High-Throughput Toxicokinetic Models
and In Vitro-In Vivo Extrapolation (IVIVE)

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US EPA CSS-HERA Board of Scientific Counselors
Chemical Safety Subcommittee Meeting

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Disclaimer: The views expressed are those of the author and do not necessarily represent the policies of the US EPA.
Many in vitro systems:

- lack consideration of biotransformation capabilities
  - Overestimation of hazard for chemicals rapidly cleared in vivo
  - Underestimation of hazard for chemicals bioactivated in vivo
- lack consideration of exposure route
- lack consideration of susceptible populations / life stages
- In vitro potency estimates are often not adjusted for chemical availability in the in vitro system (ie, in vitro disposition)

Recent Agency Case Study Finding:

TK data availability rate limiting factor in TSCA screening for chemical prioritization

*A Proof-of-Concept Case Study Integrating Publicly Available Information to Screen Candidates for Chemical Prioritization under TSCA*
**In Vitro-In Vivo Extrapolation (IVIVE)**

### I. In Vitro Toxicokinetic Assays

IVIVE originally used and vetted in pharma applications
HT-IVIVE approach uses
- hepatic clearance
- plasma protein binding
- conservative assumptions
Predictions consistently protective of human health

**Ongoing efforts will:**
- Incorporate additional TK inputs for better predictivity
- Assess impact of transporter involvement
- Evaluate extent of population variability
- Employ experimental measures to develop predictive tools
**In Vitro-In Vivo Extrapolation**

**II. Physiologically-based Toxicokinetic Modeling**

“httk”: Open-source modeling package

Modeling Platform incorporates:
- **chemical-specific** inputs (TK data, physico-chemical)
- **physiologic** inputs (blood flow rates, tissue size)
  
  into *Simulations* set up for:
  - **populations** of interest
  - **exposures** of interest
    - Capturing **variability** (within or across populations)

Based on variations in the physiologic inputs (Monte Carlo)

Pearce *et al.*, 2017, *J Statistical Software*

**Evolving Capabilities**

- Augmentation of PBTK models based on need
- Expanding to incorporate additional TK data (intestinal, renal compartments)
- Incorporating additional exposure routes
- Incorporating additional pathways (gestational)
- Incorporating demographic info to expand population-based info (variability)
NAMs for Prioritization

Integrating Hazard, TK, and Exposure

High throughput *in vitro* screening can be used to estimate doses needed to cause bioactivity.

Exposure intake rates can be inferred from biomarkers.

Wambaugh *et al.*, 2014
Wetmore *et al.*, 2015
Ring *et al.* (2017)
*And others...*
Toxicokinetics and IVIVE – Stakeholder Needs

Ongoing Development of Toxicokinetic and IVIVE Tools for use in NAMs

- **Primary goal:** to provide a human exposure-dose context for bioactive *in vitro* concentrations from NAMs for hazard testing
  - TK Methods across TSCA landscape – including *challenging chemistries, emerging contaminants*
  - Incorporating more exposure routes and pathways
  - Tools to characterize exposures to sensitive populations and life stages
  - Characterize *in vitro* disposition across TSCA landscape
  - Tools to identify, quantify and/or reduce sources of uncertainty
- **Secondary goal:** to provide *open-source data and models* for evaluation and use by the broader scientific community
  - Concomitant incorporation of above tools and data in HTTK package
  - Databases with *in vitro, in vivo* data for use in IVIVE evaluations, *in silico* tool development
Rapid Exposure Modeling and Dosimetry

**Predictive Tools**
- Plasma protein binding
- Hepatic clearance
- Transporter Involvement
- Isozyme Involvement

**Databases**
- *in vitro* TK data
- *in vivo* TK data (CvTdb)

**TK Data Generation**
- *in vitro*: More chemicals, chemistries
  - Species expansion (rat, human)
  - TK assay expansion (intestinal, renal)
- *in vivo*: Rat (cross-species extrapolation)

**Model Expansion**
- Multi-compartment; PBTK
- Exposure routes
- Gestational pathway
- Incorporating new TK data streams

**Refinement**
- IVIVE / IVIVC efforts
- *In Vitro* Disposition
- Best Practices

**Uncertainty / Variability Assessments**
- Bayesian approaches
- Experimental uncertainty
- Biologic variability

**HTTK: Open-Source Platform**

\[ C_{\text{oral dose rate}} = \frac{GFR \cdot F_{\text{ub}}}{Q_t + F_{\text{ub}} \cdot C_l_{\text{int}}} + \left( Q_t \cdot F_{\text{ub}} \cdot \frac{C_l_{\text{int}}}{Q_t + F_{\text{ub}} \cdot C_l_{\text{int}}} \right) \]

**Population Variability**
- NHANES; physiology
- Toxicokinetic variability
"...the EPA plans to use new approaches such as high throughput and computational approaches to explore different chemical categories of PFAS... to inform hazard characterization, and to promote prioritization of chemicals..."
- In Vitro Toxicokinetic Data Generation - Category-Based Analyses of Toxicokinetic Data

PFAS TK data: ~150 PFAS
- Hepatic clearance
- Plasma protein binding
- Renal transporter activity
→ IVIVE, modeling, TK NAMs

Preliminary set: Plasma protein binding data across 50+ PFAS
75% of PFAS: \( F_u < 0.05 \)
- Predictive Tool Development -

- In vitro TK measurements are being employed in model development and evaluation.
  - Plasma protein binding ($f_u$); hepatic clearance ($C_{l_{int}}$) underway; others to follow.

In silico predictions for $f_u$ (plasma protein binding)

This method uses nearest neighbors, and many evaluation chemicals are in training set.

Dawson et al. submitted
Pradeep et al., 2020
Tornero-Velez et al., underway
Sipes et al., 2017
Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation

Dustin F. Kapraun, John F. Wambaugh, R. Woodrow Setzberg, Richard S. Judson

1 National Center for Environmental Assessment, US Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America, 2 National Center for Computational Toxicology, US Environmental Protection Agency, Research Triangle Park, North Carolina, United States of America

Kapraun, Dustin f. et al., 2019 PLOS One

Table 1. Itemized comparison of selected publications that contain one or more formulas related to human gestation and pregnancy.

<table>
<thead>
<tr>
<th>Manuscript</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>Presents original data</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>N</td>
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<tr>
<td>Presents original compiled data set(s)</td>
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<td>N</td>
<td>N</td>
<td>Y</td>
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<td>Y</td>
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<td>Presents original models based on compiled data sets of Abduljalil et al.</td>
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<tr>
<td>(+) Employers and thoroughly describes rigorous statistical methods for parameter estimation</td>
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<td>N</td>
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<td>(+) Employers and thoroughly describes rigorous statistical methods for model selection</td>
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<td>N</td>
<td>N</td>
<td>N</td>
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<tr>
<td>(+) Presents original models for multiple maternal compartments</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>(+) Presents original models for multiple fetal compartments</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
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<tr>
<td>(+) Presents models that reflect a biologically accurate depiction of the fetal circulatory system</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<tr>
<td>(+) Presents explicit models for “rest of body” compartments that yield feasible (e.g., non-negative) values for all relevant time points</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>(+) Systematically compares original models with previously published models</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>(+) Presents models that contain errors or inconsistencies identified in the current manuscript</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

Fig 11. Maternal blood flow to the placenta vs. gestational age. The proportional-to-volume model (solid line) given by Eq. 12, the linear transition model given by Eq. 21, and two published models [8, 20, 22] are shown.
- Model Expansion -

Generic Gas Inhalation Model


- The structure of the inhalation model was developed from two previously published physiologically-based models from Jongeneelen et al. (2011) and Clewell et al. (2001)

- The model can be parameterized with chemical-specific \textit{in vitro} data from the HTTK package for 917 chemicals in human and 181 chemicals in rat

- Model was made publicly available with the release of httk v2.0.0 in February 2020
- Database Development -
CvTdb: An *In Vivo* TK Database

- EPA has developed a **public database** of **concentration vs. time data** across several species for building, calibrating, and evaluating TK models
- Effort ongoing, but to date includes:
  - 198 analytes (EPA, National Toxicology Program, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, and inhalation exposure
- Standardized, open-source curve fitting software invivoPKfit used to calibrate models to all data

https://github.com/USEPA/CompTox-ExpoCast-invivoPKfit

CvTdb Link: https://github.com/USEPA/CompTox-PK-CvTdb
R package “httk”

- Open source, transparent, and peer-reviewed tools and data for high throughput toxicokinetics (httk)
- Available publicly for free statistical software R
- Allows in vitro-in vivo extrapolation (IVIVE) and physiologically-based toxicokinetics (PBTK)
- Human-specific data for 987 chemicals
- Described in Pearce et al. (2017a)
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Specific <em>In Vitro</em> Measurements</td>
<td>Metabolism and protein binding for ~1000 chemicals in human and ~200 in rat</td>
<td>Wetmore et al. (2012, 2013, 2015), plus others</td>
</tr>
<tr>
<td>Chemical-Specific <em>In Silico</em> Predictions</td>
<td>Metabolism and protein binding for ~8000 Tox21 chemicals</td>
<td>Sipes et al. (2017)</td>
</tr>
<tr>
<td>Generic toxicokinetic models</td>
<td>One compartment, three compartment, physiologically-based oral, intravenous, and inhalation (PBTK)</td>
<td>Pearce et al. (2017a), Linakis et al. (2020)</td>
</tr>
<tr>
<td>Tissue partition coefficient predictors</td>
<td>Modified Schmitt (2008) method</td>
<td>Pearce et al. (2017b)</td>
</tr>
<tr>
<td>Variability Simulator</td>
<td>Based on NHANES biometrics</td>
<td>Ring et al. (2017)</td>
</tr>
<tr>
<td><em>In Vitro</em> Disposition</td>
<td>Armitage et al. (2014) model</td>
<td>Honda et al. (2019)</td>
</tr>
<tr>
<td>Uncertainty Propagation</td>
<td>Model parameters can be described by distributions reflecting uncertainty</td>
<td>Wambaugh et al. (2019)</td>
</tr>
</tbody>
</table>
An Experimental Evaluation of Mass Balance Models
describing in vitro partitioning and disposition

- Pilot study completed
- 20 chemical case study underway
- Chemical levels quantitated across 5 in vitro compartments

Armitage et al. 2014 PMID 25014875

Diagram of in vitro compartments

Figure 1. Conceptual representation of an in vitro test system.

Table 2. Plate Matrix

<table>
<thead>
<tr>
<th>Test Plate</th>
<th>Test Plate Barcode</th>
<th>Plating Condition</th>
<th>Exposure Duration (hr)</th>
<th>Measured Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TC000000013</td>
<td>Medium - cells</td>
<td>1</td>
<td>Medium</td>
</tr>
<tr>
<td>B</td>
<td>TC000000014</td>
<td>Medium - cells</td>
<td>1</td>
<td>Plastic</td>
</tr>
<tr>
<td>C</td>
<td>TC000000015</td>
<td>Medium + cells</td>
<td>1</td>
<td>Whole Well Wash</td>
</tr>
<tr>
<td>D</td>
<td>TC000000016</td>
<td>Medium - cells</td>
<td>6</td>
<td>Medium</td>
</tr>
<tr>
<td>E</td>
<td>TC000000017</td>
<td>Medium + cells</td>
<td>6</td>
<td>Plastic</td>
</tr>
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<td>F</td>
<td>TC000000018</td>
<td>Medium + cells</td>
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<td>Whole Well Wash</td>
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<tr>
<td>G</td>
<td>TC000000019</td>
<td>Medium - cells</td>
<td>24</td>
<td>Medium</td>
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<tr>
<td>H</td>
<td>TC000000020</td>
<td>Medium + cells</td>
<td>24</td>
<td>Plastic</td>
</tr>
<tr>
<td>I</td>
<td>TC000000021</td>
<td>Medium + cells</td>
<td>24</td>
<td>Whole Well Wash</td>
</tr>
</tbody>
</table>

In Vitro Disposition –
A Tox21 Cross Partner Project (EPA, NTP, FDA)

Preliminary Design and Data
Providing the Pieces for Prioritization

Informing TSCA

Target Population

Consumers

- ToxCast
  - HTTK Oral Route
  - Dermal Route Needed
  - Evaluation Data: NHANES
  - Human
    - ExpoCast/SEEM
    - Many Exposure Predictors

General Population

- ToxCast
  - HTTK Oral Route
  - Aerosol Route Needed
  - Evaluation Data: NHANES
  - Human
    - ExpoCast/SEEM
    - Many Exposure Predictors

Pathways Covered

- Consumer
- Ambient

Office of Research and Development
Highly Exposed and Sensitive Populations

Informing TSCA

Target Population

Consumers
- ToxCast
- HTTK Oral Route
- Dermal Route Needed
- Evaluation Data: NHANES
- Many Exposure Predictors
- Human ExpoCast/SEEM

General Population
- ToxCast
- HTTK Oral Route
- Aerosol Route Needed
- Evaluation Data: NHANES
- Many Exposure Predictors
- Human ExpoCast/SEEM

Workers
- ToxCast
- Aerosol Route Needed
- Evaluation Data: OSHA
- Occupational ExpoCast/SEEM
- HT ChemSTEER, others

Gestational
- ToxCast
- Gestational Model Needed
- Evaluation Data: NHANES
- Demographic Human ExpoCast / SEEM
- Many Exposure Predictors

Pathways Covered
- Consumer
- Ambient
- Occupational
- Multiple

Office of Research and Development
Highly Exposed and Sensitive Populations

Informing TSCA

- Consumers: ToxCast
  - HTTK Oral Route
  - Dermal Route Needed
  - Evaluation Data: NHANES, Human ExpoCast/SEEM, Many Exposure Predictors

- General Population: ToxCast
  - HTTK Oral Route
  - Aerosol Route Needed
  - Evaluation Data: NHANES, Human ExpoCast/SEEM, Many Exposure Predictors

- Workers: ToxCast
  - Aerosol Route Needed
  - Evaluation Data: OSHA, Occupational ExpoCast/SEEM, HT ChemSTEER, others

- Gestational: ToxCast
  - Gestational Model Needed
  - Evaluation Data: NHANES, Many Exposure Predictors

Informing EDSP

- Ecological (Fish): ToxCast + SeqaPass / LC50 Models
  - EPI Suite BCF*
  - HTTK Fish Needed
  - Evaluation Data: USGS Surface Water, 3 HT Models

Ecological (Fish) Informing TSCA

Pathways Covered

- Consumers: Ambient
- General Population: Ambient
- Workers: Occupational
- Gestational: Multiple
- Ecological (Fish): Ambient
TK and IVIVE Projects and Relationships

**CSS Products**

- 2.6.4: New Methods/Data
- 2.6.5: Exposure Routes
- 2.6.6: Life-stage and Sens. Pop.
- 2.6.7: QSAR Models
- 2.6.8: In Vitro Distribution
- 2.6.9: Uncertainty Experiments
- 2.6.10: Parent-Metabolite
- 2.6.11: HTTK Fish
- 2.6.12: HTTK-AOP Model

**Supporting Models/Data**

- Generic Dermal Model
- Generic Aerosol Model
- New Chemicals
- Generic Parent-Metabolite Model
- Generic Human Gestational
- Generic Aquatic Species Model
- TK/TD Model

**Outputs**

- Address Uncertainty
- Challenging Chemistries
- New Exposure Routes
- Sensitive Pop’s and Lifestages

**Applications**

- IVIVE for Gen. Pop. Risk Workflows (OPPT, OLEM, MN)
- New R Package “httk” Release
- Occupational Risk IVIVE
- Ecological Risk IVIVE

Office of Research and Development
In Vitro Bioactivity, HTTK, and In Vivo Toxic Doses

For ~89% of the chemicals, POD\textsubscript{NAM} was conservative. (~100-fold on average), but less conservative than a TTC

Chemicals where POD\textsubscript{NAM} was not conservative enriched in OPs/carbamates

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA

Paul-Friedman \textit{et al.} 2020
Additional Efforts and Outreach

**Additional Efforts**

- **In vitro TK data generation**: Ongoing, internal (>400 TSCA, incl. 150 PFAS) and external (>215); as needed on program office-initiated efforts (Office of Chemical Safety and Pollution Prevention, Office of Water)
- **In vivo TK**: rat in vivo studies for comparative assessments and IVIVE evaluation (Hughes *et al.*, underway)
- **Dermal Route**: permeability/partitioning models completed (Evans *et al.*); integration with HTTK begun
- **Bioavailability**: incorporation of Caco-2 data in IVIVE (Honda *et al.*, 2019; Honda *et al.*, in preparation)
- **Transporters**: TK renal transporter data generation for PFAS IVIVE modeling (Smeltz *et al.*, underway)
- **Sensitive Populations/Variability**: Isozyme-specific chemical evaluations to evaluate TK variability and supply *in silico* predictive efforts (Kreutz *et al.*, underway); Correlated Monte Carlo approach to incorporate physiologic variability (Ring *et al.*, 2017)
- **Parent-Metabolite HTTK**: NTA data for metabolism of ToxCast chemicals generated by contractor and being analyzed (Boyce *et al.* underway)

**Stakeholder Outreach and Collaborations**

- CompTox Chemicals Dashboard: Contains ADME data for >1000 chemicals.
- FIFRA SAP “The use of new approach methodologies (NAMs) to derive extrapolation factors and evaluate developmental neurotoxicity for human health risk assessment” - Incorporation of *in vitro* TK / HTTK
- Integration of high throughput hazard, exposure, and TK NAMs into proposed TSCA workflows (white paper, peer review)
- APCRA Collaborations – HTTK case study (underway) and NAM prospective case study (underway)
- Ongoing collaborations with Health Canada, US Geological Survey, and MN Department of Health
References


Office of Research and Development
ExpoCast Project
(Exposure Forecasting)

Center for Computational Toxicology and Exposure

Linda Adams
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