Overview and Principles Underpinning In Vitro to In Vivo Extrapolation (IVIVE)

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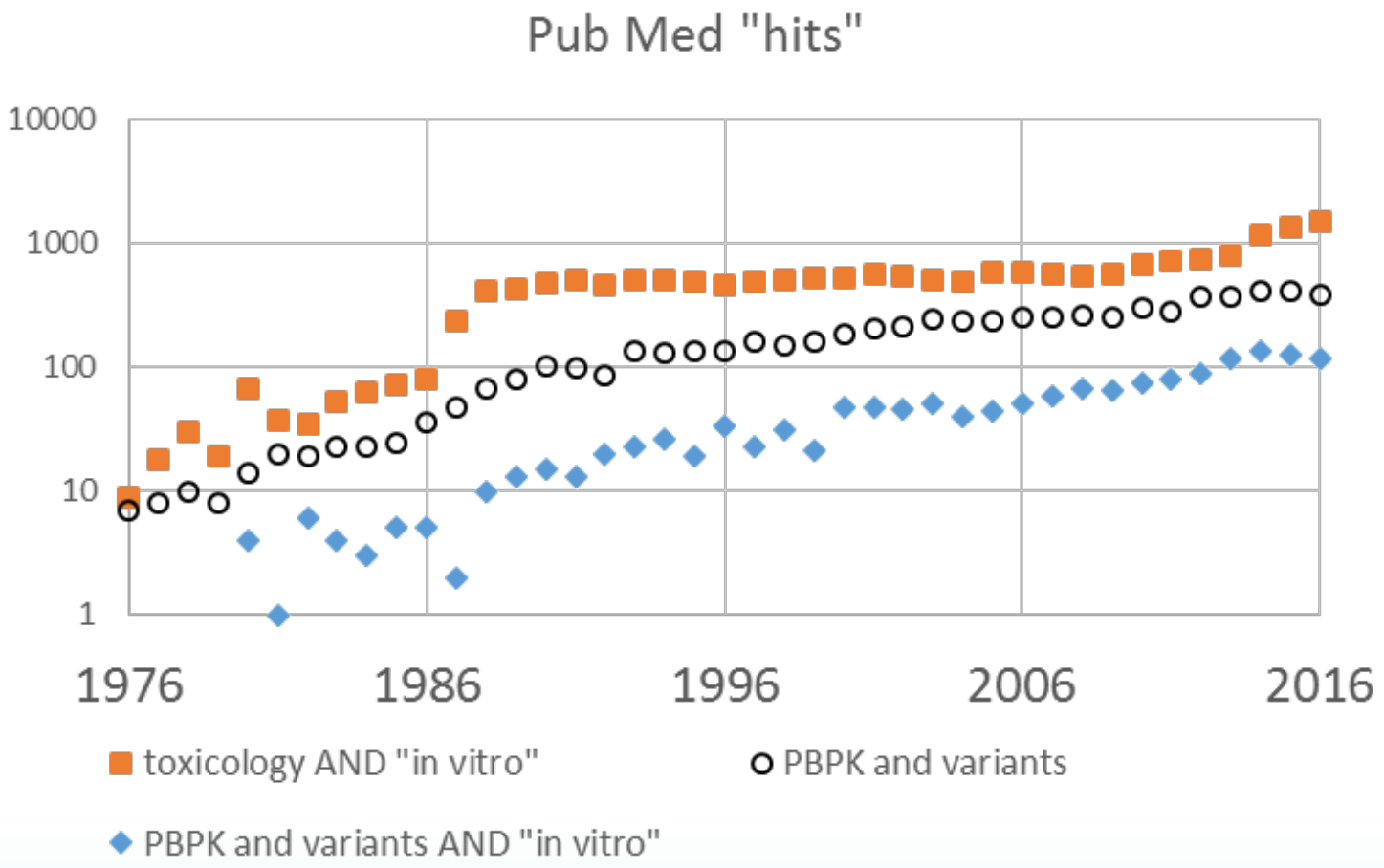
Conflict of Interest Statement

- I disclose that I have no conflicts of interest

Why This Topic?

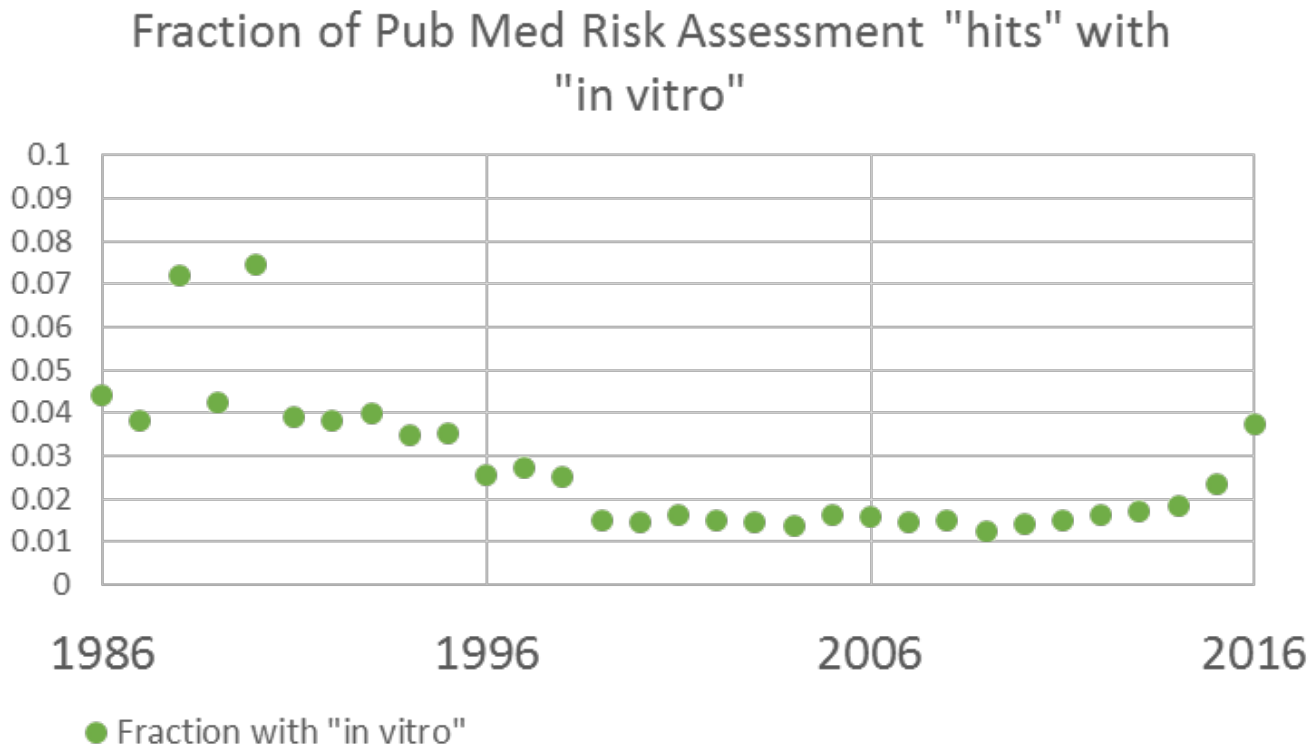
- “Methods are needed to extrapolate the concentrations found to elicit effects *in vitro* to equivalent doses in humans.”
- *In Vitro* to *In Vivo* Extrapolation (IVIVE) uses physiologically-based pharmacokinetic (PBPK) modeling to calculate oral equivalent doses (or internal circulating/target organ concentrations) in humans.
- Such IVIVE-derived doses can then be compared to modeled or measured human intakes or exposures to better understand margins of exposure.

Why now? More "*in vitro*"….



2016 metrics as of November 4, 2016

… including in Risk Assessment



2016 metrics as of November 4, 2016

Overview

- Principles of toxicology
- Physiologically based pharmacokinetic (PBPK) modeling (what, why, how; examples)
- How to evaluate models for risk-based decision making
- IVIVE
- Summary of principles underlying application of IVIVE in safety assessment

Principles and Objectives of Toxicology

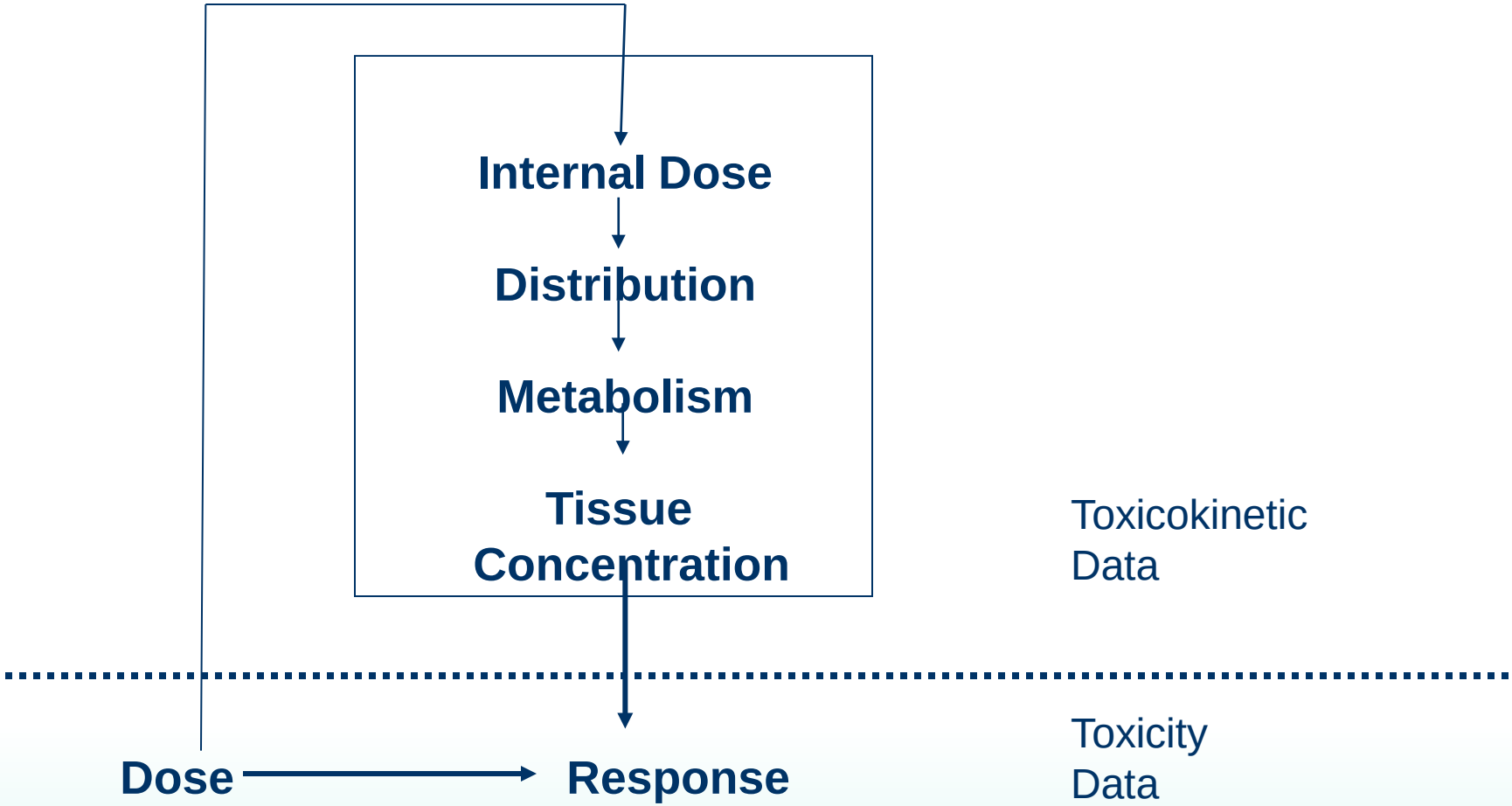
- What is toxicology? “study of poisons”
- Specifically - Toxicology is the study of the adverse effects of chemical, physical or biological agents on people, animals, and the environment
- Objective: assess nature and probability of effects on human health or the environment from exposure to toxic agents
- In synthesis with exposure assessment, the primary application of toxicology is to provide a basis for risk-based decision making

The First Law of Toxicology

The dose makes the poison

(Paracelsus, 1493-1541)

Refining Dose-Response Expression via Toxicokinetic Understanding



PBPK Model Use in Risk Assessment

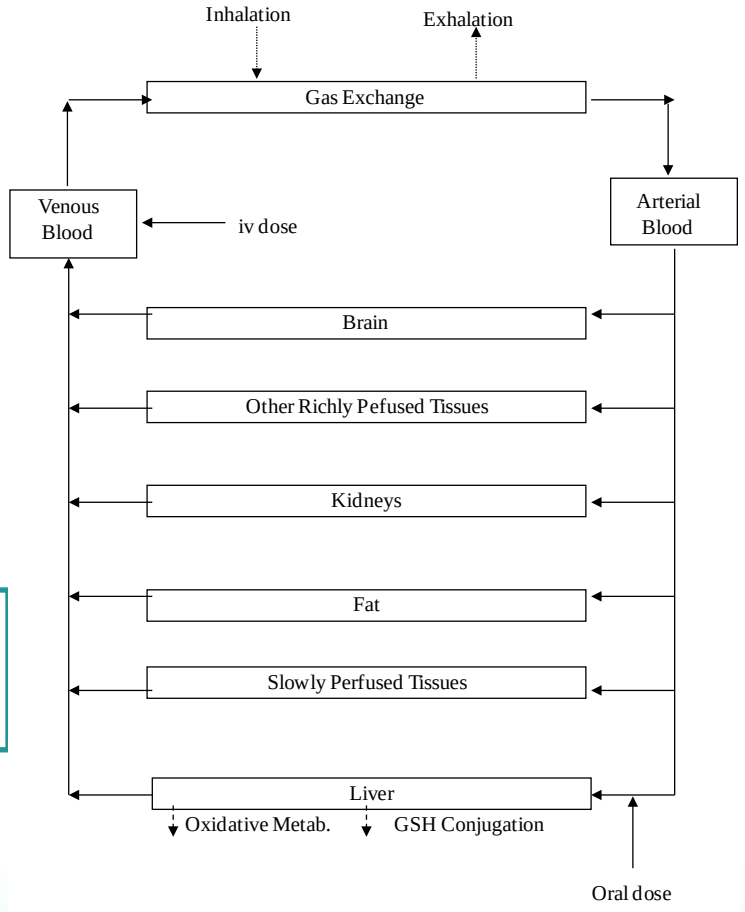
- Why would we want to use PBPK models in risk-based decision making?
  - Because chemical risk is intrinsically about “dose”—dose of an active compound at a target site.
  - PBPK modeling is a tool that describes the relationship between exposure and internal dose
  - The better the estimate of dose is, the better the characterization of the dose-response relationship.

PBPK Modeling

- **What?**
- Biology + chemistry + mathematics
  - Tissue perfusion characteristics
  - Special tissue functions (metabolism, excretion)
  - Solubility characteristics (lipophilicity)
  - Simplification of reality described via mass balance equations

- **Why?**
- Relationship of *internal* dosimetry to hazard.
- In particular: Prediction of internal dosimetry in test and target species under tested and hypothetical scenarios

- **How?**
- Lumping/splitting
- Target tissues
- Data availability (blood, tissue, saliva)



Example PBPK Modeling Equations

- Arterial blood
  - $C_a = (Q_c \times C_v + Q_p \times C_{inh})/(Q_C + Q_p/P_b)$
- Venous blood
  - $C_v = (Q_{T1} \times C_{T1} + Q_{T2} \times C_{T2} + \ldots Q_{Tn} \times C_{Tn})/ Q_C$
- Alveolar air
  - $C_{alv} = C_a/P_b$
- Metabolism
  - $dA_{met}/dt = Vmax \times C_{vT}/(Km + C_{vT})$
- Tissue
  - $V_T \times dC_T/dt = Q_T \times (C_a – C_{vT}) – dA_{met}/dt$
  - $C_{vT} = C_T/P_T$

Applications of PBPK Modeling in Risk Assessment

- Exposure assessment
  - "Reverse dosimetry"
- Dose-response modeling
  - Using internal dose to combine data from various study designs
- Route-to-route extrapolation
- Interspecies extrapolation
- Intraspecies variability
  - Subpopulations with varying susceptibility
    - Ethnicity
    - Age/lifestage
    - Health status

Example: Special Challenges of PBPK Modeling for Reproductive/Developmental Toxicity

- PBPK model application to reproductive and developmental toxicity may have particular complexity due to the dynamic nature of the test system and the exposure scenarios
- Changing physiology/anatomy
  - Fetal/neonatal development
  - Maternal changes (e.g., mammary)
  - Life-stage dependence of metabolic capacity
- Study designs
  - Methods of delivery to target change with lifestage
    - Placental transfer
    - Dietary ingestion (lactation)
    - Study-specific route of administration
  - Large number of endpoints
    - Differing modes of action
    - Differing windows of susceptibility

Case Study: Ethylbenzene

- PBPK modeling used in support of risk characterizations for the EPA Voluntary Children's Chemical Evaluation Program
- Evaluated by a peer consultation panel organized by TERA on February 22-23, 2007
- PBPK model evaluation details in report appendix, summarized in a publication (Sweeney et al., 2015)
- For more details: http://www.tera.org/Peer/VCCEP/Ethylbenzene/EBWelcome.html

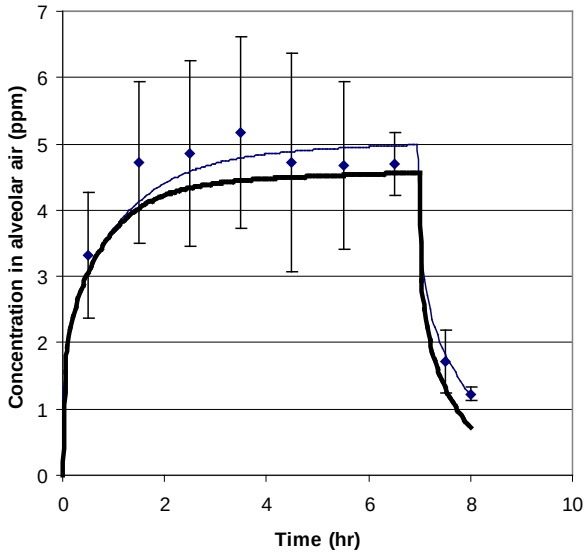
Uses of PBPK Models in EB VCCEP Assessment

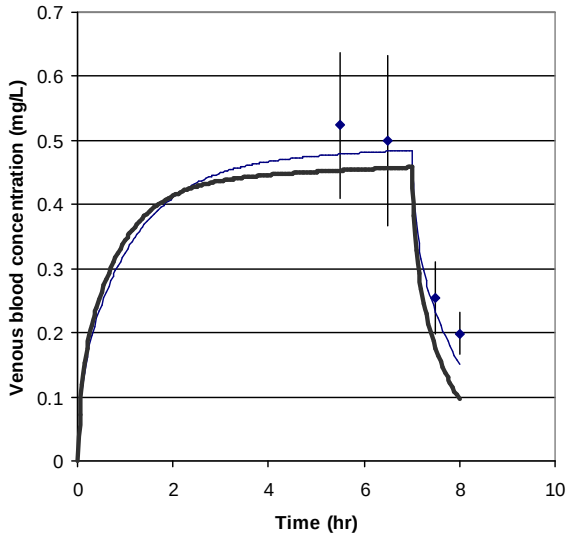
- Exposure Assessment
  - Human model used for estimation of lactational transfer of EB from mothers to infants
  - “Reality Check” for biomonitoring studies
- Used in experimental design
- Derivation of cancer and noncancer toxicity reference values based on internal dosimetry
  - Selection of “point of departure” for high-to-low dose extrapolation
  - Route-to-route extrapolation
  - Interspecies extrapolation

PBPK Models for EB

- Mouse model was developed for VCCEP program application (Nong et al., 2007)
- Models for rat and human developed previously (Tardif et al., 1997; Haddad et al., 1999, 2000; Dennison et al., 2003) were extended and modified
  - Rat model
    - Simulation of oral dosing
    - Improve simulation of high concentrations
  - Human model
    - Simulation of lactational transfer of EB to infants
    - Upper-bound estimate of formation of reactive metabolites in the lung from *in vitro* metabolism data

Human PBPK Model Fit—Initial and modified





Human exposure to 33 ppm EB (Tardif et al., 1997)
Thin line: Haddad et al. (2001) model.
Thick line: human model with scaled rat lung *in vitro* metabolism rate

Low rate of lung metabolism not likely to be identifiable from *in vivo* data, but *in vitro* data can provide bounding estimates on a lung cancer-relevant dose metric.

Model Evaluation: Introduction

- Goal: To assess model confidence for either a specific application or a spectrum of (tiered) applications
  - Prioritization vs. IRIS RfD or slope factor
  - Level of model confidence vs. acceptable margin of exposure
- We will assume a model has already been built
  - Model building is frequently iterative
  - Initial model evaluation may identify modifications required/desired for a particular purpose
- Key questions adapted from McLanahan et al. (2012)

Model Evaluation: Key Questions

- How biologically realistic is the model structure vs. how realistic does it need to be?
  - Lumping vs. splitting
- Is the model suitable for intended use? /For what uses is the model suitable?
  - Species, exposure route/scenario, suitable metrics
  - Simplified, steady-state models may not be suitable for short, dynamic life stages (e.g. pregnancy)

Model Evaluation: Key Questions

- Are the mathematical description and computational implementation adequately verifiable?
  - Reconstruction of a model from a literature description only is often challenging
- Is the model verifiable?
  - Can previous simulations be reproduced?

Model Evaluation: Key Questions

- Evaluate model performance
  - Has model been tested against all (or most) of the appropriate literature data?
    - Not all published models have been comprehensively evaluated
  - How well did the model perform?
    - How good is “good enough”?
      - One recommendation is, on average, within a factor of 2 (IPCS, 2010)
    - How well is the model known/expected to perform in the scenario of interest (e.g., low vs. high concentrations)

Model Evaluation: Key Questions

- Evaluate parameter values
  - Are values consistent with well-vetted collections?
  - Are values suitable for the scenario of interest?
    - Population: general public, workers, subpopulations, level of activity

Model Evaluation: Key Questions

- Evaluate parameters (cont'd)
  - Have the variability and/or uncertainty in the parameter values been characterized?
  - Sensitivity analysis may be very helpful in prioritizing parameters for scrutiny and will be further discussed

Model Evaluation: Key Questions

- Evaluate parameters (cont'd)
  - Are assumptions about parameters supportable?
    - Species/strain/ethnic differences minimal, or substantial?
    - Parallelogram approach (supported by values determined for another species)
    - Read across (supported by values determined for another chemical)
    - If not a "purely" predictive model (e.g., parameters were optimized), can confidence in optimized parameters be judged ("identifiability")?

Model Evaluation: Conclusions

- **Assess model applicability and confidence based on answers to previous questions and additional considerations**
  - Level of model confidence may limit application or have other implications

Sensitivity Analysis

- Sensitivity analysis involves determination of how a change in input affects the model output (prediction)
- Analysis can be done using many different approaches
- Reference point should be clearly defined
  - Sensitivity of (metric) (moiety) (compartment) (measured when) (exposed to what, when, how, how much) for what population
  - E.g., Sensitivity of the concentration of Chemical X in the venous blood after 10 years of continuous ingestion of X at the Oral Equivalent Dose by a healthy adult with no other exposure to X

Sensitivity Analyses Approaches

- Direct comparison of two groups
  - Healthy adults vs. adults with renal failure
  - People exposed for 2 years vs. 20 years
- Local sensitivity analysis
  - E.g., make a 1% change in one input parameter, determine change in output
  - Typically, the results are normalized to starting values (fractional change in output/fractional change in input) = normalized sensitivity coefficient (NSC)

$$NSC_{C:P} = \frac{\frac{C_1 - C_0}{C_0}}{\frac{P_1 - P_0}{P_0}}$$

Local Sensitivity Analysis Applications

- Prioritize parameters for uncertainty and/or variability analysis
  - Product of sensitivity and variability (or uncertainty) drives the spread of predicted possible outcomes
- Use LSA results in model variability predictions

$$CV_m = \sqrt{\sum_i ((NSC_{m:i})^2 \times CV_i)}$$

Where CV = coefficient of variation, m= model output, i = model input parameters, and inputs are normally distributed (Licata et al., 2001; Sweeney et al., 2003)

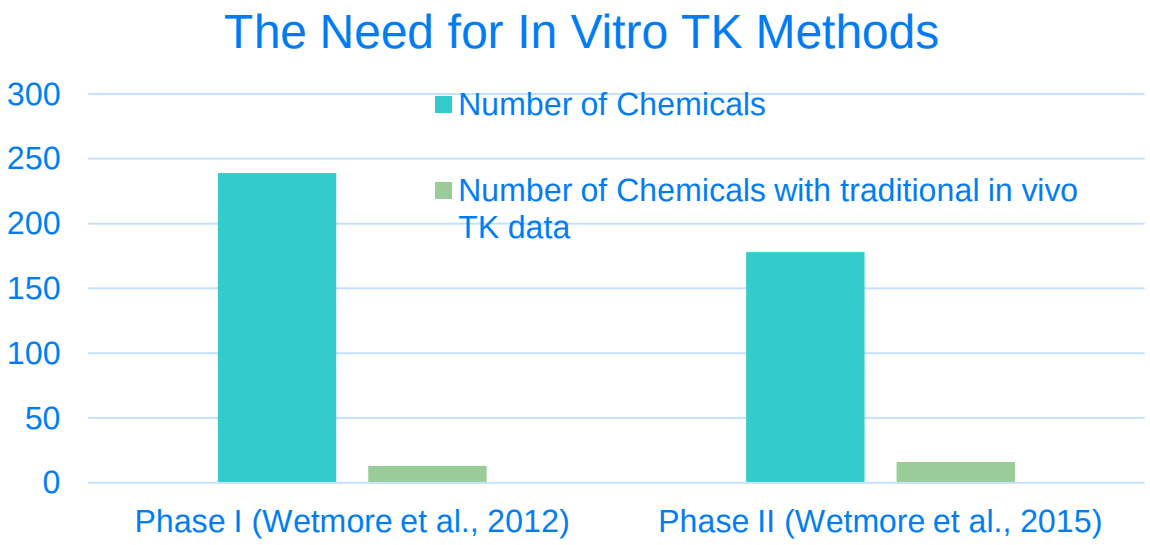
Impact of Model Sensitivity Information on Model Confidence

- Which parameters are the largest contributors to variability in model predictions?
- If key parameters were optimized/estimated, were they identifiable from fit to TK data?
- If key parameters were estimated, are the estimates supportable, and to what degree?
- With respect to IVIVE applications, are parameters determined *in vitro* key determinants of tissue dosimetry?
  - If so, what is the confidence in the methods used to derive these values?

The Need for IVIVE

- The purpose of toxicology is risk assessment
  - Risk assessment is the synthesis of exposure assessment and hazard assessment
- Those involved in ToxCast/Tox21 efforts recognized the need for context for *in vitro* effective doses (Rotroff et al., 2010; Wetmore et al., 2012, 2015)

The Need for IVIVE



- Studies like Rotroff et al. (2010), Wetmore et al. (2012, 2015) addressed the need for TK data using *in vitro* methods

IVIVE is not new: Igari et al. (1982)

- "A **physiologically based pharmacokinetic model**, which is an extension of the Bischoff-Dedrick multiorgan model, was developed to describe the kinetics of barbiturates (hexobarbital, phenobarbital, and thiopental) in the rat….
- "Michaelis-Menten constants for drug metabolism (Km, Vmax) were determined from ***in vitro* experiments using liver microsomes**.
- **"**Binding of drugs to plasma and tissue proteins was **measured *in vitro* using an equilibrium dialysis method**. **Distribution of drugs to red blood cells was measured *in vitro*.**
- **"Penetration rates** of the barbiturates **into the brain were predicted on the basis of their lipid solubilities**.
- "However, **predicted time course of drugs in plasma and brain were not in good agreement with those observed.** Therefore, the tissue to plasma distribution ratios evaluated from *in vivo* experiments were substituted for the *in vitro* values, resulting in fairly good agreement between predicted and observed values."

IVIVE History

- In vitro measurement of partition coefficients (Gargas et al., 1989 compilation)
- In vivo metabolism predicted by scaling in vitro metabolism
  - Hepatocytes: scale by cellularity (# of hepatocytes/g liver × liver weight)
  - Subcellular fractions (e.g., microsomes and cytosol): scale by yield (mg MSP/g tissue) and tissue volume
  - cDNA expressed enzymes: scale by tissue content of specific isozymes
  - Note: scaling factors may vary with demographic characteristics such as age
- Parallelogram approach used to provide confidence in human metabolism estimates in the absence of human in vivo TK data
- High(er) throughput toxicokinetics (Rotroff et al., 2010; Wetmore et al., 2012, 2015)

Simple PK models of Wetmore and co-workers

- Described in Rotroff et al. (2010), Wetmore et al. (2012, 2015) and other publications
- Determine metabolic clearance by mixed donor hepatocytes (10 donor pool; $CL_H = CL_{in\ vitro} * VH * HPGL$)
- Determine renal clearance via plasma protein binding assay (6 donor pool; $CL_R = Fub * GFR$)
- Predict steady state blood concentrations of administered compound

$CONC_{SS}$ = Dose rate * BW/Whole Body Clearance

Whole Body Clearance = $CL_H + CL_R$

Summary of Principles Underlying Application of IVIVE in Safety Assessment

- Principle No. 1: The (internal) dose makes the poison
- Principle No. 2: PBPK models are particularly well-suited to predict internal dosimetry for countless hypothetical scenarios
- Principle No. 3: Appropriate structure and parameterization are essential characteristics of any predictive model. To assess whether IVIVE models are fit-for-purpose, decision makers must understand both the specific assessment's requirements and the models' limitations.
- Principle No. 4: New higher-throughput, more physiologically-realistic in vitro assays yield parameter estimates that better reflect in vivo disposition, resulting in better model predictivity.

Additional PBPK Modeling and IVIVE Resources

- SOT FDA Symposium: Application of ADME/PK Studies to Improve Safety Assessments for Foods and Cosmetics—February 23, 2015
  - http://www.toxicology.org/events/shm/fda/fda.asp
- NTP/EPA Workshop: *In Vitro* to *In Vivo* Extrapolation for High Throughput Prioritization and Decision Making—February17-18, 2016
  - http://ntp.niehs.nih.gov/pubhealth/evalatm/3rs-meetings/past-meetings/ivive-2016/ivive-2016.html

Thank You

- Questions?
  - At this time, please limit questions to those specific to this presentation, and save general questions for the Roundtable Discussion with all the presenters.

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