Integrative Systems Toxicology for Human Health

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General discussion and conclusions

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Endocrine disrupting chemicals (EDCs) are natural or anthropogenic substances in environment, food, or consumer products that can disrupt hormonal balances in humans and wildlife, and result in adverse health effects even at low dosage. There are several challenges in quantitative prediction of the EDCs-induced adverse effects on human health associated with their complex exposure, non-linear kinetics, metabolite(s), and their complex mechanism or the complex responses of organisms over different life stage or time scales. The ECHA and the EFSA mainly focus on the development of tools, alternative to animal testing, to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. These involved development of novel in-vitro assays, in-silico tools and integrated assessment and testing approaches (IATAs) etc.

In-silico techniques have been receiving attention as alternative methods to classical approaches. Emerging high-throughput analysis, OMICS and tools such as PBPK, PD, Systems biology models and AOPs offer an opportunity to understand the chemical fate inside the body, the biological complexities and their multilevel connectivity. The successive use of the PBPK model in the field of toxicology is commendable since it offers the great advantage of predicting internal tissue dose of compounds or metabolites by utilizing the data derived from the in-vitro and in-silico tool such as QSAR, without any animal experiments. The PBPK also allows cross species extrapolation, cross-route dose interpolation, age and population specific without the need of experimental analysis. This is of great advantage to the field of the environmental toxicology enabling to test the large amount of the organic chemicals, reducing the cost and the time of analysis.

Many biological adverse effects emerge from perturbations of multiple signalling pathways. These signalling pathways involve nonlinear interactions consisting of many components which requires more information for their specification than linear interactions do, and it is hard to foresee what comes from nonlinear interactions. Systems biology suggests a solution to this problem – to reconstruct the biological behaviour in an in silico replica of the system. It is possible to reconstruct the biological emergence by translating the information about how components communicate into mathematical equations. By integrating and solving the resulting system of mathematical equations in a computer, one should be able to simulate the biological system's behaviour. Basically, with the use of Systems biology models we could be able to solve the complex biological system. In parallel, the concepts of AOPs has been developed and elaborated as an approach to predict the adverse effects of the chemicals. It presumes that the biological system's behaviour is the result of the events of the sequences of biological components' interactions. This approach was developed to reduce the inherent biological complexity where the knowledge/data are lacking. Integration of a wide range of in silico tools (QSAR, PBPK/PD, AOP, systems biology models etc.), and databases (OMICS,

epidemiological and exposure data) can directly tie the results into a predictive adverse outcomes model. This integrative approach would lead to mechanistic understanding of adverse effects vs conventional empirical end points and animal based testing.

The main objective of the thesis was to explore the use of the in-silico models such as PBPK, PD and Systems biology and to elaborate an integrated, harmonized and pragmatic methodology for the human health assessment mostly focusing on endocrine disrupting compounds (EDCs). Our case studies of integrative systems toxicology approaches were presented in 5 subsequent chapters, illustrating the potential of this innovative approach for human health risk assessment.

Chapter 1 explored the possible EDCs and their effects on human health based on their modes of action. Grouping of EDCs was proposed based on their target organs, receptors, and similar adverse outcomes. Chapter I also addressed the challenges in the quantitative risk assessment of such EDCs effects on human health such as multiple mechanisms of action, delayed responses (time lag between exposure to adverse outcomes), dynamic interactions involving crosstalk, and transgenerational effects. The transgenerational effects of EDCs were demonstrated via a case study of female fertility. The potential EDCs targets involved in life stage development from germ cell to zygote have been also identified. A conceptual model of PBPK/PD was proposed. This model involves an integrated risk assessment framework linking exposome-internal exposure-biological effect to the adverse outcome. This chapter showed the need for a dynamic model (AOPs or systems biology) in addition to the PBPK (a kinetic model) to have a complete and wider picture on predicting adverse effects of chemicals on biological systems.

Chapter 2 included the development and validation of a PBPK model for two different chemicals including their metabolite(s). The First part included di (2-ethylhexyl) phthalate (DEHP), and the second part included Flutamide. Both chemicals are categorized as non-persistent Endocrine disruptors' compounds. Two different approaches were used for the development of the PBPK model: 1) a bottom up approach and 2) a cross-species extrapolation.

DEHP is metabolized into a toxic compound, mono-(2-ethylhexyl) phthalate (MEHP) and other metabolites. In this chapter, the DEHP PBPK model including its major metabolites was developed using in-vitro metabolic data. The IVIVE (in-vitro in-vivo extrapolation) approach was used to translate the in-vitro metabolic data into their in-vivo counterpart. The tissue composition QSAR method was used to determine the distribution of the compounds and their metabolites. Both uncertainty and sensitivity analysis were performed. To our knowledge this was the first PBPK model developed with detailed metabolic kinetics. The model was validated against independent experimental data on chemical/metabolite(s) plasma and urine concentrations. The similarity of the model prediction to the experimental data showed that the integration of the data derived from the in-vitro and the in-silico sources was well enough to predict the chemical's kinetics.

The currently developed model was applied in both biomonitoring and exposome case studies for human health risk assessment (Martínez et al., 2017, 2018). Both studies were included in two major EU projects: HEALS and EuroMix.

In the second part of the chapter 2, flutamide PBPK model was developed both for the rat and for human. First the rat PBPK model was developed and validated against experimental data on rats. Then the model was extrapolated to the human using human specific data for enzyme activities, organ volumes and blood flow through each organ, keeping the other parameters the same as for the rat PBPK. Parameter uncertainties were handled by running multiple models with various values for each uncertain parameter in parallel. This led to quantitative assessment of uncertainties levels in the predicted dynamic behaviour of flutamide concentrations in the various tissues. The extrapolation of the model for predicting flutamide kinetics in humans were in a good agreement with the observed data. The metabolic data were found to be the most conducive parameters in the model, as they were not only determining the hepatic clearance of the chemicals, but also generating the metabolites. This indicates that metabolic studies are very important and it should be an integral part of a PBPK model.

In general, this chapter showed that integration of an in vitro metabolic and an in silico data into a PBPK using IVIVE (in-vitro in-vivo extrapolation) and QSAR (Quantitative structure activity relationship) approaches could predict the kinetics with minimal or no animal experiments, supporting the 3Rs strategies of minimizing animal use. Since this new whole-body PBPK model can predict chemical's concentrations not only in plasma but also in various organs, the model may have applications for safety assessment of these chemicals. Physiologically specific in nature, the current PBPK models could also be adapted to the context of a large human population by considering their metabolic variations and could be used for analysing the large human biomonitoring data.

The integration of specific dynamic physiological data into the PBPK model enables to predict the chemical kinetics for a special group of a population. Chapter 3 demonstrated this by applying it to a case study on the development of a pregnancy PBPK model for Bisphenol A. Bisphenol A is an EDC which has been associated with the developmental effects on growing fetus, an association that has been experimentally proved in animal studies. In the development of the Pregnancy-PBPK model, pregnancy growth dynamic equations were implemented into the model that mimics the physiology of the pregnant mother. The inclusion of the fetus compartment and its communication with the mother was done via placenta blood flow. The currently developed model is able to predict the concentrations of chemicals in the fetus plasma, in the placenta, the in fetus liver, and in the amniotic fluid. Detailed metabolic kinetics of BPA conjugation and deconjugation was investigated in the fetus liver. Then performance of the model was checked using five different cohort studies. The prediction of higher concentrations of BPA during the mid-gestational period in the amniotic fluid, placenta, and the fetus liver are in accordance with biomonitoring data, indicating that the mid-gestational period might be the critical

window of exposure for the fetus. To our knowledge this was the first Pregnancy PBPK model for BPA.

Chapter 4 demonstrated the complex systems biology model of reconstructing the dynamic network of oxidative stress and its application in personalized therapies for Parkinson's disease. It is based on seven design principles: design principle 1, ROS induces mitophagy and in turn provides stability to the system; design principle 2, Keap1-Nrf2 module provides homeostasis to the system through negative feed-back; design principle 3, NFkB activates recovery of damaged mitochondria and prevents necrosis at high ROS level; design principle 4, DJ-1 coordinates mitochondrial recovery and amplification of NRF2 signalling and helps to bring dynamic homeostasis close to perfect adaptation; design principle 5, strong adaptation runs out with ageing; design principle 6, preconditioning (pre-treatment by NRF2 activation) may play Parkinson's disease protective role; Design principle 7, inter-individual variations cause disease variability between individuals that provide a foundation for the development of personalized medicine. The model demonstrated that fine tuning the balance between mitochondrial recovery and mitophagy is crucial for the systems. The NRF2-KEAP pathway back regulates the ROS level and acts as ROS sensor and as the first important defense mechanism. DJ1 showed to be an additional ROS sensor. DJ1 upregulation increases the cell's robustness, and DJ1 downregulation makes the system more sensitive to oxidative stress. The model was validated against the in-vitro experimental data on antioxidant response, p62, Bclxl and ATP consequent to the addition of menadione in the model. The model showed that chronic exposure to increased ROS generation can exhaust the adaptation so that the system ultimately collapses. The model also showed that mutations in DJ1 and alpha-Synuclein make the defense system weaker, so that the systems collapses more readily with the increased accumulation of ROS. This phenomenon showed that the inter-individual variability can be a susceptibility factor for development or progression of the disease. The current detailed model of ROS management could be very useful in studying the health effects of various types of environmental chemicals/EDCs generating ROS. One case study with flutamide is shown in chapter 5 (part 2).

Chapter 5, illustrated the integrative systems toxicology approach. It included the coupling of a PBPK model to AOPs/ systems biology model. The first part of the chapter included the integration of a PBPK model with a linear mechanistic pathway model (which could be viewed as an AOP) associated with the BDNF link neuronal survivability. The second part of the chapter, included the integration of PBPK (developed in chapter 2) with the ROS System biology model (chapter 4). The first part of this chapter illustrated the ways to systems biology models in the field of toxicology via Pharmacodynamics coupled tissue dosimetry model (PBPK/PD). This was shown by applying a case study on the PFOS-induced neurotoxicity. The integration of the model included three main steps. 1: Development of a PBPK model, 2: Development of a mechanistic system model, and 3: Coupling of the PBPK model with the mechanistic

model by using a Pharmacodynamics interaction model. The development of the coupled PBPK/PD-mechanistic model allowed to quantify the dynamics of the endogenous biomolecule concentrations of different species at the different levels of PFOS exposures which perturbed key components of the system (in the miRNA model). The interaction of the PFOS with the given pathway was modelled by implementing an indirect sigmoid response model. This integrated PBPK/PD coupled mechanistic system pathway model can be called a "Systems Toxicology" that describes the kinetics of both -the chemicals and – the biomolecules, helping us to understand the dynamic and steady-state behaviours of molecular pathways under perturbed conditions.

The 2nd part of the chapter included the Integrative Systems Toxicology comprising of PBPK, QIVIVE and Systems Biology (integrating in-vitro, in-vivo and in-silico). This approach was used to evaluate the flutamide induced hepatotoxicity. PBPK was used to describe the time course of drug concentration at the cellular level. Then coupling of the estimated tissue dose of flutamide to consequent perturbation of endogenous ROS was achieved using direct pharmacodynamic response models. These perturbations (changes in the ROS level) were dynamically linked with a systems biology model of ROS. This integrative approach allowed us to predict the behaviour of several components of the ROS management model, such as antioxidant regulated genes, mitochondrial respiration and ATP level as a function of flutamide dosing. The model showed a greater induction in NRF2 levels in case of both rats and humans. However, the level of the ATP did not decrease enough and quickly recovered when drug was eliminated from the body. When flutamide induced ROS, NRF2 was upregulated. The latter activated antioxidant regulator genes and ROS concentration was decreased. This helped the system recover. For individuals with reduced superoxide dismutase activities, the extent of the perturbation was predicted to be a lot higher certainly to the extent of becoming toxic. This led us to suggest relevance of our IST for the issue of idiosyncratic toxicity.

We set out to devise a methodology to predict endocrine disruptive activities of compounds in the environment. One of the issues here is the ultralow concentrations these compounds can have in the human environment. Our results towards this idiosyncrasy plus the possibility to include PBPK-based tissue accumulation of such compounds, suggests that the IST methodology designed here may of use in EDC analyses.