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

US EPA CSS-HERA BOSC Meeting – February 2-5, 2021

CSS Session 1B Slides

The work presented within represents US EPA Office of Research and Development research activities. Material includes both peer reviewed, published results and work-in-progress research. Please do not cite or quote slides.

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United States Environmental Protection Agency

ExpoCast
exposure forecasting

High Throughput Exposure Models
and the Systematic Empirical Evaluation of Models (SEEM) Framework

John Wambaugh

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

February 2-5, 2021

Stakeholder Need
as stated in research plan

Chemical exposure scenarios and pathways:

Chemical evaluations require information to estimate exposure via a variety of high-priority pathways, including scenario-specific data and models particular to consumer products and materials in the indoor environment, as well as occupational, ambient and ecological pathways.

Exposure Pathways

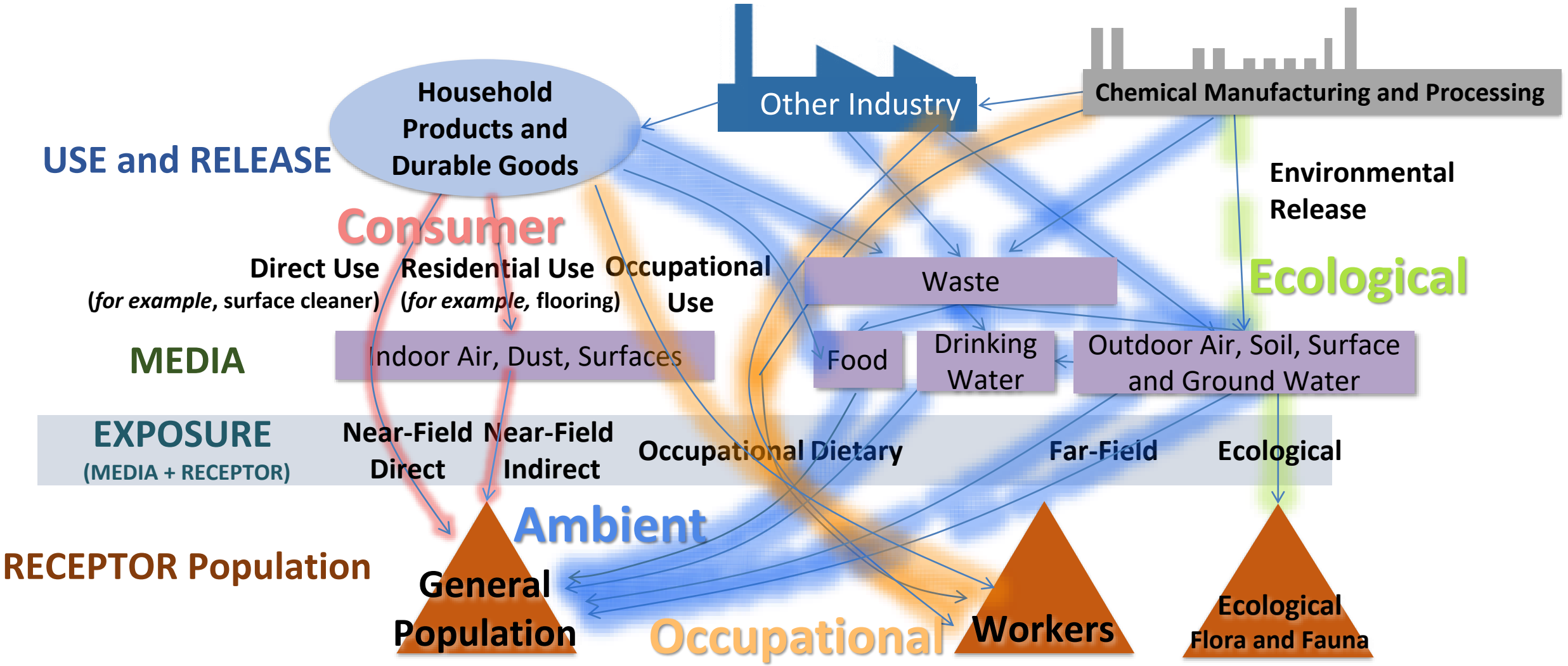


Figure from Kristin Isaacs

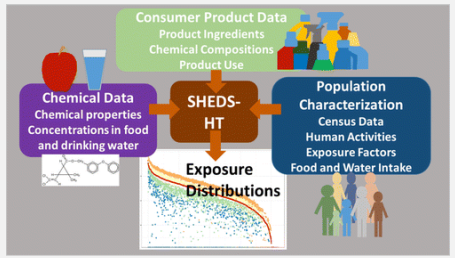
Properties of High-Throughput Exposure Models

1) Capable of handling **many chemicals with minimal descriptive information**
2) Cover **one or more relevant exposure routes**
3) **Allow for integration** with models for other pathways
4) Scientifically plausible
5) Allow for the assessment of interindividual and intraindividual variation in exposure
6) **Amenable to integration within statistical frameworks that quantify uncertainty**
7) **No more complicated than necessary**

Existing HT Models for Key Pathways

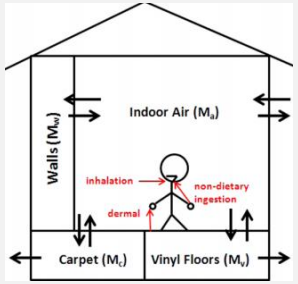
Consumer (Near-Field) Pathways

SHEDS-HT (Isaacs et al., 2014)



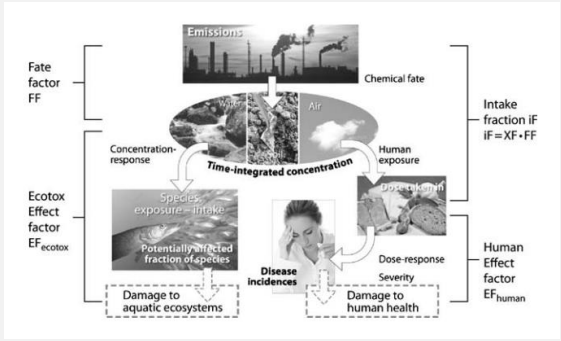
RAIDAR-ICE (Li et al., 2018)

FINE (Shin et al., 2015)

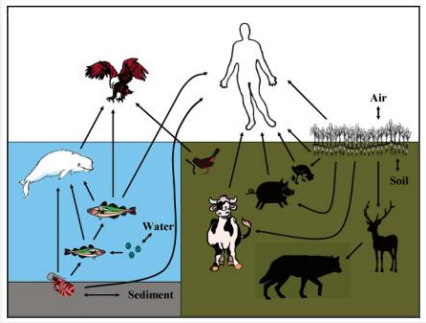


Ambient (Far-Field) Pathways

UseTox (Rosenbaum et al., 2008)

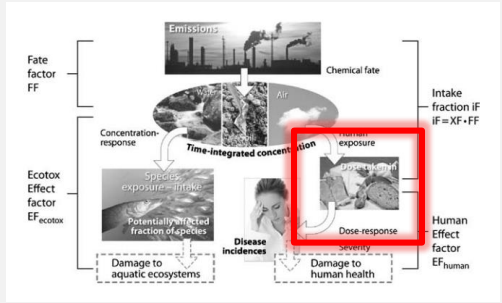


RAIDAR (Arnot et al., 2006, 2008)



Dietary Pathways

***UseTox** (Rosenbaum et al. (2008)*



SHEDS-HT (Biryol et al., 2017)

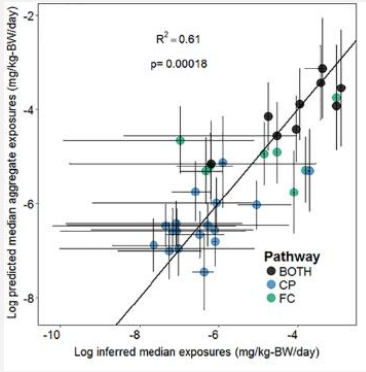
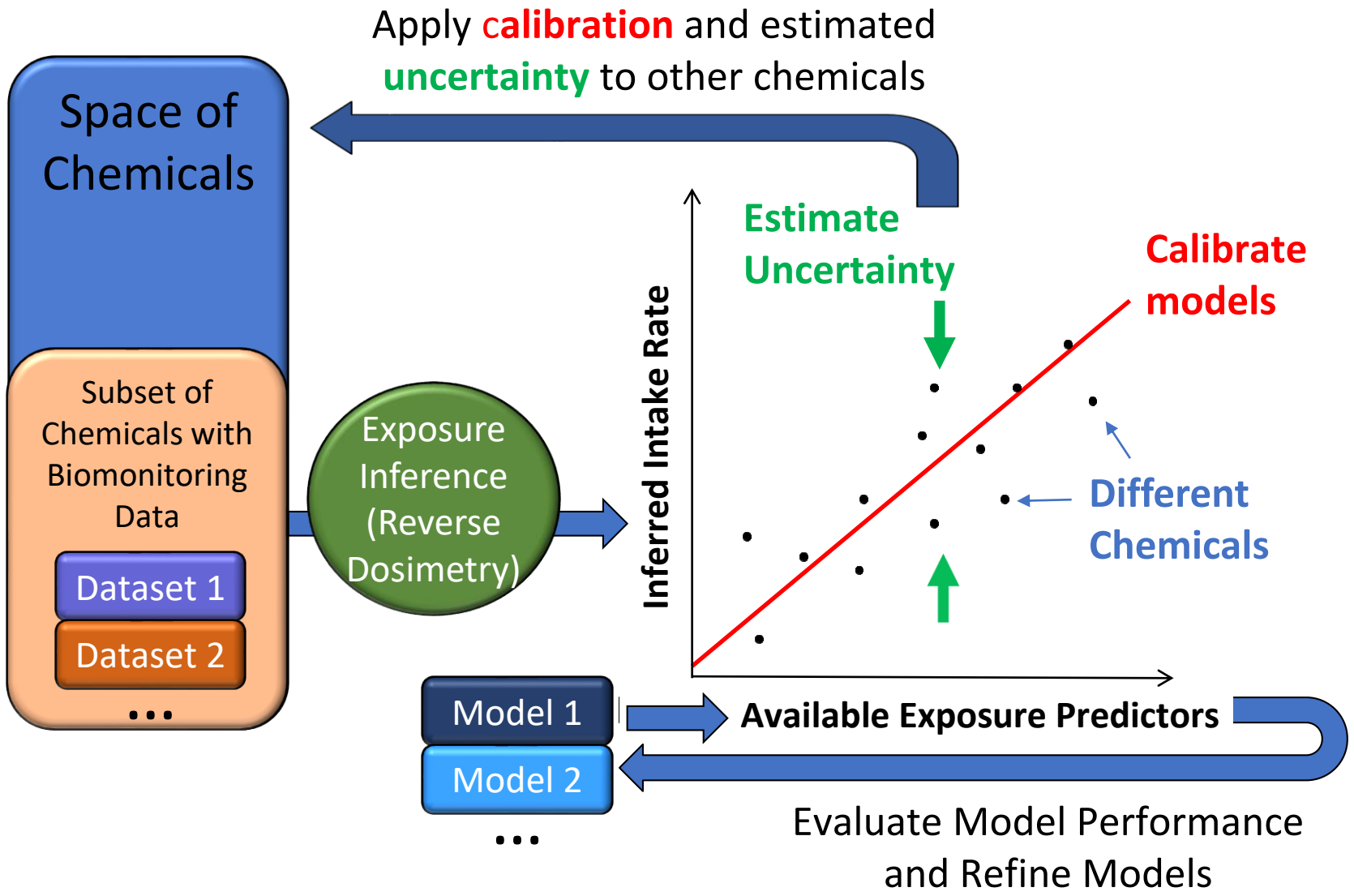


Figure from Kristin Isaacs

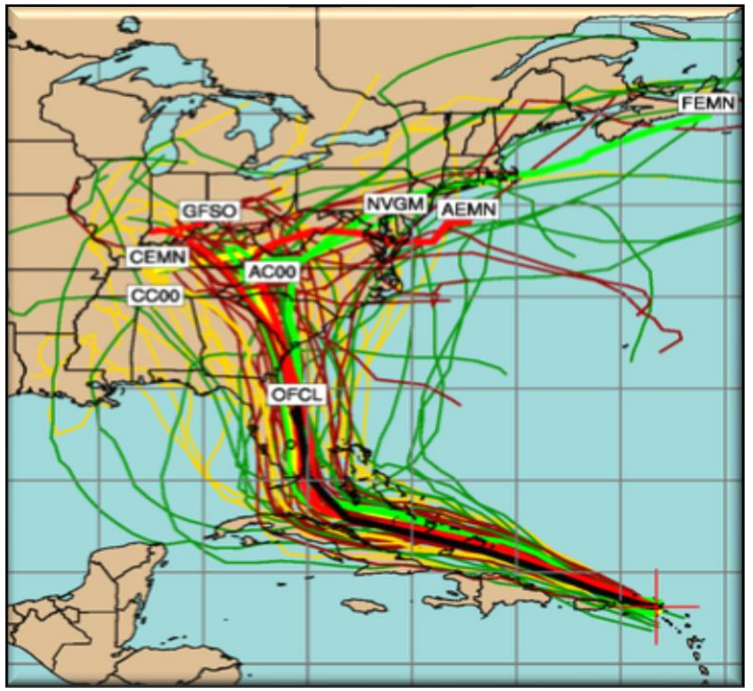
Consensus Exposure Predictions with the SEEM Framework

- Different exposure models incorporate **knowledge**, **assumptions**, and **data** (MacLeod et al., 2010)
- We incorporate multiple models (including SHEDS-HT, USEtox, RAIDAR) into consensus predictions for 1000s of chemicals within the **Systematic Empirical Evaluation of Models (SEEM)** (Wambaugh et al., 2013, 2014, Ring et al., 2019)
- Evaluation is like a sensitivity analysis: What models are working? What data are most needed?



Ensemble Predictions

- We can use ensemble methods to make more stable models and characterize uncertainty
- "Ensemble methods are learning algorithms that construct a set of classifiers and then classify new data points by taking a (weighted) vote of their predictions." Dietterich (2000)
- Ensemble systems have proven themselves to be very effective and extremely versatile in a broad spectrum of problem domains and real-world applications (Polikar, 2012)
- Ensemble learning techniques in the machine learning paradigm can be used to integrate predictions from multiple tools. Pradeep (2016)



Hurricane Path Prediction is an Example of Integrating Multiple Models

SEEM3 Collaboration

Jon Arnot, Deborah H. Bennett, Peter P. Egeghy, Peter Fantke, Lei Huang, Kristin K. Isaacs, Olivier Jolliet, Hyeong-Moo Shin, Katherine A. Phillips, Caroline Ring, R. Woodrow Setzer, John F. Wambaugh, Johnny Westgate

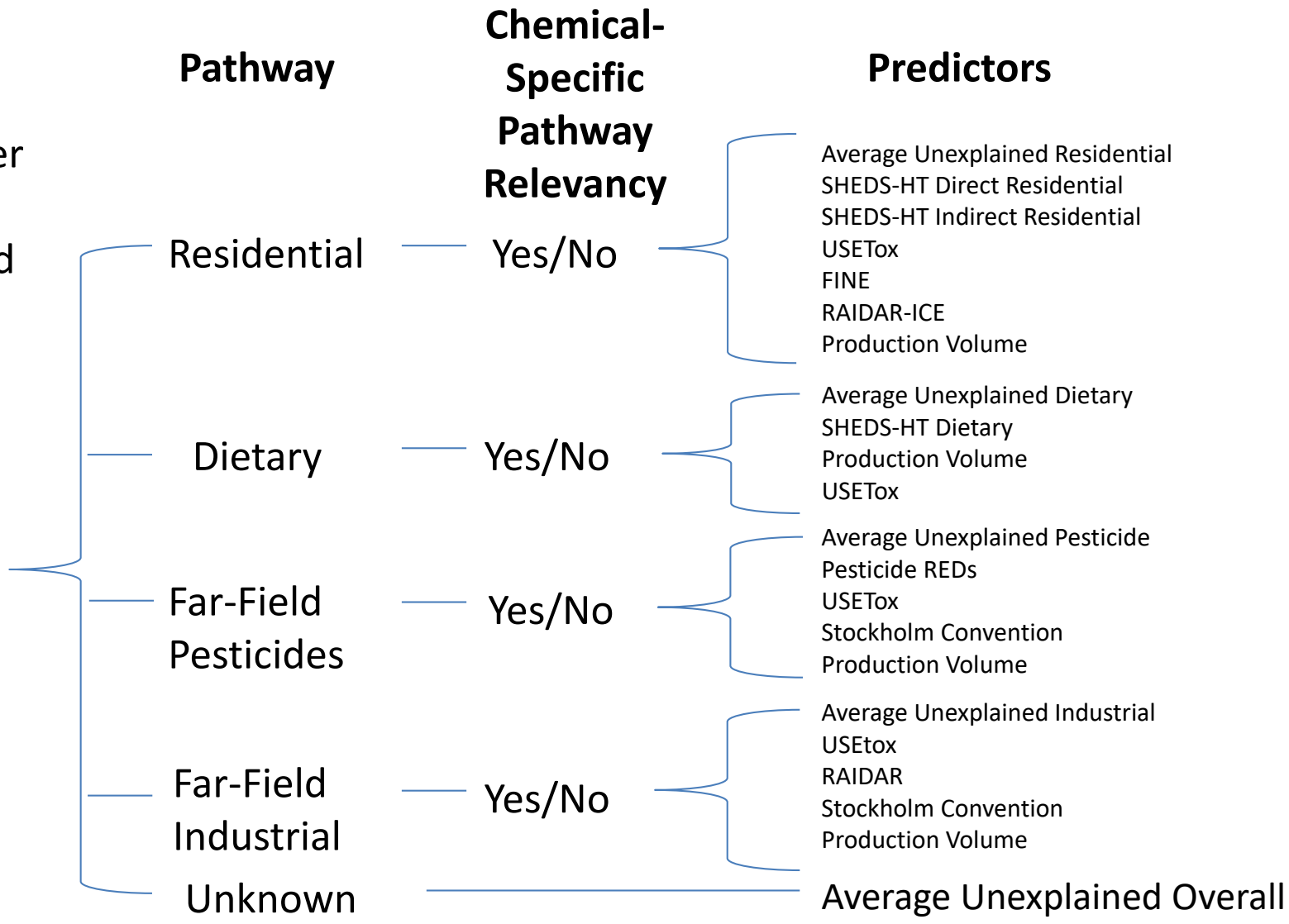
Predictor	Reference(s)	Chemicals Predicted	Pathway(s)
EPA Inventory Update Reporting and Chemical Data Reporting (CDR) (2015)	US EPA (2018)	7856	All
Stockholm Convention of Banned Persistent Organic Pollutants (2017)	Lallas (2001)	248	Far-Field Industrial and Pesticide
EPA Pesticide Reregistration Eligibility Documents (REDs) Exposure Assessments (Through 2015)	Wetmore et al. (2012, 2015)	239	Far-Field Pesticide
United Nations Environment Program and Society for Environmental Toxicology and Chemistry toxicity model (USEtox) Industrial Scenario (2.0)	Rosenbaum et al. (2008)	8167	Far-Field Industrial
USEtox Pesticide Scenario (2.0)	Fantke et al. (2011, 2012, 2016)	940	Far-Field Pesticide
Risk Assessment IDentification And Ranking (RAIDAR) Far-Field (2.02)	Arnot et al. (2008)	8167	Far-Field Pesticide
EPA Stochastic Human Exposure Dose Simulator High Throughput (SHEDS-HT) Near-Field Direct (2017)	Isaacs (2017)	7511	Far-Field Industrial and Pesticide
SHEDS-HT Near-field Indirect (2017)	Isaacs (2017)	1119	Residential
Fugacity-based INdoor Exposure (FINE) (2017)	Bennett et al. (2004), Shin et al. (2012)	645	Residential
RAIDAR-ICE Near-Field (0.803)	Arnot et al., (2014), Zhang et al. (2014)	1221	Residential
USEtox Residential Scenario (2.0)	Jolliet et al. (2015), Huang et al. (2016,2017)	615	Residential
USEtox Dietary Scenario (2.0)	Jolliet et al. (2015), Huang et al. (2016), Ernstoff et al. (2017)	8167	Dietary

Ring et al. (2018)

SEEM3 Considers Pathway of Exposure

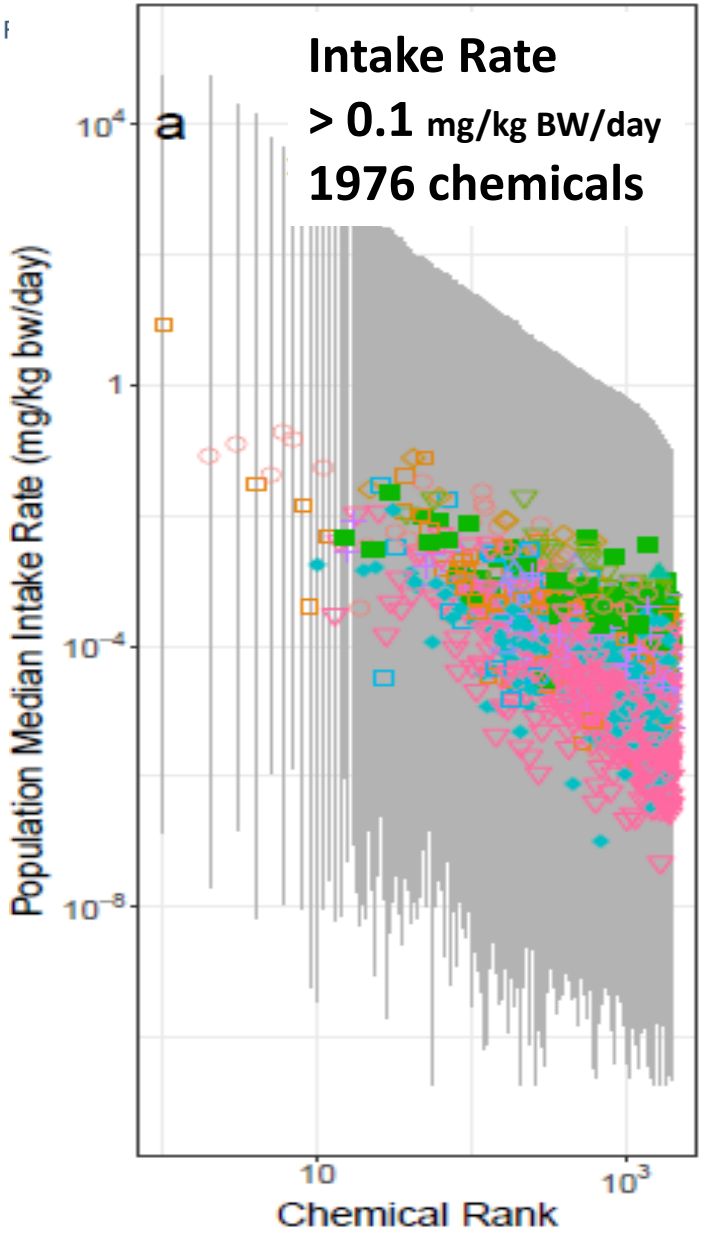
We organize models by the exposure pathways they cover

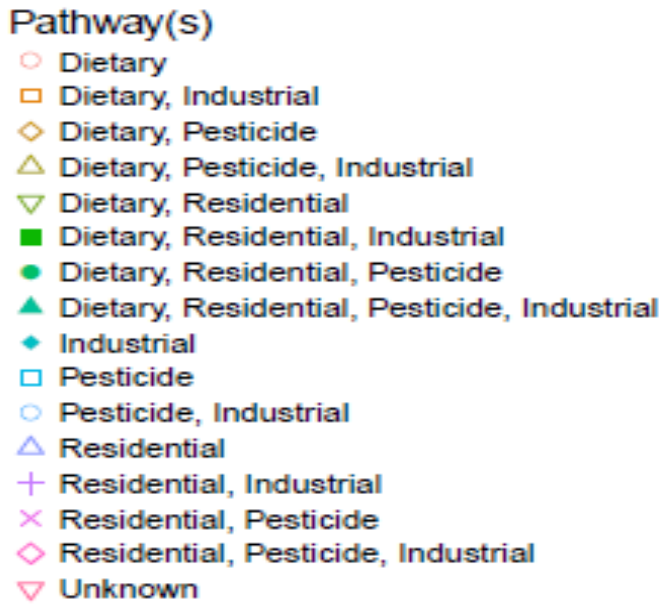
We calibrate predictors based on ability to explain median NHANES exposure rates



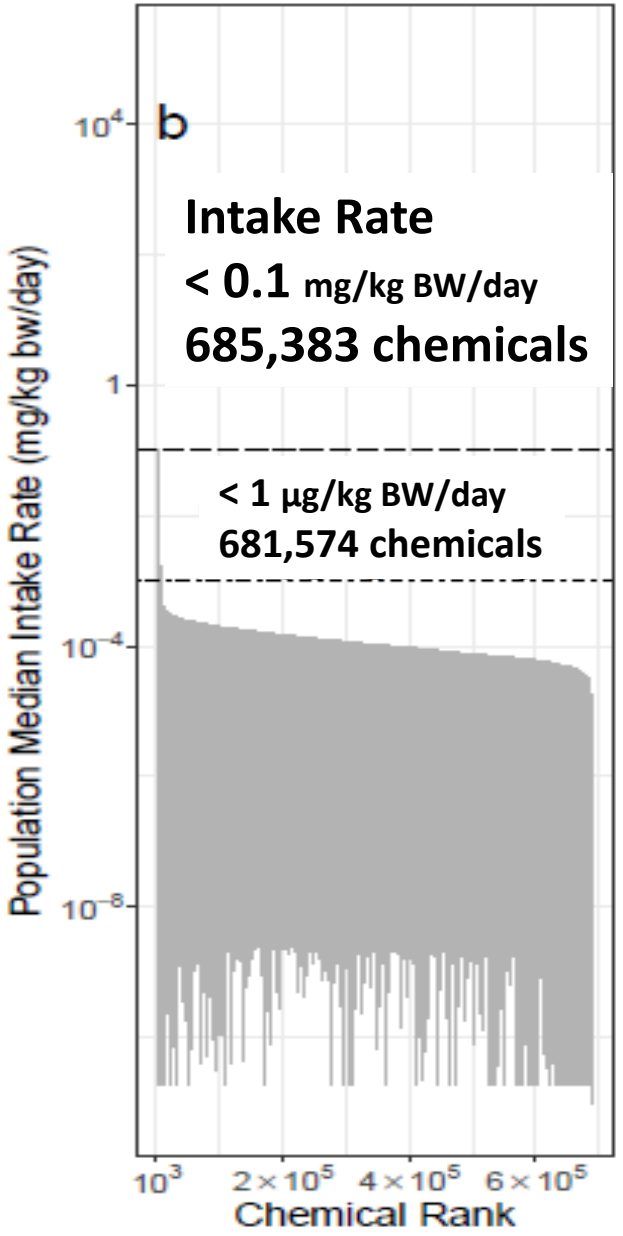
Ring et al. (2018)

Consensus Modeling of Median Chemical Intake





Of 687,359 chemicals evaluated, 30% have less than a 50% probability for exposure via any of the four pathways and are considered outside the "domain of applicability"



Ring et al. (2018)

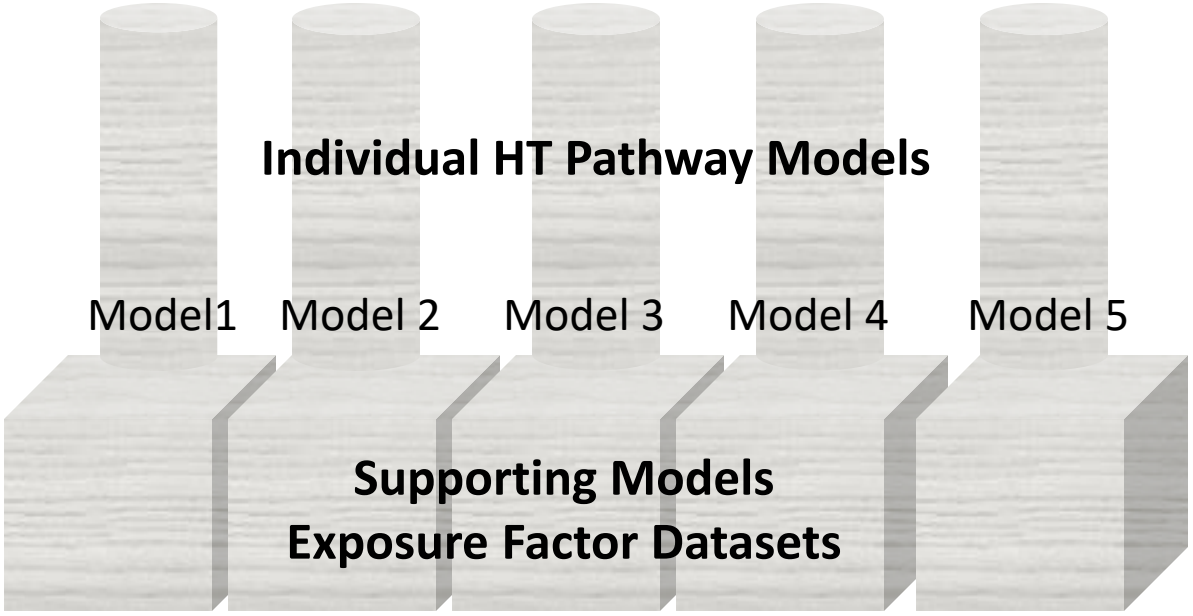
ExpoCast SEEM Models: Required Building Blocks for the Output

Machine-learning models for filling gaps from structure when no data are available

Composition and use/release data

Slide from Kristin Isaacs

ExpoCast SEEM Models: Required Building Blocks for the Output



for example, SHEDS-HT, HT ChemSteer, external models

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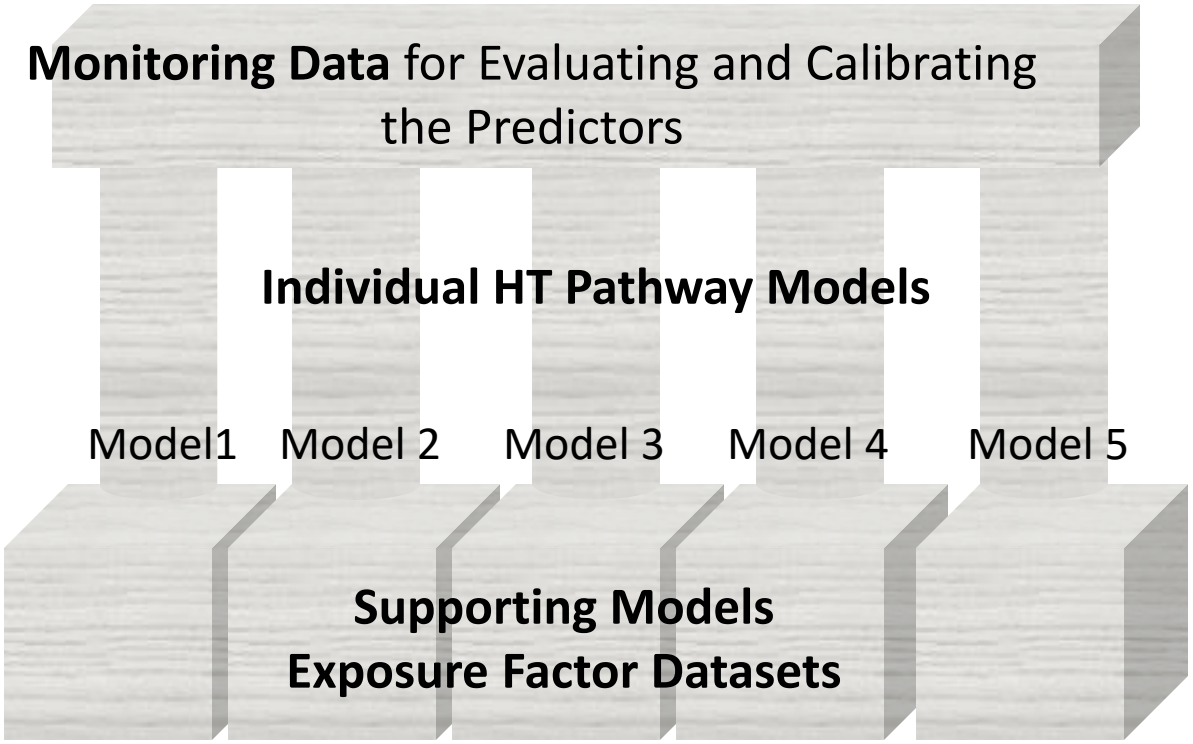
ExpoCast SEEM Models: Required Building Blocks for the Output



Including NHANES biomonitoring and USGS water datasets

for example, SHEDS-HT, HT ChemSteer, external models

Machine-learning models for filling gaps from structure when no data are available

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Slide from Kristin Isaacs

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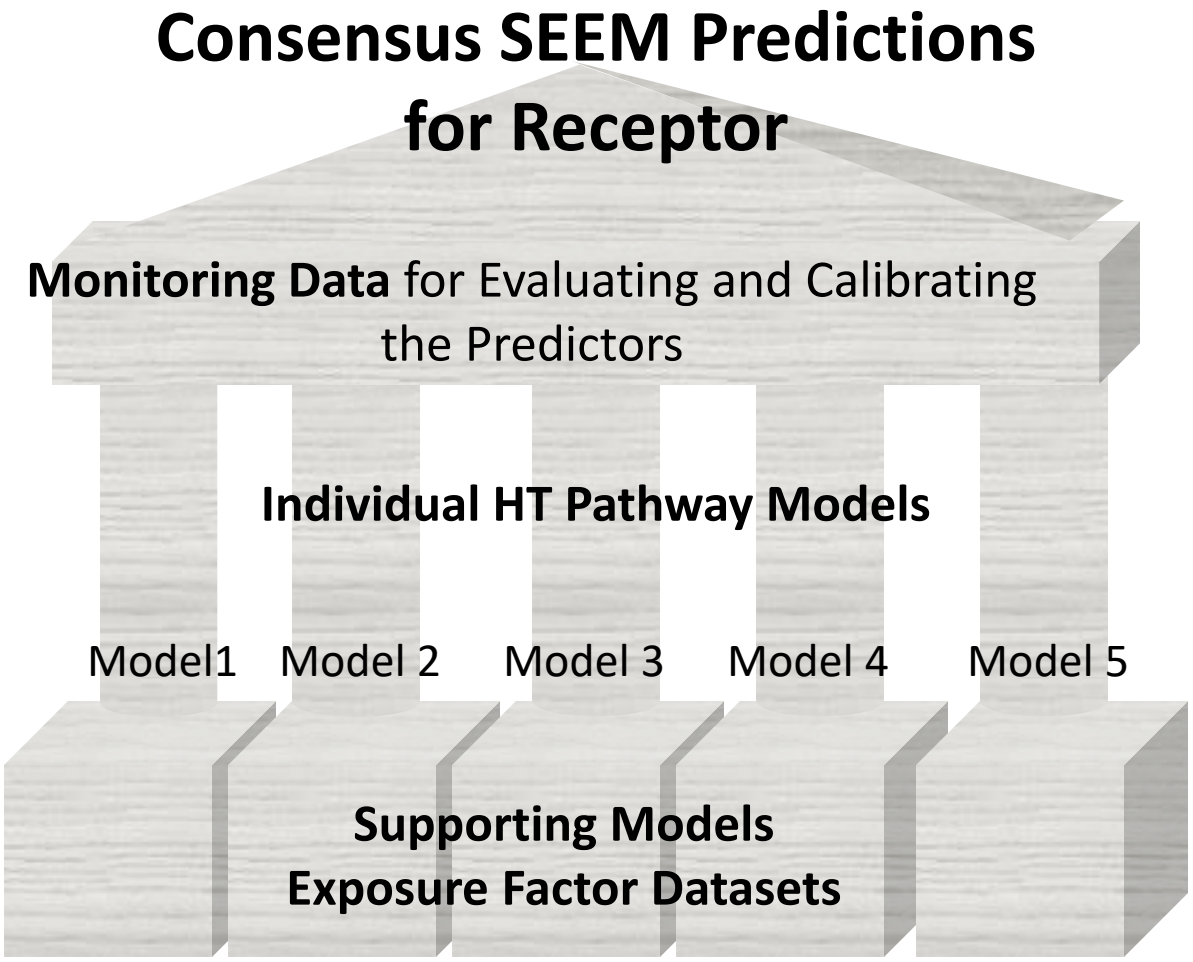

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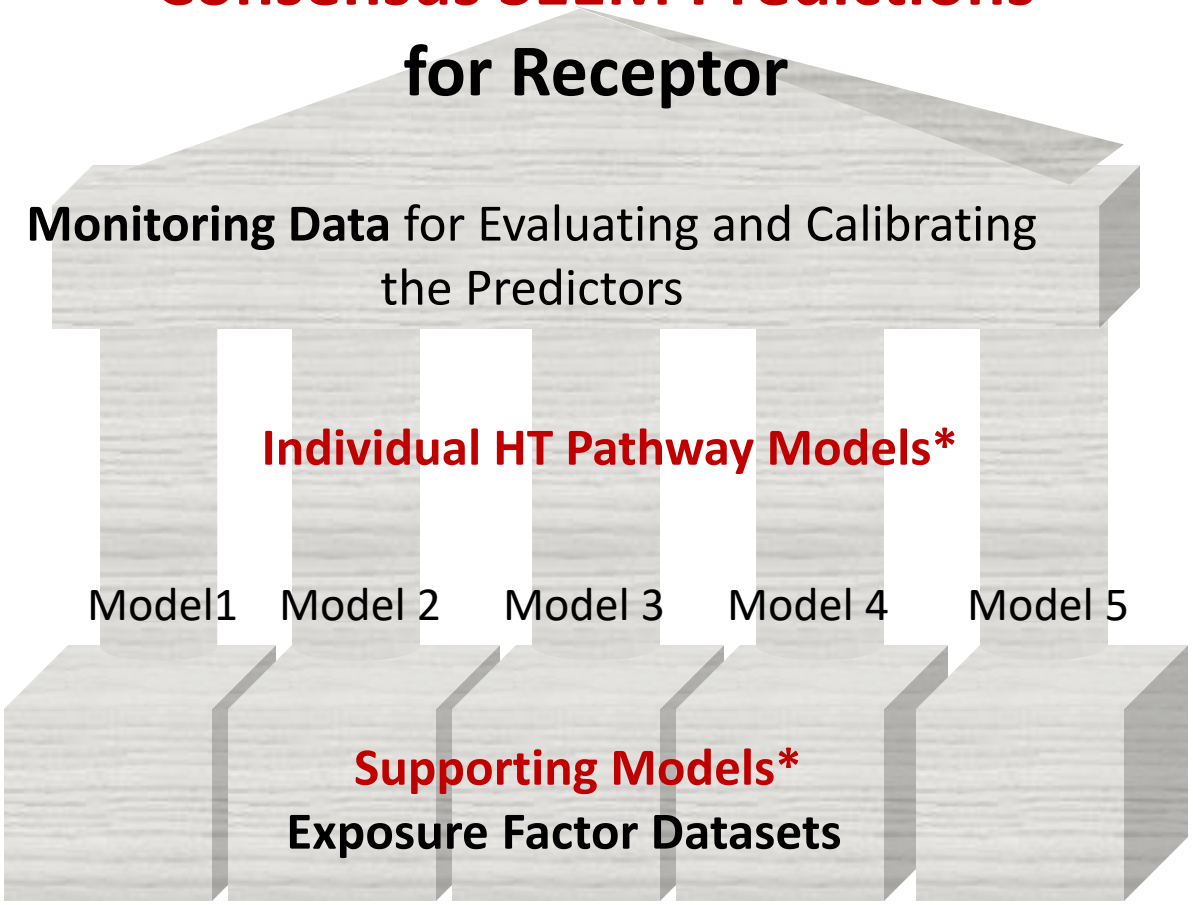


***New Approach Methodologies for Exposure**:
Application to Real Decision Contexts

Including NHANES biomonitoring and USGS water datasets

for example, SHEDS-HT, HT ChemSteer, external models

Machine-learning models for filling gaps from structure when no data are available

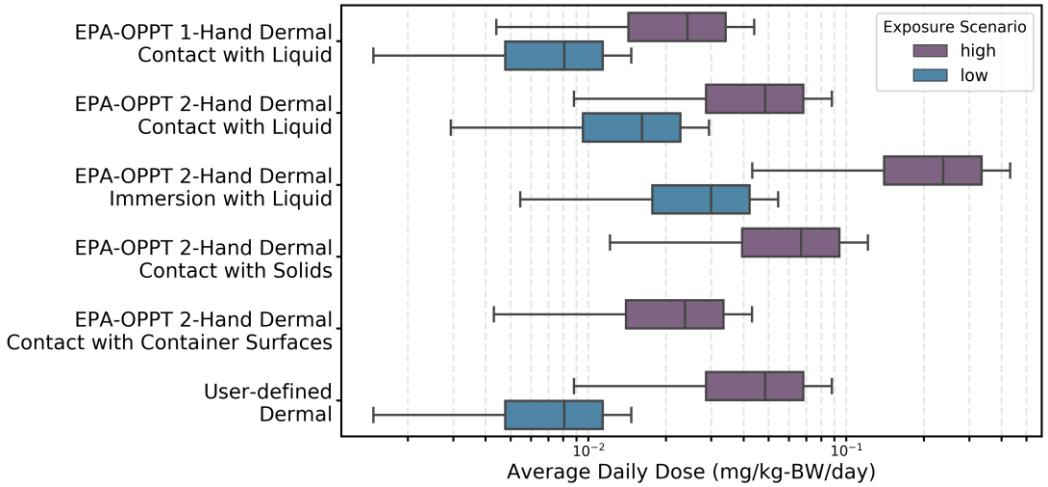
Composition and use/release data

Slide from Kristin Isaacs

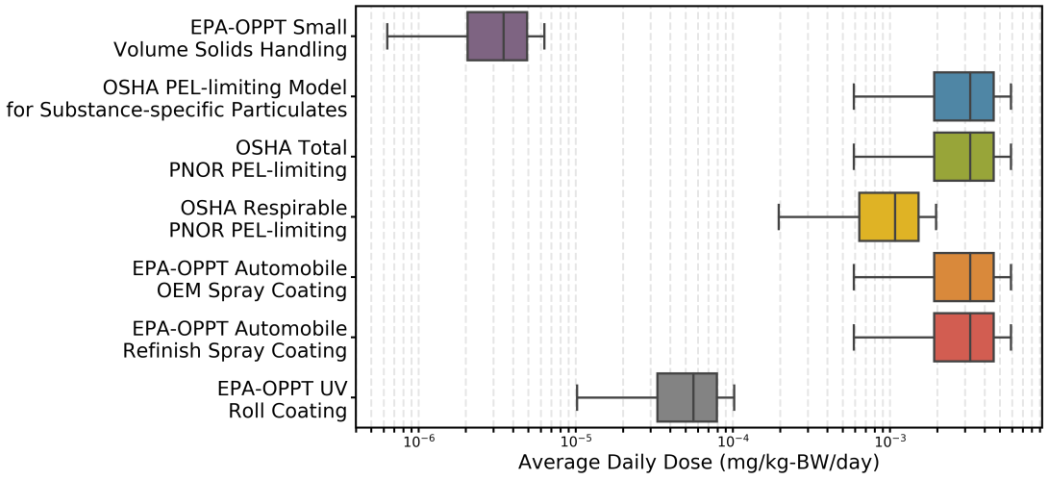
Formatting Occupational Exposure Models for HT Use

- We have developed consensus models for consumer and some ambient pathways, but ecological and occupational consensus models are ongoing
- Many predictors for these pathways exist, but they are not typically oriented for high throughput capacity, for example EPA's ChemSTEER (Chemical Screening Tool for Exposures and Environmental Releases)
- Command Line Occupational Exposure Tool (CLOET) a command line tool that allows use of ChemSTEER v3.0 in a high throughput manner
- Multiple scenarios for each model have been run and tested against ChemSTEER GUI to test for model fidelity.

Dermal Models



Inhalation Models

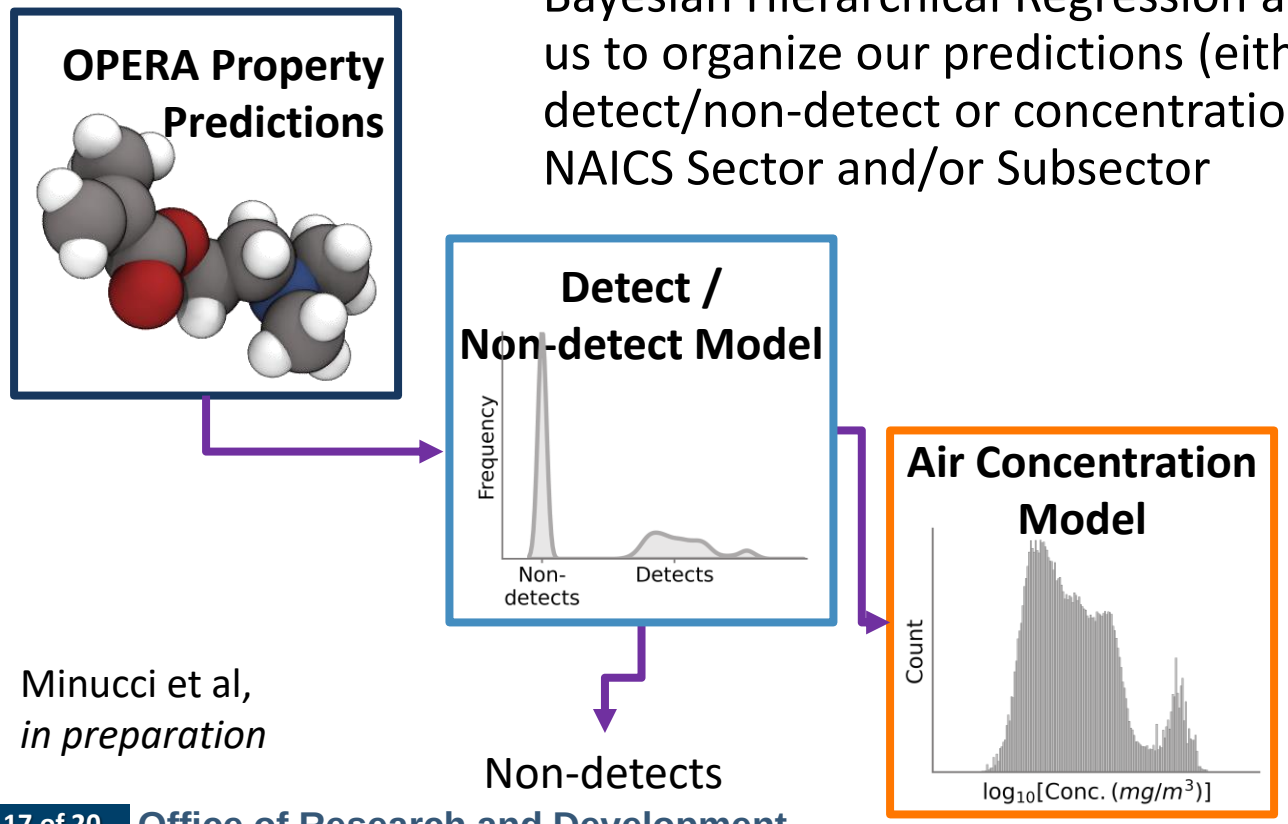


Concentrations were varied from 0.1 to 1

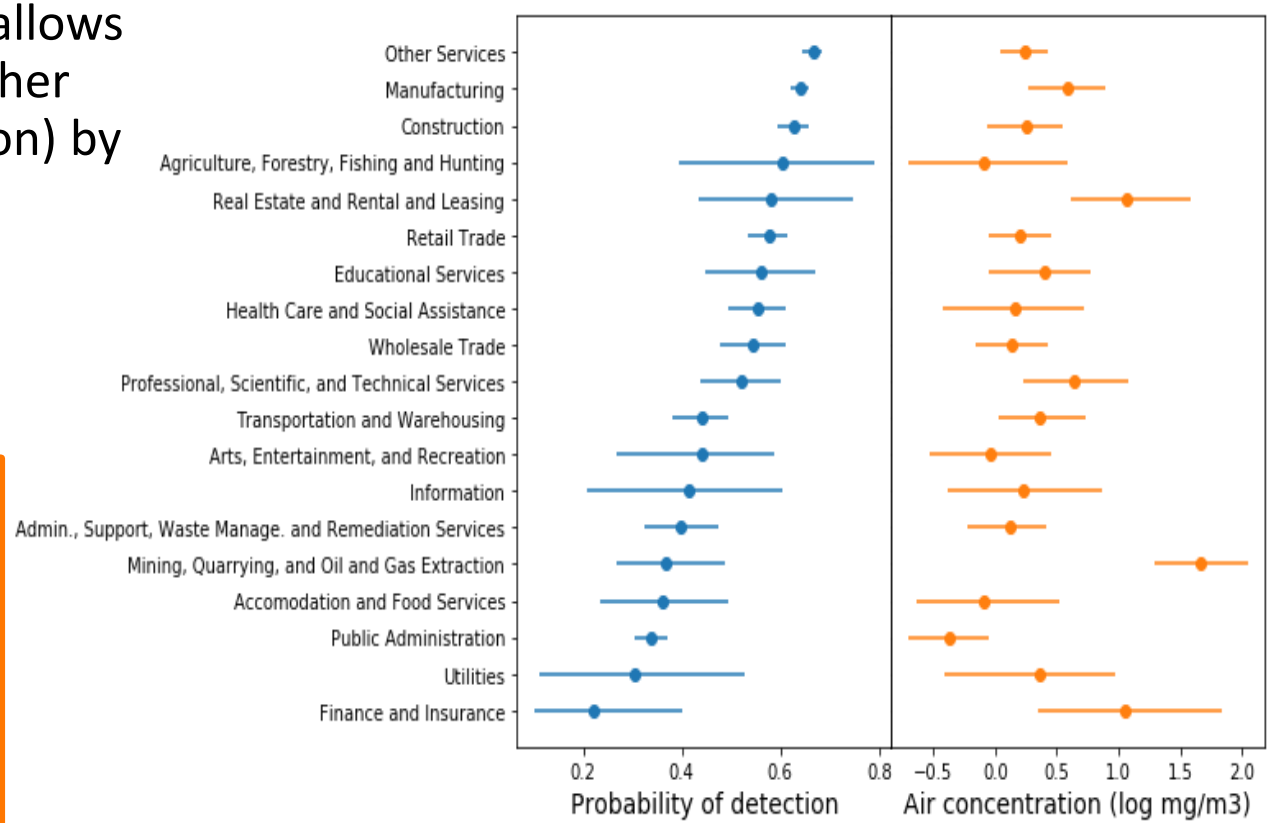
Slide from Katherine Phillips

Two-Stage Occupational Exposure Model

- OSHA's chemical exposure health data set for air samples was used to build a two-stage model that predicts 1) if a chemical is likely to be detected in air and 2) what the likely concentration would be
- OPERA physicochemical property distributions across NAICS sector and subsectors are included as input distributions to the models in addition to the OSHA data
- Bayesian Hierarchical Regression allows us to organize our predictions (either detect/non-detect or concentration) by NAICS Sector and/or Subsector

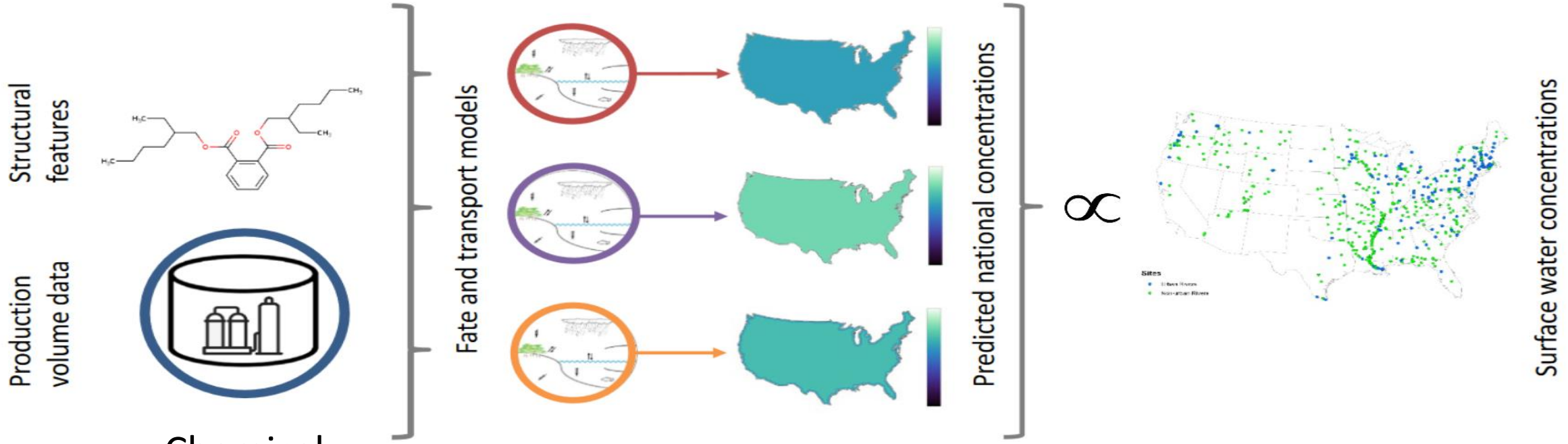


Minucci et al,
in preparation



Slide from Katherine Phillips

EcoSEEM Metamodel for Surface Water Chemical Concentrations



Chemical-specific information (*l*) → Release (loading) and fate predictors (*p*) → **EcoSEEM metamodel** (*m*) ← USGS/EPA water monitoring data (*y*)

Sayre et al,
in preparation

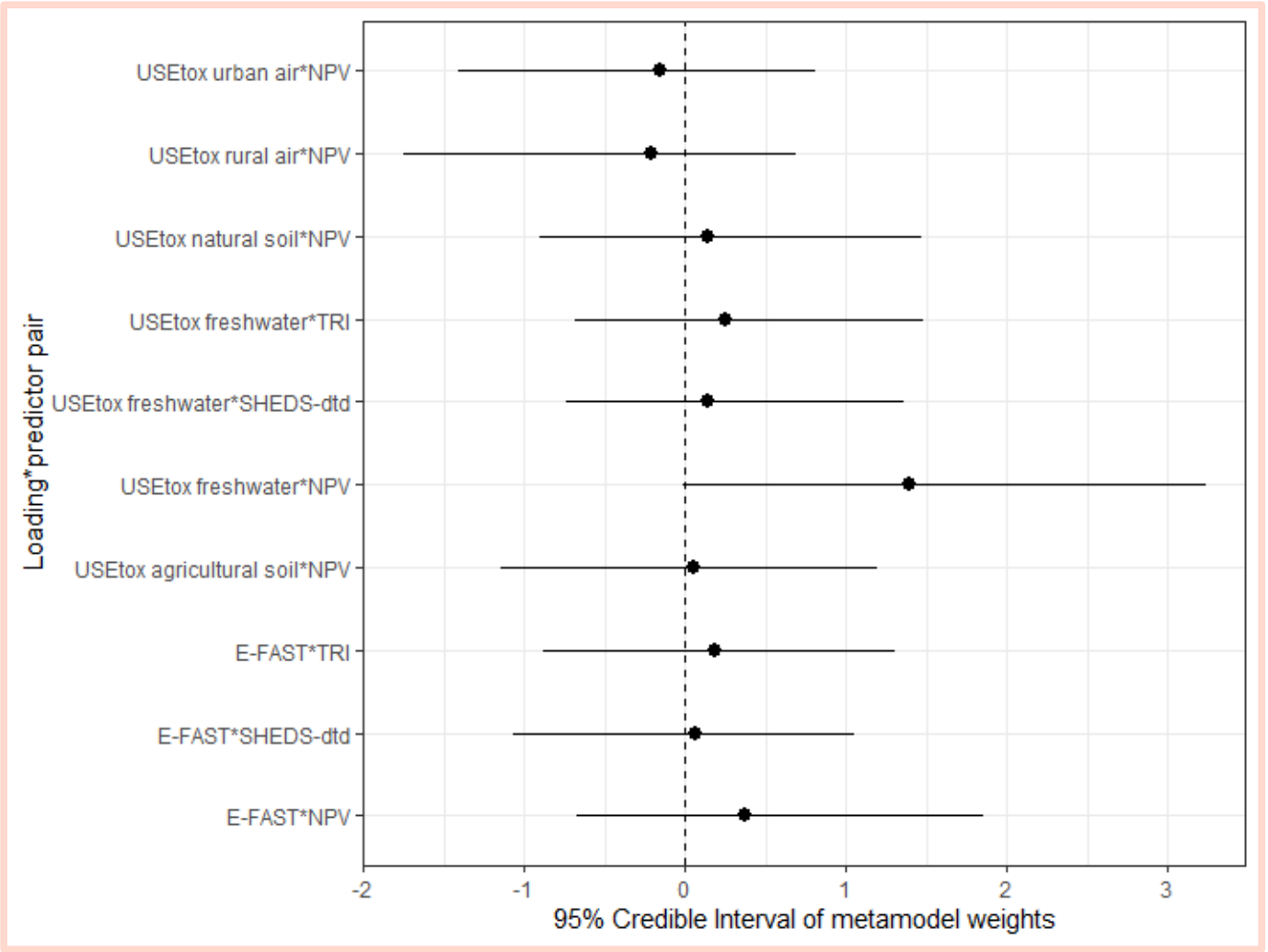
$$\ln y_i = m_0 + \sum_{j=1}^{n_j} \sum_{k=1}^{n_{kj}} m_{jk} \ln\left(l_{ji} p_{ki}\right)$$

Slide from Risa Sayre

EcoSEEM Evaluating Predictive Ability of HT Surface Water Models

- The strength of the correlation between each combination of release and fate model predictions and the observed water concentrations allows model calibration

- The most informative pair for bulk concentrations was USEtox freshwater model using loadings from NPV

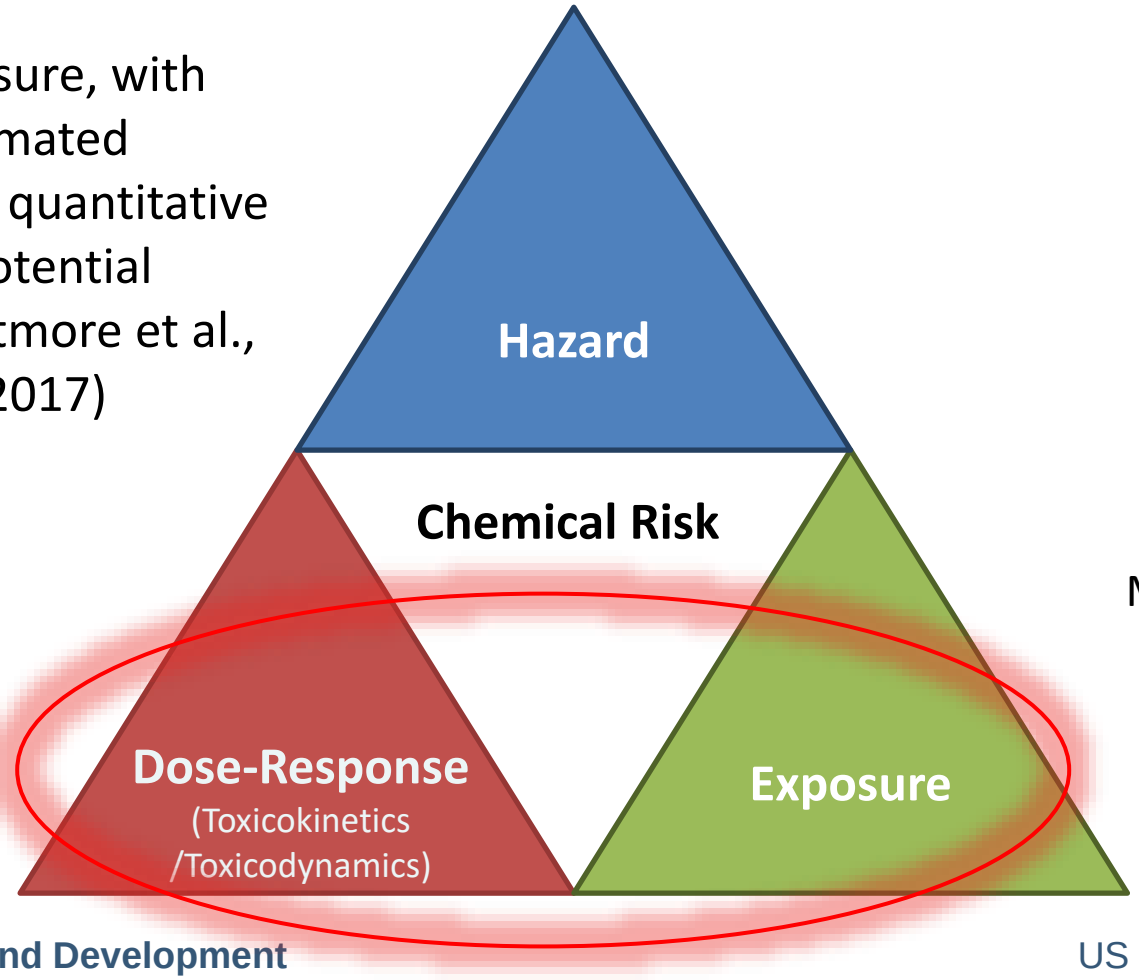


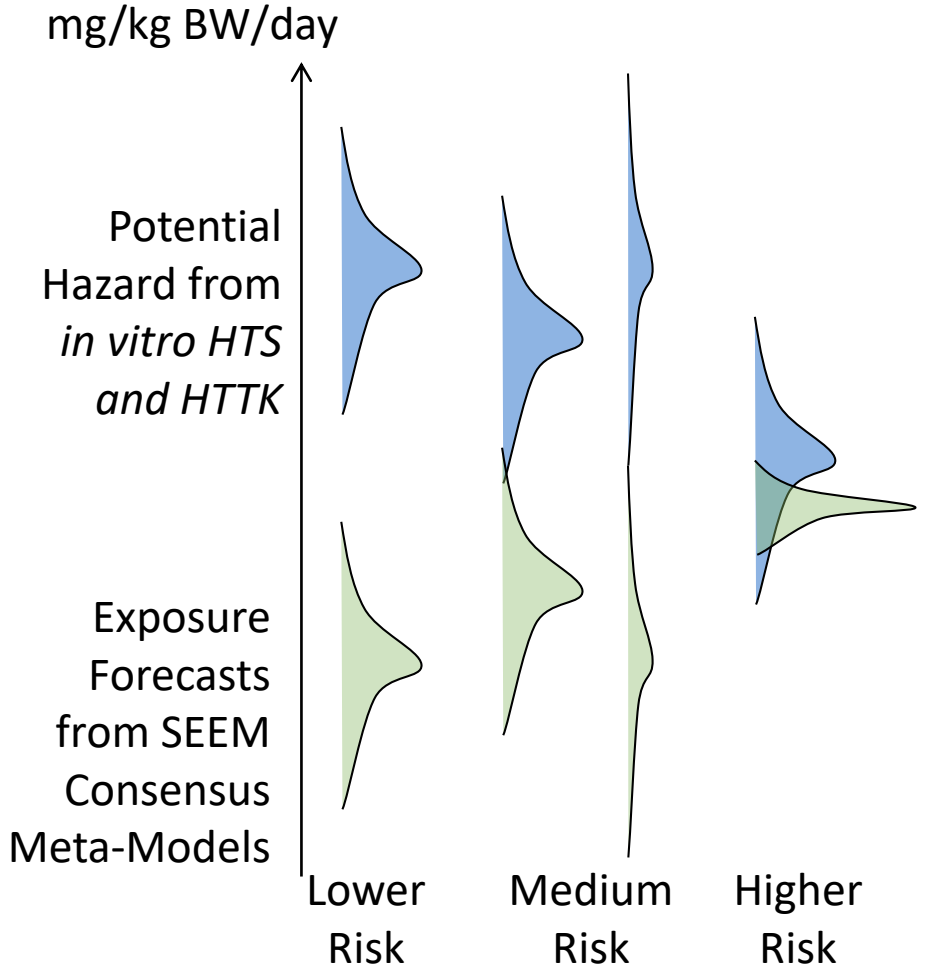
Sayre et al,
in preparation

Outlook

- SEEM metamodels have been developed for consumer and some ambient pathways (Ring et al., 2018) and ecological and occupational consensus models are in development
- Estimates of exposure, with appropriately estimated uncertainty, allow quantitative prioritization of potential chemical risk (Wetmore et al., 2015; Ring et al., 2017)

EPA's ExpoCast Project





ExpoCast Project (Exposure Forecasting)

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EPA ORD Publications in Bold

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United States Environmental Protection Agency

ExpoCast
exposure forecasting

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

Barbara A. Wetmore

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

February 2, 2021

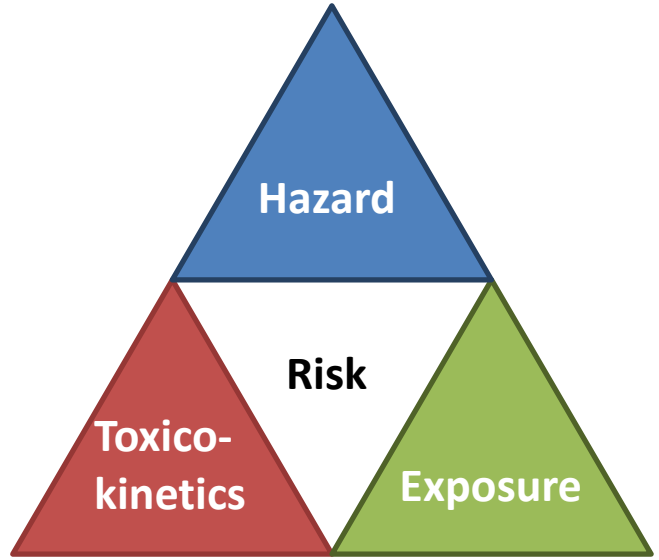
The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the US EPA.

NAMs for Exposure *Toxicokinetics*

Acceptance and use of in vitro *data for hazard identification is limited by uncertainties associated with exposure characterization and metabolism*

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)



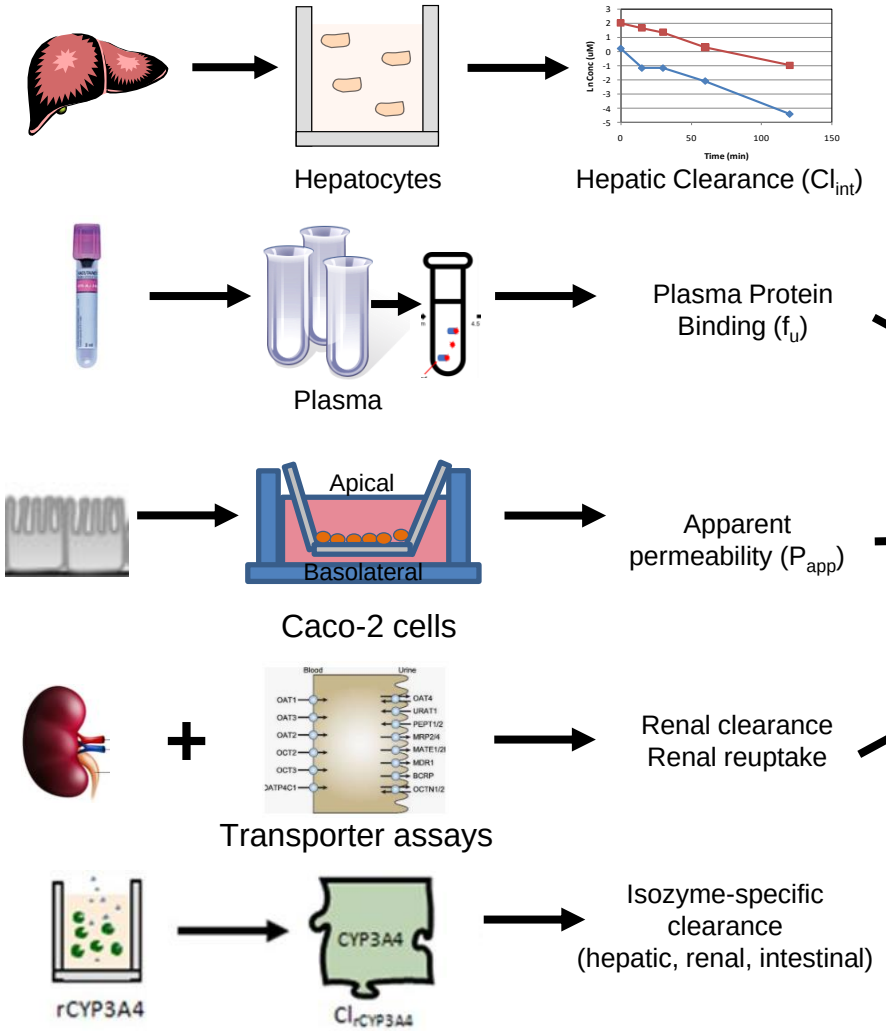
Recent Agency Case Study Finding:

→ 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

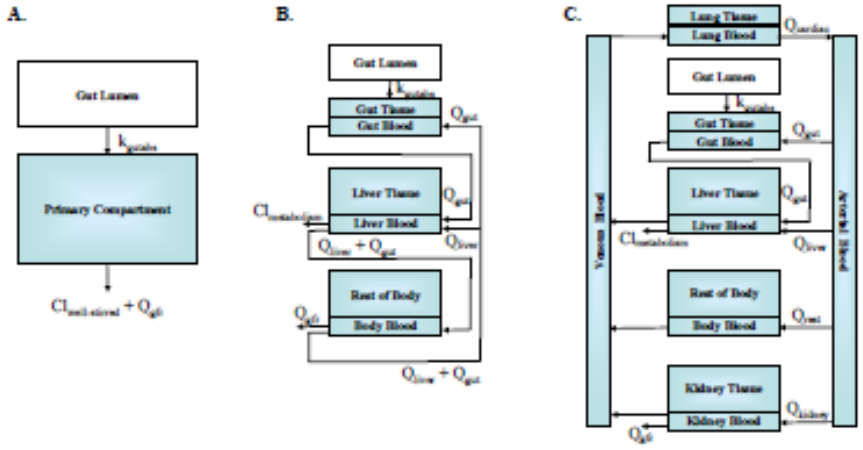
IVIVE

Internal Concentration Predictions Given a Set Administered Dose

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

Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012
Wetmore *et al.*, Tox Sci., 2014
Wetmore *et al., Tox Sci.,* 2015
Wambaugh *et al.*,*Tox Sci.,* 2015
Honda *et al.,* 2019
Wambaugh *et al.*, 2019
Smeltz *et al.*, in preparation
Kreutz *et al*., in preparation

In Vitro-In Vivo Extrapolation
II. Physiologically-based Toxicokinetic Modeling



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)

"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:

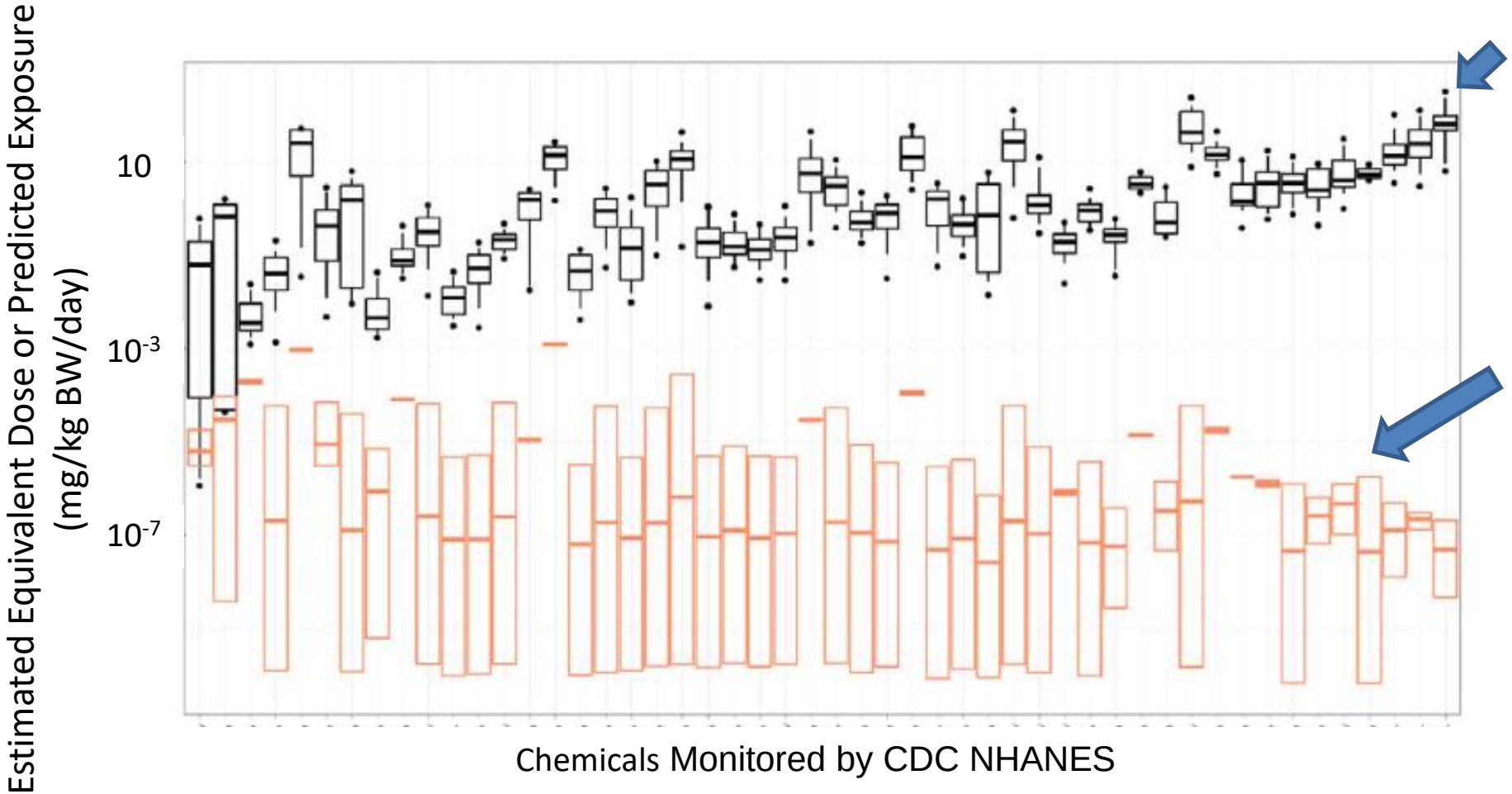
- **populations** of interest
- **exposures** of interest
  - Capturing **variability** (within or across populations)

Based on variations in the physiologic inputs (Monte Carlo)

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

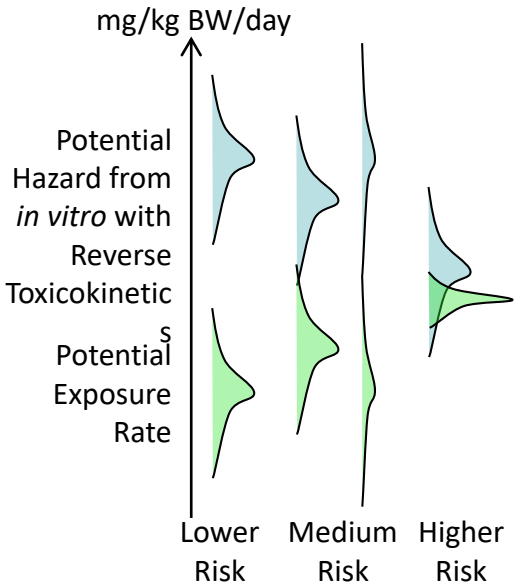
NAMs for Prioritization

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

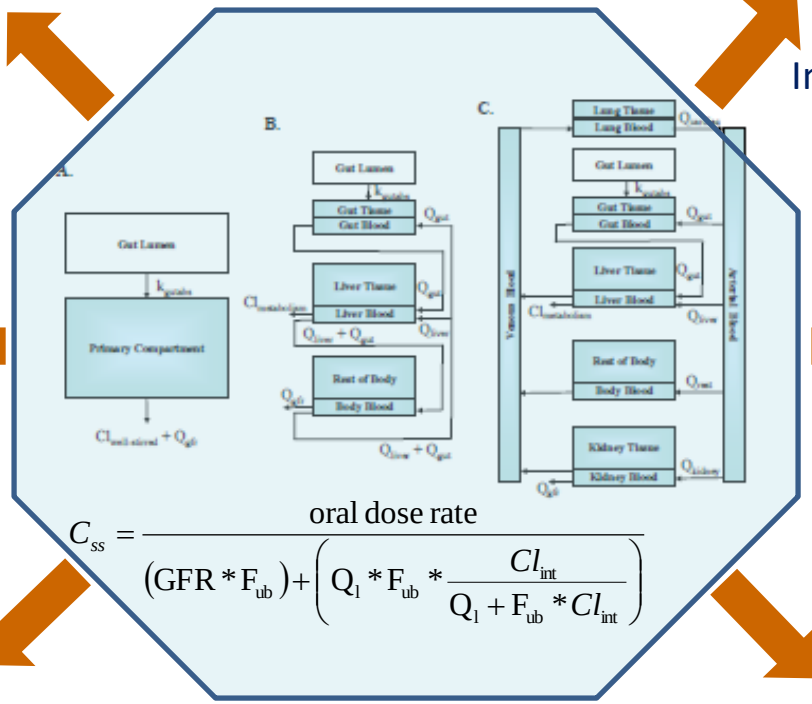
- Primary goal: to provide a human exposure-dose context for bioactive *in vitro* concentrations from NAMs for hazard testing
  - TK Methods across TSCA landscape – including *challenging chemistries*, *emerging contaminants*
  - Incorporating more exposure routes and pathways
  - Tools to characterize exposures to sensitive populations and life stages
  - Characterize *in vitro* disposition across TSCA landscape
  - Tools to identify, quantitate 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

HTTK: Open-Source Platform

Predictive Tools
Plasma protein binding
Hepatic clearance
Transporter Involvement
Isozyme Involvement

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



$$C_{ss} = \frac{\text{oral dose rate}}{(\text{GFR} * \text{F}_{ub}) + \left(\text{Q}_l * \text{F}_{ub} * \frac{Cl_{int}}{\text{Q}_l + \text{F}_{ub} * Cl_{int}}\right)}$$

Databases
in vitro TK data
In vivo TK data (CvTdb)

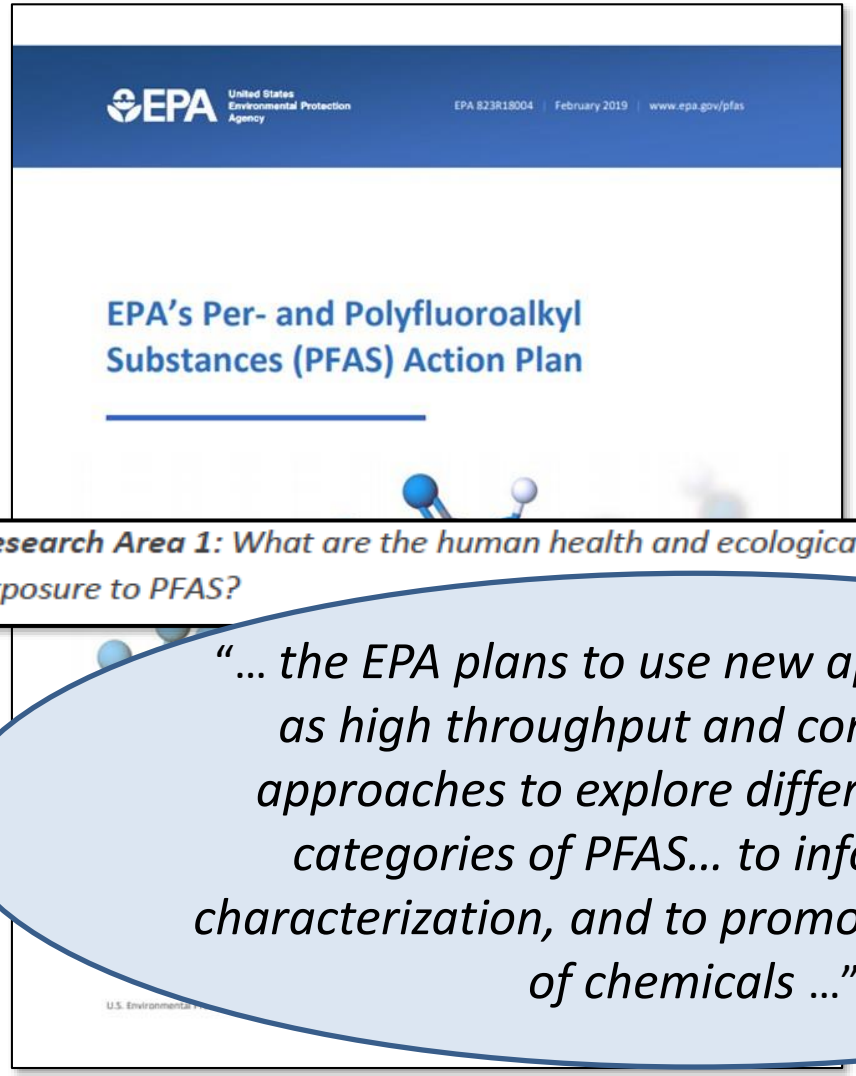
Refinement
IVIVE / IVIVC efforts
In Vitro Disposition
Best Practices

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

Population Variability
NHANES; physiology
Toxicokinetic variability

Uncertainty / Variability Assessments
Bayesian approaches
Experimental uncertainty
Biologic variability

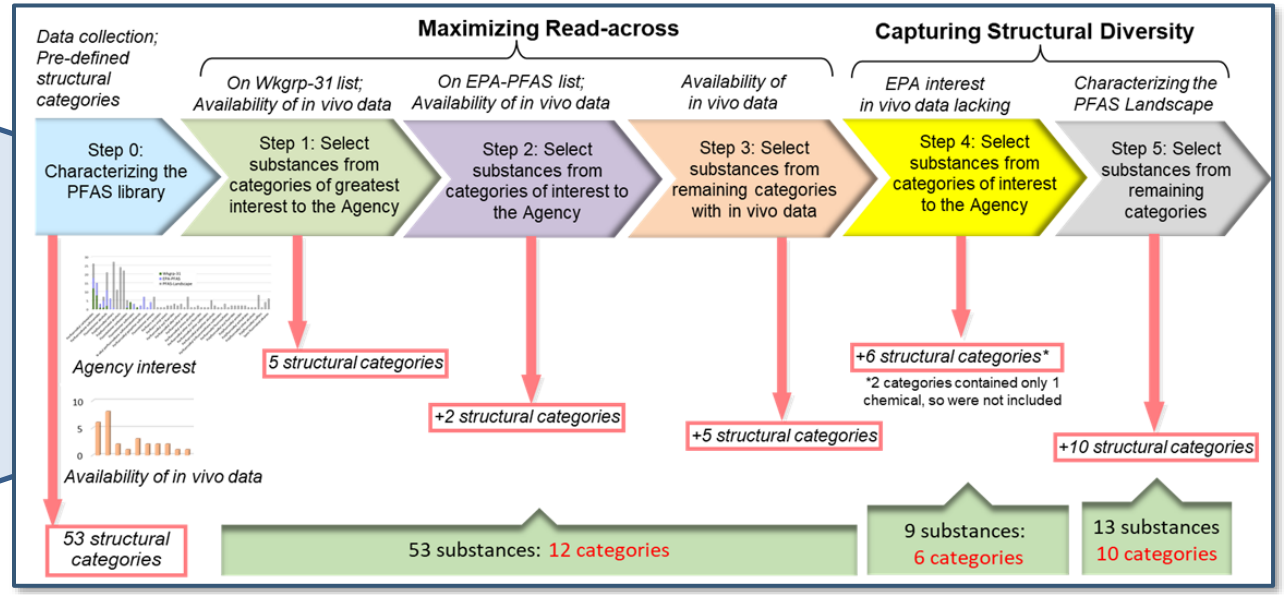
- *In Vitro* Toxicokinetic Data Generation -
PFAS: Using NAMs to Fill Information Gaps



Goals:

- Generate data to support development and refinement of categories and read-across evaluation
- Incorporate substances of interest to Agency
- Characterize mechanistic and toxicokinetic properties of the broader PFAS landscape

"… 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 …"

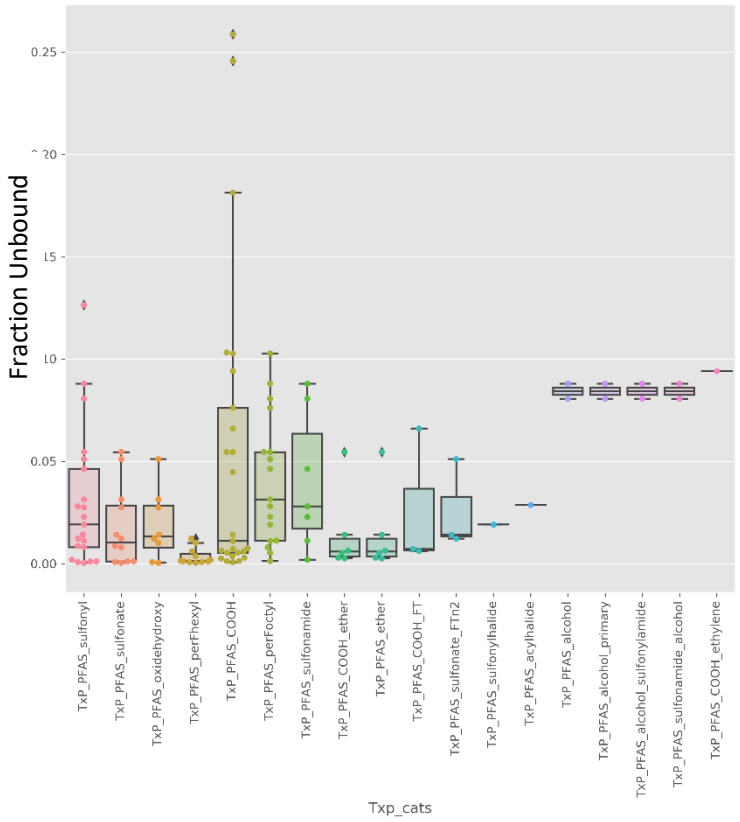


- *In Vitro* Toxicokinetic Data Generation -
Category-Based Analyses of Toxicokinetic Data

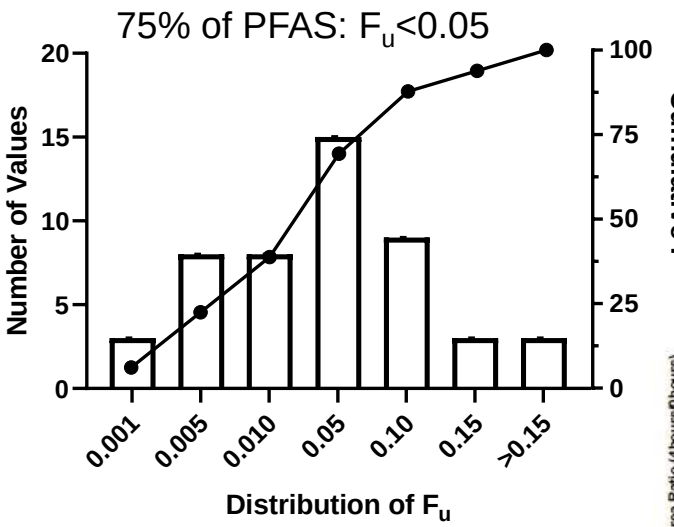
PFAS TK data: ~150 PFAS
- Hepatic clearance
- Plasma protein binding
- Renal transporter activity

→ IVIVE, modeling, TK NAMs

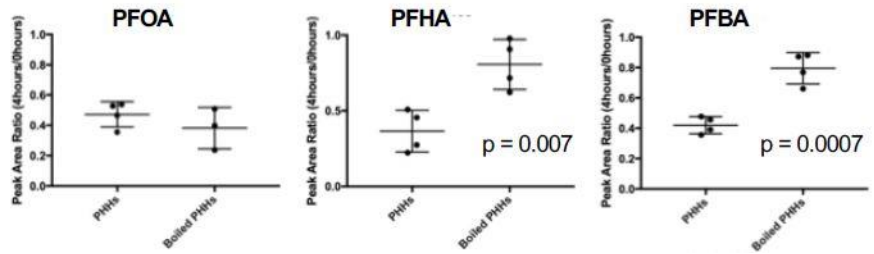
Category-Based Analysis of Plasma Protein Binding Data

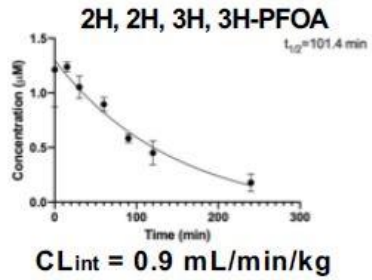


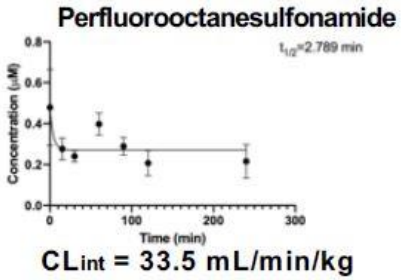
Preliminary set: Plasma protein binding data across 50+ PFAS

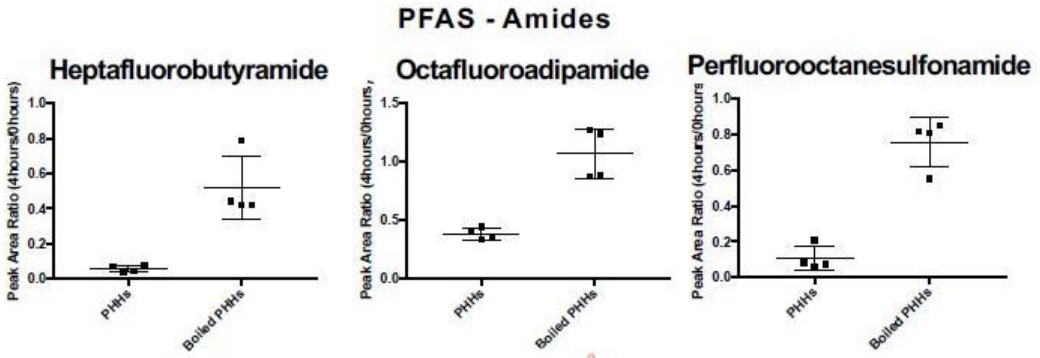


Hepatic Clearance Data





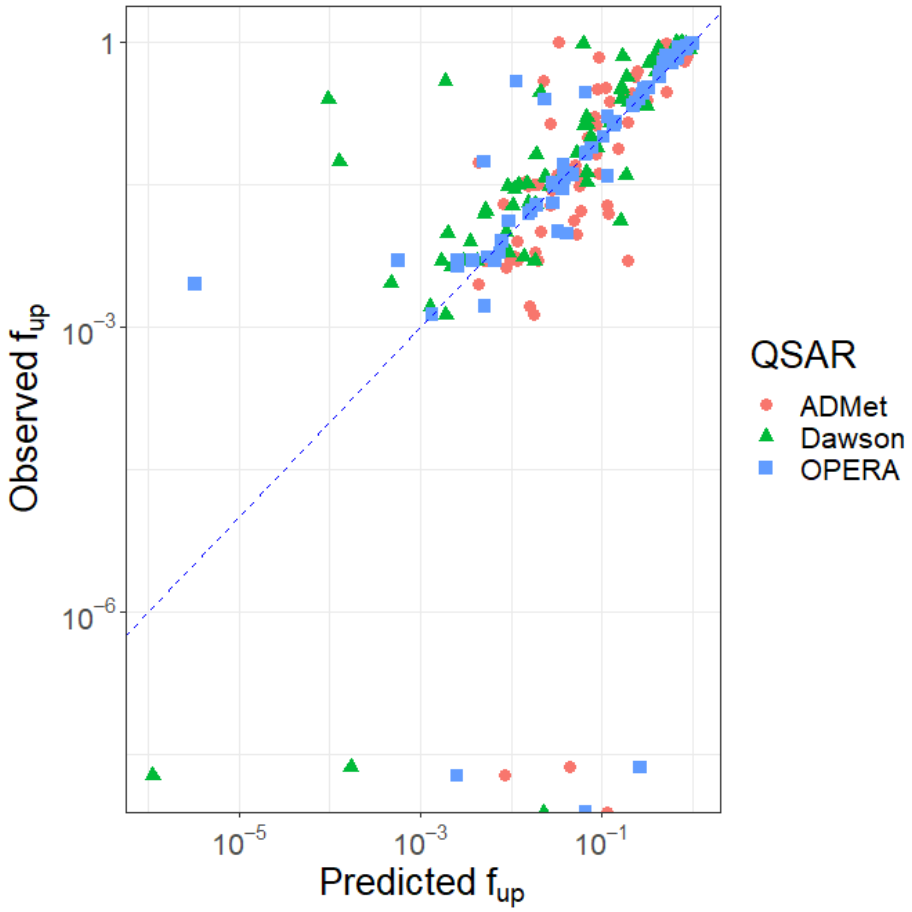


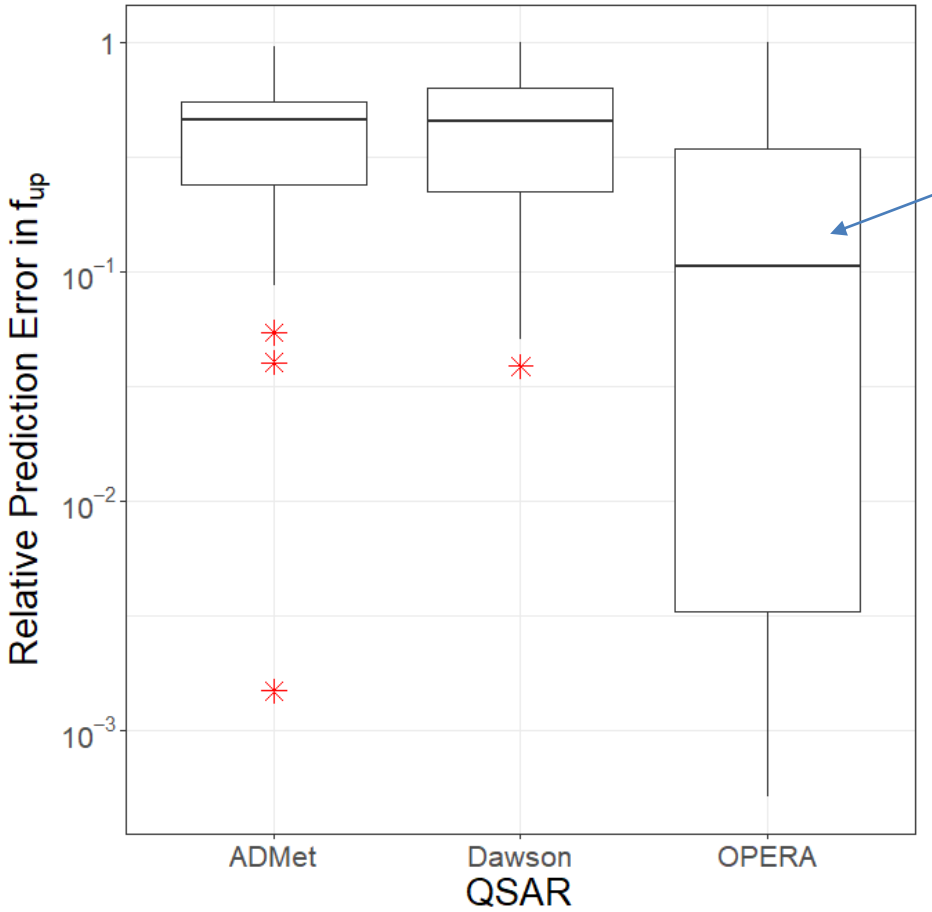


- Predictive Tool Development -

- *In vitro* TK measurements are being employed in model development and evaluation.
  - Plasma protein binding (f_u); hepatic clearance (Cl_{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

- Model Expansion - Gestational Pathway

RESEARCH ARTICLE

Empirical models for anatomical and physiological changes in a human mother and fetus during pregnancy and gestation

Dustin F. Kapraun[1]*, John F. Wambaugh[2], R. Woodrow Setzer[2], Richard S. Judson[2]

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@epa.gov

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

Manuscript	[27]	[15]	[25]	[28]	[29]	[31]	[3]	[32]
Presents original data*	N	N	N	N	N	N	N	N
Presents original compiled data* set(s)	Y	N	N	Y	N	N	Y	Y
Presents original models† based on compiled data sets of Abduljalil et al. [28]	N	N	N	Y	Y	N	N	N
Presents original models† based on compiled data sets of Abduljalil et al. [33]	N	N	N	N	N	N	N	N
(+) Employs and thoroughly describes rigorous statistical methods for parameter‡ estimation	Y	N	Y	N	N	N	Y	N
(+) Employs and thoroughly describes rigorous statistical methods for model† selection	N	N	N	N	N	N	Y	N
(+) Presents original models† for multiple maternal compartments	N	Y	N	Y	Y	Y	Y	N
(+) Presents original models† for multiple fetal compartments	N	Y	Y	N	N	Y	N	Y
(+) Presents models that reflect a biologically accurate depiction of the fetal circulatory system§	N	N	N	N	N	Y	N	N¶
(+) Presents explicit models† for "rest of body" compartments that yield feasible (e.g., non-negative) values for all relevant time points	N	N	N	N	N	N	N	Y
(+) Systematically compares original models† with previously published models†	N	N	N	N	N	N	N	N	N	Y
(-) Presents models that contain errors or inconsistencies identified in the current manuscript	N	Y	N	N	Y	N	Y	Y	Y	N

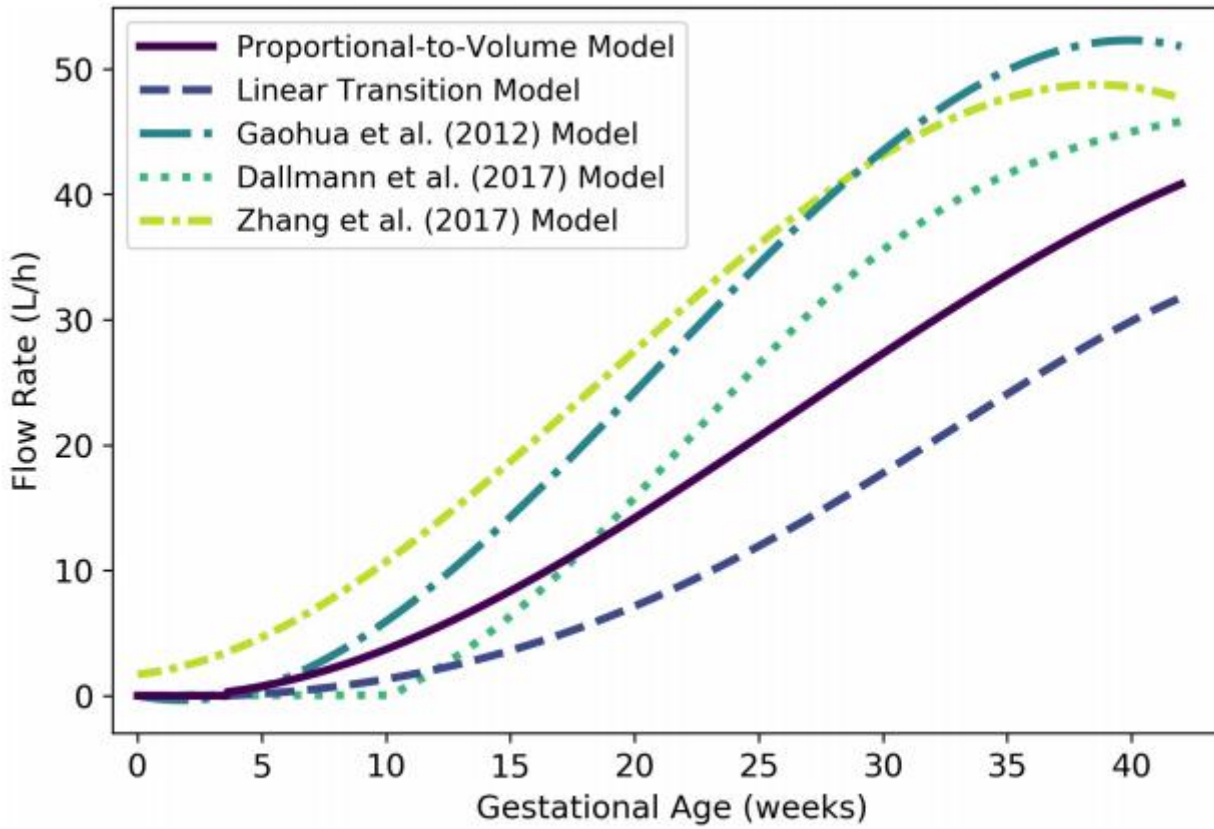


Fig 11. Maternal blood flow to the placenta vs. gestational age. The proportional-to-volume model (solid line) given by Eq 22, the linear transition model given by Eq 21, and two published models [3, 29, 32] are shown.

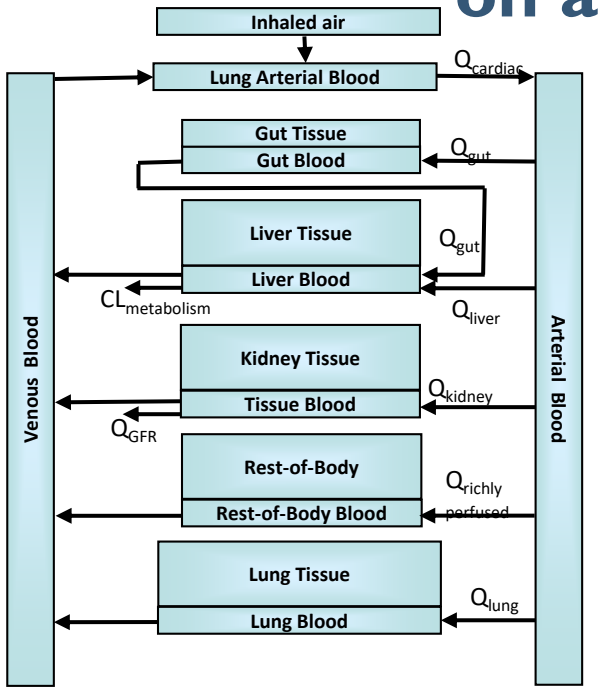
Kapraun *et al.*, 2019 PLOS One

New HT-PBTK Models: Development is Done with "Branches" on a Git Repository

United States Environmental Protection Agency

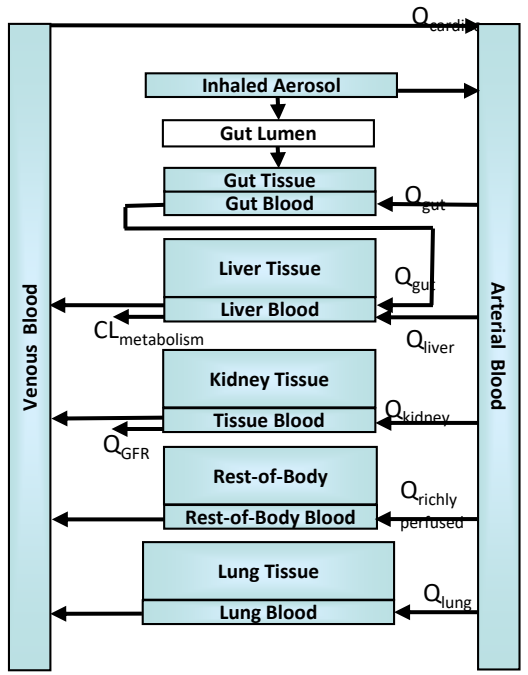
Gas Inhalation Exposure Route

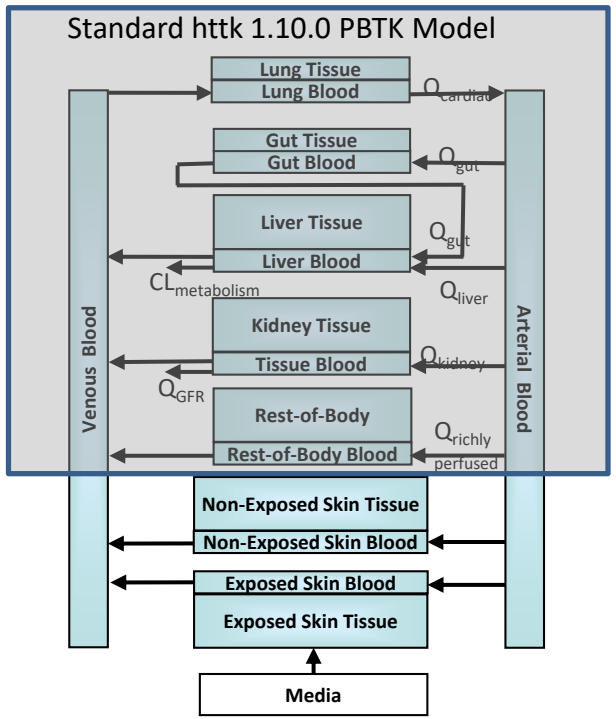
EPA, USAFSAM



Aerosol Inhalation Exposure Route (with APEX model)

EPA, USAFSAM



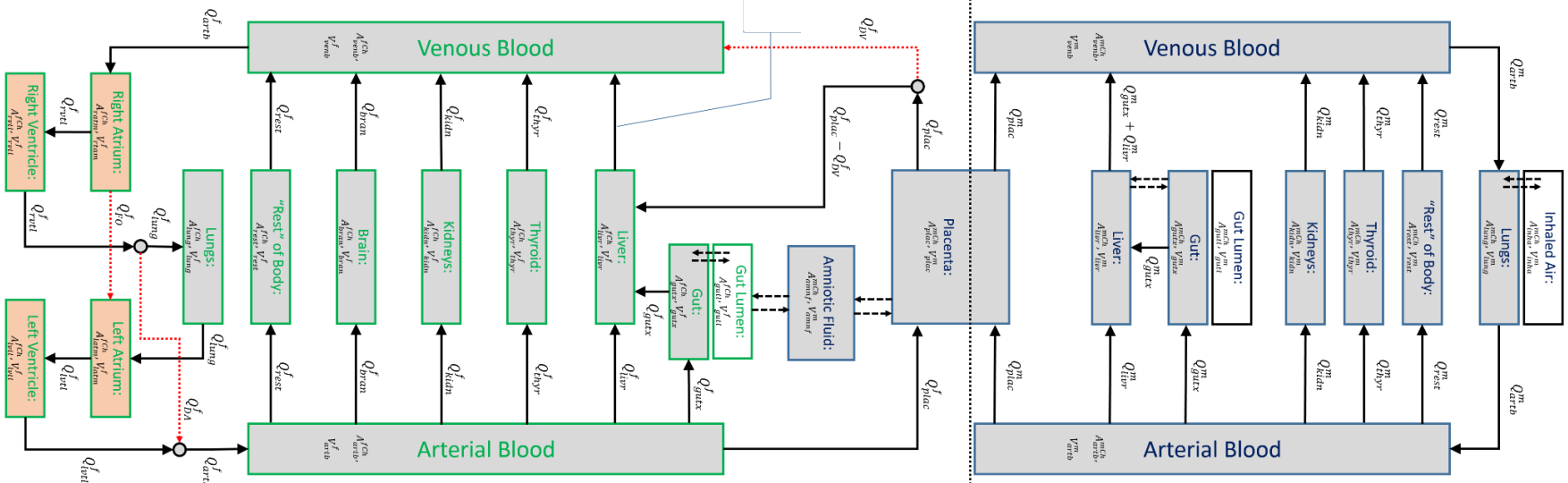


Dermal Exposure Route

EPA, Unilever, INERIS

Human Gestational Model

EPA, FDA

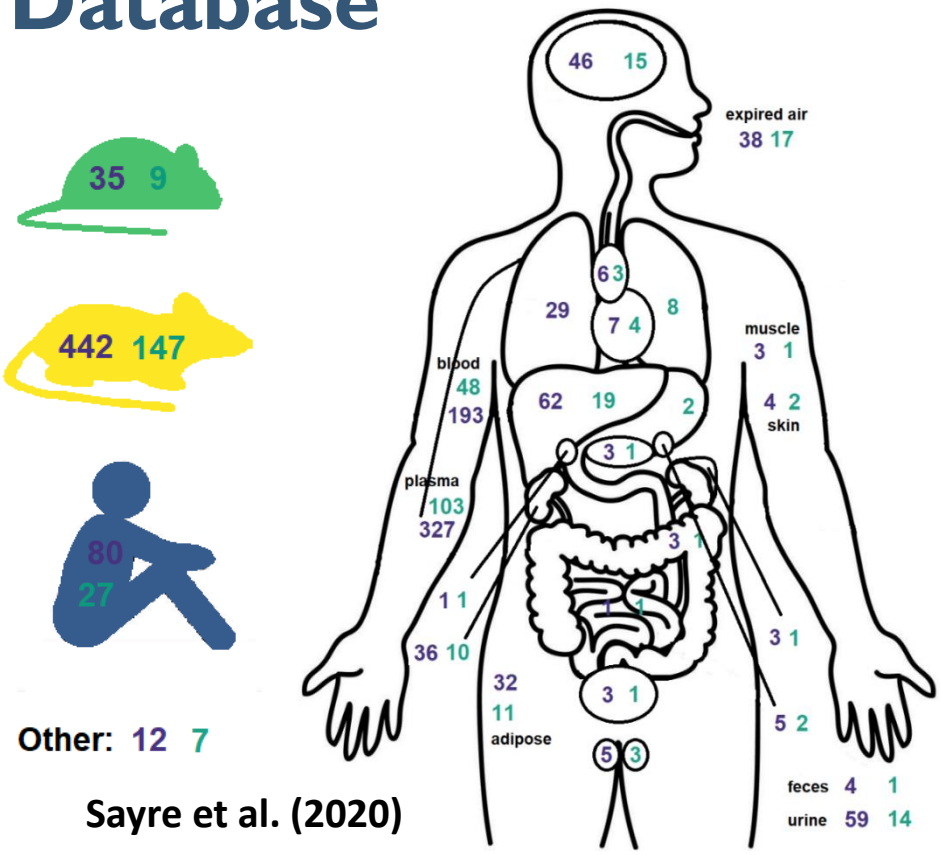


- Database Development -
CvTdb: An *In Vivo* TK Database

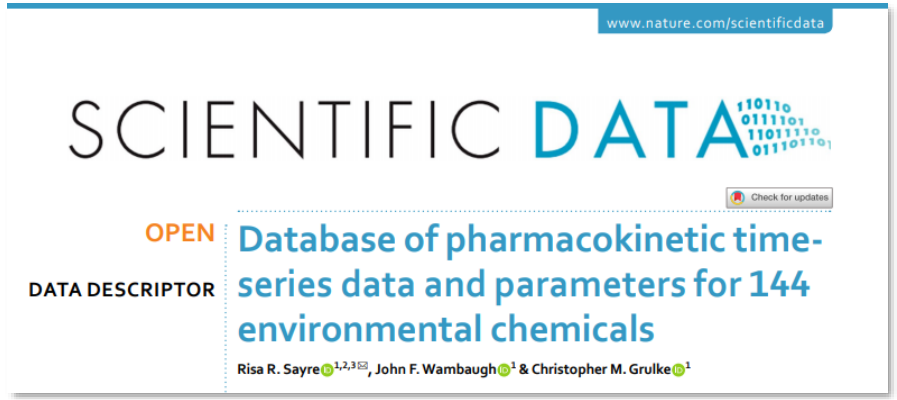
- EPA has developed a **public database** of **concentration vs. time data** across several species for building, calibrating, and evaluating TK models
- Effort ongoing, but to date includes:
  - 198 analytes (EPA, National Toxicology Program, literature)
  - Routes: Intravenous, dermal, oral, sub-cutaneous, 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

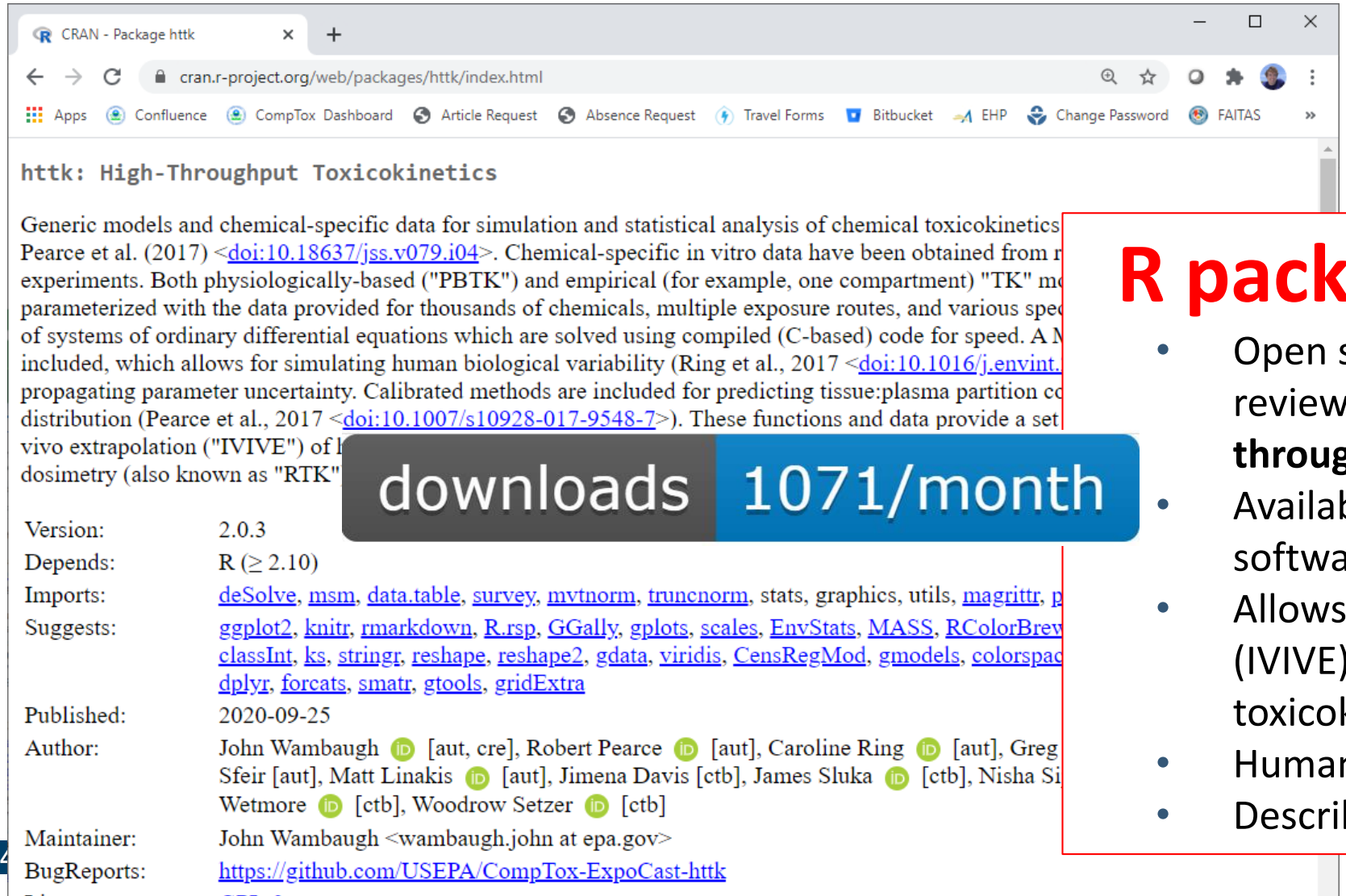


Sayre et al. (2020)



- HTTK Platform -
Open-Source Tools and Data for HTTK

https://CRAN.R-project.org/package=httk



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)

- HTTK Platform -
Modules within R Package "httk"

Feature	Description	Reference
Chemical Specific *In Vitro* Measurements	Metabolism and protein binding for ~1000 chemicals in human and ~200 in rat	Wetmore et al. (2012, 2013, 2015), plus others
Chemical-Specific *In Silico* Predictions	Metabolism and protein binding for ~8000 Tox21 chemicals	Sipes et al. (2017)
Generic toxicokinetic models	One compartment, three compartment, physiologically-based oral, intravenous, and inhalation (PBTK)	Pearce et al. (2017a), Linakis et al. (2020)
Tissue partition coefficient predictors	Modified Schmitt (2008) method	Pearce et al. (2017b)
Variability Simulator	Based on NHANES biometrics	Ring et al. (2017)
In Vitro Disposition	Armitage et al. (2014) model	Honda et al. (2019)
Uncertainty Propagation	Model parameters can be described by distributions reflecting uncertainty	Wambaugh et al. (2019)

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

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

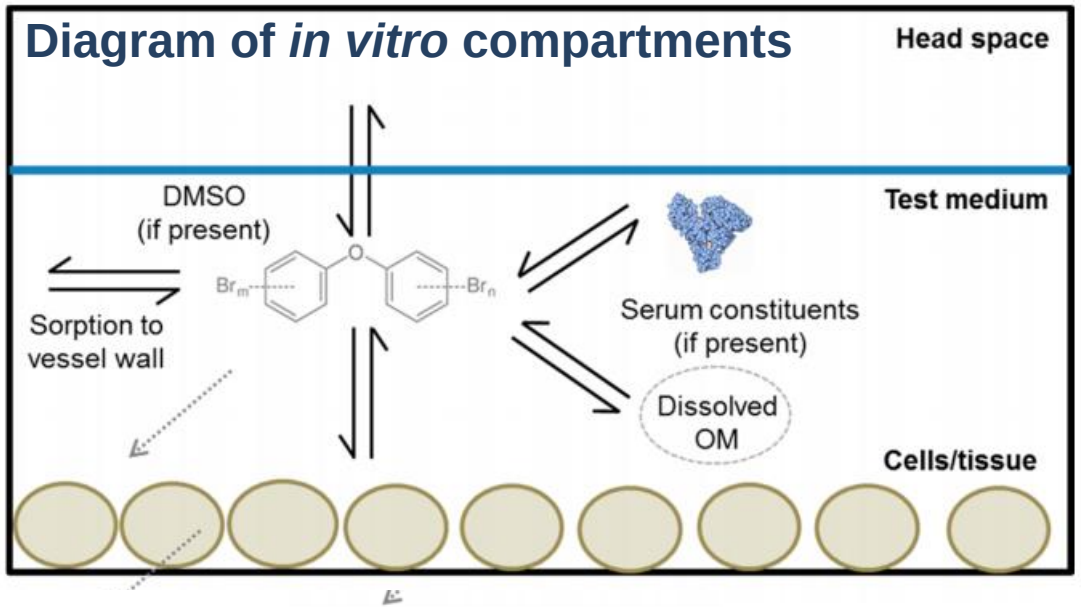
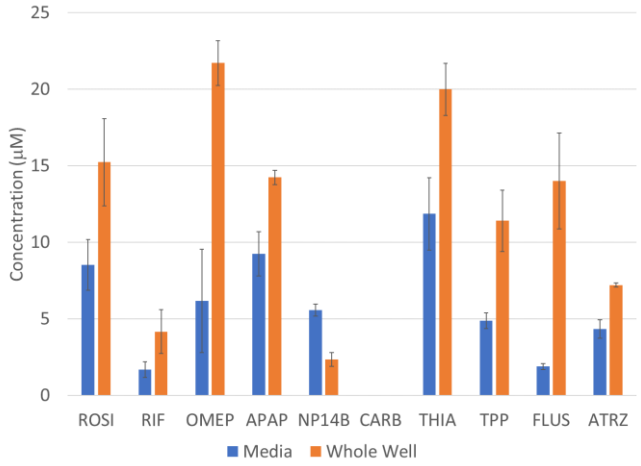


Figure 1. Conceptual representation of an in vitro test system.

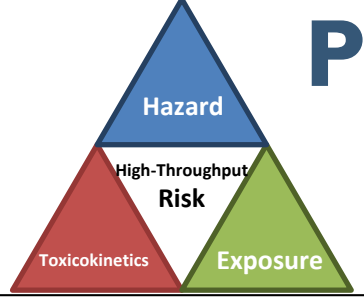
Preliminary Design and Data

Table 2. Plate Matrix

Test Plate	Test Plate Barcode	Plating Condition	Exposure Duration (hr)	Measured Compartment
A	TC00000013	Medium - cells	1	Medium
		Medium - cells	1	Plastic
B	TC00000014	Medium + cells	1	Medium
		Medium + cells	1	Plastic + Cells
C	TC00000015	Medium + cells	1	Whole Well Crash
D	TC00000016	Medium - cells	6	Medium
		Medium - cells	6	Plastic
E	TC00000017	Medium + cells	6	Medium
		Medium + cells	6	Plastic + Cells
F	TC00000018	Medium + cells	6	Whole Well Crash
G	TC00000019	Medium - cells	24	Medium
		Medium - cells	24	Plastic
H	TC00000020	Medium + cells	24	Medium
		Medium + cells	24	Plastic + Cells
I	TC00000021	Medium + cells	24	Whole Well Crash



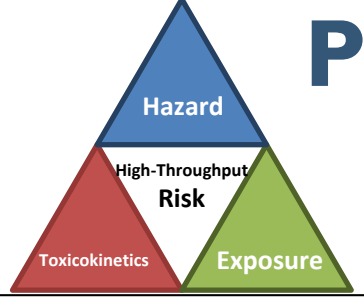
Providing the Pieces for Prioritization



Informing TSCA

Target Population	**Consumers**	**General Population**
	ToxCast	ToxCast
	HTTK Oral Route; Dermal Route Needed	HTTK Oral Route; Aerosol Route Needed
	Evaluation Data: NHANES; **Human** ExpoCast/SEEM; Many Exposure Predictors	Evaluation Data: NHANES; **Human** ExpoCast/SEEM; Many Exposure Predictors
Pathways Covered	**Consumer**	**Ambient**

Providing the Pieces for Prioritization

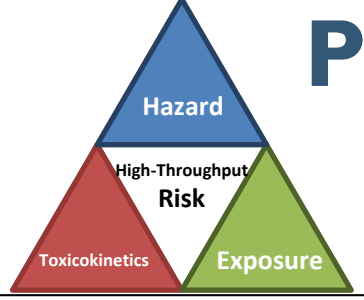


Informing TSCA

Highly Exposed and Sensitive Populations

Target Population	**Consumers**	**General Population**	**Workers**	**Gestational**
	ToxCast	ToxCast	ToxCast	ToxCast
	HTTK Oral Route; Dermal Route Needed	HTTK Oral Route; Aerosol Route Needed	Aerosol Route Needed	Gestational Model Needed
	Evaluation Data: NHANES	Evaluation Data: NHANES	Evaluation Data: OSHA	Evaluation Data: NHANES
	Human ExpoCast/SEEM	**Human** ExpoCast/SEEM	**Occupational** ExpoCast/SEEM	**Demographic Human** ExpoCast / SEEM
	Many Exposure Predictors	Many Exposure Predictors	HT ChemSTEER, others	Many Exposure Predictors
Pathways Covered	**Consumer**	**Ambient**	**Occupational**	**Multiple**

Providing the Pieces for Prioritization



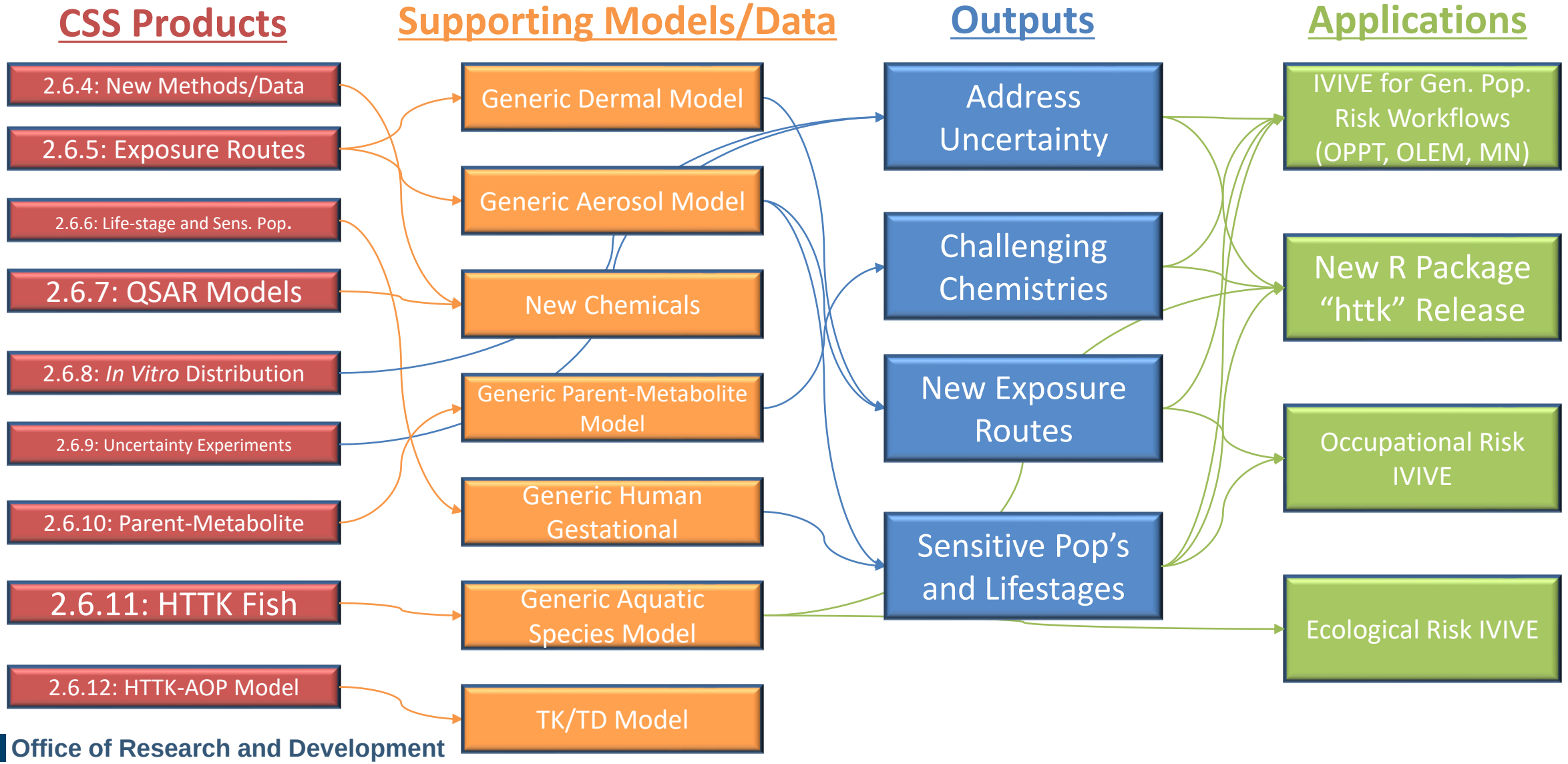
Informing TSCA

Highly Exposed and Sensitive Populations

Informing EDSP

Target Population	Consumers	General Population	Workers	Gestational	Ecological (Fish)
	ToxCast	ToxCast	ToxCast	ToxCast	ToxCast + SeqaPass / LC50 Models
	HTTK Oral Route; Dermal Route Needed	HTTK Oral Route; Aerosol Route Needed	Aerosol Route Needed	Gestational Model Needed	EPI Suite BCF*; HTTK Fish Needed
	Evaluation Data: NHANES; Human ExpoCast/SEEM; Many Exposure Predictors	Evaluation Data: NHANES; Human ExpoCast/SEEM; Many Exposure Predictors	Evaluation Data: OSHA; Occupational ExpoCast/SEEM; HT ChemSTEER, others		Evaluation Data: USGS Surface Water; Ecological ExpoCast/SEEM; 3 HT Models
Pathways Covered	Consumer	Ambient	Occupational	Multiple	Ambient

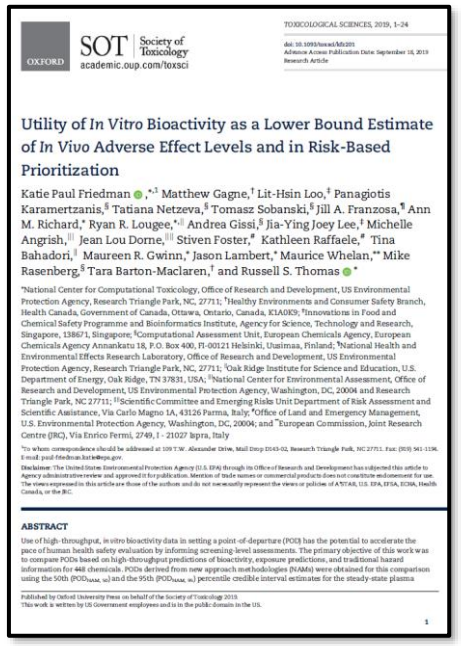
TK and IVIVE Projects and Relationships



International Collaborations

- Accelerating the Pace of Chemical Risk Assessment (APCRA) -

In Vitro Bioactivity, HTTK, and *In Vivo* Toxic Doses



SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2019, 1–24

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

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ABSTRACT

Use of high-throughput, *in vitro* bioactivity data in setting a point-of-departure (POD) has the potential to accelerate the pace of human health safety evaluation by informing screening-level assessments. The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals. PODs derived from new approach methodologies (NAMs) were obtained for this comparison using the 50th ($POD_{NAM,\ 50}$) and the 95th ($POD_{NAM,\ 95}$) percentile credible interval estimates for the steady-state plasma

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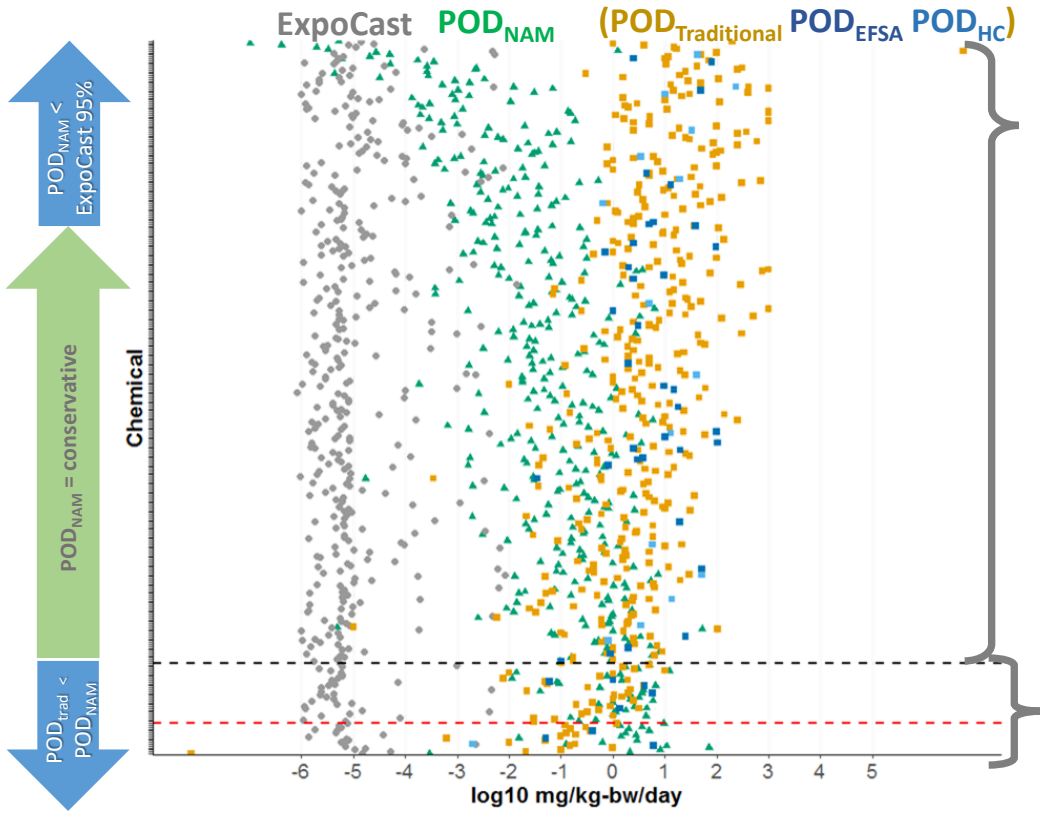
International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA



For ~89% of the chemicals, POD_{NAM} was conservative.

(~100-fold on average), but less conservative than a TTC

Chemicals where POD_{NAM} was not conservative enriched in OPs/carbamates

Paul-Friedman *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.
- 2020 SOT: “New Data and Tools for Understanding Chemical Distribution *In Vitro*” - Nynke Kramer and John Wambaugh
- 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

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ExpoCast Project (Exposure Forecasting)

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ExpoCast
exposure forecasting

NAMs for Exposure: Non-Targeted Analysis

Jon Sobus, Ph.D.

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

February 2-5, 2021

Stakeholder Needs
(OCSPP; EPA Regions)

Chemical safety evaluations require an improved understanding of chemical exposure scenarios and pathways

High-priority exposure data needs → consumer products, indoor environments, occupational settings, ambient environments, ecological pathways

Challenges

- High-quality exposure data are unavailable for many chemicals
- Measurement data traditionally generated using “targeted” methods
- Targeted analytical methods:
  - Require *a priori* knowledge of chemicals of interest
  - Produce data for few selected analytes (10s-100s)
  - Require standards for method development & compound quantitation
  - Are blind to emerging contaminants
  - Can’t keep pace with the needs of 21st century chemical safety evaluations

Research Objective

Rapid Exposure Modeling and Dosimetry Output 2.7:

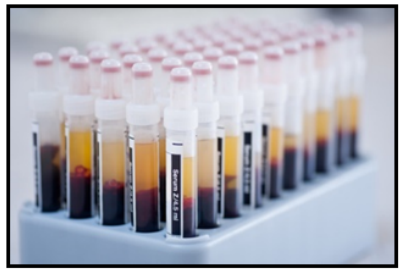
Develop, evaluate, and apply ***non-targeted analysis (NTA)*** methods, alongside targeted monitoring methods, to identify critical sources and pathways of human and ecological exposures

Key Question:

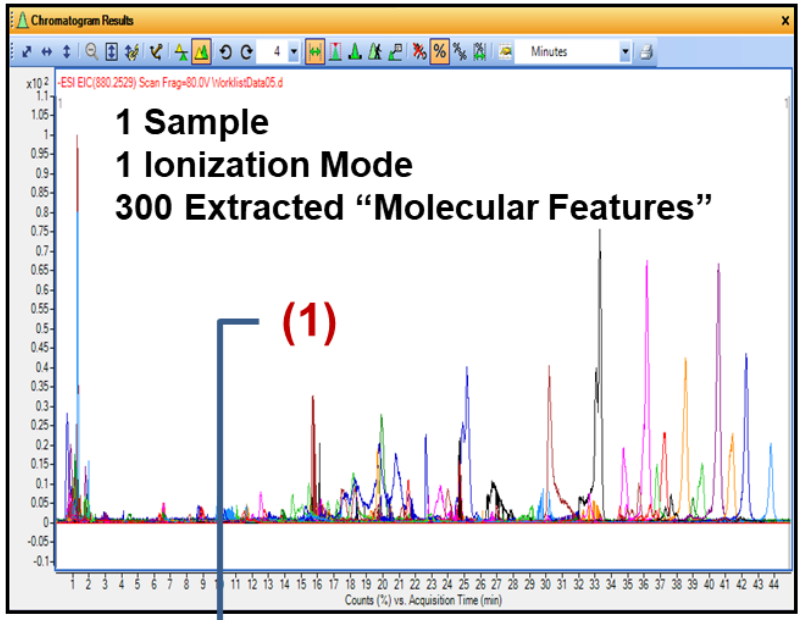
Are NTA methods suitable to meet the needs of 21st century chemical safety evaluations?

General NTA Workflow Steps

Samples



High-Resolution MS

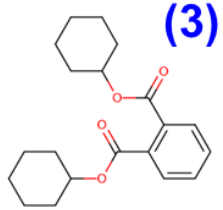


1) Prioritize "molecular features"
2) Correctly assign formulas
3) Correctly assign structures
4) Predict chemical concentrations
5) Determine chemical sources

(2)

$C_{20}H_{26}O_4$

(3)



(4)

12 µg/mL

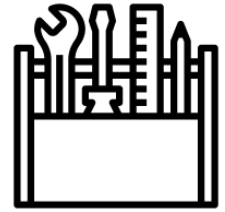
(5)

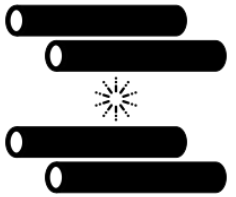
Ongoing Research Activities

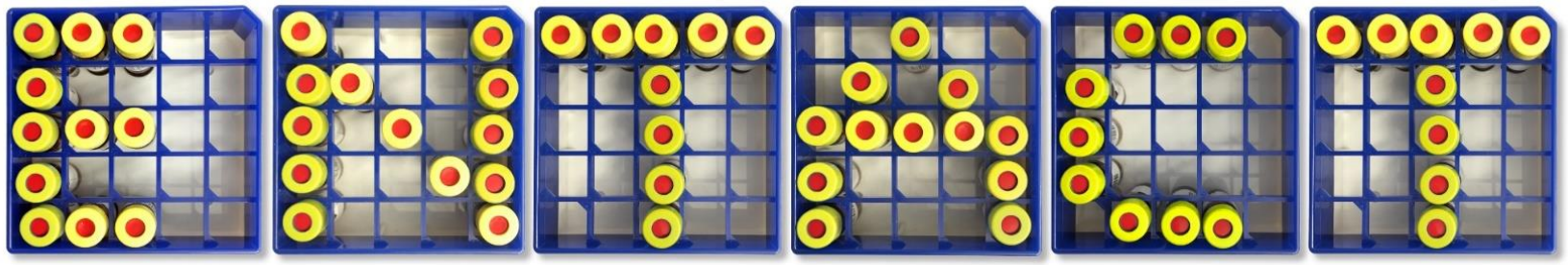
- **Evaluate NTA State-of-the-Science**
  - EPA's Non-Targeted Analysis Collaborative Trial (ENTACT)
- **Develop and Disseminate Guidance Materials**
  - Benchmarking and Publications for NTA (BP4NTA)
- **Build Tools to Ensure Transparency & Reproducibility**
  - NTA Study Reporting Tool (NTA SRT)
  - EPA NTA Web Application (NTA WebApp)
- **Address Priority Data Needs with Proof-of-Concept Applications**

Evaluating NTA Science-of-the-Science

- How variable are tools and results from lab to lab?
- Are some methods/workflows better than others?
- How does sample complexity affect performance?
- What chemical space does a given method cover?
- How sensitive are specific instruments/methods?



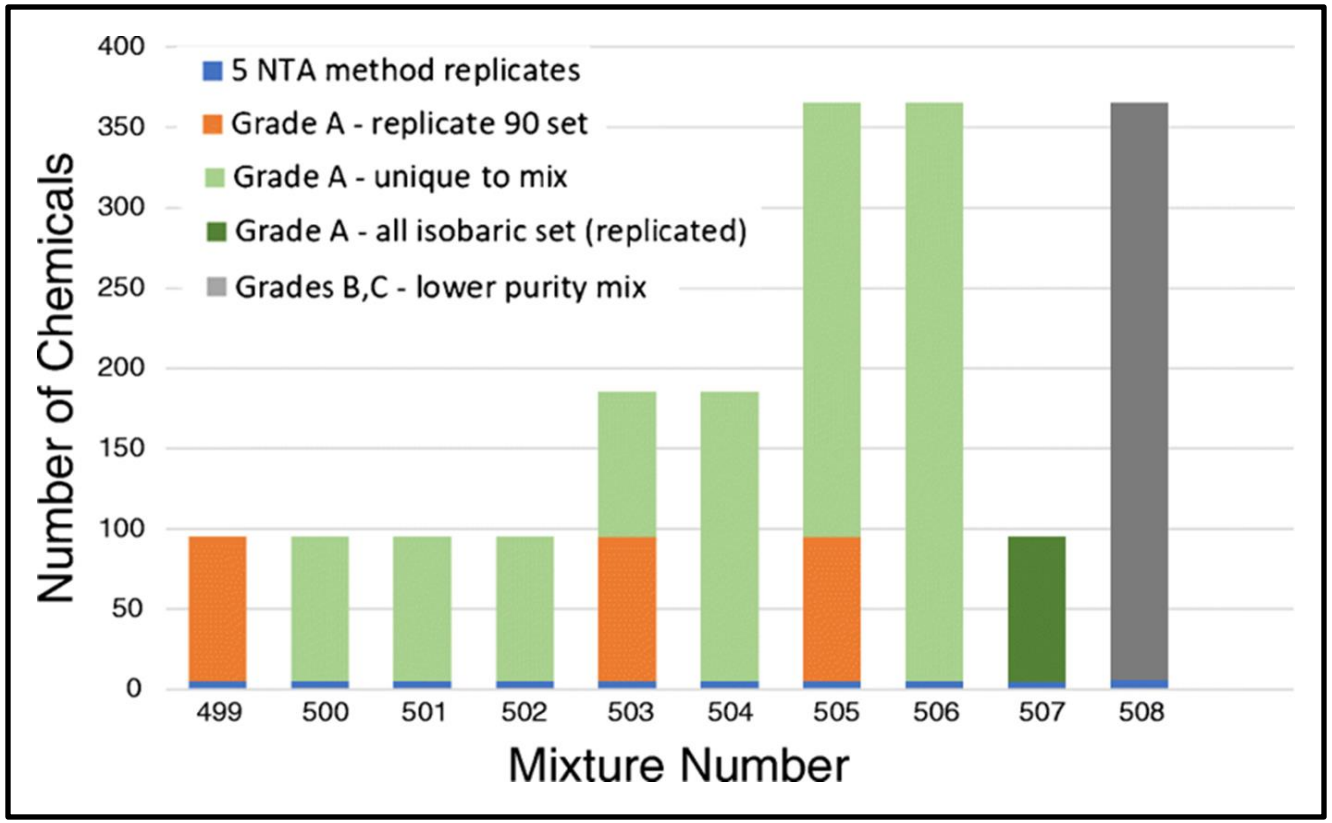




EPA's Non-Targeted Analysis Collaborative Trial

ENTACT Study Design (Part I)

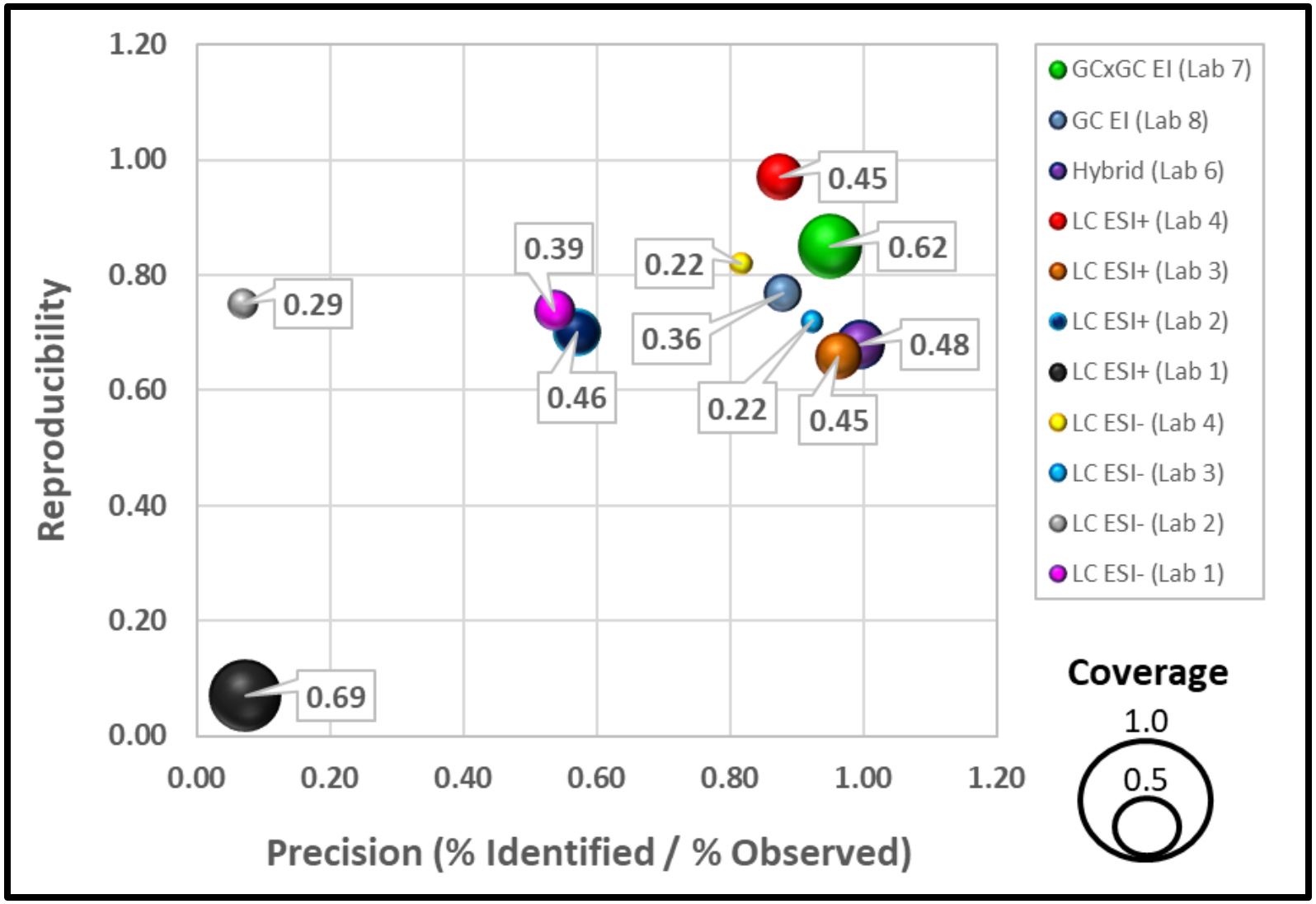
- ~30 global participants, 19 results submitted to date
- 10 synthetic mixtures of ToxCast substances (n=1269)



Replication in substance spikes offers a unique means to assess NTA method reproducibility!

Ulrich et al. 2019. doi: 10.1007/s00216-018-1435-6

Performance Comparison Across Methods



Metrics:

Bubble Size →
What % observed?
(of those spiked)

X-Axis →
What % correct?
(of those observed)

Y-Axis →
What % consistent?
(of those correct)

Take-Away Messages from ENTACT (to date…)

- Lack of transparency in methods/results reporting
- Method procedures change over short time increments
- Biased self-reporting → highlight strengths, mask weaknesses
- Blinded ToxCast mixtures allow for NTA performance assessment
- Standard performance measures highly variable across labs/methods
- Standard performance assessment methods/benchmarks must be adopted
- Benchmarks require input/consensus from NTA community
- Community focus must be on QA/QC and guidance (and innovation)

Developing and Disseminating Guidance Materials

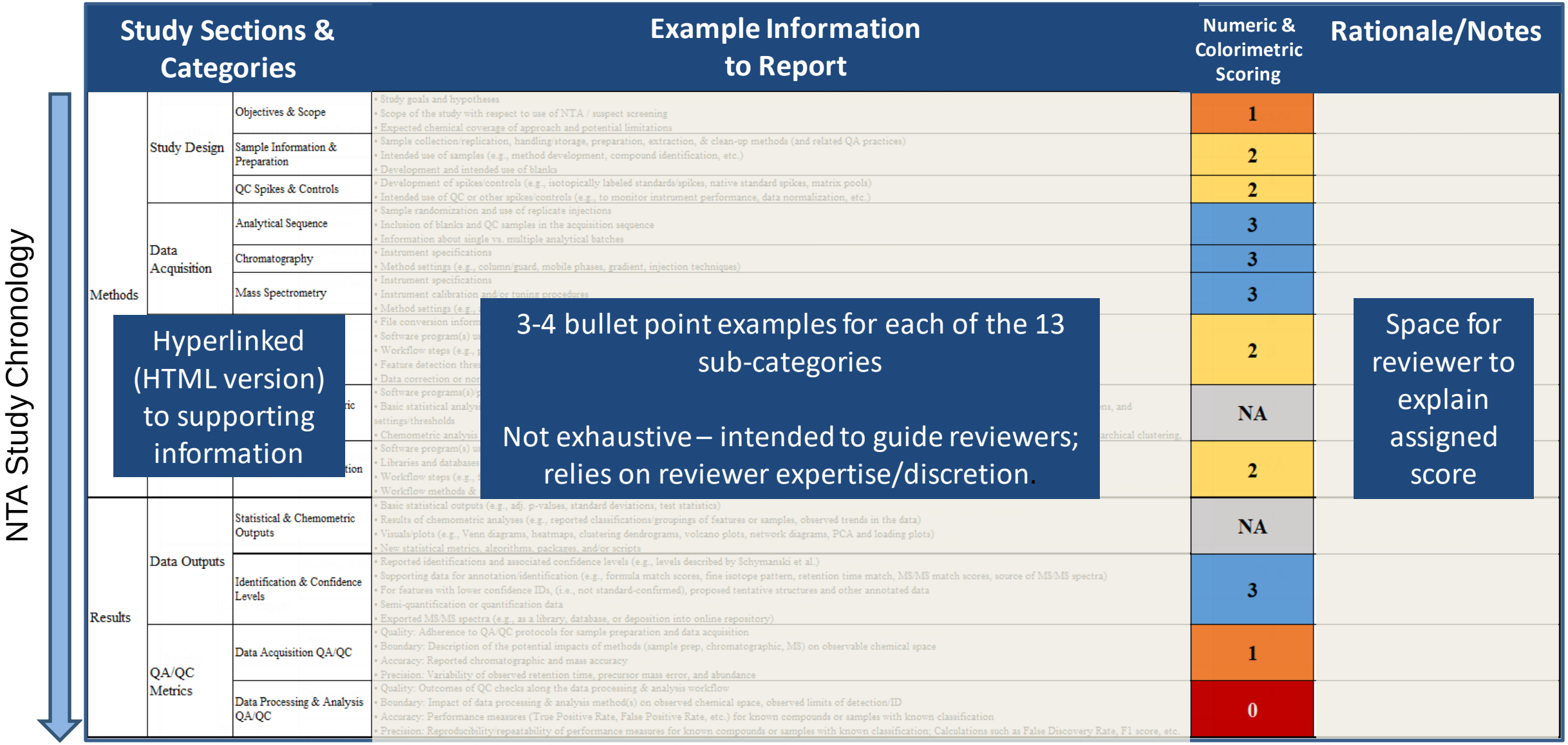
- BP4NTA → Borne out of 2018 ENTACT workshop
- ~100 U.S. and international members
  - Government, academia, and industry
- Working Group Objectives:
  - Short term → define common NTA terms, concepts, and performance metrics
  - Short term → provide recommendations on research & reporting best practices
  - Long term → establish proficiency testing levels (ASTM/ISO)
- Products (including 3 manuscripts):
  - Website with key resources and links: https://nontargetedanalysis.org/
  - Guidance documents with definitions & supporting info
  - "NTA Study Reporting Tool" to standardize reporting (proposals & manuscripts)

Building Tools to Ensure Transparency & Reproducibility

The "NTA Study Reporting Tool" (NTA SRT):

- Standardized framework for reviewing quality of NTA reporting
- Aids NTA study design and review (proposals & manuscripts)
- Follows chronology of typical NTA studies with detailed examples
- Scale-based scoring (numeric & colorimetric) for individual study attributes
- HTML interactive version via BP4NTA website (hyperlinks → supporting docs.)
- Fillable PDF version available for download (via website)
- Comment box for periodic updates/revisions (via website)
- Working with journal editors for initial testing and deployment

NTA Study Reporting Tool (draft version)



Hyperlinked (HTML version) to supporting information

3-4 bullet point examples for each of the 13 sub-categories

Not exhaustive – intended to guide reviewers; relies on reviewer expertise/discretion.

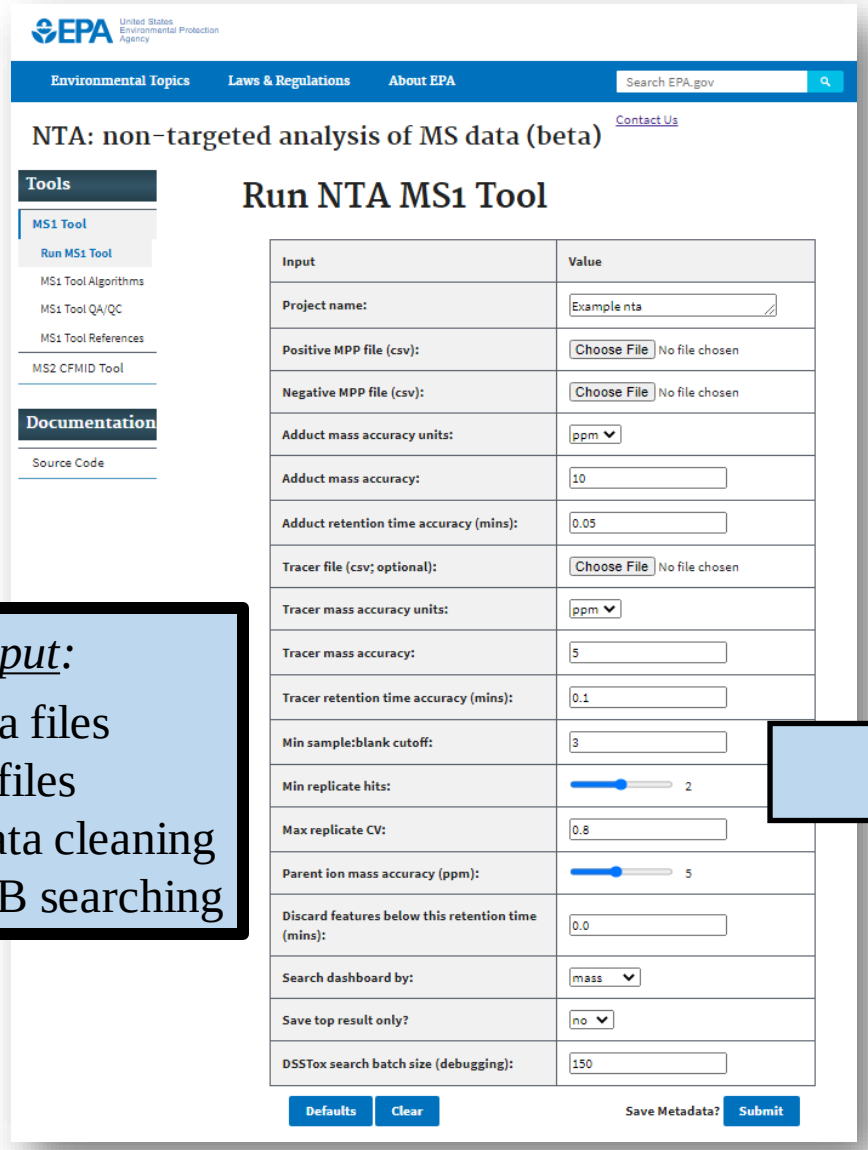
Space for reviewer to explain assigned score

Building Tools to Ensure Transparency & Reproducibility

The EPA NTA WebApp:

- Queries NTA data against DSSTox DB (~900K substances)
- Aggregates metadata to aid candidate prioritization
- Calculates match metrics to aid candidate filtering
- Provides interactive visualization of chemical candidates
- Processes data for advanced statistical analyses
- Standardizes and documents procedures for NTA data analysis
- Adheres to recommendations from BP4NTA workgroup
- Produces publication-ready output in accordance with NTA SRT

EPA's NTA WebApp

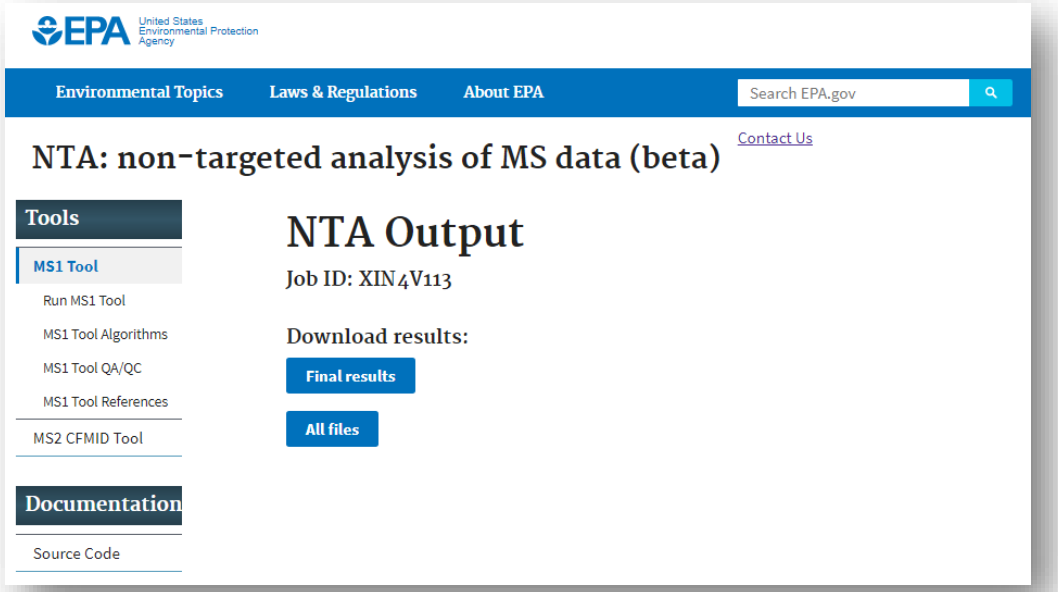


WebApp Input:

- Experimental data files
- Tracer (QA/QC) files
- Parameters for data cleaning
- Parameters for DB searching

WebApp Output:

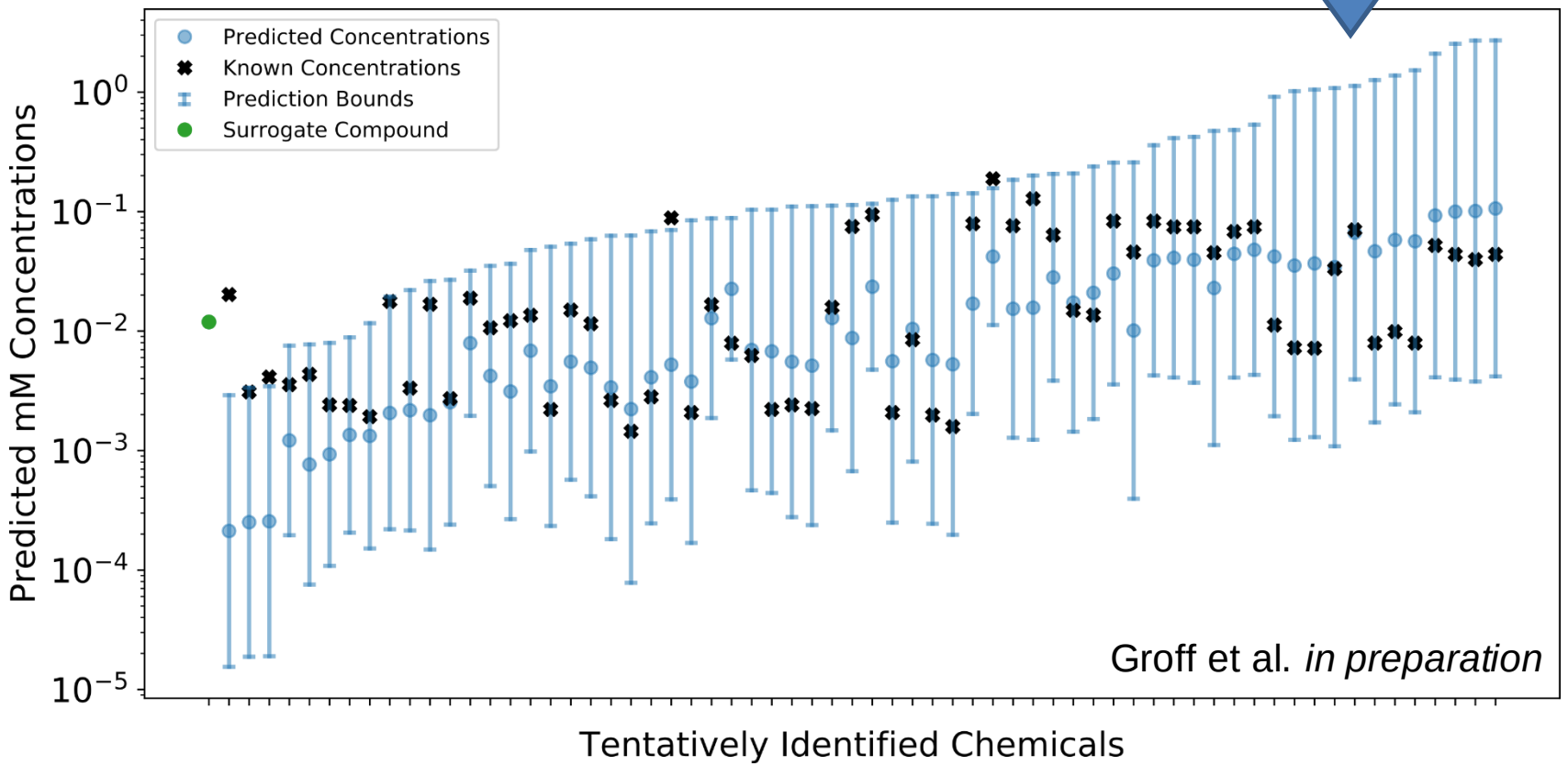
- QA/QC tracer results
- Cleaned, unannotated file for stats analysis
- Cleaned, annotated file with DSSTox chemicals
- Complete file with all chemicals & metadata



Addressing High Priority Data Needs with Proof-of-Concept Applications

- Characterizing chemical contents of products (including UVCBs)
- Characterizing data-poor xenobiotics in biological tissues & fluids
- Identifying xenobiotic metabolites produced from *in vitro* assays
- Developing semi-quantitative (SQ) methods for risk-based interpretation
- Characterizing emerging contaminants in Brita filters (SQ proof-of-concept)
- Developing a framework for rapid response NTA

SQ NTA Proof-of-Concept



- Analysis of Brita filter extracts via GC-HRMS.
- Concentration estimates can be above or below true value.
- Prediction intervals used to bound SQ concentration estimates.
- 95% prediction intervals shown; Can use 99%, 99.9%, etc.
- Tentatively identified compounds ranked by upper bound estimates.
- Upper bound estimates compared to level-of-interest to set priorities.
- Priority compounds further examined using targeted methods.

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Relevant EPA NTA Publications

1) Rager JE, Strynar MJ, Liang S, McMahen RL, Richard AM, Grulke CM, Wambaugh JF, Isaacs KK, Judson R, Williams AJ, Sobus JR. Linking high resolution mass spectrometry data with exposure and toxicity forecasts to advance high-throughput environmental monitoring. Environ Int. 2016 Mar;88:269-280.
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Questions?

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