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WP 12 - From HBM to exposure

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Entity	Name of person responsible	Short name of institution	Received [Date]
Coordinator	Marike KOLOSSA-GEHRING	UBA	05/12/2017
Grant Signatory	Theodoros Laopoulos	AUTH	01/12/2017
Pillar Leader	Robert BAROUKI	INSERM	05/12/2017
Work Package Leader	Denis SARIGIANNIS	AUTH	01/12/2017
Task leader	Milena HORVAT	JSI	01/12/2017

Responsible author	Milena HORVAT, Denis SARIGIANNIS	E-mail	milena.horvat@ijs.si
Short name of institution	JSI, AUTH	Phone	+386 1 5885 389
Co-authors	Evangelos HANDAKAS, Spyros KARAKITSIOS, Alberto GOTTI		

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1 Authors and Acknowledgements

Lead authors

Milena HORVAT (JSI), Denis SARIGIANNIS (AUTH)

Contributors

Evangelos HANDAKAS (AUTH), Spyros KARAKITSIOS (AUTH), Alberto GOTTI (IUSS)

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2 Introduction

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” (CDC, 2009). 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) (NRC, 2006).

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).

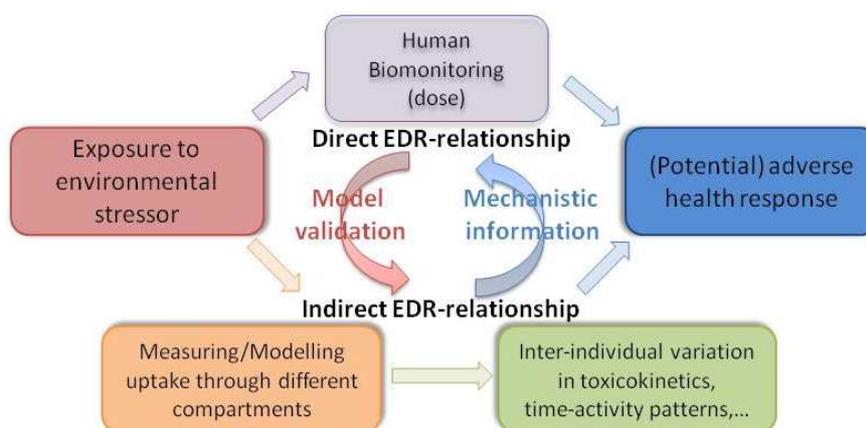


Figure 1: The Exposure-Dose-Response Triad to evaluate the potential adverse health effects of exposure to environmental agents (adapted from Smolders and Schoeters, 2007)

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.

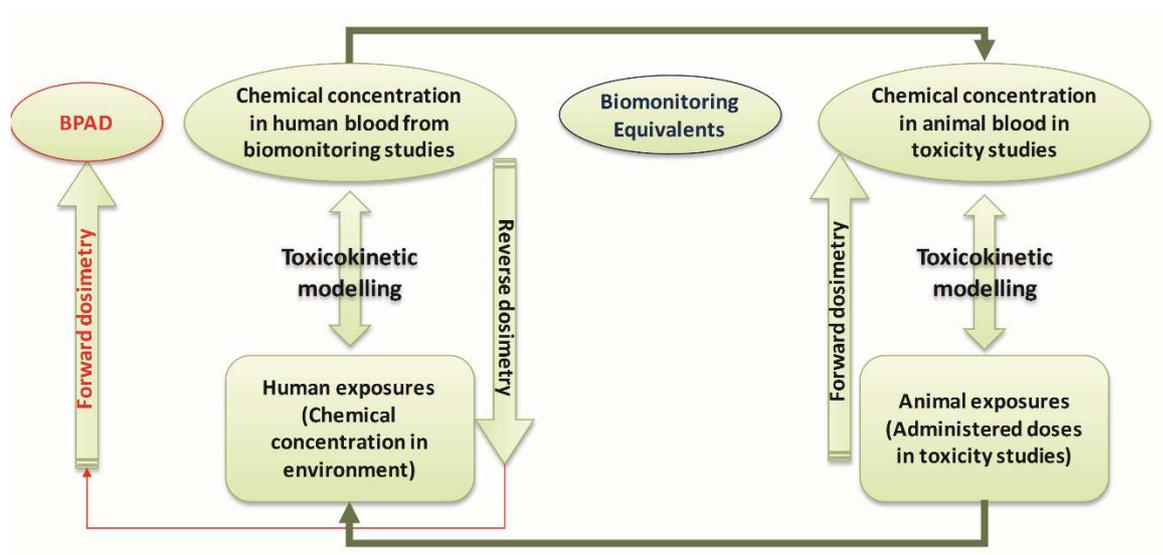


Figure 2: Interpretation of biomonitoring data

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 characterised in humans. In forward dosimetry, pharmacokinetic data in experimental animals can be used to support a direct comparison of internal exposure in humans that derives (or which derives) 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 introduction of biomonitoring equivalents. Alternatively, this concept is called reverse dosimetry. 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 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). This is similar to usual external exposure risk assessment metrics that associate exposure to response (Judson et al., 2010; Judson et al., 2011). The similarity is closest when perturbation of a pathway is a key event in the mode of action (MoA) resulting in a specified adverse outcome. BPADs are delivered from high-throughput screening (HTS) *in vitro* data, and the data are available in the Toxcast 21 database.

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3 Methodological framework for optimal use of HBM data in assessing population exposure

3.1 General recommendations for selection of an exposure reconstruction modelling framework

Human biomonitoring typically is an integrative measure of different exposure episodes along various routes and over different time scales; thus, it is often very difficult to reconstruct the primary exposure routes from HBM data alone. This uncertainty often limits the interpretative value of biomarker data. However, several mathematical approaches have been developed to reconstruct exposures related to population biomonitoring studies, and can be subdivided in a number of different approaches. Exposure reconstruction techniques combined with PBTK modeling can be subdivided into Bayesian and non-Bayesian approaches (Georgopoulos et al., 2008). Moreover, the computational inversion techniques (and exposure reconstruction techniques as well), can be classified as deterministic or stochastic (Moles et al., 2003) based on the identification of a global minimum of the error metric, the input parameters and the model setup. Based on the above, the methods for exposure reconstruction related to population HBM studies and their recommended use are summarised below:

- **Deterministic methods**
 - Intake mass balance: This is the simplest method for approximating the daily intake dose. It can give reliable results only for compounds that are rapidly metabolised and the metabolites measured in urine are eliminated within a day and when whole day urine has been collected.
 - Deterministic method using a PBTK model. This is a deterministic method that associates measured biomarkers with actual intake, using single values. This can be applied either with comprehensive PBTK model, or with a simple Pharmacokinetic (PK) model. This method can be applied also for non-rapidly metabolised compounds; however, it does not incorporate variability and uncertainty either related to the PBTK model parameterisation, or with the exposure scenario.
- **Stochastic methods**
 - Exposure conversion factor: This is a stochastic method that incorporates variability and uncertainty in both exposure scenario and PBTK model parameterisation, however it assumes a constant exposure and the expected biomarker concentration in steady-state conditions. This method cannot handle complex exposure scenarios or multiple biomarker samples within a given exposure regime.
 - Bayesian approach: This is the most comprehensive approach, able to address complex exposure scenarios, dynamic in time and provides reliable results for both rapidly and non-rapidly metabolised compounds, addressing all kinds of uncertainties and variabilities introduced in the modeling process.

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3.2 Methods for exposure reconstruction related to population HBM studies

3.2.1 Deterministic methods

3.2.1.1 Deterministic methods using a PBTK model

The deterministic methods aim to convergence towards a global minimum. The problem is solved using an “objecting function” based on biomarkers. Additionally, constraints such as bounds, equalities and inequalities are incorporated. The deterministic models have been used on several biological applications using different methods. Muzic Jr and Christian (2006) have applied a regression technique in order to estimate biokinetic parameters. Moreover, a gradient method has been used by Isukapalli et al. (2000) calculating the uncertainty in PBTK models. A good example of a the application of a maximum likelihood method for short and long term for exposure reconstruction is given by Roy et al. using a PBTK for chloroform (Roy et al., 1996).

According to Georgopoulos et al.(2009) the use of deterministic Inversion to solve the exposure reconstruction problem, results in a problem of global minimisation, related to the identification of exposures x by minimising a “cost function” J . In general, in those problems, a forward model is applied aiming to minimise the difference between the observed biomonitoring data (b) and the solution of model at each time point (t_i). The following constrains should be accounted for as well:

- exposure limits ($x^l \leq x \leq x^u$),
- equality constraints on the model ($f(x,b,t)=0$), and
- inequality constraints ($g(x,b,t) \leq 0$).

Typical examples of J include

$$J = \sum_{i=1}^{N_{meas}} (b'(t_i) - m(x, t_i))^T (b'(t_i) - m(x, t_i))$$

and

$$J = -L(m, x, b') = - \prod_{i=1}^{N_{means}} f_x(b'(t_i) | x, m(x, t_i))$$

Where the likelihood L is a function of the parameters of a statistical model given data that depends on the assumptions regarding the distribution of “errors”.

3.2.1.2 Intake mass balance

Intake mass balance is a simplified exposure reconstruction framework that can be used for rapidly metabolised and excreted compounds. This approach is based on the following equation:

$$DailyIntake = \frac{C_{urine} \cdot exc_{vol}}{BW} \cdot \frac{MB}{MB_{met}} \quad (0.1)$$

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Where C_{urine} is the concentration of the metabolite in the urine, BW is the bodyweight, exc_{vol} is the excreted urine volume, MB the molecular weight of the studied chemical and MB_{met} the molecular weight of the metabolite observed in the urine.

This method has been used to BPA exposure reconstruction using lifestyle and dietary data and associating time with biomonitoring data coming up the exposure occurring with the previous 24-h period (LaKind and Naiman, 2015). A similar approach that takes into account urinary elimination fraction has been used for the evaluation of the daily exposure to a wide range of phthalates (Kohn et al., 2000).

This exposure reconstruction framework is sensitive to the last dose before sample collection and it works better when the overall daily urine amount is collected. However, a major disadvantage is the underestimation of the daily intake dose when this approach is applied in spot samples. In this case, the overall intake might be seriously misinterpreted since the time dynamics associated to the elimination process that are so important (Sarigiannis et al., 2016), are not taken into account. Thus, this method is applicable only for rapidly metabolised compounds and provides more reliable exposure estimates if whole day urine samples have been collected.

3.2.2 Stochastic methods

3.2.2.1 Exposure conversion factor

The exposure conversion factor (ECF) method, proposed by Tan et al. (2006), proposed that the association relationship between measured biomarkers and the related dose is defined by a linear function. To implement this method, the following steps have to be implemented:

- (1) To derive synthetic biomarker data based on distributions of exposure and PBTK parameters,
- (2) To run in the forward mode the coupled exposure-PBTK model using these distributions, and
- (3) To invert the distribution of output (i.e. the synthetic biomarker levels) to derive an "ECF."

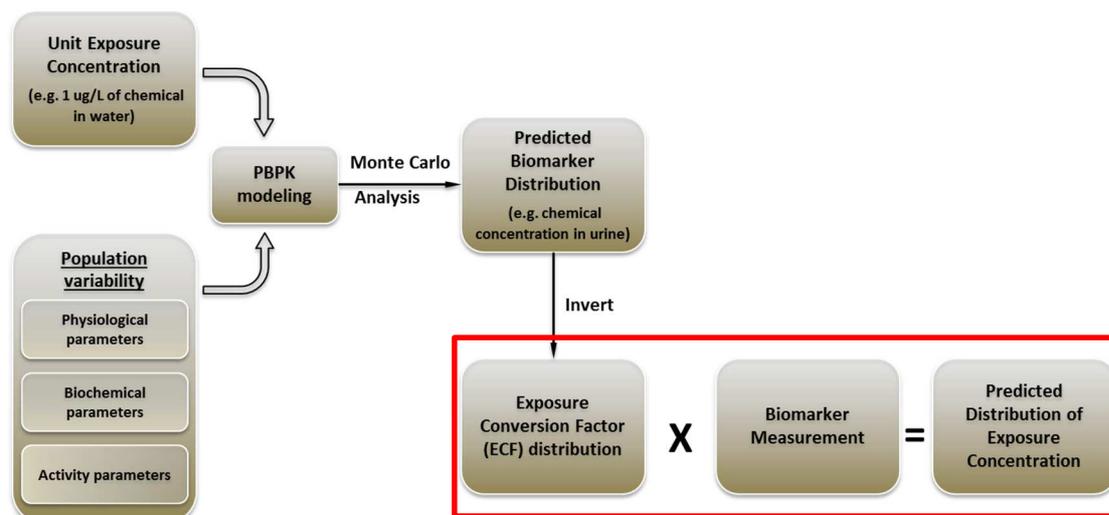


Figure 3: ECF framework. The linear function based on formula $y=ax$ is illustrated inside the red box. Where α is the exposure Conversion Factor (ECF) distribution, x is the biomarker measurement and y is the Predicted Distribution of Exposure Concentration (Tan et al., 2006).

Using the ECF and the distribution of observed biomarkers, the possible exposures for that particular biomarker distribution can then be estimated through a straightforward convolution (Tan et al., 2006). In practice, the PBTK model can be run forward using a given intake rate range, coupled with ranges of exposure modifiers, PBTK parameters and relevant biomarker sample times, in order to produce sets of synthetic biomarker data. After that, the distribution of biomarkers for a unit exposure metric is derived for inversion and calculation of the respective ECF. The ECF can then be multiplied by the values of available biomonitoring data (e.g. the ones that will be available within HBM4EU) so as to derive intake estimates. Although this method is relatively simple pretty straightforward, ECF estimates depend upon the characteristics of the prior distributions. Finally, this method cannot be used in the case of complex exposure scenarios, or when multiple biosamples have been collected for a given exposure regime.

3.2.2.2 Bayesian approach

3.2.2.2.1 General Bayesian approach

The stochastic methods aim to provide a reasonable solution. A probabilistic framework for inverse computation problem is the Bayesian approach which is based on Bayes theorem:

$$p(x | y) = \frac{p(y | x)p(x)}{\int p(y | x)p(x) dx}$$

Where x is the possible exposure and y is the amount of the biomarker $P(x)$ which is the available prior information. The relationship between x and y and inherently the relationship between the prior and theoretical knowledge is given by

$$p(x | y').$$

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Moreover,

$$P_{theory}(y | x) = P_{model}(y | x)$$

$$p(y | x) = P_{model}(y | x)$$

The posterior distribution of the biomarker measurements will be

$$P_{inferred}(x | y')$$

and

$$P_{prior}(x | y') = P_{inferred}(x | y')P_{prior}(y')$$

Hence taken into account,

$$P_{prior}(x) = \int P_{prior}(x | y) dy = P_{prior}(x) \int P_{theory}(y | x) dy$$

Therefore,

$$P_{posterior}(x | y') = \frac{P_{theory}(y' | x)P_{prior}(x)}{\int P_{theory}(y' | x)P_{prior}(x) dx}$$

And

$$P_{theory}(y | x) = \int P_{error}(y | m)P_{model}(m | x) dm$$

Where $P_{error}(y | m)$ is the probability of measuring y when the true value is m .

Therefore,

$$p(x | y) = \frac{p(x) \int P_{error}(y | m)P_{model}(m | x) dm}{\int p(x) dx \int P_{error}(y | m)P_{model}(m | x) dm}$$

Bayesian Markov Chain Monte Carlo (MCMC) has been used to for the exposure reconstruction of intakes in combination with PBTK (McNally et al., 2014). Holmes et al. (2000) applied genetic algorithms on PBTK models for biokinetic of nicotine to optimise the parameters of the model. Also, fast equivalent operational models (FEOMs) such as the deconvolution technique has been used (Sparacino et al., 2002) for exposure reconstruction in a model combined with PBTK.

3.2.2.2 Bayesian Markov Chain Monte Carlo

Markov Chain Monte Carlo techniques are numerical approximation algorithms. They originated in statistical physics and they were used in Bayesian inference to sample from probability distributions by constructing Markov chains. In Bayesian inference, the target distribution of each Markov chain is a marginal posterior distribution. Each Markov chain begins with an initial value and the algorithm attempting to maximise the logarithm of the unnormalised joint posterior distribution and eventually arriving at each target distribution by multiple iterations. Each iteration is considered a state. A Markov chain is a random process

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with a finite state-space where the next state depends only on the current state, not on the past one.

The implemented methodologies are based on Bayesian Markov Chain Monte Carlo (Gelman and Rubin, 1996; Gilks et al., 1996). The method requires defining the prior distributions, the biomonitoring data, as well as a likelihood function defining the likelihood of the data given a set of forward model parameters. The MCMC approach takes into account an acceptance criterion that considers the likelihood of the data given parameters. Also, the MCMC samples using algorithms are based on Metropolis Hastings (M-H) or on differential evolution.

Several studies have used MCMC techniques combined with PBTK models for inverse modeling (Andra et al., 2015; Chen et al., 2010; Georgopoulos et al., 2009; Lyons et al., 2008; McNally et al., 2012; McNally et al., 2014; Sarigiannis et al., 2016)

3.2.2.2.1 Metropolis Hastings (M-H)

Metropolis Hastings is the sampling algorithm of choice for the MCMC method in this work. Given a target density F that is associated with a working conditional density $q(y|x)$, a Markov kernel K is created with stationary distribution F and according this kernel a Markov chain $(X(t))$ is generated. The limiting distribution of the Markov chain is F and integrals can be approximated according to the Ergodic Theorem. M-H is used for deriving and constructing of a kernel K that is associated with an arbitrary density F (Robert and Casella, 2010). Thus, the proposed distribution typically depends on the current sample and the acceptance of the sample depends on the criteria of M-H. Then, the acceptance of the samples leads the samples to be the next element in the chain, otherwise the previous element is added again in the chain.

The acceptance probability is calculated according the following ratio:

$$a(X | X^{(t-1)}) = \min \left\{ 1, \frac{f(X)q(X^{(t-1)} | X)}{f(X^{(t-1)})q(X | X^{(t-1)})} \right\}$$

Where $q(X^{(t-1)}|X)$ is the Gaussian proposal density and $q(X|X^{(t-1)})$ its equal symmetric, $F(X)$ and $F(X^{(t-1)})$ are the calculated values for the probabilities for the current and for the candidate point. It has to be mentioned that the Metropolis sampler must have symmetric proposed distributions because the use of Markov Chain draws samples under the condition of reversibility (Robert and Casella, 2010).

The process ends when the chain has converged to its stationary distribution or enough samples have been collected in order to perform the desired statistical analysis. The chain is expected to eventually converge to the stationary distribution, which is also the target distribution but typically requires a burn-in period. The burn-in period is the number of iterations that have to be performed before the collected samples. The determination of convergence is based on the diagnostics of the Gelman-Rubin technique (Gelman and Rubin, 1992) that examines multiple MCMC chains by dividing each chain up into batches and by examining the variance between the chains.

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The sampling techniques and the generation of the proposed samples used on calculation are determined by a particular permutation of the update mode, the adaptive proposal and the delay reduction.

3.2.2.2.2 Update mode

The update mode is based on multivariate as well as on component-wise sampling.

A multivariate sampling proposal allows to each iteration the generation of proposed distributions that take into account the correlation from a multivariate normal distribution and from a proposed covariance matrix (Genz and Bretz, 2009; Roberts and Rosenthal, 2009). Hence, multivariate normal sampling proposes the generation of a sample by drawing from a multivariate normal distribution with dimension equal to the number of parameters, mean equal to the previous sample and a covariance matrix determined either by a previously converged chain, or by the computed covariance matrix of the sample chains gathered so far in the run.

Component-wise sampling proposals indicate that a proposal is made for each parameter without considering correlation and it has to evaluate the model a number of times equal to the number of parameters, per iteration. Hence, component-wise update mode samples only one parameter at a time, holding the other fixed (in a Gibbs sampling scheme). The proposed is a univariate normal with a mean equal to the last sample value and a standard deviation computed either by sampling the priors, or by adaptive tuning as the run progresses to achieve the desired acceptance rate.

3.2.2.2.3 Adaptive Proposal Variance

The adaptive MCMC algorithm corresponds to the case where a finite dimensional parameter θ depends on the whole history of the chain $(X_0, \dots, X_n, \theta_0, \dots, \theta_n)$ though in practice it is often the case that the pair process $[f(X_n; \theta_n); n > 0]$ is Markovian. The adaptive mode provides the ability of the sample to explore the parameter space and collect samples which are indicative of the target distribution. The acceptance rate is determined by the variance used in the proposal distribution. The amount of the variance controls the size of the steps between points and also it has influence to time of exploration of the parameter space. An effective proposal distribution using a random walk Metropolis algorithm has been done using the Adaptive Proposal Variance (Haario et al., 2001).

3.2.2.2.4 Delayed Rejection

The Metropolis – Hastings algorithm can be improved by the delaying rejection mechanism (Tierney, 1994) in that the resulting estimates have, uniformly, a smaller asymptotic variance on a sweep by sweep basis. When a Markov chain retains the same position over subsequent time and a candidate sample generated from the rejected proposal sample, the estimates obtained by averaging along the chain trajectory become less efficient. The solution to that problem is the reduction of the number of rejected proposals based on Mira (2001) methodology. In particular, when a sample is rejected by the Metropolis-Hastings criteria, delaying rejection technique generates a new proposal sample with smaller variance. Thus, delayed rejection is a technique wherein if a sample is rejected when applying the

Metropolis-Hastings criteria, another sample is immediately generated by using a proposal with a smaller variance. If this second sample is accepted, it is appended to the chain instead of a repeat of the previous sample. This technique provides the generation of well-mixed chains at the expense of more evaluations of the likelihood on each MCMC iteration. Moreover, it can be used as an alternative technique in case strong correlations exist between the parameters.

3.2.2.2.5 MCMC algorithms

The goal of MCMC is to design a Markov chain such that the stationary distribution of the chain is exactly the distribution that we are interested in sampling from. The combination of the sampling technique settings leads to existing Metropolis Hasting techniques. Table 1 presents the available MCMC algorithms based on Metropolis Hasting sampling that can be used.

Table 1: MCMC algorithms based on Metropolis Hasting

MCMC algorithms	Update mode:	Reference
Delayed Rejection Metropolis (DRM)	Multivariate	(Mira, 2001)
Delayed Rejection Adaptive Metropolis (DRAM)	Multivariate	(Haario et al., 2006)
Adaptive Metropolis(AM)	Multivariate	(Haario et al., 2001)
Componentwise Metropolis (CHM)	Component-wise	(Haario et al., 2005)
Random-Walk Metropolis (RWM)	Component-wise	(Gilks and Roberts, 1996)

The Delayed Rejection Metropolis (DRM or DR) algorithm is a Random-Walk Metropolis (RWM) (Mira, 2001). Whenever a proposal is rejected, the DRM selects one or more alternate proposals and corrects for the probability of this conditional acceptance. The delaying rejection enforces the decreased autocorrelation in the chains and the algorithm is encouraged to move. The additional calculations increase the computational cost of each iteration of the algorithm in which the first set of proposals is rejected, but the major benefit is the faster convergence to the optimal solution.

The Delayed Rejection Adaptive Metropolis (DRAM) algorithm is merely the combination of both Delayed Rejection Metropolis (DRM) and Adaptive Metropolis (AM) (Haario et al., 2006). DRAM has been demonstrated to be robust in extreme situations where DRM or AM fail separately. Haario et al. (2006) present an example involving ordinary differential equations in which least squares could not find a stable solution, and DRAM did well.

The Adaptive Metropolis (AM) algorithm of Haario et al. (2001) is an extension of Random-Walk Metropolis (RWM) that adapts based on the observed covariance matrix from the history of the chains. The algorithm is specified under adaptation and periodicity. Thus, the beginning of the iteration and the frequency in the periodicity in adaption have to be set. The

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adaption has to be controlled and immediate adaption has to be avoided since the algorithm is based on the observed covariance matrix of historical and accepted samples. Hence, a valid covariance matrix before adaptation has to be composed with a large number of samples. However, at the beginning of the algorithm, a small covariance matrix is commonly used to encourage a high acceptance rate.

The Component-wise Metropolis (CHM) is based on the Single Component Adaptive Metropolis (SCAM) that has been developed by Haario et al. (2005) and on the single component Metropolis – Hasting algorithm. In the SCAM the adaption is performed component by component. The chain is no more Markovian, but it remains ergodic. The SCAM can be used in many moderately high dimensional problems. Also, the algorithm does not need detailed prior knowledge of the target distribution and it can be used in numerous problems typically solved using pre-runs and hand tuning (Haario et al., 2005). Also, the algorithm resembles basic single component Metropolis algorithm with Gaussian proposal distributions, the only exception being that the variances of the one-dimensional proposal distributions depend on time and the variance is computed by a simple recursive formula. Moreover, in high dimension the updating of the proposal distribution performed demands only computations of component-wise variances. Hence, the additional computation brought in by the adaptiveness is negligible. Additionally, component-wise proposals usually indicate that a proposal is made for each parameter, without considering correlation. In case that parameters are correlated, the problem of the distribution is faced with the rotation of the proposal distribution. Thus, the covariance matrix of the chain is computed and the principal vector direction is determined and it is used as sampling directions in the SCAM-algorithm. After the burn-in period of the algorithm, the proposal direction is fixed and the sampling is continued by only updating the size of the one-dimensional Gaussian proposal distribution. Hence, the SCAM is characterised as a fully automatic algorithm. SCAM is widely applicable and general-purpose algorithm. It is appropriate to be performed to models with a small to medium number of parameters since the proposal covariance matrix grows with the number of parameters and the computation cost simultaneous increases.

The random walk algorithm of Metropolis is known to be an effective Markov chain Monte Carlo method for many diverse problems (Metropolis et al., 1953). The proposed Random-Walk Metropolis (RWM) is a multivariate extension of Metropolis-within-Gibbs (MWG) (Gilks and Roberts, 1996). RWM is an algorithm where the initials specification is not necessary though blockwise sampling. In fact RWM is a generic algorithm to draw a sample from a d-dimensional target distribution from a probability density function. The optimal scale of the proposal covariance is based on the asymptotic limit of infinite-dimensional Gaussian target distributions that are independent and identically-distributed (Gelman et al., 1996). In case of multiple parameters the existence of correlations occurrences is very common. Hence, MCMC algorithms attempt to estimate multivariate proposals from a multivariate normal distribution taking into account correlations through the covariance matrix. The convergence of the algorithm is related with the proposal density. A small variance leads to slowly converge and conversely, if the variance is too large, the Metropolis algorithm will reject too high a proportion of its proposed moves (Roberts et al., 1997).

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3.2.2.2.3 Differential Evolution Monte Carlo

Differential Evolution (DE) is a genetic algorithm for numerical global optimisation. It is a population Markov Chain Monte Carlo algorithm, in which parallel run for several chains is applied (Ter Braak, 2006). The combination of DE and MCMC is called Differential Evolution Monte Carlo (DEMC) and the field has been explored among others by Liang and Wong (2001), Liang (2002) and Laskey and Myers (2003). DEMC provides solutions to the choosing and the orientation of the jumping of the distribution that is an important practical problem in random walk Metropolis. In fact DEMC algorithm is based on a Metropolis Hasting and it is combined with a genetic algorithm called Differential Evolution (DE) with multiple chains and each chain learn from another parallel chain. The crucial idea behind DE is an innovated generation of parameter vectors. DE adds a weighted difference vector between two population members in order to generate vectors. The vector yields an objective function value. Then the value is compared with the predetermined population and if the resulting value is lower than the existent, the new vector replaces the compared vector. Moreover, the evaluation of each generation can be done with the best parameter vector in order to retain track of progress during the minimisation process. The DE is described in detail by Storn and Price (1995, 1997) and the adaptation of DE in MCMC is described and proven by Ter Braak (2006).

3.2.2.2.3.1 DEMC algorithms

The applied DEMC algorithm suggested for us is based on the Ter Braak (2006) algorithm.

Table 2: MCMC algorithms based on Differential Evolution method

MCMC algorithms	Update mode:	Reference
Differential Evolution Monte Carlo (DEMC)	Multivariate	(Ter Braak, 2006)

DEMC is similar with Metropolis-within-Gibbs (MWG) but the main difference consists in that DEMC updates by chain. The algorithm is specified under the number of chain that should be at least three and the thinning factor. The thinning factor provides the reduction of storage requirements and enhances the convergence of the chain to posterior distribution. In particular, the sampling is realised randomly and without replacement from a possibly thinned chain. Moreover, an adaptive step size can be used (ter Braak and Vrugt, 2008) with the same contribution as it has been described to section 3.2.2.2.2. Also the snooker update fraction (Gilks et al., 1994; Liang and Wong, 2001; ter Braak and Vrugt, 2008) can be specified providing to the sampler the ability to update along each coordinate axis in turn one axis at a time, with the specificity that this axis does not need to run parallel to the coordinate axes. Finally, it can be set the randomly uniform offset distribution that added to the creation of the DEMC proposal distribution.

3.2.3 Methodology in HBM4EU and selected algorithm

The exposure reconstruction approach to be applied in the HBM4EU methodology and computational platform relies upon the concept initially described by Georgopoulos et al. (2009). The analysis of the exposure reconstruction problems based on the MCMC and DEMC technique is realised according to the following steps:

1. The process starts from exposure related data which are fed into the exposure model;
2. This in turn provides input to the PBTK model, taking into account the duration and the magnitude of exposure from all the exposure routes (inhalation, skin and oral route);
3. The result of the PBTK model simulation (taking also into account the distribution of PBTK parameters, e.g. inter-individual variability in clearance), is then evaluated against the human biomonitoring data distributions. Based on the outcome of the comparison, the optimisation algorithm changes the exposure model input parameters following each iteration, so as to achieve the convergence to biomonitoring data;
4. More detailed information on exposure parameters reduces uncertainty in back-calculating doses from biomarker information, resulting in faster and more efficient convergence;
5. Several iterations are repeated, until minimising the error between the predicted and the actual biomonitored data.

The framework shown in Figure 4 is not limited to exposure reconstruction. It can also be used for estimating distributions of physiological and biochemical PBTK model parameters (under well-defined exposure conditions) for individuals and populations that are consistent with available biomarker data (typically study-specific data where exposures are adequately characterised) by combining the data with prior estimates of the parameters.

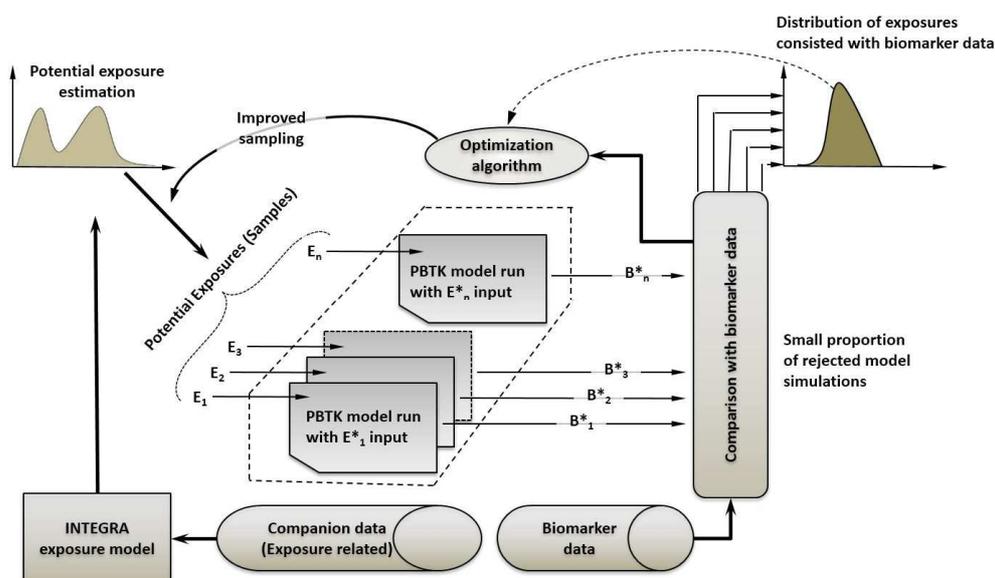


Figure 4: Optimisation-aided exposure reconstruction based on HBM data using time-evolving PBTK models (figure adapted from Georgopoulos et al. (2009))

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The Bayesian Markov Chain Monte Carlo technique described above simulates and calculates the investigated exposure conditions. The sampling is set appropriately according to the problem and to the available data for the proposal function. The flowchart diagram of the whole process is shown in Figure 5.

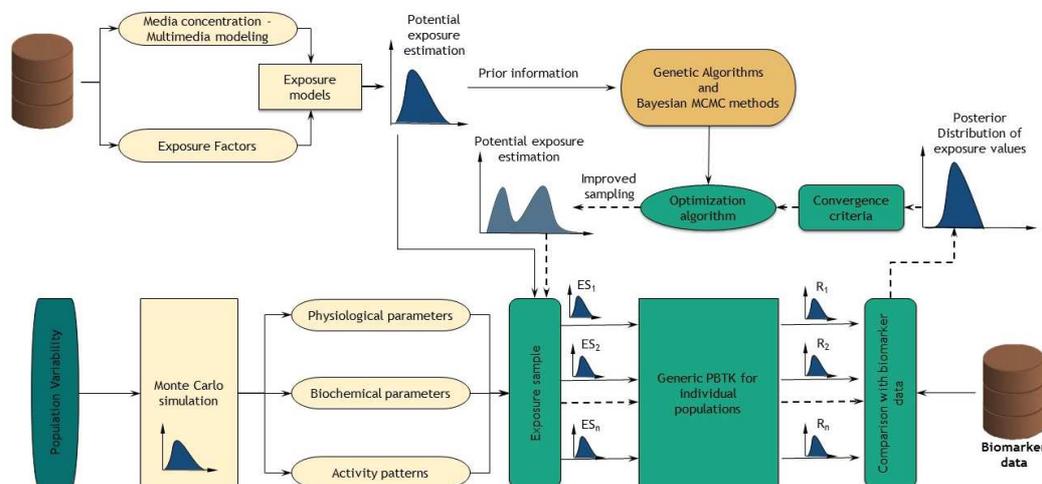


Figure 5: Exposure reconstruction, flowchart procedure

The model has been developed in acsIX[®]. The user can choose between component wise or multivariate update mode. The adaptive mode as well as the delay rejection can be set in the M functions.

3.3 Demonstration of the methodology for two priority substances using synthetic and real data

3.3.1 Average daily intake exposure reconstruction starting from synthetic HBM data (spot urine samples)

In order to validate the efficiency of the exposure reconstruction algorithm, the methodology described above has been applied for BPA and DEHP under 2 realistic exposure scenarios for both chemical substances. Based on the realistic exposure scenarios (from bottom up intake estimates), the time course of metabolites has been calculated. The aim was to reconstruct the exposure scenario, based on a hypothetical spot sample of the first morning void (7:00 a.m.). The scenarios were investigated using both the MCMC algorithm with RWM and the DEMC algorithm. The algorithms had a burn-in period of 50 iterations. The total number of iteration was set to 1000.

3.3.1.1 Exposure to BPA – single route exposure

Bisphenol A (BPA) (4,4'-(propane-2,2-diyl)diphenol) is one of the chemicals with the highest industrial production volume worldwide (Bailin et al., 2008). The major volume of BPA is used for the production of polycarbonate plastics as well as a basic component in production of the epoxy resin (Vandenberg et al., 2009). Various common consumer products contain or are made using polycarbonate plastics such as household electronics and baby bottles (Liao and Kannan, 2011). Epoxy resin is used in the majority of food and beverage cans (Erickson, 2008). Moreover, BPA is commonly used in paper industry and particularly as color

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developer in thermal and copy paper (Biedermann et al., 2010; Liao and Kannan, 2011; Mendum et al., 2011; Viñas et al., 2012). The first scenario referring to BPA is common for adult general population that is exposed to BPA 3 times per day, mainly through diet. The exposure scenario includes three oral doses (dietary exposure) during breakfast at 7:00 AM (dose 1), lunch at 2:00 PM (dose 2) and dinner at 7:00 PM (dose 3). The intake doses have been set to 14, 28 and 14 ug respectively. In addition, the exposure dose boundaries for the prior distributions were ranging between 10 and 40 ug. The prior knowledge of the distribution of the exposure time is based on the actual dietary schedule of the general population.

The generic PBTK model developed by Sarigiannis et al. (2016) was parameterised based on literature data (Edginton and Ritter, 2009). Biomonitoring data from first morning urine void were supposed to be sampled and used for the analysis. The biomonitoring data referred to BPA-glucuronide, a common type of metabolite quickly formed by liver metabolism, which is the only metabolite that has been detected in urine and blood after controlled exposure (Matthews et al., 2001; Völkel et al., 2002). The total time of the iteration was 885 seconds. The results of the exposure reconstruction (Figure 6 (a), (b) and (c)) show that the posterior distributions predict very well the actual exposure doses. Moreover, the posterior distributions have a reduced standard deviation and a mean value closer to the real. However, the MCMC model using the prior knowledge cannot achieve a posterior distribution with a sufficient confidence interval (CI) for actual exposure value. The dose 2 and 3 appear a better prediction and a high frequency of the predictive closer to the actual exposure value. The use of the DEMC algorithm demonstrated that the predictions have a similar behavior compared to the MCMC (Figure 7 (a), (b) and (c)).

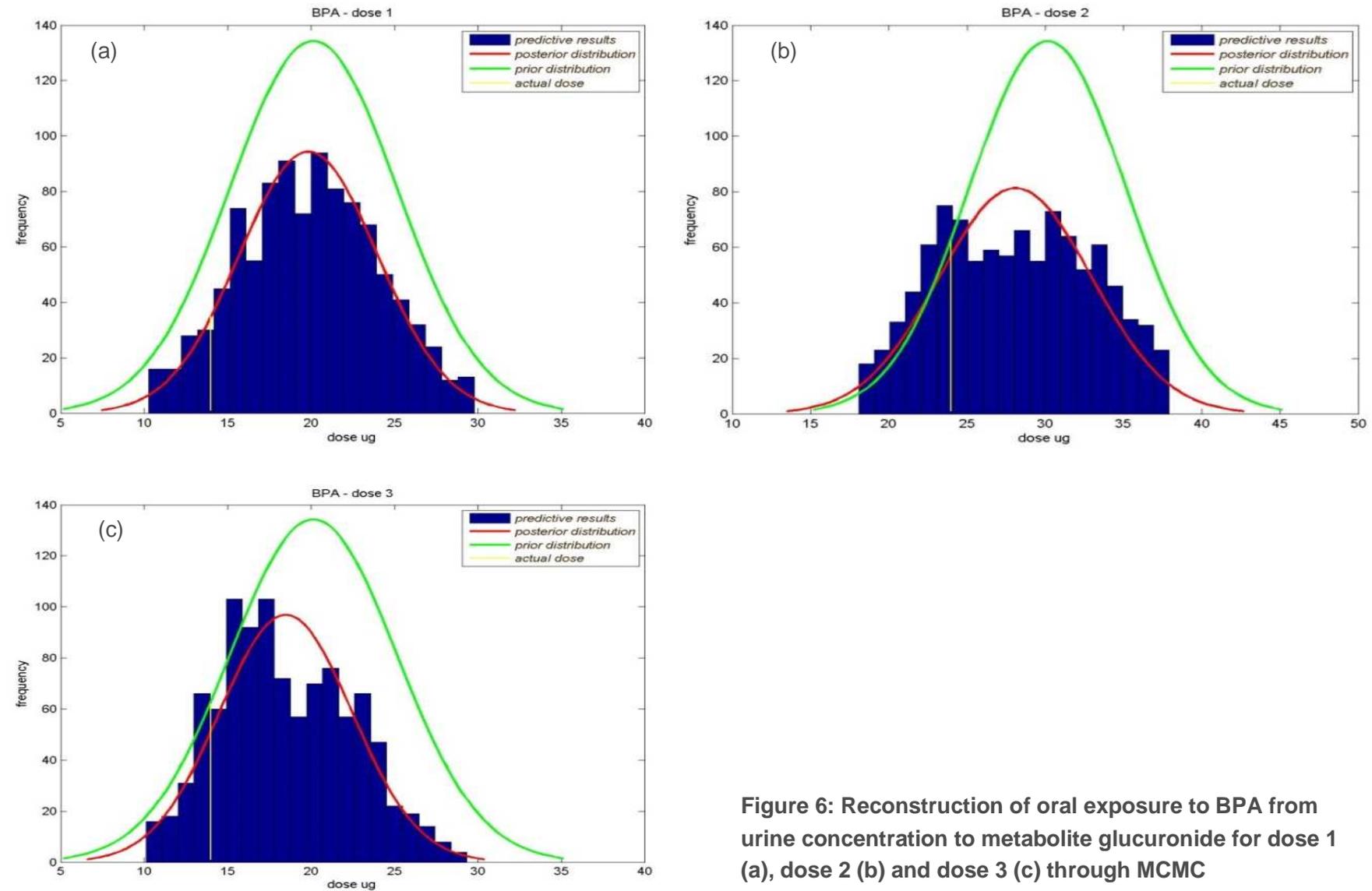


Figure 6: Reconstruction of oral exposure to BPA from urine concentration to metabolite glucuronide for dose 1 (a), dose 2 (b) and dose 3 (c) through MCMC

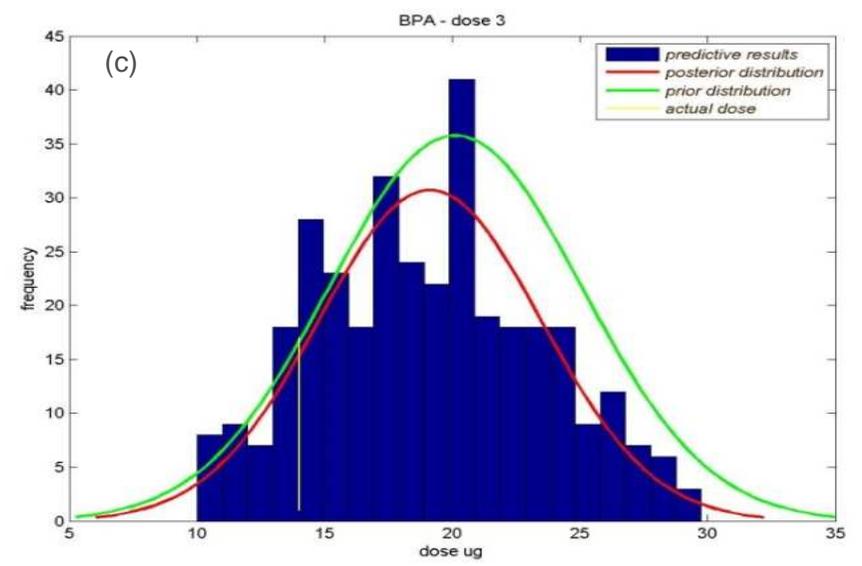
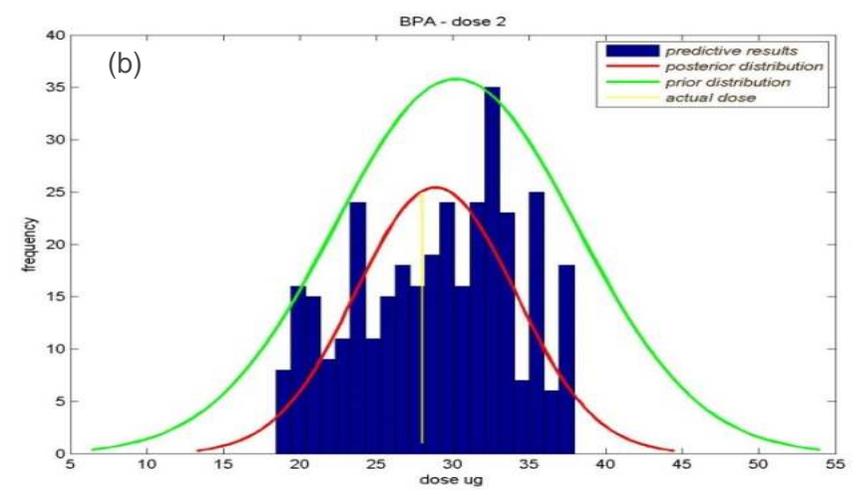
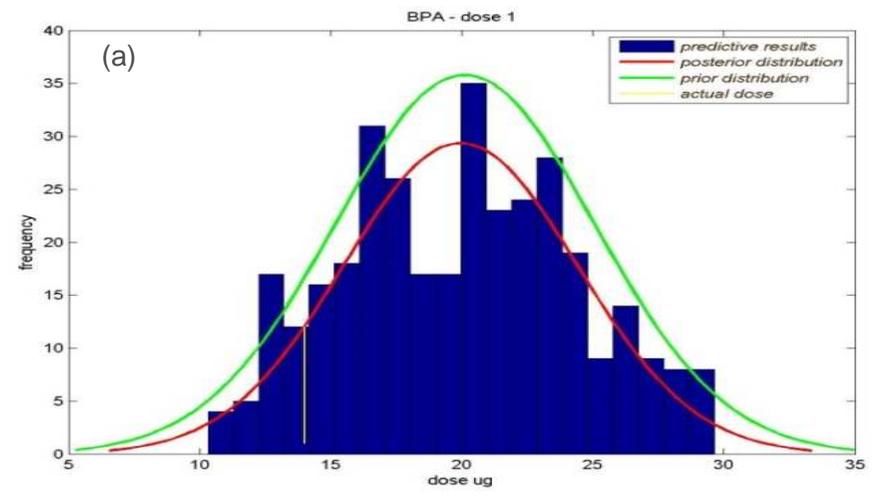


Figure 7: Reconstruction of oral exposure to BPA from urine concentration to metabolite glucuronide for dose 1 (a), dose 2 (b) and dose 3 (c) through DEMC

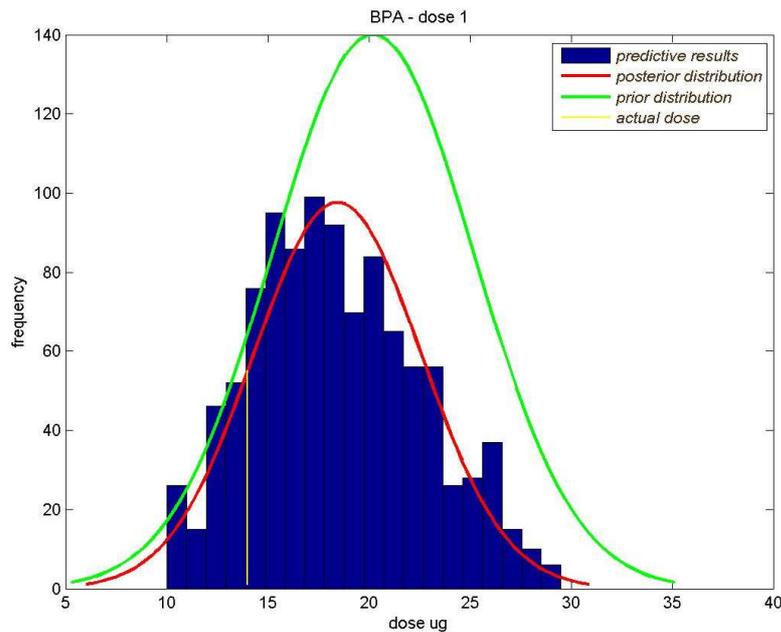


Figure 8: Reconstruction of oral exposure to BPA from urine concentration to metabolite glucuronide – dose 1 – DEMC (using one additional urine sample taken at 17:00)

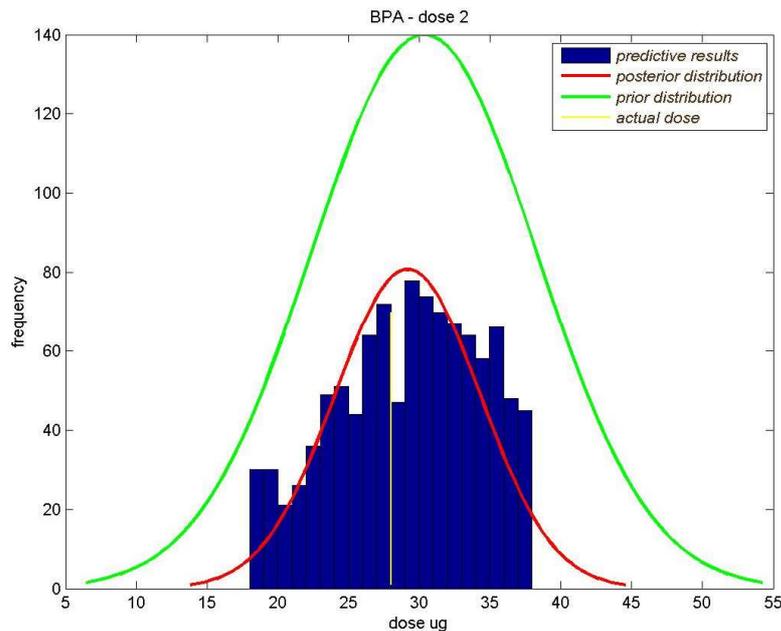


Figure 9: Reconstruction of oral exposure to BPA from urine concentration to metabolite glucuronide – dose 2 – DEMC (using one additional urine sample taken at 17:00)

The same scenario has been tested using synthetic biomonitoring data of two different spot sample measurements through the DEMC algorithm. The first one taken at 10:00 AM that is 3 hours after the first dietary exposure while the second one has been kept the same at 7:00 AM.

A third simulation was realised for exposure reconstruction of BPA using the DEMC algorithm. The biomonitoring data used in the simulation were increased by adding one sampling point between the 2nd and the 3rd exposure at 17:00. The simulation results of that the reconstructed distributions

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of the 1st and 2nd exposure were improved. This is due to the fact that the new distances of the prior information of the time exposure from the available biomonitoring data were shorter than the first simulation, as well as the new data point gave a better determination of the exposure scenario. Hence, the new information between the second and third exposure gave the ability to the Metropolis Hasting algorithm to create a better set of covariance matrix, which in turn resulted in improved predictions according to the prior information. The comparison of **Fehler! Verweisquelle konnte nicht gefunden werden.** (a) with Figure 8 as well as of **Fehler! Verweisquelle konnte nicht gefunden werden.** (b) with Figure 9 confirmed that addition of biomonitoring data enhanced the prediction capability.

The predictive result distribution of the histograms shows that density of the results is near to the actual exposure. Moreover, the mean value of the distribution of the second dose in Figure 9 is almost identical with the mean value of the posterior distribution.

3.3.1.2 Exposure to DEHP – exposure from two exposure routes

DEHP (Bis(2-ethylhexyl) phthalate) is plastic-softening phthalate of widespread use that is used to enhance the flexibility of rigid polyvinylchloride (PVC) (Lorz et al., 2007). The content of DEHP in flexible polymer materials varies but is often around 30% (w/w) (Program, 1982). The DEHP can migrate, leach, or evaporate into indoor air and atmosphere from building materials, daily and common used products such clothes, accessories, toys and also inside automobiles from plasticized components leading to exposure of phthalate esters via ingestion, inhalation and dermal pathways (Becker et al., 2004; Fromme et al., 2007; Wormuth et al., 2006). Moreover, among 25 different phthalate esters DEHP is the most common used in the production of medical devices (Tickner et al., 2001) such blood bags and dialysis equipment. However, human exposure to DEHP is age and lifestyle dependent (Franco et al., 2007). DEHP is mainly used in PVC products profiles and hoses as well as in film, wall- and roof covering and flooring. Hence, the wide use of DEHP gives rise to many possible scenarios of human exposure.

The scenario from where the synthetic biomonitoring data has been investigated refers to:

- non-dietary oral exposure from dust (hand to mouth behavior), accounting for DEHP dust concentration with prior distribution N (400, 70) ug/g dust and range between 200 and 800.
- exposure via inhalation, with a prior distribution of N (3.5, 0.7) ug/kg/day, ranging between 1 and 6.

The generic PBTK model developed by Sarigiannis et al. (2016) was parameterised and validated based on the toxicokinetic data from Cahill et al. (2003) and Lorber et al. (2010). The biomonitoring data used in this example refer to morning measurements of urine samples, referring to one major DEHP metabolite, namely MEHP.

The MCMC analysis showed that the posterior distributions include the actual exposure doses via inhalation and oral route. Moreover, the posterior distribution has a reduced standard deviation and a mean value close to the actual exposure value even though in case of inhalation exposure the movement of the mean value toward the actual one is not at a sufficient level. The results obtained applying the DEMC algorithm show how the actual exposure level is very close to the posterior distribution. In addition, the density of the predictive results from the DEMC shows a peak close to the actual exposure. The results are presented in Figure 12 and Figure 13.

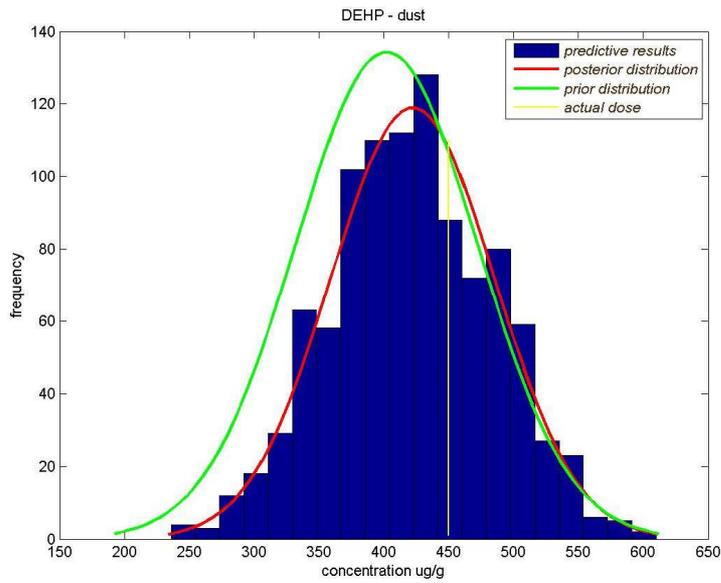


Figure 10: Reconstruction of dust exposure to DEHP from urine concentration to metabolite MEHP – dust - MCMC

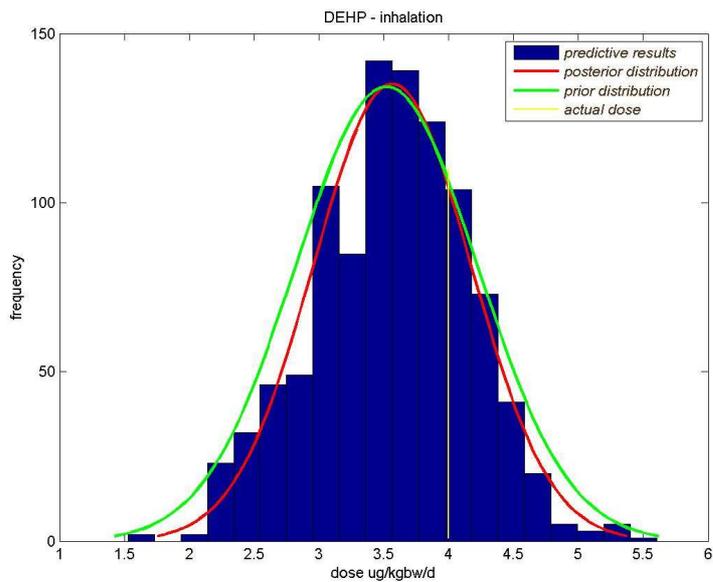


Figure 11: Reconstruction of inhalation exposure to DEHP from urine concentration to metabolite MEHP – inhalation – MCMC

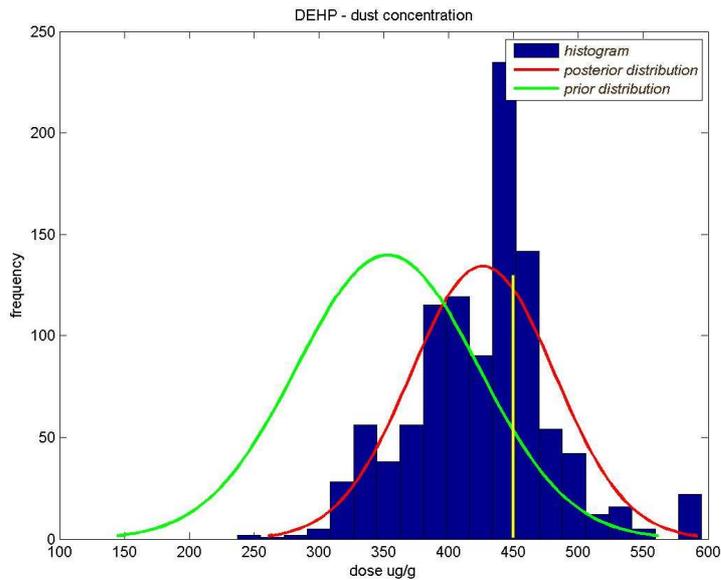


Figure 12: Reconstruction of inhalation exposure to DEHP from urine concentration to metabolite MEHP – inhalation - DEMC

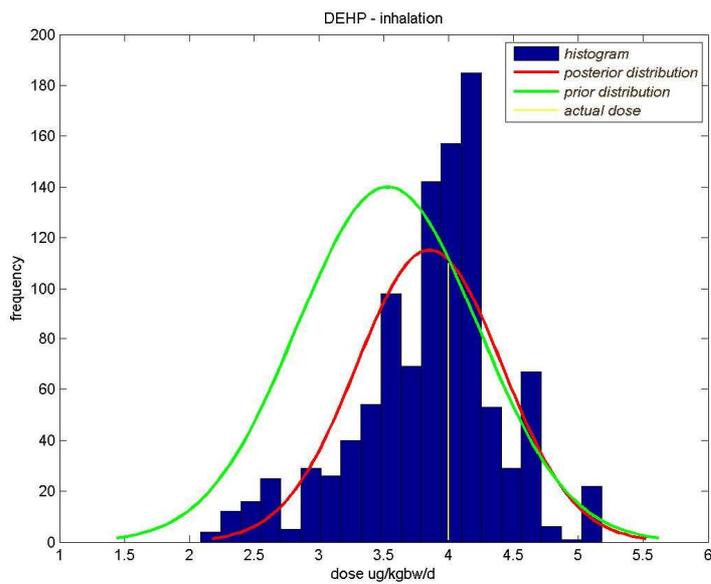


Figure 13: Reconstruction of inhalation exposure to DEHP from urine concentration to metabolite MEHP – inhalation – DEMC

3.3.2 Reconstruction of timely variable exposure from multiple samples of individualised HBM data of bisphenol A

In this modelling exercise, the aim was to investigate the capability of the model to simulate complex exposure events within the day, evaluating multiple HBM data. And, to identify the timing and the magnitude of exposure under more complex and randomised dietary schedule and intake doses within the day, using the data collected from a single individual who had a typical dietary menu, that included some variant sources of BPA (drinking beverages from plastic bottles, canned food consumption and others).

Similarly, diurnal exposure to bisphenol A through food and drink items was estimated starting from the urinary biomonitoring data. The model performed very well and the predicted time course of the urinary predicted concentrations fitted very well the actual measured data. The results indicated that overall daily exposure to bisphenol A remained below 0.1 $\mu\text{g}/\text{kg}_{\text{bw}}/\text{d}$, while internal dose of free plasma bisphenol A was in the range of few pg/L (Figure 14). It has to be noted that these data refer to a specific individual, that his urine samples were collected intentionally in purpose of testing the exposure reconstruction model and, that population HBM data used for exposure reconstruction to BPA exposure are presented in the next chapter of this report.

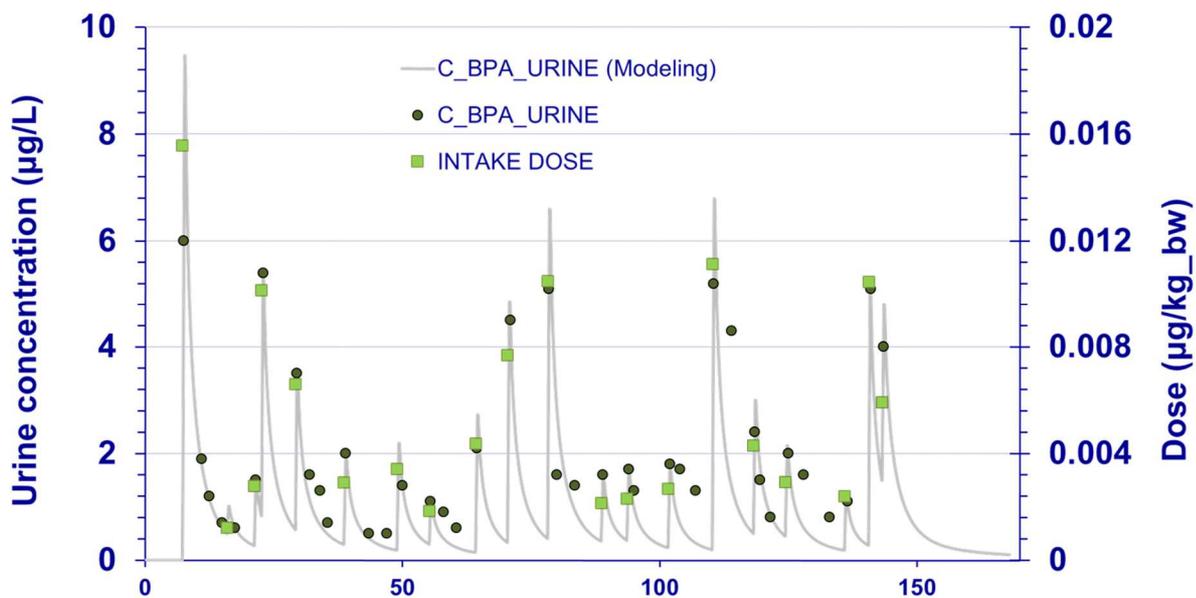


Figure 14: Measured (black dots) and modelled (grey line) urinary bisphenol A levels, and predicted dose (green dots)

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4 Assimilation of HBM data from existing cohorts

4.1 Compounds included in the assessment and scenario development

4.1.1 Overall approach

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, with regard to compounds included in the 1st set of priority substances for HBM4EU project. The applied methodology relies upon the approach developed and described by Sarigiannis et al. (2016). To this aim the generic model was adapted to the computation and methodological needs making use of advanced Quantitative Structure-Activity Relationship (QSAR) models (Papadaki et al., 2017; Sarigiannis et al., 2017) to properly parametrise it for the chemicals under investigation.

Due to the lack of suitable and complete exposure data the 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, available in the HEALS and the CROME projects. 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. When additional data will be available from HBM4EU WP5 and WP10, additional simulations will be carried on the basis of these data.

The methodology was applied to the chemicals presented in *Table 3*. Results were obtained for BDE-47, p,p'-DDT, HCB, cadmium, bisphenol A, as reported in the following chapters.

Table 3: Chemical substances tested by exposure reconstruction framework

a/a	IUPAC Name	Chemical name	CAS-number	1 st set of priority substances for HBM4EU
1	2,2',4,4'-Tetrabromodiphenyl ether	BDE 47	5436-43-1	Brominated flame retardants
2	Hexachlorobenzene	HCB	118-74-1	Pesticides mixture
3	Dichlorodiphenyltrichloroethanes	p,p'-DDT	50-29-3	Pesticides mixture
4	Cadmium	Cd		Metals
5	Bis(2-ethylhexyl) phthalate	DEHP	117-81-7	Phthalates
6	Bis(7-methyloctyl) benzene-1,2-dicarboxylate	DiNP	28553-12-0	Phthalates
7	Benzylbutylphthalate	BBzP	85-68-7	Phthalates
10	4,4'-(propane-2,2-diyl)diphenol	Bisphenol-A		Bisphenols

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4.1.2 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.

4.1.3 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.

4.2 Exposure reconstruction and target dose estimation

4.2.1 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 through 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 PBTK simulation for BDE-47 was performed based on scenario 2 for the Valencia population¹. Results show that in steady state condition, at the age of 30 years, the BDE-47 concentration in uterus has a median value of 0.2 (0.1 - 0.8) ug/L.

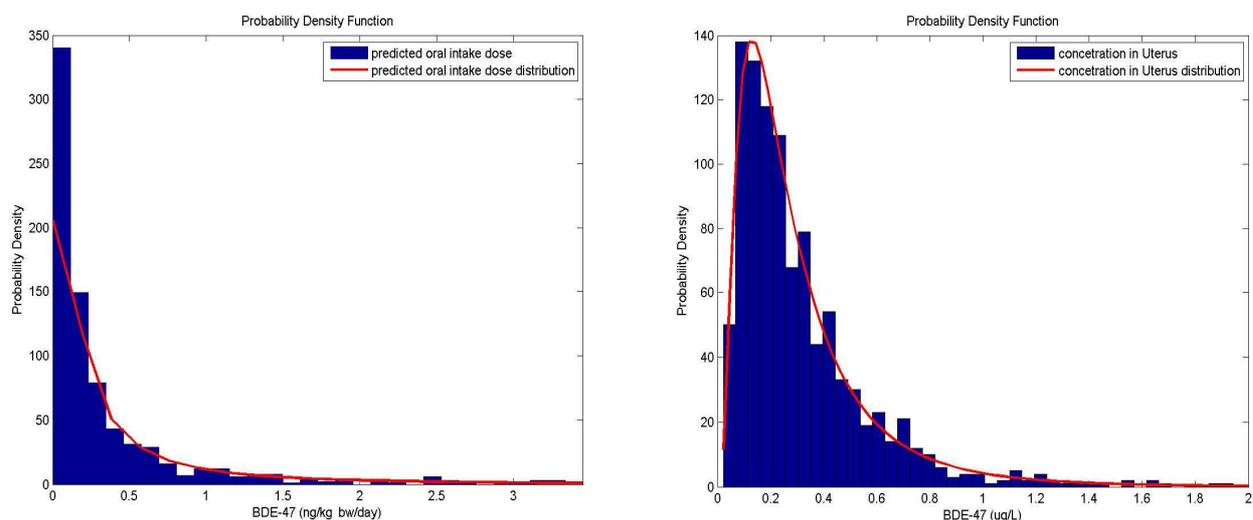


Figure 15: BDE-47 – Valencia (Spain) population – left: predicted oral intake dose. Right: predicted concentration in uterus

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

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4.2.2 Mixture of pesticides

At the moment, data on pesticides are available for hexachlorobenzene (HCB) which 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 uterus through the placenta. Workers can be exposed to organochlorines pesticides in the manufacture, formulation, or application of these chemicals.

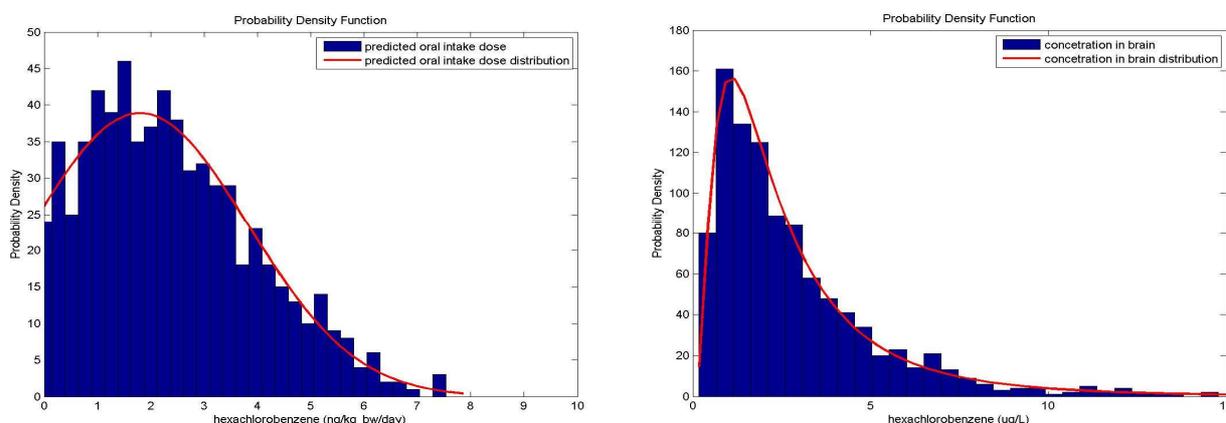


Figure 16: HCB – Valencia (Spain) population – left: predicted oral intake dose. Right: Predicted concentration in brain

Chronic exposure studies in animals have demonstrated kidney injury, immunologic abnormalities, reproductive and developmental toxicities, and liver and thyroid cancers (ATSDR, 2002). In humans, very high, acute doses produce central nervous system depression and seizures. Therefore, PBTK 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 16).

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 is possibly an endocrine disruptor (ATSDR, 2002). 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 population². 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 17).

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

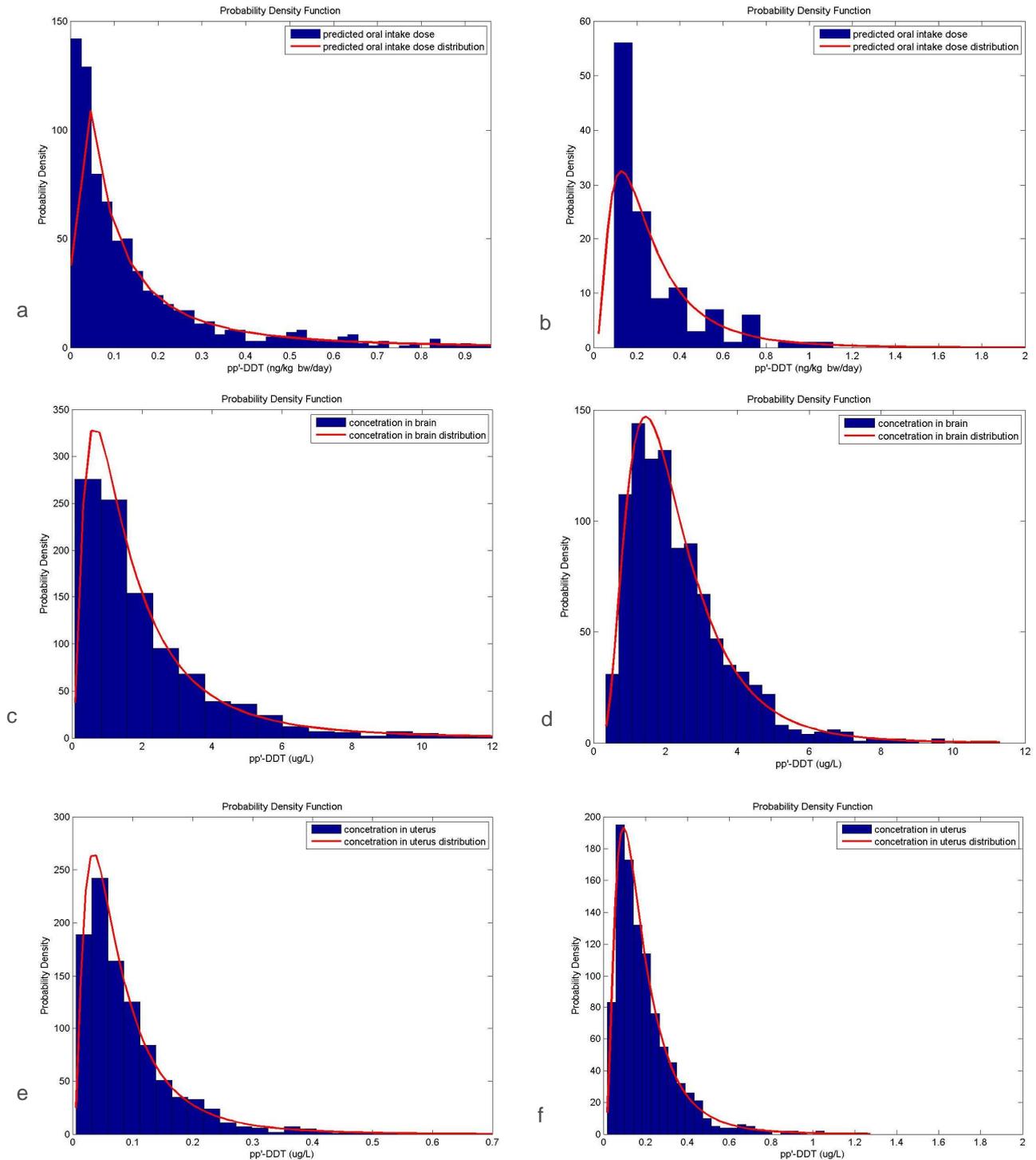


Figure 17: pp'-DDT

- a) predicted oral intake dose for Valencia (Spain) population,**
- b) predicted oral intake dose for Menorca (Spain) population,**
- 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**

4.2.3 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. Less 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). In addition, chronic cadmium inhalation is also suspected to be a possible cause of lung cancer (Sorahan and Esmen, 2004). PBTK simulations for Cadmium were based on scenario 2, for Spain population. The results of the simulations are illustrated in Figure 18.

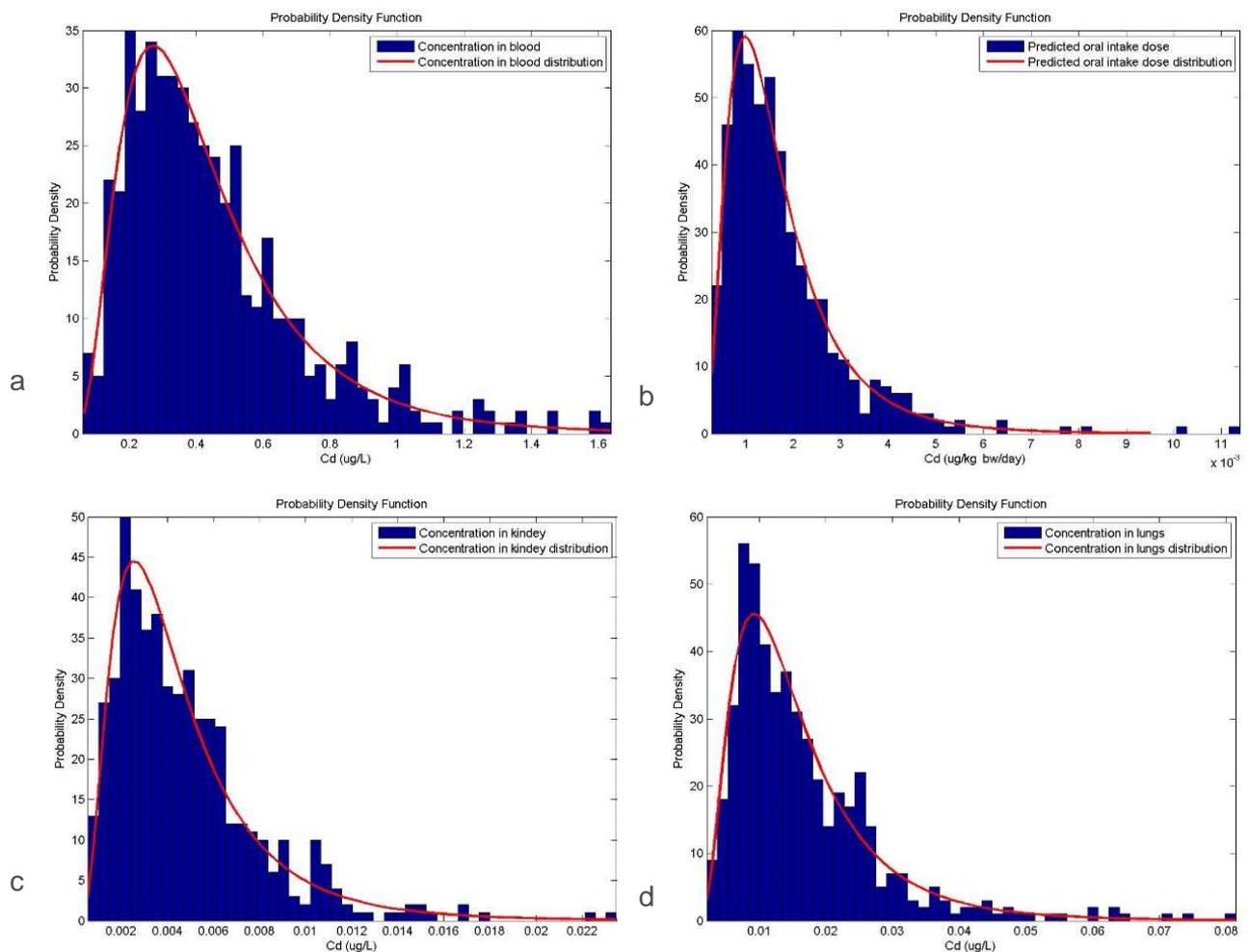


Figure 18: Cadmium

- a) concentration in blood,**
- b) predicted oral intake dose,**
- c) calculated concentration in kidney,**
- d) calculated concentration in lungs**

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4.2.4 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 particularly 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).

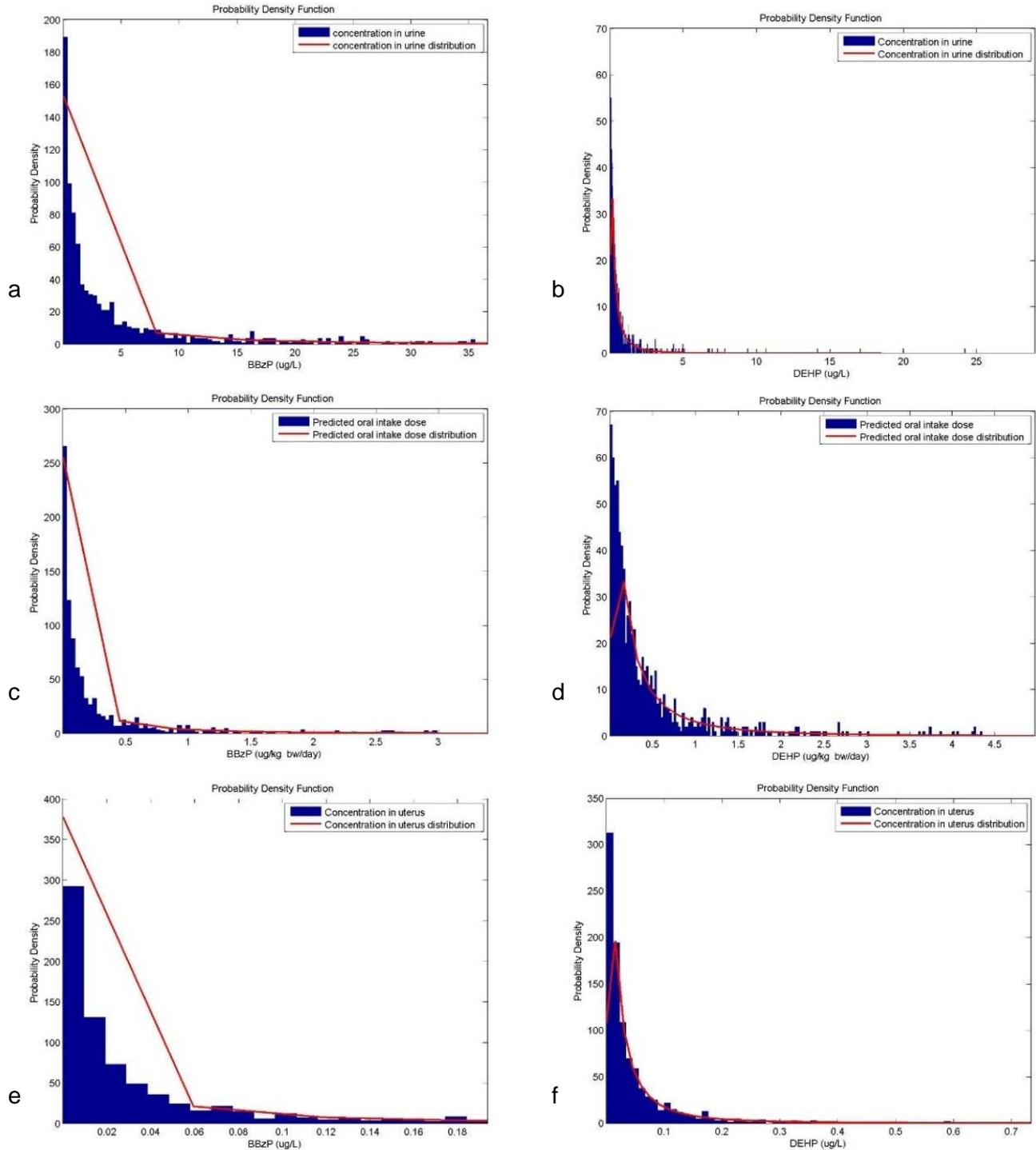


Figure 19:

- 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

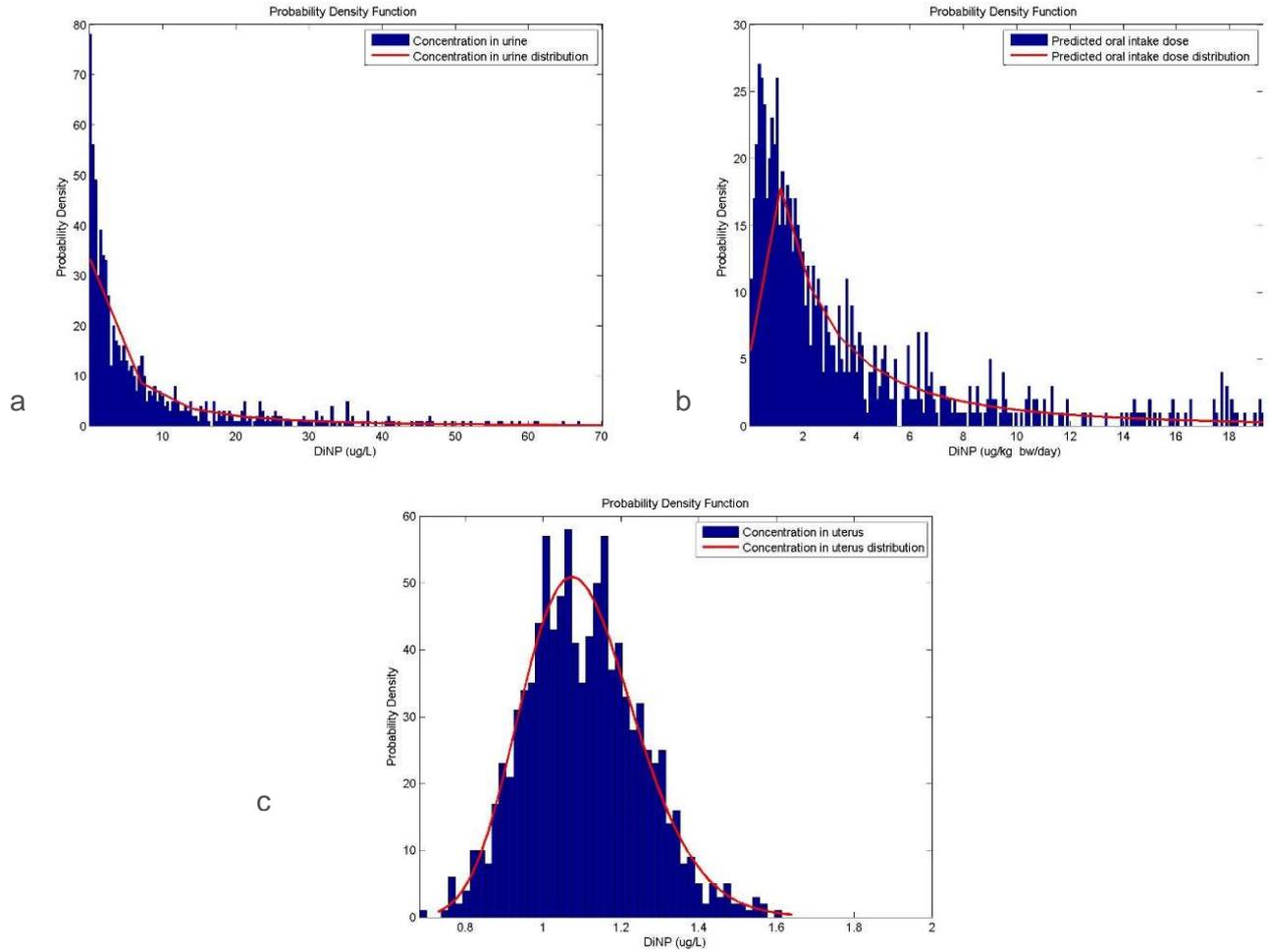


Figure 20:

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

4.2.5 BPA

Bisphenol A (BPA) is one of the highest industrial volume chemicals produced worldwide (Bailin et al., 2008). The major volume of BPA is used for the production of polycarbonate plastic as well as a basic component in production of the epoxy resin (Vandenberg et al., 2009). Various common consumer products contain or are made by polycarbonate plastic such as household electronics and baby bottles (Liao and Kannan, 2011). Epoxy resin is used in the majority of food and beverage cans (Erickson, 2008). Moreover, BPA is commonly used in paper industry and particularly as color developer in thermal and copy paper (Biedermann et al., 2010; Liao and Kannan, 2011; Mendum et al., 2011; Viñas et al., 2012). Also, BPA has been found in thermal paper of sale receipts (Biedermann et al., 2010) and banknote (Liao and Kannan, 2011).

BPA is characterised as an estrogen characterised by endocrine disrupting activities that are mediated via multiple molecular mechanisms (Alonso-Magdalena et al., 2006; Bouskine et al., 2009). Additionally, recent studies have examined the neurotoxicity of BPA, highlighting that even low maternal exposure to BPA is associated to neurodevelopmental defects (Gioiosa et al., 2007; Moser, 2011; Palanza et al., 2008; Tian et al., 2010).

BPA simulations were based on scenario 2, for Italian and Slovenian population. Simulation results showed that in steady state condition and at the age of 30 years, BPA concentration levels in skin and in lung are about 3 times higher for the population of Italy with respect to the Slovenian one.

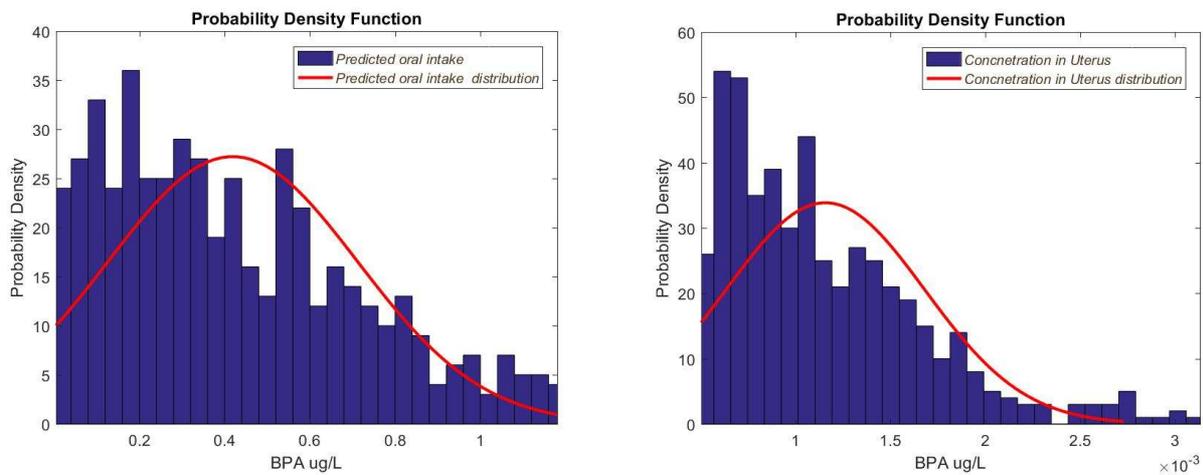


Figure 21: BPA - Slovenia population – left: predicted oral intake dose. Right: predicted concentration in uterus

4.2.6 Summary of results

The statistical summary of the concentrations of simulated chemical in the various target organs is reported in Table 4.

Table 4: Summary statistics 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	BDE47	Uterus	1.3	0.7	0.2	0.1	0.8	Spain (Valencia)
2	HCB	Brain	3.1	1.1	2.1	0.6	7.7	Spain (Valencia)
3	Cadmium	Lung	0.015	0.01	0.0135	0.008	0.05	Slovenia
4	Cadmium	Kidney	0.003	0.004	0.004	0	0.01	Slovenia
5	ppDDT	Uterus	0.12	0.05	0.1	0.0	0.3	Spain (Valencia)
6	ppDDT	Kidneys	0.21	0.08	0.2	0.0	0.8	Spain (Menorca)
7	ppDDT	Brain	3.2	1.1	2.0	0.8	5.0	Spain (Menorca)
8	ppDDT	Brain	2.1	0.9	1.5	0.3	6.2	Spain (Valencia)
9	DEHP	Uterus	0.1	0.2	0.05	0	0.4	Poland (Lodz)
10	DiNP	Uterus	1.1	0.2	1	0.8	1.4	Poland (Lodz)
11	BBzP	Uterus	0.1	0.6	0	0.01	1.0	Poland (Lodz)
12	BPA	Uterus	0.0001	0.00005	0.00008	0.00001	0.0008	Slovenia

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