

Moving Towards Version 2.0 of Toxicity Testing in the 21st Century and Application to Regulatory Decision Making

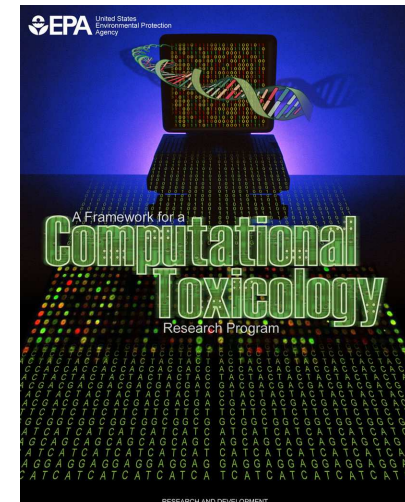
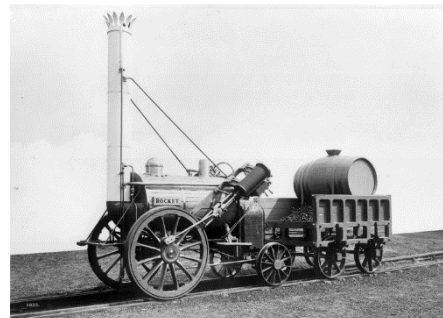
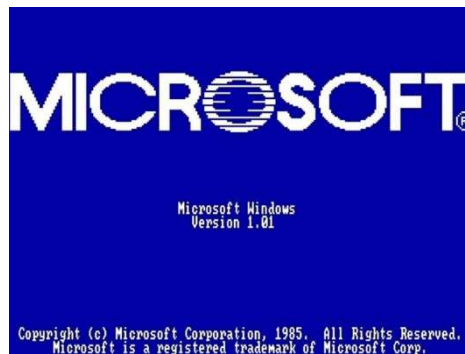
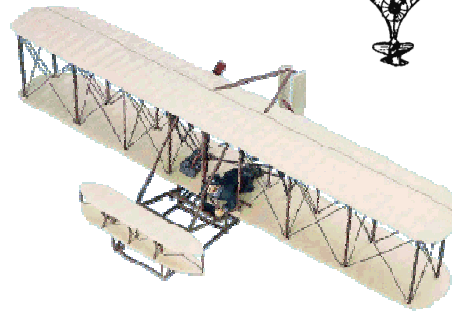
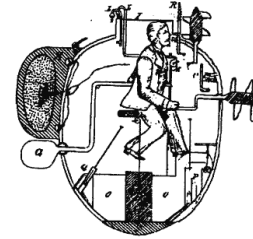
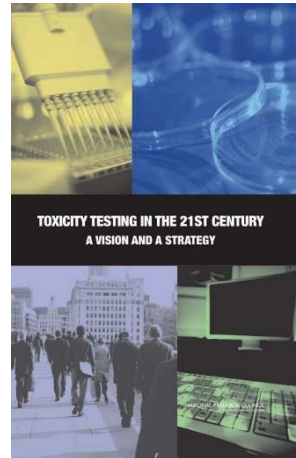


**ECHA Topical Scientific Workshop on New Approach Methodologies
April 20, 2016**

**Rusty Thomas
Director
National Center for Computational Toxicology**

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

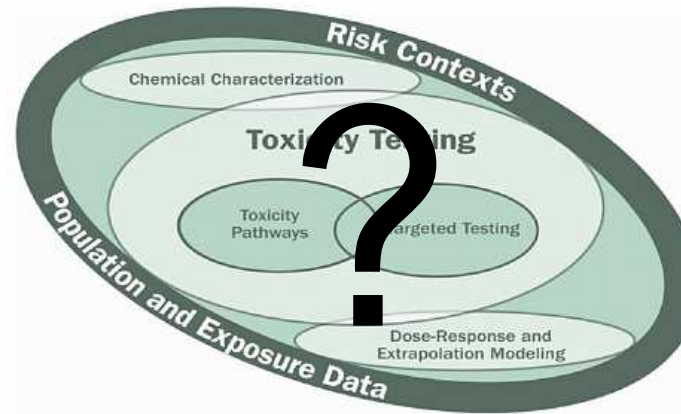
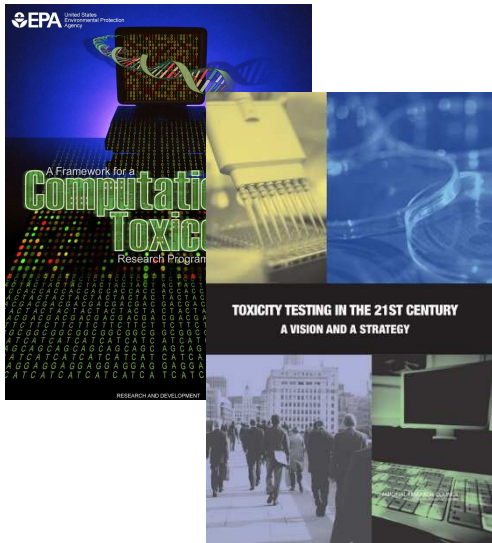
Version 1.0 is Never Perfect...



What Does Version 2.0 Look Like?



■ ■ ■

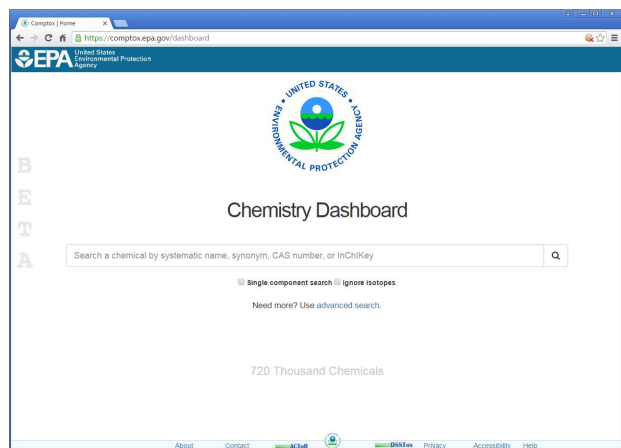


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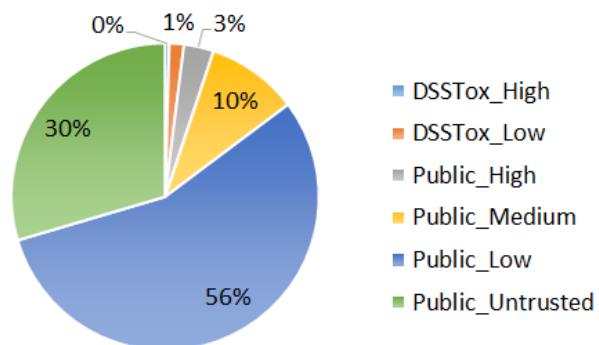
The scientific and knowledge base has evolved significantly over the past 8 – 10 years. Are the new approaches ready for regulatory decision making?

Chemical Characterization

Quality Chemistry as the Foundation



<https://comptox.epa.gov>

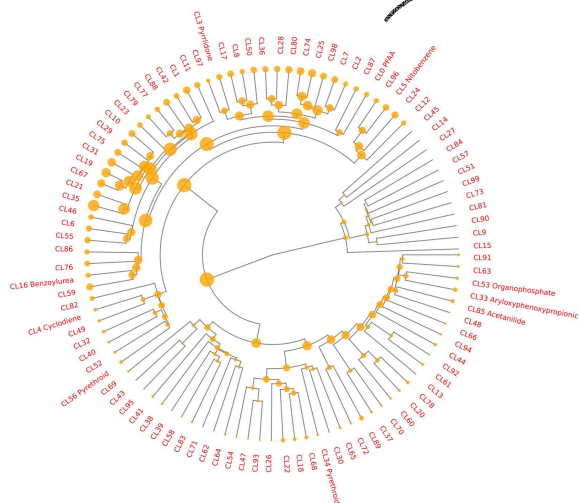


- Developing a centralized resource for curated chemical structure, identifier, and physical chemical properties of >700K unique substances with data quality flags
- Expand and curate training sets for QSAR models for phys-chem, environmental fate, and toxicological properties
- Use the centralized chemical resource as the foundation for an integrated hazard, bioactivity, pharmacokinetics, and exposure knowledgebase

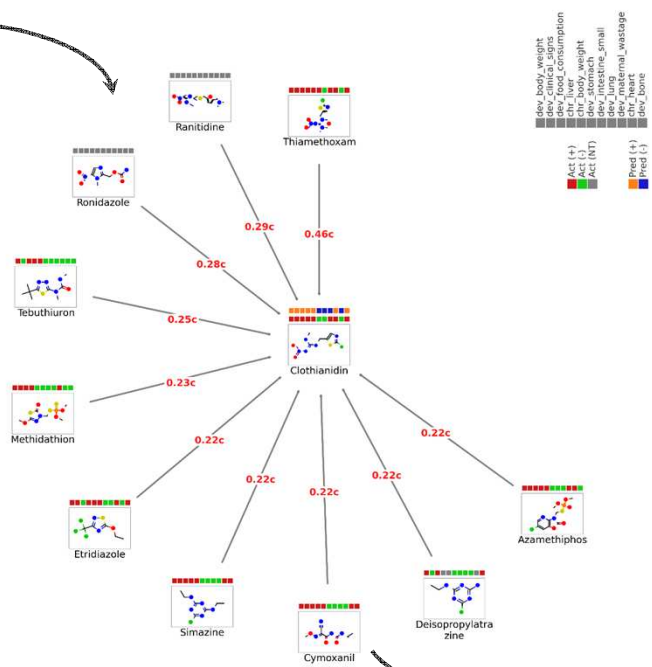
Chemical Characterization

Quantitatively Evaluating Read-Across Uncertainty

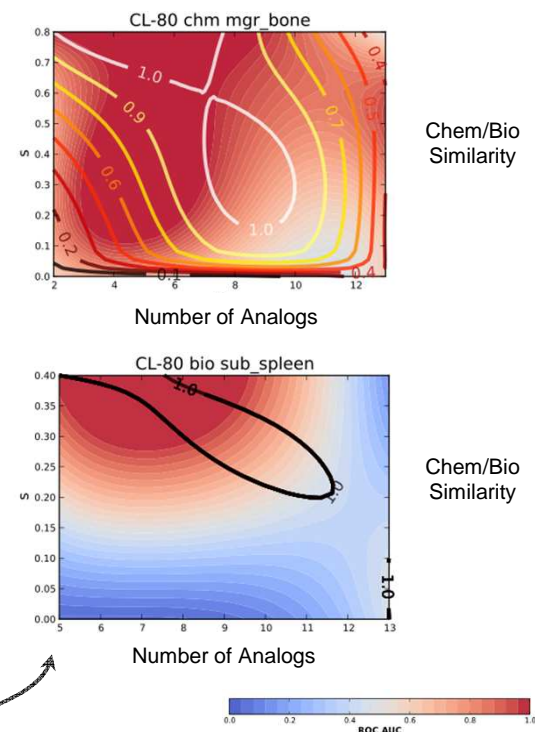
Chemicals Clustered Based on Chemotype, Structure, or Biological Descriptors



Read-across Analysis Based on Similarity-Weighted Activity of Nearest Neighbors



Uncertainty Analysis



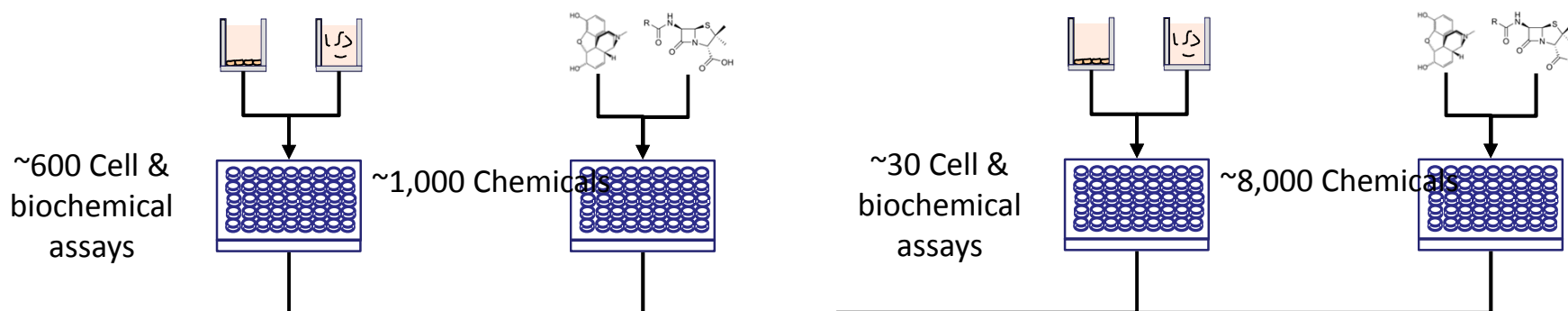
Read-across approach will allow users to define similarity and analog cut-offs while trading off uncertainty

Toxicity Testing

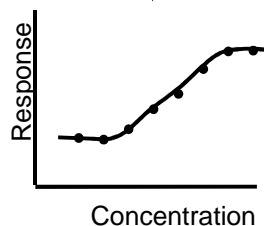
ToxCast and Tox21 High-Throughput Screening

ToxCast

Tox21

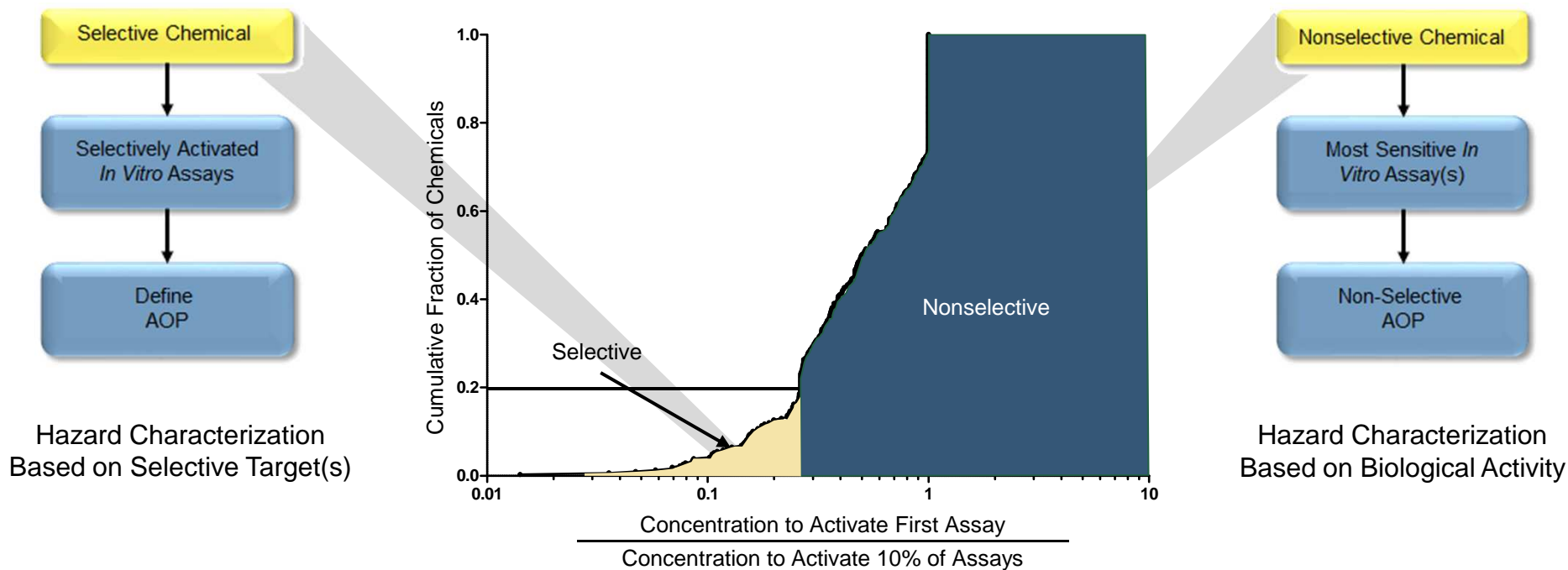


Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013



Toxicity Testing

Considering Biological Selectivity as a Starting Point for Safety Decisions

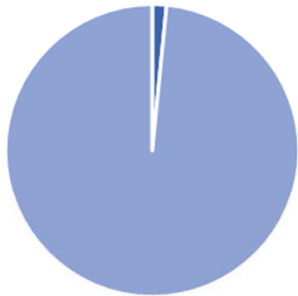


Thomas *et al.*, 2013

Toxicity Testing

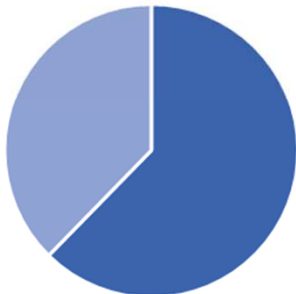
New Approaches to Comprehensively Assess Potential Biological Effects

Gene Coverage



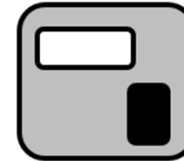
■ ToxCast
■ Not in ToxCast

Pathway Coverage*



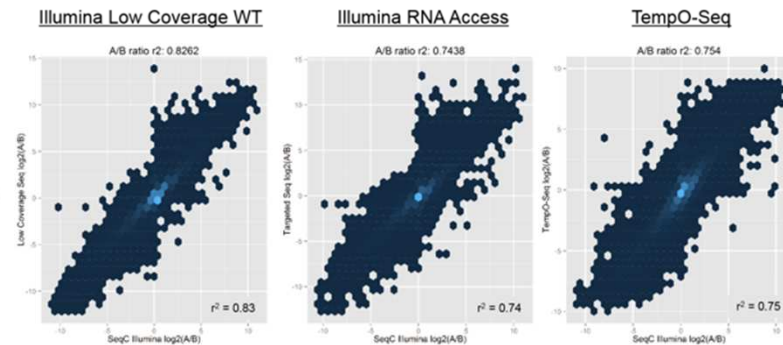
*At least one gene from pathway represented

High-Throughput
Transcriptomic
Platforms



- Low-cost
- Whole genome
- 384-well
- Automatable

Technical
Comparison



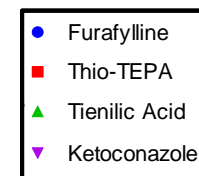
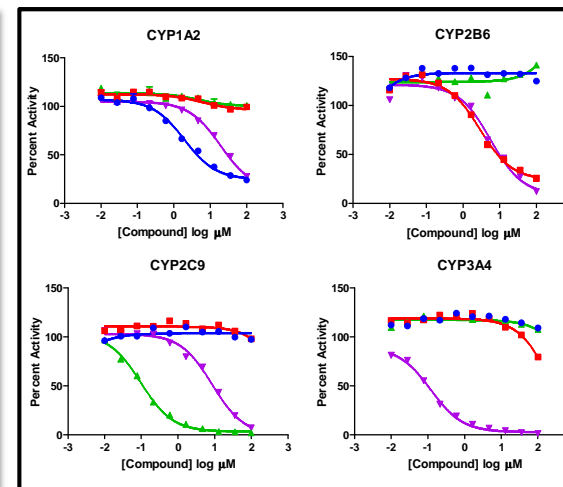
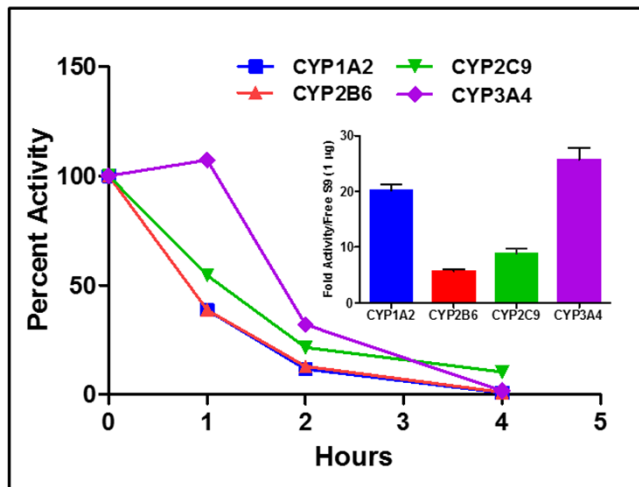
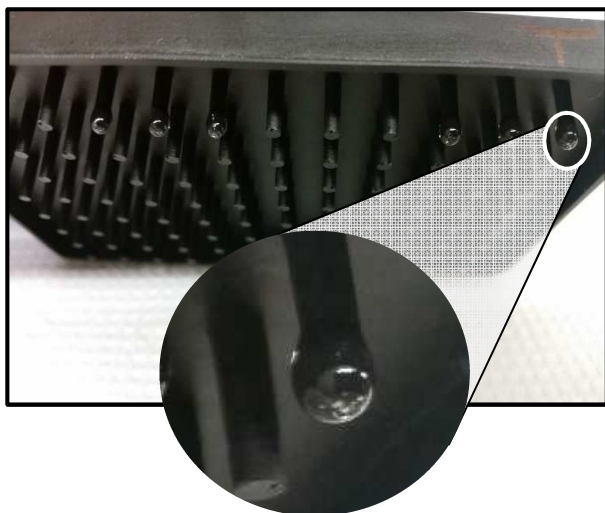
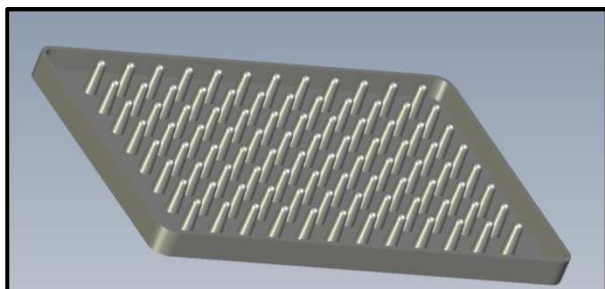
Functional
Comparison

Correct Mechanistic Match

2/5 (40%)	2/5 (40%)	5/5 (100%)
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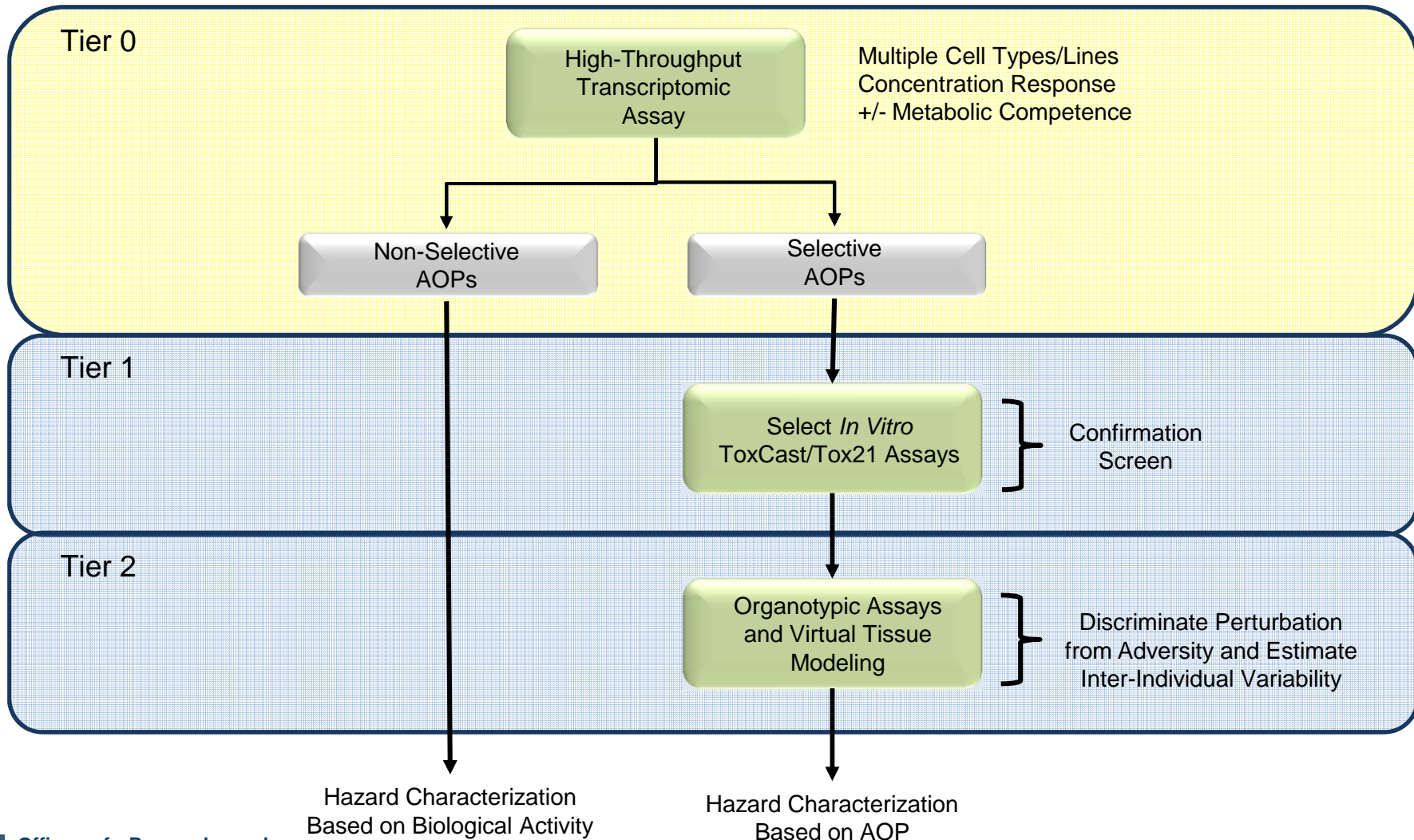
Toxicity Testing

Incorporating Metabolic Competence into In Vitro Assays



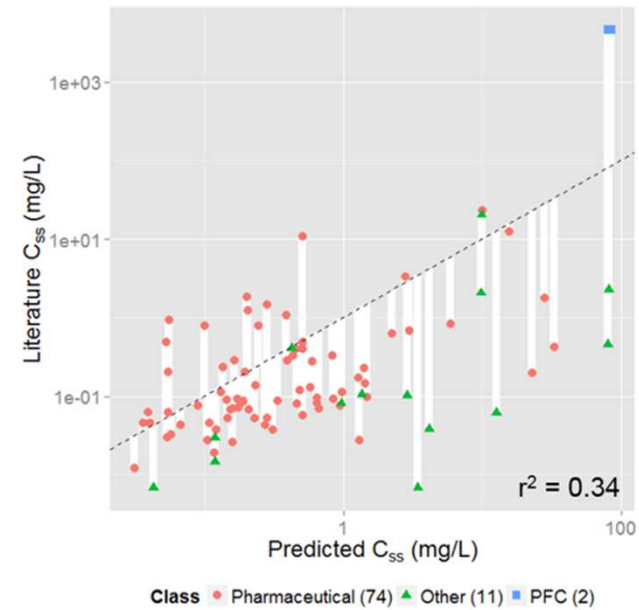
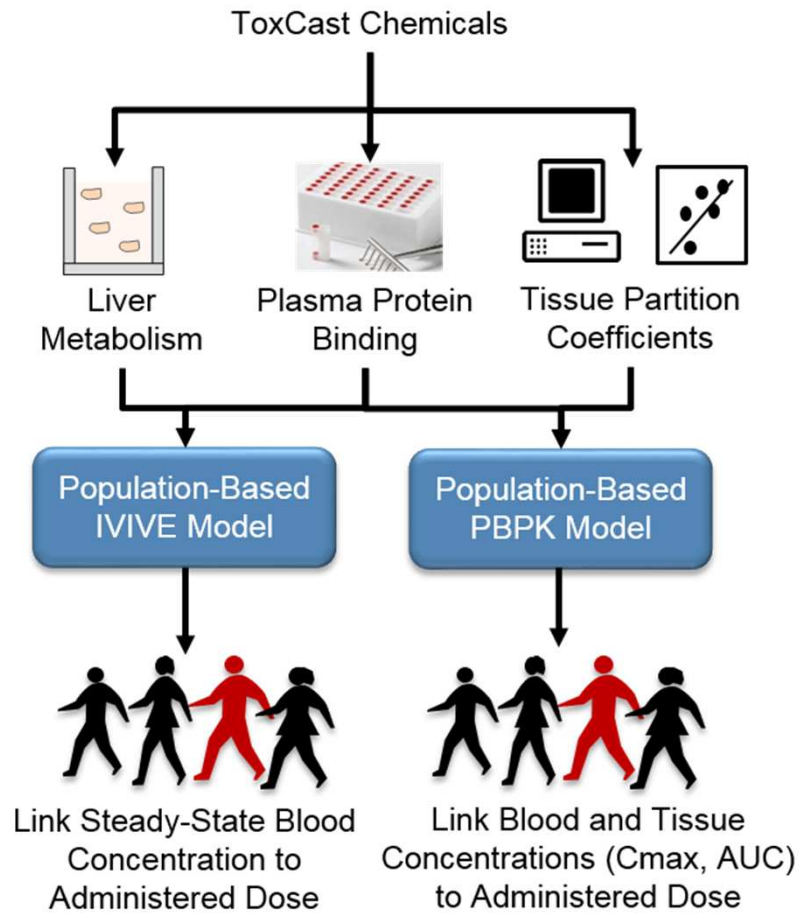
Toxicity Testing

Integrating New Thinking Into a Tiered Testing Framework

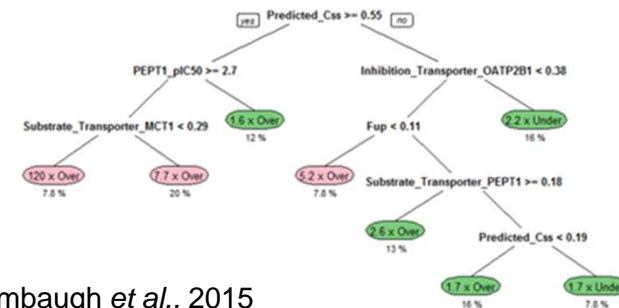


Dose and Systems Modeling

Incorporating Dosimetry and Uncertainty into In Vitro Screening



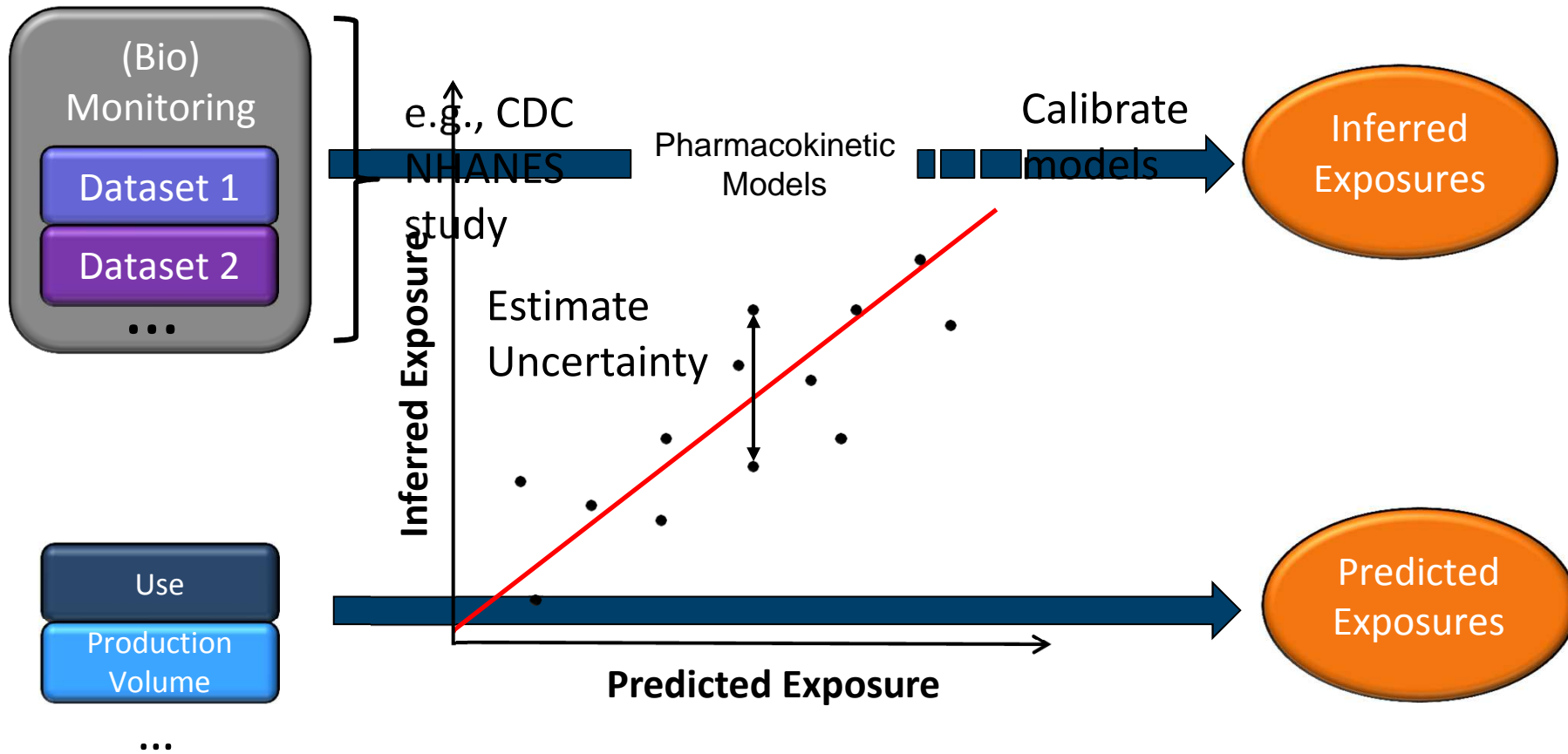
Recursive Partition Tree on Residuals



Wambaugh *et al.*, 2015

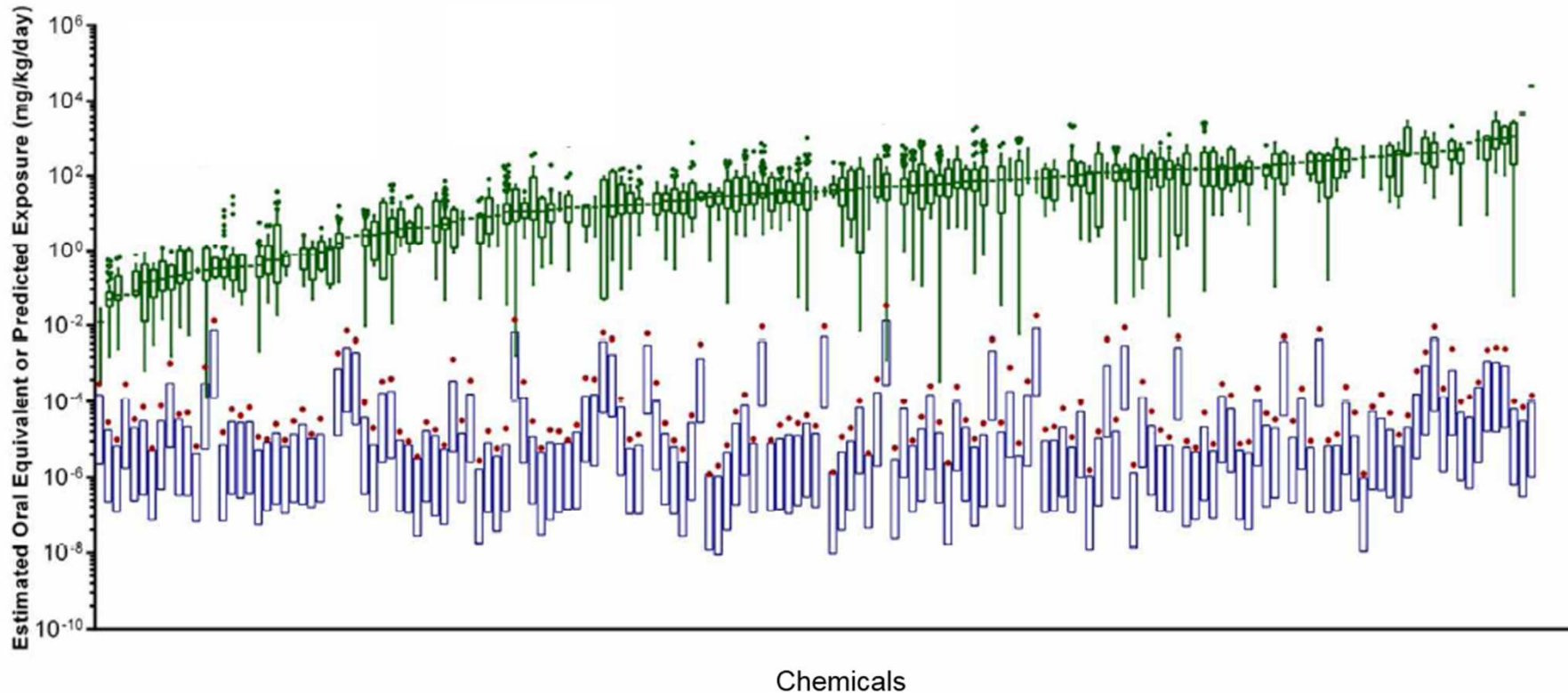
Population and Exposure Data

Estimating Exposure and Associated Uncertainty with Limited Data



Population and Exposure Data

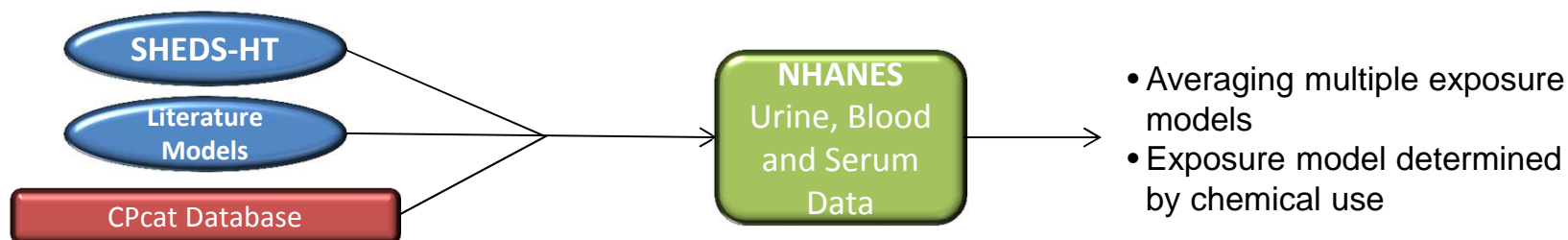
Enabling Risk-Based Chemical Safety Decisions



Wetmore *et al.*, *Tox Sci.*, 2015

Population and Exposure Data

Reducing Uncertainty in Exposure Models



Use Database
(FUSE)

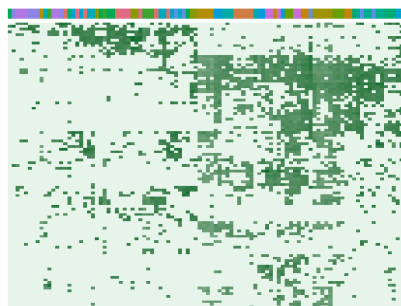


adhesive	additive for liquid systems	additive for rubber	additive for plastic	anti-microbial	anti-oxidant	antibiotic agent
buffer	catalyst	colorant	colorant	condensate	emulsifier	emulsifier
emulsion stabilizer	flow forming agent	flame retardant	fluorocarbon	flame boosting agent	foamer	fragrance
hair conditioner	hair dye	heat stabilizer	humectant	lubricating agent	masking agent	nutriment
oral care	organic pigment	oxidant	perfumer	pH stabilizer	photo-stabilizer	plasticizer
pre-sensitizer	radical	thinning modifier	ultra-conditioner	skin protectant	soluble dye	solvent
surfactant	UV absorber	UV absorber	viscosity controlling agent	wetting agent	whitener	

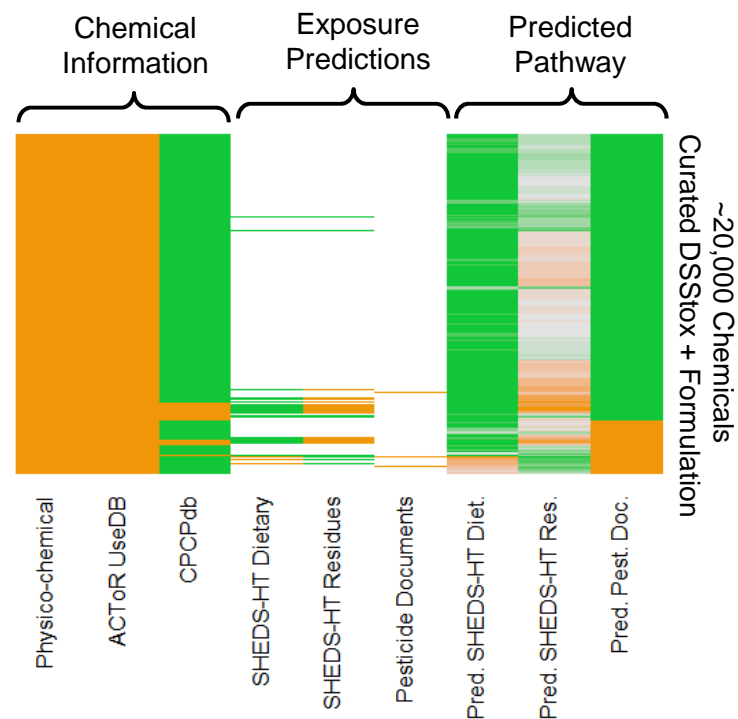
Machine Learning Approaches to Predict Chemical Use

Pilot Study

- 20 Product types x 5 products/type
- 2D GCxTOF/MS



Non-Targeted Analytical Screening of Consumer Product Composition



Risk Context

Initial Focus on Endocrine-Related Regulatory Application

Prioritization of the EDSP Universe of Chemicals

Prioritization of the Endocrine Program Universe of Chemicals: Receptor Adverse Outcome Computational Toxicology

U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program

Jointly developed by:

- Office of Chemical Safety and Pollution Prevention
Office of Science Coordination and Policy (OSCP)
Office of Pesticide Programs (OPP)
Office of Pollution Prevention and Toxics (OPPT)
- Office of Water (OW)
Washington, DC 20460
- Office of Research and Development (ORD)
National Environmental and Effects Health Research
Mid-Continent Ecology Division (MED), Duluth, MN
Toxicity Assessment Division (TAD), RTP, NC 27111
- National Center for Computational Toxicology (NCCCT)
Research Triangle Park, NC 27709

December 2012

Exposure SAP White Paper

New High-throughput Methods to Estimate Chemical Exposure

Scientific Advisory Panel Meeting, July 2014

New High-throughput Methods to Estimate Chemical Exposure 1

7/8/2014

Integrated Bioactivity and Exposure Ranking

Integrated Bioactivity and Exposure Ranking: A Computational Approach for the Prioritization and Screening of Chemicals in the Endocrine Disruptor Screening Program

Environmental Protection Agency
Endocrine Disruptor Screening Program

by:

Office of Chemical Safety and Pollution Prevention
Office of Research and Development (ORD)
Office of Water (OW)

National Center for Computational Toxicology Program Interagency Center for Toxicological Methods (NICETAM)

SAP December 2-5

35350 Federal Register / Vol. 80, No. 118 / Friday, June 19, 2015 / Notices

may claim all or part of a response confidential. EPA will issue another Federal Register document pursuant to 5 CFR 1320.5(a)(1)(iv) to announce the submission of the ICR to OMB and the opportunity to submit additional comments to OMB. If you have any questions about this ICR or the approval process, please contact the technical person listed under FOR FURTHER INFORMATION CONTACT.

Authority: 44 U.S.C. 3501 et seq.
Date: June 10, 2015.

James Janes
Assistant Administrator, Office of Chemical Safety and Pollution Prevention.
EPA Doc. 2015-11889 Final 16-11-15, 6:41 am
BILLING CODE 6560-60-P

ENVIRONMENTAL PROTECTION AGENCY
[EPA-102-OPP-2015-0205, FRL-9228-65]

Use of High Throughput Assays and Computational Tools: Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment

AGENCY: Environmental Protection Agency (EPA).
ACTION: Notice.

SUMMARY: This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will improve the Agency's ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a computational model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and computational models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and

efficient screening using alternative test methods to some assays in the Tier 1 battery to protect human health and the environment.

DATES: Comments must be received on or before August 18, 2015.

ADDRESSES: Submit your comments, identified by docket identification (ID) number EPA-102-OPP-2015-0205, by one of the following methods:

- **Federal eRulemaking Portal:** <http://www.regulations.gov>. Follow the online instructions for submitting comments. Do not submit electronically any information you consider to be Confidential Business Information (CBI) or other information whose disclosure is restricted by statute.
- **Mail:** Document Control Office (7407M), Office of Pollution Prevention and Toxics (OPPT), Environmental Protection Agency, 1200 Pennsylvania Ave. NW, Washington, DC 20460-0001.
- **Hand Delivery:** To make special arrangements for hand delivery or delivery of boxed information, please follow the instructions at <http://www.epa.gov/dockets/contacts.html>. Additional instructions on commenting or visiting the docket, along with more information about dockets generally, is available at <http://www.epa.gov/dockets>.

FOR FURTHER INFORMATION CONTACT: For technical information contact: Jane Robbins, Office of Science Coordination and Policy (OSCP), Office of Chemical Safety and Pollution Prevention, Environmental Protection Agency, 1200 Pennsylvania Ave. NW, Washington, DC 20460-0001; telephone number: (202) 564-6625; email address: robbins.jane@epa.gov.

For general information contact: The TSCA Hotline, ABVL-Goodwill, 422 South Clinton Ave, Rochester, NY 14620; telephone number: (202) 554-1404; email address: TSCA-Hotline@epa.gov.

SUPPLEMENTARY INFORMATION:

I. General Information.

A. Does this action apply to me?

This action is directed to the public in general, and may be of interest to a wide range of stakeholders including those interested in endocrine testing of chemicals (including pesticides), and the EDSP in general. Since others also may be interested, the Agency has not attempted to describe all the specific entities that may be affected by this action.

B. What is the agency authority for taking this action?

The EDSP is established under section 408(p) of the Federal Food, Drug and

Risk Context

Case Studies Applying NAMs to Chemical Assessments

OPP Case Study

Decision Context: Prioritize non-food use inert ingredients for additional study

Desired Components:

- Phys-Chem properties with environ fate and transport
- Hazard profile – GL and GL-like studies, RA, and QSAR
 - Chronic tox endpoints
- ToxCast data in AOP context
- Toxicokinetic data (*in vivo* and *in vitro*)
- Consumer and industrial use

OLEM-Region Case Study

Decision Context: Estimate toxicity values with associated uncertainty for data poor chemicals at Superfund sites

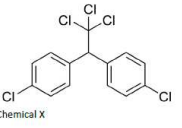
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- Hazard profile – GL and GL-like studies, RA, and QSAR
 - Acute and chronic tox endpoints
- ToxCast data in AOP context
- Toxicokinetic data (*in vivo* and *in vitro*)
- Bioavailability (sediment and Caco-2)
- Consumer and industrial use
- Screening level estimates with defined exposure scenarios
- Available analytical chemistry methods

Risk Context

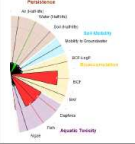
Integrating Traditional and NAMs for Regulatory Decisions

RapidTox Assessment

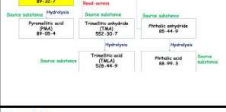
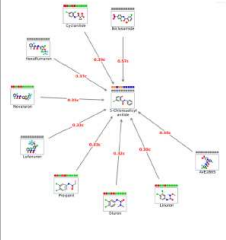
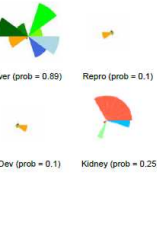


Chemical X

Physical Chemical Properties		
MW	MP	pKa
BP	VP	LogP

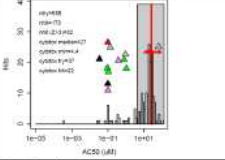


Hazard

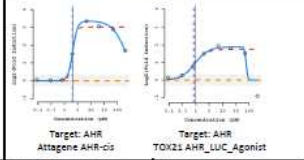
Level 1: In Vivo	Level 2: Read-Across	Level 3: In Silico and Bioactivity																																																															
<table border="1"> <thead> <tr> <th>Species</th> <th>Mouse</th> <th>Rat</th> </tr> </thead> <tbody> <tr> <td>Acute</td> <td></td> <td></td> </tr> <tr> <td>Inhalation</td> <td>100</td> <td>26</td> </tr> <tr> <td>Feed</td> <td></td> <td></td> </tr> <tr> <td>Other</td> <td></td> <td></td> </tr> <tr> <td>Subacute</td> <td></td> <td></td> </tr> <tr> <td>Inhalation</td> <td></td> <td></td> </tr> <tr> <td>Feed</td> <td>20</td> <td>16</td> </tr> <tr> <td>Other</td> <td></td> <td></td> </tr> <tr> <td>Subchronic</td> <td></td> <td></td> </tr> <tr> <td>Inhalation</td> <td></td> <td></td> </tr> <tr> <td>Feed</td> <td>15</td> <td>10</td> </tr> <tr> <td>Other</td> <td></td> <td></td> </tr> <tr> <td>Chronic Non-Cancer</td> <td></td> <td></td> </tr> <tr> <td>Inhalation</td> <td></td> <td></td> </tr> <tr> <td>Feed</td> <td></td> <td></td> </tr> <tr> <td>Other</td> <td></td> <td></td> </tr> <tr> <td>Chronic Cancer</td> <td></td> <td></td> </tr> <tr> <td>Inhalation</td> <td></td> <td></td> </tr> <tr> <td>Feed</td> <td></td> <td></td> </tr> <tr> <td>Other</td> <td></td> <td></td> </tr> </tbody> </table>	Species	Mouse	Rat	Acute			Inhalation	100	26	Feed			Other			Subacute			Inhalation			Feed	20	16	Other			Subchronic			Inhalation			Feed	15	10	Other			Chronic Non-Cancer			Inhalation			Feed			Other			Chronic Cancer			Inhalation			Feed			Other			<p>Expert Derived</p> 	<p>QSAR</p> <ul style="list-style-type: none"> Ames Mutagenicity In vitro Chromosomal Abs In vivo Micronucleus Test Skin Irritation Skin Sensitization Developmental Toxicity Acute Rat Oral Toxicity Carcinogenicity Dev/Repro Toxicity ER CERAPP AR CERAPP
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	<p>GENERA</p> 	<p>ToxCast</p>  <p>Liver (prob = 0.89) Repro (prob = 0.1)</p> <p>Dev (prob = 0.1) Kidney (prob = 0.25)</p>																																																															

Mode-of-Action/Adverse Outcome Pathway

Biological Selectivity



Concentration Response Data



Target: AHR
Attagene AHR-cis

Target: AHR
TOX21 AHR_LUC_Agonist

Top Selective Assays	Linked Target AOPs	Literature-Assay Concordance																								
<table border="1"> <thead> <tr> <th>Name</th> <th>Gene</th> <th>Z-score</th> <th>Target</th> <th>AOPs</th> <th>Enrichment</th> <th>Target</th> <th>Lit Hits</th> </tr> </thead> <tbody> <tr> <td>ATG_Ahr_cis</td> <td>AHR</td> <td>12</td> <td>AHR</td> <td>Cleft palate</td> <td>0.0001</td> <td>AHR</td> <td>Li et al., 1992</td> </tr> <tr> <td>Tox21_Ahr_LUC_Agonist</td> <td>AHR</td> <td>7.5</td> <td>AHR</td> <td>Liver Prolif</td> <td>0.0002</td> <td>AHR</td> <td>Miller et al., 1999</td> </tr> </tbody> </table>	Name	Gene	Z-score	Target	AOPs	Enrichment	Target	Lit Hits	ATG_Ahr_cis	AHR	12	AHR	Cleft palate	0.0001	AHR	Li et al., 1992	Tox21_Ahr_LUC_Agonist	AHR	7.5	AHR	Liver Prolif	0.0002	AHR	Miller et al., 1999		
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Tox21_Ahr_LUC_Agonist	AHR	7.5	AHR	Liver Prolif	0.0002	AHR	Miller et al., 1999																			

Product Use

- agricultural_omp
- airc_crafts
- automotive_care
- child_use
- cleaning_washing
- construction
- degreaser
- dietary_supplement
- drug
- electronics_batteries
- food_additive
- fragrance_consumer_use
- manufacturing_chemical
- personal_care
- pesticide
- pesticide_biocide_active_ingredient
- pesticide_inert_ingredient
- photographic
- preservatives
- risk_materials_personal_care_cosmetics
- sports_equipment
- surface_treatment
- toxic_personal_care_hair
- toys_child_use

Available Analytical Methods

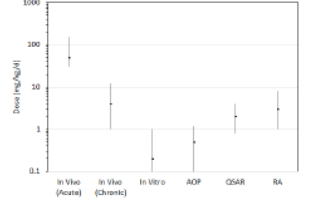
Analytical Method #112

- Media: Soil
- Extraction: DCM
- Clean-up: Silica column
- Separation: LC
- Detection: MS
- Reference: Johns et al., Anal Chem., 12:243, 2003

Analytical Method #234

- Media: Blood
- Extraction: hexane:MTBE
- Clean-up: amino propylene column
- Separation: LC
- Detection: MS
- Reference: Applehans et al., Environ Res. 93:187, 2001

Pharmacokinetics

Level 1: In Vivo Studies	Level 2: High-Throughput Pharmacokinetics																		
None Available	<table border="1"> <thead> <tr> <th>Sub</th> <th>Renal Clearance</th> <th>Met. Stability</th> </tr> </thead> <tbody> <tr> <td>0.692</td> <td>4.64</td> <td>9.612</td> </tr> <tr> <td>C₅₀/OE (Median)</td> <td>C₅₀/OE (Upper)</td> <td>C₅₀/OE (Lower)</td> </tr> <tr> <td>0.50</td> <td>0.45</td> <td>1.72</td> </tr> </tbody> </table>	Sub	Renal Clearance	Met. Stability	0.692	4.64	9.612	C ₅₀ /OE (Median)	C ₅₀ /OE (Upper)	C ₅₀ /OE (Lower)	0.50	0.45	1.72						
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Assessment Summary																			
Chemical Selectivity:	Moderate	Confidence	Moderate	UFs		RFD													
Likely Hazards:	Liver toxicity	Confidence	High	UFs															
Likely AOP/MOA:	PPARA receptor activation causing hepatocyte prolif	Confidence	High	UFs															
Point-of-Departure Estimate	4.0 mg/kg/d	UFs	X-X-X-X	RFD	0.04 mg/kg/d														
RapidTox Screening Levels																			
Resident Soil (mg/kg)	7.5	Resident Air (ug/m ³)	0.15	Tap Water (ug/L)	1.1														
Industrial Soil (mg/kg)	33	Industrial Air (ug/m ³)	0.6																
Comments																			

Mock Up – Not real data

- Semi-automated decision support tool with dashboard interface
- Combining diverse data streams into quantitative toxicity values with associated uncertainty

Challenges

- Technical limitations/obstacles associated with each technology (e.g., metabolism, volatiles, etc.)
- Moving from an apical to a molecular paradigm and defining adversity
- Predicting human safety vs. toxicity
- Combining new approaches to have adequate throughput and sufficiently capture higher levels of biological organization
- Systematically integrating multiple data streams from the new approaches in a risk-based, weight of evidence assessment
- Quantifying and incorporating uncertainty and variability
- Dealing with the “V” word
 - Defining a fit-for-purpose framework(s) that is time and resource efficient
 - Performance-based technology standards vs. traditional validation
 - Role of *in vivo* rodent studies and understanding their inherent uncertainty
- Legal defensibility of new methods and assessment products

Acknowledgements and Questions

Tox21 Colleagues:

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NCATS Collaborators

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NERL

NHEERL

NCEA



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