In Vitro to *In Vivo* Extrapolation: Optimizing Parameters for Improved Predictions

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In vitro assays provide an efficient way to identify estrogen-active chemicals. However, nominal *in vitro* assay concentrations of a chemical may not accurately reflect the blood or tissue levels that cause *in vivo* effects, mostly due to differences in bioavailability and clearance between the two systems. We evaluated the impact of critical pharmacokinetic (PK) parameters and dosimetry in two models for *in vitro* to *in vivo* extrapolation of activity in the estrogen receptor (ER) pathway. A simple one-compartment PK and a multi-compartment physiologically based pharmacokinetic (PBPK) model were used to correlate *in vitro* effective concentrations to *in vivo* blood concentrations for two reference estrogens and eight environmental chemicals with ER agonist activity. Data from 16 *in vitro* ToxCast assays that map to various key events along the ER pathway (including binding, transcription, and cell proliferation) were used in these models to estimate daily equivalent administered doses (EADs) that would result in a steady-state blood concentration (Css) or maximum blood concentration (Cmax) equivalent to lowest effective concentrations (LECs) in these assays. The EAD estimates from both models were then compared to the lowest effect levels (LELs) for each chemical in *in vivo* uterotrophic assays. We examined the impact of two key PK parameters in relating the administered dose to blood concentration: fraction of unbound chemical (Fub) and hepatic clearance. We systematically varied the values of these parameters across a range of experimentally observed values and investigated the changes in EAD estimates. In addition, to better estimate the free fraction of a chemical that is available for uptake into tissue/cells and results in a biological effect, we applied a Fub adjustment method to estimate EADs that could lead to blood concentrations of free chemicals equivalent to *in vitro* LECs. The models performed well in predicting *in vivo* lowest LELs from *in vitro* LECs for the majority of chemicals tested, particularly after Fub adjustment. This study demonstrates the ability to quantitatively predict *in vivo* effects for proper interpretation of *in vitro* data. *This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No.HHSN27320140003C.*