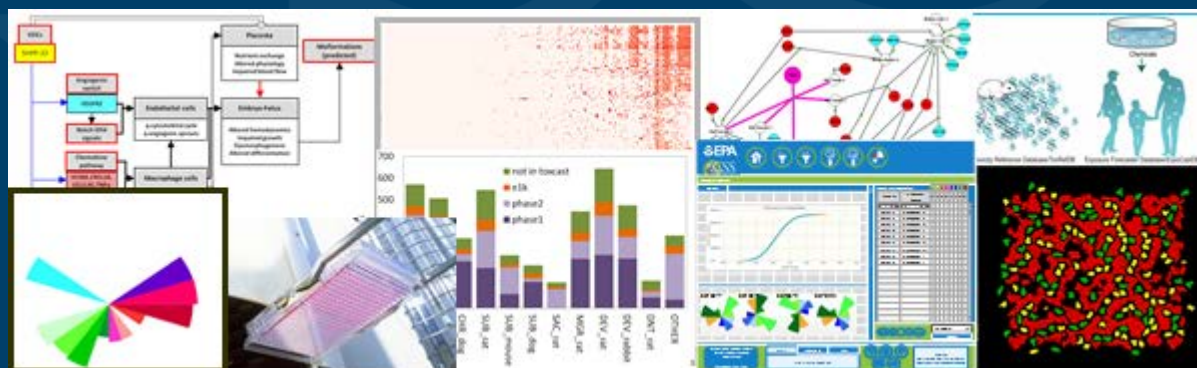


High Throughput *in vitro* Assay Testing in Hazard Assessment

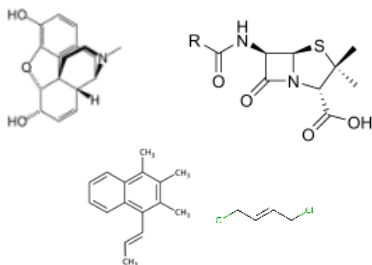


JOINT DOD TECHNICAL INTERCHANGE AND ROADMAP
DEVELOPMENT TO PROMOTE THE USE OF NEW APPROACH
METHODOLOGIES IN RAPID CHEMICAL HAZARD ASSESSMENT
August 14, 2018

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

Regulatory Agencies Make a Broad Range of Decisions on Chemicals...

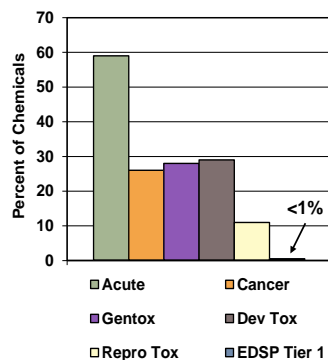
Number of Chemicals /Combinations



Ethics/Relevance Concerns

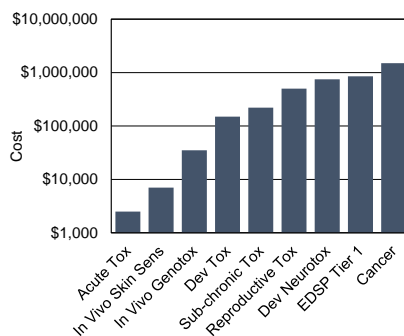


Lack of Data



Modified from Judson *et al.*, EHP 2010

Economics



- Number of chemicals and combinations of chemicals is extremely large (>20,000 substances on active TSCA inventory)
- Due to historical regulatory requirements, most chemicals lack traditional toxicity testing data
- Traditional toxicology testing is expensive and time consuming
- Traditional animal-based testing has issues related to ethics and relevance

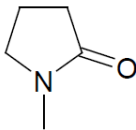
Risk Assessments Generally Contain a Standard Set of Components

EPA United States Environmental Protection Agency
EPA Document# 740-R1-5002 March 2015
Office of Chemical Safety and Pollution Prevention

TSCA Work Plan Chemical Risk Assessment

**N-Methylpyrrolidone:
Paint Stripper Use**

CASRN: 872-50-4



March 2015

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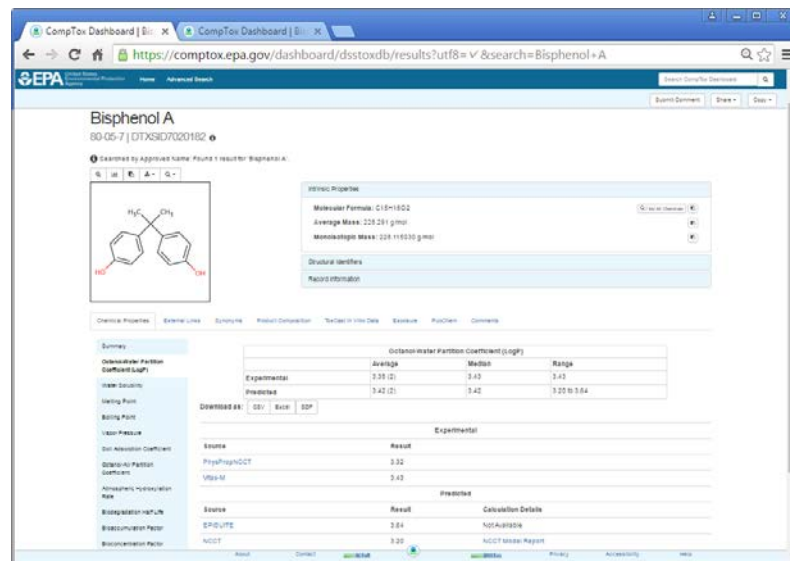
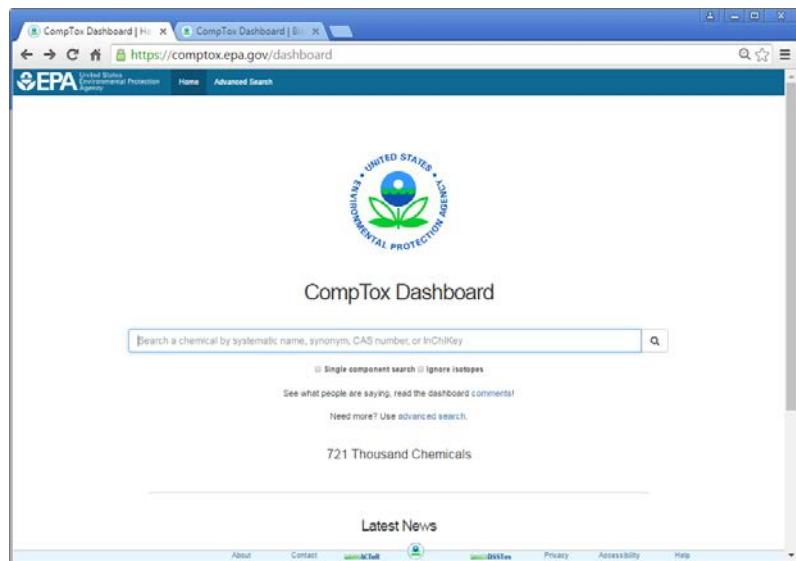
Phys Chem
Exposure
Hazard
Dose Response,
PK, and PODs

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E-5-3 NMP Scenario 3. Chest, Brush-On, Workshop, User in ROH during wait time, 0.18 ACH, 0.5 Weight Fraction	191

Variability
Risk Summary
Uncertainty

New technologies and approaches will also have to cover these basic components

It All Starts With Chemistry...

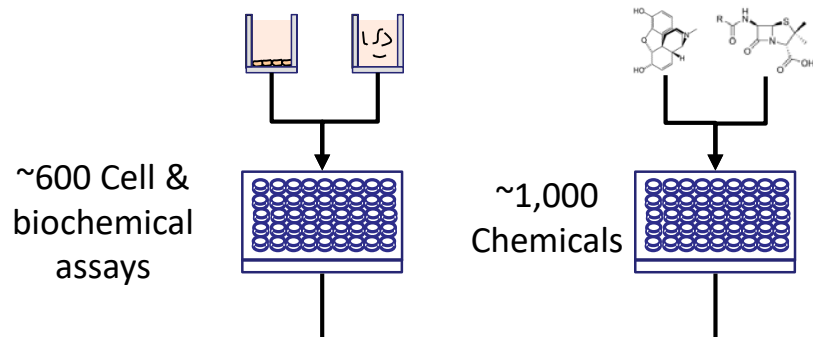


<https://comptox.epa.gov/dashboard>

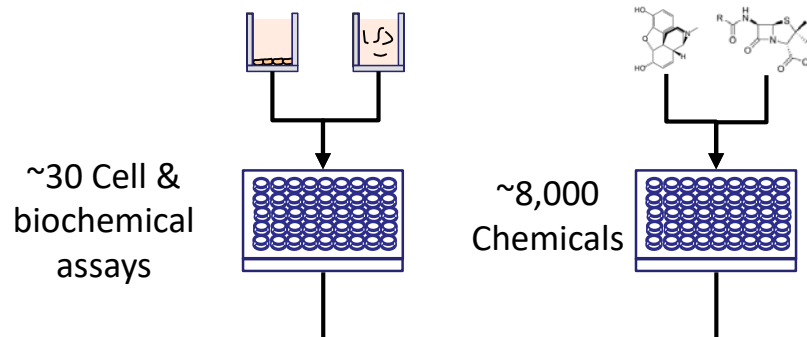
- Chemical structure database of >700,000 unique substances with QC flags to link chemical structure with names and identifiers
- Consensus QSAR models for a range of physical chemical properties, environmental fate, and hazard characteristics
- Comprehensive physical-chemical property database (experimental and predicted)

ToxCast and Tox21: Adding the High-Throughput Hazard Screening Component

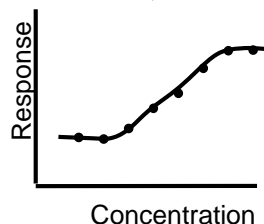
ToxCast



Tox21

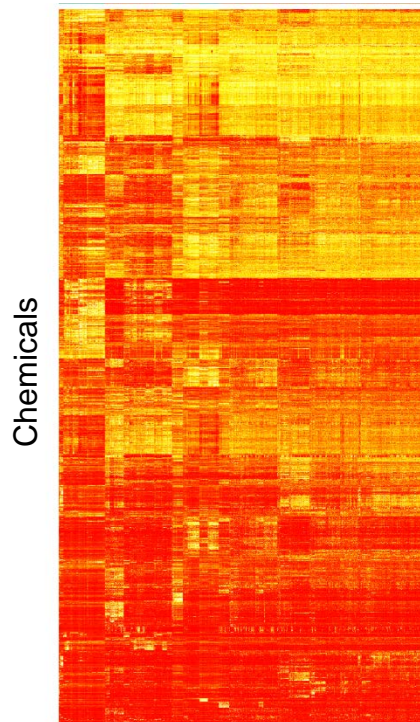


Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013



Broad Success Derived from High-Throughput Screening Approaches


Group Chemicals by
Similar Bioactivity and
Predictive Modeling



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 metabolism of DCM does occur in

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


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

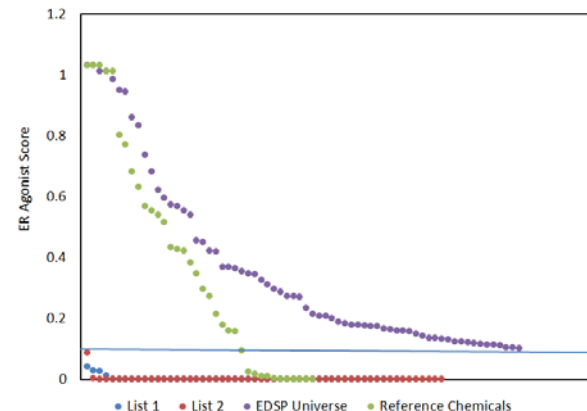
Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

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 acid. 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

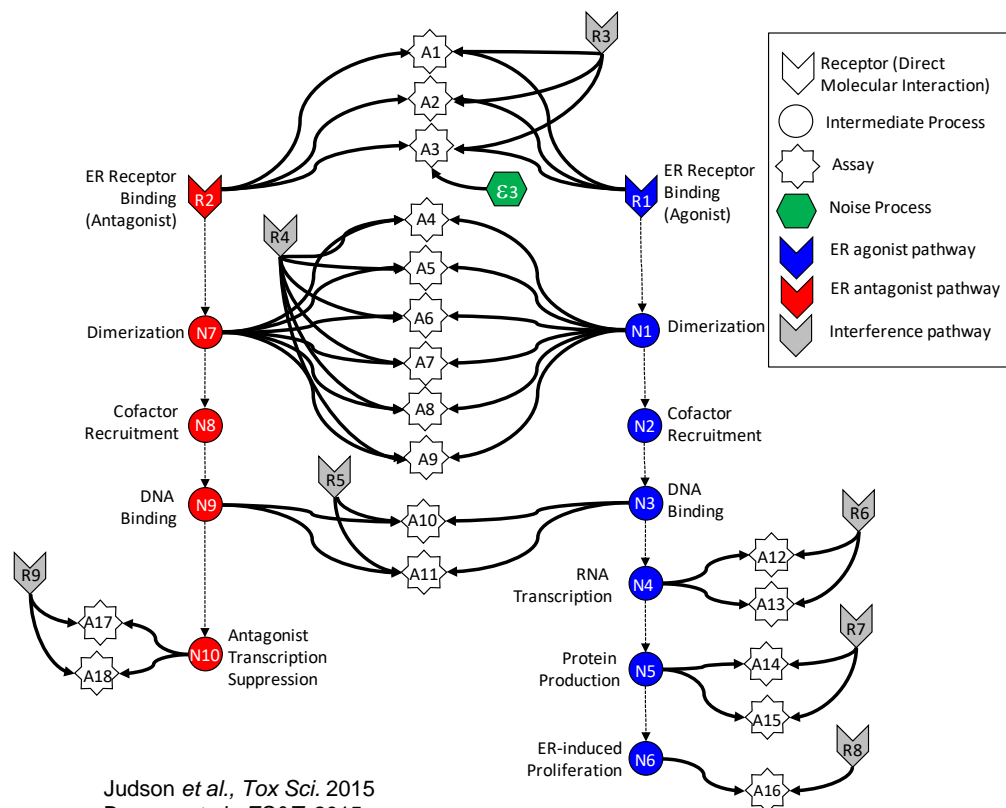
Prioritization of Chemicals
for Further Testing



FIFRA SAP, Dec 2014

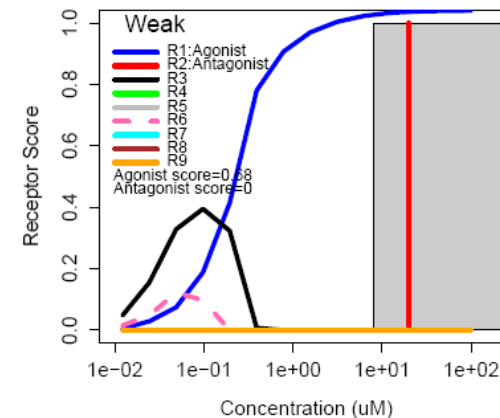
Application of High-Throughput Assays to Identify Potential Endocrine Disrupting Chemicals

18 In Vitro Assays Measure ER-Related Activity

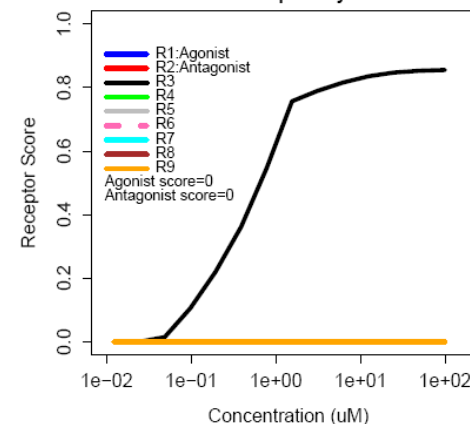


Judson *et al.*, *Tox Sci.* 2015
Browne *et al.*, *ES&T.* 2015
Kleinstreuer *et al.*, *EHP* 2016

80-05-7 : Bisphenol A



10016-20-3 : alpha-Cyclodextrin

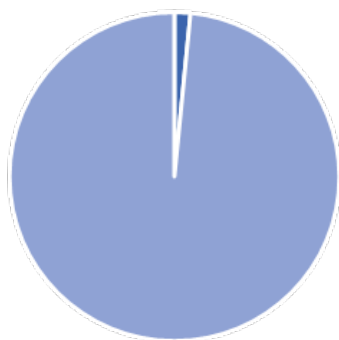


Selected Criticisms of ToxCast

- You don't include metabolism in your *in vitro* assays
- You don't measure my favorite endpoint
- You don't cover all of biological space
- *In vitro* assays are not normal biology
- Assay (x) in your battery did not get the right answer for my chemical
- My assay disagrees with your assay (x), so your approach is flawed
- You can't test my favorite chemicals because of limitations in your methods (e.g., solvents, high LogP)
- Your assay descriptions do not allow me to reproduce your results
- I get different answers when I analyze your data

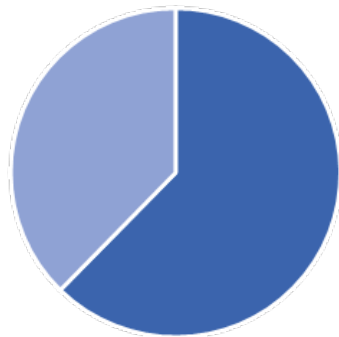
Beginning to Address Concerns for Increased Biological Coverage

Gene Coverage

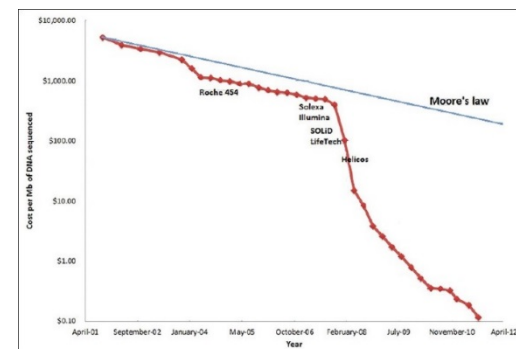


■ ToxCast
■ Not in ToxCast

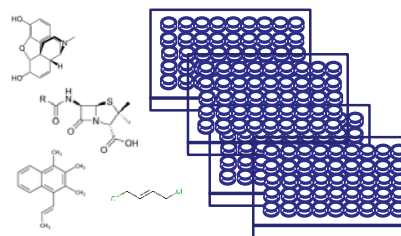
Pathway Coverage*



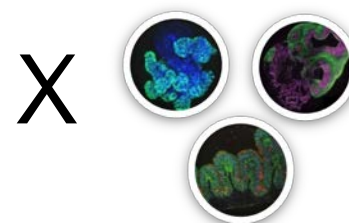
*At least one gene from pathway represented



Thousands of chemicals



Multiple Cell Types



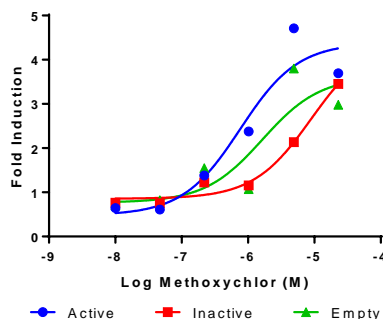
Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

Beginning to Address Metabolic Competence

“Extracellular” Approach

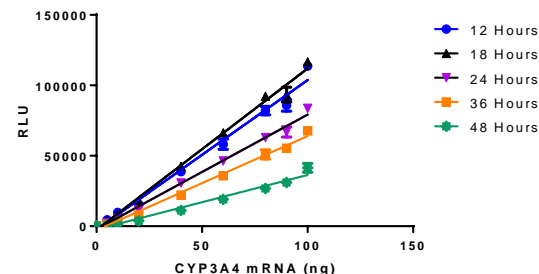
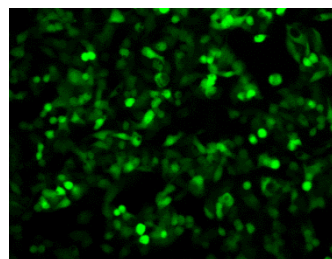
Chemicals metabolism in the media or
buffer of cell-based and cell-free assays



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

“Intracellular” Approach

Capable of metabolizing chemicals
inside the cell in cell-based assays

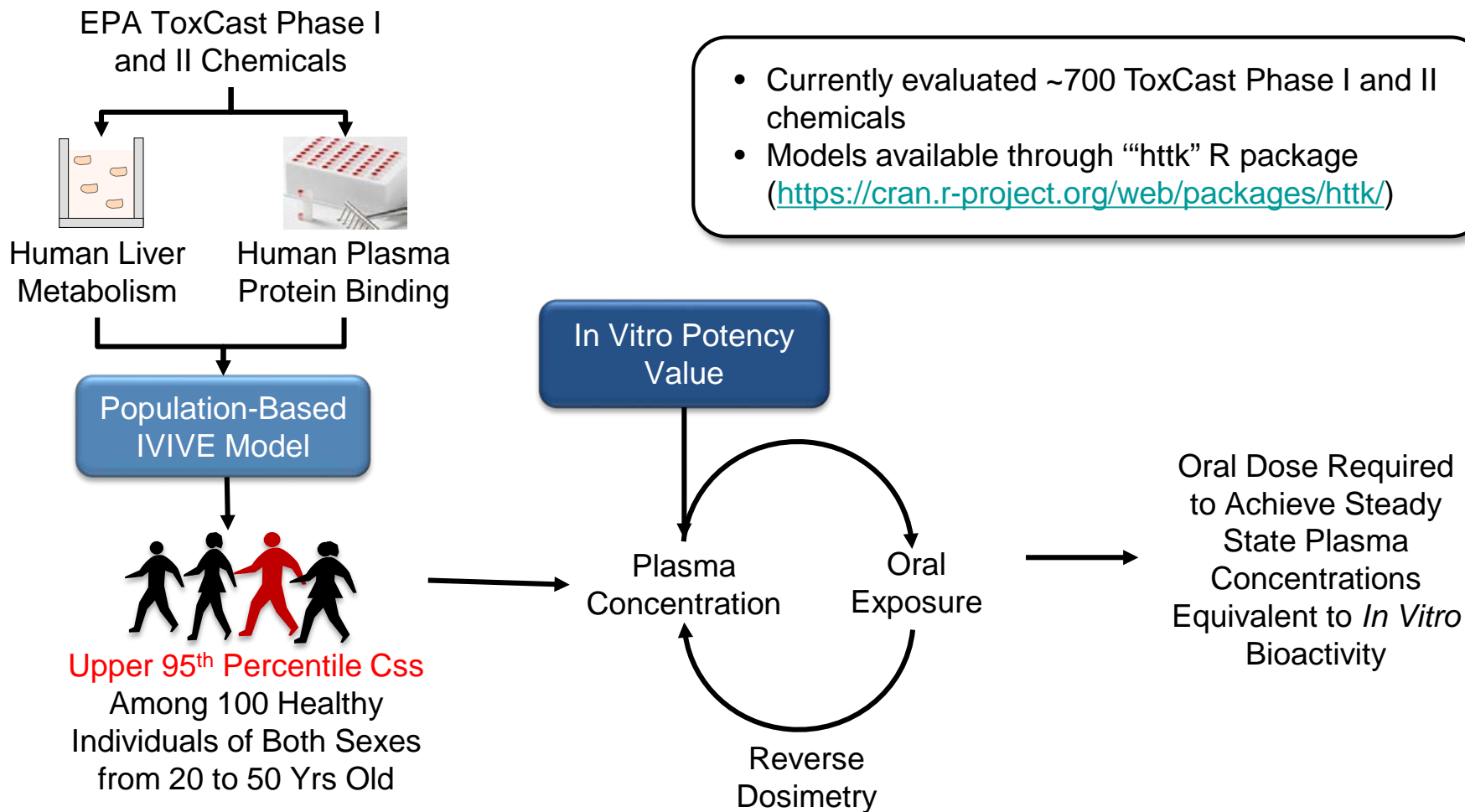


More closely models effects of target
tissue metabolism

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

Collaboration with Unilever

Adding the High-Throughput Toxicokinetic Component



Retroff *et al.*, *Tox Sci.*, 2010
Wetmore *et al.*, *Tox Sci.*, 2012

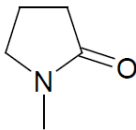
Covering All the Components of a 21st Century Risk Assessment

EPA United States Environmental Protection Agency
EPA Document# 740-R1-5002
March 2015
Office of Chemical Safety and
Pollution Prevention

TSCA Work Plan Chemical Risk Assessment

**N-Methylpyrrolidone:
Paint Stripper Use**

CASRN: 872-50-4



March 2015

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E-5-3 NMP Scenario 3. Chest, Brush-On, Workshop, User in ROH during wait time, 0.18 ACH, 0.5 Weight Fraction	191

✓
Variability

✓
Risk Summary

✓
Uncertainty

✓
Phys Chem

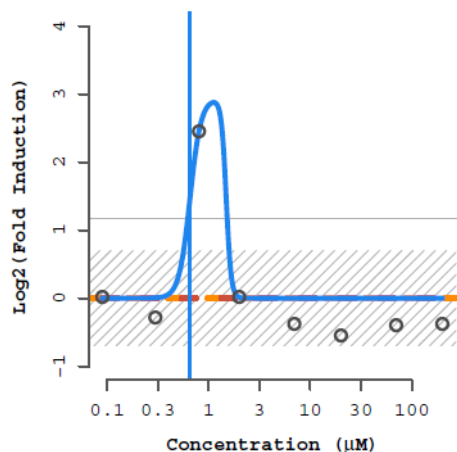
✓
Exposure

✓
Hazard

✓
Dose Response,

✓
PK, and PODs

Regulatory Applications Require More Focus on Quality and Transparency



ASSAY: ARID117 (ATQ_Era_TRANS)

NAME: Thioglycolic acid
CHID: 26141 CASRN: 68-11-1
SPID(S): TX007664
L4ID: 420385

HILL MODEL (in red):
tp ga gw
val: 3.1e-11 -2.15 0.416
sd: NaN NaN NaN

GAIN-LOSS MODEL (in blue):
tp ga gw la lw
val: 2.93 -0.184 8 0.173 18
sd: 3.56 0.334 9.48 5.82 814

	CNST	HILL	GNLS
AIC:	20.14	26.14	17.79
PROB:	0.23	0.01	0.76
RMSE:	0.92	0.92	0.32

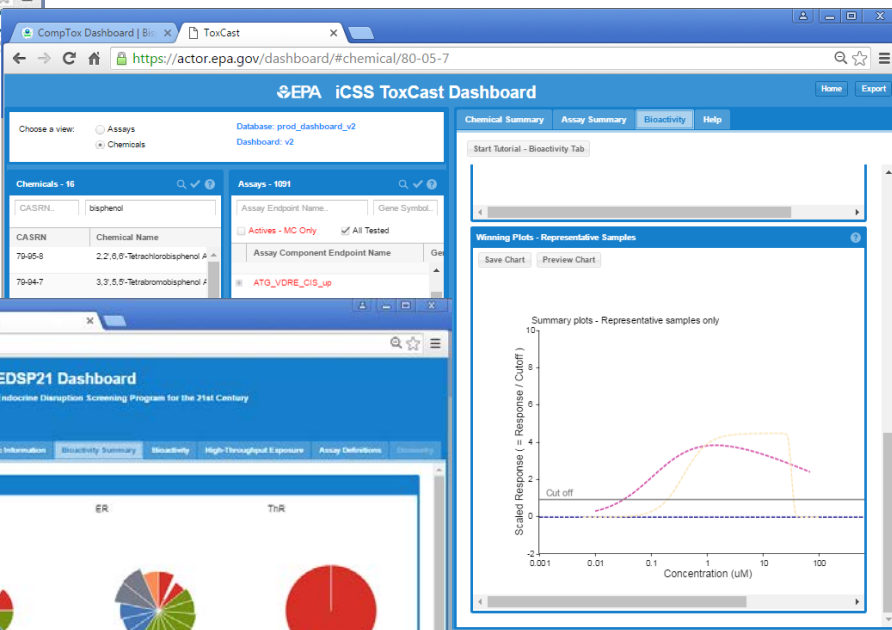
MAX_MEAN: 2.45 MAX_MED: 2.45 BMAD: 0.233

COFF: 1.17 HIT-CALL: 1 FITC: 50 ACTP: 0.77

FLAGS:

Only one conc above baseline, active
Borderline active

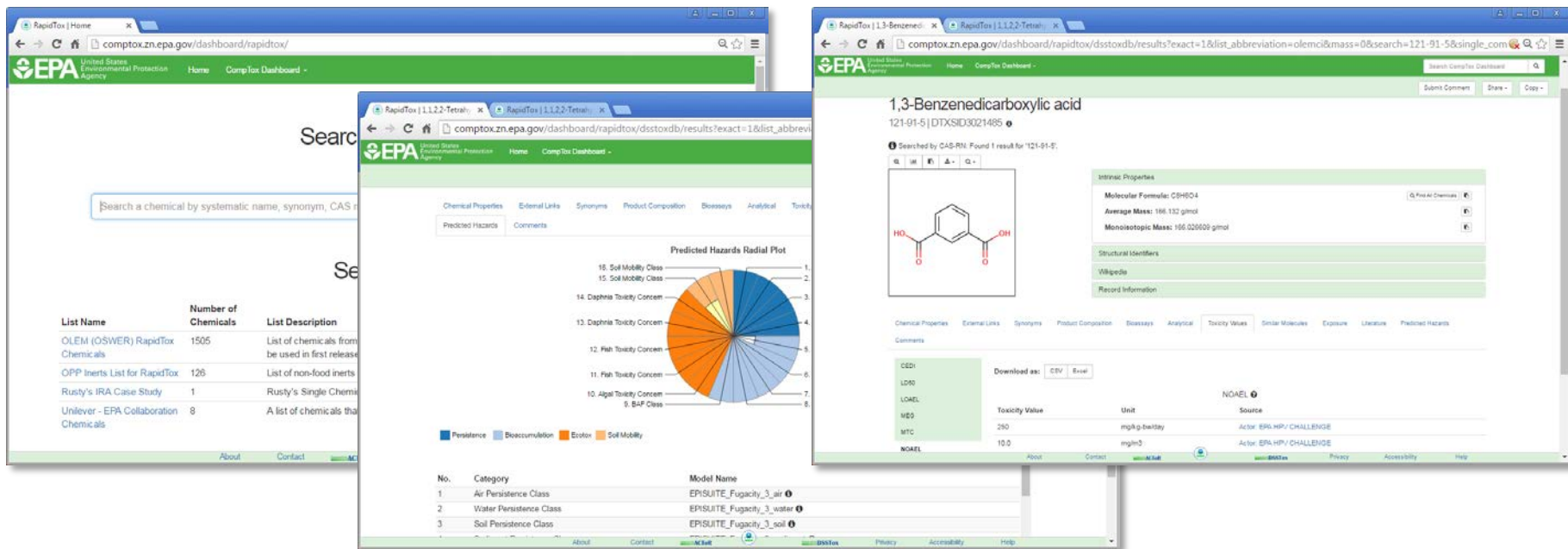
- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Publicly available ToxCast data analysis pipeline
 - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
- Tox21 and ToxCast chemical libraries have undergone analytical QC and results publicly available
- Public posting of ToxCast procedures
 - Chemical Procurement and QC
 - Data Analysis
 - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline
- Migrating ToxCast assay annotations to OECD 211 compliant format



ToxCast Dashboard
([https://actor.epa.gov/
dashboard](https://actor.epa.gov/dashboard))

**National Center for
Computational Toxicology**

RapidTox Workflow as a Focal Point for Integrating Components



- Semi-automated decision support tool with dashboard interface for high-throughput risk assessments
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure
- Transparent and interactive enough to enable expert users to review the assumptions made and refine the predictions
- Deliver quantitative toxicity values with associated estimates of uncertainty

Where do we go from here?

Barriers to progress

- Different regulatory needs
- Inconsistent characterization of data, NAMs
- Low confidence in new methods due to lack of understanding
- Culture shift needed!

Opportunities for progress

- Data sharing
- Classification systems for NAMs
- Collaborative case studies as proof of concept for use of NAMs in chemical risk assessment

Thank You for Your Attention!

Tox21 Colleagues:

NTP Crew

FDA Collaborators

NCATS Collaborators

EPA Colleagues:

NERL

NHEERL

NCEA

Collaborators:

Unilever



EPA's National Center for Computational Toxicology