

Opening Session

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.

Workshop Background and Summaries of Webinars

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Toxicokinetics (TK) provides a bridge between hazard and exposure by predicting tissue concentrations due to exposure. Higher throughput toxicokinetics (HTTK) appears to provide essential data to established context for in vitro bioactivity data obtained through high throughput screening. The National Toxicology Program has sponsored the workshop "In Vitro to In Vivo Extrapolation for High Throughput Prioritization and Decision Making." In the lead-up to the meeting, a series of four webinars was held. This presentation summarizes those webinars as an introduction to the workshop. First and foremost, we must keep in mind the purpose: simple models appear to allow meaningful prioritization of further research. A primary application of HTTK is "reverse dosimetry" or RTK, from which we can infer daily doses that produce plasma concentrations equivalent to the bioactive concentrations identified by high throughput screening. However, we must consider the reliability, parsimony, and domain of applicability of these approaches.

Session 1: Application in Risk Assessment: What Do We Need for Decision-making and Prioritization?

Using In Vitro Data in Quantitative Risk Assessments (QRAs)

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Historical use of quantitative risk assessment (QRA) to support risk management decisions is reviewed to determine how such support is changed by the use of in vitro rather than in vivo data as the basis for the hazard identification and dose response portions of QRA. The components of QRA are used as a framework to organize and evaluate the impacts of a move to in vitro data. The analysis demonstrates the following. First, the differing natures of the two types of data result in an exchange of one set of uncertainties for another. This exchange makes answering questions about the relative certainty in resulting risk findings complex. Second, the characteristics of in vivo data have directly and indirectly shaped the policies and procedures used in QRA and in chemical risk management. New policies will need to be developed and evaluated as part of any transition to in vitro. Third, the nature of the in vitro data changes how the exposure portion of QRA is performed, requiring predictions of internal concentrations rather than administered doses. As a result, an effective use of in vitro data in QRA will require novel approaches to problem formulation, exposure assessment, and risk management that build on the strengths of the data and provide cost-effective approaches for reducing uncertainties in the resulting predictions of risk. *(Although this work was reviewed by EPA and approved for presentation, it may not necessarily reflect official Agency policy.)*

Interindividual Variability in High Throughput Risk Prioritization of Environmental Chemicals

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We incorporate interindividual variability into an open-source high throughput (HT) toxicokinetics (TK) modeling framework for use in a next-generation risk prioritization approach. Risk prioritization involves rapid triage of thousands of environmental chemicals, most which have little or no existing TK data. Chemicals are prioritized based on model estimates of hazard and exposure to decide which chemicals should be first in line for further study. Hazard may be estimated with *in vitro* HT screening assays, e.g., the U.S. Environmental Protection Agency's ToxCast program. Bioactive ToxCast concentrations can be extrapolated to doses that produce equivalent concentrations in body tissues using a reverse TK approach in which generic TK models are parameterized with (1) chemical-specific parameters derived from *in vitro* measurements and predicted from chemical structure and (2) with physiological parameters for a virtual population. Here we draw physiological parameters from estimates of distributions of demographic and anthropometric quantities in the modern U.S. population based on the most recent data from the Centers for Disease Control's National Health and Nutrition Examination Survey (NHANES) study. A Monte Carlo approach, accounting for the correlation structure of physiological parameters, is used to estimate ToxCast equivalent doses for the most sensitive portion of the population. For risk prioritization, ToxCast equivalent doses are compared to population estimates of exposure rates based on Bayesian inferences drawn from NHANES urinary analyte biomonitoring data. The inclusion of interindividual variability in the TK modeling framework allows targeted risk prioritization for potentially sensitive subpopulations. *(This abstract does not necessarily represent U.S. EPA policy.)*

Quantitative Prediction of Phenotypic Change from High Throughput Assay Results

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Ted Simon, LLC

As the practice of risk assessment transitions away from animal testing to the use of high throughput in vitro assays such as those in the U.S. Environmental Protection Agency's ToxCast™ program, the hope of many risk practitioners is that adverse outcome pathways will become a means of contextualizing the results of assays for screening/prioritization and ultimately, risk prediction. Quantitative key event relationships (KERs) provide one means of understanding how assay results can possibly be used for these predictions. Genistein, a common dietary phytoestrogen, has been tested in a number of ToxCast assays and the assay results used to predict whether or not a uterotrophic response in rodents might occur. The uterotrophic response is used here as a surrogate endpoint for unwanted estrogenic effects. As an example risk assessment for genistein, both in vitro to in vivo extrapolation estimates of free genistein in blood from NHANES urinary data and measurements of steady state concentrations of free genistein in blood are used in an assessment of the likelihood of whether dietary genistein exposure will reach the levels needed for uterotrophy in rodents. This example with genistein is used to illustrate the related ideas of KERs and tipping points in which the assay results can be understood in terms of downstream key events and the adverse outcome.

Toxicokinetics in Risk Assessment: From Predictive Evaluations to Regulatory Testing

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Background: Efficient safety evaluation of new chemical products requires a variety of strategies, from in silico assessments of exposure and effects, to in vitro measurements of these endpoints, as well as in vivo studies in a variety of animal species. The accurate prediction of absorption and/or bioavailability arising from the oral, dermal and inhalation routes of exposure is one of the key elements in these chemical safety assessments.

Method: An overview will be given on the use of both predictive and interpretative toxicokinetic (TK) model types during the various stages of product development (predevelopment, development, registration and post-registration). Strategies for developing standardized predictive modeling approaches across the product development cycle will be highlighted. Specific details on our approach to validate and implement GastroPlus™ for both Predevelopment phase and high-throughput systemic exposure (in vitro to in vivo; IVIVE) evaluations will be then be presented. High throughput evaluations comprising both de novo predictions, as well as refined model simulations utilizing in vitro or in vivo compound-specific parameters will be shown. Strengths and weaknesses of IVIVE calculations for the various exposure routes (oral, inhalation, dermal) for both acute and steady-state paradigms will be discussed. Finally, an overview of the integration of IVIVE predictions into the High-throughput Exposure Assessment Tool (HEAT) will be given.

Conclusion: Substantial efficiencies can be realized by utilizing the fewest possible predictive TK modeling tools, both in reduced training requirements, as well as hopefully improved regulatory acceptance. The GastroPlus™ program was chosen as the best approach for IVIVE modeling, based on the built-in quantitative structure-activity relationship models for refined absorption by all relevant exposure routes, inclusion of cytochrome P450-based metabolism of compounds, and the ability to model in one or more animal species and include humans of varying physiology and lifestyles. Proper understanding of the benefits and limitations of predictive modeling tools such as GastroPlus™ will allow for optimum implementation of animal alternatives in novel high throughput safety assessment programs.

Development and Application of Biologically Based Dose–Response Modeling for Pregnancy Conditions: Evaluation of Thyroid Active Chemical Exposure During Sensitive Life Stages

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Disturbances in the hypothalamus–pituitary–thyroid (HPT) axis in pregnant women that lead to hypothyroxinemia and hypothyroidism have been shown to cause adverse neurodevelopmental effects in the fetus in utero and the neonate after birth. Iodide deficiency is a major cause of such disturbances, potentially predisposing individuals to further alterations in thyroid homeostasis upon exposure to perchlorate and other thyroid-active chemicals, such as thiocyanate and nitrate. To better characterize the perturbations in thyroid hormone levels due to exposure to environmental chemicals, there is a need for a quantitative tool. Recently, we developed a deterministic biologically based dose–response model for the HPT axis to evaluate the effects of iodide intake and perchlorate exposure on thyroid hormone levels, including maternal free thyroxine (fT4), in the near-term pregnant woman and fetus. In an effort to capture the response of a population of pregnant women to such perturbations, the deterministic model was extended to a probabilistic framework. Global sensitivity analysis and Monte Carlo methods were used to evaluate the effects of variability and uncertainty in the model input parameters on predicted levels of maternal fT4. The resulting model predictions provide a good representation of the maternal fT4 and urinary iodide levels observed in the pregnant population of the United States and other countries. The model was used to evaluate the effects of various perchlorate exposure scenarios on the estimated distribution of maternal fT4, including perchlorate exposure distribution estimated from food sources for pregnant women in the United States. A deterministic and probabilistic model framework was successfully developed that can be used as a quantitative risk assessment tool to better understand the risk conditions of sensitive life stages for perchlorate exposure and the associated potential adverse neurodevelopmental outcomes.

Session 2: Metabolism and Excretion

Strength and Limitations of In Vitro Xenobiotic Metabolism Assays and In Silico Models

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Significant progress has been made in the use of in vitro models to explore chemical metabolism and quantitatively estimate metabolic clearance rates. For this, human liver-derived in vitro models (i.e. microsomes, S9 fractions, primary hepatocytes) and assay systems have been particularly useful tools. In addition, the commercial availability of recombinant human drug metabolizing enzymes and specific inhibitors has led to a better understanding of important metabolic clearance pathways, improved prediction of drug clearance liabilities (e.g. poor metabolizers), and better estimation of drug-drug interaction potential. In this presentation, current approaches for predicting human xenobiotic metabolism will be discussed that highlight their strengths and limitations and the need for more organotypic models that integrate xenobiotic metabolism and tissue/organ function for toxicology applications. In addition, our efforts to predict xenobiotic metabolism in silico using ADMET-Predictor with the Tox21 10k library will be discussed.

In Vitro Models for Quantitative Prediction of Hepatobiliary Clearance

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Knowledge of hepatobiliary excretion processes is important in accurately predicting the systemic, hepatic and biliary/intestinal exposure to xenobiotics and generated metabolites in vivo. Hepatic transport proteins facilitate the hepatic uptake, basolateral efflux and biliary excretion of many xenobiotics, and may be the rate-limiting step in hepatobiliary clearance. Systemic concentrations of the compound(s) of interest may not always reflect hepatocellular concentrations, which serve as the driving force for hepatic metabolism, protein induction/inhibition, biliary excretion, and efflux transporter-mediated xenobiotic interactions. Hepatocellular accumulation of xenobiotics and derived metabolites also may be an important consideration in hepatotoxicity. Numerous in vitro models are available to support hepatic transporter evaluation, including membrane vesicle-based transporter assays, recombinant cell lines, and hepatocytes in various formats.¹ Advantages and caveats of each model will be highlighted, and issues concerning data interpretation as well as the predictive value of each system will be addressed. Intracellular concentrations of xenobiotics and generated metabolites can be determined in sandwich-cultured hepatocytes from relevant species, and biliary clearance values can be quantified using B-CLEAR[®] technology. This information can be incorporated with modeling and simulation to facilitate in vitro to in vivo extrapolation. Integration of data from in vitro transporter assays and in vivo studies is essential to elucidate the factors that influence hepatobiliary disposition of a compound and to accurately predict the liability for transporter-mediated interactions and hepatotoxicity in humans. *This research was supported by NIH R01 GM41935.*

¹*In Vitro* Methods to Support Transporter Evaluation in Drug Discovery and Development. KLR Brouwer, D Kepler, KA Hoffmaster, DAJ Bow, Y Cheng, Y Lai, JE Palm, B Stieger and R Evers (on behalf of the International Transporter Consortium) *Clin. Pharmacol. Ther.*, 94:95-112, 2013.

Session 3: In Silico Modeling

Predictive Power of PBPK Modeling and In Silico/In Vitro–In Vivo Extrapolation Using GastroPlus™ and ADMET Predictor™ Software Tools

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Physiologically based pharmacokinetic (PBPK) modeling has its beginning in the 1930s and has significantly evolved in recent years. PBPK models consist of two distinct parts: anatomical and compound-specific. The anatomical part utilizes mathematical description of the whole body defined as a number of tissues connected by blood flow. The tissues are described by physiologically relevant parameters defining their volumes, blood flows, metabolism, transport processes, and tissue components. This part is specific to each species. The compound-specific part consists of a compound's physicochemical properties that determine its absorption, distribution and elimination.

GastroPlus™ is a mechanistically based PBPK modeling and simulation software package that simulates intravenous, oral, oral cavity, ocular, pulmonary, and dermal/subcutaneous absorption, pharmacokinetics, and pharmacodynamics in human and animals. It utilizes the ACAT™ (Advanced Compartmental Absorption and Transit) model that accounts for pH-dependent solubility, ionization effects, precipitation, bile salt effects, chemical degradation, saturable metabolism, and influx/efflux transport along the gastrointestinal tract to simulate the intestinal absorption of xenobiotics. The software offers parameter sensitivity analysis and single-subject and population simulations, and the simulations can be run in single or batch mode.

ADMET Predictor™ extends the capability of GastroPlus by predicting all physicochemical, pharmacokinetic, and cytochrome P450 metabolism kinetic parameters required for the simulation. Most of these values are predicted by artificial neural network ensemble quantitative structure–activity relationship models solely from the two-dimensional structure of the molecule. The predictions are used in situations where the experimental values have not been determined.

Application of PBPK modeling to in vitro to in vivo extrapolation and in silico/in vitro to in vivo extrapolation will be discussed. Comparison of simulation results to experimental data will be provided.

In Vitro In Vivo Extrapolation and its Applications in Predicting Pharmacokinetic Population Variability

Alice Ke, Ph.D.

Simcyp, a Certara company

In a systems pharmacology paradigm, the bottom-up approach to modeling and simulation of the processes that determine the plasma concentration–time course of a chemical—namely, absorption, distribution, metabolism and excretion (ADME)—is a valuable tool in integrating available prior information and improving decision-making. Improvement in the in vitro systems which can act as surrogates for in vivo reactions relevant to ADME and advances in the understanding of the extrapolation factors (physical chemistry, biology, physiology and genetics) as well as the ability to integrate such information using mechanistic models of the human body have greatly improved our ability to conduct in vitro to in vivo extrapolation.

The key element of this approach is the separation of information on the system (i.e. human body) from that of the drug (e.g. physicochemical characteristics determining permeability through membranes, partitioning to tissues, binding to plasma proteins, or affinities towards certain enzymes and transporter proteins). Such separation allows us to develop generic pharmacokinetics models suitable for investigating various chemicals. Further, due to its extrapolation capability it facilitates predicting pharmacokinetic characteristics in a wide range of healthy or disease populations accounting for variability in age, sex, ethnicity, genetics, etc. In this presentation the principles of systems pharmacology as applied to in vitro to in vivo extrapolation and its application in predicting pharmacokinetic characteristics in various populations are discussed.