Physiologically based toxicokinetic modelling of bisphenols in zebrafish (Danio rerio) accounting for variation in metabolic rates, brain distribution and liver accumulation

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P04-06

New approaches for evaluating kidney toxicity using physiological maps

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As computational approaches advance, predictive models in toxicology are also improving their performance. Today, many models with high accuracy are available, covering most of the relevant endpoints for human toxicity [1]. However, these *in silico* predictions are often based solely on the structure of the molecules and do not consider the molecular and biological mechanisms of action that explain toxicity. A second limitation is that the current models are developed from tests performed on laboratory animals, with predictions for animal toxicity instead of human toxicity, which for some endpoints may be very different. To overcome these drawbacks, it is important to add biological properties to the model and develop methods that include human physiological data. This can be accomplished using systems biology approaches, allowing us to create a network of chemicals, genes and proteins, describing relationships among them and with their respective toxicity phenotypes. In this way, we can generate a Physiological Map (PM), a framework useful for studying possible toxicities.

For the initial setup and validation of PMs, we have developed a semi-automated method. It comprises a computational part with a parallel manual literature search, followed by an expert review. Our approach facilitates the creation of a PM from scratch and offers a standardized method for different diseases. It is based on querying ontologies (as Gene Ontology, Human Phenotype Ontology and Chemical Entities of Biological Interest), which contain information already organized and therefore easily convertible into a network [2]. Eventually, the reconstructed networks are graphically represented using the CellDesigner software and displayed on-line via MINERVA platform.

In the framework of ONTOX, a 5-year European project aiming to improve the performance of risk assessment using computational methods and avoiding the use of animal tests [3], we developed a first version of a PM for the kidney. This map is specifically designed to study tubulopathy in kidney disease. It contains the major nephron segments: glomerulus, proximal tubule, loop of Henle, distal tubule and collecting duct. The PM comprises the required mechanisms for vitamin D metabolism, for blood substances filtration, as well as for the transporters involved in secretion and reabsorption from the filtrate, eliminating the waste from our body via urine.

These PMs are developed as static (qualitative) models and can be used as benchmarks for the subsequent development of Adverse Outcome Pathways. Furthermore, these maps can be turned into dynamic (quantitative) models when adding kinetic parameters. The future goal is to develop a quantitative predictive method, describing the response of the human body to chemicals more accurately and providing a better biological explanation of toxicity.

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P04-07

Physiologically based toxicokinetic modelling of bisphenols in zebrafish (Danio rerio) accounting for variation in metabolic rates, brain distribution and liver accumulation

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Bisphenol A (BPA) is a chemical of toxicological concern due to its endocrine disruptive properties combined with its frequent detection in the environment due to widespread industrial use. Recently, many BPA-like compounds have been employed for various industrial applications leading to their distribution in the environment including in water and aquatic organisms. Although these analogs are not as well-studied as BPA, some of them show endocrine disrupting effects and toxicity in various organisms including fish.[1] In order to better assess the risk of various bisphenols in fish, it is crucial to understand their toxicokinetic properties and the dose at target organs.

A physiologically-based toxicokinetic (PBTK) model was developed such as to model the toxicokinetic behaviour of various BPA analogs in zebrafish (*Danio rerio*), a widely used model organism in toxicology research. We provide novel *in vivo* data on time-course organ specific distribution and elimination of bisphenol Z (BPZ) in female zebrafish including important targets of toxicity such as brain, liver and gonads. Additionally, we quantified *in vitro* fish-specific biotransformation rates for a subset of 11 environmentally relevant bisphenols using fish liver S9 fractions. Knowledge on biotransformation was previously lacking for fish despite being crucial for understanding species-specific toxicokinetic behaviour. Our measurements show lower biotransformation rates than previously reported human ones, indicating that using human-specific values may lead to underestimation of risk in fish.

The novel experimental data in this study, together with literature data for BPA and bisphenols AF (BPAF) were used to parameterize and validate the model. Our PBTK model predicted gonad, brain, liver and whole-body concentrations with good accuracy at a variety of exposure scenarios for BPA, BPZ and BPAF. Notably, liver concentrations predictions were greatly improved compared with previously available models.[2] The model captured the preferential distribution of bisphenols to liver rather than brain or gonads which has been previously observed in various fish species including zebrafish.[3–5] Developed PBTK model was lastly used to predict and compare bioconcentration potential of 11 bisphenols in order to increase our understanding of their potential ecotoxicological relevance. These predictions showed good agreement with literature measurements of bisphenols in a variety of fresh- and salt-water fishes, thus showing good application potential for environmental risk assessment of bisphenols.

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P04-09
Integrated hazard assessment of the carcinogenic potency of N-nitroso compounds

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N-nitrosamines are relatively common environmental contaminants as well as being present in food and drinking water. Specific concerns have also been raised recently over their presence as impurities in pharmaceuticals. The intrinsic ability of N-nitrosamines to act as electrophiles has led to numerous investigations of DNA-reactive carcinogenicity. As such, N-nitrosamines are viewed as part of the cohort of concern and are banned in many circumstances. Whilst we have many historical in vivo and in vitro data, as well as associated structure-activity relationships (SARs), which go a long way to characterise the carcinogenic potency of N-nitrosamines, there are still many data gaps. The aim of this study was to predict, with high confidence, the carcinogenic potency of N-nitrosamines through a computational approach. A sub-group of N-nitrosamines were selected for analysis, compounds were sought where there was clear evidence of the same mechanism of action, namely the transformation of the N-nitrosamine into a diazonium ion via enzymatic α-hydroxylation. The data space was populated with publicly available mutagenicity and carcinogenicity data, which were deemed to be of high quality. Molecular descriptors were calculated for the compounds in the data set, with similarity analyses (ToxPrint and RDKit fingerprint-derived Tanimoto coefficients) performed with the ChemTunes.GPS software. The analysis of the structure of N-nitrosamines confirmed that patterns, broadly associated with electrophilic reactivity, are related to carcinogenic potency. Using these as a starting point it made possible to perform read-across as well as creating computational models and SAR-derived understanding which can be used to predict potency of compounds lacking data. The similarity analysis supports this assumption and gives weight of evidence to the overall conclusions. It is important to note that these approaches are class specific and akin to read-across. However, there is high confidence in the analyses as evidenced by the assessment of uncertainties, namely due to the use of high-quality data, mechanistic interpretability and transparency and the well-defined description and applicability domain of the similarity hypotheses underpinning the read-across and models. It is intended that prediction from the models could inform, at least in a limited manner, tolerable intake of N-nitrosamines.

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P04-10
Application of Dempster-Shafer theory to estimate uncertainty in hazard assessment obtained by combining computational and experimental results

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In drug-development, *in vitro* experiments, *in vivo* animal and clinical studies are conducted to uncover human health risks associated with the therapeutic use of new drug candidates. When experimental data is not available, *in silico* methods can also be applied to fill data gaps by predicting toxic effects of compounds. Eventually, the results collected from different sources are reviewed by experts who are to integrate and translate them into a decision reflecting the overall toxicological risk. Frequently, the fate of the new drug candidate and its way to the market depends on this complex decision.

But humans are not prefficient at objective evaluation of data quality, combining evidence, and estimating risks. This is because any toxicological evidence is always associated with some degree of aleatory and epistemic uncertainty [1]. These refer to the random nature of events happening within biological systems and lack of full knowledge about the process under investigation, respectively. Thus, the consideration of random and systematic errors only does not guarantee to reduce the uncertainty in the final hazard assessment or make correct decisions.

In this work, we propose a methodology for representing multiple elements of toxicological evidence in probabilistic terms [2], which are then combined using rules based on Dempster-Shafer theory (DST) [3, 4]. Our approach allows for translating computational and experimental results into objective decisions and provides a rigorous way to consider the epistemic uncertainty, which is often neglected in scientific evaluations [5]. We tested the application of DST in a case study, where it was used for combining *in silico* predictions [6] as well as for merging several types of evidence (e.g., *in silico* and *in vitro*).

Our case study was focused on endpoints from the standard genotoxicity test battery. We compiled publicly available *in vitro* data from the Bacterial Reverse Mutation Assay, Micronucleus test, and Chromosome aberration test. These *in vitro* data were used to build *in silico* (Q)SAR models. Next, we applied Bayesian statistics to update the reliability metrics of the experimental assays and computational models, thereby obtaining the associated measures of ignorance. Then, we integrated evidence from multiple sources using combination rules based on the DST theory. The proposed approach is not endpoint-specific and can be applied to communicate the degrees of belief and plausibility for single elements of toxicological evidence and to combine toxicological results to a single hazard estimate expressed by means of probability bounds.

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