AN INTEGRATED TOOL FOR RISK ASSESSMENT FOR INDUSTRIAL CHEMICALS – THE CASE OF BISPHENOL A

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ABSTRACT
The INTEGRA project (funded by CEFIC-LRI) provides the computational platform that integrates environmental fate, exposure and internal dose dynamically in time aiming to advance risk assessment from the “exposure-based” risk assessment era. Beyond the multimedia environmental and aggregate exposure modelling framework, a major methodological component of INTEGRA is the incorporated generic Physiology Based BioKinetic (PBBK) model. The anthropometric parameters of the models are age dependent covering the whole lifespan from the moment of conception, so as to provide a lifetime internal dose assessment. The PBBK model is geared with reverse modeling algorithms in order to reconstruct exposure from human biomonitoring (HBM) data. Probabilistic techniques are used to validate the exposure outcome on the basis of actual environmental and population biomonitoring data. At the individual level, the PBBK model is combined with multimedia models and survey questionnaires to identify exposure sources, used as ancillary information, aiming to predict exposure magnitude and eventually the timing of exposure events. The generic model was applied for the assessment of a highly controversial industrial chemical with widespread applicability in consumer goods, namely bisphenol-A (BPA). Exposure scenarios were built based on an extensive literature review of BPA exposure data.

In order to associate the risk of the several exposure scenarios based on the Biologically Effective Dose (BED) derived by the PBBK model, two different exposure metrics were used, including both the conventional techniques based on Tolerable Daily Intake (TDI) of 50 μg/kg_bw/d proposed by the European Food Safety Authority, as well as to recent advances of in vitro High Throughput Screening (HTS) based on the ToxCast assays. For the majority of scenarios, the estimated internal dose was close to 0.002 μg/L and only in the case of bottle fed infants, internal exposure concentrations were up to 0.023 μg/L. This is partially explained by the neonates immaturity of the detoxification pathway, resulting to higher internal doses for the same bodyweight normalized dose compared to children older than 1 year old or adults. In addition, exposure to BPA was reconstructed based on real-life HBM data. Average urine BPA-Glu was 2.8 μg/L across Europe, covering different age groups, using an average urine BPA-Glu equal to 2.8 μg/L. The results indicated that the overall daily intake is below 1 μg/kg_bw/d and the estimated internal dose was close to 0.002 μg/L, far below any internal dose derived reference value, corresponding to the lower estimates of the already considered exposure scenarios. The only scenario of concern was the one related to premature neonates hosted in intensive care units. However, the risk characterization ratio depends on the threshold considered. If in vitro data are used, all exposure scenarios are considered as safe.

INTRODUCTION
Exposure to chemical agents originates either from environmental contamination (air, water, soil, transfer through food chain), or from consumer products (food contact materials, construction materials, cosmetics, clothes, etc.) through multiple routes, namely inhalation, ingestion and dermal contact. Aggregate exposure, i.e. the quantitative exposure assessment to a single agent from all potential exposure pathways (the physical course taken by an agent as it moves from a source to a point of contact with a person) and the related exposure routes, poses specific questions that need to be addressed. In particular, contamination sources need to be identified and contamination of different environmental media contamination estimated taking into account multi-media exchange. Human exposure needs to be reckoned based on media concentrations and contact duration and exposure mechanisms (pathways and relevant routes) need to be identified. Based on the temporal variation of exposure and accounting for the contribution of different exposure routes the internal dose in target tissue(s) needs to be calculated. The distribution of exposure to the wider population or specific susceptible groups (e.g. infants) has to be computed and apportioned to each source or possible exposure patterns when biological indices of exposure (biomarkers) are measured (reverse modelling/exposure reconstruction). Finally, available biomonitoring data either can be compared with regulatory thresholds using the concept of biomonitoring equivalent to the reference dose, or might be used for exposure reconstruction.
The current study aims to provide a description of the methodology developed in INTEGRA, applied to a chemical of wide scientific and regulatory interest. Moreover, different levels of available prior information and model complexity will be investigated, in order to determine the advantages of using a comprehensive methodology and the respective computational framework for assessing exposure at across different scales, down to human tissue concentration and the respective limitations.

**METHODOLOGY**

**Main methodological concept**

Apparently, refined aggregate exposure assessment is data-intensive, requiring detailed information at every step of the source-to-dose pathway. Based on the needs described above, the objective of INTEGRA is to bring together all available information within a coherent methodological framework for assessing the source-to-dose continuum for the entire life cycle of substances covering an extensive chemical space. Hence, the major component of INTEGRA is an integrative computational platform that integrates environmental fate, exposure and internal dose dynamically in time. A conceptual representation of the INTEGRA methodology is graphically illustrated in Figure 1. The platform will be largely validated using human biomonitoring data from Europe and the USA.

The INTEGRA computational platform incorporates the following major components:

1. Incorporation of ART (and its dermal exposure-integrated version, DART) for assessing occupational exposure, coupled to a generic PK model for linking exposure to internal dosimetry and estimating total body burden
2. Development of a multimedia model to account for multi-scale interactions affecting the environmental transport and fate of chemicals
3. Development of indoor micro-environmental modelling and detailed personal exposure assessment
4. Development of a generic PBBK model so as to incorporate life stage changes and physiological and metabolic efficiency change over an individual’s lifetime (from conception till 80 years of age). The model is able to cover perinatal exposure including exposure routes such as lactation, being practically a mother-fetus interaction model. Advanced QSAR models will be used to estimate physicochemical and biochemical parameters of the model in order to allow it to cover a large chemical space.
5. Inverse modelling for exposure reconstruction and HBM data assimilation.
6. Framework for probabilistic exposure assessment based on Markov Chain Monte Carlo simulation aiming to extend point estimates to population-relevant assessment based on Bayesian statistics.

**Conceptualization of the generic PBBK model**

The model is designed to describe as much as possible the actual ADME processes occurring in human body, so as to be easily applicable for a broad variety of chemicals under proper parameterization. The model includes the parent compound and a number of three potential metabolites. For each compound/metabolite all major organs is included and the link among the compounds and the metabolites is through the metabolizing tissues. This is mainly the liver, but also other sites of metabolism might be considered based on the presence or not of the
enzymes involved in the metabolism of the compound of interest. Both phase I and phase II metabolism are described. Both membrane and blood flow limited processes are included. In order to capture the in-utero exposure, the model is also replicated in order to describe the functional interaction of the mother and the developing fetus through the placenta. The anthropometric parameters of the models are age dependent covering the whole lifespan from the moment of conception, so as to provide a lifetime internal dose assessment [1].

![Figure 2. Conceptual representation of the Mother-Fetus PBBK model](image)

**Reverse Dosimetry Model**

The PBBK model is geared with reverse modeling algorithms in order to reconstruct exposure from human biomonitoring (HBM) data. A tiered approach is followed as a function of data availability (periodicity and size of sampling, specimen type) and requirements of the exposure reconstruction analysis (temporal analysis of exposure, contribution from different routes), ranging from Exposure Conversion Factors (ECFs), up to Markov Chain Monte Carlo analysis. Probabilistic techniques are used to validate the exposure outcome on the basis of actual environmental and population biomonitoring 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 biomarkers. 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). At the individual level, the PBBK model is combined with multimedia models and survey questionnaires to identify exposure sources, used as ancillary information, aiming to predict exposure magnitude and eventually the timing of exposure events.

More in detail, a computational framework was developed based on Bayesian Markov Chain Monte Carlo (MCMC) combined with the generic Physiological Based Biokinetic (PBBK) model to perform 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.
MCMC simulation refers to a class of iterative simulations in which the random variables of interest are drawn from a sequence, or chain, of distributions that eventually converge to a stable posterior distribution. Moreover, Differential Evolution (DE) and MCMC algorithms have been combined to this problem for the first time. Differential Evolution Markov Chain is a population MCMC algorithm, in which multiple chains run in parallel. In fact DE is a simple genetic algorithm for numerical optimization in real parameter spaces. As a result, this combined computational framework speeds up the calculation and convergence, even for nearly collinear parameters and multimodal densities. The analysis of exposure reconstruction problems is based on the MCMC and DEMC technique and it has been carried out according to the following steps: The exposure models are posed to the PBBK models, simulating human exposure via inhalation, skin and ingestion. Then, the probability model is specified according to the prior parameter distributions, as well as the calculated and/or fixed distribution of the pharmacokinetic model parameters. The PBBK model has been combined with the Bayesian MCMC [2, 3] and DEMC [4] techniques in order to simulate and calculate the expected exposure. The model has been developed and applied in the acsILX version 3.0 software. The distribution plots and histograms are created using Matlab® 2014. Sampling is set appropriate according to the problem and the available data for the proposal function. The computer used in this study was an Intel(R) Core(TM) i7- 3537U CPU 2.00 GHz 4.00 GB RAM. The flowchart diagram of the procedure is demonstrated in Figure 3.

![Figure 3. Exposure reconstruction flowchart procedure](image)

The uncertainty of the results depends on the uncertainty of the prior distributions. Also, although that the estimation of the parameters’ distribution provides a mathematical and physiological fit of the model, it can lead to a non-negligible error and especially when it is assumed to be representative for all individuals’ parameters. It has to be noted that DEMC algorithm results in better predictions. Moreover, comparing the computation needs of the algorithms, the DEMC is 3 to 4 time slower than MCMC but the predictions are closer to the actual value because of parallel running of multiple chains. Also, computational needs increase in case of the existence of multiple exposure pathway(s) and mechanisms as well as, when a high frequency of exposure events has to be simulated. Larger amount of information is needed for rapidly metabolized and eliminated compounds. Hence, additional knowledge of the metabolite prior distribution (i.e. higher sampling frequency) could decrease the uncertainty of the reconstructed exposure estimates.

**Test case – Bisphenol-A**

The methodology was applied in the case of bisphenol-A (BPA), one of the most produced industrial volume chemicals produced worldwide [5]. 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 [6]. Various common consumer products contain or are made by polycarbonate plastic such as household electronics and baby bottles [7]. Epoxy resin is used in the majority of food and beverage cans [8]. Moreover, BPA is commonly used in paper industry and particularly as color developer in thermal and copy paper [7, 9-11]. Hence, BPA has been found in thermal paper of sale receipts [9] and money [7]. In particular, the amount of BPA in the thermal paper has a mean concentration of 13.3 g/kg [9]. BPA is characterized as an estrogen characterized by endocrine disrupting activities that are mediated via multiple molecular mechanisms [12, 13]. In addition, recent studies have examined the neurotoxicity of BPA, highlighting that even low maternal exposure to BPA is associated to neurodevelopmental defects [14-17].
In order to estimate population exposure to BPA, a comprehensive methodological scheme was followed. This included the acquisition of data related to the overall production of BPA, as well as the concentration of BPA found in several food items, 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 and drinking water. All plausible scenario combinations were investigated.

Exposure to BPA and its potential adverse health effects have raised a lot heated debate in the regulatory arena. The debate focuses mainly on the definition of actual toxicological reference doses for the substance and its actual toxicokinetic behaviour. Although BPA glucuronidation (the dominant detoxification mechanism) is complete and fast, due to the reduced metabolic capacity of infants-neonates, there is still ample room for internal exposure [18, 19]. The generic PBPK model developed and incorporated into the INTEGRA platform was parameterized so as to capture the complex biokinetics of BPA. Our model captures mother-fetus interactions. The first part of the model describes maternal physiology and includes specific sub-models for breast and uterus. The second part describes fetus physiology captured from conception onwards to early infancy. Both parts account for age-dependent physiological and metabolic changes continuously in time. Gestation is a period of continuous physiological change for the fetus as well as for the mother. They are both subject to altered cardiac output, intestinal absorption, pulmonary ventilation and renal excretion [20], following the overall change of maternal weight. Nevertheless, due to the rapid first pass metabolism of BPA, the change in these parameters has an almost negligible effect to the overall free plasma BPA concentration in maternal plasma.

The concept of biomonitoring equivalent (BE) was used to derive an internal reference dose for BPA. 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) [21]. 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 of the absorption to the systemic circulation related to the route of exposure. Since BPA is characterized by rapid clearance, all BPA entering during the day is excreted in urine. Thus, urine sampling of excreted BPA is representative for the overall daily intake (from all routes).

However, it fails to capture the history of internal exposure variability. Taking into account all the above, free plasma BPA was considered as the most suitable BE for BPA. The external exposure threshold taken into account for deriving the BE value was the EFSA Tolerable Daily Intake (TDI) value of 50 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 PBPK model described above. Based on these assumptions, the corresponding BE value was calculated equal to 0.16 μg/L free plasma BPA. A detailed description of the developed model is given by Sariyannis and Karakitsios [1].

In addition, 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 regulatory threshold such as the EFSA TDI (obtained from in vivo animal NOAEL extrapolation), we can use an in vitro threshold. In vitro, the ToxCast 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 (0.64 μM for Attagene Factorial cis ERE assay) [22, 23]. This concentration (145 μg/L) is 3 orders of magnitude higher than the equivalent derived from the EFSA TDI (0.16).

RESULTS
Exposure analysis using external exposure metrics (actual daily intake) was carried out through the use of probabilistic data (food residues) and detailed multimedia environmental modelling, taking into account actual emissions to the environment rather than default values based on the overall production volume and the relevant ERCs. In addition, exposure to BPA was reconstructed based on real-life HBM data. Average urine BPA-Glu based on several European studies, was 2.8 μg/L. In particular the algorithm has been tested under the assumption that the average amount of 2.8 μg/L BPA-Glu to human’s urine is the result of an ordinary adult dietary schedule that includes 3 different meals: i) breakfast at 7:00 am (dose 1), ii) lunch at 2:00 pm (dose 2) and iii) dinner at 7:00 pm (dose 3). The results of the exposure reconstruction algorithms after 1000 iterations converge to the available biomonitoring data. All the prior distribution have been moved through to the direction of the actual exposure dose (Figure 4). The results indicated that the overall daily intake is below 1 μg/kg bw/d, which is far below the Tolerable Daily Intake (TDI) of 50 μg/kg_bw/d proposed by the European Food Safety Authority (EFSA). In addition, exposure to BPA of premature neonates hosted in intensive care units was estimated as well based on biomonitoring data. Premature infants exposure to BPA was based on the biomonitoring data of Calafat et al. [24], where BPA geometric mean urinary concentration (30.3 μg/L, s.d. 5.2
μg/L) among premature infants undergoing intensive therapeutic medical interventions corresponding to an average daily exposure of about 7 μg/kg_bw.

The results of this refined type of analysis indicated that external exposure exposure for all consumer exposure scenarios (including premature neonates and bottle fed infants) is below EFSA TDI of 50 μg/kg_bw (Figure 5) and significantly lower compared to the ones from a basic exposure analysis which is based on worst case exposure estimates and environmental contamination based on ERCs and tonnage.

**Figure 4.** Reconstruction of oral exposure to BPA – dose 1 using two different algorithms (left side MCMC – right side DEMC)

**Figure 5.** Daily uptake under all plausible exposure scenario combinations. The reference dose is 50 μg/kg_bw/d (EFSA TDI).

Incorporation of internal dosimetry alters the overall exposure assessment outcome when age- and route-dependent differences are reflected in the actual biologically effective dose (BED). Thus, specific exposure scenarios such as premature infants hosted in neonate intensive care units that under might external exposure assessment appear to be safe, exceed the corresponding equivalent safety thresholds when more biological information is incorporated in the assessment. An additional exposure scenario where exposure assessment outcome was altered when age- and route-dependent differences in internal dose are taken into account, includes bottle-fed neonates/infants, mainly due to infant formula contamination from the baby bottle. Although bottle fed infants are exposed to BPA at levels below the EFSA TDI, the equivalent exposure is almost 3.5 times higher compared to the analysis made without accounting for age-dependent metabolic variance, reflecting the immaturity of the detoxification metabolic pathway [18].
Figure 6. Free plasma BPA under all plausible exposure scenario combinations using internal dosimetry metrics

For the majority of scenarios presented in Figure 6, the estimated internal dose was close to 0.002 μg/L and only in the case of bottle fed infants, internal exposure concentrations were up to 0.023 μg/L. As already mentioned, this is explained by the neonates immaturity of the detoxification pathway, resulting to higher internal doses for the same bodyweight normalized dose compared to children older than 1 year old or adults [18]. The biologically effective dose of the developing fetus during gestation is highly linked to the one in maternal blood. According to our model and based on a conservative exposure scenario for the mother (e.g. 5 μg/kg_bw/d), free plasma BPA in maternal blood is almost 0.006-0.007 μg/L, which is slightly higher than what expected for a non-pregnant woman (0.005 μg/L). Placental concentration is 0.0013 μg/L and the corresponding fetal concentration is 0.004-0.005 μg/L. These results are in complete agreement to the ones presented by the FAO/WHO [25] study. 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. Reconstruction of dose from the actual biomonitoring data, allows as to run the model in forward mode and to estimate the biologically effective dose through the target tissue. In that case the estimated internal dose was close to 0.003 μg/l, far below any internal dose derived reference value, corresponding to the lower estimates of the already considered exposure scenarios.

Using BPAD as the internal exposure reference value, the maximum derived internal exposure values of the worst-case exposure scenarios are 400 times lower to the BPAD, indicating that there is no reason for concern for individual or aggregate scenarios of BPA exposure. The free plasma BPA values derived by our methodology, are significantly lower than the ones derived by Judson et al [22]; this is the result of using a more advanced PBBK model, instead of using a simpler PK approach as done by Judson et al [22].

CONCLUSIONS

The study presented herein describes an integrated methodological framework for risk assessment of chemicals within an integrative computational platform that takes into account the required environmental and exposure related interactions at multiple scales. The methodology was tested in a largely controversial chemical, i.e. bisphenol A. The results of the study identified that for chemicals with widespread consumer applications, the contribution of environmental contamination to total exposure is overestimated when the assessment is based on rough estimations of environmental releases. On the contrary, actual risks might be underestimated for specific population groups (e.g. neonates and infants) if the assessment does not take into account the variability in internal exposure due to genetic, physiological and developmental factors. Biology- and physiology-based models are able to give the proper solution to this problem. Thus, assessing exposure at multiple scales across the source-to-dose continuum, needs to take into account the actual complexity of the environmental and biological/physiological processes that are critical to the proper description of the phenomena involved. This results in targeted interventions and consequently more cost efficient risk management. In addition, a comprehensive integrated exposure framework estimating tissue dosimetry for the various relevant exposure scenarios, could be of great use in exploiting the in vitro HTS results rapidly produced by ToxCast21, advancing thus both exposure science and toxicology towards serving the needs of risk assessment in the 21st century.
Coupling the modeling platform developed and outlined herein with the HTS assay results of ToxCast21 for a large number of compounds of different chemical families and enhancing the INTEGRA methodology to take into account combined exposure to multiple chemicals will be the next steps in our development work.

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REFERENCES