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INTEGRA: FROM GLOBAL SCALE CONTAMINATION TO TISSUE DOSE

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Abstract: The objective of the INTEGRA project is to bring together all information necessary for assessing the source-to-dose continuum over the entire life cycle of substances covering an extensive chemical space through the use of QSARs. The major outcome of INTEGRA is a comprehensive computational platform that integrates multimedia environmental and micro-environmental fate, exposure and internal dose within a dynamic framework in time. The platform allows multimedia interactions across different spatial scales, taking into account environmental releases and related processes at global, regional and local scale, up to the level of personal microenvironment. Coupling seamlessly exposure models with refined computational tools for internal dosimetry transforms risk assessment of environmental chemicals since it allows risk characterization to be based on internal dosimetry metrics. In this way high throughput system data such as the ones generated by Tox21 in vitro testing can be used. This opens the way towards a higher level of assessment that incorporates refined exposure (tissue dosimetry) and toxicity testing (Biological Pathway Altering Dose – BPAD) associated to environmental contamination at different scales. The applicability of INTEGRA was tested on bisphenol-A. Tier 1 assessment (environmental releases based on production volumes, worst case exposure estimates and a Tolerable Daily Intake of 50μg/kg bw/d) indicated that specific exposure scenarios (i.e. bottle fed neonates and premature infants hosted in intensive care units) are close to the legislative thresholds. The refined assessment incorporating probabilistic analysis, actual environmental release data, detailed consumer exposure modelling and the use of BPAD as risk characterization metric, resulted in increased margins of safety compared to conventional risk assessment.

Keywords: exposure; modelling platform; PBTK; Bisphenol A.

1 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 can be compared with
regulatory thresholds using the concept of biomonitoring equivalent to the reference dose.
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.

2 METHODOLOGY

2.1 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 will be a unified computational platform
that integrates environmental fate, exposure and internal dose dynamically in time. The platform will
be largely validated using human biomonitoring data from Europe and the USA.
The INTEGRA computational platform is based on the existing platform developed in the frame of the
CEFiC-LRI INTERA and TAGS projects extending it to incorporate several advances:
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. Refinement of the TAGS multimedia model to account for multi-scale interactions affecting the
environmental transport and fate of chemicals
3. Refinement of the TAGS/INTERA micro-environmental modeling for improved personal
exposure assessment
4. Refinement of the TAGS/INTERA generic PBTK 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 modeling for exposure reconstruction and HBM data assimilation.

Figure 1. Conceptual representation of INTEGRA methodology

The development of the INTEGRA computational platform started with a review of the latest models
and exposure assessment computational environments and databases available in the EU and the
USA. This computational environment includes a probabilistic or activity-based exposure scenario development module, a module for multimedia environmental fate modelling of chemicals including different scales where releases to the environment occur; an exposure modelling module, an internal exposure modelling module using a PBTK/D platform to translate chemical exposure into internal dose. This allows exposure reconstruction from biomonitoring data, enhancing the usefulness of INTEGRA. A Markov chain Monte Carlo simulation module is included, aiming to extend point estimates to population-relevant assessment based on Bayesian statistics. A conceptual representation of the INTEGRA methodology is graphically illustrated in Figure 1.

2.2 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 (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). Hence, BPA has been found in thermal paper of sale receipts (Biedermann et al., 2010) and money (Liao and Kannan, 2011). In particular, the amount of BPA in the thermal paper has a mean concentration of 13.3 g/kg (Biedermann et al., 2010). BPA is characterized as an estrogen characterized by endocrine disrupting activities that are mediated via multiple molecular mechanisms (Alonso-Magdalena et al., 2006; Bouskine et al., 2009). In addition, 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).

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 (Edginton and Ritter, 2009; Ginsberg and Rice, 2009). The generic PBTK 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 subjected to altered cardiac output, intestinal absorption, pulmonary ventilation and renal excretion (Beaudouin et al., 2010), 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) (Hays et al., 2007). 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. Thus, free plasma BPA was considered as the most descriptive 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,
ToxCast assays provided six ER agonist or binding AC50 values for BPA, ranging from 0.6 to 1.7 μM for Attagene Factorial cis ERE assay.

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) (Judson et al., 2010; Judson et al., 2011). Based on our refined PBTK model, this concentration (145 μg/l) is 3 orders of magnitude higher than the equivalent derived from the EFSA TDI (0.16).

3 RESULTS

The total amount of bisphenol-A manufactured within the EU, based upon submissions to CEFIG by the manufacturers, was estimated at approximately 1,100,000 tonnes/year. For the purposes of this assessment a representative EU consumption of bisphenol-A is estimated to be approximately 1,149,870 tonnes/year from producer and end user data. ERCs (Environmental Release Categories) defined by the technical guidance document of the REACH regulation were used (ECHA, 2010). The ERC defines the fraction of tonnage (tonnes per year) assigned to the region and the fraction used by largest consumer (local assessment), as well as default release estimates. The estimated environmental concentrations of BPA based on ERCs were very high compared to actual measured concentrations. Analogically high was the environmental contribution to the respective food related pathways (meat, milk, poultry).

As illustrated in Figure 2, environmental contribution through food web contributes very significantly to the overall exposure (approximately 14.5 μg/kg_bw/d) and the respective RCR (~0.3). Exposure through air is significantly lower (1.5 μg/kg_bw/d). By combining environmental contribution to consumer exposure scenarios, adults who have a diet based mostly on canned food and canned beverages have a rather low daily uptake (16.2 μg/kg_bw/d). The highest exposure is observed for premature infants hosted in neonates intensive care units (84 μg/kg_bw/d); this is the only consumer scenario exceeding the EFSA TDI. Female cashiers (exposed dermally to 1.2 μg/kg_bw/d due to thermal paper contact) who consume canned food (canned soup, canned fruits) and canned beverages would be expected to be exposed to higher amounts of BPA (8.7 μg/kg_bw/d) through consumer uses. Nevertheless, the exposure levels are significantly below EFSA TDI (Figure 2).

![Figure 2](image_url)

Figure 2. Route contribution for the most significant exposure scenarios plausible combinations (Tier 1). The reference dose is 50 μg/kg_bw/d (EFSA TDI).

Tier 2 analysis (Figure 3) refines exposure assessment 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
The results of the refined analysis indicate that exposure for all consumer exposure scenarios (including premature neonates and bottle fed infants) is below EFSA TDI and significantly lower compared to the ones from the Tier 1 analysis. Beyond the use of distributions for food residues, overall exposure is significantly lower due to the practically negligible contribution of the environmental component (oral and inhalation) compared to the unrealistic Tier 1 estimations based on ERCs and tonnage.

Figure 3. Daily uptake under all plausible exposure scenario combinations (Tier 2a). 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 exceed the corresponding equivalent safety thresholds. Additional exposure scenarios where exposure outcome changes when age- and route-dependent differences in internal dose are taken into account include 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, in this high tier analysis, the equivalent exposure is almost 3.5 times higher (or 2 times higher, depending on the accepted level of bioavailability differences) compared to the analysis made without accounting for age-dependent metabolic variance, reflecting the immaturity of the detoxification metabolic pathway.

Figure 4. Free plasma BPA under all plausible exposure scenario combinations (refined internal exposure analysis). The corresponding BE value is 0.16 μg/l.

The biologically effective dose of the 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 6E-3 – 7E-3 μg/L, which is slightly higher than what expected for a non-pregnant woman (5E-3 μg/L). Placental concentration is 13E-3 μg/L and
the corresponding fetal concentration is 4E-3 – 5E-3 µg/L. These results are in complete agreement to the ones presented by the FAO/WHO (2010) report. 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).

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 (2011), due to our use of a more advanced PBTK model (instead of using a simpler PK model as done by Judson et al).

Figure 5. Free plasma BPA in comparison to Biological Pathway Altering Dose (focus on early developmental stages)

4 CONCLUSIONS

The study presented herein describes an integrated methodological framework for risk assessment of chemicals within a unified 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


ABBREVIATIONS

AC50: Concentration at half maximal Activity
ART: Advanced REACH Tool
BPA: Bisphenol A
BPAD: Biological Pathway Altering Dose
BE: Biomonitoring Equivalent
BED: Biologically Effective Dose
DART: Dermal Advanced REACH Tool
ECETOC TRA: European Centre for Ecotoxicology and Toxicology of Chemicals Targeted Risk Assessment
EFSA: European Food Safety Authority
ERCs: Environmental Release Categories
FAO: Food and Agriculture Organization of the United Nations
INTEGRA: Integrated External and Internal Exposure Modelling Platform
INTERA: INTegrated Exposure for Risk Assessment in indoor environments
MLR: Minimal Risk Level MRL
NOAEL: No Observed Adverse Effect Level
PBTK: Physiologically Based ToxicoKinetic
PK: Pharmacokinetic
QSAR: Quantitative structure-activity relationship
RfC: Reference Concentration
RfD: Reference Dose
TAGS: Tiered AGgregate exposure to chemical substanceS
TDI: Tolerable Daily Intake
WHO: World Health Organization