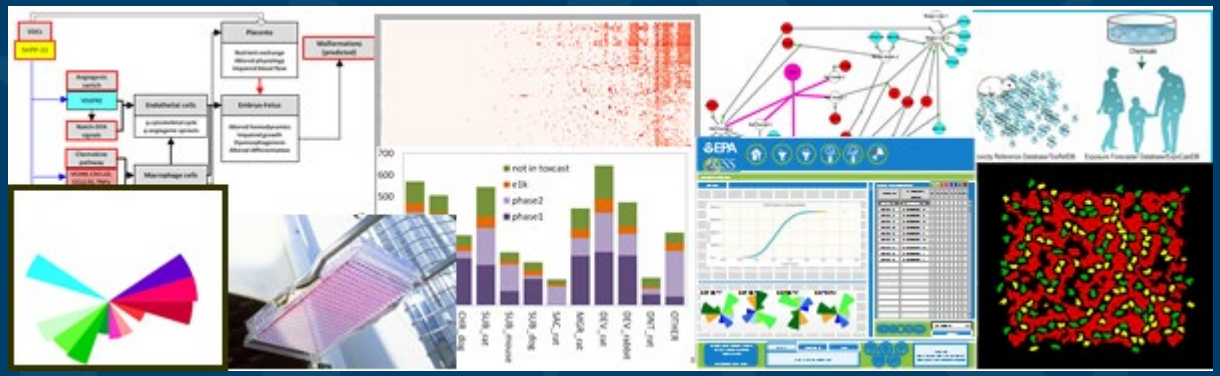
EPA
United States
Environmental Protection
Agency

Current Status of New Approach Methodologies

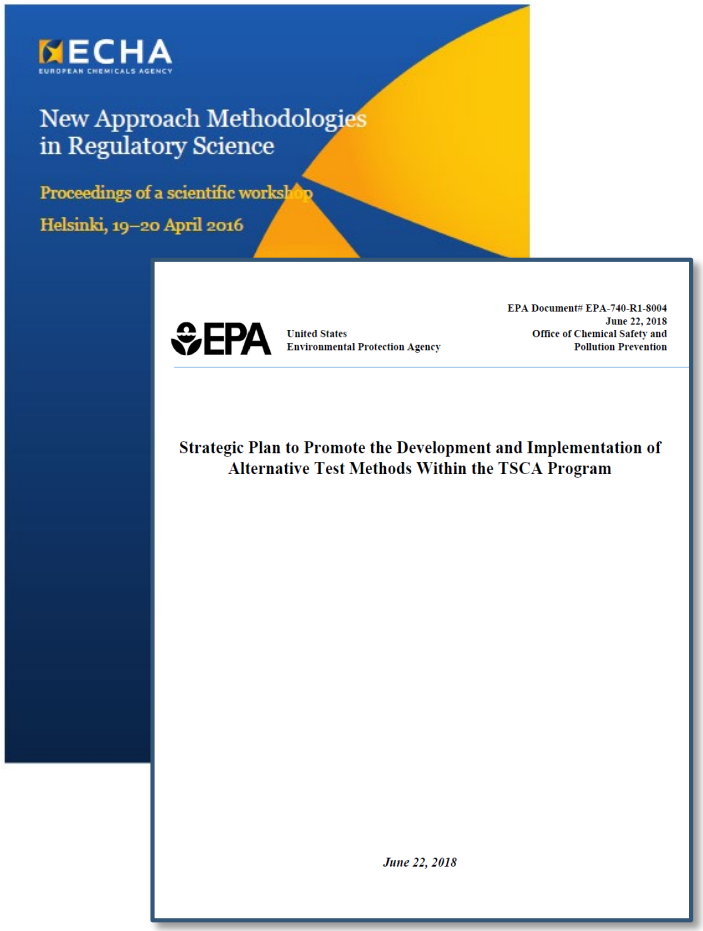


EMAP 514: Introduction to Environmental Health Risk Assessment and Management
Environmental Metrology and Policy Program
GEORGETOWN UNIVERSITY

April 29, 2019

Dr. Maureen R. Gwinn
National Center for Computational Toxicology
Office of Research and Development
US Environmental Protection Agency

New Approach Methodologies (NAMs)



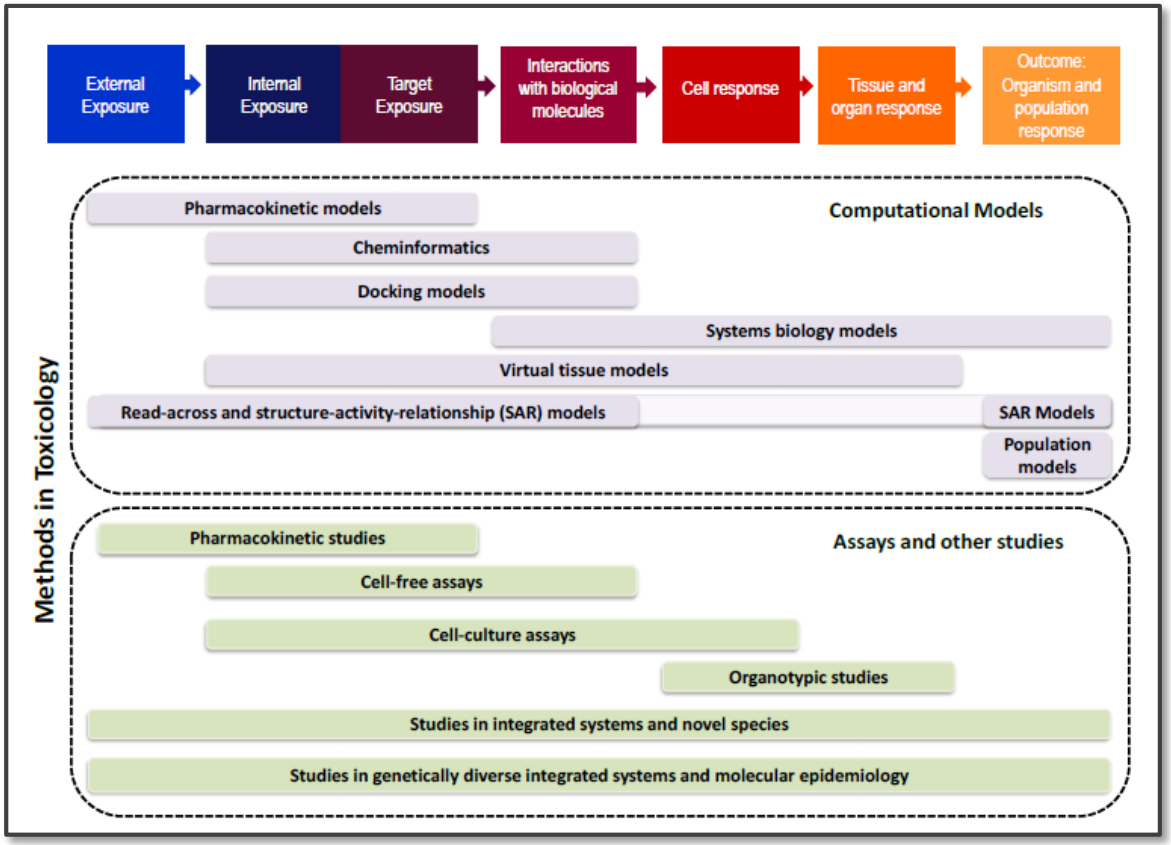
https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf

https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce

- Commonly defined to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment.

- Recently defined in the EPA's TSCA Alternative Toxicity Strategy as:
  - a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals.

Toxicology Moving to Embrace 21st Century Methods

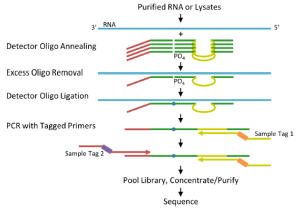


https://www.nap.edu/catalog/24635/using-21st-century-science-to-improve-risk-related-evaluations

Use of NAMs in Filling Gaps in Hazard and Exposure Information

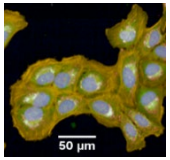
United States Environmental Protection Agency

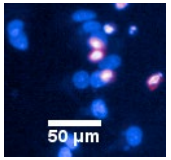
Interactions with biological molecules → Cell response

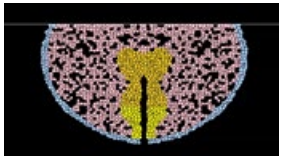


- Use of comprehensive screening to inform hazard characterization
  - High- and Medium-Throughput Screening Assays
  - High-Throughput Metabolism
  - High-Throughput Transcriptomics & Phenotypic Profiling

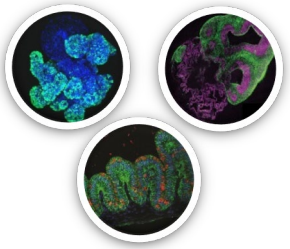
Tissue and organ response → Outcome: Organism and population response



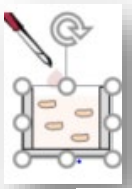




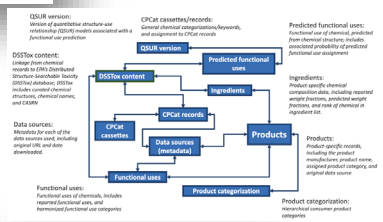
- Higher Tier Adversity
  - Organotypic Cellular Models
  - Virtual Tissue Models

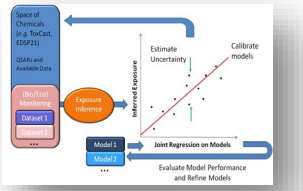


External Exposure → Internal Exposure → Target Exposure

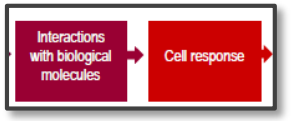


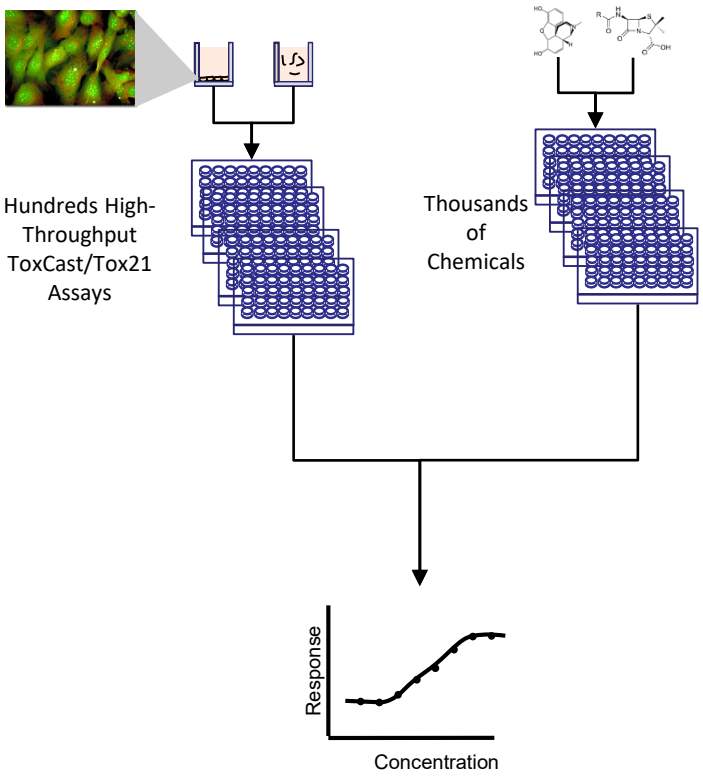
- High-throughput toxicokinetics
  - *In-vitro* studies
  - *In-silico* models and toolls
- Consensus multi-pathway modeling approaches (e.g., ExpoCast SEEM)
- Use of structure-based machine-learning QSAR models to predict exposure information
  - Functional use
  - Exposure pathways

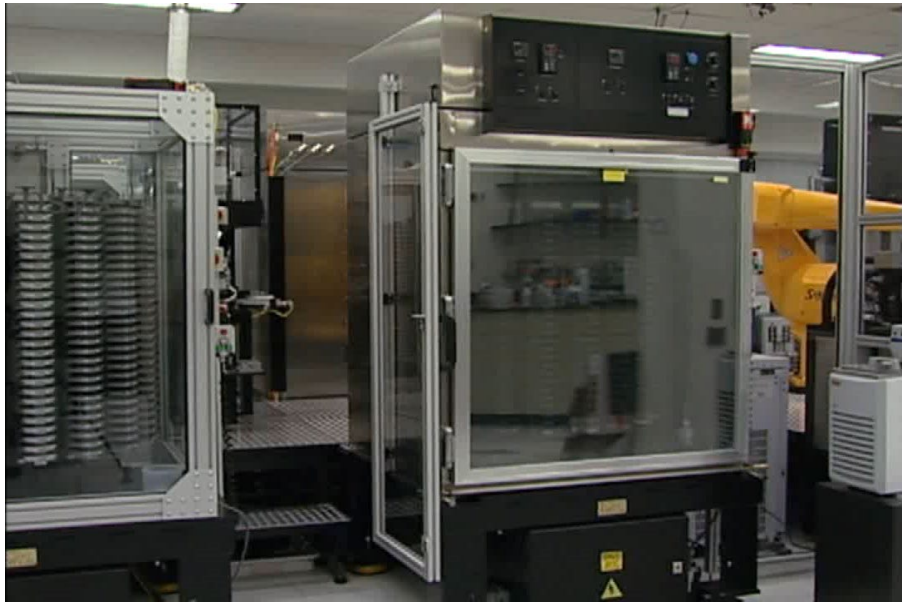




High-Throughput Assays Used to Screen Chemicals for Potential Toxicity

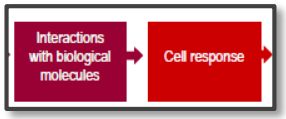






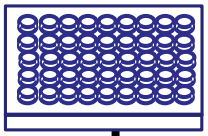
- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations

Innovations in Incorporating Xenobiotic Metabolism

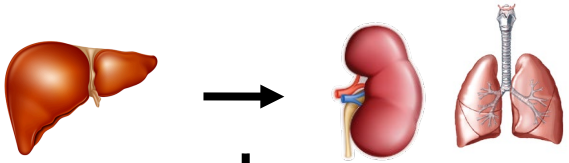


"Extracellular" Approach

Chemical metabolism in the media or buffer of cell-based and cell-free assays

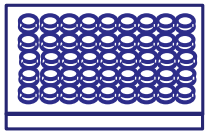


More closely models effects of hepatic metabolism and generation of circulating metabolites

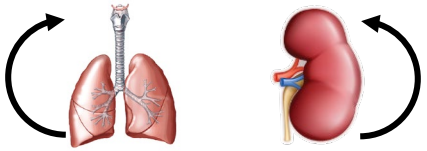


"Intracellular" Approach

Chemical metabolism inside the cell in cell-based assays

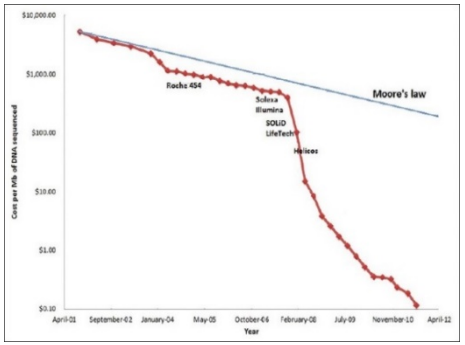


More closely models effects of target tissue metabolism

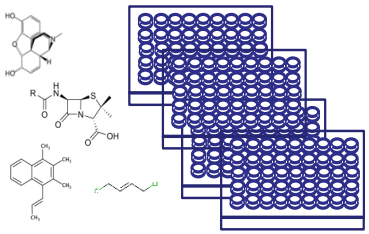


Integrated strategy to model *in vivo* metabolic bioactivation and detoxification

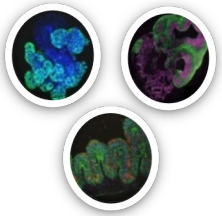
High-Throughput Transcriptomics and Phenotypic Profiling



Thousands of chemicals

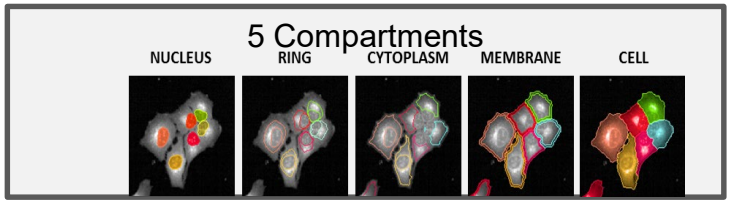


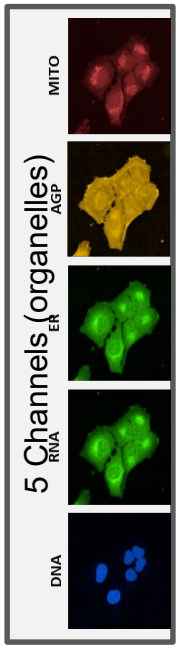
Multiple Cell Types

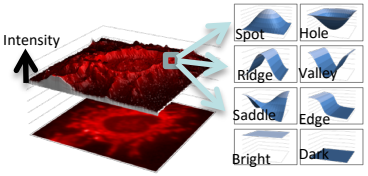


Requirements:

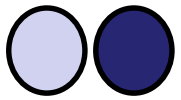
- Low cost
- Whole genome
- 384 well
- Automatable





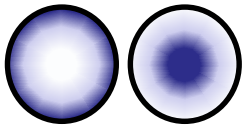


Texture

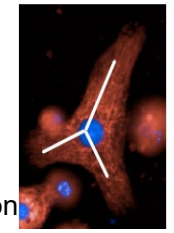


Intensity

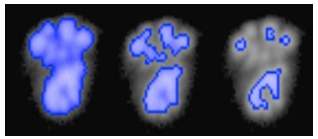
Axial



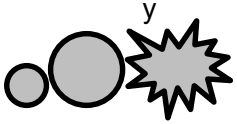
Radial distribution



Symmetry



Compactness



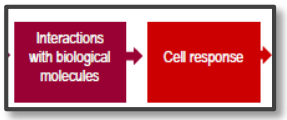
Shape

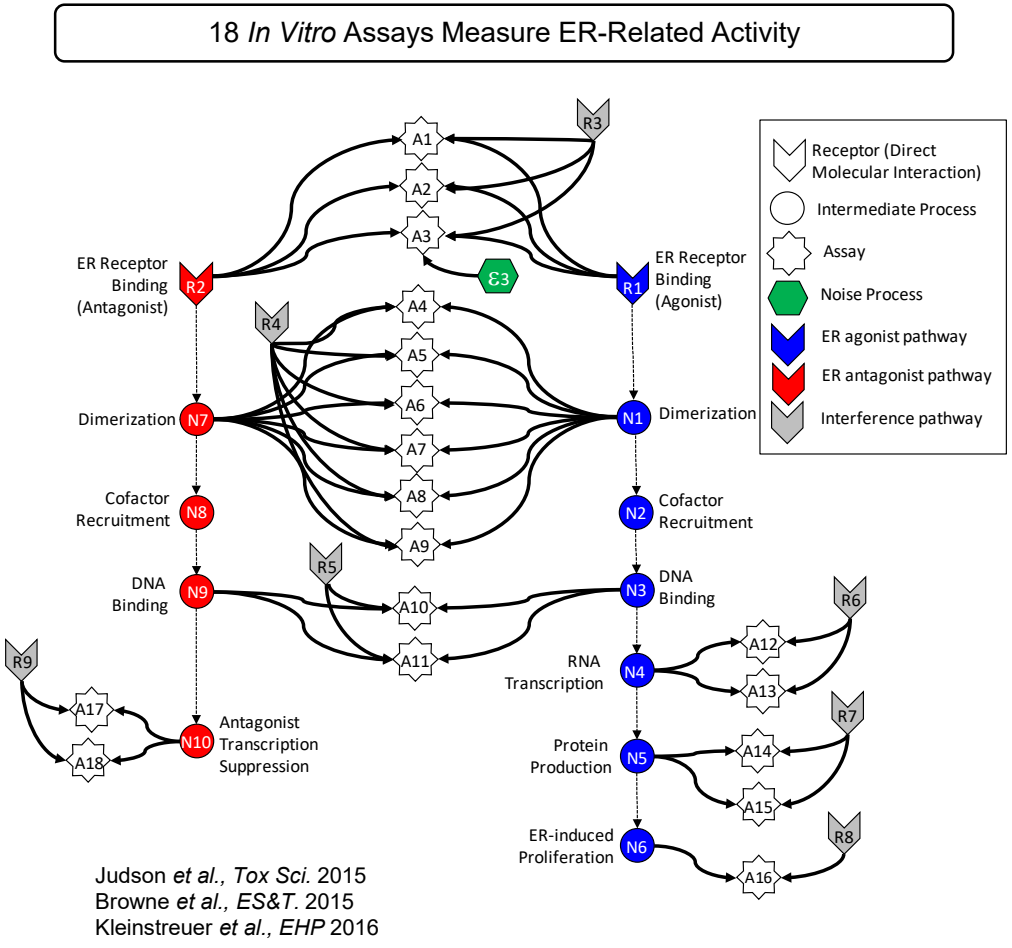
Profile

Illustrations from Perkin Elmer

~ 1300 endpoints
(tcpl: "components")

Application of High-Throughput Assays to Identify Potential Endocrine Disrupting Chemicals



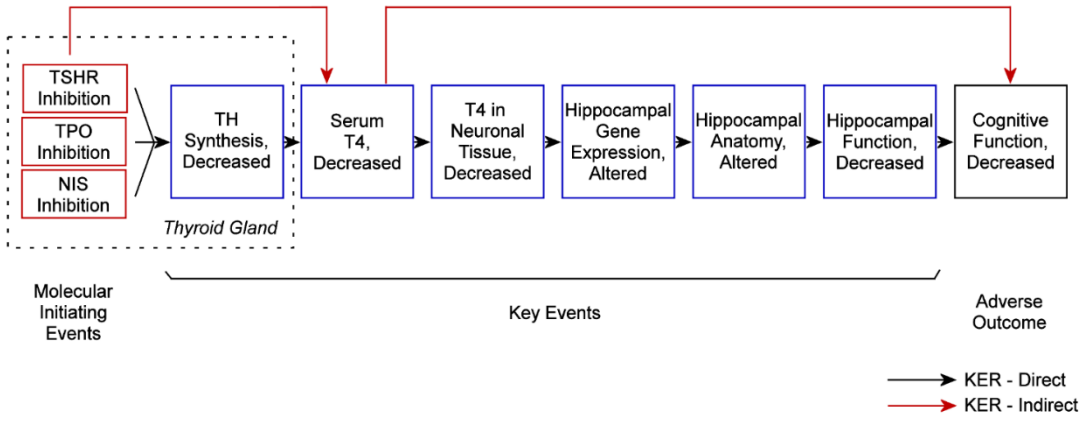


Judson *et al.*, *Tox Sci.* 2015
Browne *et al.*, *ES&T.* 2015
Kleinstreuer *et al.*, *EHP* 2016

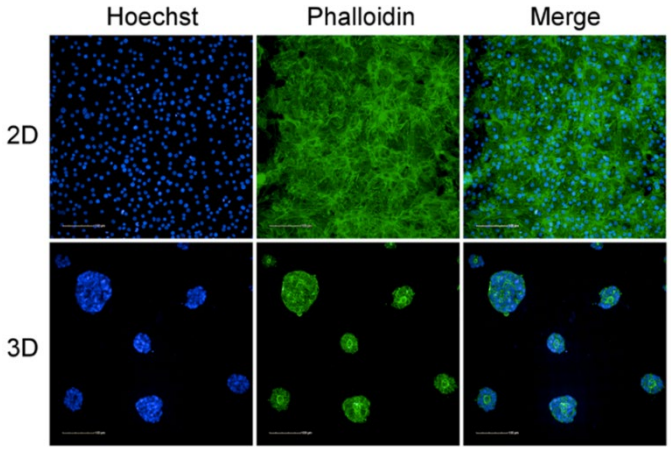
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- Use model to integrate assays
- Model creates a composite dose-response curve for each chemical to summarize results from all assays

Innovating in Organotypic Culture Models to Predict Tissue Effects

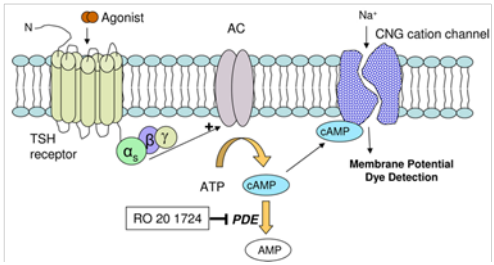
Tissue and organ response



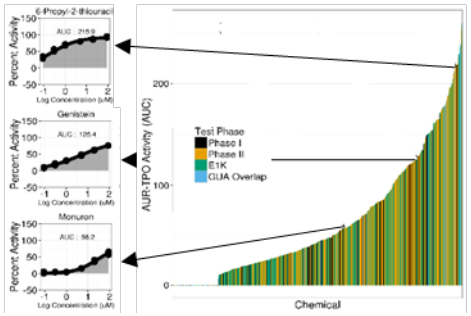
3D Microtissue Model of Primary Human Thyrocytes



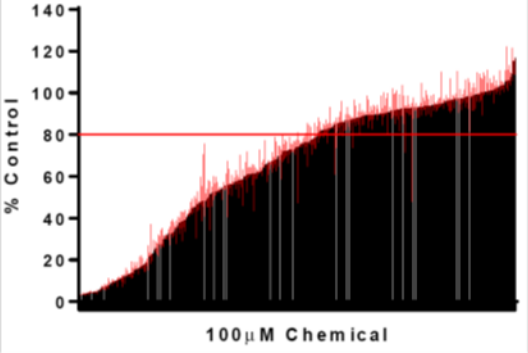
TSH Receptor (TSHR) Screen



Thyroid Peroxidase (TPO) Screen



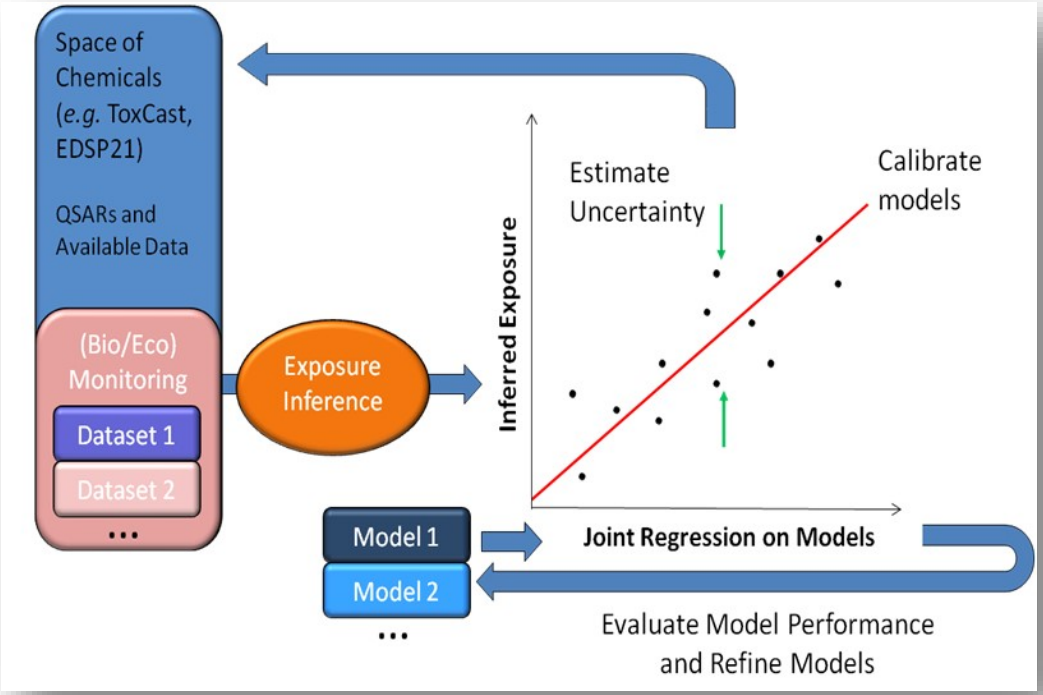
Sodium-Iodide Symporter (NIS) Screen

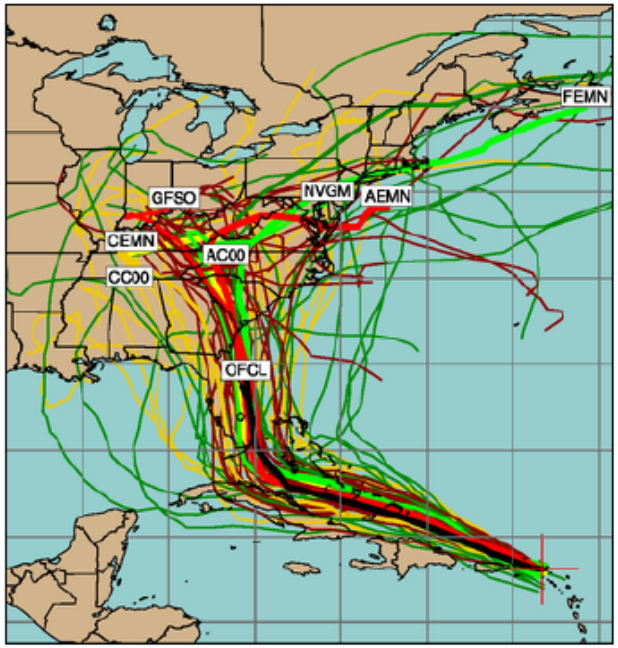


How do we generate quantitative linkages from MIE effects to immediate key events in the thyroid DNT AOP framework for hundreds of chemical "hits"?

Consensus Exposure Predictions with SEEM Framework

- Different exposure models incorporate knowledge, assumptions, and data (Macleod, et al., 2010)
- We incorporate multiple models into consensus predictions for 1000s of chemicals within the Systematic Empirical Evaluation of Models (SEEM) framework (Wambaugh et al., 2013, 2014; Ring et al., 2018).

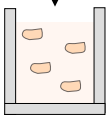




Integrating Multiple Models

High-Throughput Toxicokinetic Component

EPA ToxCast Phase I and II Chemicals



Human Liver Metabolism

Human Plasma Protein Binding

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through "'httk" R package (https://cran.r-project.org/web/packages/httk/)

Population-Based IVIVE Model

Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

In Vitro Potency Value

Plasma Concentration

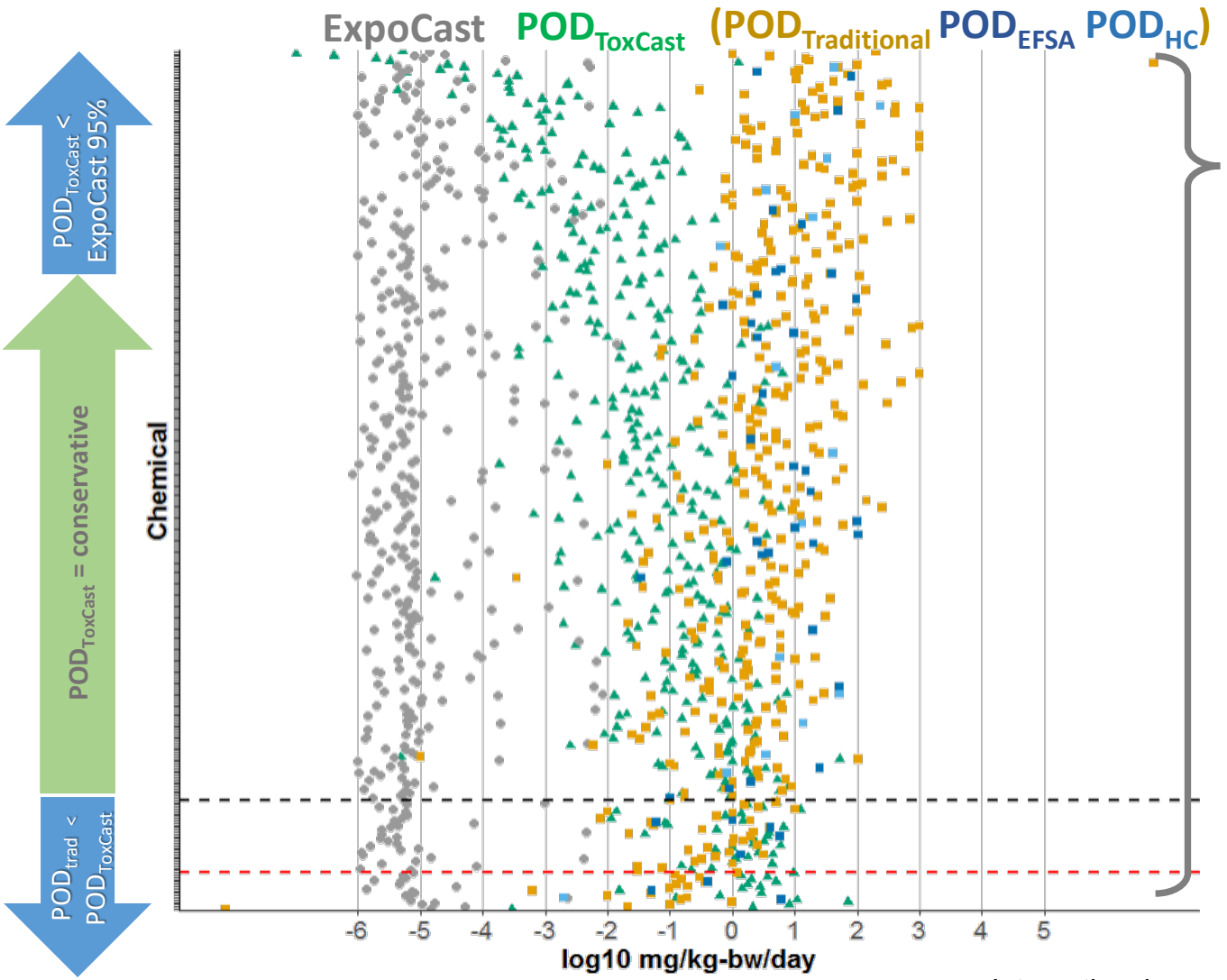
Exposure Route

Reverse Dosimetry

Administered Dose Required to Achieve Steady State Plasma Concentrations Equivalent to *In Vitro* Bioactivity

Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.*, 2012
Wetmore *et al., Tox Sci.*, 2015

Results from High Throughput Assays Provide a Conservative Estimate of Adverse Effects



Total =

448 chemicals

httk, ToxCast data, and POD value(s) currently available

For ~92% of the chemicals, $POD_{ToxCast}$ was conservative.

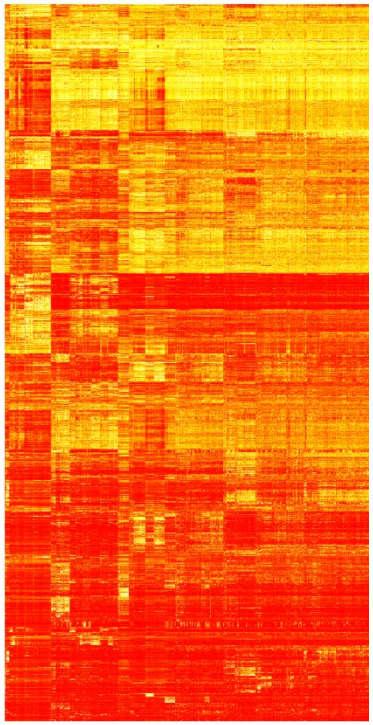
(~100-fold with human HTTK

~50-fold with rat HTTK)

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

Broad Success Derived from High-Throughput Screening Approaches

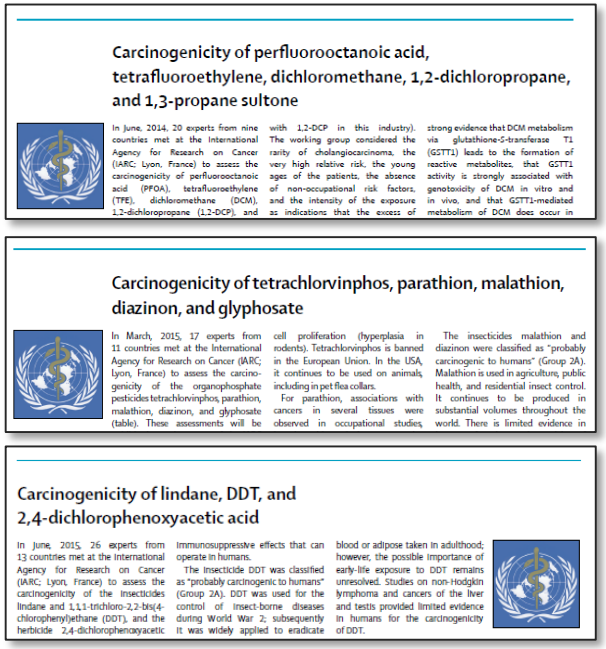
Group Chemicals by Similar Bioactivity and Predictive Modeling



Chemicals

Assays/Pathways

Provide Mechanistic Support for Hazard ID



Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone

In June, 2014, 20 experts from nine countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of perfluorooctanoic acid (PFOA), tetrafluoroethylene (TFE), dichloromethane (DCM), 1,2-dichloropropane (1,2-DCP), and with 1,2-DCP in this industry). The working group considered the rarity of cholangiocarcinoma, the very high relative risk, the young ages of the patients, the absence of non-occupational risk factors, and the intensity of the exposure as indications that the excess of strong evidence that DCM metabolism via glutathione-S-transferase T1 (GSTT1) leads to the formation of reactive metabolites, that GSTT1 activity is strongly associated with genotoxicity of DCM in vitro and in vivo, and that GSTT1-mediated metabolism of DCM does occur in

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be cell proliferation (hyperplasia in rodents). Tetrachlorvinphos is banned in the European Union. In the USA, it continues to be used on animals, including in pet flea collars.

For parathion, associations with cancers in several tissues were observed in occupational studies, The insecticides malathion and diazinon were classified as "probably carcinogenic to humans" (Group 2A). Malathion is used in agriculture, public health, and residential insect control. It continues to be produced in substantial volumes throughout the world. There is limited evidence in

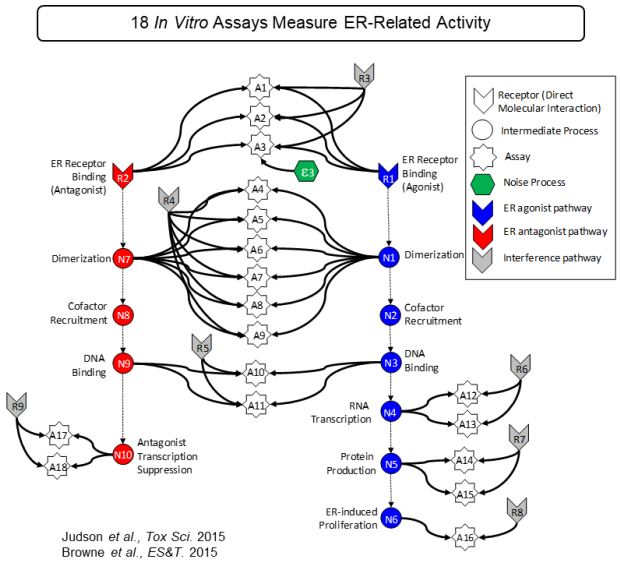
Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

In June, 2015, 26 experts from 13 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the insecticides lindane and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), and the herbicide 2,4-dichlorophenoxyacetic Immunosuppressive effects that can operate in humans.

The insecticide DDT was classified as "probably carcinogenic to humans" (Group 2A). DDT was used for the control of insect-borne diseases during World War 2; subsequently it was widely applied to eradicate blood or adipose taken in adulthood; however, the possible importance of early-life exposure to DDT remains unresolved. Studies on non-Hodgkin lymphoma and cancers of the liver and testis provided limited evidence in humans for the carcinogenicity of DDT.

IARC Monographs 110, 112, 113

Prioritization of Chemicals for Further Testing



Judson *et al.*, *Tox Sci.* 2015
Browne *et al.*, *ES&T.* 2015
Kleinstreuer *et al.*, *EHP* 2016

EPA United States Environmental Protection Agency

Conclusions

- Incorporating new technologies and innovations in toxicology can more rapidly and inexpensively screen chemicals for potential adverse biological effects
- Incorporating dosimetry and exposure provides an important dose and exposure context
- Uncertainty analysis of NAMs is an ongoing part of research and development of these new technologies
- Data management systems and decision support tools will be increasingly important for interpreting and integrating the expanding and diverse landscape of chemical safety information for use in weigh-of-evidence decisions

EPA United States Environmental Protection Agency

Thank You for Your Attention!

Tox21 Colleagues:
- NTP Crew
- FDA Collaborators
- NCATS Collaborators

EPA Colleagues:
- NERL
- NHEERL
- NCEA

EPA's National Center for Computational Toxicology

EPA United States Environmental Protection Agency

Additional slides

Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals

Dr. Maureen R. Gwinn (gwinn.maureen@epa.gov)
U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC

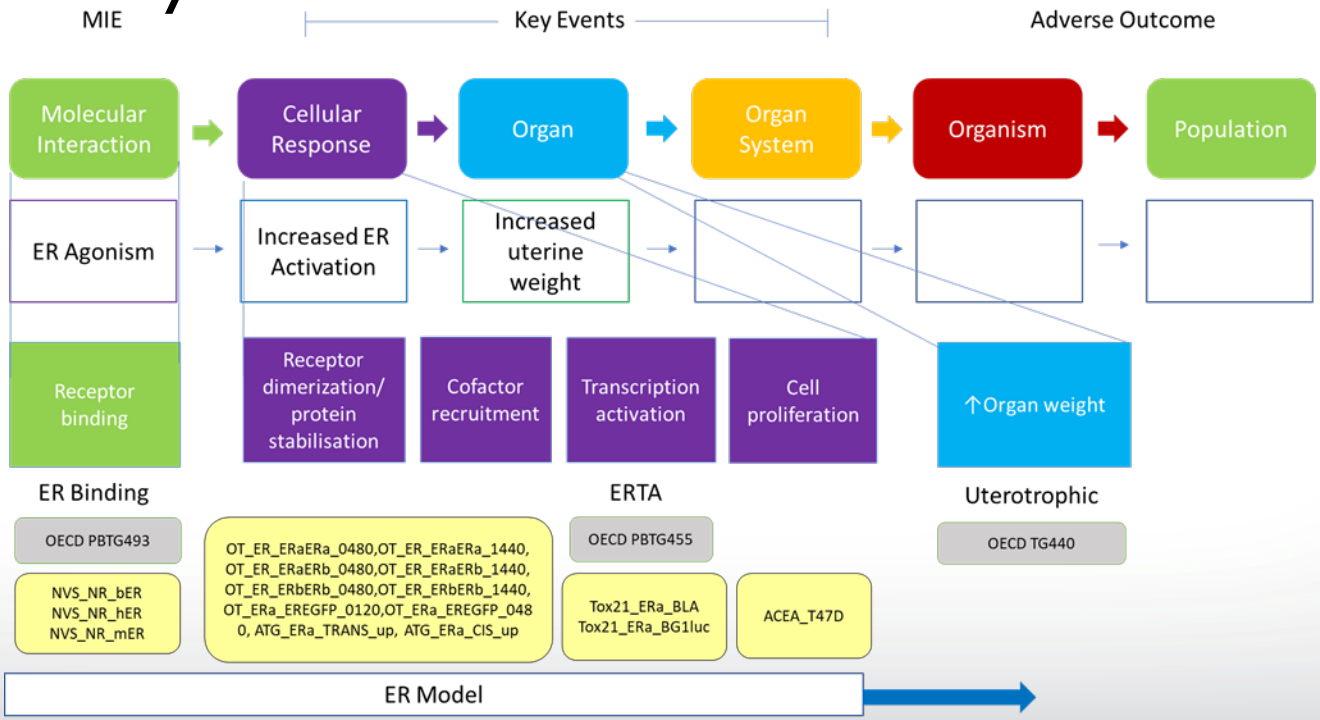
The views expressed in this presentation are that of the presenter and do not represent the views and/or policies of the US Environmental Protection Agency

Intended Application

- The intended application of this IATA is for
  - screening of environmental chemicals based on their ER agonist activity
  - determining whether further evaluation of endocrine-related activity in higher tier in vivo tests (e.g., female pubertal assay, two generation reproductive toxicity study) is needed

Purpose

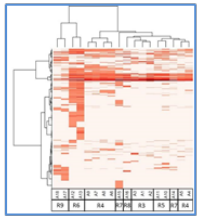
- To use a combination of 16 in vitro high throughput screening (HTS) assays and a computational model for estrogen receptor (ER) agonist activity, as an alternative to low and medium throughput in vitro and in vivo tests for ER activity.



Overall Approach

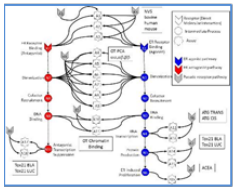
Internal

External



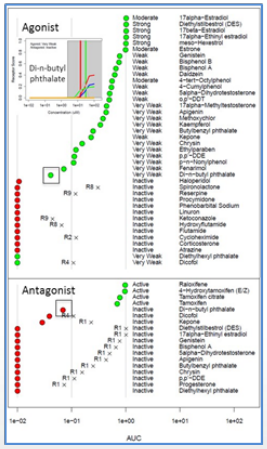
ER High-Throughput Screening Data

In vitro reference chemicals

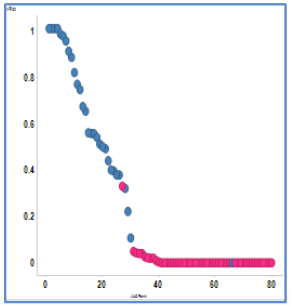


ER Pathway Computational Model

In vivo reference chemicals



Model Performance Evaluation



Validated Model for Chemical Screening

Equivalent Performance Observed for Subsets of *In Vitro* Assays

- Results of this analysis demonstrate that one could use one of multiple subset models to accurately predict estrogenic activity of a chemical.
- Subsets of as few as 4 of the original 16 agonist assays have acceptable performance against the full model, and the in vitro and in vivo reference chemicals.
- The acceptable subsets all have assays that:
  - probe diverse points in the ER pathway
  - use diverse assay reporting technologies
  - use diverse cell types

Judson et al., Reg. Tox. Pharm. (2017)

Summary of Proposed Case Study

- Outlines the curation of lists of reference chemicals for *in vitro* and *in vivo* ER activity
- Integrates results from multiple *in vitro* assays using pathway-based ER computational model as an IATA
- Evaluates performance of the IATA using the curated lists of reference chemicals
- Demonstrates equivalent performance for subsets of *in vitro* assays
- Characterizes the uncertainty associated with the *in vitro* assays and computational model
- Discusses potential application to regulatory decisions