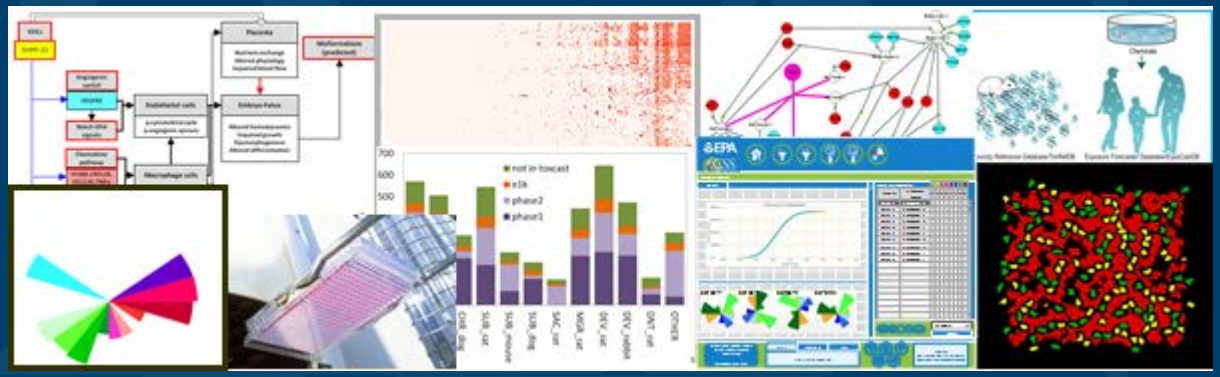
United States Environmental Protection Agency

ToxCast and the Use of Human Relevant *In Vitro* Exposures:

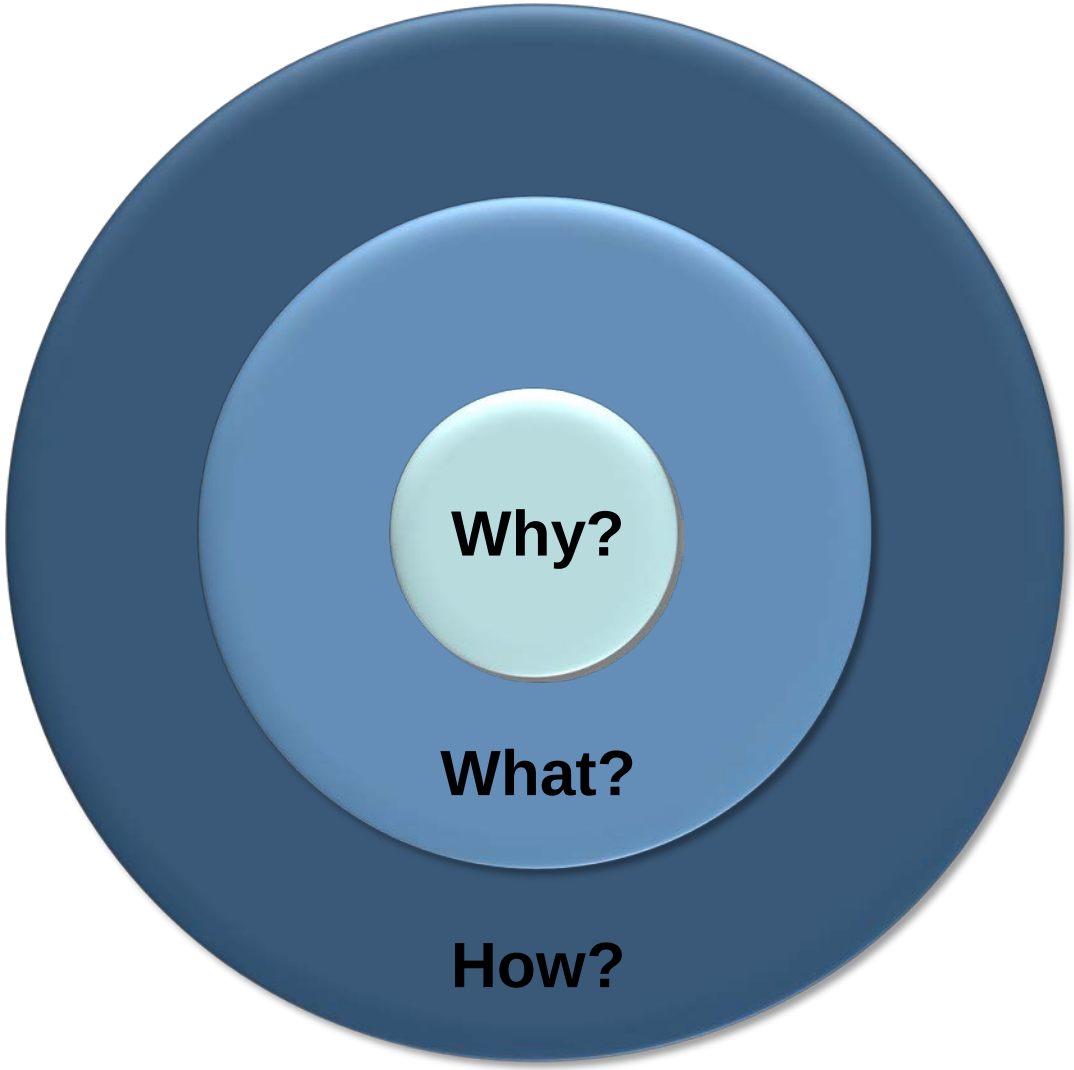
Incorporating high-throughput exposure and toxicity testing data for 21st century risk assessments



BTS
April 4, 2017

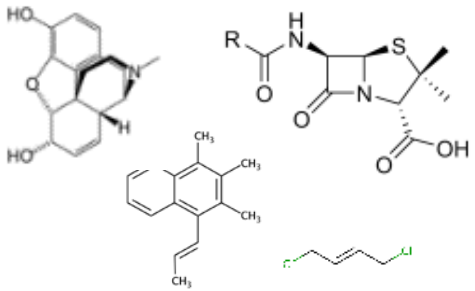
Russell Thomas
Director
National Center for Computational Toxicology

Using the 'Golden Circle' for the Transition to 21st Century Risk Assessment

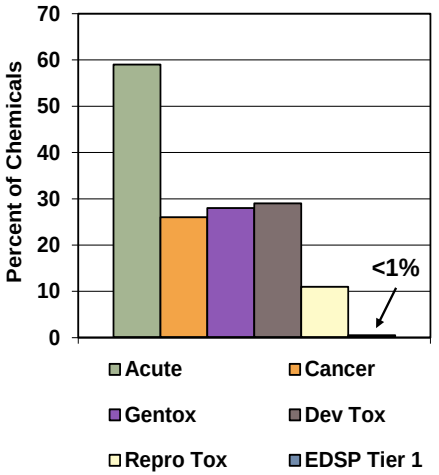


Understanding 'Why' We Need to Innovate In This Space…

Number of Chemicals /Combinations



Lack of Data

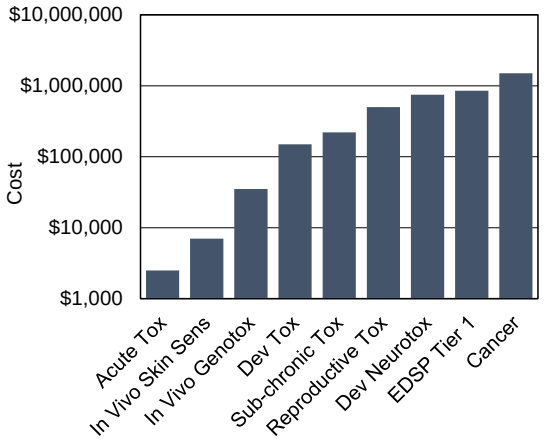


Modified from Judson *et al.*, EHP 2010

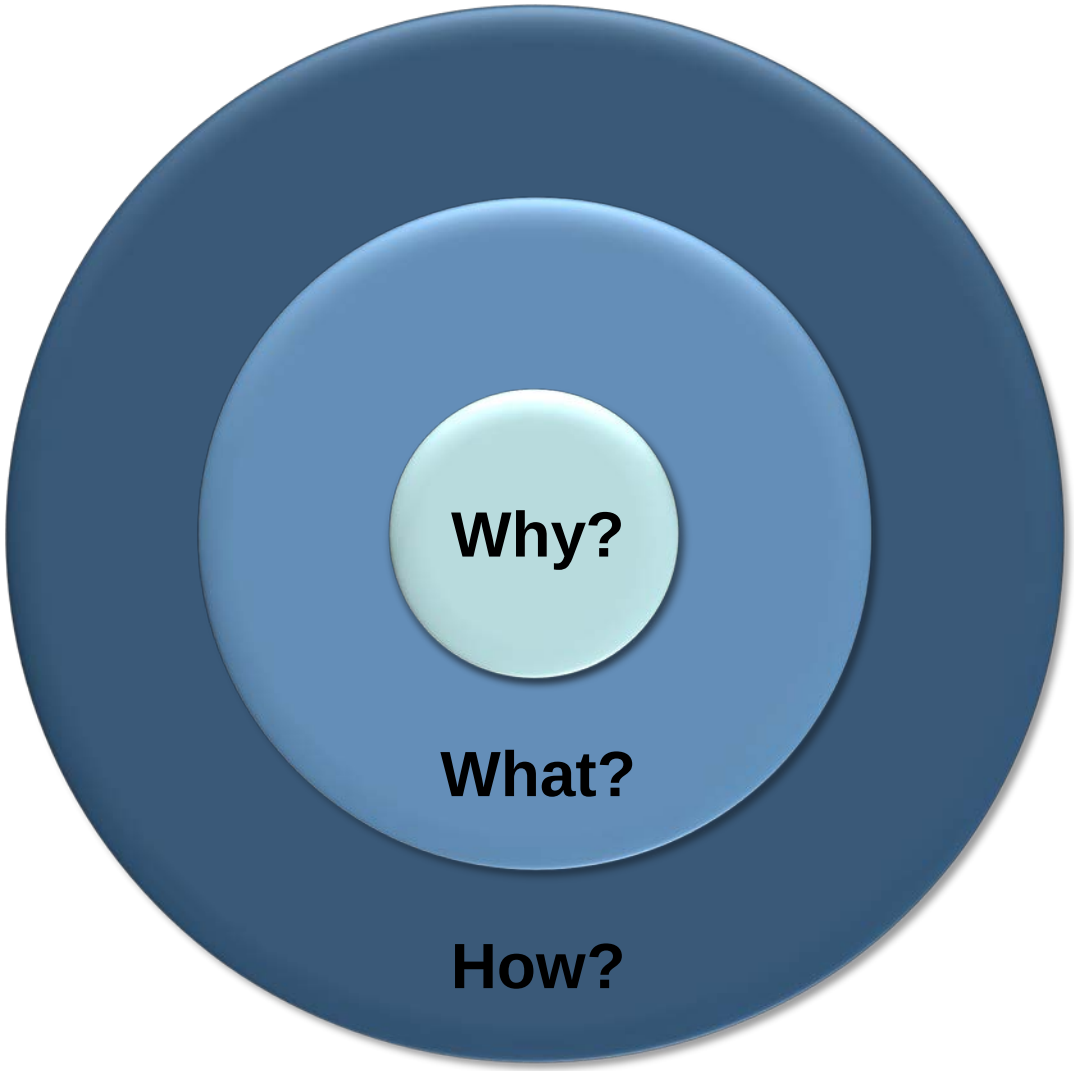
Why?

Ethics Concerns

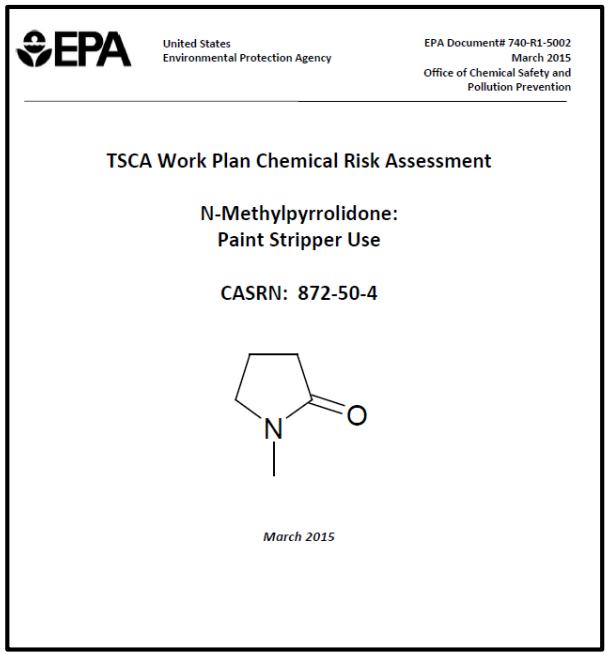
Economics



‘Golden Circle’ of 21st Century Risk Assessment



Risk Assessments Generally Contain a Standard Set of Components



United States Environmental Protection Agency

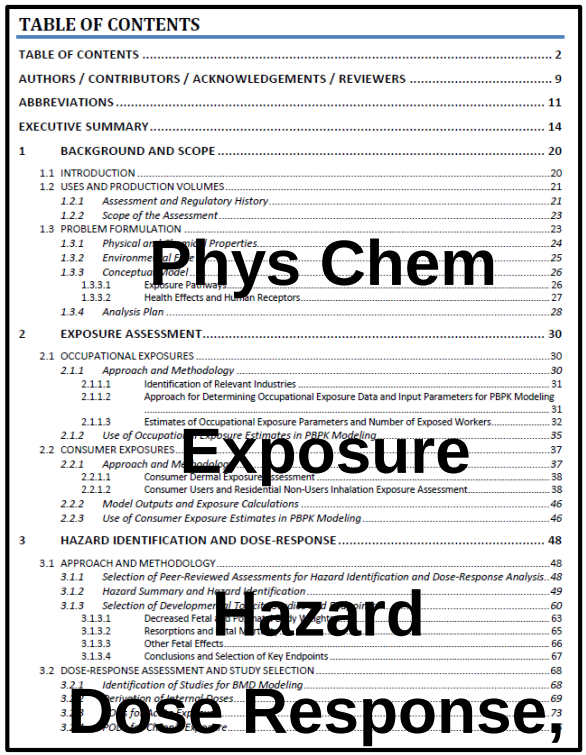
EPA Document# 740-R1-5002
March 2015
Office of Chemical Safety and Pollution Prevention

TSCA Work Plan Chemical Risk Assessment

N-Methylpyrrolidone: Paint Stripper Use

CASRN: 872-50-4

March 2015



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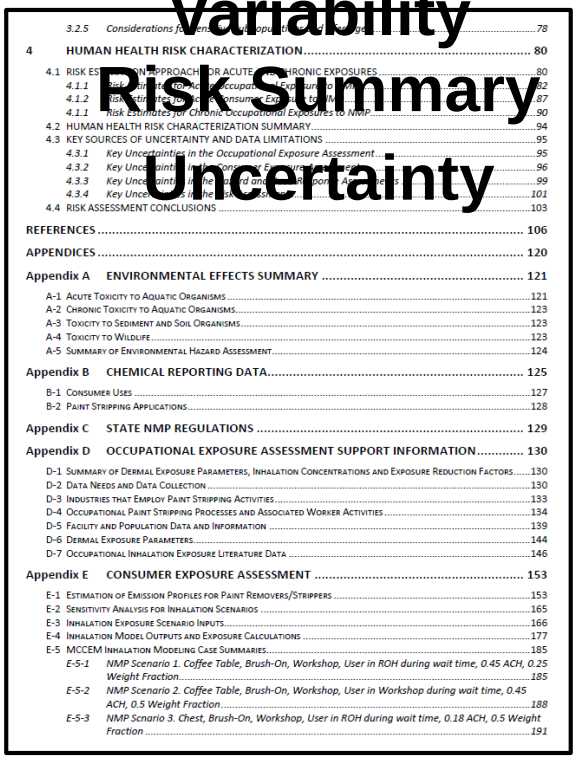


Variability

Risk Summary

Uncertainty

Phys Chem

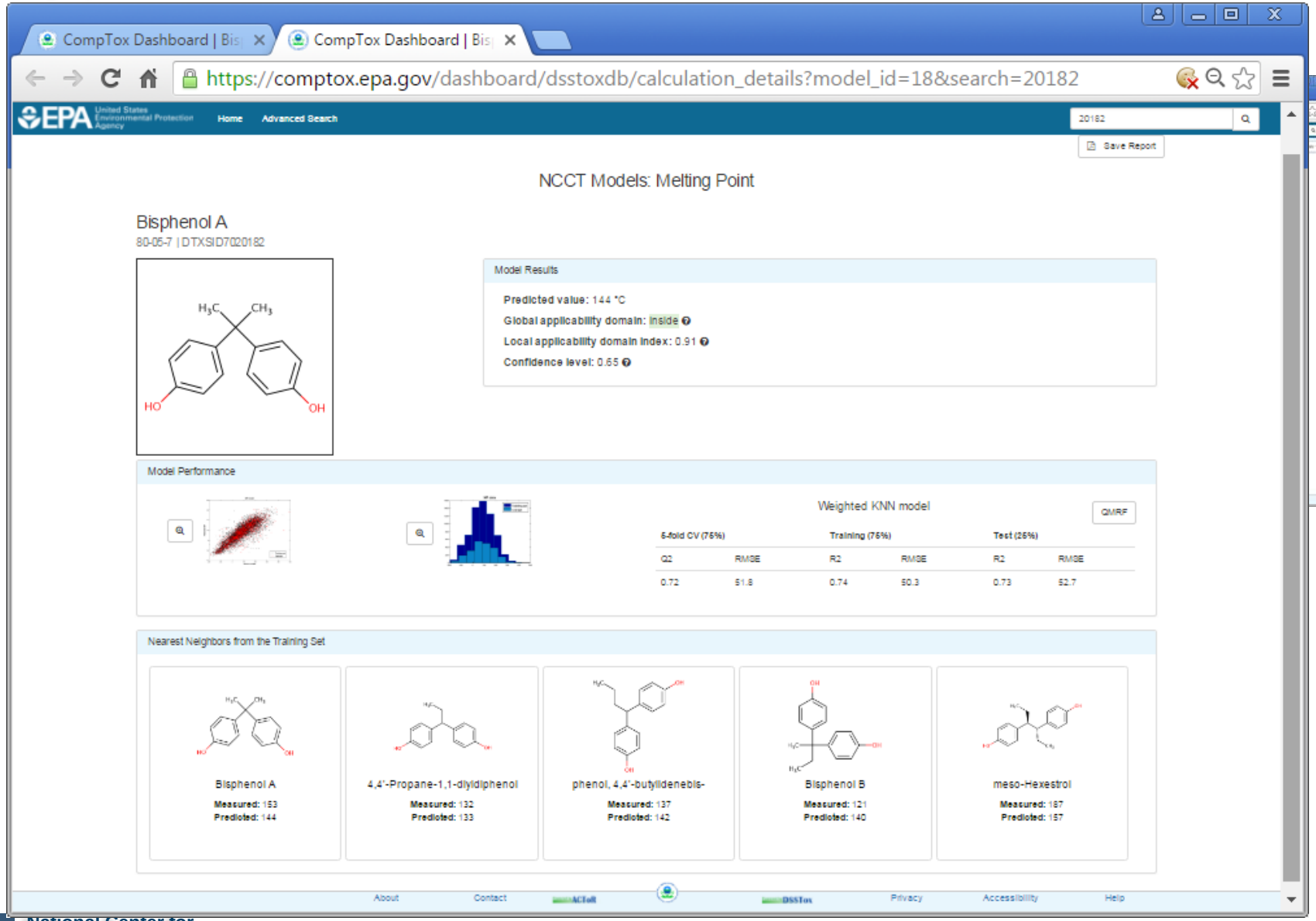
Exposure

Hazard

Dose Response, PK, and PODs

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

It All Starts With Chemistry…



Adding the High-Throughput Hazard Screening Component

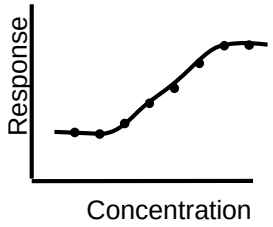
ToxCast | **Tox21**

~600 Cell & biochemical assays

~1,000 Chemicals

~30 Cell & biochemical assays

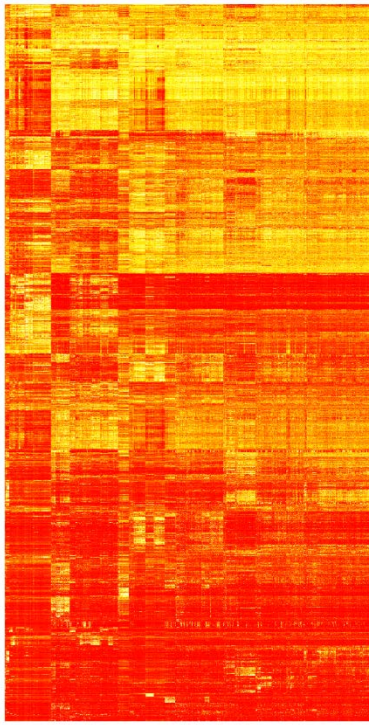
~8,000 Chemicals



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

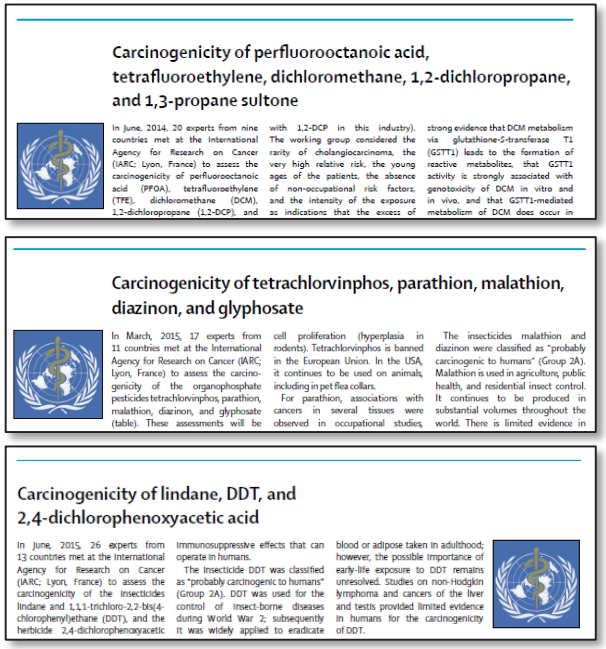
Group Chemicals by Similar Bioactivity and Predictive Modeling



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

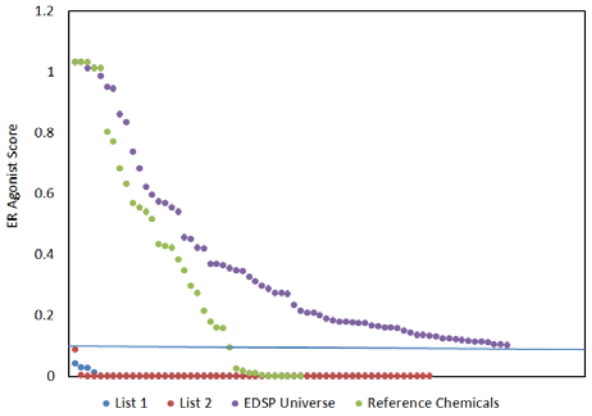
Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

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



FIFRA SAP, Dec 2014

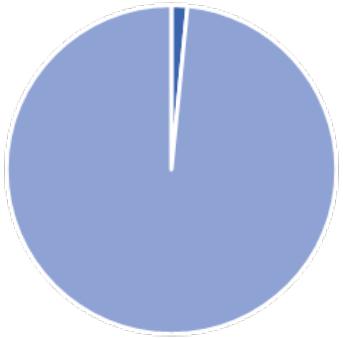
Selected Criticisms of ToxCast

- 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 to do not allow me to reproduce your results
- I get different answers when I analyze your data

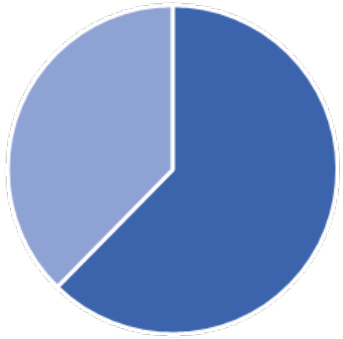
Updated from Bob K's original list

Beginning to Address Concerns for Increased Biological Coverage

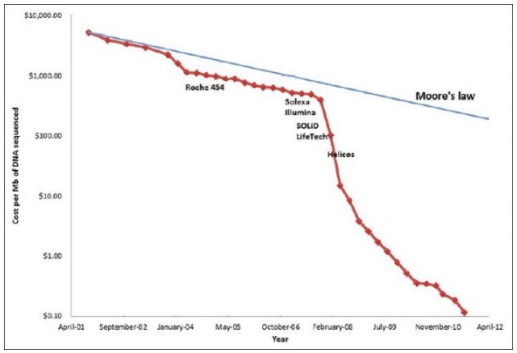
Gene Coverage



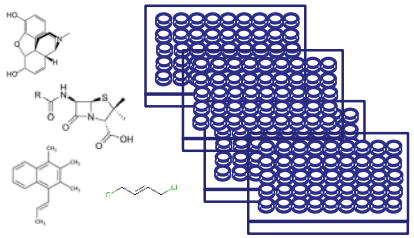
Pathway Coverage*



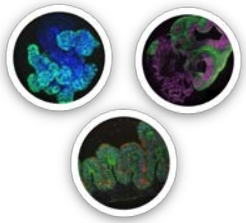
*At least one gene from pathway represented



Thousands of chemicals



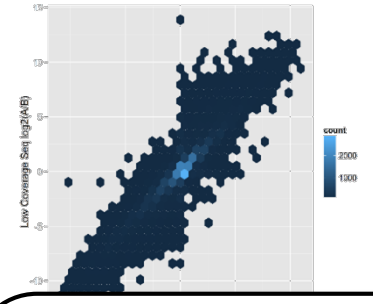
Multiple Cell Types



Requirements:

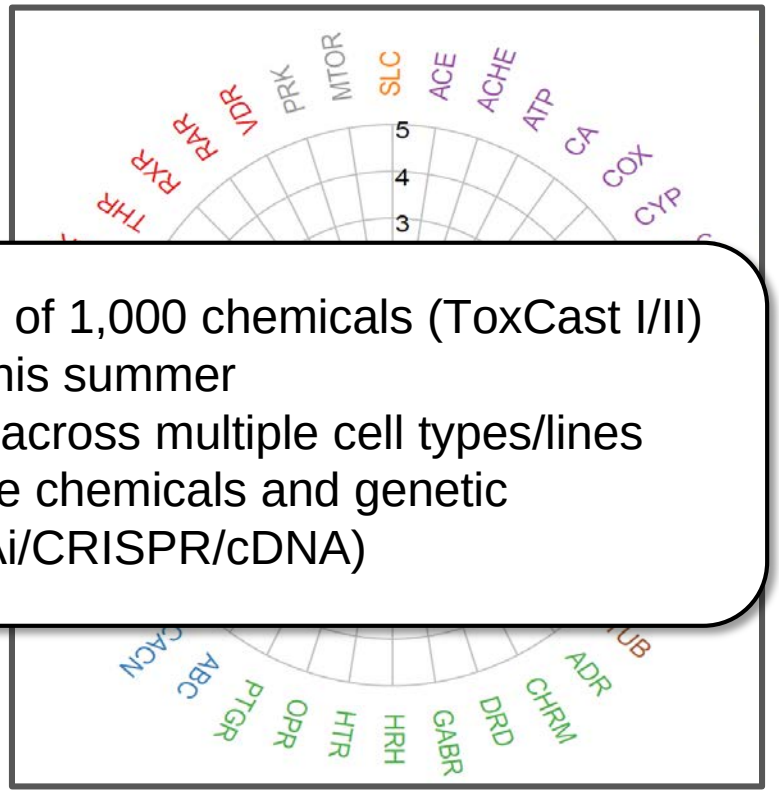
- Low cost
- Whole genome
- 384 well
- Automatable

Comparing Sequencing Platforms and Developing Analysis Approach

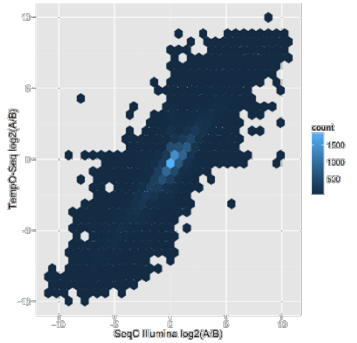


TruSeq
r^2 0.74

MOA/MIE Analysis Pipeline



- Large scale screen of 1,000 chemicals (ToxCast I/II) in single cell type this summer
- Additional screens across multiple cell types/lines
- Additional reference chemicals and genetic perturbations (RNAi/CRISPR/cDNA)



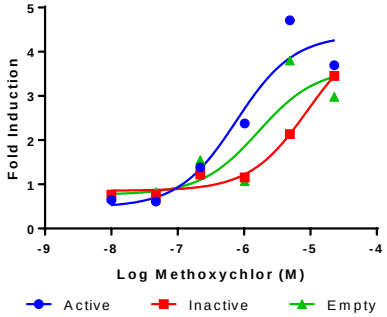
Low Coverage
r^2 0.83

Currently capable of assigning to >40 MOAs/MIEs based on transcriptional responses

Beginning to Address Metabolic Competence

"Extracellular" Approach

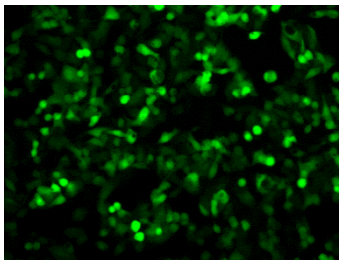
Chemicals metabolism in the media or buffer of cell-based and cell-free assays

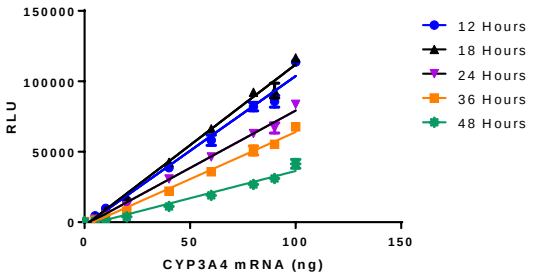


More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Approach

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



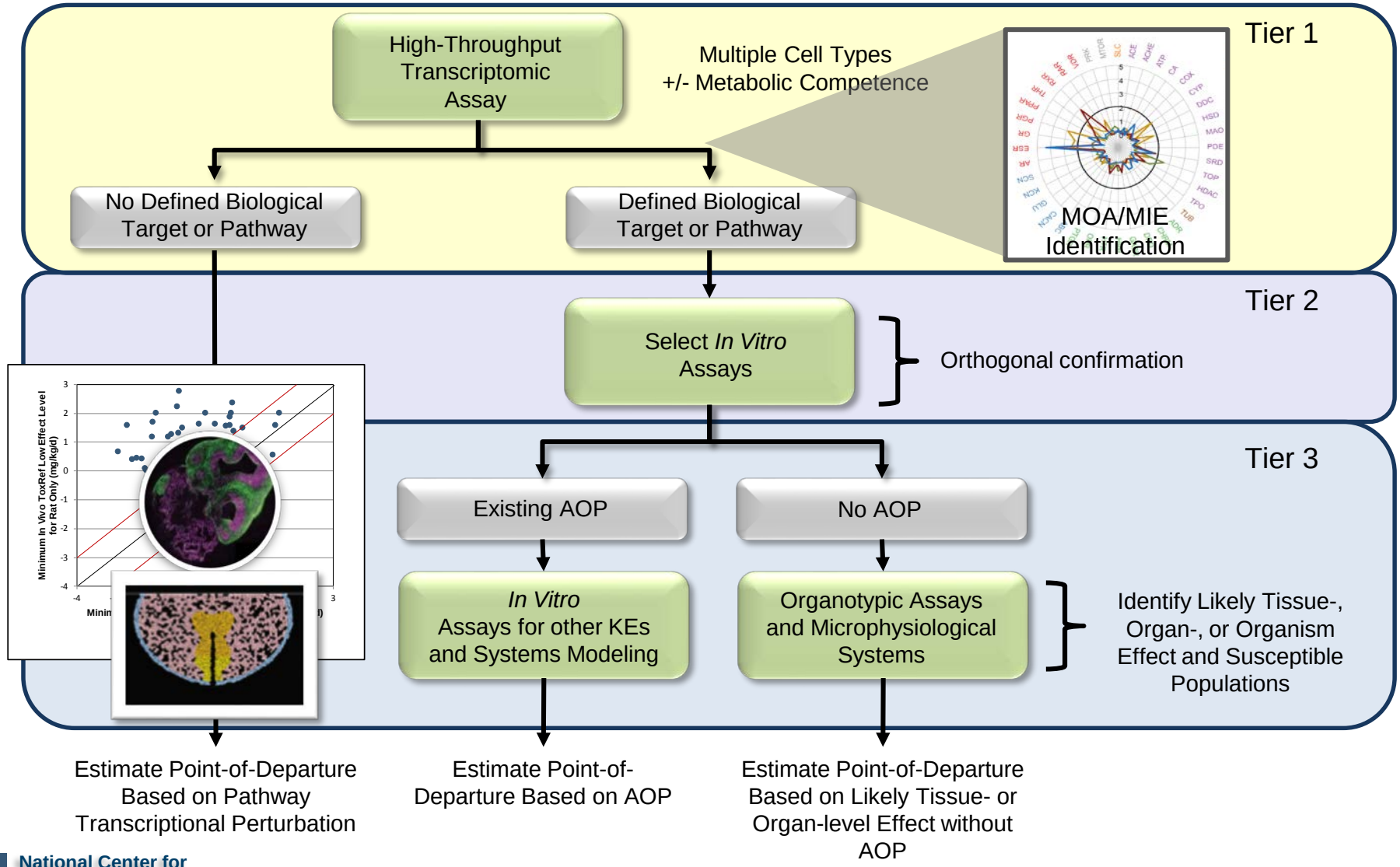


More closely models effects of target tissue metabolism

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

Collaboration with Unilever

Framework for Integrating Hazard Components…



Adding the High-Throughput Toxicokinetic Component

EPA ToxCast Phase I and II Chemicals

Human Liver Metabolism

Human Plasma Protein Binding

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

Oral Exposure

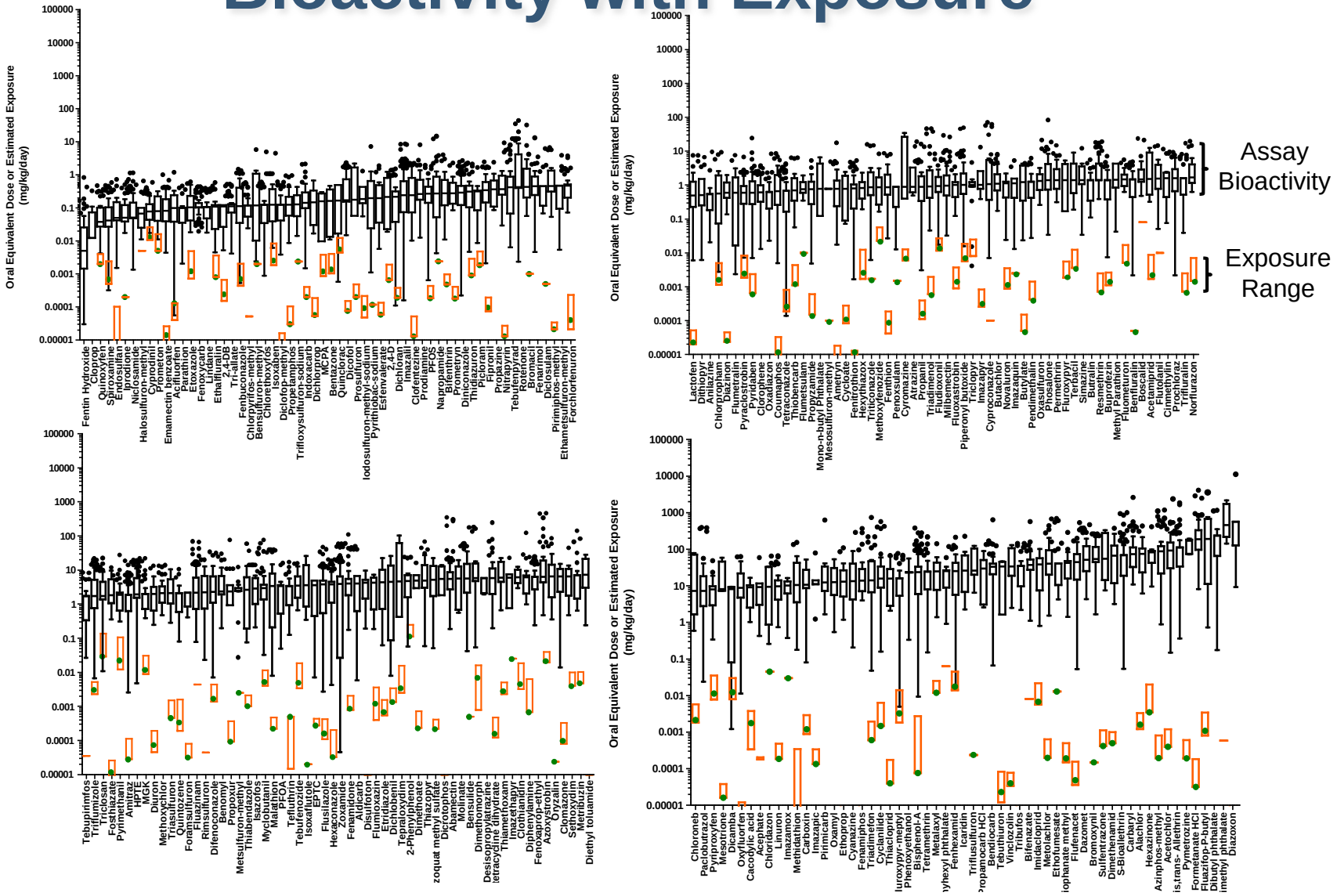
Reverse Dosimetry

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

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

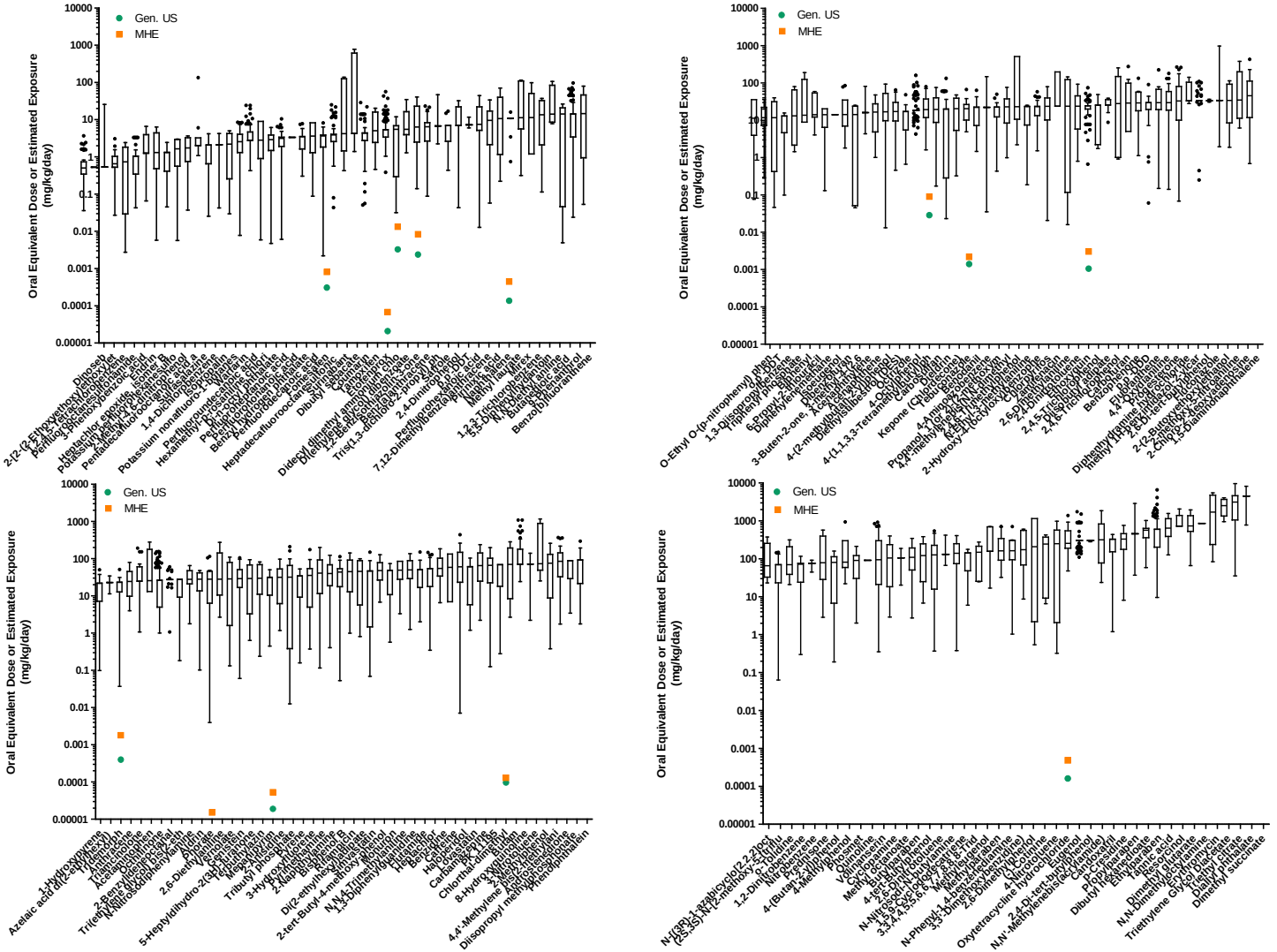
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012

Comparing Dosimetry Adjusted Bioactivity with Exposure



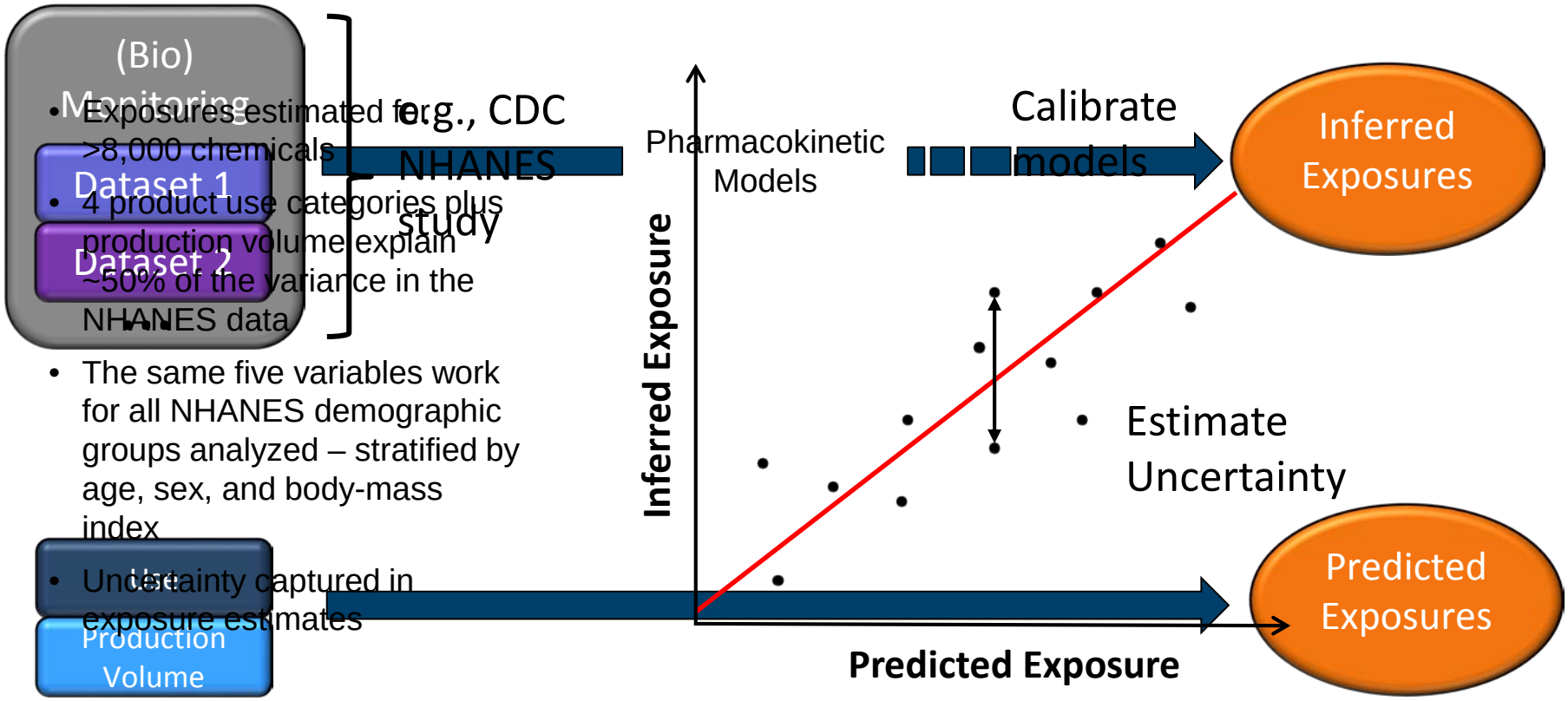
Wetmore *et al.*, *Tox Sci.*, 2012

But, Exposure Information is Lacking on Most Chemicals



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

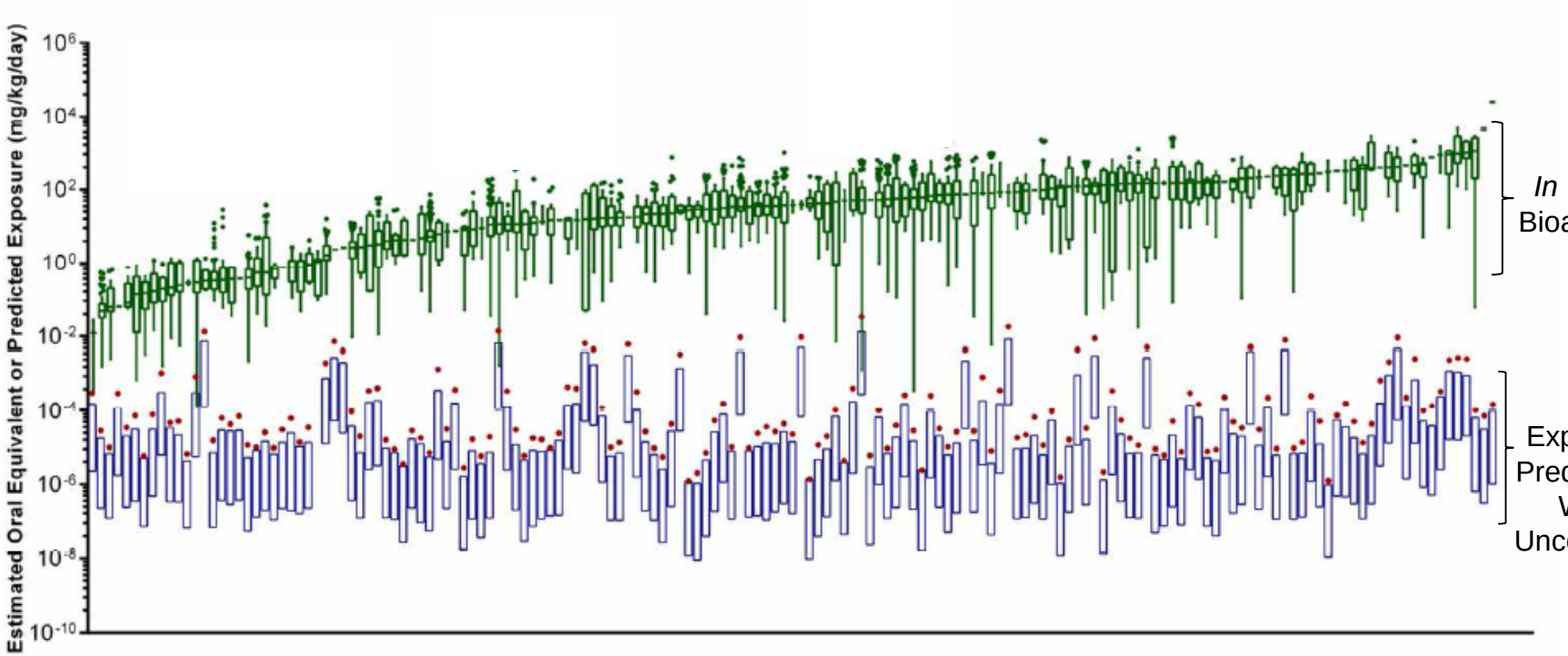
Adding the High-Throughput Exposure Component



- Exposures estimated for >8,000 chemicals
- 4 product use categories plus production volume explain ~50% of the variance in the NHANES data
- The same five variables work for all NHANES demographic groups analyzed – stratified by age, sex, and body-mass index
- Uncertainty captured in exposure estimates

Wambaugh *et al.*, 2014

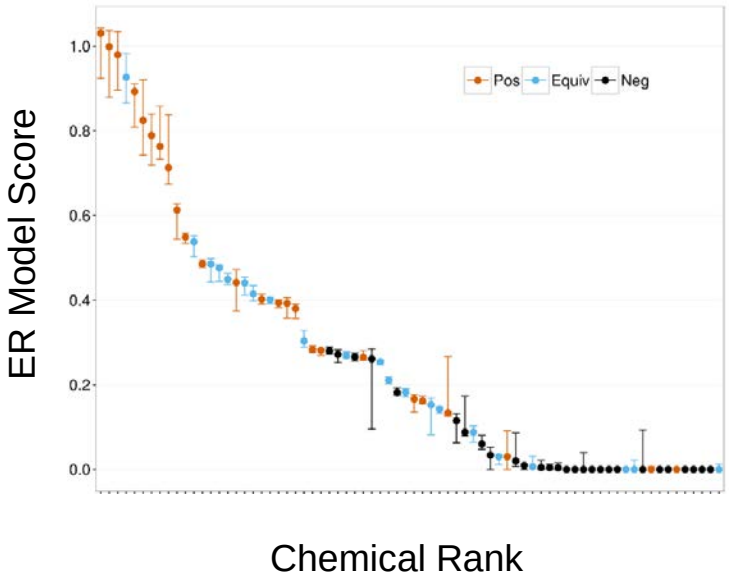
Comparing Bioactivity with Exposure Predictions for Risk Context



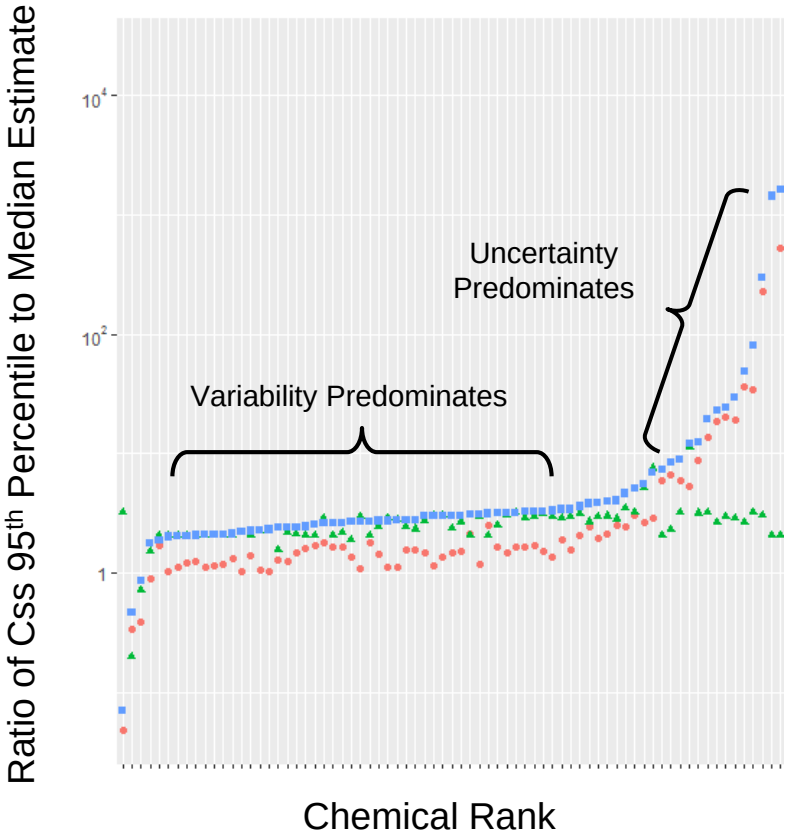
Wetmore *et al., Tox Sci.,* 2015

Adding in Uncertainty and Variability for PD and PK

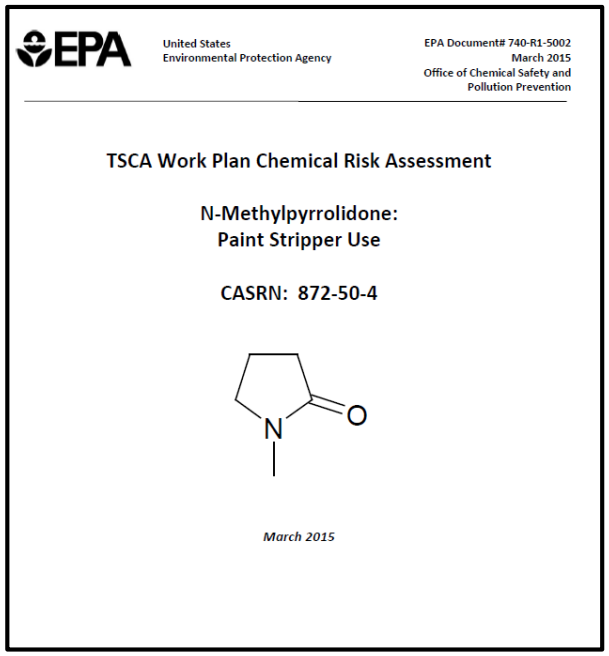
Propagation of Experimental Uncertainty in Models of ER Potency



Propagation of Experimental Uncertainty in High-Throughput Toxicokinetic Estimates



Covering All the Components of a 21st Century Risk Assessment



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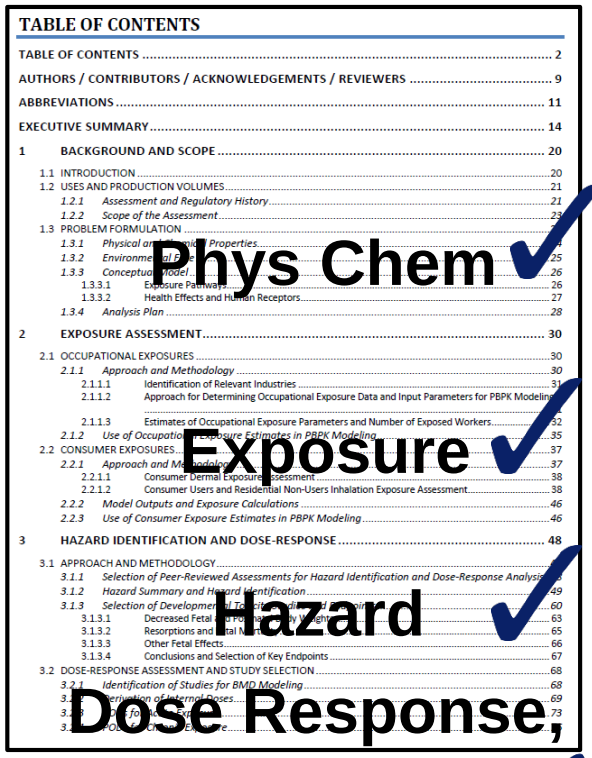

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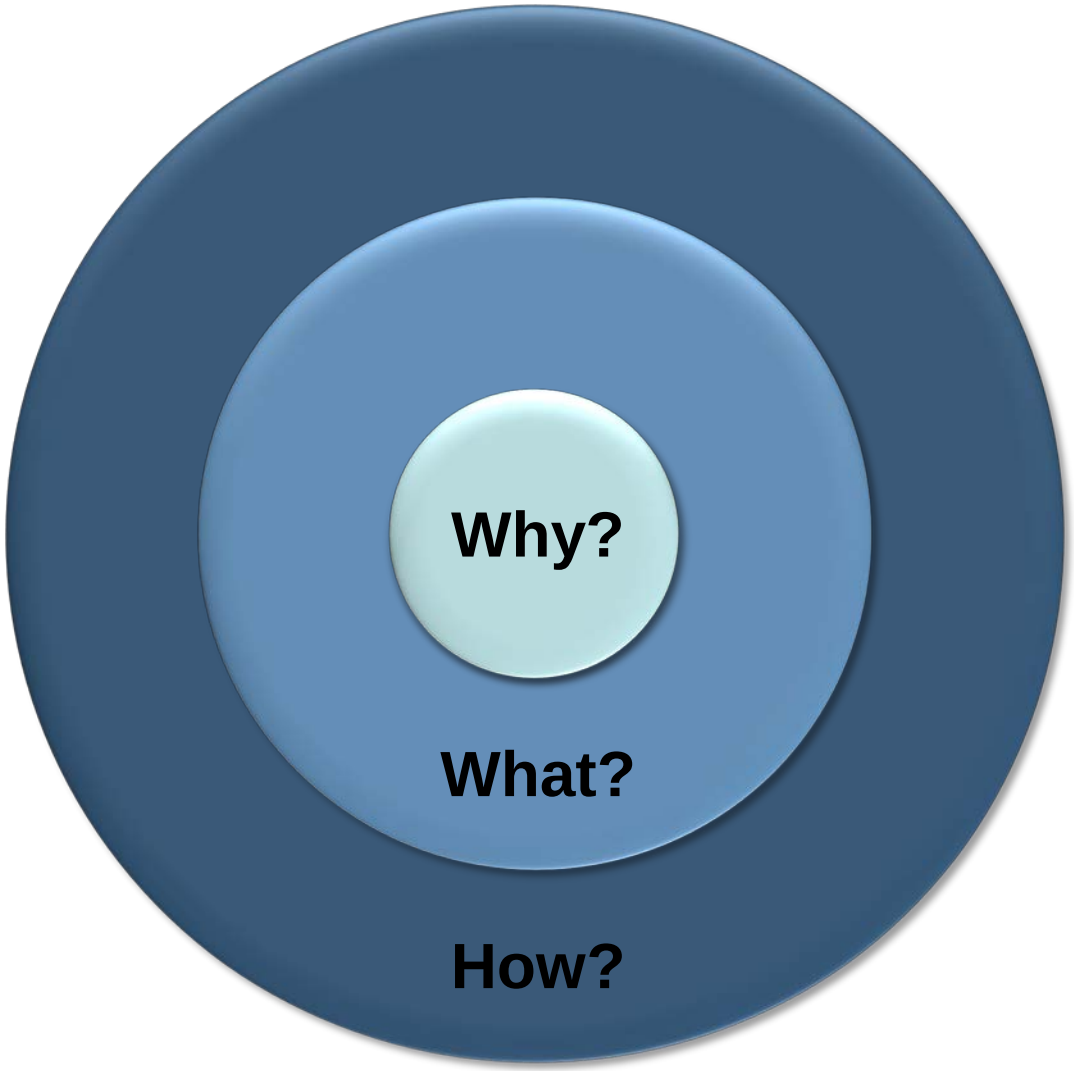
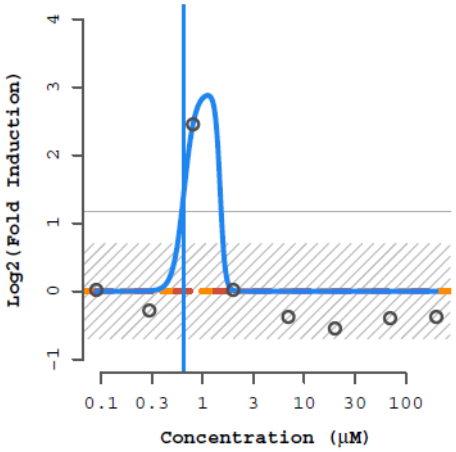
‘Golden Circle’ of 21st Century Risk Assessment



Regulatory Applications Require More Focus on Quality and Transparency

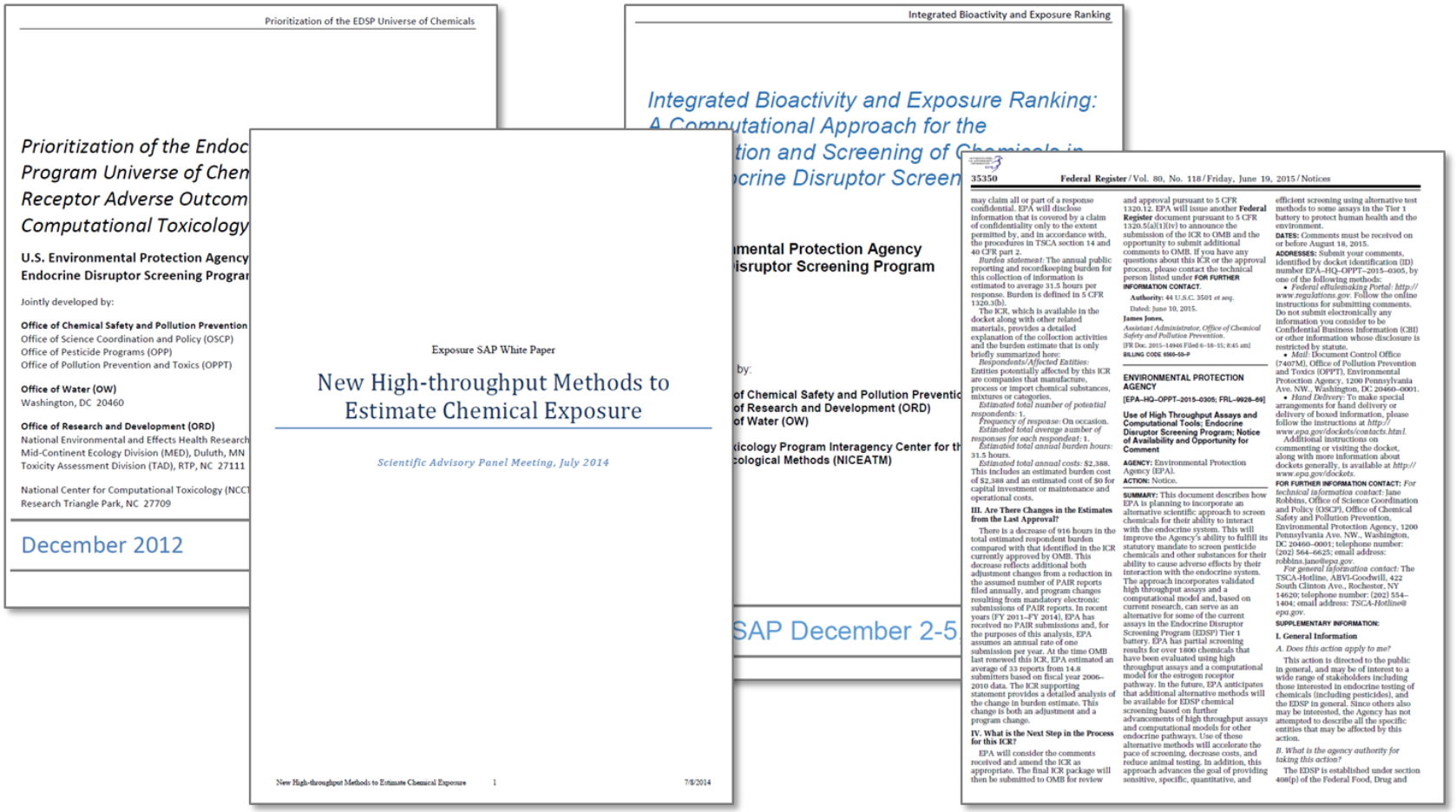



```
ASSAY:    AEID117 (ATG_ERa_TRANS)

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
```

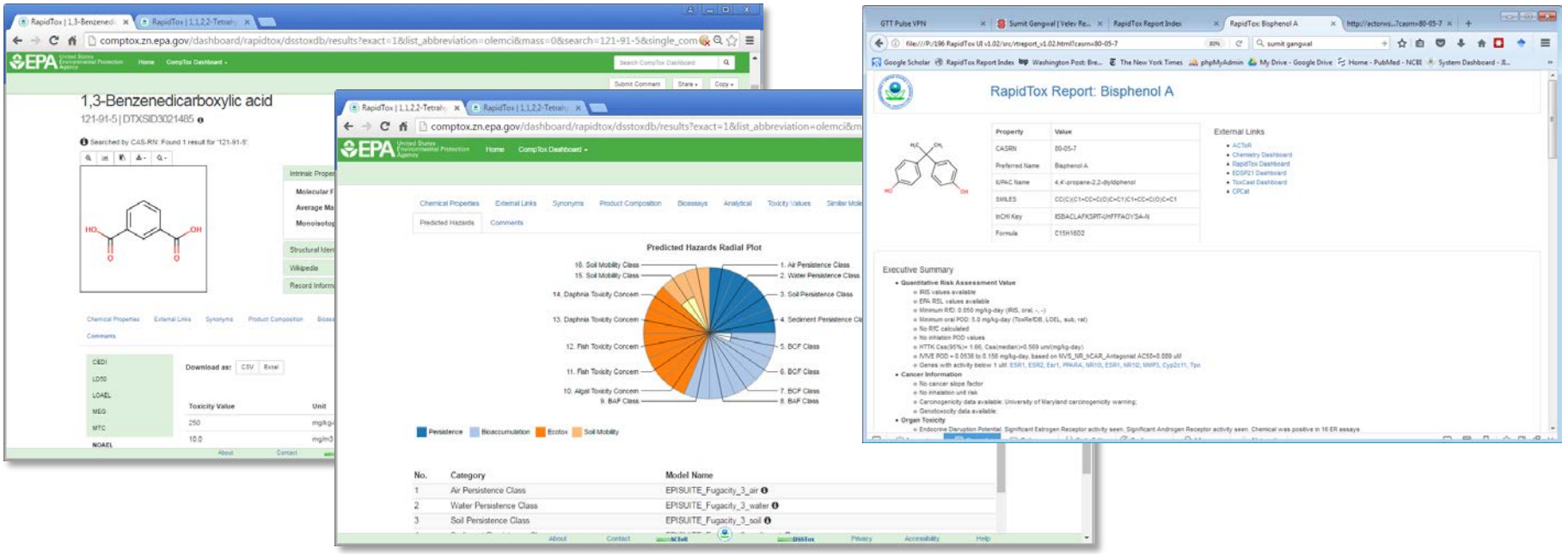
FLAGS:
Only one conc above baseline, active
Borderline active

- 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

Application to Regulatory Decisions for Endocrine Screening



Beginning Application to Quantitative Risk Assessment Through New 'RapidTox' Dashboard



- 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

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