	Glass	8.78	<0.17	<0.17
Artichokes	Can	6.31	<0.17	<0.17
	Glass	<0.17	<0.17	<0.17
Peach in syrup	Can	4.49	<0.17	<0.17
	Glass	<0.17	<0.17	<0.17
Cookies	Plastic	<0.17	<0.17	<0.17
Green beans	Can	13.02	<0.17	<0.17
	Glass	<0.17	<0.17	<0.17
Mackerel	Can	33.19	<0.33	<0.83
Salmon	Fresh	<0.33	<0.33	<0.83
Olive oil	Can	<0.17	1.25	<0.83
	Glass	<0.17	0.85	<0.83

The concentrations of BPA in canned food samples were compared with the new migration limit for BPA set recently by the European Commission in canned food (European Commission, 2018). Only canned asparagus was above 50 µg/kg (Figure 1). Although asparagus exceeded the new migration limit, probably this does not mean a risk for human health since asparagus consumption by the Spanish adult population is estimated to be only 0.67 g/day, which would mean an exposure of 0.0008 µg/kg bw/day for the general population (0.02% of contribution to the established limit) (AECOSAN, 2016). Anyway, it should be explored if this occurs in all the commercial canned asparagus brands, or it is only related to the purchased brand in this study.

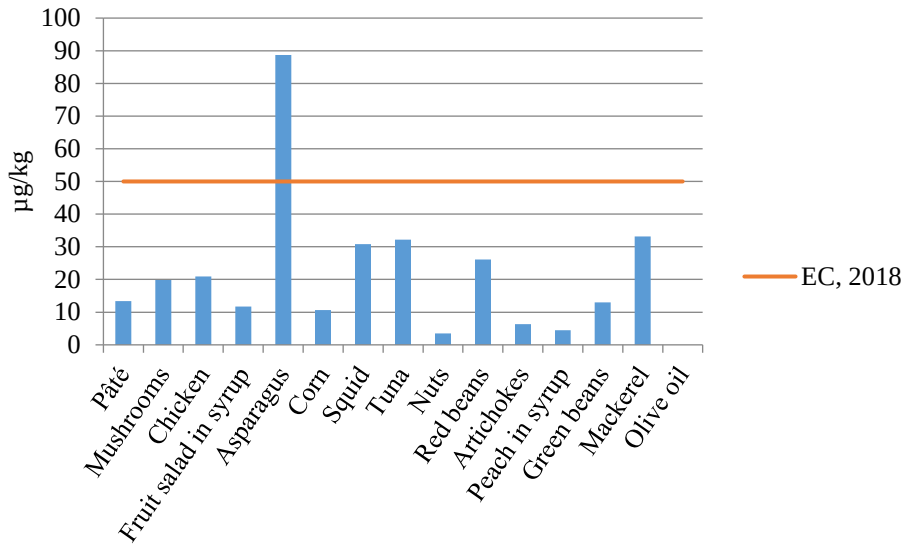

Figure 1. Comparison between detected levels of BPA in canned samples and the new migration limit established by the European Commission in 2018.

BPB was detected in four samples. Both pairs of canned and non-canned chicken and olive oil samples had BPB above their corresponding LOD. For chicken, the concentration of BPB in fresh samples was slightly higher than that found in the canned chicken (4.19 vs 3.86 µg/kg, respectively). In contrast, canned olive oil showed a higher concentration than non-canned olive oil (1.25 vs 0.85 µg/kg, respectively). Finally, BPE was found only in two food samples, both of them belonging to the non-canned group. Concentrations of BPE in mushrooms and nuts were 2.40 and 12.35 µg/kg, respectively.

As expected, canned food presented significantly higher levels of BPA than non-canned food ($p<0.01$), which is due to the fact that food is directly in contact with the can lining. Nonetheless, relevant concentrations of BPs were found in non-canned food. One explanation could be that packaging, other than cans, might also cause the migration of BPs into the food, even though these packaging are made to preserve a high-quality food (García Ibarra et al., 2019). BPs contamination in non-canned food could be the result of the migration from the coating of the caps of glass bottles, since a residual amount of BPs monomer could remain after the polymerization process (Noonan et al., 2011). Another

hypothesis would be the potential contamination during the primary production of the products (Mercogliano and Santonicola, 2018; Santonicola et al., 2018). Finally, the ubiquity of plastics elsewhere could also be related to the unexpected presence of BPs in food.

The scientific literature assessing the levels of BPA in food is extensive, but each study comprises different food samples. Consequently, the comparison of non-canned food samples between studies conducted in different countries is not always easy. Table 3 summarizes concentrations of BPA in food of different countries. The levels of BPA show a huge variation between countries due to methodological differences. Anyhow, the levels of BPA found in the current study are in the lower part of the ranges for canned food and in the upper part of the ranges for non-canned food.

In China, BPA was detected in 36% of the canned and non-canned composites, a percentage lower than the 58% of the present survey. Concentrations ranged from 0.20 to 106 µg/kg, including canned and non-canned food (Cao et al., 2011). These results are in the same range to that of the current study. In Japan, BPA mean concentration in canned food was 3.4 µg/kg, being the highest level: 30 µg/kg, which are quite lower than the mean and the maximum level of BPA in our study: 22.49 and 88.66 µg/kg, respectively. This important difference is probably due to the decrease of the polycarbonate use in Japanese manufacturers since the late 1990s, when it was replaced by polyphenylsulfone and polyethersulfone, both materials BPA-free (Kawamura et al., 2014).

In Korea, BPA was found within the range from <1.41 to 278.5 µg/kg in canned food samples (Choi et al., 2018), while in Egypt, BPA levels ranged from 6.14 to 710.59 µg/kg in canned food, and from 5.75 to 236.76 µg/kg in food packaged in plastic (Osman et al., 2018). These results are certainly higher than those found in the present study. In the United States, BPA was found in 73% and in 7% of the canned and non-canned food samples, respectively. These percentages are lower than those found the present study (93% and 36%, respectively). BPA levels found in canned food ranged between 0.31 and

149 µg/kg, while in non-canned food varied between 0.28 and 0.41 µg/kg (Lorber et al., 2015). Thus, BPA concentrations in canned food are higher than those detected in the present survey. By contrast, BPA concentrations in non-canned food are lower than those found in the current study.

In Portugal, BPA levels were determined in canned samples of tuna and sardines, with levels ranging from <1 to 63 µg/kg, which is in accordance to those found in the present study (Cunha et al., 2017). Canned vegetables and canned fruit were also analyzed. Higher detection rates for BPA (87% versus 58%) and a range of concentrations, from 3.7 to 256.6 µg/kg, which is higher than in the present study (from <0.17 to 88.66 µg/kg) were reported (Cunha and Fernandes, 2013). In turn, Sakhi and co-workers (2014) analyzed the concentrations of BPA in 37 canned and non-canned foodstuffs in Norway. Composites for each food group comprised food samples with different packaging materials. Thus, comparison was made with joint results for canned and non-canned groups. Detectable levels for the food samples ranged from <0.020 to 8.7 µg/kg, being lower than the results of the present study (Sakhi et al., 2014). On the other hand, Tzatzarakis et al. (2016) analysed the content of BPA in the two phases of the canned product (liquid and solid). They found higher levels of BPA in the solid phase than in the liquid phase (2.70 vs. 33.4 µg/kg).

Beyond BPA, studies assessing the levels of BPs analogues are limited. Moreover, most of these studies only determined the concentrations of 2 or 3 analogues (especially, BPS, BPF and BPB). The occurrence of 8 BPs have been only determined in two studies. In USA, BPAF, BPP, BPS, BPAP, BPF, BPB and BPZ were found in analyzed food samples, with detection rates varying from 0 - 11% for BPZ, to 0 – 60% for BPF. Detection rates for BPB (0 – 13%) were in accordance with those found in this study (10%). On the other hand, BPB concentrations varied from <0.013 to 0.017 µg/kg, which are lower than the current results (<0.17 to 4.19 µg/kg) (Liao and Kannan, 2013). In Belgium, no bisphenol analogues were detected in any of the ready-to-eat meal samples analyzed, with the exception of BPS and BPF, which were only present in one sample (beef ravioli) (Regueiro and Wenzl,

2015). In Korea, BPS and BPF levels were determined in canned food samples. Like in the present study, BPS and BPF were not detected in any of the samples (Choi et al., 2018).

In parallel, BPB was found in canned seafood samples purchased in Portugal and Italy, both with lower detection rates than BPA (83% vs 12%, and 75% versus 12%, respectively) (Cunha et al., 2012; Fattore et al., 2015). These results agree with the percentages of BPB detection of the present survey (13%). Lower rates for BPB were found in Portugal, where BPB was only detected in 2 of 39 samples (Cunha and Fernandes, 2013).

Table 3. Concentrations of BPs in foodstuffs from different countries: a summary of scientific literature.

Country	BPA (µg/kg)	Type of food container	Type of food	Reference
China	0.20 – 106	Canned and non-canned	Dairy, meat, poultry, fish, soup, bread and cereal, vegetable, fruit, beverage, baby food, fast food, miscellaneous	Cao et al., 2011
Japan	3.4	Canned	Fish, meat, vegetable, fruit, other cooked food, coffee, tea, other beverages	Kawamura et al., 2014
Korea	<1.41 – 278.5	Canned	Meat, fish, corn and beans, fruit, sauces, vegetables, liquor, beverages and coffee	Choi et al., 2018
Egypt	6.14 – 710.59 5.75 – 236.76	Canned Non-canned	Meat, fish, vegetables, fruits, oil, milk and beverages	Osman et al., 2018
United States	0.31 - 149 0.28 – 0.41	Canned Non-canned	Fruit, vegetables, meat, fish and dairy	Lorber et al., 2015
Portugal	<1 - 62	Canned	Seafood	Cunha et al., 2017
Norway	0.11 – 5.8	Canned and non-canned	Grain and grain products, milk and dairy products, meat and meat products, fish and fish products, fats, fruits and vegetables, ready-to-eat, snacks, beverages, condiments, others	Sakhi et al., 2014
Spain	<0.17 – 88.66 <0.17 – 9.56	Canned Non-canned	Meat, fish, vegetables, fruit, bread, dairy products and bakery	Present study
Country	**BPB (µg/kg)**	**Type of food container**	**Type of food**	**Reference**
United states	<0.013 – 0.017	Canned and non-canned	Beverages, dairy products, fats and oils, fish and seafood, cereals and cereal products, meat and meat products, fruit, vegetables, others	Liao and Kannan., 2013
Portugal	<0.4 – 21.7	Canned	Seafood	Cunha et al., 2012
Italy	<0.9 – 145.9	Canned	Tuna	Fattore et al., 2015
Spain	<0.17 – 3.86 <0.17 – 4.19	Canned Non-canned	Meat, fish, vegetables, fruit, bread, dairy products and bakery	Present study

In a recent review, Russo et al. (2019) reported BPs concentration in food from different countries and matrices. Regarding vegetables, asparagus was the food product that contained the highest level of BPA (959 µg/kg), being in accordance with the results here presented. Other types of food showed highly variable levels of BPA, being lower levels in beverages and higher in other foodstuffs (seafood, vegetables and meat). These data are in agreement with those provided by EFSA (2015), which highlighted the significant differences between canned and non-canned food, with meat, fish, grains, legumes, condiments, and snacks showing relatively higher levels (>30 µg/kg).

Estimated dietary intake of BPs through the diet

Total and daily intake through the diet of BPs analogues was assessed. Although in this study drinking water was not analyzed, exposure was calculated using concentrations of BPs taken from the literature (Zhang et al., 2018), being mean water consumption data from the ANIBES study (Nissensohn et al., 2016). BPA, BPAF, BPB, BPE, BPF and BPS exposure from drinking water was estimated to be 0.005, 0.001, 0.001, 0.0001, 0.0001 and 0.0003 µg/day, respectively.

Total BPA intake for the two-day diet for the canned group was estimated to be 24.9 µg, way above the estimated intake for the non-canned group: 3.12 µg. For BPB, a similar estimation was found for both groups: 0.46 and 0.45 µg, for canned and non-canned diet, respectively. Lastly, the estimated intakes of BPE were 0.28 and 1.16 µg, for the canned and non-canned groups, respectively.

Taking the days separately, canned group had a BPA intake of 15.7 µg/day for day one, and 9.26 µg/day for day two. On the other hand, non-canned group had an intake of 2.20 and 0.92 µg/day for the first and the second day, respectively. For BPB, canned group, had an intake of 0.31 and 0.15 µg/day for each day. Similarly, non-canned group had intakes of 0.31 and 0.14 µg/day, respectively. Finally, for the canned group BPE intake was 0.14 µg/day for both days, while for the non-canned group, the estimated intake was

calculated to be 0.71 and 0.45 µg/day, for the first and second day, respectively (Table 4).

Table 4. Estimated dietary intake of BPA, BPB and BPE for canned and non-canned diet.

Day of the diet	BPA (µg/day)		BPB (µg/day)		BPE (µg/day)	
	Canned	Non-canned	Canned	Non-canned	Canned	Non-canned
Day 1 (D1)	15.7	2.20	0.31	0.31	0.14	0.71
Day 2 (D2)	9.26	0.92	0.15	0.14	0.14	0.45
Mean ± SD	12.5 ± 4.6	1.56 ± 0.9	0.23 ± 0.11	0.22 ± 0.12	0.14 ± 0.002	0.58 ± 0.19

Based on the daily intake of BPs and the average body weight of the cohort (68 kg), two-day diet total BPA exposure was estimated to be 0.37 and 0.05 µg/kg bw for canned and non-canned diet, respectively. With respect to BPB, 0.007 µg/kg bw was the estimated exposure for both diet groups. Finally, BPE exposure was estimated to be 0.004 and 0.02 µg/kg bw, for canned and non-canned food, respectively.

Daily exposure was estimated as follows: on the first day for the canned diet, BPA, BPB and BPE exposures were 0.23, 0.004 and 0.002 µg/kg bw/day, respectively. On the second day, estimations were 0.14, 0.002 and 0.002 µg/kg bw/day for BPA, BPB and BPE, respectively. For the non-canned diet, exposures to BPA, BPB and BPE on the first day, were 0.03, 0.005 and 0.01 µg/kg bw/day, respectively, while in the second day, 0.01, 0.002 and 0.007 µg/kg bw/day were the exposures estimated for BPA, BPB and BPE, respectively.

Canned asparagus had BPA concentrations above the migration limit, being its contribution a 28% of the total exposure to BPA. However, high-BP diet group did not exceed the TDI of 4 µg/kg bw/day, which is established by the EFSA. Neither the BPA-free diet group exceeded the threshold limit (Figure 2) (EFSA, 2015). The comparison between other analogues of BP and their TDI values was not possible, because international organizations have not set threshold limits yet.

Although the estimated dietary intake of BPA is below the TDI, other exposure pathways, such as dermal absorption or air inhalation, should not be disregarded. In addition, the presence of traces of other endocrine disruptors in food could increase the total exposure and cause adverse health effects, even at low-dose exposures (Tsatsakis et al., 2016).

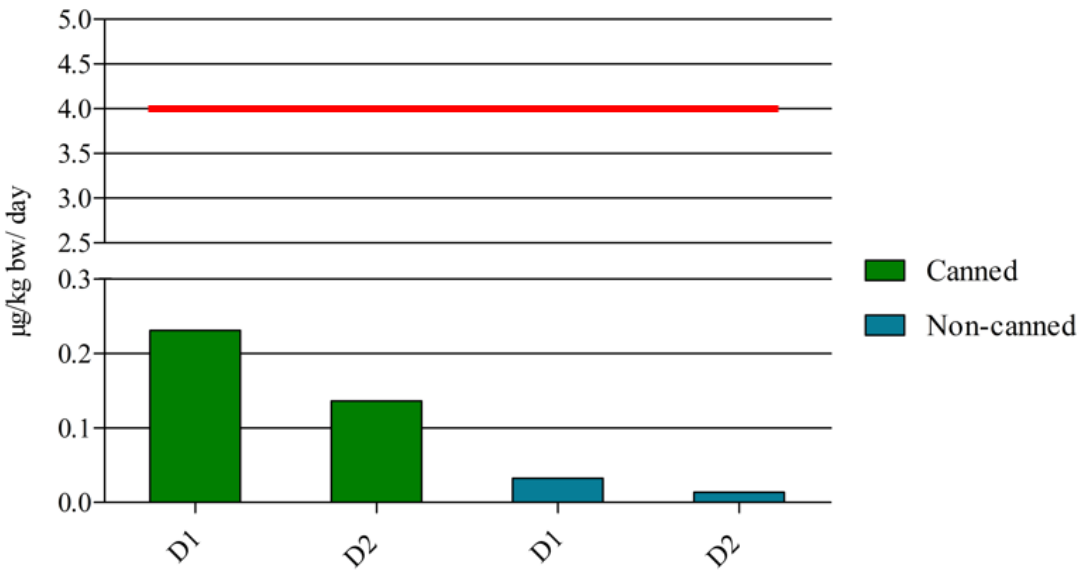

Figure 2. Estimated dietary exposure of BPA for 2 different groups, and comparison with TDI (4 µg/kg bw/ day). D1: Day 1; D2: Day 2

CONCLUSIONS

BPA is the most widespread BP analogue in both canned and non-canned foodstuff purchased in Spain. Consequently, the Spanish population is mainly exposed to this BP analogue. BPB and BPE were also detected, but at a much lower rate than BPA. The other analogues here assessed (BPS, BPF, BPAF, BPZ, BPAP and BPP) were not detected in any food sample. Nevertheless, the assessment of the BPs levels in food – regardless the food packaging – is clearly needed in order to ensure that food products do not mean a risk for human health. The estimated dietary exposure to BPA showed that none of the groups (canned and non-canned) exceeded the TDI established by the EFSA, even though

canned asparagus were above the new migration limit recently fixed by the European Commission.

Biomonitoring studies of BPs must be conducted in duplicate diet studies to explore their ADME -adsorption, distribution, metabolism and excretion- and to protect human health. These studies should not only be focused on BPA, but also on all BPs analogues. Moreover, as it has been proved that BPs analogues –other than BPA- are also used by the food industry, regulations on their occurrence in food, migration limits from food packaging materials, and TDIs are urgently required.

ACKNOWLEDGEMENTS

This study was financially supported by the Spanish Ministry of Economy and Competitiveness, through MODELBIS Project (AGL2016-78942-R). Sara C. Cunha and José O. Fernandes thanks to UID/QUI/50006/2019 with funding from FCT/MCTES. Sara C. Cunha also acknowledges FCT for the IF/01616/2015 contract.

REFERENCES

AECOSAN (2016) Encuesta nacional de alimentación en población adulta, mayores y embarazadas. Resultados sobre datos de consumo. Agencia Española de Consumo, Seguridad Alimentaria y Nutrición. Ministry of Health, Madrid, Spain. Available at: http://www.aecosan.msssi.gob.es/AECOSAN/web/seguridad_alimentaria/subdetalle/enalia_2.htm [accessed: 04/06/2019]

Cao, X.L., Perez-Locas, C., Dufresne, G., Clement, G., Popovic, S., Beraldin, F., Dabeka, R.W., Feeley, M., 2011. Concentrations of bisphenol a in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 28, 791–798. https://doi.org/10.1080/19440049.2010.513015

Choi, S.J., Yun, E.S., Shin, J.M., Kim, Y.S., Lee, J.S., Lee, J.H., Kim, D.G., Oh, Y.H., Jung, K., Kim, G.H., 2018. Concentrations of Bisphenols in Canned Foods and Their Risk Assessment in Korea. J. Food Prot. 81, 903–916. https://doi.org/10.4315/0362-028x.jfp-17-447

Cunha, S.C., Alves, R.N., Fernandes, J.O., Casal, S., Marques, A., 2017. First approach to assess the bioaccessibility of bisphenol A in canned seafood. Food Chem. 232, 501-507.

Cunha, S.C., Fernandes, J.O., 2013. Assessment of bisphenol A and bisphenol B in canned vegetables and fruits by gas chromatography–mass spectrometry after QuEChERS and dispersive liquid–liquid microextraction. Food Control 33, 549-555. https://doi.org/10.1016/j.foodcont.2013.03.028

Cunha, S.C., Cunha, C., Ferreira, A.R., Fernandes, J.O., 2012. Determination of bisphenol A and bisphenol B in canned seafood combining QuEChERS extraction with dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 404, 2453–2463. https://doi.org/10.1007/s00216-012-6389-5

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs : Executive summary. EFSA Journal 2015;13(1):3978

European Commission, 2018. Commission Regulation (EU) 2018/832 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materi. Off. J. Eur. Union 2001, 20–30. https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf

European Commission, 2018. Commission regulation (EU) 2018/213. Off. J. Eur. Communities 6-12.

European Commission, 2017. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11813/2017.

European Commission, 2011. Commission Directive 2011/8/EU. Off. J. Eur. Communities 29–32.

Fattore, M., Russo, G., Barbato, F., Grumetto, L., Albrizio, S., 2015. Monitoring of bisphenols in canned tuna from Italian markets. Food Chem. Toxicol. 83, 68–75. https://doi.org/10.1016/j.fct.2015.05.010

García Ibarra, V., Sendón, R., Bustos, J., Paseiro Losada, P., Rodríguez Bernaldo de Quirós, A., 2019. Estimates of dietary exposure of Spanish population to packaging contaminants from cereal based foods contained in plastic materials. Food Chem. Toxicol. 128, 180–192. https://doi.org/10.1016/j.fct.2019.04.003

González, N., Cunha, S.C., Monteiro, C., Fernandes, J.O., Marquès, M., Domingo, J.L., Nadal, M., 2019. Quantification of eight bisphenol analogues in blood and urine samples of workers in a hazardous waste incinerator. Environ. Res. 176, 108576. https://doi.org/10.1016/j.envres.2019.108576

Kang, J.-H., Kondo, F., Katayama, Y., 2006. Human exposure to bisphenol A (BPA). Toxicology 26, 79–89. https://doi.org/10.1016/j.reprotox.2007.07.010

Kawamura, Y., Etoh, M., Hirakawa, Y., Abe, Y., Mutsuga, M., 2014. Bisphenol A in domestic and imported canned foods in Japan. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 31, 330–340. https://doi.org/10.1080/19440049.2013.874047

Lehmler, H-S., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to Bisphenol A, Bisphenol F, and Bisphenol S in U.S. Adults and Children: The National Health and Nutrition Examination Survey 2013−2014. ACS Omega. 3, 6523-6532.

Liao, C., Kannan, K., 2013. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J. Agric. Food Chem. 4655–4662. https://doi.org/10.1021/jf400445n

Lorber, M., Schecter, A., Paepke, O., Shropshire, W., Christensen, K., Birnbaum, L., 2015. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ. Int. 77, 55–62. https://doi.org/10.1016/j.envint.2015.01.008

Mancini, F.R., Busani, L., Tait, S., La Rocca, C., 2016. The relevance of the food production chain

with regard to the population exposure to chemical substances and its role in contaminated sites. Ann Ist Super Sanità 50, 505–510. https://doi.org/10.4415/Ann

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25–34. https://doi.org/10.1016/j.envres.2018.05.008

Matuszczak, E., Komarowska, M.D., Debek, W., Hermanowicz, A., 2019. The impact of bisphenol A on fertility, reproductive system, and development: A review of the literature. Int. J. Endocrinol. 2019, 1–8. https://doi.org/10.1155/2019/4068717

Mercogliano, R., Santonicola, S., 2018. Investigation on bisphenol A levels in human milk and dairy supply chain: A review. Food Chem. Toxicol. 114, 98–107. https://doi.org/10.1016/j.fct.2018.02.021

Mirmira, P., Evans-Molina, C., 2014. Bisphenol A, obesity, and type 2 diabetes mellitus: Genuine concern or unnecessary preoccupation? Transl. Res. 164, 13–21. https://doi.org/10.1016/j.trsl.2014.03.003

Nissensohn, M., Sánchez-Villegas, A., Ortega, R.M., Aranceta-Bartrina, J., Gil, Á., González-Cross, M., Varela-Moreiras, G., Serra-Majem, L., 2016. Beverage consumption habits and association with total water and energy intakes in the Spanish population: Findings of the ANIBES Study. Nutrients. 8, 232.

Noonan, G.O., Ackerman, L.K., Begley, T.H., 2011. Concentration of bisphenol a in highly consumed canned foods on the U.S. market. J. Agric. Food Chem. 59, 7178–7185. https://doi.org/10.1021/jf201076f

Osman, M.A., Mahmoud, G.I., Elgammal, M.H., Hasan, R.S., 2018. Studying of bisphenol A levels in some canned food, feed and baby bottles in egyptian markets. Fresenius Environ. Bull. 27, 9374–9381.

Pelch, K., Wignal, J., Goldstone, A., Ross, P., Blain, R., Shapiro, A., Holmgren, S., Hsieh, J.-H., Svoboda, D., Auerbach, S., Parham, F., Masten, S., Thayer, K., 2017. NTP research report on biological activity of bisphenol A (BPA) structural analogues and functional alternatives. Natl. Toxicol. Progr. 1, 1–78.

Rather, I.A., Koh, W.Y., Paek, W.K., Lim, J., 2017. The sources of chemical contaminants in food and their health implications. Front. Pharmacol. 8. https://doi.org/10.3389/fphar.2017.00830

Regueiro, J., Wenzl, T., 2015. Development and validation of a stable-isotope dilution liquid chromatography-tandem mass spectrometry method for the determination of bisphenols in ready-made meals. J. Chromatogr. A. 1414, 110-121.

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–155. https://doi.org/10.1016/j.reprotox.2013.08.008

Russo, G., Barbato, F., Mita, D.G., Grumetto, L., 2019. Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe. Food Chem. Toxicol. 110575.

Sakhi, A.K., Lillegaard, I.T.L., Voorspoels, S., Carlsen, M.H., Løken, E.B., Brantsæter, A.L., Haugen, M., Meltzer, H.M., Thomsen, C., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ. Int. 73, 259–269. https://doi.org/10.1016/j.envint.2014.08.005

Santonicola, S., Ferrante, M.C., Murru, N., Gallo, P., Mercogliano, R., 2018. Hot topic: Bisphenol A in cow milk and dietary exposure at the farm level. J. Dairy Sci. 102, 1007–1013. https://doi.org/10.3168/jds.2018-15338

Tsatsakis, A.M., Docea, A.O., Tsitsimpikou, C., 2016. New challenges in risk assessment of chemicals when simulating real. Food Chem. Toxicol. 96, 174-176.

Tzatzarakis, M.N., Karzi, V., Vakonaki, E., Goumenou, M., Kavvalakis, M., Stivaktakis, P., Tsitsimpikou, C., Tsakiris, I., Rizos, A., Tsatsakis, A.M., 2016. Bisphenol A in soft drinks and canned foods and data evaluation. Food Addit. Contam. Part B 10, 85-90.

Usman, A., Ahmad, M., 2016. From BPA to its analogues: Is it a safe journey? Chemosphere 158, 131–142. https://doi.org/10.1016/j.chemosphere.2016.05.070

Zhang, H., Zhang, Y., Li, J., Yang, M., 2018. Occurrence and exposure assessment of bisphenol analogues in source water and drinking water in China. Sci. Total. Environ. 655, 607-613.

CHAPTER 3

Biomonitoring of co-exposure to bisphenols by consumers of canned foodstuffs

Neus González[a], Montse Marquès[a], Sara C. Cunha[b], José O. Fernandes[b], José L. Domingo[a], Martí Nadal[a,*]

Submitted to Environment International

[a] *Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Catalonia, Spain*

[b] *LAQV-REQUIMTE, Department of Bromatology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal*

UNIVERSITAT ROVIRA I VIRGILI
EXPOSURE ASSESMENT TO BISPHENOLS: COMBINING BIOMONITORING AND DUPLICATE DIET STUDIES
Nieves González Paradell

ABSTRACT

For non-occupationally exposed adults, dietary intake is the main route of exposure to bisphenols (BPs), with canned foodstuffs playing a key role. This study was aimed at biomonitoring bisphenol A (BPA) and 5 more BP analogues (BPB, BPE, BPF, BPAF and BPZ) in spot urine and blood samples of a cohort of adults, who followed a diet based on a high consumption of canned food. The estimated total dietary exposure was 0.37 and 0.045 µg/kg body weight/day, for the BP-exposed and control groups, respectively. BPA was the compound with the highest concentration in urine in comparison with the values of the remaining BPs analogues. A high detection rate of BPA was noted in urine (94%), while in blood it could be only quantified in 6% of the samples. The identification of other analogues was poor and random, being hardly related to diet. After 2 days, excretion of BPA was significantly higher in the BP-exposed subjects than those in the control group (7.02 vs. 1.89 µg/day), confirming that diet and canned foodstuffs are the main route of exposure to BPA. Anyhow, the t-TDI established by the EFSA was not exceeded, even by those consumers with a diet rich in canned food. Moreover, spot urine samples provided accurate information about exposure and excretion of BPA, being the 4 h, instead of 24 h, the optimal sampling interval. To the best of our knowledge, this is the first study aimed at assessing the co-exposure of BPs analogues in food and biological samples after a two-day duplicate diet study.

Keywords: BPA; duplicate diet; biomonitoring; bisphenol analogues; urine.

INTRODUCTION

Human biomonitoring is an analysis method widely used to measure concentrations of chemical substances and metabolites in biological tissues (Albertini et al., 2006; Ibarluzea et al., 2016). It is a good mechanism to assess exposure to environmental pollutants and to identify potential risks for human health. Traditionally, a number of biological matrices, including blood, urine, hair and breast milk, among others, have been used for human biomonitoring (Ashrap et al., 2018; Esplugas et al., 2019, 2020; Ibarluzea et al., 2016; Katsikantami et al., 2016; Nadal et al., 2019; Quinete et al., 2016; Schuhmacher et al., 2019; Song et al., 2018; Velázquez-Gómez and Lacorte, 2019).

In recent years, the biomonitoring of endocrine disruptors has been increasing (Cullen et al., 2017; Karrer et al., 2020; Rodríguez-Gómez et al., 2017; Tordjman et al., 2016; Vela-Soria et al., 2016). Specifically, many efforts have been made to assess the occurrence of bisphenols (BPs) in biological matrices. BPs are a family of organic compounds that have a hydroxyl residue directly bound to an aromatic ring, obtained by the condensation of a phenol with a ketone or an aldehyde (Corrales et al., 2015; Geens et al., 2012; Michałowicz, 2014). Bisphenol A (BPA) is the most used analogue among the 24 BPs described in the literature (Pelch et al., 2017). BPs are not persistent in the human body, being usually excreted through urine around 6 h after ingestion (Oh et al., 2018; Thayer et al., 2015). Although BPs are not bioaccumulative, human exposure is ubiquitous. BPs are contained in many daily-life products, such as coatings for food packaging, metal jar lids, automobile parts, thermal paper, dental sealants, toys, household appliances, adhesive plastics and clothes (Vandenberg et al., 2007; Chen et al., 2016a; Kang et al., 2006; Freire et al., 2019; Xue et al., 2017).

BPs have been found in trace amounts in a wide range of samples, including food (Choi et al., 2018; Cunha et al., 2012; Fattore et al., 2015; González et al., 2019a; Liao and Kannan, 2014), biological tissues (Cunha and Fernandes, 2010; Genuis et al., 2012; González et al., 2019b; Kolatorova Sosvorova et al., 2017; Martínez et al., 2019; Morgan

et al., 2018; Tzatzarakis et al., 2015), as well as in different environmental compartments (Česen et al., 2018; Hu et al., 2019; Jin and Zhu, 2016; Liao et al., 2012; Liu et al., 2017).

For non-occupationally exposed adults, exposure to BPA occurs mainly through the diet, which means more than 99% of the total exposure (Martínez et al., 2017, 2018). After ingestion, BPs are quickly metabolized in the liver, transformed into an inactive form, and subsequently excreted through urine (Gramec Skledar and Peterlin Mašič, 2016). In turn, although the contribution of other routes of exposure (i.e.: dermal, inhalation) is minor, its relative importance should not be ignored (Geens et al., 2012; Lu et al., 2018; Porras et al., 2014; von Goetz et al., 2017). Taking into account that dietary intake is the most important route of exposure to this BPs, the monitoring of these chemicals in food, as well as the exposure assessment, should be periodically performed for public health protection. To date, many investigations have already been focused on assessing the dietary exposure to BPA (Abou Omar et al., 2017; Chen et al., 2016b; Teeguarden et al., 2011), but the knowledge on the occurrence and co-exposure of other BPs analogues is scarce (Husøy et al., 2019).

The present study was aimed at assessing the levels of BPA and 5 more BP analogues in blood and urine samples of an adult cohort of population, who followed a diet with a high content of canned food. The dietary intake of these BPs analogues was also estimated and compared with previous values (Gonzalez et al., 2019a). To the best of our knowledge, this is the first time that the dietary co-exposure to 6 BPs analogues is assessed by means of a duplicate diet study, considering two perspectives: the occurrence of BPs in both, food (intake) and biological matrices (excretion). Previous investigations were basically focused on the single exposure to BPA.

MATERIALS AND METHODS

Standards and chemicals

BPA (99% purity), BPB (98% purity), BPF (98% purity), BPE (98% purity), BPAF (98% purity), and BPZ (99% purity) were purchased from Sigma-Aldrich (West Chester, PA, USA). In

addition, d_{16}-bisphenol A ($BPAd_{16}$; 98 atom % D), used as internal standard, was purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA). Individual standard solutions and internal standards were prepared in methanol (HPLC grade from Sigma-Aldrich) at concentrations of 1000 µg/L. Acetonitrile (MeCN, gradient grade for HPLC), acetic anhydride (AA; >99% purity) and tetrachloroethylene (T4CE, >99% purity) were acquired from Sigma-Aldrich. Potassium carbonate (analytical grade) was obtained from Panreac Quimica (Barcelona, Catalonia, Spain). β-Glucuronidase (Type 1 from *Helix pomatia*, 100000 U/g solid glucuronidase) was purchased from Sigma-Aldrich.

Instrumental equipment

BP analyses were performed in a gas chromatograph 6890 (Agilent, Little Falls, DE, USA) equipped with a Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland) and a mass selective detector (5975B, Agilent), with an electron ionization (EI) chamber. The separation was performed on a DB-5MS column (30 m × 0.25 mm I.D. × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Chromatographic and detection specifications have been described elsewhere (González et al., 2019b).

Quality Control/Quality Assurance

Procedural blanks were measured each batch of 10 samples. Blank samples were spiked with both recovery and internal standards to evaluate linearity, linear range, sensitivity, precision and accuracy, according to EU guidelines (European Commission, 2017). A multilevel matrix-matched calibration with nine calibration levels, was generated by the least squares' linear regression model. The peak area ratios of each target analyte, as well as the internal standard vs. the concentration of each target compound, were plotted. Detection limits were calculated using low level points to achieve signal-to-noise ratios of 3. In turn, the quantification limits were established as the lowest concentration assayed with acceptable accuracy and precision, corresponding to the lowest calibration level of the calibration curve.

Duplicate diet study

A 2-day duplicate diet study was performed to assess exposure to BPs of adults mainly consuming canned foodstuffs. Briefly, a cohort of 26 individuals was divided into two groups: 1) those following a potential high-BPA diet based on canned foodstuffs, (exposed), and 2) those following a BPA-free diet, made of fresh foodstuffs and food products packed in glass containers and other BP-free materials (control). Both groups of subjects followed a balanced diet, which was revised and approved by a dietitian. Additional details of the diet were previously given (González et al., 2019a). The cohort characteristics are described in Table 1. One of the participants (No. 19) was excluded from the study because he declared the consumption of food that was not in the provided food basket. Therefore, the cohort of the study consisted of 25 individuals. The study was approved by the Ethical Committee for Clinical Investigation of the Institut d'Investigació Sanitària Pere Virgili (IISPV, Reus/Tarragona, Spain, Ref. CEIm: 112/2018). A written informed consent was obtained from all the participants.

Table 1. Cohort characteristics (n=25)

%	**Exposed** (n= 14)	**Control** (n=11)
Gender		
Male	29	36
Female	71	64
Body Mass Index		
Underweight (<19 kg/m^2)	0	0
Normal (19-25 kg/m^2)	64	45
Overweight (>25 kg/m^2)	29	55
Obese (>30 kg/m^2)	7	0
Smoking		
Yes	14	0
No	86	100

Sample collection

Urine and blood samples were collected to further analyse the concentrations of BPs. During the whole 48-h study, each participant collected spot urine samples and the first void urine (FVU) of the third day. On the other hand, blood samples were extracted in heparin tubes during the first day of the study. Extraction was done at four time spots: 8 AM (fasting), 11 AM, 2 PM and 5 PM. Fasting samples were considered as a baseline exposure, while the schedule for the other blood extractions was designed taking into account that the maximum concentration of BPs in serum is reached 1 h after their intakes (Thayer et al., 2015; Oh et al., 2018). Firstly, 2 mL of whole blood were taken separately. Blood samples were then centrifuged at 1300 g for 10 min in order to obtain plasma and the red blood cell (RBC) fraction. All the biological samples were stored at -20ºC until analysis. In order to complete the information about the cohort, food frequency and lifestyle questionnaires, as well as a journal to record the total volume of each urine sample, were filled up by each subject. Participants were also asked for noting any other activities with a potential exposure and excretion of BPs, as well as incidents occurred during the study.

BPs analysis in urine

After collection, urine samples were thawed at room temperature and homogenized by vortex. The analysis of free BPs was performed following a previously developed method (González et al., 2019b). Five mL of sample were transferred to a glass tube with conical bottom and spiked with 50 µL of BPA_{d16}. Rapidly, 1325 µL of MeCN, 85 µL of T4CE and 125 µL of AA were added to the sample. Tubes were shaken and centrifuged for 10 min at 2500 rpm and 4ºC. Finally, 70 µL of the lower phase were transferred to a 100 µL insert, being 1 µL injected into the GC-MS equipment. In turn, for the determination of total (free + conjugated) BPs, 5 mL of sample were transferred to a clear vial, to which were added 100 µL of β-glucoronidase solution (20,000 U/mL in 1M ammonium acetate buffer pH 5.0). The mixture was then incubated overnight at 37ºC in order to guarantee the hydrolysis. After cooling at room temperature, BPs were extracted as above stated.

BPs analysis in whole blood, RBC and plasma

The preparation of the samples and the extractive procedures were performed according to González et al. (2019b). Firstly, whole blood, RBC and plasma samples were thawed at room temperature and homogenized by vortex. For the analysis of free BPs, 1.5 mL of MeCN was added to 500 µL of samples of total blood, RBC and plasma. Each sample was firstly spiked with 40 µL of BPA_{d16}, kept at room temperature for 10 min, and then centrifuged at 3500 rpm for 4 min. Subsequently, 1 mL of sample was transferred to a clean vial, adding 85 µL of T4CE and 100 µL of AA to the sample. In a glass tube with conical bottom, 3 mL of deionized water and 300 µL of K2CO3 (to achieve a pH ≥ 10) were added. Rapidly, the sample was transferred to the glass tube and vortexed. Samples were then centrifuged at 2100 rpm for 4 min. Finally, 70 µL of the lower phase were transferred to a 100 µL insert, being 1 µL injected into the GC-MS system. In turn, for the analysis of total (free + conjugated) BPs, 1.5 mL of sample was transferred to a clean vial and 30 µL of β-glucoronidase solution (20,000 U/mL in 1M ammonium acetate buffered to pH 5.0) were added. Samples were incubated overnight at 37ºC to guarantee the hydrolysis. After cooling at room temperature, BPs were extracted as previously described.

Calculation of BPs urinary elimination

Total BPs elimination was calculated for each individual by multiplying the BP analogue concentration of each spot urine sample (µg/mL) by the volume of the urine sample of the corresponding spot sample (mL). To calculate the elimination of BPs on a daily basis, the following assumptions were considered: i) the first void urine (FVU) contained urine samples collected before the breakfast of the day 1; ii) day 1 data included urine samples collected between the breakfast of day 1 and the breakfast of day 2; iii) day 2 contained urine samples collected after the breakfast of day 2 until the FVU sample of day 3.

Statistical analysis

To evaluate significant differences between exposed and control groups, as well as through time, the statistical package SPSS 20.0 was used. Firstly, a Kolmogorov-Smirnov test was applied to compare the homogeneity of the variances. Then, a Student's t-test was applied to determine statistical differences. In turn, the effect of consuming canned food on the exposure to BPA was assessed by building a mixed effects linear model on: i) the quantity of excreted BPA as a function of time; ii) the dichotomous factor of ingestion of canned food as a fixed effect; and iii) the baseline excretion of each participant as a random effect. This analysis was carried out by means of the statistical package R version 3.5.3.

RESULTS AND DISCUSSION

BPs in urine samples

Concentrations of BPA

Free BPA was found only in 19 out of 189 urine samples of the BP-exposed group and 7 out of 138 urine samples of the control group. Hence, detection rates were only 10% and 5% for the BP-exposed and control groups, respectively (Supplementary Material, Tables S1-S2). Mean concentrations of free BPA were similar in both groups, independently on the consumption of canned foodstuffs (0.21 vs. 0.12 μg/L for the BP-exposed and control groups, respectively). Low detection rates of free BPA might be related to the fact that BPA is mostly eliminated in the conjugated form, because of its rapid conjugation upon intake (Völkel et al., 2002). In fact, our results are in good agreement with those reported in a German study, in which less than 15% of the urine samples showed unconjugated BPA levels (Koch et al., 2012).

As expected, a high detection rate of total BPA (free + conjugated) was observed in urine. Total BPA was found in 306 out of 327 urine samples (94%) of both BP-exposed and control groups. The mean concentrations of total BPA per individual participant and per diet group (BP-exposed and control) over the study are summarized in Table 2. The mean

concentrations of total BPA in the FVU of exposed and control groups were not significantly different (3.29 vs. 2.11 µg/L; $p>0.05$). Afterwards, the excretion of total BPA by the BP-exposed population significantly increased in days 1 and 2 (5.24 and 6.21 µg/L, respectively). In contrast, the excretion of total BPA in the control group over the two days of the study was similar to their FVU, being 2.45 and 1.82 µg/l after 24 h and 48 h, respectively. The variability was substantially higher between individuals of the BP-exposed group than in those of the control group. It was probably due to the increase of BPA ingestion and the inherently different metabolism of the participants.

Detection rate of the current study is in accordance with those found in other investigations. In Norway, Husøy et al., (2019) detected BPA in 96% of 24-h urine samples from 144 adult volunteers who kept detailed diaries on their food consumption, the use of personal care products and handling of cash receipts. In the USA, BPA was detected in 98% of urine samples from 50 adults aged between 19 and 50 (Morgan et al., 2018), while slightly lower detection rates to those here observed were previously reported in Portugal (85%) and China (84%) (Cunha and Fernandes, 2010; Zhang et al., 2013).

Regarding urinary BPA concentrations, the geometric mean (GM) of the control group (1.64 µg/L) is in agreement with the results reported by Covaci et al. (2015), who analysed the urinary concentrations of total BPA in six European countries (Belgium, Denmark, Luxembourg, Slovenia, Spain and Sweden). The GM of BPA levels ranged between 2.55 and 1.30 µg/L, with Belgium and Sweden showing the highest and lowest GM, respectively. For the control group, the current results would fit well within the range. Contrastingly, as a potentially high-BPA diet was designed, the GM of BPA in urine of the BP-exposed group (3.73 µg/L) was notably higher than the data relative to 6 European countries (Covaci et al., 2015).

Table 2. Mean levels of total BPA in urine (µg/L) in BP-exposed and control groups during the study.

No. Participant	FVU	Day 1	Day 2
	BP-EXPOSED		
1	2.91	1.50	2.80
2	<0.2	1.96	1.76
3	1.45	6.08	10.6
5	0.95	2.52	3.43
8	4.53	7.72	8.31
9	15.7	3.25	6.75
10	0.48	3.85	3.59
18	1.78	5.01	4.30
20	2.06	3.89	6.02
21	5.74	8.31	9.73
22	3.64	8.42	14.6
23	1.94	10.5	2.60
24	3.47	3.55	6.32
25	1.45	6.75	6.06
Mean	**3.29** [a]	**5.24**[**, b]	**6.21**[***, b]
Standard deviation	3.91	2.76	3.61
Geometric mean	2.43	3.31	4.51
	CONTROL		
4	0.76	1.83	1.93
6	3.58	1.46	1.28
7	3.34	3.32	2.92
11	2.35	3.38	2.09
12	1.44	1.88	1.80
13	0.71	2.41	1.82
14	2.26	1.40	1.01
15	<0.2	1.40	1.36
16	2.39	1.84	1.15
17	3.10	3.88	1.59
26	3.29	4.14	3.10
Mean	**2.11** [a]	**2.45** [a]	**1.82** [a]
Standard deviation	1.22	1.04	0.68
Geometric mean	2.03	1.72	1.57

[*] Asterisks indicate significant differences between BP-exposed and control groups at **P < 0.01 and ***P < 0.001.
[abc] Different superscripts indicate significant differences between days (p<0.05).
FVU: First void urine.

Amount of BPA excreted

The amount of total urinary BPA, estimated from the concentration of total BPA and the volume of urine, is shown in Table 3. Before the beginning of the study, all participants of both groups, BP-exposed and control, excreted a non-significantly different amount of BPA (0.81 and 0.53 µg/day, respectively). Afterwards, people eating canned foodstuffs significantly increased the amount of total BPA excreted during the rest of the study ($p<0.001$), with urine amounts of 7.11 and 7.02 µg obtained in days 1 and 2, respectively. Unexpectedly, the control group also excreted a higher amount of total BPA through the study, reaching values of 2.84 µg and 1.89 µg after 24 h and 48 h, respectively. The analysis of total BPA levels in FVU samples in the first day confirmed that both groups were similarly exposed to BPA before starting the duplicate diet study. However, the excreted levels for the BP-exposed group were significantly higher than those corresponding to the control group. Consequently, it can be confirmed that the consumption of canned foodstuffs and food packed in polycarbonate plastics significantly increase the exposure to BPA.

The average daily exposure to BPA in the BP-exposed and control groups is summarized in Table 3. The mean daily exposure to BPA of the BP-exposed group was constant through the study: 0.11 µg/kg bw/day. This value is slightly lower than that calculated by Gonzalez et al. (2019a) for the same population, in which data on food frequencies and BPA concentrations in foodstuffs was taken into account (0.23 µg/kg bw/day in day 1, and 0.14 µg/kg bw/day in day 2). In contrast, no differences were found in the control group when comparing the current dietary exposure with that previously estimated (González et al., 2019a): 0.04 vs. 0.03 µg/kg bw/day, and 0.03 vs. 0.01 µg/kg bw/day in days 1 and 2, respectively. Differences in both estimated BPA exposures could be the result of BPA exposure through other routes, either individual or combined (e.g., dermal absorption, air inhalation, etc.), and/or the fact that BPA may be excreted through other routes, such as sweat (Genuis et al., 2012). Although BPA is mostly eliminated through

urine, it must be remarked that BPA is a lipophilic compound and, consequently, it might be partly accumulated in adipose tissue (Artacho-Cordón et al., 2018).

Table 3. Total BPA elimination and exposure for the BP-exposed and control groups through the study.

No. Participant	Total BPA elimination (µg)			Total BPA exposure (µg/kg bw/day)		
	FVU	Day 1	Day 2	FVU	Day 1	Day 2
			BP-EXPOSED			
1	1.02	3.48	3.60	0.013	0.045	0.046
2	ND	3.10	3.49	ND	0.055	0.062
3	0.39	6.59	5.73	0.006	0.100	0.087
5	0.38	7.88	7.26	0.007	0.141	0.130
8	0.79	7.69	10.6	0.012	0.118	0.162
9	3.93	6.30	5.45	0.058	0.093	0.080
10	0.087	8.52	5.75	0.002	0.158	0.106
18	0.58	3.64	6.42	0.007	0.046	0.080
20	0.41	5.89	8.60	0.008	0.118	0.172
21	0.29	14.9	11.8	0.005	0.276	0.218
22	0.87	4.77	14.3	0.013	0.071	0.213
23	1.16	9.20	3.79	0.015	0.118	0.049
24	1.09	4.89	5.80	0.014	0.061	0.073
25	0.32	12.7	5.74	0.004	0.149	0.068
Mean	**0.81**[a]	**7.11**[***, b]	**7.02**[***, b]	**0.01**	**0.11**	**0.11**
St. Dev.	0.97	3.42	3.21	0.01	0.06	0.06
			CONTROL			
4	0.08	2.19	2.25	0.001	0.035	0.036
6	1.07	2.73	2.10	0.016	0.040	0.030
7	1.09	4.17	3.34	0.012	0.048	0.038
11	0.47	3.42	1.61	0.008	0.057	0.027
12	0.30	1.97	1.05	0.004	0.029	0.015
13	0.11	1.75	1.38	0.002	0.025	0.020
14	0.59	1.97	0.50	0.011	0.037	0.009

15	ND	3.24	1.41	ND	0.044	0.019
16	0.36	2.54	1.48	0.005	0.036	0.021
17	0.50	3.31	2.36	0.009	0.060	0.043
26	1.31	3.92	3.26	0.014	0.043	0.036
Mean	**0.53**[a]	**2.84**[b]	**1.89**[c]	**0.01**	**0.04**	**0.03**
St. Dev.	0.44	0.83	0.88	0.01	0.01	0.01

[***] Asterisks indicate significant differences between BP-exposed and control groups at P < 0.001.
[abc] Different superscripts indicate significant differences between days (p<0.05).
FVU: First void urine.

In 2015, the European Food Safety Authority (EFSA) reduced the tolerable daily intake (TDI) of BPA from 50 to 4 µg/kg bw/day (EFSA, 2015). Considering this last value, none of the groups here evaluated would exceed the threshold value. However, it must be noticed that the EFSA-TDI is still temporary, being the EFSA updated assessment scheduled for 2020. Furthermore, it cannot be neglected that a number of studies have reported adverse effects of BPA - even at low doses - such as disturbed mammary gland development, changes in normal behavioural parameters, or interference in brain development and functions (Negri-Cesi, 2015; Rosenmai et al., 2014).

Finally, for the BPA exposure group, the values are similar to those reported during a 24-hour high-dietary BPA exposure study performed in the United States with 20 volunteers, who consumed a diet rich in canned foods and juices (Teeguarden et al., 2011). The volunteers' average consumption of BPA, estimated from the urinary excretion of total BPA, was 0.27 µg/kg bw/day. On the other hand, the dietary exposure to BPA of the control group would be similar to other worldwide reported values. Huang et al. (2017) estimated the levels of human BPA exposure considering urinary concentrations. Results reported exposure ranges of 0.0079-0.065 µg/kg bw/day for people living in 30 countries, with Italy and Tunisia showing the maximum and minimum levels, respectively. However, these authors found that European countries showed relatively higher exposure levels, with Italy, Sweden, Denmark, France and Cyprus in the top five (Huang et al., 2017).

The total BPA elimination curve of each participant over the study is depicted in Figure 1. Although participants who followed a diet rich in canned foods excreted higher amounts of total BPA than those in the control group, the elimination curve of both groups had a similar pattern, with a higher peak of BPA between 1 AM and 9 AM of day 2.

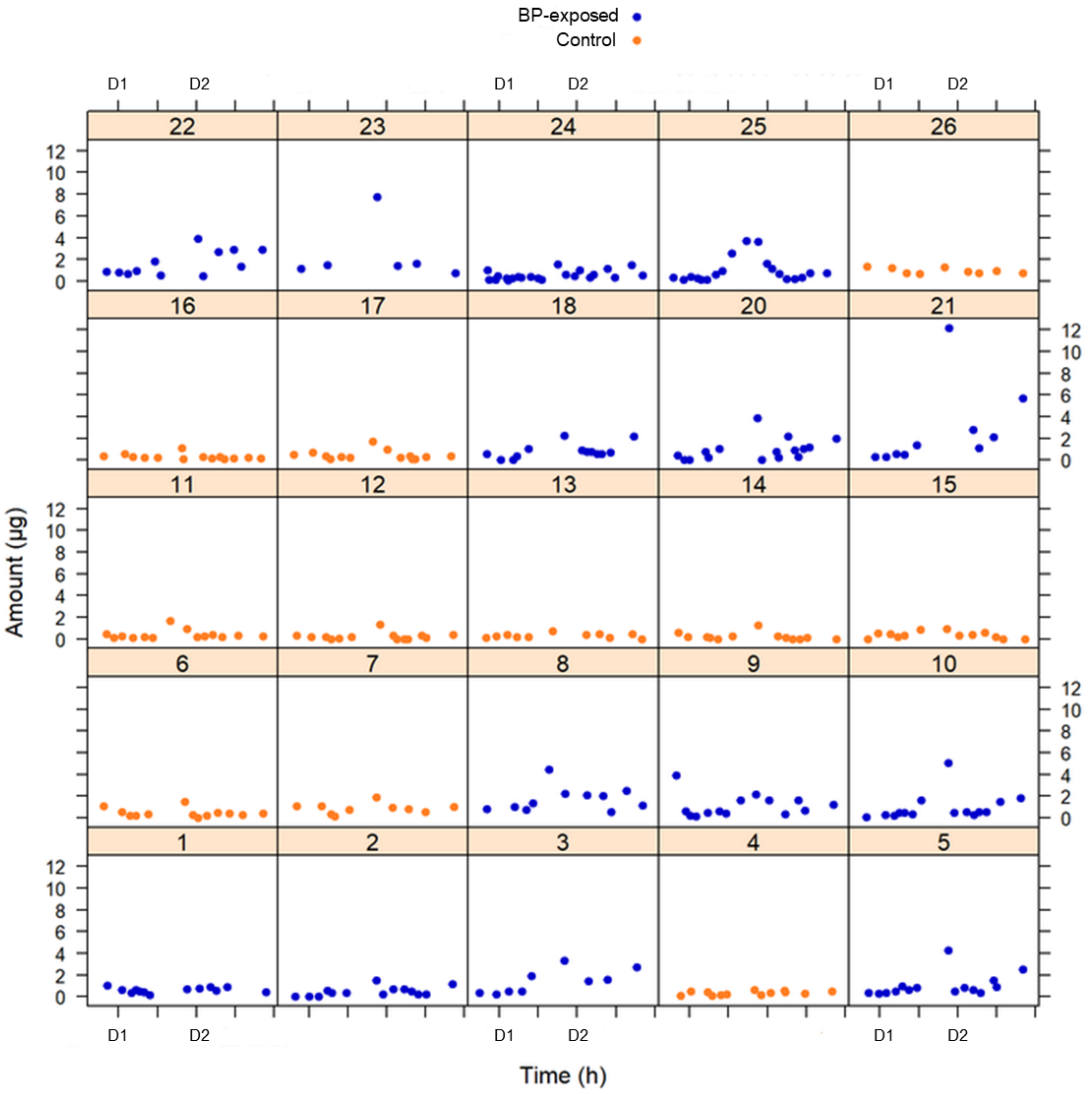

Figure 7. Total urinary BPA (µg) elimination curve for individual participants of BP-exposed and control groups during the 2-days duplicate diet study. D1: Day 1; D2: Day 2.

The comparison in the levels of total BPA excretion between both exposure groups, classified according to 4-, 12-, and 24-h intervals, are shown in Figure 2. In general terms, the wider the interval is, the lower the sensitivity to exhibit changes during the duplicate diet study, being 4 h the optimal. The R^2 of each mixed model worsens as the interval increases, proving that the optimization of the model increases inversely with the time interval. Hence, the shorter the interval, the better the excretion of total BPA is modelled (Supplementary Material, Tables S3-S4). Consequently, these results clearly confirm that

the BPA analysis of spot urine samples provides much more information than the determination of BPA in composite samples of urine. Moreover, when the collection of spot samples is not possible, urine samples should be collected in short intervals of at least 4 h or less.

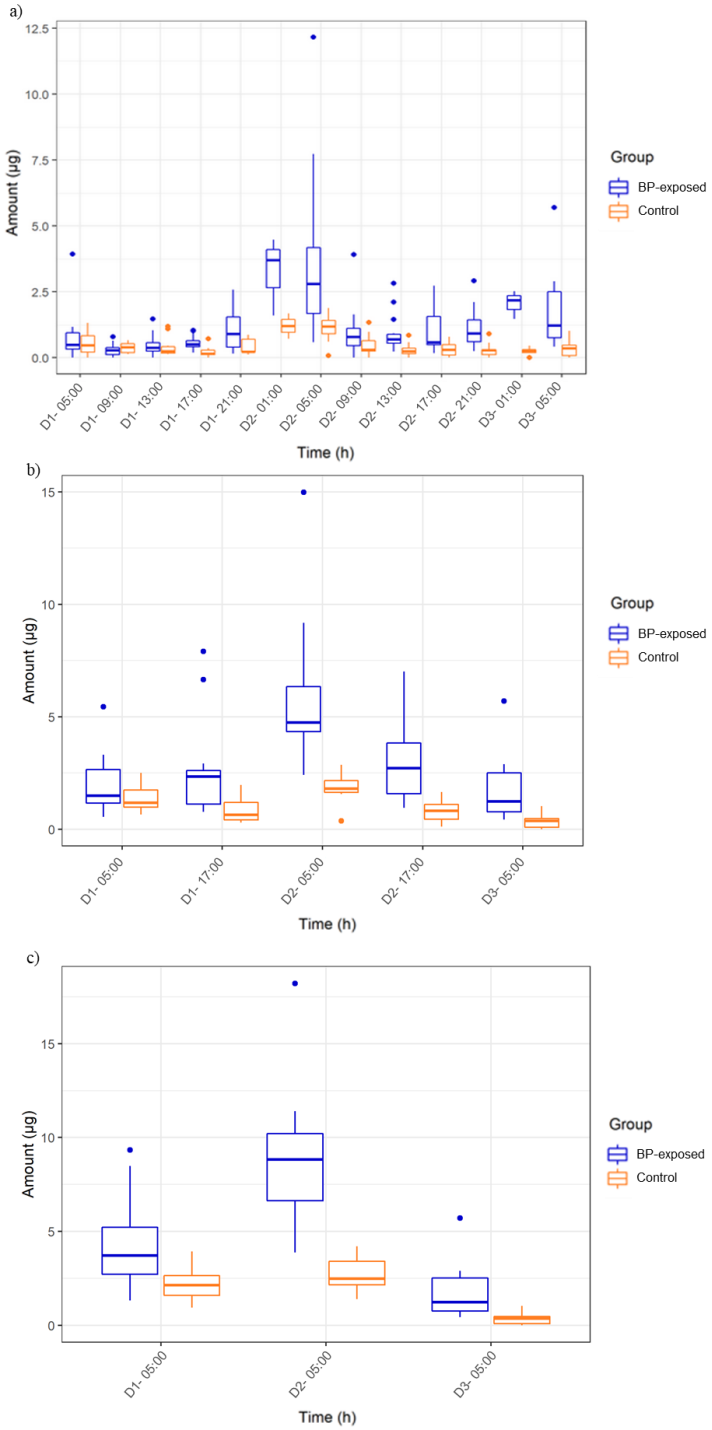

Figure 8. Total BPA elimination for BP-exposed and control groups considering a) 4-hour; b) 12-hour and c) 24-hour intervals during the duplicate diet study. D1: Day 1; D2: Day 2; D3: Day 3.

The comparison between the dietary intake and the excretion of total BPA is depicted in Figure 3. During day 1, both groups showed similar levels of total BPA. However, at the night of day 1 and, especially in the morning of day 2, the difference between both groups clearly increased. This high peak might be related to the higher intake of BPA at dinner of day 1. It must be highlighted that the sample of canned asparagus consumed by the BP-exposed population contained a BPA level that exceeded the specific migration limit of BPA (González et al., 2019a), set at 50 µg/kg by the EC (2018). On the other hand, the excretion curve of total BPA for the control group also showed a peak in the morning of day 2, which could be related to the intake of glass-packed corn containing BPA at a concentration of 4.21 µg/kg. Subsequently, the amount of excreted BPA decreased in both groups. However the BP-exposed group continued to excrete a higher amount of total BPA than those in the control group until the end of the study. The mixed model between exposed and control groups (Table 4) showed significant differences on the time-slots 1 AM – 5 AM and 5 AM – 9 AM of day 2 ($p<0.001$), confirming the strong correlation between diet and BPA exposure. Finally, the mixed model explained up to 71% of the variability (σ^2), thus proving that the inter-individual variability (τ_{00}) only contributed 17%.

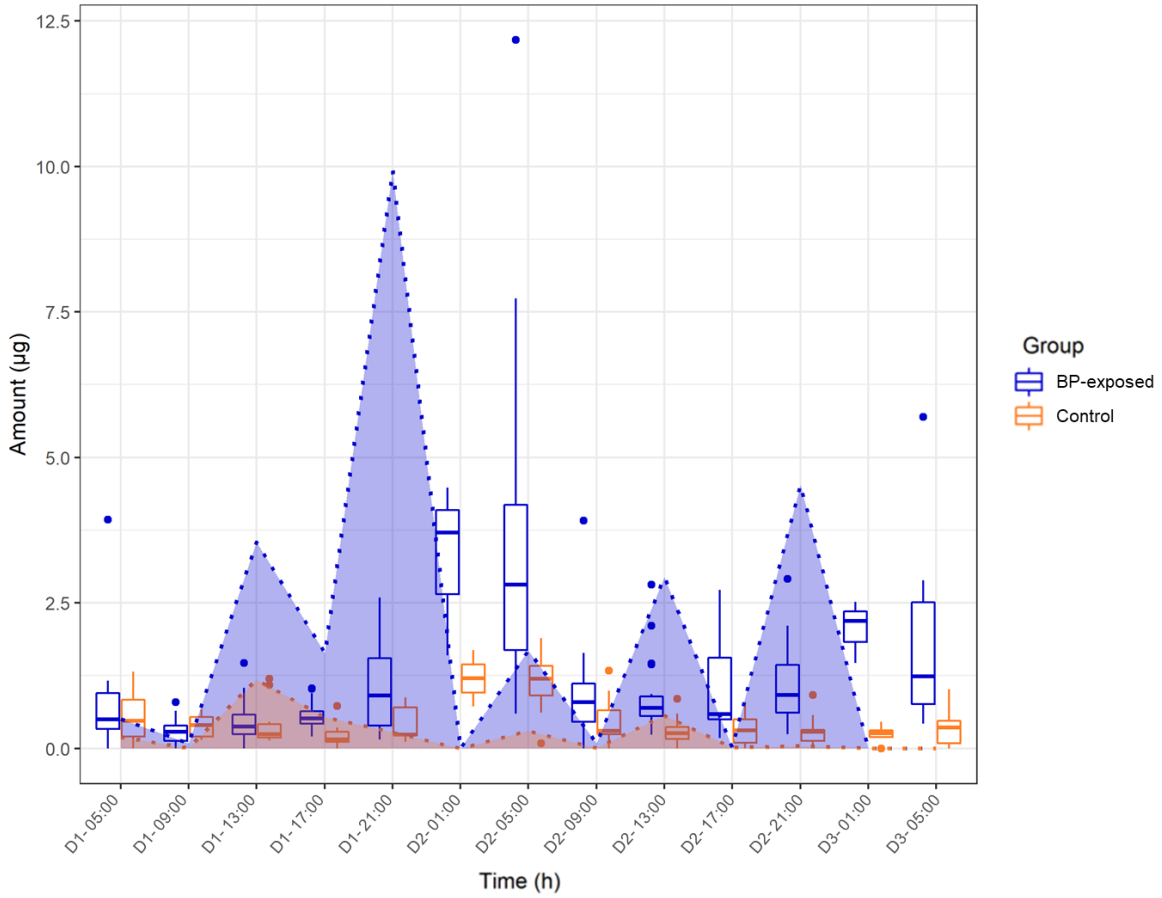

Figure 9. Total BPA elimination plus dietary intake of BPA (in dots) for BP-exposed and control groups during the duplicate diet study. D1: Day 1; D2: Day 2; D3: Day 3

Table 4. Mixed model between BP-exposed and control groups considering 4-hour intervals.

Predictors	Amount (µg)		
	Estimates	CI	*P*
Intercept	1.08	0.68 – 1.49	**<0.001**
Non-canned	-0.78	-1.15 – -0.40	**<0.001**
Day 1 09:00	-0.44	-0.90 – 0.01	0.056
Day 1 13:00	-0.29	-0.72 – 0.14	0.187
Day 1 17:00	-0.23	-0.68 – 0.21	0.297
Day 1 21:00	0.02	-0.47 – 0.50	0.947
Day 2 01:00	1.71	0.89 – 2.54	**<0.001**
Day 2 05:00	1.89	1.41 – 2.36	**<0.001**
Day 2 09:00	0.12	-0.35 – 0.59	0.613
Day 2 13:00	-0.03	-0.47 – 0.42	0.904
Day 2 17:00	-0.01	-0.45 – 0.43	0.974
Day 2 21:00	0.00	-0.46 – 0.46	0.997
Day 3 01:00	0.52	-0.20 – 1.24	0.158
Day 3 05:00	0.41	-0.06 – 0.88	0.090
Random effects			
σ^2	0.71		
τ_{00} person	0.17		
ICC person	0.19		
Observations	327		
Marginal R^2 / Conditional R^2	0.355 / 0.477		

Levels of other BPs analogues

Unconjugated BPs analogues, other than BPA, were not detected in any of the urine samples from both, BP-exposed and control groups. In turn, total BPAF, BPF and BPE could be quantified in some urine samples from individuals with a diet based on canned foodstuffs (Supplementary Material, Tables S1-S2). Traces of BPAF were found in two punctual samples (1.05 and 0.50 µg/L) of two BP-exposed participants (No. 1 and 18). These subjects indicated they had been in contact with personal care products (PCPs), which have been identified as an additional exposure pathway of relative importance for some BPs (Lu et al., 2018).

Eight samples from four participants had quantifiable levels of BPF, with a mean concentration of 6.56 µg/L. It must be remarked that one of the volunteers (No. 23) had detectable amounts of BPF in two-thirds of his urine samples, with a mean concentration

of 4.96 µg/L. According to his diary record, this subject had been handling thermal paper products, (i.e., bus tickets) and using PCPs, such as face cleansers, toothpaste or moisturizers, which potentially contain BPs (Lu et al., 2018; Rochester and Bolden, 2015).

BPE was also found in some specific samples of three participants, being the mean level of these 0.28 µg/L. Surprisingly, three of the five positive samples belonged to one single participant. However, exposure to BPE was unlikely to be linked to diet, since the food items that contained BPE (mushrooms and nuts) were consumed in the first day 1 of the duplicate diet study (González et al., 2019a), while BPE was detected in urine samples of the second day. Unlike BPAF and BPF, no information on the occurrence of BPE in daily-life products is available in the scientific literature. Therefore, further research focused on determining sources and presence of BPE in market basket products is required.

BPs in blood samples

The concentrations of BPs in blood were determined as a complementary analysis to confirm whether BPs tend to remain in blood, or they are rapidly excreted through urine Unfortunately, the detection rates of BPA and the remaining 5 BPs analogues in 3 different blood matrices (whole blood, plasma and RBC) were extremely low and random (Supplementary Material, Tables S5-S10). Therefore, it would be hard to relate these concentrations to the dietary exposure of BPs. Briefly, total BPA was detected in the whole blood of 4 participants of the BP-exposed group and 2 volunteers of the control group, while free BPA was only detected in a sample of an individual consuming canned foodstuffs. As the participant with the highest concentrations of BPA reported to smoke and take medication during the study, some interference might have occurred (He et al., 2009). In turn, BPAF was detected in the first blood sample of one participant belonging to the control group. Although BPs have been scarcely determined in whole blood, the current detection rates are much lower than those reported elsewhere (González et al., 2019b; Yamamoto et al., 2016, Zhang et al., 2013). Regarding the occurrence of BPs in plasma, total BPA was only found in three BP-exposed participants, while total BPB was detected in two samples of the BP-exposed group and one in the control group (first blood extraction). Once more, the

current detection rates of BPs in plasma were notably lower than those previously reported in the scientific literature (Cambien et al., 2020; Wiraagni et al., 2019; Kolatorova Sosvorova et al., 2017). Finally, BPA and BPAF were only detected in one RBC sample – each analogue in a different RBC sample – of the control group belonging to two different participants, being these detection rates also lower than those recently reported in Chinese adults (Jin et al., 2018).

On one hand, our uncommonly low detection rates might be related to the fact that most of the above-mentioned studies were performed by means of LC-MS/MS. Anyhow, scarce results found in whole blood, plasma and RBC fractions could be explained by the fact that BPA is a non-persistent compound in the body that is quickly metabolized. Consequently, the occurrence of BPA in blood may be several orders of magnitude lower than that in urine (Calafat et al., 2013), being concluded that blood is not a suitable biological matrix to assess the dietary exposure to BPs.

CONCLUSIONS

To the best of our knowledge, this is the first duplicate diet study assessing co-exposure to BPA and five more BP analogues from a double perspective. The dietary intake of BPs was estimated by analysing the food consumed during the duplicate diet study, while their excretion was evaluated by determining BPs in urine. Interestingly, spot urine samples, instead of 24 h composite samples, were collected and individually analysed. The results of the present investigation mean valuable information for future studies, as an optimal time-slot for urine sampling is also recommended. Additionally, BPs levels in three blood fractions were also determined as a complementary analysis.

Urine was confirmed to be the best matrix to assess the dietary exposure to BPs, especially BPA. Detection rates in urine reached up to 94%, while BPA could be only found in 6% of the blood samples. Trace levels of other BPs analogues were found only promptly, but at much lower quantities than those of BPA. FVU indicated that both groups – BP-exposed and control – were similarly exposed to BPA before starting the study, meaning that

avoiding the consumption of canned food could reduce substantially the exposure to BPs. Afterwards, exposure to BPA of the participants following a canned foodstuffs-based diet, significantly increased through time, supporting that diet plays an essential role in the exposure to BPA. BPA is a compound with a relatively short half-life in the body, since a high dietary intake of BPA is consistent with a subsequent high BPA elimination through urine. Moreover, the sampling and analysis of spot urine samples allows a better sensitivity to detect slight changes, providing more accurate information than 24 h composite samples. If individual samples cannot be collected, urine should be sampled in short periods (every 4 h or less) to avoid any potential loss of information.

Although participants following a diet rich in canned food products were more exposed to BPA than those in the control group, the temporary TDI established by the EFSA was not exceeded. However, further actions are required to ensure human health protection because the exposure to BPs has been linked to adverse health effects, even at acceptable doses.

ACKNOWLEDGEMENTS

This study was financially supported by the Spanish Ministry of Economy and Competitiveness, through MODELBIS Project (AGL2016-78942-R), as well as by the European Union, through HBM4EU project (H2020, grant agreement No. 733032). Additional funding to Sara C. Cunha and José O. Fernandes was provided by CTM/MCTES (UID/QUI/50006/2019) and FCT (IF/01616/2015). The authors thank Eudald Correig, for his excellence assistance in the statistical analysis, Dr. Sònia Abelló and Dr. Irene Maijó, for their valuable guidance and assistance in GC-MS analysis, and IISPV nurses, who carried out the blood extractions and, of course, all the study participants.

REFERENCES

Abou Omar, T., Sukhn, C., Fares, S.A., Abiad, M.G., Habib, R.R., Dhaini, H.R., 2017. Bisphenol A exposure assessment from olive oil consumption. Environ. Monit. Assess. 189. https://10.1007/s10661-017-6048-6

Albertini, R., Bird, M., Doerrer, N., Needham, L., Robinson, S., Sheldon, L., Zenick, H., 2006. The use of biomonitoring data in exposure and human health risk assessments. Environ. Health Perspect. 114, 1755–1762. https://doi.org/10.1289/ehp.9056

Artacho-Cordón, F., Fernández, M.F., Frederiksen, H., Iribarne-Durán, L.M., Jiménez-Díaz, I., Vela-Soria, F., Andersson, A.M., Martin-Olmedo, P., Peinado, F.M., Olea, N., Arrebola, J.P., 2018. Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. Environ. Int. 119, 203-2011. https://doi.org/10.1016/j.envint.2018.05.052

Ashrap, P., Watkins, D.J., Calafat, A.M., Ye, X., Rosario, Z., Brown, P., Vélez-Vega, C.M., Alshawabkeh, A., Cordero, J.F., Meeker, J.D., 2018. Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends. Environ. Int., 121, 990-1002.

Calafat, A.M., Koch, H.M., Swan, S.H., Hauser, R., Goldman, L.R., Lanphear, B.P., Longnecker, M.P., Rudel, R.A., Teitelbaum, S.L., Whyatt, R.M., Wolff, M.S., 2013. Misuse of blood serum to assess exposure to bisphenol A and phthalates. Breast Cancer Res. 15, 1–2. https://doi.org/10.1186/bcr3494

Cambien, G., Venisse, N., Migeot, V., Rabouan, S., Belmouaz, M., Binson, G., Albouy-Llaty, M., Ayraud-Thevenot, S., Dupuis, A., 2020. Simultaneous determination of bisphenol A and its chlorinated derivatives in human plasma: Development, validation and application of a UHPLC–MS/MS method. Chemosphere 242, 125236. https://doi.org/10.1016/j.chemosphere.2019.125236

Česen, M., Lenarčič, K., Mislej, V., Levstek, M., Kovačič, A., Cimrmančič, B., Uranjek, N., Kosjek, T., Heath, D., Dolenc, M.S., Heath, E., 2018. The occurrence and source identification of bisphenol compounds in wastewaters. Sci. Total Environ. 616–617, 744–752. https://doi.org/10.1016/j.scitotenv.2017.10.252

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016a. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity - A review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387

Chen, W., Shen, Y., Chen, S., 2016b. Assessing bisphenol A (BPA) exposure risk from long-term dietary intakes in Taiwan. Sci. Total Environ. 543, 140-146. https:// 10.1016/j.scitotenv.2015.11.029

Choi, S.J., Yun, E.S., Shin, J.M., Kim, Y.S., Lee, J.S., Lee, J.H., Kim, D.G., Oh, Y.H., Jung, K., Kim, G.H., 2018. Concentrations of bisphenols in canned foods and their risk assessment in Korea. J. Food Prot. 81, 903–916. https://doi.org/10.4315/0362-028x.jfp-17-447

Corrales, J., Kristofco, L.A., Baylor Steele, W., Yates, B.S., Breed, C.S., Spencer Williams, E., Brooks,

B.W., 2015. Global assessment of bisphenol a in the environment: Review and analysis of its occurrence and bioaccumulation. Dose-Response 13, 1–29. https://doi.org/10.1177/1559325815598308

Covaci, A., Hond, E. Den, Geens, T., Govarts, E., Koppen, G., Frederiksen, H., Knudsen, L.E., Mørck, T.A., Gutleb, A.C., Guignard, C., Cocco, E., Horvat, M., Heath, E., Kosjek, T., Mazej, D., Tratnik, J.S., Castaño, A., Esteban, M., Cutanda, F., Ramos, J.J., Berglund, M., Larsson, K., Jönsson, B.A.G., Biot, P., Casteleyn, L., Joas, R., Joas, A., Bloemen, L., Sepai, O., Exley, K., Schoeters, G., Angerer, J., Kolossa-Gehring, M., Fiddicke, U., Aerts, D., Koch, H.M., 2015. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. Environ. Res. 141, 77–85. https://doi.org/10.1016/j.envres.2014.08.008

Cullen, E., Evans, D., Griffin, C., Burke, P., Mannion, R., Burns, D., Flanagan, A., Kellegher, A., Schoeters, G., Govarts, E., Biot, P., Casteleyn, L., Castaño, A., Kolossa-Gehring, M., Esteban, M., Schwedler, G., Koch, H.M., Angerer, J., Knudsen, L.E., Joas, R., Joas, A., Dumez, B., Sepai, O., Exley, K., Aerts, D., 2017. Urinary phthalate concentrations in mothers and their children in Ireland: Results of the DEMOCOPHES human biomonitoring study. Int. J. Environ. Res. Public Health 14. https://doi.org/10.3390/ijerph14121456

Cunha, S.C., Cunha, C., Ferreira, A.R., Fernandes, J.O., 2012. Determination of bisphenol A and bisphenol B in canned seafood combining QuEChERS extraction with dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 404, 2453–2463. https://doi.org/10.1007/s00216-012-6389-5

Cunha, S.C., Fernandes, J.O., 2010. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta 83, 117–125. https://doi.org/10.1016/j.talanta.2010.08.048

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs : Executive summary. EFSA Journal 2015;13(1):3978

Esplugas, R., Mari, M., Marquès, M., Schuhmacher, M., Domingo, J.L, Nadal, M., 2019. Biomonitoring of trace elements in hair of schoolchildren living near a hazardous waste incinerator - A 20 years follow-up. Toxics 7, 52.

Esplugas, R., Serra, N., Marquès, M., Schuhmacher, M., Nadal, M., Domingo, J.L., 2020. Trace Elements in blood of the population living near a hazardous waste incinerator in Catalonia, Spain. Biological Trace Element Research, in press. doi: 10.1007/s12011-020-02051-9

European Commission, 2018. Commission regulation (EU) 2018/213. Off. J. Eur. Communities 6-12.

European Commission, 2017. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11813/2017.

Fattore, M., Russo, G., Barbato, F., Grumetto, L., Albrizio, S., 2015. Monitoring of bisphenols in canned tuna from Italian markets. Food Chem. Toxicol. 83, 68–75. https://doi.org/10.1016/j.fct.2015.05.010

Freire, C., Molina-Molina, J.M., Iribarne-Durán, L.M., Jiménez-Díaz, I., Vela-Soria, F., Mustieles, V., Arrebola, J.P., Fernández, M.F., Artacho-Cordón, F., Olea, N., 2019. Concentrations of bisphenol A and parabens in socks for infants and young children in Spain and their hormone-like activities. Environ. Int. 127, 592–600. https://doi.org/10.1016/j.envint.2019.04.013

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. 50, 3725–3740. https://doi.org/10.1016/j.fct.2012.07.059

Genuis, S.J., Beesoon, S., Birkholz, D., Lobo, R.A., 2012. Human excretion of bisphenol A: Blood, urine, and sweat (BUS) study. J. Environ. Public Health 2012, 185731. https://doi.org/10.1155/2012/185731

González, N., Cunha, S.C., Ferreira, R., Fernandes, J.O., Marquès, M., Nadal, M., Domingo, J.L., 2019a. Concentrations of nine bisphenol analogues in food purchased from Catalonia (Spain): Comparison of canned and non-canned foodstuffs. Food Chem. Toxicol. 110992. https://doi.org/10.1016/j.fct.2019.110992

González, N., Cunha, S.C., Monteiro, C., Fernandes, J.O., Marquès, M., Domingo, J.L., Nadal, M., 2019b. Quantification of eight bisphenol analogues in blood and urine samples of workers in a hazardous waste incinerator. Environ. Res. 176, 108576. https://doi.org/10.1016/j.envres.2019.108576

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. https://doi.org/10.1016/j.etap.2016.09.014

He, Y., Miao, M., Herrinton, L.J., Wu, C., Yuan, W., Zhou, Z., Li, D.K., 2009. Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. Environ. Res. 109, 629–633. https://doi.org/10.1016/j.envres.2009.04.003

Hu, Y., Zhu, Q., Yan, X., Liao, C., Jian, G., 2019. Occurrence, fate and risk assessment of BPA and its

substituents in wastewater treatment plant: A review. Environ. Res. 178, 108732. https://doi.org/10.1016/j.envres.2019.108732

Huang, R.-P., Liu, Z.-H., Yuan, S.-F., Yin, H., Dang, Z., Wu, P.-X., 2017. Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000–2016) and its risk analysis. Environ. Pollut. 230, 143–152. https://doi.org/10.1016/j.envpol.2017.06.026

Husøy, T., Andreassen, M., Hjertholm, H., Carlsen, M.H., Norberg, N., Sprong, C., Papadopoulou, E., Sakhi, A.K., Sabaredzovic, A., Dirven, H.A.A.M., 2019. The Norwegian biomonitoring study from the EU project EuroMix: Levels of phenols and phthalates in 24-hour urine samples and exposure sources from food and personal care products. Environ. Int. 132, 105103. https://doi.org/10.1016/j.envint.2019.105103

Ibarluzea, J., Aurrekoetxea, J.J., Porta, M., Sunyer, J., Ballester, F., 2016. La biomonitorización de sustancias tóxicas en muestras biológicas de población general. Gac. Sanit. 30, 45–54. https://doi.org/10.1016/j.gaceta.2016.02.012

Jin, H., Zhu, J., Chen, Z., Hong, Y., Cai, Z., 2018. Occurrence and partitioning of bisphenol analogues in adults' blood from China. Environ. Sci. Technol. 52, 812–820. https://doi.org/10.1021/acs.est.7b03958

Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from Liaohe River Basin and Taihu Lake, China. Water Res. 103, 343–351. https://doi.org/10.1016/j.watres.2016.07.059

Kang, J.-H., Kondo, F., Katayama, Y., 2006. Human exposure to bisphenol A (BPA). Toxicology 26, 79–89. https://doi.org/10.1016/j.reprotox.2007.07.010

Karrer, C., Andreassen, M., von Goetz, N., Sonnet, F., Sakhi, A.K., Hungerbühler, K., Dirven, H., Husøy, T., 2020. The EuroMix human biomonitoring study: Source-to-dose modeling of cumulative and aggregate exposure for the bisphenols BPA, BPS, and BPF and comparison with measured urinary levels. Environ. Int. 136, 105397.

Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.-I., Tsatsakis, A.M., Rizos, A.K., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97, 212-236.

Koch, H.M., Kolossa-Gehring, M., Schröter-Kermani, C., Angerer, J., Brüning, T., 2012. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: A retrospective exposure evaluation. J. Expo. Sci. Environ. Epidemiol. 22, 610–616. https://doi.org/10.1038/jes.2012.39

Kolatorova Sosvorova, L., Chlupacova, T., Vitku, J., Vlk, M., Heracek, J., Starka, L., Saman, D., Simkova, M., Hampl, R., 2017. Determination of selected bisphenols, parabens and estrogens in human plasma using LC-MS/MS. Talanta 174, 21–28. https://doi.org/10.1016/j.talanta.2017.05.070

Liao, C., Kannan, K., 2014. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 31, 319–329. https://doi.org/10.1080/19440049.2013.868611

Liao, C., Liu, F., Moon, H.-B., Yamashita, N., Yun, S., Kannan, K., 2012. Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: spatial and temporal distributions. Environ. Sci. Technol. 46, 11558–11565. https://doi.org/10.1021/es303191g

Liu, Y., Zhang, S., Song, N., Guo, R., Chen, M., Mai, D., Yan, Z., Han, Z., Chen, J., 2017. Occurrence, distribution and sources of bisphenol analogues in a shallow Chinese freshwater lake (Taihu Lake): Implications for ecological and human health risk. Sci. Total Environ. 599–600, 1090–1098. https://doi.org/10.1016/j.scitotenv.2017.05.069

Lu, S., Yu, Yuling, Ren, L., Zhang, X., Liu, G., Yu, Yingxin, 2018. Estimation of intake and uptake of bisphenols and triclosan from personal care products by dermal contact. Sci. Total Environ. 621, 1389–1396. https://doi.org/10.1016/j.scitotenv.2017.10.088

Martínez, M.A., Rovira, J., Sharma, R.P., Nadal, M., Schuhmacher, M., Kumar, V., 2017. Prenatal exposure estimation of BPA and DEHP using integrated external and internal dosimetry: A case study. Environ. Res. 158, 566–575. https://doi.org/10.1016/j.envres.2017.07.016

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25-34. https://doi.org/10.1016/j.envres.2018.05.008

Martínez, M., Castro, I., Rovira, J., Ares, S., Rodríguez, J.M., Cunha, S.C., Casal, S., Fernandes, J.O., Schuhmacher, M., Nadal, M., 2019. Early-life intake of major trace elements, bisphenol A, tetrabromobisphenol A and fatty acids: Comparing human milk and commercial infant formulas. Environ. Res. 169, 246-255.

Michałowicz, J., 2014. Bisphenol A - Sources, toxicity and biotransformation. Environ. Toxicol. Pharmacol. 37, 738–758. https://doi.org/10.1016/j.etap.2014.02.003

Morgan, M.K., Nash, M., Barr, D.B., Starr, J.M., Scott Clifton, M., Sobus, J.R., 2018. Distribution, variability, and predictors of urinary bisphenol A levels in 50 North Carolina adults over a six-week monitoring period. Environ. Int. 112, 85–99. https://doi.org/10.1016/j.envint.2017.12.014

Nadal, M., Mari, M., Schuhmacher, M., Domingo, J.L. Monitoring dioxins and furans in plasma of individuals living near a hazardous waste incinerator: Temporal trend after 20 years. Environ. Res. 173, 207-211. DOI: 10.1016/j.envres.2019.03.051

Negri-Cesi, P., 2015. Bisphenol A interaction with brain development and functions. Dose-Response 13, 1–12. https://doi.org/10.1177/1559325815590394

Oh, J., Choi, J.W., Ahn, Y.A., Kim, S., 2018. Pharmacokinetics of bisphenol S in humans after single oral administration. Environ. Int. 112, 127–133. https://doi.org/10.1016/j.envint.2017.11.020

Pelch, K., Wignal, J., Goldstone, A., Ross, P., Blain, R., Shapiro, A., Holmgren, S., Hsieh, J.-H., Svoboda, D., Auerbach, S., Parham, F., Masten, S., Thayer, K., 2017. NTP research report on biological activity of bisphenol A (BPA) structural analogues and functional alternatives. Natl. Toxicol. Progr. 1, 1–78.

Porras, S.P., Heinälä, M., Santonen, T., 2014. Bisphenol A exposure via thermal paper receipts. Toxicol. Lett. 230, 413–420. https://doi.org/10.1016/j.toxlet.2014.08.020

Quinete, N., Esser, A., Kraus, T., Schettgen, T., 2016. Determination of hydroxylated polychlorinated biphenyls (OH-PCBs) in human urine in a highly occupationally exposed German cohort: New prospects for urinary biomarkers of PCB exposure. Environ. Int. 97, 171-179.

Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ. Health Perspect. 123, 643–650.

Rodríguez-Gómez, R., Martín, J., Zafra-Gómez, A., Alonso, E., Vílchez, J.L., Navalón, A., 2017. Biomonitoring of 21 endocrine disrupting chemicals in human hair samples using ultra-high performance liquid chromatography–tandem mass spectrometry. Chemosphere 168, 676–684. https://doi.org/10.1016/j.chemosphere.2016.11.008

Rosenmai, A.K., Dybdahl, M., Pedersen, M., van Vugt-Lussenburg, B.M.A., Wedebye, E.B., Taxvig, C., Vinggaard, A.M., 2014. Are structural analogues to bisphenol a safe alternatives? Toxicol. Sci. 139, 35–47. https://doi.org/10.1093/toxsci/kfu030

Schuhmacher, M., Mari, M., Nadal, M., Domingo, J.L., 2019. Concentrations of dioxins and furans in breast milk of women living near a hazardous waste incinerator in Catalonia, Spain. Environ. Int. 125, 334-341. DOI: 10.1016/j.envint.2019.01.074

Song, X., Tang, S., Zhu, H., Chen, Z., Zang, Z., Zhang, Y., Niu, X., Wang, X., Yin, H., Zeng, F., He, C., 2018. Biomonitoring PFAAs in blood and semen samples: Investigation of a potential link between PFAAs exposure and semen mobility in China. Environ. Int. 113, 50-54.

Teeguarden, J.G., Calafat, A.M., Ye, X., Doerge, D.R., Churchwell, M.I., Gunawan, R., Graham, M.K.,

2011. Twenty-four hour human urine and serum profiles of bisphenol A during high-dietary exposure. Toxicol. Sci. 123, 48–57. https://doi.org/10.1093/toxsci/kfr160

Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ. Int. 83, 107–15. https://doi.org/10.1016/j.envint.2015.06.008

Tordjman, K., Grinshpan, L., Novack, L., Göen, T., Segev, D., Beacher, L., Stern, N., Berman, T., 2016. Exposure to endocrine disrupting chemicals among residents of a rural vegetarian/vegan community. Environ. Int. 97, 68-75.

Tzatzarakis, M.N., Vakonaki, E., Kavvalakis, M.P., Barmpas, M., Kokkinakis, E.N., Xenos, K., Tsatsakis, A.M., 2015. Biomonitoring of bisphenol A in hair of Greek population. Chemosphere 118, 336–341. https://doi.org/10.1016/j.chemosphere.2014.10.044

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W. V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24, 139–177. https://doi.org/10.1016/j.reprotox.2007.07.010

Vela-Soria, F., Jiménez-Díaz, I., Díaz, C., Pérez, J., Iribarne-Durán, L.M., Serrano-López, L., Arrebola, J.P., Fernández, M.F., Olea, N., 2016. Determination of endocrine-disrupting chemicals in human milk by dispersive liquid-liquid microextraction. Bioanalysis 8, 1777–1791. https://doi.org/10.4155/bio-2016-0073

Velázquez-Gómez, M., Lacorte, S., 2019. Nasal lavages as a tool for monitoring exposure to organic pollutants. Environ. Res. 178, 108726. https://doi.org/10.1016/j.envres.2019.108726

Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287. https://doi.org/10.1021/tx025548t

von Goetz, N., Pirow, R., Hart, A., Bradley, E., Poças, F., Arcella, D., Lillegard, I.T.L., Simoneau, C., van Engelen, J., Husoy, T., Theobald, A., Leclercq, C., 2017. Including non-dietary sources into an exposure assessment of the European Food Safety Authority: The challenge of multi-sector chemicals such as Bisphenol A. Regul. Toxicol. Pharmacol. 85, 70–78. https://doi.org/10.1016/j.yrtph.2017.02.004

Wiraagni, I.A., Mohd, M.A., Rashid, R. bin A., Mohamad Haron, D.E.b., 2019. Validation of a simple extraction procedure for bisphenol A identification from human plasma. PLoS One 14, 1–8. https://doi.org/10.1371/journal.pone.0221774

Xue, J., Liu, W., Kannan, K., 2017. Bisphenols, benzophenones, and bisphenol A diglycidyl ethers in

textiles and infant clothing. Environ. Sci. Technol. 51, 5279–5286. https://doi.org/10.1021/acs.est.7b00701

Yamamoto, J., Minatoya, M., Sasaki, S., Araki, A., Miyashita, C., Matsumura, T., Kishi, R., 2016. Quantifying bisphenol A in maternal and cord whole blood using isotope dilution liquid chromatography/tandem mass spectrometry and maternal characteristics associated with bisphenol A. Chemosphere 164, 25–31. https://doi.org/10.1016/j.chemosphere.2016.08.001

Zhang, T., Sun, H., Kannan, K., 2013. Blood and urinary bisphenol A concentrations in children, adults, and pregnant women from China: partitioning between blood and urine and maternal and fetal cord blood. Sci. Total Environ. 47, 4686–4694. https://doi.org/10.1016/j.scitotenv.2013.04.07

OVERALL DISCUSSION

UNIVERSITAT ROVIRA I VIRGILI
EXPOSURE ASSESMENT TO BISPHENOLS: COMBINING BIOMONITORING AND DUPLICATE DIET STUDIES
Nieves González Paradell

Bisphenols (BPs) are chemical compounds widely used in the industry for the manufacture of polycarbonate plastic, epoxy resins or thermal paper. In fact, many daily used products – such as cans, dental fillings, medical devices or toys, among others – incorporate BPs (Chen et al., 2016). BPs are chemicals of concern because of its structural similarity to 17β-estradiol. This similar structure allows BPs to interact with the hormone receptors, disrupting endocrine pathways. This hormonal interference causes a dysfunctionality of the endocrine system, leading to adverse outcomes for the human health (Rochester, 2013).

The present doctoral thesis was aimed at assessing the dietary co-exposure to BPA and other BP analogues in an adult cohort by means of a duplicate diet study.

Firstly, in order to improve the analytical method of BPs in whole blood, extraction and derivatization of samples were optimized. Thus, different sample volumes, the use of ultrasonic bath, salts, and derivatizing agents were tested. Our results showed that 1000 µL did not provide a proportional increase of the peaks, being 500 µL the most suitable sample volume. MeCN was selected as a dispersive agent in the DLLME procedure, according to the results of Cunha and Fernandes (2010). The use of ultrasonic bath was unsuccessfully tested in the dissolution step of the sample, mainly because ultrasounds break down the molecules of BPs. Besides, the addition of salts ($MgSO_4$ and $C_2H_3NaO_2$) did not improve the quantification peaks. On the other hand, acetic anhydride (AA), N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), trifluoroacetamide (TFA) and BSTFA with trimethylchlorosilane-pyridine (BSTFA:TMCS-pyridine) were tested as derivatizing agents, being AA the one showing the best performance.

GC-MS of urine samples showed better linearity for BPA, BPAF, BPE, BPF, BPB, BPZ and BPAP than BPS, while in blood the linearity of BPA, BPAF, BPE, BPF and BPB was more satisfactory than those of BPS, BPAP and BPZ.

Summarizing, it was concluded that the analysis by means of GC-MS is appropriate and it shows a good sensitivity for most BPs analogues. However, this technique would be somehow limited to analyse some specific BP analogues such as BPA. This is a polar

molecule with a sulfonyl group in its structure, even after the derivatizing of the hydroxyl groups. Thus, HPLC is better technique for BPS determination.

After the optimization, the analytical method was validated by determining the concentrations of the selected BPs in urine and blood samples from a cohort of workers from a hazardous waste incinerator (HWI). This cohort was chosen because they are already included in a parallel biomonitoring program of an occupationally-exposed population. No potential correlations between BPs and trace elements in blood and urine were found. Anyhow, results indicated that the occurrence of trace elements in urine and blood is not correlated to that of BPs.

In general terms, the exposure to BPs was not different according to the workplace or the gender. Differences in BP levels in urine or blood could be the result of different dietary patterns, since food intake – especially canned food or packed in polycarbonate plastic – has been described as the major route of exposure to this compounds. Unfortunately, information regarding their dietary habits was not available to accurately correlate the results with their food intake. Anyhow, the first chapter was aimed at validating the analytical method, which was successfully achieved.

Subsequently, the duplicate diet study was initiated in order to evaluate: i) the levels of BPs in food samples; and ii) the real dietary exposure to these compounds in a population group. As mentioned before, food (especially canned) is the most important source of BPs for the general population. In canned foodstuffs, BPs are being used as the lining that protects the food from the direct contact with the tin. Although this can lining is made to protect the food, BPs can migrate to the food and enter to the human body through the diet. Therefore, accurate analysis of food samples is needed to estimate the exposure to BPs.

With this purpose, up to 26 individuals were recruited to build the cohort, which were divided into two groups: 1) a potential high-BPA diet, consisting of the “canned food basket”, and 2) BPA-free diet, made of fresh food and food packed in glass containers and other BP-free materials, consisting of the “non-canned food basket”, and used as a control.

The cohort followed two days of balanced diet and they were able to drink as much water as they wished, always recording the source of the water (tap, bottled, etc.).

In Chapter 2, food samples from both canned and control group were analysed. Only BPA, BPB and BPE were detected, being BPA the most widespread BP analogue. In fact, BPA was detected in 14 out of 15 food items. Only canned asparagus was above the EU limit (50 µg/kg), something to explore in the future, as it could be a single event or related to a commercial brand. In fact, asparagus, because of its high BPA concentration, was likely the main responsible of the high levels of BPA found in the urine samples at night of day 1 and in the morning of day 2, as presented in Chapter 3. In the diet provided to the cohort, the dinner of day 1, contained asparagus. Afterwards, the amount of total BPA excreted decreased in both groups, but those participants of the canned group kept excreting a higher amount of total BPA than the control group until the end of the study. The mixed model between exposed and control groups showed significant differences on the time-slots 1:00am – 5:00 am and 5:00 am – 9:00am of day 2 ($p<0.001$), confirming the strong correlation between diet and urinary BPA.

Unexpectedly, BPA was found in 36% of the non-canned food samples, being non-canned mushrooms the food item with the highest concentration. In any case, the levels of BPA in canned food were higher than those observed in non-canned food ($p<0.01$), mainly because food is in direct contact with the can lining. Regarding other BPs analogues, BPB was detected in chicken and olive oil, regardless the packaging, while BPE was only found in non-canned mushrooms and nuts.

The relevant concentrations of BPs in non-canned food might be due to: i) the migration from the coating of the caps of glass bottles (Noonan et al., 2011); ii) the potential contamination during the primary production of the products (Mercogliano & Santonicola, 2018; Santonicola et al., 2018); iii) the ubiquity of plastics, related to an unexpected presence of BPs in food. Finally, BPs – other than BPA – were found at relatively low quantitites in food samples, meaning that BPA is not being replaced by BP analogues, despite the increasing regulations against this compound.

As expected, the total BPA intake for the whole two-day diet was higher for people eating canned food than for the controls. In contrast, the intake of BPB was similar, regardless the packaging of the food, while the control group ingested more BPE than the exposed group. Although the concentration of BPA in canned asparagus was above the EU limit, contributing up to 28% of the total exposure, the temporary TDI established by the EFSA was not exceeded. However, as for many EDCs, low doses are no guarantee of safety, so the potential harmful effects of BPs should not be disregarded (Tsatsakis et al., 2016).

Unfortunately, the comparison of the estimated exposure to BPB and BPE with TDI values was not possible, since threshold values have not been established for BP analogues, other than BPA. Since some BP analogues are becoming serious alternatives to BPA, international organizations should consider to start specific regulation for these new chemicals.

Subsequently, Chapter 3 was based on the analysis of BPs in biological samples of participants in the duplicate diet study. One of the 26 participants was discarded because he declared to consume food that was not provided in the food basket. This fact made clear the relevance of using food questionnaires and diaries to obtain the most detailed information about each participant. Therefore, the results of the third chapter are based on a cohort of 25 participants.

Free BPA was found only in few urine samples, being their concentrations similar in both groups, exposed and control. It also supports that BPA is mostly eliminated in the conjugated form because of its rapid conjugation upon ingestion (Völkel et al., 2002; Koch et al., 2012). The high detection rate (94%) of total BPA in urine was not surprising. Since they were told to avoid the consumption of canned foodstuffs before starting the study, participants of both groups excreted a similar concentration of total BPA in the FVU. Afterwards, the excretion of total BPA significantly increased in those participants eating canned foodstuffs, while no significant changes were noted in the control group. Anyhow, the variability was substantially higher among individuals of the exposed group than in those not consuming canned foodstuffs, very probably due to the increase of BPA ingestion, as well as metabolism variability.

The detection rate of total BPA here found in the control group is in accordance with that observed in previous studies (Husøy et al., 2019; Morgan et al., 2018; Cunha & Fernandes, 2010; Zhang et al., 2013). In turn, the urinary concentrations of BPA for the exposed participants are higher than those reported corresponding to typical calues in Europe (Covaci et al., 2015). It is likely to be because a potentially high-BPA diet was hereby designed. The amount of total BPA excreted through urine of exposed participants was significantly higher than that of the control, confirming that the consumption of canned food and foodstuffs packed in polycarbonate plastics significantly increase the exposure to BPA. Interestingly, the participants of the control group excreted a higher amount of BPA during the study than in the FVU.

The estimated dietary exposure (Chapter 2) for the exposed group was 2-fold higher in day 1 and 1.3-fold higher in day 2 than the real exposure calculated from the urinary BPA (Chapter 3). On the other hand, for the control group, urinary BPA values revealed that 75% of the total exposure in day 1 came from the diet. The dietary contribution to total exposure lowered to 33% in day 2. Differences in both estimated and real BPA exposures could be the result of: i) the potential exposure to BPA through other routes, such as dermal absortion or air inhalation; ii) the potential excretion of BPA through other elimination pathways, like sweat (Genuis et al., 2012); and/or iii) the potential bioaccumulation of BPA in adipose tissue (Artacho-Cordón et al., 2018).

Finally, the dietary exposure to BPA of the exposed population was similar to values reported worldwide, while that of the control group was similar to countries with diet based on traditional cuisine, such as Tunisia (Huang et al., 2017). This substantial difference might be related to the fact that the diet of the control group avoids the consumption of canned food, while that of other studies might contain both canned and non-canned foodstuffs. Moreover, the exposure to this group of compounds – mostly used in food products – is more likely to happen in developed countries compared to developing countries, because of the higher consumption rates of canned or processed food. Therefore, it is reasonable that European countries present higher exposure levels to BPA. Anyhow, even when a

potentially high-BPA diet was designed, the average daily exposures to BPA are clearly below the temporary TDI (4 µg/kg bw/day) established by EFSA, (2015). Although this threshold is not definitive, with the actual TDI value, we can consider that the current exposure to BPA does not pose a risk for the human health, even when consuming a potentially high-BPA diet.

Currently, EFSA is undergoing a re-evaluation of the TDI for the BPA along with its exposure effects to the human population which is expected to be delivered later in 2020.

Anyway, it is also remarkable that low doses of BPA have been reported to cause some adverse health effects, such as allergic pulmonary inflammation, airway hyperresponsiveness and hormone receptor downregulation (Yanagisawa et al., 2019). In addition, a feminization of the male offspring along with a decreased sperm count was also reported (Mandrup et al., 2016).

Furthermore, the chronic exposure to BPA should be also taken into account because humans are permanently exposed to low concentrations of BPA. This chronic exposure to low doses of BPA has been found to affect sperm production and motility. Moreover, male hormones, like testosterone can be reduced, while female hormones, like estradiol can be increased (Ullah et al., 2018).

In duplicate diet studes, the socioeconomic level is not an important factor because all the consumed food is provided in a food basket. Although this issue should be a critical consideration when performing larger biomonitoring studies. In general, people with lower incomes have higher BPA levels (Nelson et al., 2012). This could be the result of a limited access to fresh food, and a higher consumption of ready-to-eat food or processed food, such as canned food (Ruiz et al., 2017; Nelson et al., 2012). Higher BPA levels were even reported for people who had low food security and received emergency food assistance, which is likely to contain canned food (Nelson et al., 2012). Therefore, attention should be paid to the most vulnerable part of the society.

When modelling urinary BPA elimination, the amount of total BPA excretion in spot urine samples, as well as 4- 12- and 24-hours intervals was compared. It was observed that the wider the interval is, the lower the sensitivity to exhibit changes during the duplicate diet study. Thus, the 4h-interval was the optimal interval of urine sampling. In parallel, R^2 of the mixed model worsens as the interval increases, proving that the shorter the interval is, the better the excretion of total BPA is modelled. This finding was relevant and should be further considered when assessing BPA exposure. Most studies consider only 24-h urine composites samples, instead of point sample. In these, the elimination curve might not be as accurate as with shorter intervals. According to our calculations, urine samples of 4-h or less should be collected and alaysed in order to avoid any loss of sensitivity.

Biomonitoring data could be useful to validate physiologically based pharmacokinetic (PBPK) models, which predicts the absortion, distribution, metabolism and excretion (ADME) of BPA in the human body compartments without the need to analyse biological tissues (Martínez et al., 2018; Sharma et al., 2018).

Unconjugated BPs analogues, other than BPA, were not detected in any of the urine samples. In turn, total BPAF, BPF and BPE were detected in few samples from the exposed group. Anyhow, the results of BPs in foodstuffs indicated that the occurrence of these BPs analogues was not related to the consumption of canned products, so it is likely to happen as a consequence of using personal care products – such as face cleansers, toothpaste or moisturizers – or handling thermal paper, like bus tickets (Lu et al., 2018; Rochester and Bolden, 2015).

Finally, the assessment of BPs in blood, being differentiated in three matrices, was carried out as a complementary analysis to understand whether certain BPs tend to remain in blood, instead of being rapidly excreted through urine. However, the detection rate of the 6 BPs analogues (BPA, BPE, BPB, BPF, BPAF, BPZ) in the three blood matrices was extremely low and random, being hardly related to the dietary exposure. Anyhow, it should be noted that the participant with the highest concentrations of BPA declared to smoke and take medication during the study, so, some kind of interference might have occurred (He et al.,

2009). Probably, non-smokers and healthy population should have been an additional eligibility criterion in order to avoid potential interferences coming from these type of sources.

Our uncommonly low detection rates might be related to the fact that in the scientific literature BPs analysis are usually performed by means of LC-MS/MS instead of GC-MS. However, scarce results found in whole blood, plasma and RBC fractions could be explained by the fact that BPA is a non-persistent compound in the body and it is quickly metabolized. Consequently, the occurrence of BPA in blood can be several orders of magnitude lower than in urine (Calafat et al., 2015). Because of that, it can be concluded that blood is not a suitable biological matrix to assess the exposure to BPs.

In general, BP analogues – other than BPA – are starting to be detected in several matrices, like urine or food, according to recent data from the scientific literature. The increasing trend of replacing BPA with other analogues is likely to increase the human exposure to a mixtures of BPs and possibly other endocrine-disrupting chemicals. The combined exposure to chemical “cocktails”, including BPs and many other chemical agents, is the current reality, so understanding of this co-exposure is a need to highlight BPs mechanisms of action and the potential adverse health effects resulting from this co-exposure. Moreover, taking into account that some of BPA alternatives have been found to be as toxic as BPA, it is not unreasonable to demand a proper regulation taking all bisphenols as a group and not only as individual compounds. Great efforts have been done to regulate BPA in Europe, including a new report re-evaluating toxicological data expected to be ready later in 2020. Nonetheless, because of a lack of reliable toxicological data, the regulation of other bisphenol analogues is not expected in a short term.

CONCLUSIONS

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Specific conclusions

1. GC-MS is a suitable analytical technique to identify and quantify BPs in biological and food tissues. However, it may be less sensitive for certain BPs analogues than other analytical techniques.
2. BPA is a largely widespread food processing contaminant, regardless the material of food packaging. Anyhow, its occurrence in canned foodstuffs is higher than in fresh or glass-packed food.
3. Canned asparagus surpassed the migration limit set by the European Commission. This fact should be further investigated to confirm whether it is a single or common event, so proper decisions must be made accordingly.
4. BPB and BPE were also detected in food, although in a much lower rate than BPA. No traces of other BP analogues (BPS, BPAF, BPZ, BPF, BPAP) could be found in any food sample.
5. BPA was also the main compound excreted through urine during the duplicate diet study. Traces of the other BPs were detected in point and random urine samples, being not related to dietary exposure. However, the use of personal care products and handling thermal paper could have been a role.
6. The low detection rate of BPs in blood confirmed that this biological matrix is not suitable for the biomonitoring of BPs.
7. The dietary intake of BPA through canned food was significantly correlated with a higher exposure to BPA.
8. The real exposure, calculated from urinary BPA, was similar to that estimated from the dietary ingestion for both exposure groups. Little differences between real and estimated values could be the result of the potential exposure and/or excretion of BPA through other routes.
9. When the collection and analysis of spot urine samples is not possible, urine should be collected by considering 4-h intervals. Wider intervals (12- and 24-h) show less sensitivity to detect potential changes in the excretion of BPA.

10. The calculated exposure to BPA accounted less than the 3% of the t-TDI. Therefore, according to EFSA, a diet rich in canned foodstuffs should not pose a risk to human health. Anyhow, this t-TDI will be updated in 2020, and the conclusion of no-risk should be revised. In any case, it is well known that a chronic exposure to BPs may represent a long-term risk for human health, even at low doses.
11. Since BPs analogues may own a similar structure and estrogenic effects to BPA, regulation should be aimed at BPs as a mixture instead of only BPA. Moreover, synergistic, additive and antagonistic effects derived from the co-exposure to BPs should be also considered.

General conclusion

BPA is ubiquitously present in foodstuffs, being its content higher in canned than in fresh or glass-packed food. The consumption of canned food leads to a higher exposure to BPA, although at levels below the TDI established by the EFSA. In any case, special attention must be paid to BPA and other BP analogues because their exposure has been related to adverse health effects, even at low doses. Interestingly, the occurrence of other analogues in food and urine is hardly linked to canned foodstuffs and their consumption, being the use of personal care products and thermal paper other potential exposure pathways. Finally, the rising occurrence of other unregulated BPs is likely to increase human exposure to a mixture of BPs, this increasing their potential endocrine-disrupting effects. Consequently, an integral legislation covering not only BPA but also other BP analogues should be developed by regulatory organizations.

REFERENCES

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Almeida, S., Raposo, A., Almeida-González, M., Carrascosa, C., 2018. Bisphenol A: Food Exposure and Impact on Human Health. Compr. Rev. Food Sci. Food Saf. 17, 1503–1517. https://doi.org/10.1111/1541-4337.12388

Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F., 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. J. AOAC Int. 86, 412–431.

Anatomy medicine, 2019. The endocrine system. Available online at: https://anatomy-medicine.com/endocrine-system/88-the-endocrine-system.html. Accessed on: 20/02/2020.

Andra, S.S., Charisiadis, P., Arora, M., van Vliet-Ostaptchouk, J. V., Makris, K.C., 2015. Biomonitoring of human exposures to chlorinated derivatives and structural analogs of bisphenol A. Environ. Int. 85, 352–379. https://doi.org/10.1016/j.envint.2015.09.011

Apel, P., Angerer, J., Wilhelm, M., Kolossa-Gehring, M., 2017. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German Human Biomonitoring Commission. Int. J. Hyg. Environ. Health 220, 152–166. https://doi.org/10.1016/j.ijheh.2016.09.007

Artacho-Cordón, F., Fernández, M.F., Frederiksen, H., Iribarne-Durán, L.M., Jiménez-Díaz, I., Vela-Soria, F., Andersson, A.M., Martin-Olmedo, P., Peinado, F.M., Olea, N., Arrebola, J.P., 2018. Environmental phenols and parabens in adipose tissue from hospitalized adults in Southern Spain. Environ. Int. 119, 203–211. https://doi.org/10.1016/j.envint.2018.05.052

Azzouz, A., Rascón, A.J., Ballesteros, E., 2016. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. J. Pharm. Biomed. Anal. 119, 16–26. https://doi.org/10.1016/j.jpba.2015.11.024

Bertoli, S., Leone, A., Battezzati, A., 2015. Human Bisphenol A Exposure and the "'Diabesity Phenotype.'" Dose-Response 13, 1–12. https://doi.org/10.1177/1559325815599173

Biles, J.E., McNeal, T.P., Begley, T.H., Hollifield, H.C., 1997. Determination of bisphenol-A in reusable polycarbonate food-contact plastics and migration to to food-simulating liquids. J. Agric. Food Chem. 45, 3541–3544. https://doi.org/10.1021/jf970072i

Björnsdotter, M.K., de Boer, J., Ballesteros-Gómez, A., 2017a. Bisphenol A and replacements in thermal paper: A review. Chemosphere 182, 691–706. https://doi.org/10.1016/j.chemosphere.2017.05.070

Björnsdotter, M.K., Jonker, W., Legradi, J., Kool, J., Ballesteros-Gómez, A., 2017b. Bisphenol A alternatives in thermal paper from the Netherlands, Spain, Sweden and Norway. Screening and potential toxicity. Sci. Total Environ. 601–602, 210–221. https://doi.org/10.1016/j.scitotenv.2017.05.171

Boucher, J.G., Boudreau, A., Ahmed, S., Atlas, E., 2015. In Vitro Effects of Bisphenol A β-D-Glucuronide (BPA-G) on Adipogenesis in Human and Murine Preadipocytes. Env. Heal. Perspect 123, 1287–1293.

Bramwell, L., Mortimer, D., Rose, M., Fernandes, A., Harrad, S., Pless-Mulloli, T., 2017. UK dietary exposure to PCDD/Fs, PCBs, PBDD/Fs, PBBs and PBDEs: comparison of results from 24-h duplicate diets and total diet studies. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 34, 65–77. https://doi.org/10.1080/19440049.2016.1258493

Brotons, J.A., Olea-serrano, M.F., Villalobos, M., Pedraza, V., Olea, N., 1995. Xenoestrogens Released from Lacquer Coatings in Food Cans. Environ. Health Perspect. 103, 608–612.

Calafat, A.M., Koch, H.M., Swan, S.H., Hauser, R., Goldman, L.R., Lanphear, B.P., Longnecker, M.P., Rudel, R.A., Teitelbaum, S.L., Whyatt, R.M., Wolff, M.S., 2013. Misuse of blood serum to assess exposure to bisphenol A and phthalates. Breast Cancer Res. 15, 1–2. https://doi.org/10.1186/bcr3494

Cao, X.L., Perez-Locas, C., Dufresne, G., Clement, G., Popovic, S., Beraldin, F., Dabeka, R.W., Feeley, M., 2011. Concentrations of bisphenol a in the composite food samples from the 2008 Canadian total diet study in Quebec City and dietary intake estimates. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 28, 791–798. https://doi.org/10.1080/19440049.2010.513015

Careghini, A., Mastorgio, A.F., Saponaro, S., Sezenna, E., 2015. Bisphenol A, nonylphenols, benzophenones, and benzotriazoles in soils, groundwater, surface water, sediments, and food: a review. Environ. Sci. Pollut. Res. 22, 5711–5741. https://doi.org/10.1007/s11356-014-3974-5

Česen, M., Lenarčič, K., Mislej, V., Levstek, M., Kovačič, A., Cimrmančič, B., Uranjek, N., Kosjek, T., Heath, D., Dolenc, M.S., Heath, E., 2018. The occurrence and source identification of bisphenol compounds in wastewaters. Sci. Total Environ. 616–617, 744–752. https://doi.org/10.1016/j.scitotenv.2017.10.252

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.L., Wu, Y., Widelka, M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity - A review. Environ. Sci. Technol. 50, 5438–5453. https://doi.org/10.1021/acs.est.5b05387

Chen, M.Y., Ike, M., Fujita, M., 2002. Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. Environ. Toxicol. 17, 80–86. https://doi.org/10.1002/tox.10035

Choi, S.J., Yun, E.S., Shin, J.M., Kim, Y.S., Lee, J.S., Lee, J.H., Kim, D.G., Oh, Y.H., Jung, K., Kim, G.H., 2018. Concentrations of Bisphenols in Canned Foods and Their Risk Assessment in Korea. J. Food Prot. 81, 903–916. https://doi.org/10.4315/0362-028x.jfp-17-447

Combarnous, Y., Diep Nguyen, T.M., 2019. Comparative overview of the mechanisms of action of hormones and endocrine disruptor compounds. Toxics 7, 5–14. https://doi.org/10.3390/toxics7010005

Conroy-Ben, O., Garcia, I., Teske, S.S., 2018. In silico binding of 4,4'-bisphenols predicts in vitro estrogenic and antiandrogenic activity. Environ. Toxicol. 33, 569–578. https://doi.org/10.1002/tox.22539

Corrales, J., Kristofco, L.A., Baylor Steele, W., Yates, B.S., Breed, C.S., Spencer Williams, E., Brooks, B.W., 2015. Global assessment of bisphenol a in the environment: Review and analysis of its occurrence and bioaccumulation. Dose-Response 13, 1–29. https://doi.org/10.1177/1559325815598308

Covaci, A., Hond, E. Den, Geens, T., Govarts, E., Koppen, G., Frederiksen, H., Knudsen, L.E., Mørck, T.A., Gutleb, A.C., Guignard, C., Cocco, E., Horvat, M., Heath, E., Kosjek, T., Mazej, D., Tratnik, J.S., Castaño, A., Esteban, M., Cutanda, F., Ramos, J.J., Berglund, M., Larsson, K., Jönsson, B.A.G., Biot, P., Casteleyn, L., Joas, R., Joas, A., Bloemen, L., Sepai, O., Exley, K., Schoeters, G., Angerer, J., Kolossa-Gehring, M., Fiddicke, U., Aerts, D., Koch, H.M., 2015. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. Environ. Res. 141, 77–85.

https://doi.org/10.1016/j.envres.2014.08.008

Cunha, S.C., Alves, R.N., Fernandes, J.O., Casal, S., Marques, A., 2017. First approach to assess the bioaccessibility of bisphenol A in canned seafood. Food Chem. 232, 501–507. https://doi.org/10.1016/j.foodchem.2017.04.006

Cunha, S.C., Cunha, C., Ferreira, A.R., Fernandes, J.O., 2012. Determination of bisphenol A and bisphenol B in canned seafood combining QuEChERS extraction with dispersive liquid-liquid microextraction followed by gas chromatography-mass spectrometry. Anal. Bioanal. Chem. 404, 2453–2463. https://doi.org/10.1007/s00216-012-6389-5

Cunha, S.C., Fernandes, J.O., 2010. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta 83, 117–125. https://doi.org/10.1016/j.talanta.2010.08.048

De Blasio, C., 2019. Chromatography. Fundam. Biofuels Eng. Technol. 103–125. https://doi.org/10.1007/978-3-030-11599-9

Demierre, A.L., Peter, R., Oberli, A., Bourqui-Pittet, M., 2012. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. Toxicol. Lett. 213, 305–308. https://doi.org/10.1016/j.toxlet.2012.07.001

DG GROW (Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs), 2015. Meeting with PlasticsEurope on BPA.

Dodds, E.C., Lawson, W., 1938. Molecular structure in relation to oestrogenic activity. Compounds without a phenanthrene nucleus. Proc. R. Soc. London. Ser. B - Biol. Sci. 125, 222–232. https://doi.org/10.1098/rspb.1938.0023

Dodds, E.C., Lawson, W., 1936. Synthetic strogenic agents without the phenanthrene nucleus. Nature 996.

Dualde, P., Pardo, O., Corpas-Burgos, F., Kuligowski, J., Gormaz, M., Vento, M., Pastor, A., Yusà, V., 2019. Biomonitoring of bisphenols A, F, S in human milk and probabilistic risk assessment for breastfed infants. Sci. Total Environ. 668, 797–805. https://doi.org/10.1016/j.scitotenv.2019.03.024

Duan, Y., Yao, Y., Wang, B., Han, L., Wang, L., Sun, H., Chen, L., 2018. Association of urinary concentrations of bisphenols with type 2 diabetes mellitus: A case-control study. Environ. Pollut. 1719–1726. https://doi.org/10.1016/j.envpol.2018.09.093

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA J. 13, 3978. https://doi.org/10.2903/j.efsa.2015.3978

EFSA, 2006. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). EFSA J. 428, 1–75.

EFSA , 2011. Towards a harmonised total diet study approach : A guidance document. EFSA.

Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S., Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol A story: Bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil. Steril. 103, 11–21. https://doi.org/10.1016/j.fertnstert.2014.11.005

Errico, S., Bianco, M., Mita, L., Migliaccio, M., Rossi, S., Nicolucci, C., Menale, C., Portaccio, M., Gallo, P., Mita, D.G., Diano, N., 2014. Migration of bisphenol A into canned tomatoes produced in Italy: Dependence on temperature and storage conditions. Food Chem. 160, 157–164. https://doi.org/10.1016/j.foodchem.2014.03.085

European Comission, 2016. COMMISSION REGULATION (EU) 2016/2235 of 12 December 2016 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regar. Off. J. Eur. Union 2001, 20–30. https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf

European Commission, 2018. Commission Regulation (EU) 2018/832 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materi. Off. J. Eur. Union 2001, 20–30. https://doi.org/http://eur-lex.europa.eu/pri/en/oj/dat/2003/l_285/l_28520031101en00330037.pdf

European Commission, 2017. Commission Directive (EU) 2017/898 of 24 May 2017 amending, for the purpose of adopting specific limit values for chemicals used in toys, Appendix C to Annex II to Directive 2009/48/EC of the European Parliament and of the Council on the safety of toys, a. Off. J. Eur. Union 138, 128–130.

European Commission, 2011. Commission Directive 2011/8/EU. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE (Bisphenol A). Off. J. Eur. Communities 29–32.

Fattore, M., Russo, G., Barbato, F., Grumetto, L., Albrizio, S., 2015. Monitoring of bisphenols in canned tuna from Italian markets. Food Chem. Toxicol. 83, 68–75. https://doi.org/10.1016/j.fct.2015.05.010

Feng, Y., Jiao, Z., Shi, J., Li, M., Guo, Q., Shao, B., 2016. Effects of bisphenol analogues on steroidogenic gene expression and hormone synthesis in H295R cells. Chemosphere 147, 9–19. https://doi.org/10.1016/j.chemosphere.2015.12.081

Freire, C., Molina-Molina, J.M., Iribarne-Durán, L.M., Jiménez-Díaz, I., Vela-Soria, F., Mustieles, V., Arrebola, J.P., Fernández, M.F., Artacho-Cordón, F., Olea, N., 2019. Concentrations of bisphenol A and parabens in socks for infants and young children in Spain and their hormone-like activities. Environ. Int. 127, 592–600. https://doi.org/10.1016/j.envint.2019.04.013

Fromme, H., Schlummer, M., Möller, A., Gruber, L., Wolz, G., Ungewiss, J., Böhmer, S., Dekant, W., Mayer, R., Liebl, B., Twardella, D., 2007. Exposure of an adult population to perfluorinated substances using duplicate diet portions and biomonitoring data. Environ. Sci. Technol. 41, 7928–7933. https://doi.org/10.1021/es071244n

Gao, H., Yang, B.-J., Li, N., Feng, L.-M., Shi, X.-Y., Zhao, W.-H., Liu, S.-J., 2015. Bisphenol A and hormone-associated cancers: current progress and perspectives. Medicine (Baltimore). 94, e211. https://doi.org/10.1097/MD.0000000000000211

García Ibarra, V., Sendón, R., Bustos, J., Paseiro Losada, P., Rodríguez Bernaldo de Quirós, A., 2019. Estimates of dietary exposure of Spanish population to packaging contaminants from cereal based foods contained in plastic materials. Food Chem. Toxicol. 128, 180–192. https://doi.org/10.1016/j.fct.2019.04.003

Garcia, T., Schreiber, E., Kumar, V., Prasad, R., Sirvent, J.J., Domingo, J.L., Gómez, M., 2017. Effects on the reproductive system of young male rats of subcutaneous exposure to n-butylparaben. Food Chem. Toxicol. 106, 47–57. https://doi.org/10.1016/j.fct.2017.05.031

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J., Covaci, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem. Toxicol. 50, 3725–3740. https://doi.org/10.1016/j.fct.2012.07.059

Geens, T., Apelbaum, T.Z., Goeyens, L., Neels, H., Covaci, A., 2010. Intake of bisphenol A from canned beverages and foods on the Belgian market. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 27, 1627–1637. https://doi.org/10.1080/19440049.2010.508183

Genuis, S.J., Beesoon, S., Birkholz, D., Lobo, R.A., 2012. Human excretion of bisphenol A: Blood, urine, and sweat (BUS) study. J. Environ. Public Health 2012. https://doi.org/10.1155/2012/185731

Gerona, R.R., Pan, J., Zota, A.R., Schwartz, J.M., Friesen, M., Taylor, J.A., Hunt, P.A., Woodruff, T.J., 2016. Direct measurement of Bisphenol A (BPA), BPA glucuronide and BPA sulfate in a diverse and low-income population of pregnant women reveals high exposure, with potential implications for previous exposure estimates: A cross-sectional study. Environ. Heal. A Glob. Access Sci. Source 15, 1–14. https://doi.org/10.1186/s12940-016-0131-2

González, N., Calderón, J., Rúbies, A., Timoner, I., Castell, V., Domingo, J.L., Nadal, M., 2019. Dietary intake of arsenic , cadmium , mercury and lead by the population of Catalonia , Spain : Analysis of the temporal trend. Food Chem. Toxicol. 132, 110721. https://doi.org/10.1016/j.fct.2019.110721

Gostner, J.M., Raggl, E., Becker, K., Überall, F., Schennach, H., Pease, J.E., Fuchs, D., 2015. Bisphenol A suppresses Th1-type immune response in human peripheral blood mononuclear cells in vitro. Immunol. Lett. 168, 285–292. https://doi.org/10.1016/j.imlet.2015.10.006

Government of Canada. 2010. Canada Gazette, Part II: Final Order Adding Bisphenol A, a Substance in Batch 2 of the Challenge, to Schedule 1 to the Canadian Environmental Protection Act, 1999

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199.

https://doi.org/10.1016/j.etap.2016.09.014

Grześkowiak, T., Czarczyńska-Goślińska, B., Zgoła-Grześ Kowiak, A., 2018. Biodegradation of selected endocrine disrupting compounds 1–27. https://doi.org/10.1007/978-1-4939-7425-2

Guerriero, G., 2009. Vertebrate sex steroid receptors: Evolution, ligands, and neurodistribution. Ann. N. Y. Acad. Sci. 1163, 154–168. https://doi.org/10.1111/j.1749-6632.2009.04460.x

Hampl, R., Kubátová, J., Stárka, L., 2016. Steroids and endocrine disruptors - History, recent state of art and open questions. J. Steroid Biochem. Mol. Biol. 155, 217–223. https://doi.org/10.1016/j.jsbmb.2014.04.013

Hashemi, B., Zohrabi, P., Kim, K.H., Shamsipur, M., Deep, A., Hong, J., 2017. Recent advances in liquid-phase microextraction techniques for the analysis of environmental pollutants. TrAC - Trends Anal. Chem. 97, 83–95. https://doi.org/10.1016/j.trac.2017.08.014

Hayashi, A., Sato, F., Imai, T., Yoshinaga, J., 2019. Daily intake of total and inorganic arsenic, lead, and aluminum of the Japanese: Duplicate diet study. J. Food Compos. Anal. 77, 77–83. https://doi.org/10.1016/j.jfca.2019.01.009

He, Y., Miao, M., Herrinton, L.J., Wu, C., Yuan, W., Zhou, Z., Li, D.K., 2009. Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. Environ. Res. 109, 629–633. https://doi.org/10.1016/j.envres.2009.04.003

Healy, B.F., English, K.R., Jagals, P., Sly, P.D., 2015. Bisphenol A exposure pathways in early childhood: Reviewing the need for improved risk assessment models. J. Expo. Sci. Environ. Epidemiol. 25, 544–556. https://doi.org/10.1038/jes.2015.49

Hu, J., Zhao, H., Braun, J.M., Zheng, T., Zhang, B., Xia, W., Zhang, W., Li, Jiufeng, Zhou, Y., Li, H., Li, Jun, Zhou, A., Zhang, Y., Buka, S.L., Liu, S., Peng, Y., Wu, C., Jiang, M., Huo, W., Zhu, Y., Shi, K., Li, Y., Cai, Z., Xu, S., 2019. Associations of Trimester-Specific Exposure to Bisphenols with Size at Birth: A Chinese Prenatal Cohort Study. Environ. Health Perspect. 127, 107001. https://doi.org/10.1289/ehp4664

Huang, R. ping, Liu, Z. hua, Yuan, S. fen, Yin, H., Dang, Z., Wu, P. xiao, 2017. Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000–2016) and its risk analysis. Environ. Pollut. 230, 143–152.

https://doi.org/10.1016/j.envpol.2017.06.026

Huang, S., Li, J., Xu, S., Zhao, H., Li, Y., Zhou, Y., Fang, J., Liao, J., Cai, Z., Xia, W., 2019a. Bisphenol A and bisphenol S exposures during pregnancy and gestational age – A longitudinal study in China. Chemosphere 237, 124426. https://doi.org/10.1016/j.chemosphere.2019.124426

Huang, T., Danaher, L.A., Brüschweiler, B.J., Kass, G.E.N., Merten, C., 2019b. Naturally occurring bisphenol F in plants used in traditional medicine. Arch. Toxicol. 93, 1485–1490. https://doi.org/10.1007/s00204-019-02442-5

Husøy, T., Andreassen, M., Hjertholm, H., Carlsen, M.H., Norberg, N., Sprong, C., Papadopoulou, E., Sakhi, A.K., Sabaredzovic, A., Dirven, H.A.A.M., 2019. The Norwegian biomonitoring study from the EU project EuroMix: Levels of phenols and phthalates in 24-hour urine samples and exposure sources from food and personal care products. Environ. Int. 132, 105103. https://doi.org/10.1016/j.envint.2019.105103

Ibarluzea, J., Aurrekoetxea, J.J., Porta, M., Sunyer, J., Ballester, F., 2016. La biomonitorización de sustancias tóxicas en muestras biológicas de población general. Gac. Sanit. 30, 45–54. https://doi.org/10.1016/j.gaceta.2016.02.012

Ike, M., Chen, M.Y., Danzl, E., Sei, K., Fujita, M., 2006. Biodegradation of a variety of bisphenols under aerobic and anaerobic conditions. Water Sci. Technol. 53, 153–159. https://doi.org/10.2166/wst.2006.189

Ikeda, K., Horie-Inoue, K., Inoue, S., 2019. Functions of estrogen and estrogen receptor signaling on skeletal muscle. J. Steroid Biochem. Mol. Biol. 191, 105375. https://doi.org/10.1016/j.jsbmb.2019.105375

Jin, H., Zhu, J., Chen, Z., Hong, Y., Cai, Z., 2018. Occurrence and partitioning of bisphenol analogues in adults' blood from China. Environ. Sci. Technol. 52, 812–820. https://doi.org/10.1021/acs.est.7b03958

Jin, H., Zhu, L., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from Liaohe River Basin and Taihu Lake, China. Water Res. 103, 343–351. https://doi.org/10.1016/j.watres.2016.07.059

Journal Officiel de la République Française, 2012. Loi n° 2012-1442 du 24 décembre 2012 visant à la

suspension de la fabrication, de l'importation, de l'exportation et de la mise sur le marché de tout conditionnement à vocation alimentaire contenant du bisphénol A 2011, 2012–2013.

Kabir, E.R., Rahman, M.S., Rahman, I., 2015. A review on endocrine disruptors and their possible impacts on human health. Environ. Toxicol. Pharmacol. 40, 241–258. https://doi.org/10.1016/j.etap.2015.06.009

Kang, J.-H., Kondo, F., Katayama, Y., 2006. Human exposure to bisphenol A (BPA). Toxicology 26, 79–89. https://doi.org/10.1016/j.reprotox.2007.07.010

Karrer, C., Roiss, T., von Goetz, N., Skledar, D.G., Mašič, L.P., Hungerbühler, K., 2018. Physiologically based pharmacokinetic (PBPK) modeling of the bisphenols BPA, BPS, BPF, and BPAF with new experimental metabolic parameters: Comparing the pharmacokinetic behavior of BPA with its substitutes. Environ. Health Perspect. 126, 1–17. https://doi.org/10.1289/EHP2739

Kawamura, Y., Etoh, M., Hirakawa, Y., Abe, Y., Mutsuga, M., 2014. Bisphenol A in domestic and imported canned foods in Japan. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 31, 330–340. https://doi.org/10.1080/19440049.2013.874047

Kim, D., Yoo, E.R., Li, A.A., Cholankeril, G., Tighe, S.P., Kim, W., Harrison, S.A., Ahmed, A., 2019. Elevated urinary bisphenol A levels are associated with non-alcoholic fatty liver disease among adults in the United States. Liver Int. 39, 1335–1342. https://doi.org/10.1111/liv.14110

Kinch, C.D., Ibhazehiebo, K., Jeong, J.H., Habibi, H.R., Kurrasch, D.M., 2015. Low-dose exposure to bisphenol A and replacement bisphenol S induces precocious hypothalamic neurogenesis in embryonic zebrafish. Proc. Natl. Acad. Sci. U. S. A. 112, 1475–1480. https://doi.org/10.1073/pnas.1417731112

Koch, H.M., Kolossa-Gehring, M., Schröter-Kermani, C., Angerer, J., Brüning, T., 2012. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: A retrospective exposure evaluation. J. Expo. Sci. Environ. Epidemiol. 22, 610–616. https://doi.org/10.1038/jes.2012.39

Krishnan, A. V, Stathis, P., Permuth, S.F., Tokes, L., Feldman, D., 1993. Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology 132, 2279–2286.

Lauretta, R., Sansone, A., Sansone, M., Romanelli, F., Appetecchia, M., 2019. Endocrine disrupting chemicals: Effects on endocrine glands. Front. Endocrinol. (Lausanne). 10, 1–7. https://doi.org/10.3389/fendo.2019.00178

Lee, I., Kim, Sunmi, Kim, K.T., Kim, Sungkyoon, Park, S., Lee, H., Jeong, Y., Lim, J.E., Moon, H.B., Choi, K., 2018. Bisphenol A exposure through receipt handling and its association with insulin resistance among female cashiers. Environ. Int. 117, 268–275. https://doi.org/10.1016/j.envint.2018.05.013

Lehmler, H.J., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to Bisphenol A, Bisphenol F, and Bisphenol S in U.S. Adults and Children: The National Health and Nutrition Examination Survey 2013-2014. ACS Omega 3, 6523–6532. https://doi.org/10.1021/acsomega.8b00824

Li, A.J., Xue, J., Lin, S., Al-Malki, A.L., Al-Ghamdi, M.A., Kumosani, T.A., Kannan, K., 2018. Urinary concentrations of environmental phenols and their association with type 2 diabetes in a population in Jeddah, Saudi Arabia. Environ. Res. 166, 544–552. https://doi.org/10.1016/j.envres.2018.06.040

Liao, C., Kannan, K., 2014. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 31, 319–329. https://doi.org/10.1080/19440049.2013.868611

Liao, C., Kannan, K., 2013. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J. Agric. Food Chem. 4655–4662. https://doi.org/10.1021/jf400445n

Lim, D.S., Kwack, S.J., Kim, K.B., Kim, H.S., Lee, B.M., 2009. Risk assessment of bisphenol A migrated from canned foods in Korea. J. Toxicol. Environ. Heal. - Part A Curr. Issues 72, 1327–1335. https://doi.org/10.1080/15287390903212444

Lintelmann, J., Katayama, A., Kurihara, N., Shore, L., Wenzel, A., 2003. Endocrine disruptors in the environment: IUPAC technical report. Pure Appl. Chem. 75, 631–681. https://doi.org/10.1351/pac200375050631

Liu, Y., Zhang, S., Song, N., Guo, R., Chen, M., Mai, D., Yan, Z., Han, Z., Chen, J., 2017. Occurrence, distribution and sources of bisphenol analogues in a shallow Chinese freshwater lake (Taihu

Lake): Implications for ecological and human health risk. Sci. Total Environ. 599–600, 1090–1098. https://doi.org/10.1016/j.scitotenv.2017.05.069

Llobet, J.M., Falcó, G., Casas, C., Teixidó, A., Domingo, J.L., 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. J. Agric. Food Chem. 51, 838–842. https://doi.org/10.1021/jf020734q

Loganathan, S.N., Kannan, K., 2011. Occurrence of bisphenol a in indoor dust from two locations in the Eastern United States and implications for human exposures. Arch. Environ. Contam. Toxicol. 61, 68–73. https://doi.org/10.1007/s00244-010-9634-y

López-Blanco, R., Nortes-Méndez, R., Robles-Molina, J., Moreno-González, D., Gilbert-López, B., García-Reyes, J.F., Molina-Díaz, A., 2016. Evaluation of different cleanup sorbents for multiresidue pesticide analysis in fatty vegetable matrices by liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1456, 89–104. https://doi.org/10.1016/j.chroma.2016.06.019

Lozano, A., Rajski, Ł., Uclés, S., Belmonte-Valles, N., Mezcua, M., Fernández-Alba, A.R., 2014. Evaluation of zirconium dioxide-based sorbents to decrease the matrix effect in avocado and almond multiresidue pesticide analysis followed by gas chromatography tandem mass spectrometry. Talanta 118, 68–83. https://doi.org/10.1016/j.talanta.2013.09.053

Lu, S., Yu, Yuling, Ren, L., Zhang, X., Liu, G., Yu, Yingxin, 2018. Estimation of intake and uptake of bisphenols and triclosan from personal care products by dermal contact. Sci. Total Environ. 621, 1389–1396. https://doi.org/10.1016/j.scitotenv.2017.10.088

Malespin, M., Nassri, A., 2019. Endocrine siseases and the liver: An update. Clin. Liver Dis. 23, 233–246. https://doi.org/10.1016/j.cld.2018.12.006

Mandrup, K., Boberg, J., Isling, L.K., Christiansen, S., Hass, U., 2016. Low-dose effects of bisphenol A on mammary gland development in rats. Andrology 4, 673–683. https://doi.org/10.1111/andr.12193

Mantovani, A., 2015. Endocrine disrupters and the safety of food chains. Horm. Res. Paediatr. 86, 279–288. https://doi.org/10.1159/000441496

Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2008. Dietary intake of arsenic, cadmium, mercury, and lead by the population of catalonia, Spain. Biol. Trace Elem. Res. 125, 120–132. https://doi.org/10.1007/s12011-008-8162-3

Martín-Pozo, L., Cantarero-Malagón, S., Hidalgo, F., Navalón, A., Zafra-Gómez, A., 2020. Determination of endocrine disrupting chemicals in human nails using an alkaline digestion prior to ultra-high performance liquid chromatography–tandem mass spectrometry. Talanta 208, 120429. https://doi.org/10.1016/j.talanta.2019.120429

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2016. Analytical method for biomonitoring of endocrine-disrupting compounds (bisphenol A, parabens, perfluoroalkyl compounds and a brominated flame retardant) in human hair by liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta 945, 95–101. https://doi.org/10.1016/j.aca.2016.10.004

Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V., 2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ. Res. 166, 25–34. https://doi.org/10.1016/j.envres.2018.05.008

Martorell, I., Nieto, A., Nadal, M., Perelló, G., Marcé, R.M., Domingo, J.L., 2012. Human exposure to polycyclic aromatic hydrocarbons (PAHs) using data from a duplicate diet study in Catalonia , Spain. Food Chem. Toxicol. 50, 4103–4108. https://doi.org/10.1016/j.fct.2012.08.011

Martorell, I., Perelló, G., Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2011. Human exposure to arsenic, cadmium, mercury, and lead from foods in catalonia, Spain: Temporal trend. Biol. Trace Elem. Res. 142, 309–322. https://doi.org/10.1007/s12011-010-8787-x

Matuszczak, E., Komarowska, M.D., Debek, W., Hermanowicz, A., 2019. The impact of bisphenol A on fertility, reproductive system, and development: A review of the literature. Int. J. Endocrinol. 2019, 1–8. https://doi.org/10.1155/2019/4068717

Meng, Z., Tian, S., Yan, J., Jia, M., Yan, S., Li, R., Zhang, R., Zhu, W., Zhou, Z., 2019. Effects of perinatal exposure to BPA, BPF and BPAF on liver function in male mouse offspring involving in oxidative damage and metabolic disorder. Environ. Pollut. 247, 935–943. https://doi.org/10.1016/j.envpol.2019.01.116

Mercogliano, R., Santonicola, S., 2018. Investigation on bisphenol A levels in human milk and dairy supply chain: A review. Food Chem. Toxicol. 114, 98–107. https://doi.org/10.1016/j.fct.2018.02.021

Mersha, M.D., Patel, B.M., Patel, D., Richardson, B.N., Dhillon, H.S., 2015. Effects of BPA and BPS exposure limited to early embryogenesis persist to impair non-associative learning in adults. Behav. Brain Funct. 11, 1–5. https://doi.org/10.1186/s12993-015-0071-y

Michałowicz, J., 2014. Bisphenol A - Sources, toxicity and biotransformation. Environ. Toxicol. Pharmacol. 37, 738–758. https://doi.org/10.1016/j.etap.2014.02.003

Mikołajewska, K., Stragierowicz, J., Gromadzińska, J., 2015. Bisphenol A – Application, sources of exposure and potential risks in infants, children and pregnant women. Int. J. Occup. Med. Environ. Health 28, 209–241. https://doi.org/10.13075/ijomeh.1896.00343

Morgan, M.K., Nash, M., Barr, D.B., Starr, J.M., Scott Clifton, M., Sobus, J.R., 2018. Distribution, variability, and predictors of urinary bisphenol A levels in 50 North Carolina adults over a six-week monitoring period. Environ. Int. 112, 85–99. https://doi.org/10.1016/j.envint.2017.12.014

Muros, J.J., Cabrera-Vique, C., Briones, M., Seiquer, I., 2019. Assessing the dietary intake of calcium, magnesium, iron, zinc and copper in institutionalised children and adolescents from Guatemala. Contribution of nutritional supplements. J. Trace Elem. Med. Biol. 53, 91–97. https://doi.org/10.1016/j.jtemb.2019.02.009

Murphy, L., Mérida-Ortega, Á., Cebrián, M.E., Hernández-Garciadiego, L., Gómez-Ruiz, H., Gamboa-Loira, B., López-Carrillo, L., 2019. Exposure to bisphenol A and diabetes risk in Mexican women. Environ. Sci. Pollut. Res. 26, 26332–26338. https://doi.org/10.1007/s11356-019-05731-9

Ndaw, S., Remy, A., Jargot, D., Robert, A., 2016. Occupational exposure of cashiers to bisphenol A via thermal paper: urinary biomonitoring study. Int. Arch. Occup. Environ. Health 89, 935–946. https://doi.org/10.1007/s00420-016-1132-8

Nelson, J.W., Scammell, M.K., Hatch, E.E., Webster, T.F., 2012. Social disparities in exposures to bisphenol A and polyfluoroalkyl chemicals: A cross-sectional study within NHANES 2003-2006. Environ. Heal. A Glob. Access Sci. Source 11, 10. https://doi.org/10.1186/1476-069X-11-10

Nie, J., Shi, J., Duan, X., Wang, B., Huang, N., Zhao, X., 2014. Health risk assessment of dietary exposure to polycyclic aromatic hydrocarbons in Taiyuan, China. J. Environ. Sci. (China) 26, 432–439. https://doi.org/10.1016/S1001-0742(13)60424-6

Nie, Q., Xing, M., Hu, J., Hu, X., Nie, S., Xie, M., 2015. Metabolism and health effects of phyto-estrogens. Crit. Rev. Food Sci. Nutr. 57, 2432–2454. https://doi.org/10.1080/10408398.2015.1077194

Noonan, G.O., Ackerman, L.K., Begley, T.H., 2011. Concentration of bisphenol a in highly consumed canned foods on the U.S. market. J. Agric. Food Chem. 59, 7178–7185. https://doi.org/10.1021/jf201076f

Nowak, K., Jabłońska, E., Ratajczak-Wrona, W., 2019. Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells. Environ. Int. 125, 350–364. https://doi.org/10.1016/j.envint.2019.01.078

Oh, J., Choi, J.W., Ahn, Y.A., Kim, S., 2018. Pharmacokinetics of bisphenol S in humans after single oral administration. Environ. Int. 112, 127–133. https://doi.org/10.1016/j.envint.2017.11.020

Olea, N., Pulgar, R., Pérez, P., Olea-serrano, F., Rivas, A., Novillo-Fertrell, A., Pedraza, V., Soto, A.M., Sonnenschein, C., 1996. Estrogenicity of resin-based composites and sealants used in dentistry 104.

Osman, M.A., Mahmoud, G.I., Elgammal, M.H., Hasan, R.S., 2018. Studying of bisphenol A levels in some canned food, feed and baby bottles in egyptian markets. Fresenius Environ. Bull. 27, 9374–9381.

Papadopoulou, E., Poothong, S., Koekkoek, J., Lucattini, L., Padilla-Sánchez, J.A., Haugen, M., Herzke, D., Valdersnes, S., Maage, A., Cousins, I.T., Leonards, P.E.G., Småstuen Haug, L., 2017. Estimating human exposure to perfluoroalkyl acids via solid food and drinks: Implementation and comparison of different dietary assessment methods. Environ. Res. 158, 269–276. https://doi.org/10.1016/j.envres.2017.06.011

Paterni, I., Granchi, C., Minutolo, F., 2016. Risks and benefits related to alimentary exposure to xenoestrogens. Crit. Rev. Food Sci. Nutr. 57, 3384–3404. https://doi.org/10.1080/10408398.2015.1126547

Patisaul, H.B., 2016kabir. Endocrine disruption by dietary phyto-oestrogens: Impact on dimorphic sexual systems and behaviours. Proc. Nutr. Soc. 76, 130–144. https://doi.org/10.1017/S0029665116000677

Pelch, K., Wignal, J., Goldstone, A., Ross, P., Blain, R., Shapiro, A., Holmgren, S., Hsieh, J.-H., Svoboda, D., Auerbach, S., Parham, F., Masten, S., Thayer, K., 2017. NTP research report on biological activity of bisphenol A (BPA) structural analogues and functional alternatives. Natl. Toxicol. Progr. 1, 1–78.

Perelló, G., Llobet, J.M., Gómez-Catalán, J., Castell, V., Centrich, F., Nadal, M., Domingo, J.L., 2014. Human health risks derived from dietary exposure to toxic metals in Catalonia, Spain: Temporal trend. Biol. Trace Elem. Res. 162, 26–37. https://doi.org/10.1007/s12011-014-0138-x

Pivnenko, K., Laner, D., Astrup, T.F., 2018. Dynamics of bisphenol A (BPA) and bisphenol S (BPS) in the European paper cycle: Need for concern? Resour. Conserv. Recycl. 133, 278–287. https://doi.org/10.1016/j.resconrec.2018.01.021

Porras, S.P., Heinälä, M., Santonen, T., 2014. Bisphenol A exposure via thermal paper receipts. Toxicol. Lett. 230, 413–420. https://doi.org/10.1016/j.toxlet.2014.08.020

Qiu, W., Zhan, H., Hu, J., Zhang, T., Xu, H., Wong, M., Xu, B., Zheng, C., 2019. The occurrence, potential toxicity, and toxicity mechanism of bisphenol S, a substitute of bisphenol A: A critical review of recent progress. Ecotoxicol. Environ. Saf. 173, 192–202. https://doi.org/10.1016/j.ecoenv.2019.01.114

QPC, 2015. Décision n°2015-480 visant à la suspension de la fabrication, de l'importation, de l'exportation et de la mise sur le marché de tout conditionnement à vocation alimentaire contenant du bisphénol A.

Radwan, M., Wielgomas, B., Dziewirska, E., Radwan, P., Kałużny, P., Klimowska, A., Hanke, W., Jurewicz, J., 2018. Urinary bisphenol A levels and male fertility. Am. J. Mens. Health 12, 2144–2151. https://doi.org/10.1177/1557988318799163

Rahman, M.S., Pang, M.-G., 2019. Understanding the molecular mechanisms of bisphenol A action in spermatozoa. Clin. Exp. Reprod. Med. 46, 99–106. https://doi.org/10.5653/cerm.2019.00276

Rahmani, S., Pour Khalili, N., Khan, F., Hassani, S., Ghafour-Boroujerdi, E., Abdollahi, M., 2018.

Bisphenol A: What lies beneath its induced diabetes and the epigenetic modulation? Life Sci. 214, 136–144. https://doi.org/10.1016/j.lfs.2018.10.044

Rezaee, M., Assadi, Y., Milani Hosseini, M.R., Aghaee, E., Ahmadi, F., Berijani, S., 2006. Determination of organic compounds in water using dispersive liquid-liquid microextraction. J. Chromatogr. A 1116, 1–9. https://doi.org/10.1016/j.chroma.2006.03.007

RIVM (National Institute for Public Health and the Environment). Ministry of Health, Welfare and Sport. Infographic possible sources of BPA exposure for people and the environment. Available online at: https://www.rivm.nl/en/bisphenol-a/infographic-possible-sources-of-bpa-exposure-for-people-and-environment. Accessed: 20/10/2019

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–155. https://doi.org/10.1016/j.reprotox.2013.08.008

Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ. Health Perspect. 123, 643–650.

Rodríguez-Gómez, R., Martín, J., Zafra-Gómez, A., Alonso, E., Vílchez, J.L., Navalón, A., 2017. Biomonitoring of 21 endocrine disrupting chemicals in human hair samples using ultra-high performance liquid chromatography–tandem mass spectrometry. Chemosphere 168, 676–684. https://doi.org/10.1016/j.chemosphere.2016.11.008

Ruiz, D., Becerra, M., Jagai, J.S., Ard, K., Sargis, R.M., 2017. Disparities in environmental exposures to endocrine-disrupting chemicals and diabetes risk in vulnerable populations. Diabetes Care 41, 193–205. https://doi.org/10.2337/dc16-2765

Sakhi, A.K., Lillegaard, I.T.L., Voorspoels, S., Carlsen, M.H., Løken, E.B., Brantsæter, A.L., Haugen, M., Meltzer, H.M., Thomsen, C., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ. Int. 73, 259–269. https://doi.org/10.1016/j.envint.2014.08.005

Santana-Mayor, Á., Socas-Rodríguez, B., Herrera-Herrera, A. V., Rodríguez-Delgado, M.Á., 2019. Current trends in QuEChERS method. A versatile procedure for food, environmental and biological analysis. TrAC - Trends Anal. Chem. 116, 214–235. https://doi.org/10.1016/j.trac.2019.04.018

Santonicola, S., Ferrante, M.C., Murru, N., Gallo, P., Mercogliano, R., 2018. Hot topic: Bisphenol A in cow milk and dietary exposure at the farm level. J. Dairy Sci. 102, 1007–1013. https://doi.org/10.3168/jds.2018-15338

Schreiber, E., Alfageme, O., Garcia, T., González, N., Sirvent, J.J., Torrente, M., Gómez, M., Domingo, J.L., 2019. Oral exposure of rats to dienestrol during gestation and lactation: Effects on the reproductive system of male offspring. Food Chem. Toxicol. 128, 193–201. https://doi.org/10.1016/j.fct.2019.04.013

Sharma, R.P., Schuhmacher, M., Kumar, V., 2018. The development of a pregnancy PBPK Model for Bisphenol A and its evaluation with the available biomonitoring data. Sci. Total. Environ. 624, 55-68. https://doi.org/10.1016/j.scitotenv.2017.12.023

Song, S., Duan, Y., Zhang, T., Zhang, B., Zhao, Z., Bai, X., Xie, L., He, Y., Ouyang, J. ping, Huang, X., Sun, H., 2019. Serum concentrations of bisphenol A and its alternatives in elderly population living around e-waste recycling facilities in China: Associations with fasting blood glucose. Ecotoxicol. Environ. Saf. 169, 822–828. https://doi.org/10.1016/j.ecoenv.2018.11.101

Sparkman, D.O., Penton, Z., Kitson, F.G., 2011. Gas chromatography and mass spectrometry: A practical guide. Academic Press. Book. ISBN 978-0-08-092015-3

Teeguarden, J.G., Twaddle, N.C., Churchwell, M.I., Yang, X., Fisher, J.W., Seryak, L.M., Doerge, D.R., 2015. 24-hour human urine and serum profiles of bisphenol A: Evidence against sublingual absorption following ingestion in soup. Toxicol. Appl. Pharmacol. 288, 131–142. https://doi.org/10.1016/j.taap.2015.01.009

Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ. Int. 83, 107–15. https://doi.org/10.1016/j.envint.2015.06.008

Tinggi, U., Schoendorfer, N., 2018. Analysis of lead and cadmium in cereal products and duplicate diets of a small group of selected Brisbane children for estimation of daily metal exposure. J. Trace Elem. Med. Biol. 50, 671–675. https://doi.org/10.1016/j.jtemb.2018.06.022

Tsatsakis, A.M., Docea, A.O., Tsitsimpikou, C., 2016. New challenges in risk assessment of chemicals

when simulating real exposure scenarios; simultaneous multi-chemicals' low dose exposure. Food Chem. Toxicol. 96, 174–176. https://doi.org/10.1016/j.fct.2016.08.011

Tyl, R.W., 2014. Abbreviated assessment of bisphenol A toxicology literature. Semin. Fetal Neonatal Med. 19, 195–202. https://doi.org/10.1016/j.siny.2013.11.010

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Khan, M.J., 2019. Bisphenol A analogues bisphenol B, bisphenol F, and bisphenol S induce oxidative stress, disrupt daily sperm production, and damage DNA in rat spermatozoa: a comparative in vitro and in vivo study. Toxicol. Ind. Health 35, 294–303. https://doi.org/10.1177/0748233719831528

Ullah, A., Pirzada, M., Jahan, S., Ullah, H., Turi, N., Ullah, W., Siddiqui, M.F., Zakria, M., Lodhi, K.Z., Khan, M.M., 2018. Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: A focus on the possible hormonal mode of action. Food Chem. Toxicol. 121, 24–36. https://doi.org/10.1016/j.fct.2018.08.024

Usman, A., Ahmad, M., 2016. From BPA to its analogues: Is it a safe journey? Chemosphere 158, 131–142. https://doi.org/10.1016/j.chemosphere.2016.05.070

Usman, A., Ikhlas, S., Ahmad, M., 2019. Occurrence, toxicity and endocrine disrupting potential of Bisphenol-B and Bisphenol-F: A mini-review. Toxicol. Lett. 312, 222–227. https://doi.org/10.1016/j.toxlet.2019.05.018

Vela-Soria, F., Jiménez-Díaz, I., Díaz, C., Pérez, J., Iribarne-Durán, L.M., Serrano-López, L., Arrebola, J.P., Fernández, M.F., Olea, N., 2016. Determination of endocrine-disrupting chemicals in human milk by dispersive liquid-liquid microextraction. Bioanalysis 8, 1777–1791. https://doi.org/10.4155/bio-2016-0073

Velázquez-Gómez, M., Lacorte, S., 2019. Nasal lavages as a tool for monitoring exposure to organic pollutants. Environ. Res. 178, 108726. https://doi.org/10.1016/j.envres.2019.108726

Vervliet, P., Gys, C., Caballero-Casero, N., Covaci, A., 2019. Current-use of developers in thermal paper from 14 countries using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. Toxicology 416, 54–61. https://doi.org/10.1016/j.tox.2019.02.003

Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and kinetics of

bisphenol A in humans at low doses following oral administration. Chem. Res. Toxicol. 15, 1281–1287. https://doi.org/10.1021/tx025548t

WHO, 1985. Guidelines for the study of dietary intakes of chemical contaminants. WHO Offset Publ. 87, 102.

WHO/IPCS, International Programme on Chemical Safety, 2002. Global Assessment of the State-of-the-science of Endocrine Disruptors.

Wierman, M.E., 2007. Sex steroid effects at target tissues: Mechanisms of action. Am. J. Physiol. - Adv. Physiol. Educ. 31, 26–33. https://doi.org/10.1152/advan.00086.2006

Wu, L.H., Zhang, X.M., Wang, F., Gao, C.J., Chen, D., Palumbo, J.R., Guo, Y., Zeng, E.Y., 2018. Occurrence of bisphenol S in the environment and implications for human exposure: A short review. Sci. Total Environ. 615, 87–98. https://doi.org/10.1016/j.scitotenv.2017.09.194

Xue, J., Liu, W., Kannan, K., 2017a. Bisphenols, Benzophenones, and Bisphenol A Diglycidyl Ethers in Textiles and Infant Clothing. Environ. Sci. Technol. 51, 5279–5286. https://doi.org/10.1021/acs.est.7b00701

Xue, J., Wan, Y., Kannan, K., 2017b. Occurrence of bisphenols, bisphenol A diglycidyl ethers (BADGEs), and novolac glycidyl ethers (NOGEs) in indoor air from Albany, New York, USA, and its implications for inhalation exposure. Chemosphere 151, 1–8. https://doi.org/10.1016/j.chemosphere.2016.02.038

Yanagisawa, R., Koike, E., Win-Shwe, T.T., Takano, H., 2019. Oral exposure to low dose bisphenol A aggravates allergic airway inflammation in mice. Toxicol. Reports 6, 1253–1262. https://doi.org/10.1016/j.toxrep.2019.11.012

Yang, Y., Guan, J., Yin, J., Shao, B., Li, H., 2014. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere 112, 481–486. https://doi.org/10.1016/j.chemosphere.2014.05.004

Ye, X., Kuklenyik, Z., Needham, L.L., Calafat, A.M., 2005. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 383, 638–644. https://doi.org/10.1007/s00216-005-0019-4

Yu, X., Xue, J., Yao, H., Wu, Q., Venkatesan, A.K., Halden, R.U., Kannan, K., 2015. Occurrence and estrogenic potency of eight bisphenol analogs in sewage sludge from the U.S. EPA targeted national sewage sludge survey. J. Hazard. Mater. 299, 733–739. https://doi.org/10.1016/j.jhazmat.2015.07.012

Zalko, D., Jacques, C., Duplan, H., Bruel, S., Perdu, E., 2011. Viable skin efficiently absorbs and metabolizes bisphenol A. Chemosphere 82, 424–430. https://doi.org/10.1016/j.chemosphere.2010.09.058

Zhang, R., Liu, R., Zong, W., 2016. Bisphenol S interacts with catalase and induces oxidative stress in mouse liver and renal cells, Journal of Agricultural and Food Chemistry. https://doi.org/10.1021/acs.jafc.6b02656

Zhang, T., Sun, H., Kannan, K., 2013. Blood and urinary bisphenol A concentrations in children, adults, and pregnant women from China: partitioning between blood and urine and maternal and fetal cord blood. Sci. Total Environ. 47, 4686–4694. https://doi.org/10.1016/j.scitotenv.2013.04.074

Zhu, X., Wu, G., Xing, Y., Wang, C., Yuan, X., Li, B., 2020. Evaluation of single and combined toxicity of bisphenol A and its analogues using a highly-sensitive micro-biosensor. J. Hazard. Mater. 381, 120908. https://doi.org/10.1016/j.jhazmat.2019.120908

Zoller, O., Brüschweiler, B.J., Magnin, R., Reinhard, H., Rhyn, P., Rupp, H., Zeltner, S., Felleisen, R., 2016. Natural occurrence of bisphenol F in mustard. Food Addit. Contam. - Part A Chem. Anal. Control. Expo. Risk Assess. 33, 137–146. https://doi.org/10.1080/19440049.2015.1110623

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ANNEX

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ANNEX 1 – Publications, congresses, other projects and research stays

Publications

González, N., Cunha, S.C., Ferreira, R., Fernandes, J.O., Marquès, M., Domingo, J.L., Nadal, M. 2020. Concentrations of nine bisphenol analogues in food purchased from Catalonia (Spain): Comparison of canned and non-canned foodstuffs. *Food and Chemical Toxicology* 136, 110992. https://doi.org/10.1016/j.fct.2019.110992 **Quartile 1. Impact Factor: 3.775. SJR: 0.916 [Year: 2018/2019]**

González, N., Calderón, J., Rúbies, A., Timoner, I., Castell, V., Domingo, J.L., Nadal, M. 2019. Dietary intake of arsenic, cadmium, mercury and lead by the population of Catalonia, Spain: Analysis of the temporal trend. *Food and Chemical Toxicology* 132, 110721. https://doi.org/10.1016/j.fct.2019.110721 **Quartile 1. Impact Factor: 3.775. SJR: 0.916 [Year: 2018/2019]**

González, N., Cunha, S.C., Monteiro, C., Fernandes, J.O., Marquès, M., Domingo, J.L., Nadal, M. 2019. Quantification of eight bisphenol analogues in blood and urine samples of workers in a hazardous waste incinerator. *Environmental Research* 176, 108576. https://doi.org/10.1016/j.envres.2019.108576 **Quartile 1. Impact Factor: 5.026. SJR: 1.567. [Year: 2018/2019]**

Schreiber, E., Alfageme, O., Garcia, T., **González, N**., Sirvent, J.J., Torrente, M., Gómez, M., Domingo, J.L., 2019. Oral exposure of rats to dienestrol during gestation and lactation: Effects on the reproductive system of male offspring. *Food and Chemical Toxicology* 128, 193-201. https://doi.org/10.1016/j.fct.2019.04.013 **Quartile 1. Impact Factor: 3.775. SJR: 0.916 [Year: 2018/2019]**

González, N., Marquès, M., Nadal, M., Domingo, J.L. 2019. Occurrence of environmental pollutants in foodstuffs: A review of organic vs. conventional food. *Food and Chemical Toxicology* 125, 370-375. https://doi.org/10.1016/j.fct.2019.01.021 **Quartile 1. Impact Factor: 3.775. SJR: 0.916 [Year: 2018/2019]**

González, N., Marquès, M., Nadal, M., Domingo, J.L. 2018. Levels of PCDD/Fs in foodstuffs in Tarragona County (Catalonia, Spain): Spectacular decrease in the dietary intake of

PCDD/Fs in the last 20 years. *Food and Chemical Toxicology* 121, 109-114. https://doi.org/10.1016/j.fct.2018.08.035 **Quartile 1. Impact Factor: 3.775. SJR: 0.916 [Year: 2018/2019]**

Attendance to congresses

54th Congress of the European Societies of Toxicology (EUROTOX 2018). González N, Marquès M, Nadal M, Domingo JL. Dietary intake of PCDD/Fs by the population of Tarragona County, Catalonia, Spain. Temporal trends (1998-2018). Brussels, Belgium, 2018.

54th Congress of the European Societies of Toxicology (EUROTOX 2018). González N, Schreiber E, Alfageme O, Torrente M, Domingo JL, Gómez M. Assessment of reproductive toxicity of male rats through maternal exposure to dienestrol. Brussels, Belgium, 2018.

XXI Congreso Internacional de Medicina Legal. García F, **González N**, Marquès M, Domingo JL. Ingesta dietética de PCDD/Fs en la población de Tarragona, Cataluña, España. Evolución temporal (1998-2018). La Habana, Cuba, 2018.

SETAC Europe 29th Annual Meeting. González N, Cunha S, Monteiro C, Fernandes J, Marquès M, Nadal M, Domingo JL. Quantification of 9 bisphenol analogues in total blood, plasma and urine samples from a cohort of workers through a DLLME extraction followed by GC-MS determination. Helsinki, Finland, 2019.

SETAC Europe 29th Annual Meeting. Schreiber E, González N, Kumar V, Torrente M, Domingo JL, Gómez M. Feminization effects on male offspring rats through maternal exposure to flutamide, linuron and dienestrol. Helsinki, Finland, 2019.

15th International Congress of Toxicology (IUTOX 2019). González N, Cunha S, Fernandes J, Marquès M, Domingo JL, Nadal M. Are canned food containers safe? Exposure to 9 bisphenol analogues in a duplicate diet study. Hawaii, Unites States of America, 2019.

55th Congress of the European Societies of Toxicology (EUROTOX 2019). Domingo JL, **González N**, Cunha S, Monteiro C, Fernandes J, Marquès M, Nadal M. Food levels of 9

bisphenol analogues in Catalonia (Spain): Comparing canned vs non-canned foodstuffs. Helsinki, Finland, 2019.

Participation in other projects

EuroMix: European Test and Risk Assessment Strategies for Mixtures. European project from the Horizon 2020 (Horizon 2020 Framework Programme) and funded by the European Union (2015-2019), subvention nº 633172.

Modelización farmacocinética y farmacodinámica de bisfenol A y sus análogos: aproximación a las mezclas (MODELBIS). Funded by the Ministry of Economy and Competitiveness (2017-2019), subvention nº AGL2016-78942-R.

Research stays

15/09/2018 – 20/12/2018. Faculty of Pharmacy – University of Porto (Portugal)

Development of analytical chromatographic methods for the determination of bisphenol compounds in biological matrices.

11/03/2019 – 28/06/2019. Faculty of Pharmacy – University of Porto (Portugal)

Chromatographic determination by GC-MS of several bisphenol compounds in biological and food matrices.

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ANNEX 2 – Supporting information chapter 3

Table S1. Levels of BP analogues in urine samples from the canned group. In bold, levels above the LOD. $LOD_{BPF, BPA, BPAF, BPE}$: 0.2 µg/l; $LOQ_{BPF, BPA, BPAF, BPE}$: 0.07 µg/l

Participant	Day	Sample	Bisphenol analogues (µg/l)							
			BPAF		BPF		BPE		BPA	
			Free	Total	Free	Total	Free	Total	Free	Total
1	1	1	<LOD	**1.05**	<LOD	<LOD	<LOD	<LOD	<LOD	**2.91**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.41**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.14**	**0.82**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.33**	**1.85**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.62**	**2.01**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.92**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.45**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.09**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.55**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.74**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.29**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.56**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.84**
2	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.20**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.93**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.37**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.82**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.51**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.72**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.89**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.12**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.09**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.22**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.55**
3	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.45**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.12**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.29**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.72**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**8.86**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**13.4**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.063**	**12.1**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.9**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.78**
5	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.95**

		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.97**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.37**	**1.15**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.16**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.11**	**1.90**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.56**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.11**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**7.80**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.52**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.11**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.71**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	**0.094**	<LOD	**1.29**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.25**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.12**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.01**
8	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.53**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.16**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.47**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**11.1**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**11.2**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.7**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**8.43**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**13.4**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.97**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.92**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.46**
9	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**15.7**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.22**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.79**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.44**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.60**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.91**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.34**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**3.99**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**8.68**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**10.6**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**6.97**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.34**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.67**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.11**
10	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.48**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**0.82**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**0.59**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**1.22**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**2.44**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**1.29**

		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**9.43**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**11.2**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.40**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**1.14**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.70**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**2.64**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.84**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**8.41**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.97**
18	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.78**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.10**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.88**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.05**
		2	<LOD	**0.50**	<LOD	<LOD	<LOD	<LOD	<LOD	**3.51**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.02**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.83**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.54**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.90**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.94**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.38**
20	1	1	<LOD	<LOD	<LOD	**3.88**	<LOD	<LOD	<LOD	**2.06**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	**2.51**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.51**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	**0.33**	**0.16**	**5.11**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.44**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.56**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.80**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	**0.28**	<LOD	**7.36**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	0.22	**<LOQ**	**3.29**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.032**	**1.37**
		8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.6**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**14.8**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.38**
21	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.74**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.78**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.23**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.38**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.40**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**34.8**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.63**

		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.04**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	**0.48**	<LOD	**8.44**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**22.8**
22	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.64**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.68**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.44**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**9.66**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.3**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**10.1**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**13.5**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.2**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**17.0**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**16.2**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.5**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**16.1**
23	1	1	<LOD	<LOD	<LOD	**3.26**	<LOD	<LOD	<LOD	**1.94**
		2	<LOD	<LOD	<LOD	**0.91**	<LOD	<LOD	<LOD	**3.87**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**17.2**
		2	<LOD	<LOD	<LOD	**11.9**	<LOD	<LOD	<LOD	**3.40**
		3	<LOD	<LOD	<LOD	**3.73**	<LOD	<LOD	<LOD	**3.38**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.03**
24	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.67**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.28**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.43**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**8.75**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.25**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.26**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.95**
		8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.90**
		9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.02**
		10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.52**
		11	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.62**
		12	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.57**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.35**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.96**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.96**
		4	<LOD	<LOD	<LOD	**13.5**	<LOD	<LOD	<LOD	**19.9**
		5	<LOD	<LOD	<LOD	**3.03**	<LOD	<LOD	<LOD	**6.09**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.15**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.08**
		8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**6.12**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.66**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.63**
25	1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.45**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.60**

		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**2.72**
		4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.93**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.88**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.33**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.51**
		8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**4.83**
	2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**12.9**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**18.6**
		3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**18.2**
		4	<LOD	<LOD	<LOD	**12.3**	<LOD	<LOD	<LOD	**11.7**
		5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**7.22**
		6	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**5.86**
		7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.50**
		8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.85**
		9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.18**
	3	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**9.53**
		2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**7.62**

Table S2. Levels of BP analogues in urine samples from the control group. In bold, levels above the LOD. $LOD_{BPF, BPA}$: 0.2 µg/l; $LOQ_{BPF, BPA}$: 0.07 µg/l

Participant	Day	Sample	Bisphenol analogues (µg/l)			
			BPF		BPA	
			Free	Total	Free	Total
4	1	1	<LOD	<LOD	<LOD	**0.76**
		2	<LOD	<LOD	<LOD	**2.62**
		3	<LOD	<LOD	**0.12**	**1.37**
		4	<LOD	<LOD	<LOD	**0.57**
		5	<LOD	<LOD	<LOD	**1.05**
		6	<LOD	<LOD	<LOD	**2.35**
	2	1	<LOD	<LOD	<LOD	**4.06**
		2	<LOD	**4.08**	<LOD	**0.78**
		3	<LOD	<LOD	<LOD	**1.25**
		4	<LOD	<LOD	<LOD	**1.70**
		5	<LOD	<LOD	<LOD	**3.07**
		6	<LOD	<LOD	<LOD	**2.10**
	3	1	<LOD	<LOD	<LOD	**1.51**
6	1	1	<LOD	<LOD	<LOD	**3.58**
		2	<LOD	<LOD	<LOD	**2.05**
		3	<LOD	<LOD	**0.043**	**0.77**
		4	<LOD	<LOD	**0.14**	**0.65**
		5	<LOD	<LOD	**0.26**	**1.38**
	2	1	<LOD	<LOD	<LOD	**2.43**
		2	<LOD	<LOD	<LOD	**1.02**
		3	<LOD	<LOD	<LOD	<LOD

		4	<LOD	<LOD	<LOD	**1.43**
		5	<LOD	<LOD	<LOD	**1.73**
		6	<LOD	<LOD	<LOD	**2.08**
	3	1	<LOD	<LOD	<LOD	**0.57**
		2	<LOD	<LOD	<LOD	**0.86**
7	1	1	<LOD	<LOD	<LOD	**3.34**
		2	<LOD	<LOD	<LOD	**3.62**
		3	<LOD	<LOD	<LOD	**0.89**
		4	<LOD	<LOD	<LOD	**0.89**
		5	<LOD	<LOD	<LOD	**4.34**
	2	1	<LOD	<LOD	<LOD	**6.87**
		2	<LOD	<LOD	<LOD	**2.73**
		3	<LOD	<LOD	<LOD	**3.04**
		4	<LOD	<LOD	<LOD	**2.53**
	3	1	<LOD	<LOD	<LOD	**3.40**
11	1	1	<LOD	<LOD	<LOD	**2.35**
		2	<LOD	<LOD	<LOD	**1.43**
		3	<LOD	<LOD	<LOD	**1.26**
		4	<LOD	<LOD	<LOD	**0.93**
		5	<LOD	<LOD	<LOD	**1.30**
		6	<LOD	<LOD	<LOD	**1.13**
	2	1	<LOD	<LOD	<LOD	**11.2**
		2	<LOD	<LOD	<LOD	**6.34**
		3	<LOD	<LOD	<LOD	**2.14**
		4	<LOD	<LOD	<LOD	**2.68**
		5	<LOD	<LOD	<LOD	**2.45**
		6	<LOD	<LOD	<LOD	**1.96**
	3	1	<LOD	<LOD	<LOD	**1.43**
		2	<LOD	<LOD	<LOD	**1.87**
12	1	1	<LOD	<LOD	<LOD	**1.44**
		2	<LOD	<LOD	<LOD	**1.10**
		3	<LOD	<LOD	<LOD	**0.68**
		4	<LOD	<LOD	<LOD	<LOD
		5	<LOD	<LOD	<LOD	**0.17**
	2	1	<LOD	<LOD	<LOD	**2.32**
		2	<LOD	<LOD	<LOD	**5.13**
		3	<LOD	<LOD	<LOD	**1.51**
		4	<LOD	<LOD	<LOD	<LOD
		5	<LOD	<LOD	<LOD	<LOD
		6	<LOD	<LOD	<LOD	<LOD
		7	<LOD	<LOD	<LOD	**2.09**
		8	<LOD	<LOD	<LOD	**2.46**
	3	1	<LOD	<LOD	<LOD	**1.13**
13	1	1	<LOD	<LOD	<LOD	**0.71**
		2	<LOD	<LOD	<LOD	**1.81**

		3	<LOD	<LOD	<LOD	**4.13**
		4	<LOD	<LOD	<LOD	**3.68**
		5	<LOD	<LOD	<LOD	**0.65**
	2	1	<LOD	<LOD	<LOD	**1.80**
		2	<LOD	<LOD	<LOD	**2.12**
		3	<LOD	<LOD	<LOD	**2.85**
		4	<LOD	<LOD	<LOD	**1.28**
	3	1	<LOD	<LOD	<LOD	**1.02**
		2	<LOD	<LOD	<LOD	<LOD
14	1	1	<LOD	<LOD	<LOD	**2.26**
		2	<LOD	<LOD	<LOD	**2.55**
		3	<LOD	<LOD	<LOD	**0.51**
		4	<LOD	<LOD	<LOD	**0.46**
		5	<LOD	<LOD	<LOD	<LOD
	2	1	<LOD	<LOD	<LOD	**0.91**
		2	<LOD	<LOD	<LOD	**2.57**
		3	<LOD	<LOD	<LOD	**1.31**
		4	<LOD	<LOD	<LOD	**0.64**
		5	<LOD	<LOD	<LOD	<LOD
		6	<LOD	<LOD	<LOD	<LOD
		7	<LOD	<LOD	<LOD	**1.09**
	3	1	<LOD	<LOD	<LOD	<LOD
15	1	1	<LOD	<LOD	<LOD	<LOD
		2	<LOD	<LOD	<LOD	**3.86**
		3	<LOD	<LOD	<LOD	**0.83**
		4	<LOD	<LOD	<LOD	**0.37**
		5	<LOD	<LOD	**0.087**	**0.54**
		6	<LOD	<LOD	<LOD	**1.46**
	2	1	<LOD	<LOD	<LOD	**1.32**
		2	<LOD	<LOD	<LOD	**2.07**
		3	<LOD	<LOD	<LOD	**2.08**
		4	<LOD	<LOD	<LOD	**0.95**
		5	<LOD	<LOD	<LOD	**0.34**
	3	1	<LOD	<LOD	<LOD	<LOD
		2	<LOD	<LOD	<LOD	<LOD
16	1	1	<LOD	<LOD	<LOD	**2.39**
		2	<LOD	<LOD	<LOD	**3.36**
		3	<LOD	<LOD	<LOD	**2.32**
		4	<LOD	<LOD	<LOD	**1.40**
		5	<LOD	<LOD	<LOD	**0.65**
	2	1	<LOD	<LOD	<LOD	**1.88**
		2	<LOD	<LOD	<LOD	**1.41**
		3	<LOD	<LOD	<LOD	**1.52**
		4	<LOD	<LOD	<LOD	**1.47**
		5	<LOD	<LOD	**0.058**	**1.23**

		6	<LOD	<LOD	**0.13**	**1.05**
		7	<LOD	<LOD	<LOD	**1.22**
	3	1	<LOD	<LOD	<LOD	**0.52**
		2	<LOD	<LOD	<LOD	**1.02**
17	1	1	<LOD	<LOD	<LOD	**3.10**
		2	<LOD	<LOD	<LOD	**4.45**
		3	<LOD	<LOD	<LOD	**1.69**
		4	<LOD	<LOD	<LOD	**0.47**
		5	<LOD	<LOD	<LOD	**1.27**
		6	<LOD	<LOD	<LOD	**4.71**
	2	1	<LOD	<LOD	<LOD	**10.7**
		2	<LOD	<LOD	<LOD	**4.12**
		3	<LOD	<LOD	<LOD	**0.82**
		4	<LOD	<LOD	<LOD	**1.01**
		5	<LOD	<LOD	<LOD	**0.45**
		6	<LOD	<LOD	<LOD	**0.56**
		7	<LOD	<LOD	<LOD	**1.87**
	3	1	<LOD	<LOD	<LOD	**2.30**
26	1	1	<LOD	<LOD	<LOD	**3.29**
		2	<LOD	<LOD	<LOD	**4.34**
		3	<LOD	<LOD	<LOD	**3.23**
		4	<LOD	<LOD	<LOD	**4.67**
	2	1	<LOD	<LOD	<LOD	**4.34**
		2	<LOD	<LOD	<LOD	**3.08**
		3	<LOD	**3.13**	<LOD	**4.92**
		4	<LOD	<LOD	<LOD	**3.05**
	3	1	<LOD	<LOD	<LOD	**1.34**

Table S3. Mixed model between canned and non-canned diet groups considering 12-hour intervals

Predictors	Amount (µg)		
	Estimates	CI	*p*
Intercept	0.82	0.52 – 1.13	**<0.001**
Non-canned	-0.78	-1.17 – -0.39	**0.001**
Day 1 17:00	0.28	-0.04 – 0.60	0.086
Day 2 05:00	0.85	0.56 – 1.14	**<0.001**
Day 2 17:00	0.31	0.00 – 0.62	**0.049**
Day 3 05:00	0.67	0.23 – 1.11	**0.003**
Random effects			
σ^2	0.95		
τ_{00} person	0.17		
ICC person	0.15		
Observations	327		
Marginal R^2 / Conditional R^2	0.179 / 0.301		

Table S4. Mixed model between canned and non-canned diet groups considering 24-hour intervals

Predictors	Amount (µg)		
	Estimates	CI	*p*
Intercept	0.93	0.65 – 1.20	**<0.001**
Non-canned	-0.77	-1.15 – -0.38	**0.001**
Day 2 05:00	0.49	0.27 – 0.72	**<0.001**
Day 3 05:00	0.56	0.13 – 0.99	**0.011**
Random effects			
σ^2	0.99		
τ_{00} person	0.16		
ICC person	0.14		
Observations	327		
Marginal R^2 / Conditional R^2	0.149 / 0.266		

Table S5. Levels of BP analogues in whole blood samples from the canned diet group. In bold, levels above the LOD. $LOD_{BPAF, BPA}$: 0.1 µg/l; LOD_{BPF}: 0.2 µg/l; $LOD_{BPE, BPS}$: 0.5 µg/l; LOD_{BPB}: 2.5 µg/l. $LOQ_{BPAF, BPA}$: 0.03 µg/l; LOQ_{BPF}: 0.07 µg/l; $LOQ_{BPE, BPS}$: 0.17 µg/l; LOQ_{BPB}: 0.83 µg/l

Participant	Extraction	Bisphenol analogues (µg/l)													
		BPAF		BPF		BPE		BPA		BPB		BPS		BPZ	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
8	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
9	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
10	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**1.87**	<LOD	**1.85**	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
18	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.51**	**21.7**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**25.2**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
21	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
22	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
23	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
24	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.25**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
25	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S6. Levels of BP analogues in whole blood samples from the control diet group. In bold, levels above the LOD. $LOD_{BPAF,\ BPA}$: 0.1 µg/l; LOD_{BPF}: 0.2 µg/l; $LOD_{BPE,\ BPS}$: 0.5 µg/l; LOD_{BPB}: 2.5 µg/l. $LOQ_{BPAF,\ BPA}$: 0.03 µg/l; LOQ_{BPF}: 0.07 µg/l; $LOQ_{BPE,\ BPS}$: 0.17 µg/l; LOQ_{BPB}: 0.83 µg/l

Participant	Extraction	Bisphenol analogues (µg/l)													
		BPAF		BPF		BPE		BPA		BPB		BPS		BPZ	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
4	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
11	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
12	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
13	1	<LOD	**32.3**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
14	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
15	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
16	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
17	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
26	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**0.73**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S7. Levels of BP analogues in plasma samples from the canned diet group. In bold, levels above the LOD. $LOD_{BPAF, BPA}$: 0.1 µg/l; LOD_{BPF}: 0.2 µg/l; $LOD_{BPE, BPS}$: 0.5 µg/l; LOD_{BPB}: 2.5 µg/l. $LOQ_{BPAF, BPA}$: 0.03 µg/l; LOQ_{BPF}: 0.07 µg/l; $LOQ_{BPE, BPS}$: 0.17 µg/l; LOQ_{BPB}: 0.83 µg/l

Participant	Extraction	Bisphenol analogues (µg/l)													
		BPAF		BPF		BPE		BPA		BPB		BPS		BPZ	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
8	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
9	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
10	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
18	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
21	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
22	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
23	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**<LOQ**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
24	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.07**	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
25	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**3.84**	<LOD	<LOD	<LOD	<LOD

Table S8. Levels of BP analogues in plasma samples from the control diet group. In bold, levels above the LOD. $LOD_{BPAF, BPA}$: 0.1 µg/l; LOD_{BPF}: 0.2 µg/l; $LOD_{BPE, BPS}$: 0.5 µg/l; LOD_{BPB}: 2.5 µg/l. $LOQ_{BPAF, BPA}$: 0.03 µg/l; LOQ_{BPF}: 0.07 µg/l; $LOQ_{BPE, BPS}$: 0.17 µg/l; LOQ_{BPB}: 0.83 µg/l

Participant	Extraction	Bisphenol analogues (µg/l)													
		BPAF		BPF		BPE		BPA		BPB		BPS		BPZ	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
4	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
11	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
12	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
13	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
14	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
15	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
16	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
17	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
26	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	**16.2**	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S9. Levels of BP analogues in RBC samples from the canned diet group. In bold, levels above the LOD. $LOD_{BPAF,\ BPA}$: 0.1 µg/l; LOD_{BPF}: 0.2 µg/l; $LOD_{BPE,\ BPS}$: 0.5 µg/l; LOD_{BPB}: 2.5 µg/l. $LOQ_{BPAF,\ BPA}$: 0.03 µg/l; LOQ_{BPF}: 0.07 µg/l; $LOQ_{BPE,\ BPS}$: 0.17 µg/l; LOQ_{BPB}: 0.83 µg/l

Participant	Extraction	Bisphenol analogues (µg/l)													
		BPAF		BPF		BPE		BPA		BPB		BPS		BPZ	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
1	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
2	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
8	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
9	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
10	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
18	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
20	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
21	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
22	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
23	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
24	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
25	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Table S10. Levels of BP analogues in RBC samples from the control diet group. In bold, levels above the LOD. $LOD_{BPAF,\ BPA}$: 0.1 µg/l; LOD_{BPF}: 0.2 µg/l; $LOD_{BPE,\ BPS}$: 0.5 µg/l; LOD_{BPB}: 2.5 µg/l. $LOQ_{BPAF,\ BPA}$: 0.03 µg/l; LOQ_{BPF}: 0.07 µg/l; $LOQ_{BPE,\ BPS}$: 0.17 µg/l; LOQ_{BPB}: 0.83 µg/l

Participant	Extraction	Bisphenol analogues (µg/l)													
		BPAF		BPF		BPE		BPA		BPB		BPS		BPZ	
		Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
4	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
11	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
12	1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
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