Implementation of human and zebrafish physiologically based pharmacokinetic modeling for developmental neurotoxicity hazard assessment

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The developmental neurotoxicity in vitro battery (DNT-IVB) was developed with the goal of increasing the throughput of chemical testing for DNT. While the battery was endorsed by OECD in 2023, the number of chemicals tested in and regulatory implementation of the battery has remained limited. To enhance application of the DNT-IVB, the Division of Translational Toxicology at the National Institute of Environmental Health Sciences (NIEHS) supported screening of hundreds of stakeholder-nominated chemicals through the DNT-IVB. In addition to the DNT-IVB, chemicals were tested in zebrafish embryo behavioral assays. To facilitate risk assessment application, bioactive concentrations must be translated into in vivo-relevant doses. Therefore, in vitro to in vivo extrapolation (IVIVE) was performed using physiologically based pharmacokinetic (PBPK) modeling to derive equivalent administered doses (EADs) for 95 of the chemicals that were screened. While human PBPK models are readily available, few zebrafish PBPK models exist. Following a literature review of available zebrafish PBPK models, we selected one published by Simeon et al. (2020) due to its focus on zebrafish embryonic development over the same window as that assessed in the zebrafish behavioral assays conducted here, prior application on a relatively broad chemical space, inclusion of a brain compartment, and publicly accessible code. This model accounts for zebrafish organ growth, in vitro intracellular disposition, and metabolism. We implemented the zebrafish embryo PBPK model in the simulation language MCSim, compiled and ran the models within an R environment, and conducted additional postprocessing using Python. We parameterized the PBPK model for the experimental conditions of the zebrafish embryo assays and batched modeling. We further refined and validated the model using parameter estimation based on bioavailability data collected during the screening from zebrafish medium and embryo concentrations taken at the end of testing. Using the zebrafish PBPK model, we conducted IVIVE using benchmark concentrations and zebrafish embryo and embryonic brain-specific tissue concentrations to derive EADs for zebrafish for all chemicals bioactive in the zebrafish behavioral assay. Similarly, we derived human EADs using chemical bioactive concentrations for the in vitro assays based on our previously established DNT-IVIVE approach that considers chemical partitioning into the fetus and brain during the critical period of neurodevelopment. PBPK modeling was performed with the US Environmental Protection Agency's open-source R package, httk. The human gestational PBPK model was used to predict tissue

concentrations in the fetus during pregnancy, and the complex brain-adipose model for httk we have developed was used to predict brain concentrations at six months of age. Here we have also accounted for in vitro disposition to evaluate the importance of this additional step. Human EADs were benchmarked against in vivo data available for a subset of chemicals to evaluate our predictive approach. EADs were compared against exposure estimates to provide bioactivity exposure ratios, a metric that can be used in risk assessment prioritization. This approach allows for prioritization of chemicals for further testing and facilitates application of the DNT-IVB for risk assessment.

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