HEALS

Health and Environment-wide Associations based on Large population Surveys

FP7-ENV-2013- 603946

http://www.heals-eu.eu/

6.2 Modeling module for assessing population and individual risk from biomonitoring data

WP 6 Physiology based biokinetic modeling for internal dose and exposure reconstruction

Version 2 (31/10/2017)

Lead beneficiary: AUTH

Date: 22/02/2018

Nature: Report

Dissemination level: Public

Document Information

Grant Agreement Number	ENV-603946	**Acronym**	HEALS
Full title	Health and Environment-wide Associations based on Large population Surveys		
Project URL	http://www.heals-eu.eu/		
EU Project Officer	Tuomo Karjalainen,- Tuomo.KARJALAINEN@ec.europa.eu		

Deliverable	**Number**	6.2	**Title**	Modeling module for assessing population and individual risk from biomonitoring data
Work Package	**Number**	6	**Title**	Physiology based biokinetic modeling for internal dose and exposure reconstruction

Delivery date	**Contractual**	M40	**Actual**	22/02/2018
Status	Draft ☐		Final ☒	
Nature	Demonstrator ☐	Report ☒	Prototype ☐	Other ☐
Dissemination level	Confidential ☐	Public ☒		

Author (Partners)	Denis A. Sarigiannis, S. Karakitsios, E. Handakas, A. Gotti			
Responsible Author	Denis A. Sarigiannis		**Email**	denis@eng.auth.gr
	Partner	AUTH	**Phone**	+30-2310-994562

Document History

Name	Date	Version	Desciption
AUTH	29/03/2017	1	Draft report
AUTH	31/10/2017	2	Final report
	22/02/2018	2	Submitted

TABLE OF CONTENTS

1 Introduction

This deliverable aims at providing a comprehensive methodology for associating human biomonitoring data with biologically effective dose. This provides very significant opportunities for advancing risk assessment considering that human biomonitoring, although they provide an indication about exposure magnitude, they do not actually provide an data about the time integral of the biologically effective dose at the target tissues. However, the latest is of particular importance for associating time variable exposure regimes with perturbations observed at different levels of biological organization, that are monitored through multi-omics data, as proposed in the HEALS methodology. Thus, by properly reconstructing exposure from HBM data, we have the capability to re-run in forward mode the respective PBPK model and to translate HBM data into Biologically Effective Dose (BED) at the tissue dose; this will allow the interpretation to mechanism based hazard assessment based metrics such as the Biological Pathway Altering Dose (BPAD). This quantity, the biological pathway altering dose (BPAD), is analogous to current risk assessment metrics in that it combines dose−response data with analysis of uncertainty and population variability so as to derive exposure limits. The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome. BPADs are derived from relatively inexpensive, high-throughput screening (HTS) *in vitro* data. Use of as detailed as possible PBBK modeling is the key component so as to estimate the *in vivo* doses required to achieve the BPAD in the target tissue. Uncertainty and variability will be incorporated in both the BPAD and the PBBK parameters and then combined to yield a probability distribution for the dose required to perturb the critical pathway. Thus, the more confident and explicit we are about the biokinetic behavior of the compound, the less conservative we have to be when translating BPAD into external exposure metrics. Information about BPAD for several chemicals can be easily obtained from the publicly available ToxCastDB (Judson et al., 2011). The methodology proposed by HEALS for associating HBM data with BPAD for refined risk assessment has been recently highlighted in the study of Sipes et al. , were exposure comparisons were carried out using the entirety of the Tox21 federal collaboration chemical screening data.

- .

2 Methodological framework for assessing population and individual risk from biomonitoring data

2.1 General info

One particularly well-suited source of information on exposure to environmental agents is human biomonitoring (HBM). Human biomonitoring can be defined as "the method for assessing human exposure or their effect to chemicals by measuring these chemicals, their metabolites or reaction products in human species, such as blood or urine" (Sipes et al., 2017). HBM includes (1) biomarkers that allow assessment of exposure to a chemical on the basis of its measurement in a biological matrix (biomarker of exposure) , (2) changes that have occurred in the biochemical or physiological makeup of an individual because of this exposure (biomarker of effect), or (3) biomarkers that assess a person's susceptibility to alter the progression along the exposure-effect continuum (biomarker of susceptibility) (CDC, 2009).

Most likely the main achievement of HBM data is that it provides an integrated overview of the pollutant load any participant is exposed to, and hence serves as an excellent approximation of aggregate exposure. The internal dose of a chemical, following aggregate exposure has a much greater value for environmental health impact assessment as the internal body concentration is much more relevant to the impact on human health than mere exposure data (direct EDR-relationship in Figure 1).

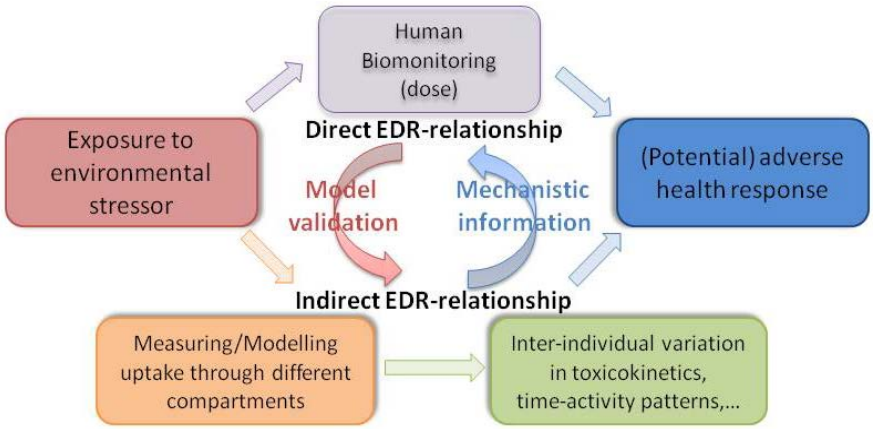


Figure 1. The Exposure-Dose-Response Triad to evaluate the potential adverse health effects of exposure to environmental agents (adapted from Smolders and Schoeters (2006))

However, it needs to be stressed that HBM in itself cannot replace environmental monitoring and modeling data. Most often, environmental monitoring data for different environmental compartments (air, water, food, soil, settled dust) provide better insight into potential sources,

hence allowing the development of more informed and appropriate risk reduction strategies. At the same time, mathematical approaches to describe the pharmacokinetic and toxicokinetic behavior of environmental agents (generally referred to as Physiologically-based Toxicokinetic - PBTK models) offer a more mechanistic insight into the behavior and fate of environmental agents following exposure (Indirect EDR-relationship in Figure 2). As biomarker data also reflect individual accumulation, distribution, metabolism and excretion (ADME) characteristics of chemicals, HBM data offer an excellent opportunity for the validation of these PBTK models. Ultimately, combining both lines of evidence to assess exposure prove to be optimal for relating complex exposure to environmental agents to potential adverse health effects assessment.

There are three approaches (Figure 2) for linking biomonitoring data to health outcomes: direct comparison to toxicity values, forward and reverse dosimetry.

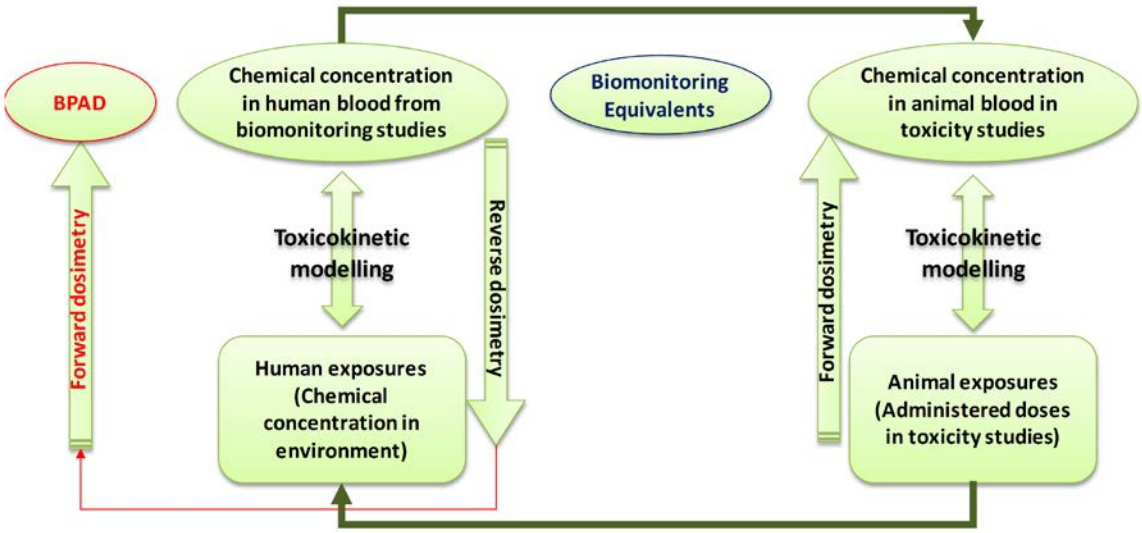


Figure 2. Interpretation of biomonitoring data

Biomonitoring data can be directly compared to toxicity values in the case where the relationship of the biomarker to the health effect of concern has been characterized in the human. In forward dosimetry, pharmacokinetic data in the experimental animal can be used to support a direct comparison of internal exposure in humans derived through the application of PBTK models, providing an estimate of the Margin of Safety (MoS) in humans. It is possible to determine the relationship between biomarker concentration and effects observed in animal studies. An evolution of this concept is the biomonitoring equivalents.

Alternatively, reverse dosimetry can be performed to estimate the external exposure that is consistent with the measured biomonitoring data through the backward application of PBTK models. In this case the PBTK model is geared with reverse modeling algorithms in order to reconstruct exposure from human biomonitoring (HBM) data. Assimilation of human

biomonitoring data and their translation into intake distribution amounts to a computational inversion problem, where the objective is to identify the specific input distributions that best explain the observed outputs while minimizing the residual error. Inputs involve spatial and temporal information on micro-environmental media concentrations of xenobiotics and corresponding information on human activities, food intake patterns or consumer product use that result in intakes; outputs are the observed biomonitored levels. The error metric can be defined in terms of population variation (the latter has to be lower than the intra-individual variation, which may be associated to measurement or other random error source).

More in detail, a computational framework was developed based on Bayesian Markov Chain Monte Carlo (MCMC) combined with the generic Physiological Based Pharmacokinetic (PBTK) model aiming at performing accurate exposure reconstruction (ER). The ER framework developed consists of 3 basic steps:

- At first the prior parameter distribution, the joint probability distribution, the population model and the determination of the measurement model have to be specified.
- At the next step exposure is calculated using MCMC simulation considering the observed biomonitoring data.
- Finally, the evaluation of the results is realized using MC simulation, with emphasis to the comparison of prior and posterior distribution as well as parameter independence

In a more elaborate scheme, the reconstructed exposure, could be used to run the PBTK model in forward mode, so as to estimate the Biologically Effective Dose (BED) at the target tissue. The estimated BED can be evaluated against the respective biological pathway altering dose (BPAD), which is analogous to current risk assessment metrics in that it combines dose−response data with analysis of uncertainty and population variability so as to derive exposure limits (Judson et al., 2010; Smolders and Schoeters, 2007). The analogy is closest when perturbation of a pathway is a key event in the mode of action (MoA) leading to a specified adverse outcome. BPADs are derived from relatively inexpensive, high-throughput screening (HTS) *in vitro* data, publicly available from the Toxcast 21 database.

3 Risk assessment and health impact based on biomonitoring data

3.1 Use of health-based guidance values as screening tool for HBM data

3.1.1 The German HBM-I and -II values

Biomonitoring data can be interpreted by comparing the measured biomarker levels to health relevant biomonitoring reference values. In this context, the German Human Biomonitoring Commission has derived health-based guidance values for several compounds (Judson et al., 2011). These are HBM values determined either based on exposure-effect relationships (for cadmium, lead, mercury and pentachlorophenol) or derived from tolerable daily intake values (as for DEHP). There are two levels of HBM values derived by the German Human Biomonitoring Commission, namely HBM-I and HBM-II:

- The HBM-I value represents the concentration of a substance in human biological material below which – according to the knowledge and judgment of the German HBM Commission – there is no risk for adverse health effects and, consequently, no need for action;
- The HBM-II value represents the concentration of a substance in a human biological material above which there is an increased risk for adverse health effects and, consequently, an acute need for exposure reduction measures and the provision of biomedical advice. The HBM-II-value should thus be regarded as an intervention or action level.

At a concentration level higher than the HBM-I- and lower than the HBM-II-value the result should be verified by further measurements. If these measurements confirm the initial result a search for potential sources of exposure should be undertaken. Exposure to such sources should be minimized or eliminated where necessary and achievable with an acceptable level of input. The HBM-I-value should thus be regarded as a verification or control value.

Up to 2014 the derivation of toxicologically founded HBM values was based on studies which allowed a correlation between the concentration of a substance or its metabolites in human body fluids und the occurrence of adverse effects. Yet, as such studies are lacking for most chemicals, the German HBM Commission decided to derive also HBM values on the basis of toxicologically justified tolerable daily intakes or other suitable parameters from animal studies. Being well aware of the uncertainties of such derivation and estimates, the HBM Commission considers this new approach a possibility to derive urgently needed HBM values for substances or their metabolites for which no appropriate studies on health effects of low dose environmental exposure are currently available. A comprehensive overview of these values is given in Table 7.

Table 1. German Human Biomonitoring Commission reference biomonitoring values for various compounds (Schulz et al., 2007). The HBM-I value is more of a control value while the HBM-II value is defined as an action level

Parameter and medium	Population group (age range)	HBM I value	HBM II value
Based on epidemiological studies			
Cadmium in urine	Children and adolescents	0.5 µg/L	2 µg/L
	Adults	1 µg/L	4 µg/L
Lead in blood	General population incl. children <12 years, women of reproductive age	Suspended	Suspended
Mercury in urine	Children and adults	7 µg/l 5 µg/ g Cr	25 µg/l 20 µg/g Cr
Mercury in blood	Children and adults* Derived from women of reproductive age. The value is recommended for other groups	5 µg/L	15 µg/L
Thallium in urine	General population	5 µg/l	/
Pentachlorophenol in serum	General population	40 µg/L	70 µg/L
Pentachlorophenol in urine		25 µg/L 20 µg/g Cr	40 µg/L 30 µg/g Cr
Sum of PCBs (138+153+180) in serum	Infants, small children and women of child-bearing age	3.5 µg/l	7 µg/l
Based on TDIs			
Sum of the metabolites of di(2-ethylhexyl)phthalate DEHP: 5-oxo- and 5-OH-MEHP in urine	Children aged 6–13	500 µg/L	***/***
	Women of reproductive age	300 µg/L	***/***
	Males ~14 years, general population	750 µg/L	/
Bisphenol A in urine	Children Adults	1.5 mg/l 2.5 mg/l	/
Glykolether which are metabolized to methgoxy acetic acid (MAA)	General population	0.4 mg MAA/G Cr	1.6 mg MMA/g Cr
∑ DINCH-metabolites (OH-MINCH + cx-MINCH)	Children Adults	3 mg/l 4.5 mg/l	/
∑ DPHP-metabolites OH-MPHP + oxo-MPHP	Children Adults	1 mg/l 1.5 mg/l	/

3.1.2 Biomonitoring equivalents

Biomonitoring Equivalents (BEs) are defined as the concentration of a chemical or metabolite in a biological matrix (blood, urine, human milk, etc.) consistent with defined exposure guidance values or toxicity criteria, including reference doses and reference concentrations (RfD and RfCs), minimal risk levels (MRLs) and tolerable daily intakes (TDI), using the knowledge about the toxicokinetic properties of the chemical (Schulz et al., 2011). The application of BEs is based on the assumption that intake and excretion are in equilibrium, in

order to ensure coherence between the targeted chronic exposure reference value and the respective estimated BE. However, real life exposure is rarely constant or periodically repeated, and this requires some additional need for caution in the interpretation of the biomonitoring data. Requirements for using the above methods involve ensuring the specificity and sensitivity of the biomarker, quantitative risk evaluation, estimation of the proper uncertainty factors for translating the external dose that corresponds to the Point of Departure (PoD) in an animal to a human biomarker concentration (Boogaard et al., 2011), as well as some knowledge of the toxicokinetic behavior of the respective biomarker. Use of reliable PBTK models is the most convenient way to translate external exposure reference values into BEs. The biomonitoring equivalents derived for several compounds are presented in Table 8.

Table 2. Biomonitoring Equivalent values for several compounds

Environmental Chemical	Matrix	Analyte	BE value	Reference value	Reference
DDT/DDE/DDD	Blood	(DDT only) (RDDT/DDE/DDD)	30,000 ng/g lipid 40,000 ng/g lipid	FAO/WHO (10 µg/kg/day)	(Angerer et al., 2011)
Hexachlorobenzene	Blood	Hexachlorobenzene	16 ng/g lipid	Health Canada (0.05 µg/kg/day)	(Kirman et al., 2011)
Dioxin TEQ	Blood	Dioxin TEQ	15 ng/g lipid	ATSDR LOAEL (0.12 ng/kg/day)	(Aylward et al., 2010)
Hexabromocyclododecane	Blood, breast milk	Hexabromocyclododecane	190,000 ng/g lipid	EU Draft (POD) (2 mg/kg/day)	(Aylward et al., 2008)
Deltamethrin	Blood Urine	Deltamethrin Dimethylcyclopropane carboxylic acid	20 µg/L[1] and 2µg/L[2] 50 µg/L[1] and 7µg/L[2]	EC (10 µg/kg/day)	(Aylward and Hays, 2011)
PBDE 99	Blood	PBDE 99	520 ng/g lipid	US EPA (0.1 µg/kg/day)	(Aylward et al., 2011)
Cyfluthrin	Urine	4-fluoro-3-phenoxybenzoic acid	400 µg/L	FAO/WHO ADI (10 µg/kg/day)	(Krishnan et al., 2011)
Triclosan	Urine	total triclosan (free plus conjugates)	2600 µg/L	EC (120 µg/kg/day)	(Hays et al., 2009)
Bisphenol A	Urine	BPA-glu	2000 µg/L	EFSA (50 µg/kg/day)	(Krishnan et al., 2010b)
Di-2(ethylhexyl) phthalate - DEHP	Urine	MEHP, MEHHP, and MEOHP MEHP, MEHHP, MEOHP, and 5cx-MEPP MEHP, MEHHP, MEOHP, 5cx-MEPP, and 2cx-MMHP	660 µg/L 1000 µg/L 1100 µg/L	EFSA (50 µg/kg/day)	(Krishnan et al., 2010a)
Diisononyl phthalate - DiNP	Urine	Oxidative (OH-, oxo-, and carboxy-MINP) metabolites MiNP	15 µg/L[3] 10.7 µg/L[4] 12.7 µg/L[5] 10.6 µg/L[6] 0.7 µg/L[3] 0.5 µg/L[4] 0.6 µg/L[5]	EFSA (150 µg/kg/day)	(Aylward et al., 2009b)

Environmental Chemical	Matrix	Analyte	BE value	Reference value	Reference
			0.5 µg/L[6]		
di-n-butyl phthalate - DBP	Urine	MBP	0.2 µg/L	EFSA (10 µg/kg/day)	(Hays et al., 2011)
benzylbutyl phthalate - BzBP	Urine	MBzP	12 µg/L	EFSA (500 µg/kg/day)	(Aylward et al., 2009a)
diethyl phthalate - DEP	Urine	MEP	18 µg/L	EPA (800 µg/kg/day)	(Aylward et al., 2009a)
Benzene (for chronic non-cancer exposure)	Blood	benzene	0.15 µg/L	USEPA Chronic RfC	
	Urine	Unmetabolized benzene	0.16 µg/L		
	Blood	benzene	1.29 µg/L	TCEQ ReV	
	Urine	Unmetabolized benzene	1.42 µg/L		
	Blood	benzene	0.29 µg/L	CA REL	
	Urine	Unmetabolized benzene	0.33 µg/L		
	Blood	benzene	0.04 µg/L	ATSDR chronic inh. MRL	
	Urine	Unmetabolized benzene	0.05 µg/L		
Benzene cancer risk-specific exposure levels	Blood	benzene	0.058–0.204 µg/L	USEPA, risk-specific concentrations (1E-04 risk - 13.0–45.0 µg/m^3)	(Aylward et al., 2009a)
	Urine	Unmetabolized benzene	0.125–0.286 µg/L		
	Blood	benzene	0.058–0.204 ng/L		
	Urine	Unmetabolized benzene	Not calculated	USEPA, risk-specific concentrations (1E-06 risk - 0.13–0.45 µg/m^3)	
	Blood	benzene	0.15 µg/L		
	Urine	Unmetabolized benzene	0.16 µg/L		
	Blood	benzene	0.204 µg/L	TCEQ, ESL cancer (1E-04 risk - 44.6 µg/m^3)	
	Urine	Unmetabolized benzene	0.286 µg/L		
	Blood	benzene	0.204 ng/L	TCEQ, ESL cancer (1E-04 risk – 0.446 µg/m^3)	
	Urine	Unmetabolized benzene	Not calculated		
Toluene	Blood	Toluene	50 µg/L	USEPA chronic RfC (128 mg/m^3)	
			40 µg/L	Health Canada chronic inhalation TDI (150 mg/m^3)	
			3 µg/L	WHO air quality guideline (332 mg/m^3)	
			3 µg/L	ATSDR chronic inhalation MRL (132 mg/m^3)	
			30 µg/L	ATSDR acute MRL (150 mg/m^3)	
Cadmium	Urine	Cadmium	1.2 µg/L	FAO/WHO (10 µg/kg/day)	(Hays et al., 2012)
Arsenic, inorganic	Urine	Inorganic arsenic, monomethylated arsenic, and dimethylated arsenic	6.4 µg/L	ATSDR (0.3 µg/kg/day)	(Hays et al., 2008)

[1] adults
[2] children
[3] children 6-11 years

[4] adolescents 11-16 years
[5] men >16 years
[6] women >16 years

In a more elaborate scheme, BEs can be derived by usig as an input to the PBBK models the external exposure reference levels, for estimating the levels that correspond to the target tissue. This is more informative that just reconstructing exposure from the HBM data, because uncertainties and susceptibilities related to interindividual differences in toxicokinetics (such as the age dependent differences of the detoxification pathways).

3.2 Assessing population and individual risk from biomonitoring data through the estimation of Biologically Effective Dose (BED)

A more comprehensive way to understand the risks associated to environmental exposure to chemical stressors is to use the biomonitoring data as the basis for estimating the actual internal dose in the target tissue. This requires (a) the mechanistic translation of this data into internal exposure estimates in the target tissue, or the respective biologically effective dose (BED) and (b) the use of an internal dose reference level (IDRL); the actual risk, will be derived as the quotient of the BED/IDRL

Regarding the estimation of BED from biomonitoring data, the following steps should be follow:

- Reconstructing exposure profiles starting from biomonitoring data, by coupling the compound-specific parameterized generic PBBK model with the exposure reconstruction scheme described in detail in D6.1 and
- Re-running forward the estimated exposure / intakes through the PBBK model for estimating the internal dose profiles in the target tissues, or the so called biologically effective dose.

For deriving internal dose reference level, there are two options:

- The first option, includes the use of regulatory accepted reference doses and reference concentrations (RfD and RfCs), minimal risk levels (MRLs) and tolerable daily intakes (TDI) into IDRL; this is a pretty straight forward process, where the respective reference dose is used in a typical adult exposure scenario, and the IDRL is estimated by the compound specific PBBK model.
- The second option is to use an *in vitro* derived Biological Pathway Altering Dose (BPAD). BPAD is analogous to current risk assessment metrics in that it combines dose−response data with analysis of uncertainty and population variability so as to derive exposure limits (Hays et al., 2007; Judson et al., 2011). The analogy is closest when perturbation of a pathway is a key event in the mode of action (MOA) leading to a specified adverse outcome. The approach is very challenging and promising considering the wealth of data continuously produced by ToxCast21 and the fact that BED is actually what has to be directly associated with BPAD.

However, two main issues have to be taken into account before the widespread use of BPAD for risk assessment; the first one is that BED might be different to the actual *in vitro* dose if the experimental conditions of the assays are not well described and, *in vitro* assays neither capture systemic effects, nor systemic homeostasis, resulting in underestimation or overestimation of toxicity. Given these limitations, within the scope of HEALS, the first option will be widely used, while the feasibility of the second one will be demonstrated in the exhaustive case study of bisphenol A.

4 Demonstration of the HEALS approach: Assessing bisphenol A related risks starting from European human biomonitoring data

4.1 INTRODUCTION

Exposure to BPA and the potential adverse health effects constitute one of the hottest public health topic. The main controversy regards the toxicokinetic behavior of BPA; although BPA glucuronidation (the dominant detoxification mechanism) is complete and rapid, due to the reduced metabolic capacity of infants-neonates, there is still ample opportunity for internal exposure (Ginsberg and Rice, 2009; Hays et al., 2010). Existing biomonitoring studies regarding BPA exposure cannot provide valuable information with regard to toxicokinetics, because almost all of them track only urinary metabolites (BPA-glu), rendering them useful only for assessing the overall uptake. Due to the rapid metabolism of BPA to BPA-glu, the extent of binding (a fraction of 0.95) to red blood cells and plasma proteins, and limitations of the analytical techniques, the related biomonitoring studies either fail to detect free-BPA in the plasma or the detected values are attributed to background contamination from labware and indoor dust (Ginsberg and Rice, 2009). The latter hypothesis is, however, strongly contested (Dekant and Völkel, 2008). Contradiction is further amplified by toxicity testing results. BPA was found to produce adverse neurodevelopmental effects in rats given an oral dose that was considered environmentally relevant; the results were, however, seriously criticized by regulatory authorities (EFSA, 2006; Vandenberg et al., 2010) mainly based on the validity of the applied methods (not GLP compliant). Additional arguments included the relevance of bioavailability for the same normalized oral dose among rodents and humans, due to substantial differences in the BPA excretion mechanism (for humans only via urine, for rodents via feces and urine due to hepatobilic recirculation) and the consequent rates of elimination (elimination half-life of 5.3 h and 10.5h for humans and rodents respectively).

There are four critical reviews targeting the main controversies about BPA. Ginsberg and Rice (2008) focused on the toxicokinetic arguments, providing a comprehensive review on biomonitoring data and the uncertainties that relate to perinatal and infancy detoxification pathways. They report that beside the detoxification pathways of glucuronidation and sulfation, the presence of enzymes necessary for infant development is also responsible for BPA-Glu deconjugation, possibly increasing the bioavailability of BPA. Vandenberg et al. (2009), besides their own contribution to BPA toxicity testing, compiled an informative review on the overall controversies (exposure, toxicokinetics and toxicity testing) and the way in which regulatory authorities evaluate the research findings, highlighting the importance of non-monotonic dose-response relationships and the related effects of low doses, as well as the increased susceptibility during the critical periods of perinatal and neonatal exposure. Beronius et al. (2009) published a critical review on the BPA risk assessment reports issued by regulatory authorities worldwide, illustrating the impact of differences in risk assessment

policy and expert judgment and highlighting the importance of transparency in the risk assessment process. The latest review paper written by Vandenberg (2010) relies on the widespread exposure to BPA evaluating more than 80 biomonitoring studies including a variety of biological fluids and BPA forms (conjugated or free), and focusing on the reliability of studies measuring free BPA in the plasma. The authors concluded that the majority of the studies detecting non negligible free-plasma BPA concentrations in blood fulfill the criteria for considering them as reliable and as such they should be taken into account in the risk assessment process.

Preliminary efforts to develop PBTK models for BPA were made by Shin et al. (2010) and Teeguarden et al. (2004), but the most comprehensive model, which clearly illustrated metabolic scaling from adults to neonates-infants was developed by Edginton and Ritter (2005). The core finding of their study was that BPA plasma concentrations could be approximately elevent times greater in newborns than in adults exposed to the same weight-normalized dose. In the latest published BPA PBTK model (Mielke and Gundert-Remy, 2009), sulfation was included as an additional metabolic pathway, considering that sulfation activity is well expressed in newborns and it is at least as high as in adults, or even higher. Besides the importance of the additional clearance pathway, this model was much more simplified than the one developed by Edginton and Ritter, with significantly fewer compartments, without considering BPA binding to red blood cells, and without estimating the fate of conjugated metabolites. The estimated steady-state free plasma BPA concentrations in neonates and infants were lower than the ones estimated by Edginton and Ritter due to the addition of sulfation.

The present study aims to quantify external and internal exposure to BPA and to assimilate biomonitoring data in Europe. The overall modelling framework is compiled in a software package (asclXtreme) which allows dynamic simulations through time (not only steady state estimates), as well as implementation of Monte Carlo (MC) sensitivity, uncertainty and variability analysis. Continuous simulation through time allowed the development of a mother-fetus PBTK model parameterized for BPA and its glucuronidated form (BPA-Glu) taking into account the changes in physiologic and metabolic parameters that occur during gestation and early in life. Absorption processes through all potential exposure routes (oral, inhalation and dermal) have been incorporated aiming at investigating route dependent bioavailability differences in BPA. Finally, exposure was reconstructed based on recent European biomonitoring data using an exposure reconstruction algorithm.

4.2 Approach

4.2.1 Particular considerations regarding BPA toxicokinetics

The main detoxification pathway of BPA is phase II glucuronidation (and sulfation at early developmental stages). Thus, in our model, we account for the parent compound (BPA) and

one metabolite (BPA-Glu and BPA-Sulf for fetus and early infancy). Glucuronidation and sulfation capacities throughout the several developmental stages are critical parameters of the BPA toxicokinetic model. Taking into account the physiologically-based approach for scaling to children (Sarigiannis et al., 2014) and the later findings regarding the ontogeny of enzymes involved in BPA detoxification (Edginton et al., 2006; Leeder, 2009), clearance rates were adjusted taking into account the findings of Fisher et al. (2012) and assuming an age-dependent bioavailability difference factor of 2. Additional parameters such as tissue:blood partition coefficients, intrinsic clearance and renal clearance, that are needed for the parameterization of the generic model so as to capture BPA toxicokinetics, have been obtained from Edginton and Ritter (2011). The most recent *ex vivo* and *in vivo* experiments have shown that BPA freely diffuses across the human placenta (Balakrishnan et al., 2010). De-conjugation kinetics in the placenta was determined empirically based on experimental findings (Balakrishnan et al., 2010; Schonfelder et al., 2002), where concentration in the placenta was found to be almost twice the one in maternal blood. Regarding lactation, in order to estimate the total amount of BPA to which the infant is orally exposed, the sum of BPA and BPA-Glu in breast milk has to be accounted for. Finally, metabolism in the skin was also taken into account; indeed based on experimental results that compared BPA diffusion in fresh human explants, it was found that BPA was extensively metabolized in viable epidermis, accounting for 27% of the daily administered dose (Takahashi and Oishi, 2000).

4.2.2 Exposure characterization

4.2.2.1 Exposure scenario definition

The identification of exposure scenarios was based on the evaluation of (a) emissions from manufacturing comprising production of polycarbonate, epoxy resins, PVC and thermal paper production and processing and (b) releases and exposure from consumer products comprising polycarbonate bottles, epoxy resin coating, medical equipment and thermal paper. The overview was based on the EU risk assessment reports (EU, 2003; Zalko et al., 2011), but not limited to these data.

Based on the above, several individual exposure scenarios and their possible combinations were derived as explicitly described in Table 1. Environmental (far field) components of BPA exposure are present in all plausible exposure scenario aggregation schemes, including (a) inhalation exposure through ambient air contamination, (b) dietary exposure through food web contamination and (c) non-dietary oral exposure of soil ingestion. Near field exposure included (a) inhalation exposure through indoor air contamination; (b) dietary exposure through canned food items and canned beverages; (c) non-dietary oral exposure of settled dust ingestion; and (d) dermal exposure through contact with thermal paper for the cashiers. Limitations in the plausibility of consumer scenarios are related to age groups considered (e.g. breast feeding neonates/infants, premature infants hosted in intensive care units). The detailed individual dietary scenarios for adults related to several types of canned food (i.e.

soup, meat, tuna, fruits and vegetables) were also investigated for toddlers, children and cashiers, but for parsimony reasons they are presented in detail only for adults.

Table 3. Exposure scenarios and plausible combinations

Age group and scenario	**Near field exposure**			**Far field exposure**		
	Oral	**Skin**	**Inhalation**	**Oral**	**Skin**	**Inhalation**
Infants premature hosted in intensive	X		X			X
Infants/neonates breast fed	X		X	X		X
Infants bottle fed/infant formula	X		X	X		X
Infants consuming canned food	X		X	X		X
Infants consuming canned food and	X		X	X		X
Toddlers consuming canned food	X		X	X		X
Toddlers consuming canned beverages	X		X	X		X
Toddlers consuming canned food and	X		X	X		X
Children consuming canned food	X		X	X		X
Children consuming canned beverages	X		X	X		X
Children consuming canned food and	X		X	X		X
Teens consuming canned food	X		X	X		X
Teens consuming canned beverages	X		X	X		X
Teens consuming canned food and	X		X	X		X
Adult consuming canned soup	**X**		**X**	**X**		**X**
Adult consuming canned meat	**X**		**X**	**X**		**X**
Adult consuming tuna	**X**		**X**	**X**		**X**
Adult consuming canned fruits	**X**		**X**	**X**		**X**
Adult consuming canned vegetables	**X**		**X**	**X**		**X**
Adult consuming canned food	X		X	X		X
Adult consuming canned beverages	X		X	X		X
Adult consuming canned food and	X		X	X		X
Cashier		X	X	X		X
Cashier, consuming canned food	X	X	X	X		X
Cashier, consuming canned beverages	X	X	X	X		X
Cashier, consuming canned food and	X	X	X	X		X

4.2.2.2 Acquisition of exposure related data

4.2.2.2.1 Intake estimates

In order to estimate population exposure to BPA, a comprehensive methodological scheme was followed. This included the acquisition of data related to environmental releases of BPA, as well as BPA food residues, stemming either through the food web (transfer through the environment) or by food contact materials (e.g. cans and polycarbonate bottles). Environmental contamination included also pathways such as air, soil, drinking water and settled dust. Data for environmental releases with regard to the several manufacturing processes were retrieved from the EU risk assessment reports (EU, 2003, 2010). Indicatively we report that indoor dust concentration in Belgium ranged from 535 to 9729 ng/g (EU, 2010) and in Germany from 117 to 1486 ng/g (Geens et al., 2009).

Exposure to breast fed infants was estimated based on data related to BPA found in human milk. Through lactation, besides free BPA, BPA-glu is also excreted. For estimating the total amount of BPA that the infant is orally exposed, the sum of free BPA and BPA-glu needs to be taken into account, since all conjugated BPA is cleaved in the gastrointestinal tract. BPA levels in breast milk have been found to range from 0.05 to 1.16 μg/kg (Volkel et al., 2008). The average daily breast milk intake is 13 g/kg bw on the first day of life, increases gradually 98 g/kg bw on day 3, and reaches a relatively constant level of 155 g/kg bw from day 5 (Deceuninck et al., 2015).

With regard to the main sources of exposure, a wide variety of BPA concentration levels were found in infant formula, ranging from 0 to 0.384 μg/g based on samples from Italy and Spain (Casey et al., 1986), Greece (Ferrer et al., 2011) and UK (Maragou et al., 2006). The concentrations of BPA found in commercial canned milk samples from Greece ranged from <0.0017 to 0.0152 μg/g (Goodson et al., 2002). The calculated mean concentration of evaporated milk from UK was 0.0498 ± 0.0109 μg/g (Maragou et al., 2006), while in the same study canned baby food mean BPA levels were in the range of 0.03 μg/g. BPA concentration values of powdered milk (skimmed milk) from Spain (Goodson et al., 2004) were higher (0.8 μg/g) than in the other countries.

BPA levels were determined in peeled canned tomatoes of different brands found in Italian supermarkets. Of the 42 tomato samples that were tested, BPA was detected only in 22 samples (52.4%). This implies that the presence of bisphenol A in peeled tomatoes does not necessarily arise from their packaging in cans. Tomato samples analyzed were packaged in cans coated with either epoxyphenolic lacquer or low BADGE enamel. However, no significant difference in bisphenol content exists between epoxyphenolic and low BADGE coated cans (Ferrer et al., 2011). BPA values from other European countries are in consistent with the Italian values from cans with epoxyphenolic lining (Braunrath et al., 2005; Goodson et al., 2002; Grumetto et al., 2008). BPA concentrations were determined in canned corn as well (Braunrath et al., 2005; Geens et al., 2010; Goodson et al., 2002).

Values varied from 0.016 (in UK) to 0.0674 μg/g (in Belgium). The maximum value of 0.103 ± 0.003 μg/g BPA appeared in green beans in Spain (García-Prieto et al., 2008).

Different canned fruit samples were analyzed by García-Prieto et al. (2008) in Spain, Braunrath et al. (2008) in Austria and Geens et al. (2005) in Belgium in order to determine BPA in the solid content of fruit salad, peaches, pears, pineapples, lychees and mango. BPA was present at concentrations in the range from 0.005 to 0.0244 μg/g in canned fruits. A significantly high BPA value of 0.024 μg/g was found in mango fruit by García-Prieto et al. (2010) and Braunrath et al. (2008) while a very low value of 0.0002 μg/g was detected in apple sauce in Belgium (Braunrath et al., 2005).

Various studies have focused on BPA concentration in canned fish. In the case of tuna, mackerel and sardines BPA concentrations were determined in both solid and liquid portions, although solid portions comprise the parts of the can content that are actually consumed. The highest BPA values were found in tuna in brine (0.1693 μg/g) and in tuna in oil (0.1264 μg/g) (in fish portion) in Belgium (Geens et al., 2010). The lowest values (0.0009 μg/g) were found in salmon (0.0007 μg/ml, in liquid portion) and in anchovy (0.0009 μg/g) (Geens et al., 2010). A variety of canned products such as soups, meat, spaghettis and desserts have been examined for BPA migration. Concentrations ranged from <0.001 to 0.1 μg/g, except for canned soup, ham and spaghetti Bolognese (heated in water) which were 0.4, 0.38 and 0.12 μg/g, respectively (Geens et al., 2010; Goodson et al., 2004; Goodson et al., 2002). Geens et al. (2005) examined similar types of food items packed in materials other than cans, such as paper, plastic, glass and Tetra Pak for comparison purposes. The food in these packing materials had clearly lower BPA contamination (average concentration 0.00046 μg/g) compared to similar canned food.

In terms of canned beverages, low BPA levels ranging from non-detectable to <0.007 μg/ml were found in beer (Braunrath et al., 2005; Geens et al., 2010; Goodson et al., 2002). BPA concentrations in cola ranged between 0.000225 and 0.0007 ± 0.0001 μg/ml (Braunrath et al., 2005; Gallart-Ayala et al., 2011; Geens et al., 2010), while Goodson et al. (2010) had not detected BPA in bottles containing cola. According to Ballesteros-Gómez et al. (2002), BPA was detected only in one tea sample (0.0023 ± 0.0001 μg/ml) out of the two that were examined. Gallart-Ayala et al. (2009) confirmed the presence of BPA at MLD (Method limits of detection) level (5 ng/lit) in tea samples and Geens et al. (2011) detected very small values (0.00069 μg/ml). Very low BPA concentrations were also detected in soda drinks (Ballesteros-Gómez et al., 2009; Gallart-Ayala et al., 2011; Geens et al., 2010). Only Braunrath et al. (2010) had detected BPA in small quantities in soft drinks. Neither Ballesteros-Gómez et al. (2005) nor Goodson et al. (2009) had detected BPA in similar samples. Geens et al. (2002) also examined beverages in PET and Tetra Pak for comparison purposes and BPA could not be detected above LOQ (0.02 ng/ml) in these bottles.

Dermal exposure to BPA was based on the study carried out by Biedermann et al. (2010), indicating a maximum additional uptake up to 71 μg of BPA during a 10 hours shift of a cashier. This result is derived by exposing skin to thermal paper and it is considered as the upper part of exposure distribution through contact to thermal paper.

4.2.2.3 Human biomonitoring data

An additional way to estimate exposure and intake is the use of biomonitoring data. Similarly to above, data were collected from a literature review. BPA urinary concentrations are age-dependent, reflecting the differences in consumer exposure related to food packaging material (canned food, milk formula, use of plastic baby bottles). The restriction of BPA use in baby bottles in EU countries in 2011, accompanied by the increasing public awareness about potential adverse health effects of BPA resulted in a decline of measured BPA levels. In the most recent studies, urinary BPA (in the form of the glucuronidated metabolite) measured levels are about 2 μg/L. BPA urinary concentrations measured in Germany (GerES) depend on children's age: they were 3.5, 2.8, 2.1 and 2.6 μg/L for children aged 3-5, 6-8, 9-11 and 12-14 years old respectively. Similar levels (2.5 μg/L) were recorded in France (Vandentorren et al., 2011) for pregnant women,. According to the German ESB (Vandentorren et al., 2011) urinary concentrations of BPA declined from 2 μg/L in 1995 to 1.3 μg/L in 2009. The DEMOCOPHES (2012) study provided results for urinary BPA levels in Belgium (2.6 μg/L), Denmark (2.2 μg/L), Luxembourg (1.9 μg/L), Slovenia (2.1 μg/L), Spain (2.1 μg/L) and Sweden (1.4 μg/L) (DEMOCOPHES, 2013).

A specific vulnerable group that had to be included in the study is premature infants hosted in intensive care units. Exposure of premature infants to BPA was based on the biomonitoring data of Calafat et al. (2014), where the BPA geometric mean urinary concentration (30.3 μg/L) among premature infants undergoing intensive therapeutic medical interventions was one order of magnitude higher than that among the general population. Considering a urine volume of about 0.3L, this corresponds to an average daily intake up to 2.6 μg/kg-bw. Considering the similarities of medical practices and equipment used between USA and Europe, although not European the data of Calafat et al (2009) were included in our review, since their study is the only one providing information on this vulnerable group as well as.

Table 4. Bisphenol A biomonitoring levels from several European national surveys

Country – study name	Population group	Mean	Median	Reference
Belgium - Democophes	Mothers (≤45 years)		2.6	(Calafat et al., 2009)
Denmark - Democophes	Mothers (≤45 years)		2.2	
Denmark - Copenhagen	Children and adolescents	2.3		(Covaci et al.,

Country – study name	Population group	Mean	Median	Reference
Puberty Study	(5-9 years)			2014)
	Children and adolescents (10-13 years)	1.5		
	Children and adolescents (14-20 years)	0.7		
Denmark - Copenhagen Study on Male Reproductive Health	Young men	3.2		
Denmark - Odense Child Cohort	Pregnant women	1.5		
France - ELFE	Pregnant women	2.5	2	(Frederiksen et al., 2014)
Germany - ESB	Students (<2000) - Münster		2.0	(Vandentorren et al., 2011)
Germany - ESB	Students (≥2000) - Münster		1.4	
Germany - GerES	3-14 years	2.7	2.7	(UBA, 2012)
Germany - GerES	3-5 years	3.5	3.6	
Germany - GerES	6-8 years	2.8	2.7	
Germany - GerES	9-11 years	2.1	2.2	
Germany - GerES	12-14 years	2.6	2.4	
Italy - InCHIANTI	20-40 years	4.4	4.3	(Becker et al., 2009)
	41-65 years	3.9	3.7	
	66-74 years	3.3	3.2	
Luxembourg - Democophes	Mothers (≤45 years)		1.9	(Covaci et al., 2014)
Netherlands -	Pregnant women (18-41	1.2	1.1	(Covaci et al.,

Country – study name	Population group	Mean	Median	Reference
Generation R	years)			2014)
Slovenia - Democophes	Mothers (≤45 years)		1.2	(Covaci et al., 2014)
Spain - INMA	Pregnant women	2.2		(Covaci et al., 2014)
Spain - INMA	Children (4 years)	4.2		
Spain - Democophes	Mothers (≤45 years)		2.1	(Casas et al., 2011)
Sweden - Democophes	Mothers (≤45 years)		1.4	
	Premature infants	30.3		(Covaci et al., 2014)

4.2.3 Risk characterization

Risk characterization of BPA was carried out employing several tools related to external and internal exposure assessment. As a starting point the EFSA temporary Tolerable Daily Intake (t-TDI) value of 4 μg/kg_bw/d was used (Calafat et al., 2009). The options for evaluating exposure levels included:

a. Direct comparison of intake estimates to EFSA t-TDI of 4 μg/kg_bw/d.

b. Use of a biomonitoring equivalent (BE) value for urinary data. A BE is defined as the concentration of a chemical or metabolite in a biological medium that is consistent with an existing exposure guidance value criteria including reference doses and reference concentrations (RfD and RfCs), minimal risk levels (MRLs), or tolerable daily intakes (TDIs) (EFSA, 2015a). The external exposure threshold used for deriving the BE value was the EFSA t-TDI of 4 μg/kg_bw/d. To derive the BE value, we assumed that this dose is given orally to an adult of 70 kg at a constant rate during the day. This dose was then used as input for the PBTK model described above. Based on these assumptions, the corresponding BE value was calculated equal to 320 μg/L urinary BPA-Glu.

Since BPA is characterized by rapid clearance, all BPA entering during the day is excreted in urine. Although urine sampling of excreted BPA is representative for the overall daily intake (from all routes), it does not provide any information about internal exposure variability. In order to come up with a more realistic metric for associating intake to internal exposure, free plasma BPA was considered as the most descriptive metric, directly associated to

Biologically Effective Dose (BED). This was used in order to capture discrepancies between internal and external exposure due to age-dependent differences in the rate of clearance, bioavailability differences based on the route of exposure and intraday variability of internal dose due to the complexity of exposure scenarios and the differences in absorption to systemic circulation, related to the route of exposure. In order to associate the risk of the several exposure scenarios based on the Biologically Effective Dose (BED) derived by the PBTK model, two different exposure metrics were used:

1. The EFSA t-TDI of 4 μg/kg_bw/d was translated into internal exposure, found to correspond to a concentration of 0.013 μg/L of free plasma BPA.

2. The use of internal dosimetry metrics allows the use of *in vitro* toxicological data for risk characterization. In this case, instead of translating an external reference dose such as the EFSA t-TDI (obtained from *in vivo* animal NOAEL extrapolation), an *in vitro* reference dose (BPAD) was used. The ToxCast BPA *in vitro* assays provided six ER agonist or binding AC50 values for BPA, ranging from 0.6 to 1.7 μM. To calculate a conservative Biological Pathway Altering Dose (BPAD), the lowest ToxCast AC50 was selected, which is 0.64 μM for Attagene Factorial cis ERE assay (Judson et al., 2010; Judson et al., 2011). Incorporating uncertainty factors related to population response to xenobiotics, two different values are produced, namely the $BPAD_{99}$, which is the permissible exposure level that accounts for population variability, and $BPADL_{99}$, which is the permissible exposure level additionally accounting for uncertainty. By using the reverse toxicokinetics approach that accounts for the concentration at steady state divided by the dose rate, the respective estimated population parameters give a $BPAD_{99}$ of 0.44 μg/kg_bw/d, with lower one-sided confidence limit, $BPADL_{99}$, of 0.16 mg/kg/day (Judson et al., 2010). Using these external exposure values in our PBTK model, we derive equivalent internal dose of 1.44 and 0.52 μg/L respectively. These concentrations are almost two orders of magnitude higher than the BED derived from the EFSA t-TDI (0.013 μg/L).

4.3 RESULTS

4.3.1 External exposure estimates

4.3.1.1 External exposure estimation based on exposure scenarios

Exposure analysis in this study was carried out through the use of probabilistic data (food residues) and detailed multimedia environmental modelling, taking into account actual emissions to the environment for estimating far field exposure, rather than default values based on the overall production volume and the relevant environmental release categories (ERCs), as described by ECHA (Judson et al., 2011). Calculated daily intake for humans

exposed via the environmental media includes oral exposure from drinking water, fish, root crops, meat, milk, and inhalation. The aggregate (total) daily dose from all environmental media is in general very low, indicating an almost negligible contribution to the overall uptake (0.0009 μg/kg_bw/d for oral and 1E-10 μg/kg_bw/d for inhalation). With regard to near field exposure, non-dietary ingestion of dust contributes to an exposure of almost 0.01 μg/kg_bw/day to infants, due to the high amount of dust daily ingested (up to 0.5 g/d) by this population group (ECHA, 2012). For the rest of the age groups, BPA intake through settled dust is below 0.001 μg/kg_bw/d.

Among the remaining exposure scenarios, the higher intake values regarded premature infants hosted in intensive care units (1.6 μg/kg_bw/d). For infants consuming milk formula or canned food and canned beverages, intake was around 0.5 μg/kg_bw/day. Toddlers consuming canned food and canned beverages had a higher intake up to 0.7 μg/kg_bw/d which is still significantly below the EFSA t-TDI. The higher intake of toddlers is the result of the higher daily amount of food consumed compared to the other age groups, when these values are normalized with bodyweight. Among the adult scenarios, cashiers had the highest intake, with an average value of 0.32 μg/kg_bw/d.

4.3.1.2 Validation of external exposure estimates using human biomonitoring data

The conservative nature of the bottom-up intake calculation is also verified by estimating intake from real-life HBM data. Using the measured urinary concentration of total BPA $\boldsymbol{C_{BPA}}$ (μg/L), the daily bodyweight normalized BPA intake ***D*** was calculated by the following formula:

$$D = \frac{C_{\mathrm{BPA}} \times V_{urine}}{BW}$$

where V_{urine} is the urinary output rate and *BW* is the body weight (LaKind and Naiman, 2008). It has to be noted that although the method provides sufficient results when daily intake is based on total daily urine, because of the non-persistent nature and short elimination half-life of BPA, as well as the intra-day exposure dynamics, the C_{BPA} value of an individual spot urine sample cannot be used to reliably estimate daily BPA intake. Using the HBM data collated in Table 1, it was found that the overall BPA daily intake is very low with an average value of 0.040 μg/kg_bw/d. This is much lower than bottom-up exposure estimates and far below the t-TDI of 4 μg/kg_bw/d proposed by EFSA.

4.3.2 Internal exposure estimates

4.3.2.1 Estimation of internal dose based on exposure scenarios

The importance of internal dosimetry in the refinement of exposure scenarios is illustrated in Figure 3.

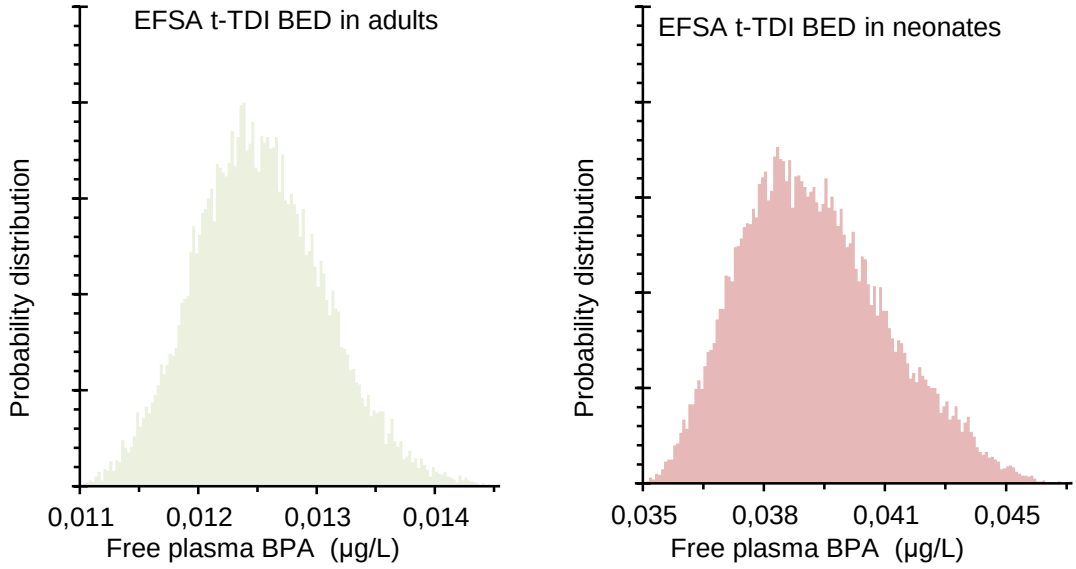


Figure 3. Differences in internal exposure to free plasma BPA between adults and neonates, when both are orally exposed to the EFSA t-TDI.

In adults, a chronic intake of 4 µg/kg_bw/d results in a mean internal exposure equal to 0.013 µg/L, while for neonates, internal dose for the same bodyweight normalized chronic intake results in twice as high levels of internal exposure (0.026 µg/L). This result shows that for the same external bodyweight normalized exposure levels between adults and neonates, internal exposure will be always higher, due to the immaturity of the detoxification pathway. This is actually reflected in reduced margins of safety for specific exposure scenarios relevant for neonates and infants.

The calculated exposure estimates were used as input to the PBTK model in order to translate external exposure into internal dose estimates. The summary statistics of the outcomes are given in table 3 below.

Table 3. Internal dose of free BPA in the plasma according to different exposure scenarios and human biomonitoring data in the EU

Scenarios	**Internal dose (μg/L)**		
	5%	**Median**	**95%**
In utero exposure	0.001265	0.002012	0.005753
Premature neonates hosted in intensive care units	0.00314	0.00810	0.01418
Infants/neonates breast fed	0.00005	0.00019	0.00034
Infants bottle fed/infant formula	0.00231	0.00320	0.00943
Infants consuming canned food and canned beverages	0.00112	0.00373	0.01050
Toddlers consuming canned food and canned beverages	0.00108	0.00215	0.00836
Children consuming canned food and canned beverages	0.00086	0.00128	0.00469
Adult consuming canned food and canned beverages	0.00053	0.00074	0.00282
Cashier, consuming canned food and canned beverages	0.00067	0.00106	0.00303
EU HBM data reconstruction	0.00019	0.00055	0.00116

An important aspect of this approach is the capability to investigate exposure scenarios that pertain to time windows of increased susceptibility such as *in utero* exposure. For assessing fetal exposure, a maternal overall BPA daily intake equal to 0.5 μg/kg_bw/d was assumed, which is a relatively conservative estimate for adults. In this case, free plasma BPA in maternal blood is almost 0.002 μg/L, i.e. slightly higher than the one expected for a non-pregnant woman (0.0016 μg/L). Placental concentration is 0.004 μg/L and the corresponding fetal free plasma BPA concentration is 0.0015 μg/L. Although placenta has almost twice the concentration of free BPA compared to the other maternal tissue compartments, fetal concentration is lower because the whole fetus acts as compartment with limited metabolic capacity, due to the presence of active enzymes able to sulfate BPA. Were sulfation negligible, the fetus would be an additional non-metabolizing compartment to the overall fetus – placental system, and the steady state concentration would be slightly higher (around 0.0025 μg/L) than the maternal one, yet lower than the placental concentration. Thus, free plasma BPA in the fetus is sensitive to the extent to which sulfation develops, as well as to the effect of β–glucuronidase in the placenta that results in BPA-Glu deconjugation. If the effect of β–glucuronidase were negligible, maternal concentration would be 0.0015 μg/L, a value that is slightly lower than the one of a non-pregnant woman. Placental concentration would be even lower (0.0013 μg/L) and the corresponding fetal exposure would be 0.0005 μg/L. However, this hypothesis is not supported by relevant experimental data. The

modelling results obtained in this study are in complete agreement to the ones presented in the FAO/WHO (2010) report. This analysis comes to the conclusion that fetal internal exposure is practically similar to the maternal one.

Maternal BPA-Glu bioavailability is also very important in the case of breast-fed infants. Transfer of BPA through milk is not sufficient enough to explain exposure of breast-fed infants; the overall BPA exposure through breast-feeding can only be explained by BPA-Glu cleavage in the gastrointestinal tract. Even when the worst-case scenario is taken into account, breast fed infants seem to be significantly less exposed compared to the bottle fed infants and neonates. This finding is corroborated by the conclusions of the FAO/WHO (2010) report.

4.3.3 Validation of internal dose using human biomonitoring data

The use of internal dosimetry coupled to the exposure reconstruction algorithm allowed the calculation of external and subsequently internal dose starting from the available HBM data. Daily intake was estimated assuming (a) an average urinary BPA-Glu concentration equal to 2.8 μg/L and (b) an ordinary dietary schedule that includes 4 different meals: (a) breakfast at 7:00 am (dose 1), (b) light snack at 11:00 am (dose 2) (c) lunch at 2:00 pm (dose 3) and (d) dinner at 7:00 pm (dose 4). Overall daily intake was considered to be equally distributed among these meals. The exposure reconstruction algorithm converged to the available biomonitoring data after 1000 iterations and the average intake estimates were 0.18 μg/kg_bw/d, which is higher than the ones estimated by the simple formula that relates intake and observed urinary levels, but still far below the EFSA t-TDI. Reconstruction of intake dose from the actual biomonitoring data, allowed us to run the model in forward mode and to estimate the biologically effective dose at the target tissues. The median estimate of the internal dose was close to 0.0006 μg/L. This value is about 25% lower than the most conservative non-occupational exposure scenario for adults (i.e. exposure of an adult who consumes continuously canned food and beverages). Given the overly conservative exposure assumptions underlying this scenario we consider that the biomonitoring measurements validate the estimates of the integrated exposure model.

4.4 Risk characterization

4.4.1 Risk characterization based on external exposure estimates

The evaluation of risk of the most important scenarios based on external exposure estimates is given in Figure 4. Our analysis indicates that exposure for all consumer exposure scenarios (including premature neonates and bottle-fed infants) is below the EFSA t-TDI, with the exception of the upper part of the exposure distribution of premature neonates (which exceeds the t-TDI value). It has also to be noted that the values of the upper part of

the intake distributions (95%, max) are the result of the highest food residues identified in some studies from the literature review. These values do not significantly affect the mean of the food residue distribution; yet they result in significant deviation from the mean in the overall distribution, thereby reducing the margin of safety for a small part of the population. Nevertheless, we need to take also into account that these estimates are quite conservative. In practice it is very unlikely that someone consumes only canned food and beverages.

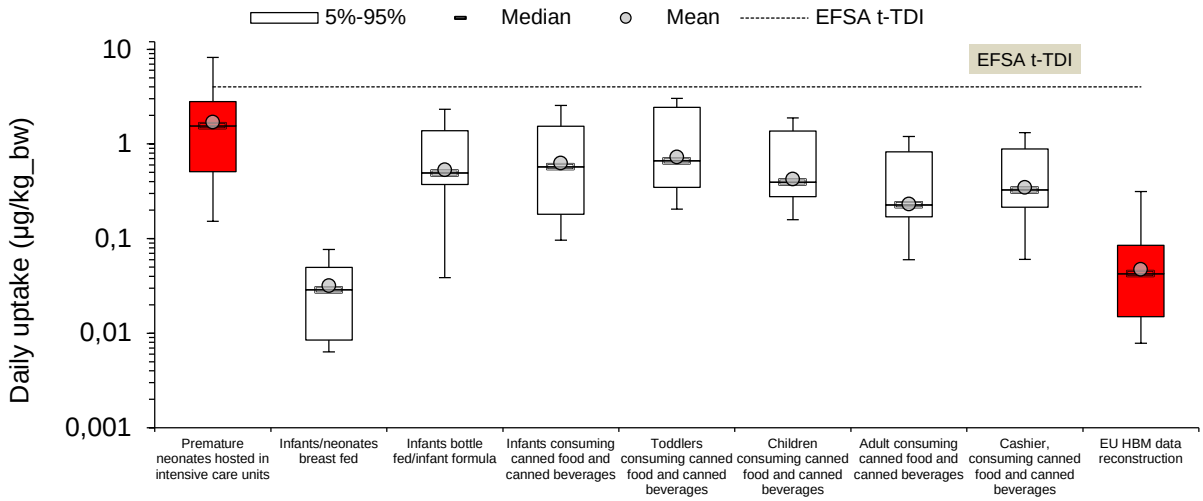


Figure 4. Daily uptake for the most important exposure scenario combinations. The reference dose is 4 µg/kg_bw/d (EFSA t-TDI)

4.4.2 Risk characterization based on internal exposure estimates

Evaluating risk based on internal exposure estimates resulted in reduced margins of safety for the scenarios involving neonates and infants; in this case, the upper distribution parts of internal exposure are closer to the respective BED derived from the EFSA t-TDI, but significantly lower compared to the BED that corresponded to the former TDI of 50 µg/kg_bw/d. Moreover, the median of the concentration of free BPA in the plasma as reckoned from the human biomonitoring data in Europe is far below the internal dose reference value (0.013 µg/L) derived by the EFSA t-TDI. This outcome corresponds to the lower estimates of the already considered exposure scenarios (Figure 5). Incorporation of internal dosimetry alters the overall exposure assessment outcome when age- and route-dependent differences are reflected in the actual internal exposure. Thus, when toxicokinetics is taken into account, the outcome of specific exposure scenarios such as premature neonates hosted in intensive care units is differentiated; accounting for the immaturity of the detoxification pathway, results in a higher probability of neonates to be exposed to an internal dose higher that the BED derived from EFSA t-TDI. Other exposure

scenarios, where exposure outcome changes when age- and route-dependent differences in internal dose are taken into account, include neonates/infants consuming milk formula and canned food and beverages. However, for these scenarios the internal dose remains significantly lower than the BED derived from the EFSA t-TDI.

Using the biological pathway altering dose (BPAD) derived from *in vitro* BPA toxicity assessment as the internal exposure reference value, the maximum derived internal exposure values of the worst-case exposure scenarios (premature neonates) are 10 times lower than the $BPADL_{99}$. This indicates that there is no reason for concern based on either individual or aggregate scenarios of BPA exposure.

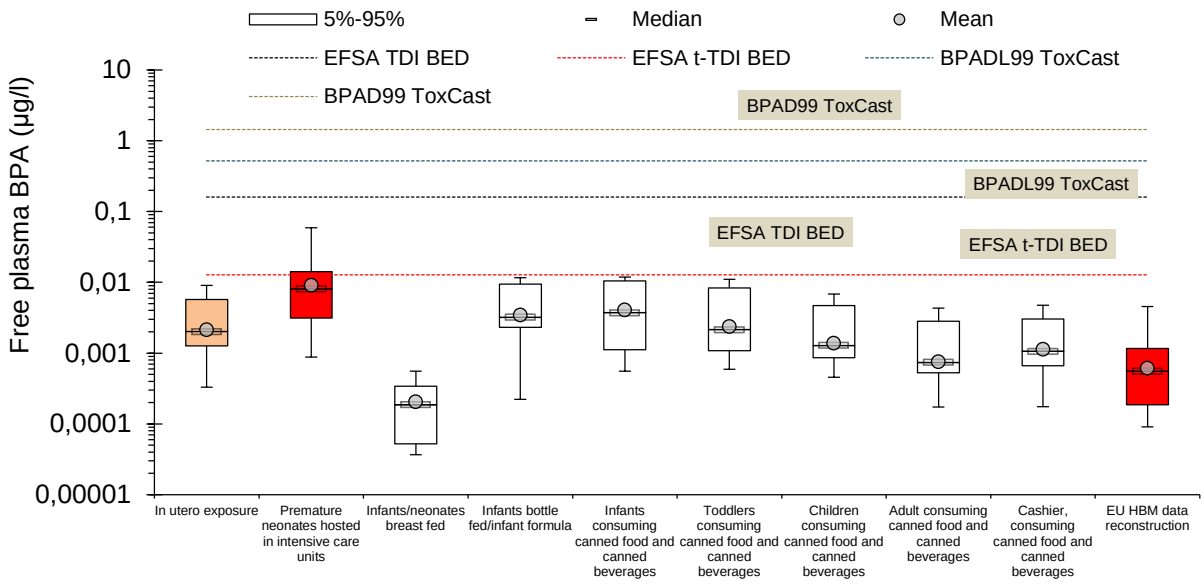


Figure 5. Free plasma BPA under most important plausible exposure scenario combinations (refined internal exposure analysis)

As already mentioned above, the use of the internal dosimetry module allowed the translation of the EFSA t-TDI into a biomonitoring equivalent urinary BPA-Glu concentration of 320 µg/L. Comparison of this value to the collected biomonitoring data shows that the current existing levels of BPA in EU are two orders of magnitude lower than the EFSA t-TDI; this margin of safety is in the same order of magnitude as the one estimated when daily intake is estimated from HBM data. Table 4 summarizes the risk characterization ratios as derived considering the free BPA levels in the plasma against the corresponding biologically effective dose of BPA based on the EFSA t-TDI and the $BPADL_{99}$.

Table 4. Risk characterization ratios for BPA exposure scenarios (median and 95th percentile)

Scenarios	**RCR**			
	RCR t-TDI (EFSA)		**BPADL99 (ToxCast)**	
	Median	**95%**	**Median**	**95%**
In utero exposure	0.16	0.45	0.004	0.011
Premature neonates hosted in intensive care units	0.63	1.11	0.016	0.027
Infants/neonates breast fed	0.01	0.03	0.000	0.001
Infants bottle fed/infant formula	0.25	0.74	0.006	0.018
Infants consuming canned food and canned beverages	0.29	0.82	0.007	0.020
Toddlers consuming canned food and canned beverages	0.17	0.65	0.004	0.016
Children consuming canned food and canned beverages	0.10	0.37	0.002	0.009
Adult consuming canned food and canned beverages	0.06	0.22	0.001	0.005
Cashier, consuming canned food and canned beverages	0.08	0.24	0.002	0.006
EU HBM data reconstruction	0.04	0.09	0.001	0.002

4.5 DISCUSSION

The study described the application of an integrated modelling framework for assessing exposure to BPA in the EU. Refined analysis of this study, was greatly facilitated by (a) the development of a comprehensive environmental and exposure modelling framework, able to estimate the contribution of all related pathways and routes of exposure and (b) the functional link to a generic PBTK model, that allows the estimation of internal dose under different exposure scenarios, as well as the assimilation of HBM data. A very important element was the development of the generic mother-fetus PBTK model that was properly parameterized for BPA, paying special attention in reducing the uncertainty regarding perinatal exposure. The use of time-dependent physiology parameterization of the evolving fetus, resulted in a mother-fetus model. Additional capabilities of the developed modeling

platform include Monte Carlo analysis for exploring variability and uncertainty, consideration of sulfation as an additional clearance pathway during early developmental stages and incorporation of inhalation and dermal routes of exposure among the pathways of exposure to BPA.

Exposure analysis results showed that chronic intake of BPA in the EU is significantly below the recently proposed t-TDI by EFSA. This is of particular importance considering the conservative nature of the bottom-up calculation of intake, where we assumed that alimentary needs for specific types of food items and beverages are covered only by canned food. Another important finding is that when incorporating the inter-individual differences in toxicokinetics, and especially the one related to the immaturity of detoxification pathways for neonates and infants, the margins of safety are reduced significantly for specific exposure scenarios. This refined type of exposure analysis proved to be a valuable tool for discriminating among scenarios of:

(a) immediate concern (i.e. use of BPA in neonates medical equipment);

(b) little concern, i.e. the scenarios involving milk formula and canned food for infants and eventually toddlers. The combination of high BPA residues in a sub-set of samples within the EU with the reduced detoxification capacity of infants result in lower margins of safety for this sensitive population group; and

(c) no concern, such as the scenarios that involve canned food and beverages consumption or exposure to thermal paper for adults. With regard to adults the low daily intake combined with the full detoxification capacity result in high margins of safety under all exposure scenarios.

In conclusion, integrated all-source and all-pathway exposure assessment allowed us to determine clearly the scenarios that might pose a public health risk. This may help design targeted interventions, e.g. by restricting the use of BPA in specific applications that relate to neonates and infants, but not for applications that relate to adults; the introduction of Commission Directive 2011/8/EU (FAO/WHO, 2010), which restricted the use of BPA in infant feeding bottles, is a typical example of targeted, well justified and successful intervention.

The use of internal dosimetry in exposure analysis provides additional insights into key questions that cannot be addressed solely by external exposure assessment, such as the extent of exposure *in utero*. Internal exposure of the developing fetus during gestation is highly linked to the maternal one, due to the high lipophilicity and the relatively small molecular weight of BPA that favors free diffusion across the placenta. Fetal internal exposure increases through placental BPA-Glu and BPA-Sulf deconjugation by β-glucuronidase and arylsulfatace-C respectively, enzymes that are present in high concentrations in the placenta. Free plasma BPA in fetal blood increases similarly to

maternal plasma after exposure, but it decreases at a slower pace since half of the intake is given intravenously due to the complexity of the uterus-placenta-fetus exchange system and erythrocyte and plasma protein binding. To date there are no adequate pharmacokinetic data to support the full quantification of the effect that β-glucuronidase has on BPA-GLU deconjugation and, consequently, on fetal exposure. Global sensitivity analysis performed on our model showed that this parameter is quite significant for placental concentration; it affects fetal exposure with a sensitivity coefficient of 0.67, which is higher than the contribution of fetal intrinsic clearance (sensitivity coefficient of 0.4). Our results corroborate the dependence of fetal exposure on de-conjugation activities originally reported by Ginsberg and Rice (EC, 2011). With regard to the relative importance of the dissociation of the two de-conjugation activities, the effect of arylsulfatace-C is found to be limited. Sulfation is considered as an alternative metabolic pathway for BPA when glucuronidation is not fully developed, and as such it is important only in fetuses and infants. Thus, the overall amount of BPA–Sulf subjected to possible de-conjucation in the placenta is mainly the one produced by the fetus, which is considered negligible compared to the amount of BPA–Glu circulating in the placenta, which comes from maternal clearance. Based on the above, β-glucuronidase is expected to have a significantly higher importance for fetal exposure.

Maternal BPA-Glu bioavailability is also very important in the case of breast-fed infants. Transfer of BPA through milk is not sufficient enough to explain exposure of breast fed infants and the overall BPA exposure through breast feeding can only be explained by BPA-Glu cleaved in the gastrointestinal tract. Even when the worst-case scenario is taken into account, breast-fed infants seem to be significantly less exposed compared to neonates and infants fed with milk formula. Based on the biomonitoring findings of Calafat et al (2009), it is clear that premature neonates hosted in intensive care units constitute the most vulnerable to BPA population group.

Incorporation of toxicokinetics, allows also the estimation of potential accumulation in the adipose tissue. BPA has a tissue:blood partition coefficient of 8.3 - the highest among all other tissue types; yet, it is still one order of magnitude lower compared to the tissue:blood partition coefficient of typical POPs, that range between 200 and 300. This tendency to partition in the adipose may protect other organs and tissues from BPA overload (Calafat et al., 2009). However, this protective function could prove to be a threat in the long run (La Merrill et al., 2013), related to the slow release into the systemic circulation, especially during weight loss.

Despite the inherent uncertainty in many parameters regarding BPA toxicokinetics (especially during gestation), our life-time PBTK model facilitates the assessment of realistic exposure scenarios, giving additional weight of evidence by translating quantitative hypotheses (e.g. the effect of β–glucuronidase) to estimates with known boundaries of uncertainties. In fact, we consider this work an initial point for a more comprehensive interaction among investigators of different disciplines, bridging exposure scenarios,

biomonitoring, toxicological and epidemiological data iteratively in order to identify and fill the current knowledge gaps (Barouki, 2013).

From the methodological point of view, using an integrated exposure framework that links mechanistically external and internal exposure, provides a comprehensive overview on how realistic exposure scenarios are translated into internal dose to humans, accounting for age-dependent and route-specific bioavailability differences. An additional capability of this integrated modelling framework is the assimilation of HBM data in two different ways; for screening purposes, the PBTK model allows the translation of external exposure/intake reference values into BE, while in a more elaborate scheme, starting from HBM data and using some ancillary information about the exposure scenario such as time activity patterns or daily schedule of meals, exposure magnitude can be back-calculated (Mattison et al., 2014). This is of particular importance, since simple mass balance approaches for estimating intake might result in significant misinterpretations when based on spot samples. To properly estimate the magnitude of an exposure event prior to the time of sampling, toxicokinetics have to be accounted for, especially for compounds that are rapidly metabolized. It has also to be noticed that the outcome of both the bottom up assessment starting from far field and moving gradually to near field exposure and internal dosimetry was consistent with the assessment starting from HBM data. This further supports the validity of the integrated modelling framework and its capability to mechanistically associate different type of data relevant for exposure assessment.

Finally, the use of this integrated modelling framework that incorporates internal dosimetry allows the direct mechanistic interpretation of environmental and consumer exposure scenarios against *in vitro* toxicological data. Using the results of the ToxCast21 BPA toxicity evaluation and comparing them with the estimated internal dose we concluded that exposure to BPA does not pose any significant threat according to most realistic exposure scenarios. These results are in agreement to the opinion that typical serum BPA concentrations are orders of magnitude lower than levels measurable by modern analytical methods and below concentrations required to occupy more than 0.0009% of Type II Estrogen Binding Sites, GPR30, ERα or ERβ receptors (Georgopoulos et al., 2009). In any case, complex mechanisms are employed in BPA toxicity that are not always captured by a single *in vitro* test and additional mechanisms and the related internal dosimetry metrics have to be taken into account, e.g. it has been found that GPR30 plays an important role in the BPA-induced activation of Erk1/2 in a manner distinct from that in ERα-mediated signaling (Teeguarden et al., 2013). On the other hand, t-TDI was derived from an oral equivalent dose for humans of 609 μg/kg bw/day, divided by an overall uncertainty factor of 150, where 25 stands for the differences between species and the differences between individual persons and 6 stands for the uncertainty in the database related to effects on mammary gland and reproductive, neurobehavioural, immune and metabolic systems (Dong et al., 2011). As a result, the more conservative nature of EFSA t-TDI is also considering systemic toxicity rather than focusing

on the activation of a single molecular mechanism that might result in adversity, thus providing a more comprehensive reference dose for public health protection.

The risk assessment estimates presented above, reflect how external and internal dosimetry metrics stand against the current regulatory threshold. The integrated methodology presented herein is independent of the thresholds and is able to provide revised risk estimates as soon as exposure, toxicity or regulatory data are available. Although the scope of the study is not to evaluate the validity of the t-TDI proposed by EFSA, it has to be mentioned that there is still an ongoing debate about low-dose effects of BPA, related either to the potential hormetic effects (EFSA, 2015a), or to cascade effects of hormonal balance disruption (Vandenberg et al., 2009).

5 Assimilation of biomonitoring data from HEALS cohorts

5.1 Compounds included in the assessment and scenario development

The main process is based on an integrated framework aiming at determining internal doses of xenobiotics, based on a realistic exposure scenario for different life stages. The applied methodology relies upon the approach developed and described by Sarigiannis et al. (Sarigiannis et al., 2016). To this aim the PBBK model was making use of advanced QSARs models to properly parametrize it for the chemicals under investigation.

Due to the lack of suitable and complete exposure data the HEALS methodological framework was applied to first derive, through reverse dosimetry modelling, exposure probability distributions which were consistent with the human biomonitoring data collected from existing cohorts in the Mediterranean region. Then, these exposure estimates were used to feed the PBTK model which was executed in forward-mode to derive internal doses of chemicals in target tissues.

The HEALS methodological framework was applied to the chemicals presented in Table 6**.** Results were obtained for BDE-47, PCB153, p,p'-DDT, HCB, Arsenic and Methylmercury, as reported in the following chapters.

Table 5. Chemical substances were tested by exposure framework

a/a	IUPAC Name	Chemical name	CAS-number	Chemical classification
1	2,4,4'-Trichlorobiphenyl	PCB 28	7012-37-5	Non-dioxin-like polychlorinated biphenyls
2	2,2',5,5'-Tetrachlorobiphenyl	PCB 52	35693-99-3	Non-dioxin-like polychlorinated biphenyls
3	2,2',4,4',5-Pentachlorobiphenyl	PCB 99	38380-01-7	Non-dioxin-like polychlorinated biphenyls
4	2,2',4,5,5'-Pentachlorobiphenyl	PCB 101	37680-73-2	Non-dioxin-like polychlorinated biphenyls
5	2,2',4,4',5,5'-Hexachlorobiphenyl	PCB 153	35065-27-1	Non-dioxin-like polychlorinated biphenyls
6	2,2',3,4,4',5,5'-Heptachlorobiphenyl	PCB 180	35065-29-3	Non-dioxin-like polychlorinated biphenyls

a/a	IUPAC Name	Chemical name	CAS-number	Chemical classification
7	2,4,4'-Tribromodiphenyl ether	BDE 28	41318-75-6	Polybrominated Diphenyl Ethers
8	2,2',4,4'-Tetrabromodiphenyl ether	BDE 47	5436-43-1	Polybrominated Diphenyl Ethers
9	2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE 153	68631-49-2	Polybrominated Diphenyl Ethers
10	2,2',4,4',5,6'-Hexabromodiphenyl ether	BDE 154	207122-15-4	Polybrominated Diphenyl Ethers
11	Hexachlorobenzene	HCB	118-74-1	Organochlorine Pesticide
12	Dichlorodiphenyltrichloroethanes	p,p'-DDT	50-29-3	Organochlorine Pesticide
13	Arsenic	As	7440-38-2	Metal
14	Methylmercury	$HgCH_3$	016056-34-1	Organometalic

5.1.1 Scenario 1 (infant-children)

The first exposure scenario consisted of one exposure event for newborn until the 4th year of his/her life. The main assumption for the newborns until the first six months of age was that they were fed exclusively with breast milk. Then, it was assumed that from 6th month until the 18th month the daily diet consisted of 6 different meals (each one every 2.5 hours) and from the age of 18 months to 4 years it consisted of differentiated meals every 3 hours. The starting time of the first meal was set to 7:00 AM. It was also assumed that the contribution of each meal to the daily intake dose is the same. This assumption was based to the fact that the modelled chemicals have long half-life time, and consequently they are accumulated for years in the human body.

5.1.2 Scenario 2 (adult)

This exposure scenario consisted of one exposure event lasting 30 years assuming a daily food consumption based on the actual dietary schedule of the generic European adult population. In this case the main assumption was that the generic population consumes 3 daily meals. The time of these three basic dietary has been set at 7:00 AM for the breakfast, 2:00 PM for the lunch and 7:00 PM for the dinner. It was assumed also that the contribution

of the three meals in the daily exposure is respectively 30%, 50% and 20%. The exposure scenario of the simulation was assumed to start at the age of 15 years.

5.2 Exposure reconstruction and target dose estimation

5.2.1 Polybrominated Diphenyl Ethers

BDE-47 is a Polybrominated diphenyl ether (PBDE). PBDEs are a class of synthetic chemicals used for the production of padding, textiles or plastics to retard combustion. PBDEs are generally persistent in the environment and have been measured in aquatic sediments as well as in aquatic and terrestrial animals and fishes. The main human exposure is occurring thought diet and mother's milk.

BDEs have been associated with neurodevelopment effects that could potentially enhance on the neurological disruptions. However, the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) indicate that PBDEs are not considered genotoxic with respect to human carcinogenicity (Eriksson et al., 2001).

A PBBK simulation for BDE-47 was performed based on scenario 2 for the Valencia population[1]. Results show that in steady state condition, at the age of 30 year, the BDE-47 concentration in uterus has a median value of 0.2 (0.1 - 0.8) ug/L.

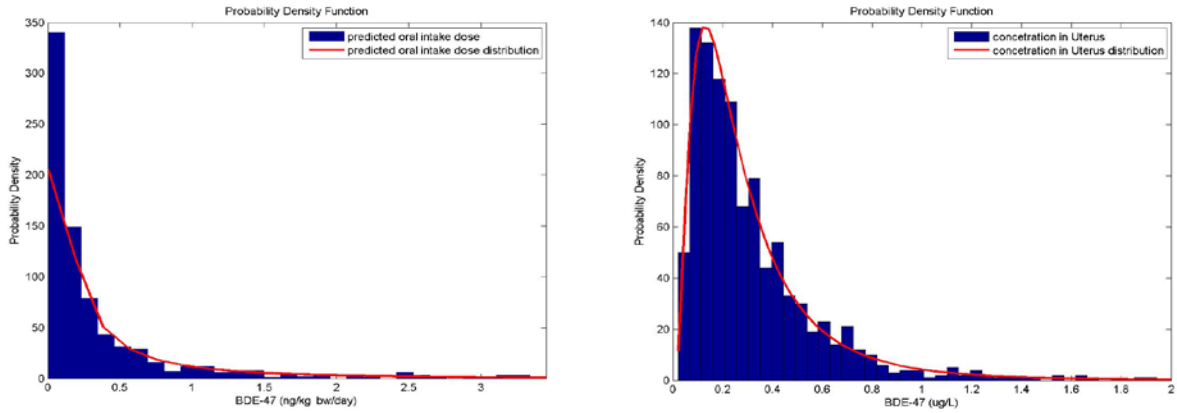


Figure 6. BDE-47 –Valencia (Spain) population – left: predicted oral intake dose. Right: predicted concentration in uterus

5.2.2 Non-dioxin-like polychlorinated biphenyls

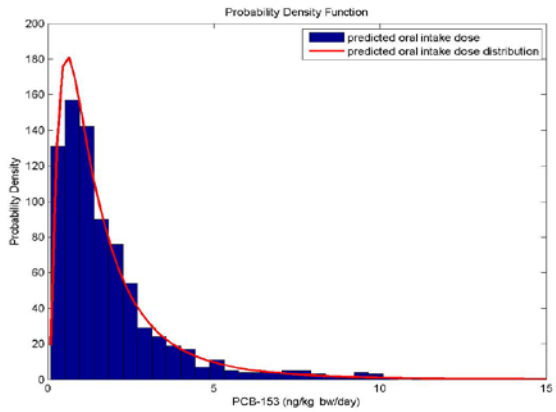
PCBs are a group of organochlorine compounds that are synthesized by catalyzed chlorination of biphenyl. The different position of ring and the position of the chlorine atoms (1-10) can give 209 individual PCB congeners. PCBs accumulate in the food chain and are

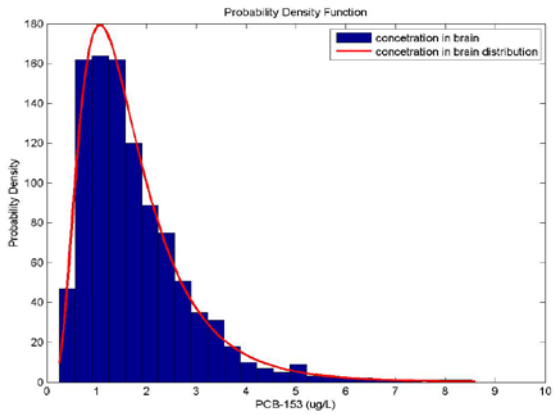
[1] More details for the population are presented in Deliverable 5.1.

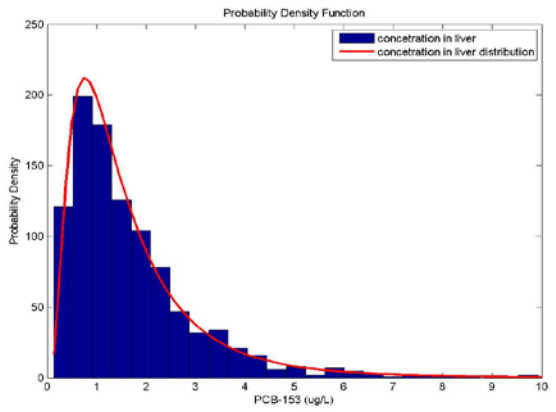
stored in fatty tissues because of their lipophilicity. The main issue is that the environmental fate of the congeners may vary because of the various transportations and partial eliminations linked to biological metabolisms (Beyer and Biziuk, 2009).

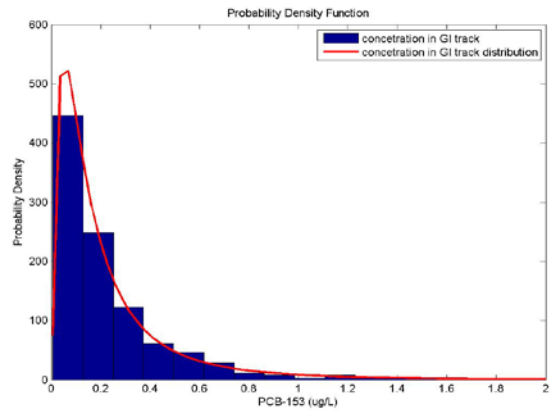
PCBs are classified as probable human carcinogens by IARC and are classified by NTP as reasonably anticipated to be carcinogens (Beyer and Biziuk, 2009). Early studies associated workplace PCB exposures with increased deaths from cancer of the liver, gastrointestinal tract and brain (Knerr and Schrenk, 2006).

Therefore, PBBK simulations for PCB-153 were performed based on scenario 1 for the Valencia population[2]. Results of the simulation show that in steady state condition and at the age of 4 years, the PCB-153 concentration in the liver and in the brain are similar with a median value respectively of 1.3 (0.4 – 4.2) ug/L and 1.5 (0.6 – 3.8) ug/L while the concentration levels in gastrointestinal tract are one order of magnitude lower (median 0.1 (0.01 – 0.7) ug/L).









[2] More details about the population are presented in Deliverable 5.1.

Figure 7. PCB-153 – Valencia (Spain) population – left above: predicted oral intake dose, right above: predicted concentration in brain, left below: predicted concentration in liver, right below: predicted concentration in gastrointestinal.

5.2.3 Organochlorine Pesticides

Hexachlorobenzene (HCB) is an organochlorine pesticide. Organochlorine pesticides, an older class of pesticides, are effective against a variety of insects. They enter in the environment after pesticide application, disposal of contaminated waste into landfills, and release from manufacturing plants. Usage restrictions have been associated with a general decrease in serum organochlorine levels in the U.S. population and other developed countries (Knerr and Schrenk, 2006). For the general population, oral is the main exposure route, primarily through the ingestion of fatty foods such as dairy products and fish (Hagmar et al., 2006). Infants are exposed through breast milk, and fetuses can be exposed in utero through the placenta. Workers can be exposed to organochlorines pesticides in the manufacture, formulation, or application of these chemicals

Chronic feeding studies in animals have demonstrated kidney injury, immunologic abnormalities, reproductive and developmental toxicities, and liver and thyroid cancers (ATSDR, 2002a). In humans, very high, acute doses produce central nervous system depression and seizures.

Therefore, PBBK simulations for HCB were performed based on scenario 2 for Valencia population. Results of the simulations show that in steady state condition and at the age of 30 years, the HCB concentration in brain has a median value of 2.1(0.6 - 7.7) ug/L (Figure 15).

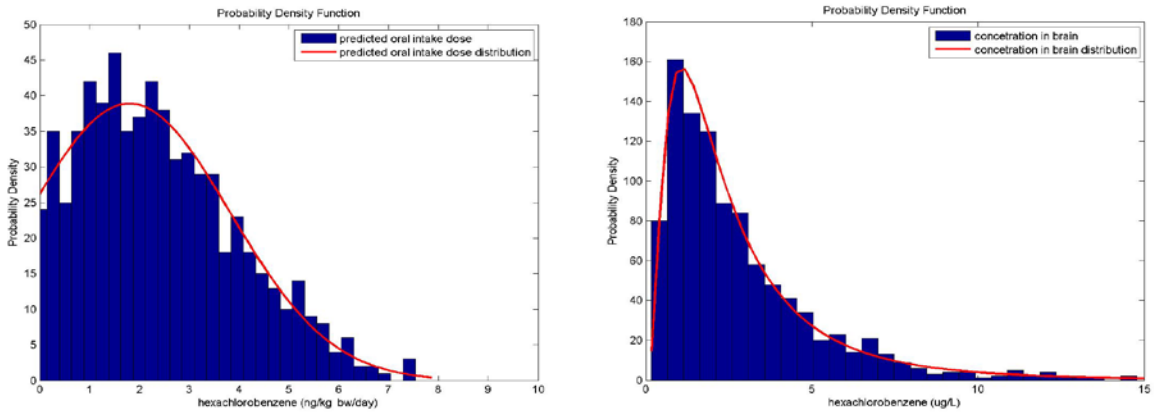


Figure 8. HCB – Valencia (Spain) population – left: predicted oral intake dose. Right: Predicted concentration in brain

Dichlorodiphenyltrichloroethane (pp'-DDT) is an organochlorine pesticide. It has been used widely as a broad-spectrum insecticide in agriculture and for control of vector-borne diseases.

DDT probably contributes to the increment of risks for cancers at various sites and its possible role as an endocrine disruptor (ATSDR, 2002a). Particularly, DDE and DDT has been strongly associated with the Cancer of breast (Turusov et al., 2002). Moreover, DDT has been reported as toxicant that induce neurotoxic effects (Eriksson et al., 1992; Wolff et al., 1993) and it has been related with disruptions on brain functions.

Therefore PBTK simulations for pp'-DDT were carried out based on scenario 1 for the Valencia and for the Menorca population[3]. Results show that in steady state condition and at the age of 30 years, the concentration of pp'-DDT in brain and in uterus are about 1.5 - 2 times higher for the population of Menorca than in Valencia (Figure 16).

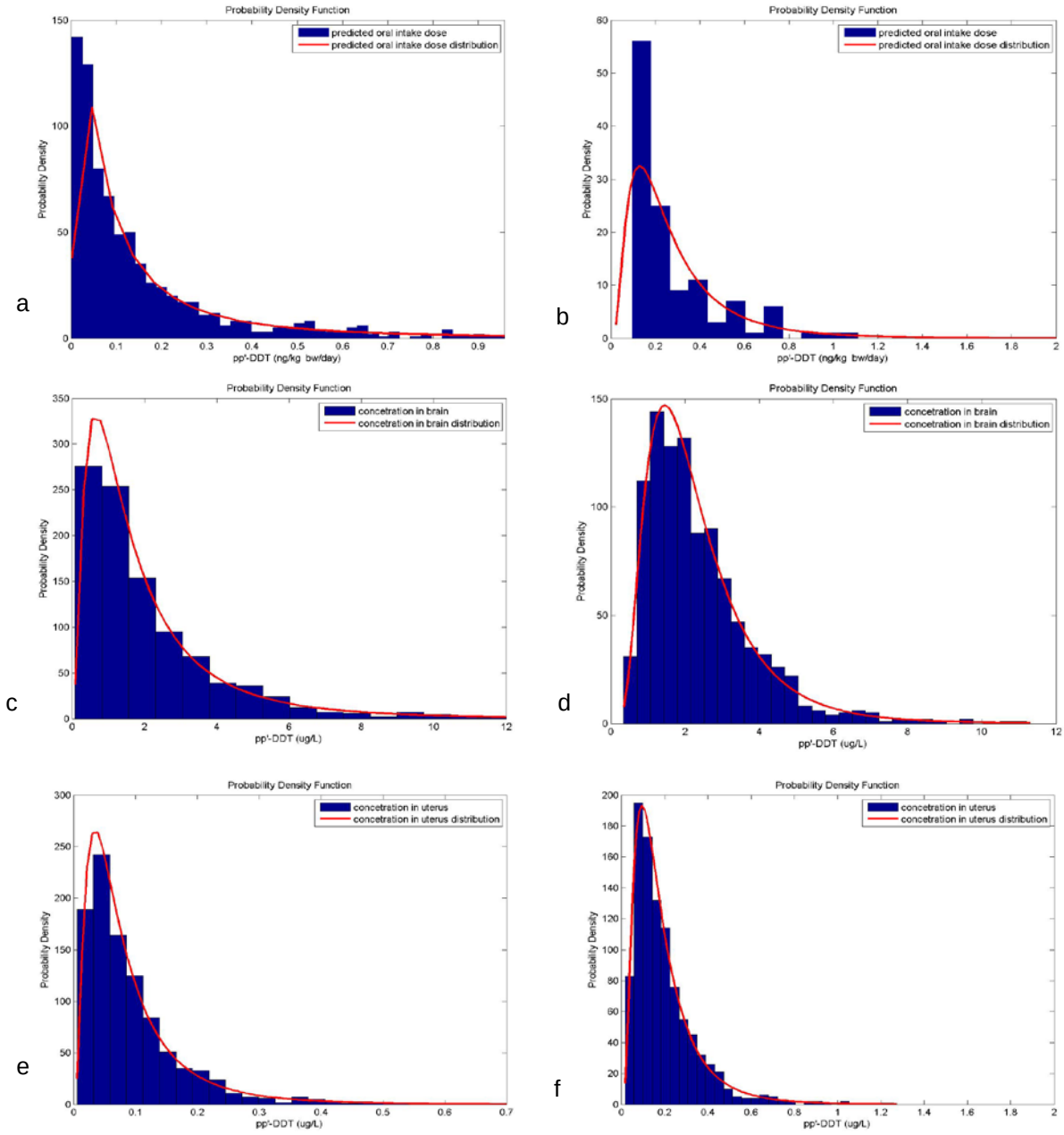


Figure 9. pp'-DDT – a) predicted oral intake dose for Valencia (Spain) population, b) predicted oral intake dose for Menorca (Spain) population,

[3] More details about the population are presented in Deliverable 5.1.

c) predicted concentration in brain for Valencia (Spain) population,
d) predicted concentration in brain for Menorca (Spain) population,
e) predicted concentration in uterus for Valencia (Spain) population and
f) predicted concentration in uterus for Menorca (Spain) population

5.2.4 Mercury and Methylmercury

Mercury is a naturally occurring metal which has several forms. The metallic mercury is a shiny, silver-white, odorless liquid. If heated, it is a colorless, odorless gas. Mercury combines with other elements, such as chlorine, sulfur, or oxygen, to form inorganic mercury compounds or "salts," which are usually white powders or crystals. Mercury also combines with carbon to make organic mercury compounds. The most common one, methylmercury, is produced mainly by microscopic organisms in the water and soil. More mercury in the environment can increase the amounts of methylmercury that these small organisms make.

Metallic mercury is used to produce chlorine gas and caustic soda, and is also used in thermometers, dental fillings, and batteries. Mercury salts are sometimes used in skin lightening creams and as antiseptic creams and ointments.

Methylmercury is the form most readily incorporated into biological tissues and most toxic to humans. The conversion of inorganic mercury to methylmercury is important for two reasons: (1) methylmercury is much more toxic than inorganic mercury, and (2) organisms require considerably longer to eliminate methylmercury.

The health effects of mercury depend on the form of the mercury, the dose as well as duration that a person is exposed. Lower levels of prenatal exposure due to maternal seafood consumption have been associated with an increased risk for abnormal neurocognitive test results in children (Eriksson, 1992; Rice, 2004). It has to be underlined that the during gestation, the fetus is more vulnerable to neurochemical disruption from methylmercury exposure via the mother and because of the susceptibility of the developing brain, this exposure has high risk (WHO, 1990).

PBTK simulations for methylmercury were based on scenario 2 for Slovenian, Croatian and Poland population. Simulation results show that the concentration of MeHg in brain and in gastrointestinal tract are 3-4 times higher for the population of Croatia than for the one of Slovenia (Figure 10).

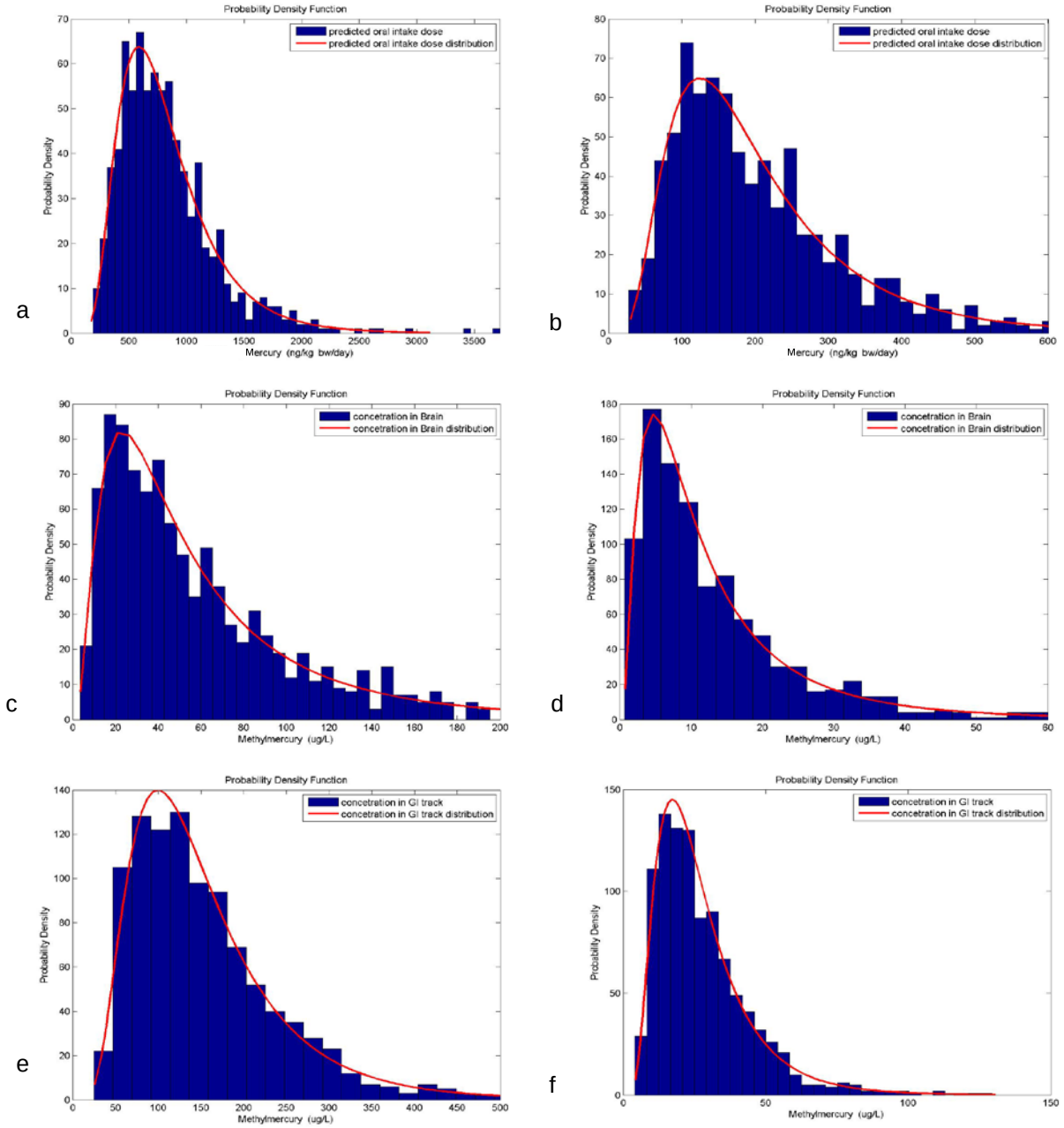


Figure 10. Mercury – a) predicted oral intake dose for Croatian population,
b) Mercury oral intake dose for Slovenian population,
c) Methylmercury – predicted concentration in brain for Croatian population,
d) Methylmercury – predicted concentration in brain for Slovenian population,
e) Methylmercury – predicted concentration in gastrointestinal for Croatian population and
f) Methylmercury – predicted concentration in gastrointestinal for Slovenian population

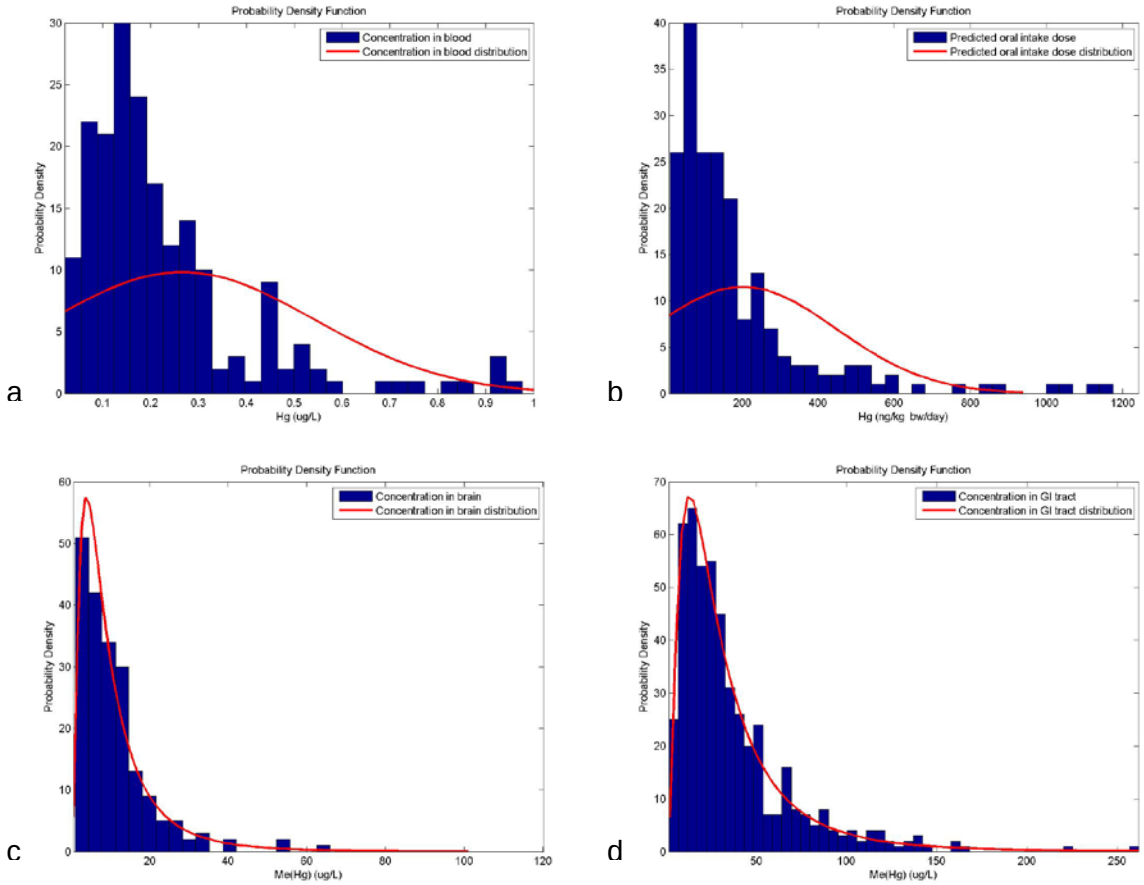


Figure 11 a) Mercury – blood concertation for Poland population,
b) Mercury – predicted oral intake dose for Poland population,
c) Methylmercury – predicted concentration in brain for Poland population,
d) Methylmercury – concentration in gastrointestinal for Poland population

5.2.5 Arsenic

Arsenic is an element that is widely distributed in the earth's surface in small amounts. Arsenic and its compounds have had many uses in the past and present as medicines, pesticides, alloys, semiconductors, and as homicidal poisons. The exposure to inorganic arsenic occurs through consumption of drinking water and, to a lesser extent, meats and grain (Organization, 1990).

An association between lung cancer and occupational exposure to inorganic arsenic has been confirmed in several epidemiologic studies (Abernathy et al., 2003), and arsenic is considered a cause of lung (ENTERLINE et al., 1987; Smith et al., 2006) as well as skin cancer (Eriksson et al., 2001). In arsenic-exposed workers, there is a systematic gradient in lung cancer mortality rates, depending upon duration and intensity of exposure (Karagas et al., 2001)

PBBK simulations for arsenic were based on scenario 2, for Italian and Slovenian population. Simulation results show that in steady state condition and at the age of 30 years, Arsenic

concentration levels in skin and in lung are about 3 times higher for the population of Italy with respect to the Slovenia one (Figure 12).

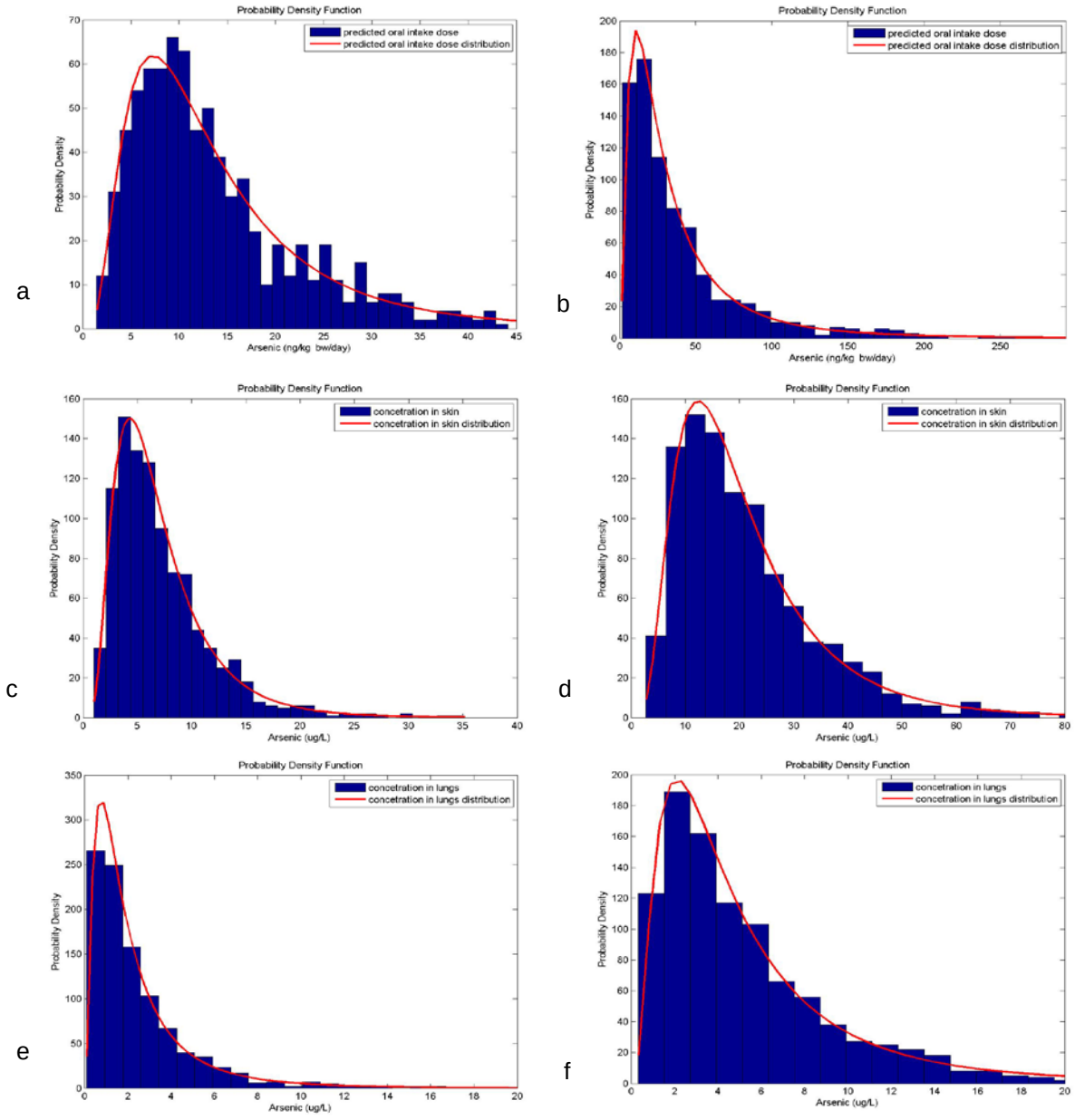


Figure 12. Arsenic – a) predicted oral intake dose for Slovenian population,
b) predicted oral intake dose for Italian population,
c) predicted concentration in skin for Slovenian population,
d) predicted concentration in skin for Italian population,
e) predicted concentration in lung for Slovenian population and
f) predicted concentration in lung for Italian population

5.2.6 Lead

Lead is a metal that is denser than most common materials, soft and malleable, and has a relatively low melting point. When freshly cut, lead is bluish-white. Lead has a variety of uses in the manufacture of storage batteries, solders, metal alloys, plastics, leaded glass, ceramic glazes, ammunition and shielding used as protection from radiation sources. sources. For children, the major sources of exposure are from deteriorated lead-based paint and the resulting dust and soil contamination (ATSDR, 2012). Exposure to lead and lead chemicals can occur through inhalation, ingestion, dermal absorption, absorption from retained or embedded leaded foreign body, and trans-placental (endogenous) routes.

Lead exerts multisystemic toxic effects through a variety of mechanisms, including interference in the function of essential cations such as; inhibition of certain enzymes, generation of reactive oxygen species and alteration in gene expression. Large amounts of lead exposure effects kidney to cause chronic kidney diseases (Navas-Acien et al., 2009) and the brain and central nervous system to cause abdominal pain, seizures and paralysis (Bechara et al., 1993). It has to be noted that in children's brain development resulting in reduced intelligence quotient and behavioural changes. Last but not least, lead exposure also causes anaemia (Wasserman et al., 1992), hypertension, renal impairment (Muntner et al., 2003), immunotoxicity and toxicity to the reproductive organs (Xuezhi et al., 1992). PBBK simulations for Lead were based on scenario 2, for Poland population. The results of the simulations are illustrated in Figure 13.

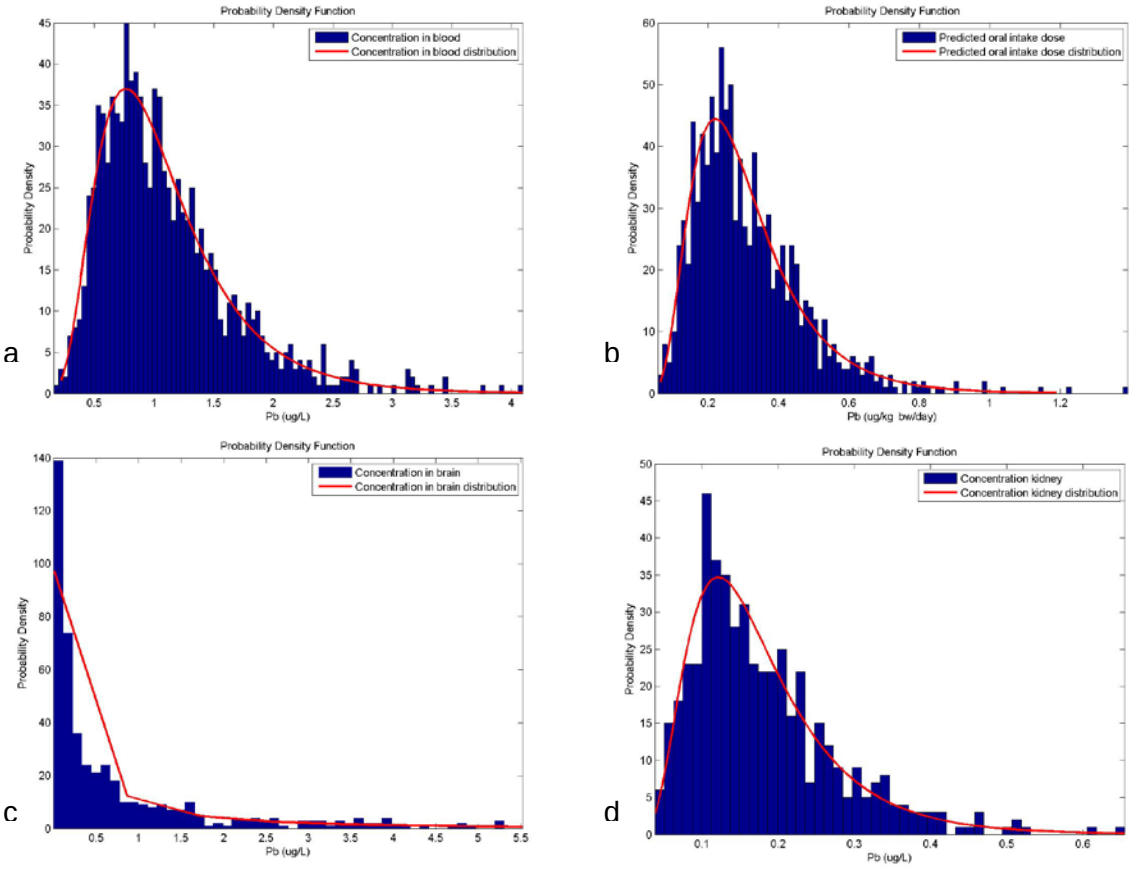


Figure 13. Pb – a) concentration in blood, b) predicted oral intake dose, c) calculated concentration in kidney, d) calculated concentration in brain

5.2.7 Cadmium

Cadmium is a metal that is obtained chiefly as a by-product during the processing of zinc-containing ores and to a lesser extent during the refining of lead and copper from sulfide ore. Exposure to cadmium can occur through multiple sources, including smoking, with food accounting for approximately 90% of cadmium exposure in the non-smoking general population. Les than 10% of total exposure of the non-smoking general population occurs due to inhalation of low levels of cadmium in ambient air (Vahter et al., 1991) and through drinking water (Olsson et al., 2002). The kidney is a critical target for cadmium and chronic exposure to cadmium can cause nephrotoxicity (Goyer, 1989; Nordberg et al., 1975). Addtionally, chronic cadmium inhalation is also suspected to be a possible cause of lung cancer(Sorahan and Esmen, 2004).

PBBK simulations for Cadmium were based on scenario 2, for Spain population. The results of the simulations are illustrated in Figure 14.

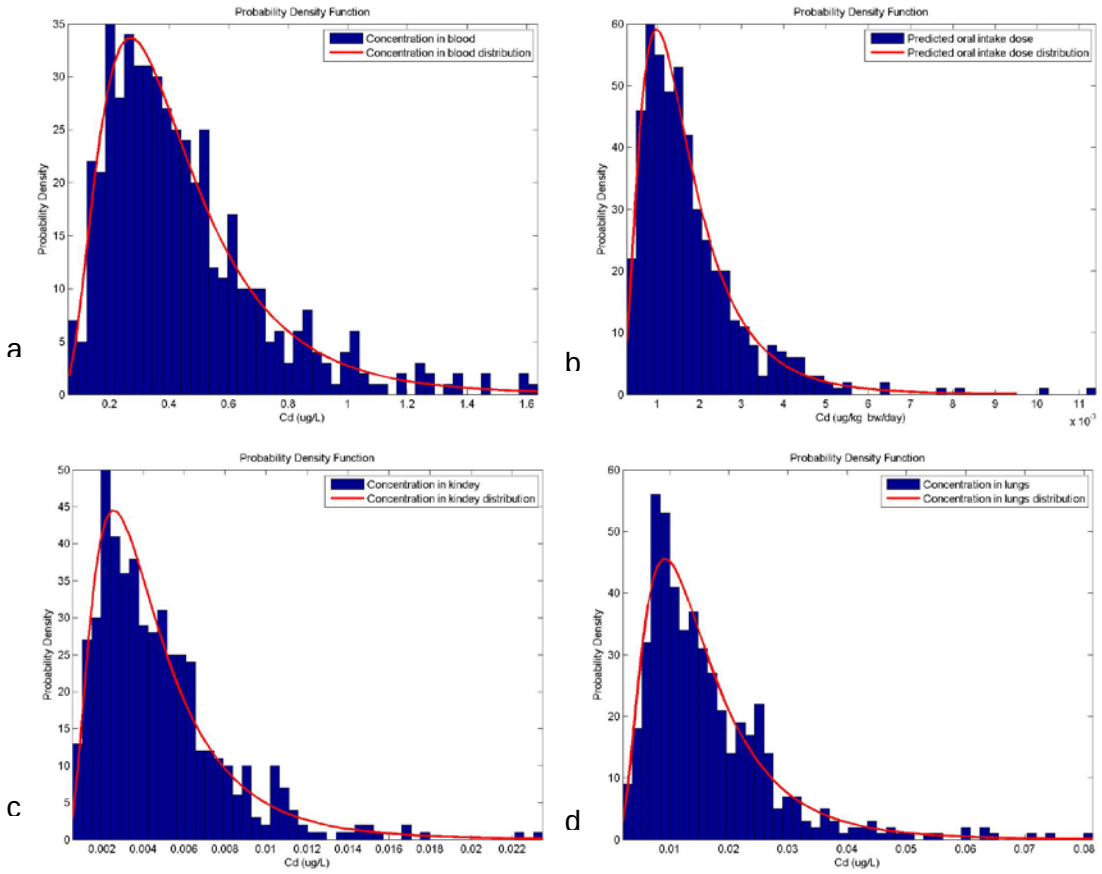


Figure 14 Cadmium – a) concentration in blood,
b) predicted oral intake dose,
c) calculated concentration in kidney,
d) calculated concentration in lungs

5.2.8 Phthalates

Phthalates are chemicals that commonly are used as plasticizers providing impart flexibility and resilience. Products that contain phthalates are vinyl flooring, adhesives, detergents, lubricating oils, solvents, automotive plastics, plastic clothing, personal-care products (Koniecki et al., 2011) and medical products. Phthalates are widely used in flexible polyvinyl chloride plastics and particuraly in plastic bags, food packaging, garden hoses, inflatable recreational toys, blood-storage bags, intravenous medical tubing, and children's toys. Soil and water contamination can be greatest in areas of industrial use and waste disposal (Schettler, 2006). In 2003, more than 800 000 tons of phthalates have been used in Western Europe, 24% DEHP and more than 50% DINP (di-iso-nonylphthalate) and DIDP (di-iso-decylphthalate)(Heudorf et al., 2007). Also other phthalates such as di-ethyl-phthalate (DEP), di-*n*-butyl phthalate (DBP), butyl benzyl phthalate (BBP), and di-*n*-octyl phthalate (DnOP) are widely used (Heudorf et al., 2007). People are mainly exposed through the oral route through

food that is in contact with packaging that contains phthalates or through skin exposure through products that contain phthalates (Schettler, 2006).

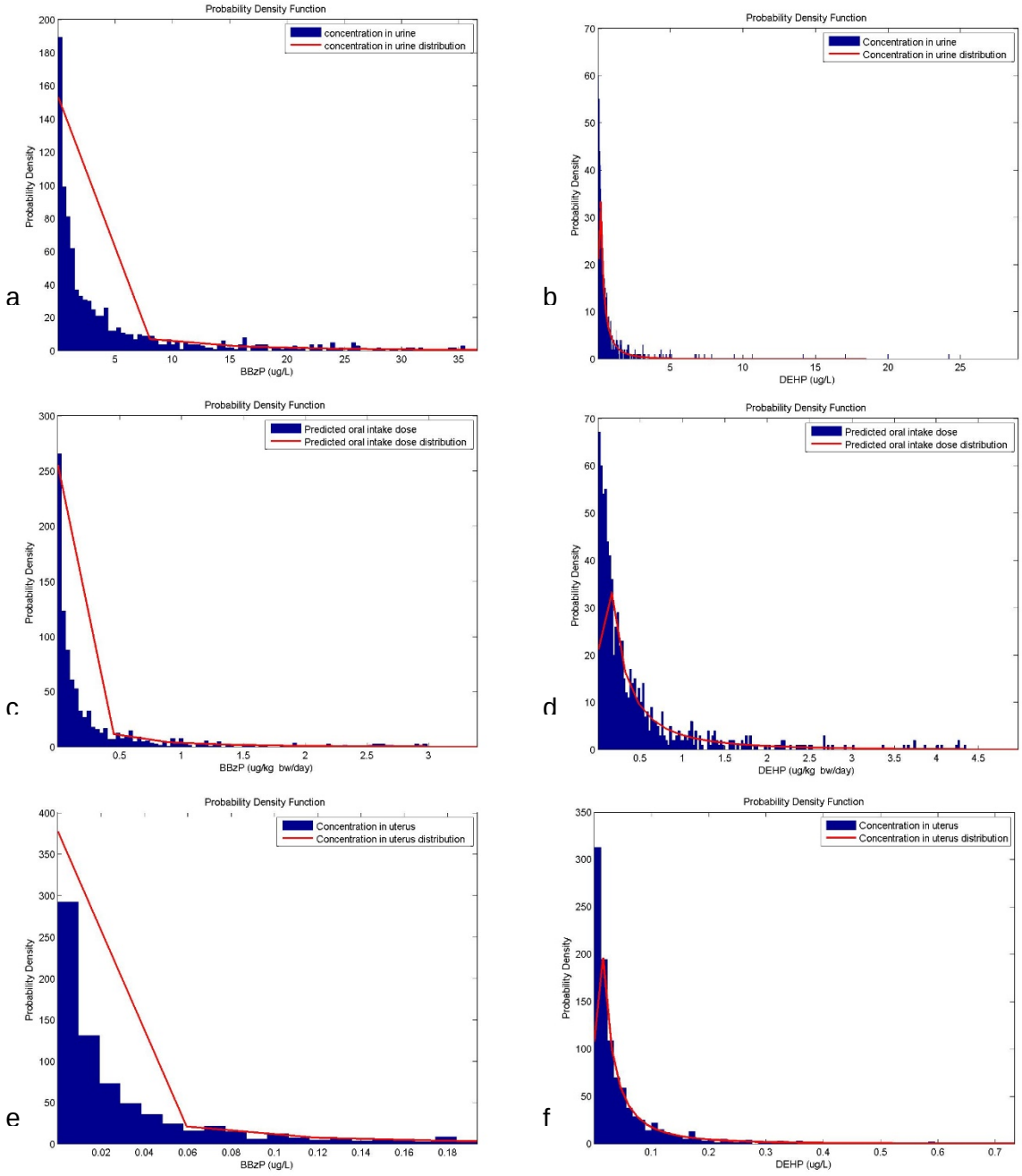


Figure 15. a) BBzP – concentration in urine,
b) DEHP -concentration in urine
c) BBzP – predicted oral intake dose,
d) DEHP – predicted oral intake dose,
e) BBzP – concentration in uterus,
f) DEHP – concentration in uterus

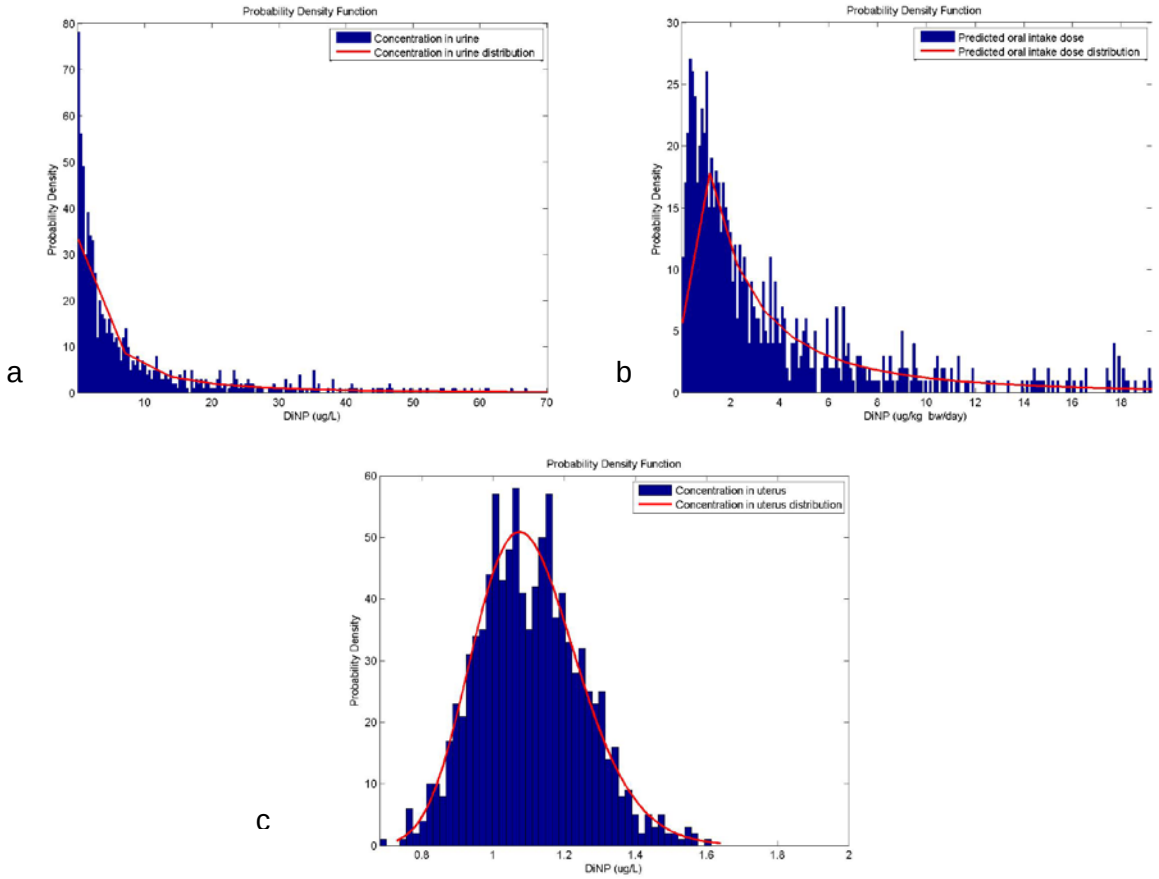


Figure 16 a) DiNP – concentration in urine,
b) DiNP – predicted oral intake dose,
c) DiNP – concentration in uterus

5.2.9 Summary of results

The statistical summary of the concentrations of simulated chemical in the various target organs is reported in Table 7.

Table 6. Summary statistic of concentrations of simulated chemical in the target tissues

	Chemical	Tissue	mean	std (ug/L)	median	Q 0.05 (ug/L)	Q 0.95 (ug/L)	location
1	Arsenic	Skin	8.4	3.8	5.5	1.0	29.7	Slovenia
2	Arsenic	Lungs	3.9	1.8	1.7	0.4	7.1	Slovenia
3	Arsenic	Skin	24.8	8.7	18.0	6.8	47.3	Italy

	Chemical	Tissue	mean	std (ug/L)	median	Q 0.05 (ug/L)	Q 0.95 (ug/L)	location
4	Arsenic	Lungs	6.8	3.9	4.1	1.1	15.2	Italy
5	BDE47	Uterus	1.3	0.7	0.2	0.1	0.8	Spain (Valencia)
6	HCB	Brain	3.1	1.1	2.1	0.6	7.7	Spain (Valencia)
7	Mercury	Brain	16.9	12	9.2	2.8	29.0	Slovenia
8	Mercury	GI tract	37.5	15.4	23.8	9.6	58.4	Slovenia
9	Mercury	Brain	70.5	16.4	46.5	12.1	174.8	Croatia
10	Mercury	GI tract	180.5	18.5	134.9	56.0	321.1	Croatia
11	Mercury	Brain	17.5	22.4	8.6	13.9	40.17	Poland (Lodz)
12	Mercury	GI tract	31.8	22.1	27.5	4.3	134.3	Poland (Lodz)
13	Lead	Brain	1.7	4.4	0.1	0.4	11.8	Poland (Lodz)
14	Lead	Kidney	0.2	0.1	0.1	0.05	0.4	Poland (Lodz)
15	Cadmium	Lung	0.015	0.01	0.0135	0.008	0.05	Slovenia
16	Cadmium	Kidney	0.003	0.004	0.004	0	0.01	Slovenia
17	PCB153	Liver	2.1	0.72	1.3	0.4	4.2	Spain (Valencia)
18	PCB153	GI tract	0.28	0.02	0.1	0.0	0.7	Spain (Valencia)
19	PCB153	Brain	2.1	0.4	1.5	0.6	3.8	Spain (Valencia)
20	ppDDT	Uterus	0.12	0.05	0.1	0.0	0.3	Spain (Valencia)
21	ppDDT	Kidneys	0.21	0.08	0.2	0.0	0.8	Spain (Menorca)

	Chemical	Tissue	mean	std (ug/L)	median	Q 0.05 (ug/L)	Q 0.95 (ug/L)	location
22	ppDDT	Brain	3.2	1.1	2.0	0.8	5.0	Spain (Menorca)
23	ppDDT	Brain	2.1	0.9	1.5	0.3	6.2	Spain (Valencia)
24	DEHP	Uterus	0.1	0.2	0.05	0	0.4	Poland (Lodz)
25	DiNP	Uterus	1.1	0.2	1	0.8	1.4	Poland (Lodz)
26	BBzP	Uterus	0.1	0.6	0	0.01	1.0	Poland (Lodz)
27	BPA	Uterus	0.0001	0.00005	0.00008	0.00001	0.0008	Slovenia

6 Risk characterization assessment results

Based on what has been described in the methodology section, the respective internal dose reference level (IDRL) were derived for the several xenobiotics involved in the respective HEALS cohorts. The results are illustrated in Table 7.

Table 7. Tolerable daily intake dose, reference, calculated internal dose in blood and in the target tissue.

Chemical	TDI	UNITS	Reference	Equivalent Internal dose in blood (ug/L)	Equivalent Internal dose in target tissue ($IDRL_{TDI}$) (ug/L)	Target tissue
Arsenic	1.0	µg/kg/day	(Baars et al., 2001)	56	157	skin
Arsenic	2.1	µg/kg bw/day	(WHO, 2011b)	118	329	skin
Arsenic	1.0	µg/kg/day	*(Baars et al., 2001)*	56	60	lungs
Arsenic	2.1	µg/kg bw/day	(WHO, 2011b)	118	122	lungs
BBzP	500	ug/kg_bd/day	(EFSA, 2005a)	168	67.3	uterus
BDE47	100	ng/kg bw/day	(Dimitriadou et al., 2016)	8.0	7.1	brain
BPA	4.0	ug/kg bw/day	(EFSA, 2015b)	0.013	0.016	uterus
Cadmium	0.833	ug/kg bw/day	(WHO, 2011a)	208	428	kidney
Cadmium	0.833	ug/kg bw/day	(WHO, 2011a)	208	189	lungs
DEHP	50	ug/kg bw/day	(EFSA, 2005b)	4.9	16.5	uterus
DiNP	150	ug/kg bw/day	(ECHA, 2015)	23	65	uterus
HCB	2	ug/kg bw/day	(USEPA)	21	21	brain
Lead	4	ug/kg bw/day	(WHO, 2011c)	12	5	brain
Lead	4	ug/kg bw/day	(WHO, 2011c)	12	2	GI tract
Mercury	0.571	mg/kg bw/day	(WHO, 2011d)	2	85	brain
Mercury	0.571	mg/kg bw/day	(WHO, 2011d)	2	215	GI tract
PCB153	10.0	ng/kg bw/day	(Afssa, 2008)	19	15	brain
PCB153	20.0	ng/kg bw/day	(EFSA, 2005c)	38	30	brain
PCB153	10.0	ng/kg bw/day	(Afssa, 2008)	19	17	liver
PCB153	20.0	ng/kg bw/day	(EFSA, 2005c)	38	34	liver

PCB153	10.0	ng/kg bw/day	(EFSA, 2005c)	19	1	GI tract
PCB153	20.0	ng/kg bw/day	(Afssa, 2008)	38	2	GI tract
ppDDT	20.0	ug/kg bw/day	(ATSDR, 2002b)	3	375	brain
ppDDT	20.0	ug/kg bw/day	(ATSDR, 2002b)	3	125	uterus

Using the $IDRL_{TDI}$ for the respective xenobiotics derived above, the respective risk characterization levels are derived. In Table 8, the respective $BED/IDRL_{TDI}$ ratios for the median exposure levels are given below.

Table 8. Internal dose of As, Cd, BBzP, DEHP, DiNP, HCB, PB, Hg, PCB153 and ppDDT in blood according to respective TDI and the collected human biomonitoring data within the frame of HEALS.

Chemical	Country	BED levels (BED)	Internal dose reference levels ($IDRL_{TDI}$) based on TDI	Units	Ratio $BED/IDRL_{TDI}$
Arsenic	Slovenia	0.18	**118**	ug/L	0.002
Arsenic	Croatia	1.3	**118**	ug/L	0.011
Cadmium	Italy	0.35	**208**	ug/L	0.002
Cadmium	Croatia	0.39	**208**	ug/L	0.002
Cadmium	Slovenia	0.15	**208**	ug/L	0.001
BBzP	Poland	0.47	**168**	ug/L	0.003
DEHP	Poland	11.18	**51**	ug/L	0.219
DiNP	Poland	8.9	**75**	ug/L	0.119
BPA	Slovenia	0.0001	**0.016**	ug/L	0.01
HCB	Spain (Valencia)	0.56	**21**	ug/L	0.027
HCB	Spain (Menorca)	0.86	**21**	ug/L	0.041
Pb	Slovenia	9.81	**120**	ug/L	0.082
Pb	Poland	1.14	**120**	ug/L	0.006
Pb	Italy	23.9	**120**	ug/L	0.199
Mercury	Slovenia	1.99	**1025**	ug/L	0.002
Mercury	Italy	1.51	**1025**	ug/L	0.001
Mercury	Croatia	4.25	**1025**	ug/L	0.004
Mercury	Poland	0.44	**912**	ug/L	0.0005
PCB153	Spain (Menorca)	0.22	**38**	ug/L	0.006
PCB153	Spain (Valencia)	0.33	**38**	ug/L	0.009
ppDDT	Spain (Menorca)	0.17	**3**	ug/L	0.057
ppDDT	Spain (Valencia)	0.12	**3**	ug/L	0.040

Based on the above, it is evident that the mean exposure levels of the European population are below the levels related to toxic effects, while small fractions of the population in specific areas are exposed to levels above concern for ppDDT in Menorca (Spain). However, epidemiological studies have identified associations of environmentally relevant exposure levels to these compounds with several adverse outcomes and this is something that will be investigated in WPs 14 to WP17. In any case, the method described herein, allows us to understand better the population and individual variability in internal dose for phenomenally similar biomonitored levels.

7 Conclusions and discussion

Biomonitoring, which is the measurement of chemicals in human tissues or fluids such as blood or urine, is continuously gaining place in modern risk assessment and molecular epidemiology. However, there is still lack of consistency for methods able to associate biomonitored data to the actual exposure and more importantly to the biologically effective dose that relates to adverse outcomes. This deliverable, provides the methodological framework for associating biomonitored levels of parent compounds or their metabolites in various biological matrices into internal dose in the target tissue through a two-step approach:

- Reconstructing exposure profiles starting from biomonitoring data, by coupling the compound-specific parameterized generic PBBK model with the exposure reconstruction algorithm and
- Re-running forward the estimated exposure / intakes through the PBBK model for estimating the internal dose profiles in the target tissues, or the so called biologically effective dose (BED).

BED is a more relevant exposure metric than the biomonitoring data itself, because can be associated mechanistically to doses that have been linked to perturbations (e.g. BPAD) that are eventually related to adverse health effects or they can be used for epi or EWAS analysis. An additional advantage of using the actual BED for deriving environment health associations, is that additional information affecting the inter-individual variability can be ascribed and reflecting the BED on individual level, relative to age, gender, bodyweight and body mass index, and most importantly the genetic differences associated to CYP isoforms related to xenobiotics metabolism.

This can be clearly illustrated on the following example on BPA. The effect of age is related to differences in (a) physiological parameters such as tissue composition, blood flows, cardiac and respiratory rate and (b) the maturity of the conjugation activity. Taking into account the physiologically-based approach for scaling to children (Edginton et al., 2006) and the most recent findings regarding the ontogeny of enzymes involved in BPA detoxification (Court et al., 2012; 2011; Leeder, 2009) an age-dependent bioavailability difference factor of 2 to 3.5 is considered between infants and adults. Similarly, significant bioavailability differences occur based on the route of exposure; In the case of inhalation, BPA enters directly in the blood stream from the alveoli and the lack of first pass metabolism results in bioavailability differences up to 6 times compared to a similar dose administrated orally. In the case of dermal exposure, the lack of first pass metabolism is somehow decompensated by slower absorption. Finally, a major factor that differentiates BED within a population is the presence of genetic variants of key enzymes involved in BPA conjugation; *in vitro* kinetic studies have identified that D85Y substitution in UGT2B15 decreases enzymatic function (Hanioka et al., 2011) and that the polymorphic alleles of UGT2B15 are translated in variations in the metabolism of BPA (Partosch et al., 2013). The effect of all the described

above parameters is summarized in *Figure 17*, where for the same bodyweight normalized dose, free plasma BPA is estimated accounting for differences in age, route of exposure and genetics. Clearly, the combination of early infancy and the presence of alleles associated to slower metabolism could result in a biologically effective dose in a neonate or an infant up to 15 times higher compared to the one of an adult. This leads to the conclusion, that a phenomenally limited variability in biomonitored data could be realistically translated into a wider BED variability. In the case that additional information had been available (such as genetic polymorphisms) or younger children and infants were enrolled in the study, wider differences would had been identified in the respective RCR outcomes among the different approaches.

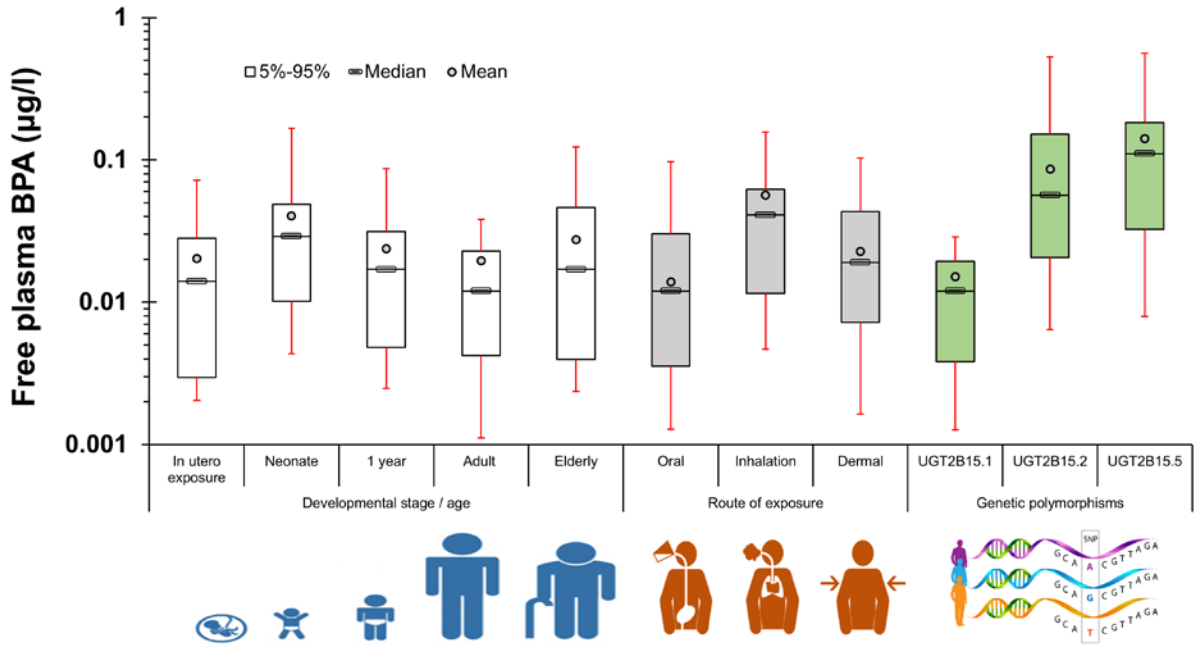


Figure 17. Understanding the major parameters inducing bioavailability differences

8 References

Abernathy, C., Thomas, D., Calderon, R., 2003. Health effects and risk assessment of arsenic J Nutr 133 (suppl 1): 1536S–1538S. Find this article online.

Afssa, O.o.t.F.F.S.A., 2008. Opinion of the French Food Safety Agency on the assessment of the risk of consuming pork contaminated with PCDD/Fs and DL-PCBs (dioxin-like PCBs)

Angerer, J., Aylward, L.L., Hays, S.M., Heinzow, B., Wilhelm, M., 2011. Human biomonitoring assessment values: Approaches and data requirements. International Journal of Hygiene and Environmental Health.

ATSDR, 2002a. Toxicological profile for hexachlorobenzene update. Agency for Toxic Substances and Disease Registry Atlanta, GA.

ATSDR, A.f.T.S.a.D.R., 2002b. Toxicological profile for DDT, DDE, and DDD.

ATSDR, U., 2012. Lead Toxicity. Agency for Toxic Substances and Disease Registry, 1, 582.

Aylward, L.L., Hays, S.M., 2011. Biomonitoring-based risk assessment for hexabromocyclododecane (HBCD). Int J Hyg Environ Health 214, 179-187.

Aylward, L.L., Hays, S.M., Gagné, M., Krishnan, K., 2009a. Derivation of Biomonitoring Equivalents for di-n-butyl phthalate (DBP), benzylbutyl phthalate (BzBP), and diethyl phthalate (DEP). Regulatory Toxicology and Pharmacology 55, 259-267.

Aylward, L.L., Hays, S.M., Gagné, M., Krishnan, K., 2009b. Derivation of Biomonitoring Equivalents for di(2-ethylhexyl)phthalate (CAS No. 117-81-7). Regulatory Toxicology and Pharmacology 55, 249-258.

Aylward, L.L., Hays, S.M., Gagne, M., Nong, A., Krishnan, K., 2010. Biomonitoring equivalents for hexachlorobenzene. Regulatory toxicology and pharmacology : RTP 58, 25-32.

Aylward, L.L., Krishnan, K., Kirman, C.R., Nong, A., Hays, S.M., 2011. Biomonitoring equivalents for deltamethrin. Regulatory toxicology and pharmacology : RTP 60, 189-199.

Aylward, L.L., Lakind, J.S., Hays, S.M., 2008. Derivation of biomonitoring equivalent (BE) values for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds: a screening tool for interpretation of biomonitoring data in a risk assessment context. Journal of toxicology and environmental health. Part A 71, 1499-1508.

Baars, A.J., Theelen, R.M.C., Janssen, P.J.C.M., 2001. RIVM report 711701 025. Re-evaluation of human-toxicological maxi- mum permissible risk levels. .

Balakrishnan, B., Henare, K., Thorstensen, E.B., Ponnampalam, A.P., Mitchell, M.D., 2010. Transfer of bisphenol A across the human placenta. American Journal of Obstetrics and Gynecology 202, 393.e391-393.e397.

Ballesteros-Gómez, A., Rubio, S., Pérez-Bendito, D., 2009. Potential of supramolecular solvents for the extraction of contaminants in liquid foods. Journal of Chromatography A 1216, 530-539.

Barouki, R., 2013. [Can xenobiotics accumulated in adipose tissue contribute to a carcinogenic risk?]. Annales d'endocrinologie 74, 154-155.

Bechara, E.J., Medeiros, M.H., Monteiro, H.P., Hermes-Lima, M., Pereira, B., Demasi, M., Costa, C.A., Abdalla, D.S., Onuki, J., Wendel, C.M., 1993. A free radical hypothesis of lead poisoning and inborn porphyrias associated with 5-aminolevulinic acid overload. Quim Nova 16, 385-392.

Becker, K., Göen, T., Seiwert, M., Conrad, A., Pick-Fuß, H., Müller, J., Wittassek, M., Schulz, C., Kolossa-Gehring, M., 2009. GerES IV: Phthalate metabolites and bisphenol A in urine of German children. International Journal of Hygiene and Environmental Health 212, 685-692.

Beronius, A., Ruden, C., Hakansson, H., Hanberg, A., 2010. Risk to all or none?. A comparative analysis of controversies in the health risk assessment of Bisphenol A. Reproductive Toxicology 29, 132-146.

Beyer, A., Biziuk, M., 2009. Environmental fate and global distribution of polychlorinated biphenyls, Reviews of Environmental Contamination and Toxicology Vol 201. Springer, pp. 137-158.

Boogaard, P.J., Hays, S.M., Aylward, L.L., 2011. Human biomonitoring as a pragmatic tool to support health risk management of chemicals - Examples under the EU REACH programme. Regulatory Toxicology and Pharmacology 59, 125-132.

Braunrath, R., Podlipna, D., Padlesak, S., Cichna-Markl, M., 2005. Determination of bisphenol A in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection. Journal of Agricultural and Food Chemistry 53, 8911-8917.

Calafat, A.M., Weuve, J., Ye, X., Jia, L.T., Hu, H., Ringer, S., Huttner, K., Hauser, R., 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environmental Health Perspectives 117, 639-644.

Casas, L., Fernandez, M.F., Llop, S., Guxens, M., Ballester, F., Olea, N., Irurzun, M.B., Rodriguez, L.S., Riano, I., Tardon, A., Vrijheid, M., Calafat, A.M., Sunyer, J., 2011. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int 37, 858-866.

Casey, C.E., Neifert, M.R., Seacat, J.M., Neville, M.C., 1986. Nutrient intake by breast-fed infants during the first five days after birth. American Journal of Diseases of Children 140, 933-936.

CDC, 2009. Fourth National Report on Human Exposure to Environmental Chemicals. Department of Health and Human Services Centers for Disease Control and Prevention., Atlanta, GA.

Court, M.H., Zhang, X., Ding, X., Yee, K.K., Hesse, L.M., Finel, M., 2012. Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. Xenobiotica 42, 266-277.

Covaci, A., Hond, E.D., 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., 2014. Urinary BPA measurements in children and mothers from six European member states: Overall results and determinants of exposure. Environmental Research *in press*.

Deceuninck, Y., Bichon, E., Marchand, P., Boquien, C.Y., Legrand, A., Boscher, C., Antignac, J.P., Le Bizec, B., 2015. Determination of bisphenol A and related substitutes/analogues in human breast milk using gas chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry.

Dekant, W., Völkel, W., 2008. Human exposure to bisphenol A by biomonitoring: Methods, results and assessment of environmental exposures. Toxicology and Applied Pharmacology 228, 114-134.

DEMOCOPHES, 2013. DEMOCOPHES Layman's report - Human biomonitoring on a European scale.

Dimitriadou, L., Malarvannan, G., Covaci, A., Iossifidou, E., Tzafettas, J., Zournatzi-Koiou, V., Kalantzi, O.-I., 2016. Levels and profiles of brominated and chlorinated contaminants in human breast milk from Thessaloniki, Greece. Science of The Total Environment 539, 350-358.

Dong, S., Terasaka, S., Kiyama, R., 2011. Bisphenol A induces a rapid activation of Erk1/2 through GPR30 in human breast cancer cells. Environ Pollut 159, 212-218.

EC, 2011. Commission Directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles, OJ L 26, 29.1.2011, p. 11–14.

ECHA, 2012. Guidance on information requirements and chemical safety assessment. Chapter R.16: Environmental Exposure Estimation Version 2.1, Helsinki, Finland.

ECHA, E.C.A., 2015. Evaluation of new scientific evidence concerning DINP and DIDP. In relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006.

Edginton, A.N., Schmitt, W., Voith, B., Willmann, S., 2006. A mechanistic approach for the scaling of clearance in children. Clinical Pharmacokinetics 45, 683-704.

EFSA, 2005a. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Butylbenzylphthalate (BBP) for use in food contact materials. The EFSA Journal.

EFSA, 2005b. 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). Question number EFSA-Q-2005-100. The EFSA Journal.

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) European Food Safety Agency.

EFSA, 2008. Toxicokinetics of Bisphenol A - Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food European Food Safety Agency.

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

EFSA, E.F.S.A., 2005c. Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to Endosulfan as undesirable substance in animal feed. EFSA Journal 3, 234-n/a.

EFSA, E.F.S.A., 2015b. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal 13.

ENTERLINE, P.E., HENDERSON, V.L., MARSH, G.M., 1987. Exposure to arsenic and respiratory cancer a reanalysis. American journal of epidemiology 125, 929-938.

Eriksson, P., 1992. Neuroreceptor and behavioral effects of DDT and pyrethroids in immature and adult mammals, The vulnerable brain and environmental risks. Springer, pp. 235-251.

Eriksson, P., Ahlbom, J., Fredriksson, A., 1992. Exposure to DDT during a defined period in neonatal life induces permanent changes in brain muscarinic receptors and behaviour in adult mice. Brain research 582, 277-281.

Eriksson, P., Jakobsson, E., Fredriksson, A., 2001. Brominated flame retardants: a novel class of developmental neurotoxicants in our environment? Environmental health perspectives 109, 903.

EU, 2003. European Union Risk Assessment Report 4,4 '-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A). JRC-ECB.

EU, 2010. European Union Risk Assessment Report 4,4 '-ISOPROPYLIDENEDIPHENOL (BISPHENOL-A). JRC-ECB.

FAO/WHO, 2010. Toxicological and Health Aspects of Bisphenol A - Report of Joint FAO/WHO Expert Meeting.

Ferrer, E., Santoni, E., Vittori, S., Font, G., Mañes, J., Sagratini, G., 2011. Simultaneous determination of bisphenol A, octylphenol, and nonylphenol by pressurised liquid extraction and liquid chromatography-tandem mass spectrometry in powdered milk and infant formulas. Food Chemistry 126, 360-367.

Fisher, J.W., Twaddle, N.C., Vanlandingham, M., Doerge, D.R., 2011. Pharmacokinetic modeling: Prediction and evaluation of route dependent dosimetry of bisphenol A in monkeys with extrapolation to humans. Toxicology and Applied Pharmacology 257, 122-136.

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

Gallart-Ayala, H., Moyano, E., Galceran, M.T., 2011. Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry. Analytica Chimica Acta 683, 227-233.

García-Prieto, A., Lunar, L., Rubio, S., Pérez-Bendito, D., 2008. Decanoic acid reverse micelle-based coacervates for the microextraction of bisphenol A from canned vegetables and fruits. Analytica Chimica Acta 617, 51-58.

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 Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 27, 1627-1637.

Geens, T., Roosens, L., Neels, H., Covaci, A., 2009. Assessment of human exposure to Bisphenol-A, Triclosan and Tetrabromobisphenol-A through indoor dust intake in Belgium. Chemosphere 76, 755-760.

Georgopoulos, P.G., Sasso, A.F., Isukapalli, S.S., Lioy, P.J., Vallero, D.A., Okino, M., Reiter, L., 2009. Reconstructing population exposures to environmental chemicals from biomarkers: Challenges and opportunities. Journal of Exposure Science and Environmental Epidemiology 19, 149-171.

Ginsberg, G., Rice, D.C., 2009. Does rapid metabolism ensure negligible risk from bisphenol A? Environmental Health Perspectives 117, 1639-1643.

Goodson, A., Robin, H., Summerfield, W., Cooper, I., 2004. Migration of bisphenol A from can coatings - Effects of damage, storage conditions and heating. Food Additives and Contaminants 21, 1015-1026.

Goodson, A., Summerfield, W., Cooper, I., 2002. Survey of bisphenol A and bisphenol F in canned foods. Food Additives and Contaminants 19, 796-802.

Goyer, R., 1989. Mechanisms of lead and cadmium nephrotoxicity. Toxicology letters 46, 153-162.

Grumetto, L., Montesano, D., Seccia, S., Albrizio, S., Barbato, F., 2008. Determination of bisphenol A and bisphenol B residues in canned peeled tomatoes by reversed-phase liquid chromatography. Journal of Agricultural and Food Chemistry 56, 10633-10637.

Hagmar, L., Wallin, E., Vessby, B., Jönsson, B.A., Bergman, Å., Rylander, L., 2006. Intra-individual variations and time trends 1991−2001 in human serum levels of PCB, DDE and hexachlorobenzene. Chemosphere 64, 1507-1513.

Hanioka, N., Oka, H., Nagaoka, K., Ikushiro, S., Narimatsu, S., 2011. Effect of UDP-glucuronosyltransferase 2B15 polymorphism on bisphenol A glucuronidation. Arch Toxicol 85, 1373-1381.

Hays, S.M., Aylward, L.L., Gagne, M., Krishnan, K., 2009. Derivation of Biomonitoring Equivalents for cyfluthrin. Regulatory toxicology and pharmacology : RTP 55, 268-275.

Hays, S.M., Aylward, L.L., Gagne, M., Nong, A., Krishnan, K., 2010. Biomonitoring equivalents for inorganic arsenic. Regulatory toxicology and pharmacology : RTP 58, 1-9.

Hays, S.M., Aylward, L.L., Kirman, C.R., Krishnan, K., Nong, A., 2011. Biomonitoring Equivalents for di-isononyl phthalate (DINP). Regulatory Toxicology and Pharmacology 60, 181-188.

Hays, S.M., Becker, R.A., Leung, H.W., Aylward, L.L., Pyatt, D.W., 2007. Biomonitoring equivalents: A screening approach for interpreting biomonitoring results from a public health risk perspective. Regulatory Toxicology and Pharmacology 47, 96-109.

Hays, S.M., Nordberg, M., Yager, J.W., Aylward, L.L., 2008. Biomonitoring Equivalents (BE) dossier for cadmium (Cd) (CAS No. 7440-43-9). Regulatory Toxicology and Pharmacology 51, S49-S56.

Hays, S.M., Pyatt, D.W., Kirman, C.R., Aylward, L.L., 2012. Biomonitoring Equivalents for benzene. Regulatory Toxicology and Pharmacology 62, 62-73.

Heudorf, U., Mersch-Sundermann, V., Angerer, J., 2007. Phthalates: Toxicology and exposure. International Journal of Hygiene and Environmental Health 210, 623-634.

Judson, R.S., Houck, K.A., Kavlock, R.J., Knudsen, T.B., Martin, M.T., Mortensen, H.M., Reif, D.M., Rotroff, D.M., Shah, I., Richard, A.M., Dix, D.J., 2010. In vitro screening of environmental chemicals for targeted testing prioritization: The toxcast project. Environmental Health Perspectives 118, 485-492.

Judson, R.S., Kavlock, R.J., Setzer, R.W., Cohen Hubal, E.A., Martin, M.T., Knudsen, T.B., Houck, K.A., Thomas, R.S., Wetmore, B.A., Dix, D.J., 2011. Estimating toxicity-related biological pathway altering doses for high-throughput chemical risk assessment. Chemical Research in Toxicology 24, 451-462.

Karagas, M.R., Stukel, T.A., Morris, J.S., Tosteson, T.D., Weiss, J.E., Spencer, S.K., Greenberg, E.R., 2001. Skin cancer risk in relation to toenail arsenic concentrations in a US population-based case-control study. American journal of epidemiology 153, 559-565.

Kirman, C.R., Aylward, L.L., Hays, S.M., Krishnan, K., Nong, A., 2011. Biomonitoring Equivalents for DDT/DDE. Regulatory Toxicology and Pharmacology 60, 172-180.

Knerr, S., Schrenk, D., 2006. Carcinogenicity of "non-dioxinlike" polychlorinated biphenyls. Critical Reviews in Toxicology 36, 663-694.

Koniecki, D., Wang, R., Moody, R.P., Zhu, J., 2011. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. Environmental research 111, 329-336.

Krishnan, K., Adamou, T., Aylward, L.L., Hays, S.M., Kirman, C.R., Nong, A., 2011. Biomonitoring equivalents for 2,2',4,4',5-pentabromodiphenylether (PBDE-99). Regulatory toxicology and pharmacology : RTP 60, 165-171.

Krishnan, K., Gagné, M., Nong, A., Aylward, L.L., Hays, S.M., 2010a. Biomonitoring Equivalents for bisphenol A (BPA). Regulatory Toxicology and Pharmacology 58, 18-24.

Krishnan, K., Gagné, M., Nong, A., Aylward, L.L., Hays, S.M., 2010b. Biomonitoring Equivalents for triclosan. Regulatory Toxicology and Pharmacology 58, 10-17.

La Merrill, M., Emond, C., Kim, M.J., Antignac, J.P., Le Bizec, B., Clement, K., Birnbaum, L.S., Barouki, R., 2013. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ Health Perspect 121, 162-169.

LaKind, J.S., Naiman, D.Q., 2008. Bisphenol A (BPA) daily intakes in the United States: Estimates from the 2003-2004 NHANES urinary BPA data. Journal of Exposure Science and Environmental Epidemiology 18, 608-615.

Leeder, J.S., 2009. Developmental pharmacogenetics: A general paradigm for application to neonatal pharmacology and toxicology. Clinical Pharmacology and Therapeutics 86, 678-682.

Maragou, N.C., Lampi, E.N., Thomaidis, N.S., Koupparis, M.A., 2006. Determination of bisphenol A in milk by solid phase extraction and liquid chromatography-mass spectrometry. Journal of Chromatography A 1129, 165-173.

Mattison, D.R., Karyakina, N., Goodman, M., Lakind, J.S., 2014. Pharmaco-and toxicokinetics of selected exogenous and endogenous estrogens: A review of the data and identification of knowledge gaps. Critical Reviews in Toxicology 44, 696-724.

Mielke, H., Gundert-Remy, U., 2009. Bisphenol A levels in blood depend on age and exposure. Toxicology Letters 190, 32-40.

Muntner, P., He, J., Vupputuri, S., Coresh, J., Batuman, V., 2003. Blood lead and chronic kidney disease in the general United States population: results from NHANES III. Kidney international 63, 1044-1050.

Navas-Acien, A., Tellez-Plaza, M., Guallar, E., Muntner, P., Silbergeld, E., Jaar, B., Weaver, V., 2009. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. American journal of epidemiology 170, 1156-1164.

Nordberg, G., Goyer, R., Nordberg, M., 1975. Comparative toxicity of cadmium-metallothionein and cadmium chloride on mouse kidney. Archives of pathology 99, 192-197.

NRC, 2006. Human Biomonitoring for Environmental Chemicals. The National Academies Press, Washington, DC.

Olsson, M., Bensryd, I., Lundh, T., Ottosson, H., Skerfving, S., Oskarsson, A., 2002. Cadmium in blood and urine--impact of sex, age, dietary intake, iron status, and former smoking--association of renal effects. Environmental health perspectives 110, 1185.

Organization, W.H., 1990. Methylmercury.

Partosch, F., Mielke, H., Gundert-Remy, U., 2013. Functional UDP-glucuronyltransferase 2B15 polymorphism and bisphenol A concentrations in blood: results from physiologically based kinetic modelling. Archives of Toxicology, 1-8.

Rice, D.C., 2004. The US EPA reference dose for methylmercury: sources of uncertainty. Environmental research 95, 406-413.

Sarigiannis, D., Karakitsios, S., Gotti, A., Loizou, G., Cherrie, J., Smolders, R., De Brouwere, K., Galea, K., Jones, K., Handakas, E., Papadaki, K., Sleeuwenhoek, A., 2014. Integra: From global scale contamination to tissue dose, Proceedings - 7th International Congress on Environmental Modelling and Software: Bold Visions for Environmental Modeling, iEMSs 2014, pp. 1001-1008.

Sarigiannis, D.A., Karakitsios, S.P., Handakas, E., Simou, K., Solomou, E., Gotti, A., 2016. Integrated exposure and risk characterization of bisphenol-A in Europe. Food and Chemical Toxicology 98, 134-147.

Schettler, T., 2006. Human exposure to phthalates via consumer products. International journal of andrology 29, 134-139.

Schonfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M., Chahoud, I., 2002. Parent bisphenol a accumulation in the human maternal-fetal-placental unit. Environmental Health Perspectives 110, A703-A707.

Schulz, C., Angerer, J., Ewers, U., Kolossa-Gehring, M., 2007. The German Human Biomonitoring Commission. International Journal of Hygiene and Environmental Health 210, 373-382.

Schulz, C., Wilhelm, M., Heudorf, U., Kolossa-Gehring, M., 2011. Update of the reference and HBM values derived by the German Human Biomonitoring Commission. Int J Hyg Environ Health 215, 26-35.

Shin, B.S., Kim, C.H., Jun, Y.S., Kim, D.H., Lee, B.M., Yoon, C.H., Park, E.H., Lee, B.M., Yoon, C.H., Park, E.H., Lee, K.C., Han, S.Y., Park, K.L., Kim, H.S., Yoo, S.D., 2004. Physiologically based pharmacokinetics of bisphenol A. Journal of Toxicology and Environmental Health - Part A 67, 1971-1985.

Sipes, N.S., Wambaugh, J.F., Pearce, R., Auerbach, S.S., Wetmore, B.A., Hsieh, J.-H., Shapiro, A.J., Svoboda, D., DeVito, M.J., Ferguson, S.S., 2017. An Intuitive Approach for Predicting Potential Human Health Risk with the Tox21 10k Library. Environmental Science & Technology.

Smith, A.H., Marshall, G., Yuan, Y., Ferreccio, C., Liaw, J., von Ehrenstein, O., Steinmaus, C., Bates, M.N., Selvin, S., 2006. Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. Environmental health perspectives, 1293-1296.

Smolders, R., Schoeters, G., 2007. Identifying opportunities and gaps for establishing an integrated EDR-triad at a European level. International Journal of Hygiene and Environmental Health 210, 253-257.

Sorahan, T., Esmen, N., 2004. Lung cancer mortality in UK nickel-cadmium battery workers, 1947–2000. Occupational and environmental medicine 61, 108-116.

Takahashi, O., Oishi, S., 2000. Disposition of orally administered 2,2-bis(4-hydroxyphenyl)propane (bisphenol A) in pregnant rats and the placental transfer to fetuses. Environmental Health Perspectives 108, 931-935.

Teeguarden, J., Hanson-Drury, S., Fisher, J.W., Doerge, D.R., 2013. Are typical human serum BPA concentrations measurable and sufficient to be estrogenic in the general population? Food and Chemical Toxicology 62, 949-963.

Teeguarden, J.G., Waechter Jr, J.M., Clewell III, H.J., Covington, T.R., Barton, H.A., 2005. Evaluation of oral and intravenous route pharmacokinetics, plasma protein binding, and uterine tissue dose metrics of bisphenol A: A physiologically based pharmacokinetic approach. Toxicological Sciences 85, 823-838.

Turusov, V., Rakitsky, V., Tomatis, L., 2002. Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. Environmental Health Perspectives 110, 125-128.

UBA, 2012, UBA (Federal Environment Agency), 2012b. The German Environment Specimen Bank, http://www.umweltprobenbank.de,

USEPA, ToxCast™ Data, http://www.epa.gov/ncct/toxcast/data.html, 26/4/2013, 2013

USEPA, U.E.P.A., Non-Carcinogen Tolerable Daily Intake (TDI) Values from US EPA.

Vahter, M., Berglund, M., Lind, B., Jorhem, L., Slorach, S., Friberg, L., 1991. Personal monitoring of lead and cadmium exposure—a Swedish study with special reference to methodological aspects. Scandinavian journal of work, environment & health, 65-74.

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2010. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environmental Health Perspectives 118, 1055-1070.

Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S., Soto, A.M., 2009. Bisphenol-a and the great divide: A review of controversies in the field of endocrine disruption. Endocrine Reviews 30, 75-95.

Vandentorren, S., Zeman, F., Morin, L., Sarter, H., Bidondo, M.L., Oleko, A., Leridon, H., 2011. Bisphenol-A and phthalates contamination of urine samples by catheters in the Elfe pilot study: implications for large-scale biomonitoring studies. Environ Res 111, 761-764.

Volkel, W., Kiranoglu, M., Fromme, H., 2008. Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. Toxicology Letters 179, 155-162.

Wasserman, G., Graziano, J., Factor-Litvak, P., Popovac, D., Morina, N., Musabegovic, A., Vrenezi, N., Capuni-Paracka, S., Lekic, V., Preteni-Redjepi, E., et al., 1992. Independent effects of lead exposure and iron deficiency anemia on developmental outcome at age 2 years. The Journal of pediatrics 121, 695-703.

WHO, W.H.O., 1990. Methylmercury.

WHO, W.H.O., 2011a, CADMIUM, Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1376,

WHO, W.H.O., 2011b, Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1863,

WHO, W.H.O., 2011c, Lead, Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=3511,

WHO, W.H.O., 2011d, Mercury, Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), http://apps.who.int/food-additives-contaminants-jecfa-database/chemical.aspx?chemID=1806,

Wolff, M.S., Toniolo, P.G., Lee, E.W., Rivera, M., Dubin, N., 1993. Blood levels of organochlorine residues and risk of breast cancer. Journal of the National Cancer Institute 85, 648-652.

Xuezhi, J., Youxin, L., Yilan, W., 1992. Studies of lead exposure on reproductive system: a review of work in China. Biomedical and environmental sciences: BES 5, 266-275.

Zalko, D., Jacques, C., Duplan, H., Bruel, S., Perdu, E., 2011. Viable skin efficiently absorbs and metabolizes bisphenol A. Chemosphere 82, 424-430.