

Chemical Risk Analysis with Bayesian Support (CRABS) In the IVIVE Context

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The context of the chemical challenge drives the types of risk-based decisions that must be made in chemical safety policy. For instance, environmental risk managers make different decisions (e.g., site cleanup, post-hoc exposure/emissions control) when compared to their chemical manufacturing risk management counterparts. However, the goals of chemical risk managers across these fields remain the same: limit chemical-induced disease/injury. I propose that Bayesian Decision Theory can be a unifying foundation for both environmental chemical and chemical manufacturing risk managers when considering In Vitro to In Vivo Extrapolation (IVIVE). Under the proposed Chemical Risk Analysis with Bayesian Support (CRABS) approach, risk management decisions are made based on minimizing the risk (and thus the cost) of a decision under uncertainty using Bayes theorem. Under CRABS, the severity scores for different diseases from the Global Burden of Disease (GBD) study are used to weight chemical-induced diseases, and thus chemicals, in combination with chemical risk information. CRABS calculates the risk of a chemical resulting in a disease based on the risk of disease given a chemical's dose/concentration and the probability of a chemical exposure. Risk managers then use CRABS to choose the decision rule that minimizes the risk adjusted for disease severity from the GBD. This will result in science and disease based policies that will address chemical prioritization, policy-based risk management and global stewardship needs. I will demonstrate how CRABS and the AOPXplorer can work together to facilitate the extrapolation from in vitro to in vivo to facilitate risk decisions.

In Vitro to In Vivo Extrapolation for Estrogenic Activity of Environmental Chemicals

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In vitro high-throughput screening (HTS) assays provide an efficient way to identify potential 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. In this study, we evaluated the impact of critical pharmacokinetic parameters, dosimetry, and modeling approaches on in vitro to in vivo extrapolation of estrogenic activity. In vitro lowest effective concentrations (LECs) for ten chemicals with estrogen receptor (ER) agonist activity were obtained from each of 16 ToxCast and/or Tox21 HTS assays mapping to various key events along the ER pathway (including binding, transcription, and cell proliferation). A simple one-compartment PK and a multi-compartment physiologically based pharmacokinetic (PBPK) model were used to estimate daily equivalent administered doses (EADs) that would result in a steady-state blood concentration (C_{ss}) or maximum blood concentration (C_{max}) equivalent to the in vitro LECs. The estimated EADs from both models were compared to the lowest effect level (LELs) from in vivo uterotrophic assays for each chemical. To better estimate the free fraction of a chemical available for tissue/cell uptake, we also applied a Fub adjustment method to estimate EADs that could lead to blood concentrations of free chemicals equivalent to *in vitro* LECs. Our models performed well in predicting in vivo LELs from in vitro LECs for the majority of chemicals tested, particularly after Fub adjustment. This study demonstrates an optimized approach for using in vitro data to quantitatively predict in vivo effects. *This project was funded in whole or in part with Federal funds from the NIEHS, NIH under Contract No. HHSN273201500010C.*

Assessment of In Vitro High Throughput Pharmacokinetic Data to Predict In Vivo Pharmacokinetic Data Of Environmental Chemicals

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Assessing the health risks of the thousands of chemicals in use requires both toxicology and pharmacokinetic (PK) data that can be generated more quickly. For PK, in vitro clearance assays with hepatocytes and serum protein binding assays provide a means to generate high throughput (HT) PK data. A PK model is used to extrapolate the in vitro data to in vivo exposures. In this study, we assessed the ability of current in vitro HTPK models to accurately predict in vivo PK parameters, such as peak plasma concentrations (C_{max}), volume of distribution (V_d), and half-life. Twenty-six ToxCast chemicals were evaluated, including a variety of pesticides (e.g., carbofuran) and industrial chemicals (e.g., bisphenol A). Adult male Sprague-Dawley rats with an indwelling jugular vein cannula were dosed by oral gavage (po) or intravenously (iv) via the tail vein to doses corresponding to those that HTPK predicted would produce serum concentrations in the range of bioactivity observed in ToxCast bioassays. Serial blood samples were collected out to 96 h. Plasma was isolated by centrifugation and stored at -80° C until analyzed for parent compound by HPLC-MS/MS. For po exposure, HTPK predictions for C_{max} were within 3-fold of measured values for 36% of observations. HTPK over-predicted C_{max} (a risk-conservative error) for 71% of observations. The prediction of V_d was within 3-fold for 43% of observations, but half-life was >3-fold over-predicted for 87% of observations. The data suggest that models based on *in vitro* HTPK data may be sufficient for ~40% of the chemicals analyzed in this study. However, for most of the discordant chemicals, the bias present for the HTPK data was conservative in a risk context. The data suggest that the discordance for many chemicals may be due to overestimation of half-life. Additional data including bioavailability and effect of transporters may aid in rendering these assays and models more predictive in the future. (*This abstract does not represent US EPA policy.*)

Unique Challenges of Toxicity Prediction for Environmentally Relevant Chemicals: Prediction of Human Plasma Protein Binding Through Quantitative Structure-Activity Relationship (QSAR) Models

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Physiologically based pharmacokinetic (PBPK) models bridge the gap between in vitro assays and in vivo effects by accounting for the adsorption, distribution, metabolism, and excretion of xenobiotics, which is especially useful in the assessment of human toxicity. Quantitative structure-activity relationships (QSAR) serve as a vital tool for the high-throughput prediction of chemical-specific PBPK parameters, such as the fraction of a chemical unbound by plasma protein (Fub). The presented work explores the merit of utilizing experimental pharmaceutical Fub data for the construction of a universal QSAR model, in order to compensate for the limited range of high-quality experimental Fub data for environmentally relevant chemicals, such as pollutants, pesticides, and consumer products. Independent QSAR models were constructed with three machine-learning algorithms, k nearest neighbors (kNN), random forest (RF), and support vector machine (SVM) regression, from a large pharmaceutical training set (~1000) and assessed with independent test sets of pharmaceuticals (~200) and environmentally relevant chemicals in the ToxCast program (~400). Small descriptor sets yielded the optimal balance of model complexity and performance, providing insight into the biochemical factors of plasma protein binding, while preventing over fitting to the training set. Overlaps in chemical space between pharmaceutical and environmental compounds were considered through applicability of domain (AD) assessment and quantified through reliability estimates. The pharmaceutical and environmental test sets exhibited similar variance and predictability, indicating the combination of a large pharmaceutical training set with a small feature space yields a generalized model for Fub with adequate coverage of environmentally relevant chemical space. Thus, the presented work provides a reliable model for the high-throughput predictions of Fub in environmentally relevant chemicals, a critical component in of in silico toxicity screening. *(The presented findings and conclusions are those of the authors and do not necessarily reflect the view of the U.S. Environmental Protection Agency.)*

Intracellular Concentrations of Endogenous Bile Acids in Sandwich-Cultured Hepatocytes: Relevance for Prediction of Cholestasis

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In vivo, concentrations of bile acids (BA) are tightly regulated through synthesis, metabolism, and transport mechanisms. Impaired canalicular BA efflux has been postulated to play a role in drug-induced liver injury. Many compounds that have been shown to inhibit bile salt export pump (BSEP)-mediated efflux of bile acids also inhibit their uptake into hepatocytes. Inhibition studies in whole-cell models using exogenously administered bile acids can be confounded if the compound significantly inhibits the uptake of bile acids. Cyclosporine A (CsA) inhibits both the uptake and efflux of bile acids. Analysis of endogenously generated bile acids may allow for separation of the uptake and efflux effects in interaction studies. Transporter Certified™ sandwich-cultured hepatocytes and B-CLEAR® technology were used to evaluate changes in the hepatobiliary disposition of endogenous bile acids following treatment with 10 μM CsA for 10 minutes to 12 hours. Dimethyl sulfoxide (DMSO) treatment was used as a solvent control. The mRNA content of multiple uptake and efflux transporters and bile acid synthetic enzymes was evaluated using TaqMan qRT-PCR. Concentrations of four endogenous bile acids (taurocholic acid [TCA], glycocholic acid [GCA], taurochenodeoxycholic acid [TCDCA], and glycochenodeoxycholic acid [GCDCA]) and CsA were determined by liquid chromatography-mass spectrometry. No changes in gene expression were detected following exposure to CsA. Intracellular concentrations of CsA were relatively constant over 12 hours. The total endogenous bile acid pool (cell + bile + media) increased over time in the DMSO control, but was not affected by CsA. The total chenodeoxycholic acid (CDCA; TCDCA + GCDCA) to total cholic acid (CA; TCA + GCA) intracellular concentration ratio decreased with time from 11.1 to 0.47 in the presence of CsA suggesting a change in the intracellular composition of the endogenous bile acid pool from CDCA conjugates to CA conjugates. A similar shift in the ratio was observed in the total endogenous bile acid pool. Consistent with inhibition of BSEP by CsA, the biliary excretion index for all bile acids decreased over time, with approximately 80% of the decrease occurring in the first 120 minutes. The intracellular concentration of total endogenous bile acids was increased in the presence of CsA. The increase in the intracellular concentration of GCA was greatest, and increased in a time dependent manner from 359% of control (10 min) to 1000% of control at 4 hours. Endogenous bile acid concentrations (e.g. GCA) may provide a sensitive tool to evaluate the intracellular effects of compounds that alter the hepatobiliary disposition of bile acids.

Assessing Interval Estimation Methods for Hill Model Parameters in a High-Throughput Screening Context

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The Hill model of concentration-response is ubiquitous in toxicology, perhaps because its parameters directly relate to biologically significant metrics of toxicity such as efficacy and potency. Point estimates of these parameters obtained through least-squares regression or maximum likelihood are commonly used in high-throughput risk assessment, but such estimates typically fail to include reliable information concerning confidence in (or precision of) the estimates. To address this issue, we examined methods for assessing uncertainty in Hill model parameter estimates derived from concentration-response data. In particular, using a sample of ToxCast concentration-response data sets, we applied four methods for obtaining interval estimates that are based on asymptotic theory, bootstrapping (two varieties), and Bayesian parameter estimation, and then compared the results. These interval estimation methods generally did not agree, so we devised a simulation study to assess their relative performance. We generated simulated data by constructing four statistical error models capable of producing concentration-response data sets comparable to those observed in ToxCast. We then applied the four interval estimation methods to the simulated data and compared the actual coverage of the interval estimates to the nominal coverage (e.g., 95%) in order to quantify performance of each of the methods in a variety of cases (i.e., different values of the true Hill model parameters). In general, we found that although confidence intervals produced by the various methods tended to have similar widths, certain interval estimation methods tended to be more reliable (in that actual coverage matched nominal coverage) in certain categories of situations (which we have characterized). No single method, however, tended to be more reliable than others in all situations. This work demonstrates a framework for obtaining interval estimates for potency and efficacy parameters, and thus provides a better means for quantifying uncertainty in risk decisions. (*This abstract does not represent US EPA policy.*)

Integrating High-Throughput Exposure and Hazard Data Using a Physiologically Based Pharmacokinetic/Pharmacodynamic Model for Thyroid Peroxidase Inhibitors

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High throughput (HT) in vitro toxicity testing, along with HT in silico exposure predictions, hold the promise of conducting human health risk assessment with greater speed and require less resources. The lack of a complete biological system in these HT approaches, however, precludes the incorporation of pharmacokinetics. In this study, a physiologically based pharmacokinetic (PBPK) model was developed and linked to a pharmacodynamic (PD) model to integrate in vitro potency data, in silico exposure predictions, and pharmacokinetic characteristics for estimating margin of exposure (MOE) for six thyroid peroxidase (TPO) inhibitors. These chemicals ranged in potency from high (methimazole and 6-propylthiouracil) to moderate (benzophenone-2 and 2-mercaptobenzothiazole) to low (resorcinol and triclosan). The PBPK/PD model described TPO inhibition as a function of binding affinity, which was determined through in vitro testing, and chemical concentration within the thyroid gland. The model was then used to estimate human equivalent intake doses able to inhibit thyroid hormone levels by 10% (ED10) for each chemical. Next, MOE was calculated by comparing each ED10 value to population exposure levels predicted from a HT exposure model. Results of this case example revealed that chemical potency and MOE estimates might not necessarily be related. For example, benzophenone-2 and triclosan exhibited small MOE values due to slow clearance or high exposure potential, respectively, for each chemical. Our study suggests that prioritizing chemicals based on hazard or exposure alone may not be sufficient, and that consideration of pharmacokinetics is warranted. *(This abstract does not represent US EPA policy.)*

A Comprehensive Physiologically Based Pharmacokinetic Knowledgebase and Web-Based Interface for Rapid Model Ranking and Querying

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Published physiologically based pharmacokinetic (PBPK) models from peer-reviewed articles are often well-parameterized, thoroughly vetted, and can be utilized as excellent resources for the construction of models pertaining to related chemicals. Specifically, chemical-specific parameters and in vivo pharmacokinetic data used to calibrate these published models can act as valuable starting points for model development of new chemicals with similar molecular structures. A knowledgebase for published PBPK-related articles was compiled to support PBPK model construction for new chemicals based on their close analogues within the knowledgebase, and a web-based interface was developed to allow users to query those close analogues. A list of 689 unique chemicals and their corresponding 1751 articles was created after analysis of 2,245 PBPK-related articles. For each model, the PubMed identifier, chemical name, major metabolites, species, gender, life stages and tissue compartments were extracted from the published articles. PaDEL-Descriptor, a Chemistry Development Kit based software, was used to calculate molecular fingerprints. Tanimoto index was implemented in the user interface as measurement of structural similarity. The utility of the PBPK knowledgebase and web-based user interface was demonstrated using two case studies with ethylbenzene and gefitinib. Our PBPK knowledgebase is a novel tool for ranking chemicals based on similarities to other chemicals associated with existing PBPK models and for rapid accessing of model-constraining publications. *(This abstract has been cleared by the EPA but solely expresses the view of the authors.)*

**Culture of Pig Embryo Kidney Cells (SPEV-2) as a Model for
Studying the Cytotoxic Effects of Antitumor Agents
on the Example of *Gratiola officinalis***

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Active dry extract solution *Gratiola officinalis* L. investigated on pigs embryo kidney cells in culture SPEV-2 using propidium iodide (PI). Concentration of 32 and 320 ug/ml had a cytostatic effect on cells SPEV-2. Concentrations of 3,2 and 32 mg/ml had a cytotoxic effect. During the day the extract induced apoptosis in cells at concentrations of 32 and 320 ug/ml. Overlaying still images allowed us to describe in different microscopic scattering and fluorescence registration, cytomorphological parameters under the influence of the extract, marking process of cell death. These indicators reflect the DNA damage in cells under conditions in vitro. We described the following indicators: the appearance of dark structures in the cytoplasm, indistinct outlines of the cells themselves and their acquisition of star-shaped or rounded preservation, but the fragmentation of the cell membrane and spillage; formation of conglomerates dead cells, Reducing the emission intensity of propidium iodide indicates the decay of the nucleus DNA. Index numbers of dead cells (CMC) can be used an index of cytotoxicity.

High Throughput Determination of Critical Human Dosing Parameters

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High throughput toxicokinetics (HTTK) is an approach that allows for rapid estimations of TK for hundreds of environmental chemicals. HTTK-based reverse dosimetry (i.e. reverse toxicokinetics or RTK) is used in order to convert high throughput in vitro toxicity screening (HTS) data into predicted human equivalent doses, which can be linked with biologically relevant exposure scenarios. Therefore, HTTK provides critical data in order to prioritize the risk for thousands of chemicals that lack TK data. The unbound fraction of a chemical in plasma (Fub) is a critical HTTK parameter that can be measured in vitro. However, for current methods whereby Fub is measured at 100% plasma concentration, Fub is below the limits of quantitation (LOQ) for high throughput analytical chemistry for chemicals that bind strongly to plasma, and therefore cannot be quantified. In order to quantify Fub, a novel method was implemented for 85 strategically selected chemicals: Fub was measured at 10%, 30%, and 100% of physiological plasma concentrations using rapid equilibrium dialysis assays. Chemicals were selected based on their capacity to be potent in vitro estrogen signaling disruptors (Rotroff et al. 2014), having data from the National Health and Nutrition Examination Survey, or either having no HTTK data or a failed Fub assay. Including plasma concentrations substantially lower than physiological levels allows the direct measurement of unbound chemical concentrations. The consequent Fub estimates at lower protein concentration can be extrapolated to physiological levels. At 100% plasma concentration, assays yielded values below LOQ for 34 chemicals. Fub could be quantified for 12 of these 34 chemicals at 10% and/or 30% plasma concentrations, which suggests that assay failure at 100% plasma concentration was caused by plasma protein binding for these chemicals. For the remaining 22 chemicals, assay failure may be due to chemical insolubility, susceptibility to enzymatic or other degradation, and ability to bind to RED device constituents such as assay plate walls or dialysis membrane. As a result of using this new approach, ~35% of missing Fub values were captured and would have been missing with the use of previous HTTK protocols. (*This abstract does not represent U.S. Environmental Protection Agency policy.*)

EURL ECVAM Strategy on Toxicokinetics

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The EURL ECVAM Strategy document on Toxicokinetics outlines objectives and activities needed to achieve a 3Rs impact in the area of toxicokinetics and systemic toxicity, with a view to developing a risk assessment approach that is increasingly based on human data. The importance of physiologically-based kinetic (PBK) modelling is a central feature in the strategy document. In order to facilitate the generation, acceptance and use of PBK models in the regulatory domain, four main objectives are identified. The first concerns the development of standards to characterize in vitro and in silico methods that measure individual absorption, distribution, metabolism and excretion (ADME) parameters. The second objective aims to establish good kinetic modeling practices, including a web portal for PBK modelling approaches. The third objective expresses the need for publicly available databases to facilitate access to anatomical and physiological (chemical-independent) information to create PBK models, to store in vitro ADME data and in vivo toxicokinetic data. The fourth objective expresses the need to develop guidance on how to generate and use these data in a regulatory setting. This strategy builds on other European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) strategy documents, and in particular the strategy to replace, reduce, and refine the use of animals in the assessment of acute mammalian systemic toxicity. The strategy also builds on work carried out in European Union research projects, such as the FP7 COSMOS project, which has been developing publicly available computational workflows based on the integrated use of open-access and open-source models for the prediction of repeated-dose toxicity.

Predictive Data-Driven Framework for Endocrine Prioritization: Triazole Fungicide Case Study

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Use of high-throughput screening (HTS) human health hazard and exposure predictions enables efficient prioritization of chemicals subject to the U.S. Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program (EDSP). However, for chemicals with extensive and available endocrine testing information, such as pesticide active ingredients (PAIs), the prioritization approach should be based on an integrated assessment inclusive of all available data. Evaluation of relevant data for three triazole fungicides, triadimefon, propiconazole, and myclobutanil, is presented as a case study in this analysis to demonstrate an integrated assessment for prioritizing PAIs for further endocrine testing related to mammalian toxicology. The evaluation included HTS results, EDSP Tier 1 testing, and other scientifically relevant information, including standard toxicology guideline studies. Systematic comparison of all the data confirmed concordance between endocrine-related HTS bioactivity and the more biologically-complex information that satisfied the EDSP Tier 1 data requirements. Similar margins (3-5 orders of magnitude) were observed between HTS-predicted bioactivity and exposure values and in vivo biological activity and EPA chronic exposure estimates for these products' registered uses. The current risk assessment process for PAIs was protective for all in vivo effects observed, endocrine or not. Combined HTS hazard and high throughput exposure predictions (ExpoCast) suggest low endocrine testing priority for human health risk of these triazoles. Further, comparison with the mammalian toxicology database for each molecule indicated that this HTS-based prioritization would have been protective for any potentially endocrine-mediated in vivo effects (if present). This example demonstrates an effective, human health-protective roadmap for EDSP evaluation of PAIs via prioritization using HTS, available exposure estimates, and guideline toxicology information.

Evaluation of Pharmacokinetic Assumptions Using a 443-Chemical Library

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With the increasing availability of high-throughput and in vitro data for untested chemicals, there is a need for pharmacokinetic (PK) models for in vitro to in vivo extrapolation (IVIVE). Though some physiologically based PK (PBPK) models have been created for individual compounds using in vivo data, we are now able to rapidly parameterize generic PBPK models using in vitro data to allow IVIVE for chemicals tested for bioactivity via high-throughput screening. We evaluated the assumptions and performance of a generic PBPK model (R package “httk”) parameterized by a library of in vitro PK data for 443 chemicals. We evaluated and calibrated Schmitt’s method by comparing the predicted volume of distribution (Vd) and tissue partition coefficients to in vivo measurements. The partition coefficients were initially overpredicted due to overestimation of partitioning into phospholipids in tissues and the lack of lipid partitioning in the in vitro measurements of the fraction unbound in plasma. Correcting for phospholipids and plasma binding improved the predictive ability (R² of 0.52 for partition coefficients and 0.34 for Vd). We lacked enough data to evaluate the accuracy of changing the model structure to include tissue blood volumes and/or separate compartments for richly/poorly perfused tissues. Therefore we evaluated the impact of these changes on model outputs. After looking at the duration and concentration at the end of the distribution phase, elimination rate, area under the curve (AUC), maximum concentration, number of days to steady state, and time elapsed for 90% of chemical eliminated, we found that the only significant change in model outputs is in the duration of the distribution phase. The richly/poorly perfused correction doubled the duration of the distribution phase while the duration for the blood volume correction was $\frac{3}{4}$ of the original. We also determined that the elimination rate was slightly smaller than for a one-compartment model, and AUC was accurately predicted with the analytic steady-state solution, suggesting equivalence to a one-compartment model for many compounds and a two-compartment model for the remaining. Overall, comparison to in vivo data identified discrepancies that we reduced by refining our phospholipid partitioning and plasma-binding models, which improved the accuracy of the partition coefficient predictions. However, separation of the rest of body into richly/poorly perfused compartments and consideration of blood volumes only made a significant difference in the duration of the distribution phase. *(This abstract does not necessarily reflect U.S. Environmental Protection Agency policy.)*

Computational Models to Estimate In Vivo Activity Concentrations from Tox21 HTS Data

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Tox21 and ToxCast efforts provide in vitro high-throughput screening (HTS) AC50 values for thousands of chemicals. Moving these data to predict whether chemicalbiological interactions observed in vitro are likely to occur in vivo is challenging. We hypothesize using a modified approach applied by the FDA for drug interaction studies, Cmax/AC50 (maximal in vivo blood concentrations over in vitro mechanistic Tox21 assay potencies), will provide useful approximations of blood concentrations required to produce in vivo interactions. 7381 Cmax values were estimated for Tox21 chemicals using physiologically based toxicokinetics models for HTS data (HTTK R-package), with both in silico (ADMET-Predictor) and in vitro (measured) parameters of intrinsic clearance and fraction unbound to human plasma. 339 chemicals with measured human Cmax values correlated with estimated Cmax values, R2=0.6. A preliminary focus on 16 chemicals with PPAR-gamma agonist activity, human exposure and Cmax data showed that the Cmax/AC50 estimates using in vitro or in silico parameters were similar and accurately identified three glitazones as likely for in vivo interaction at therapeutic doses. As these doses will not be common for environmental compounds, real-life environmental exposure predictions were obtained from a rapid heuristic method using information from urine analytes, which does not explicitly differentiate pharmaceutical exposure (Wambaugh JF et al 2014). Cmax levels where Tox21 assay responses are predicted to interact in vivo were calculated across 3706 chemicals and X Tox21 assay endpoints. The Cmax values were generally found to far exceed environmental exposures (one-time), but repeat cumulative exposures and certain occupational situations could increase exposure levels to these ranges. Model uncertainties and out-of-domain compounds also exist and need to be characterized. This approach has shown promise toward estimating in vivo interaction concentrations for HTS data. *This abstract does not reflect official NTP or EPA views.*

Using Physiologically Based Pharmacokinetic Modelling for In Vitro In Vivo Extrapolation to Predict Chemical Exposure

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Mechanistic absorption and physiologically based pharmacokinetic (MA/PBPK) models are useful tools in risk assessment. These models incorporate complex processes related to compounds' disposition such as dissolution, absorption, metabolism, and protein binding in plasma and tissues, and also offer possibility of predicting the target tissue concentrations. In this study, we are presenting the use of MA/PBPK model in GastroPlus™ version 9.0 (Simulations Plus, Inc., Lancaster, CA) to predict the exposure of eighteen chemicals with reported exposure in human after oral administration. The physicochemical and biopharmaceutical properties of all compounds were predicted from structure using ADMET Predictor™ version 7.2 (Simulations Plus, Inc., Lancaster, CA). The effects of physiological pH and bile salt concentrations on solubility/dissolution/precipitation, as well as effects of physiological pH and surface areas on passive absorption rates were included in the model to predict the fraction absorbed. The distribution in systemic circulation was predicted by PBPK model based on physiological tissue sizes for human physiology combined with tissue/plasma partition coefficients calculated from tissue compositions and physicochemical properties using default method in GastroPlus 9.0. The in vivo metabolism was estimated by two methods: (1) using in silico predictions (ADMET Predictor 7.2) of K_m and V_{max} values for five CYP enzymes, along with physiological expression levels of these enzymes in intestine and liver; (2) using in vitro metabolic clearance measured in human hepatocytes (CL_{in vitro}) (Wetmore et al., 2012 and 2015). Experimentally measured plasma protein binding (f_{up}) (Wetmore et al., 2012 and 2015) was used in both methods. The model with in silico metabolism inputs (method 1) predicted AUC of 5 and 10 compounds within 2- and 5-fold of the observed data, respectively. The model with in vitro hepatic metabolic clearance (method 2) predicted AUC of 7 and 11 compounds within 2- and 5-fold of the observed data. The higher prediction errors were further investigated and the non-hepatic clearances (e.g. active urine excretion) and elimination via bile were identified as the main reasons for the mismatch between the predicted and measured AUCs.