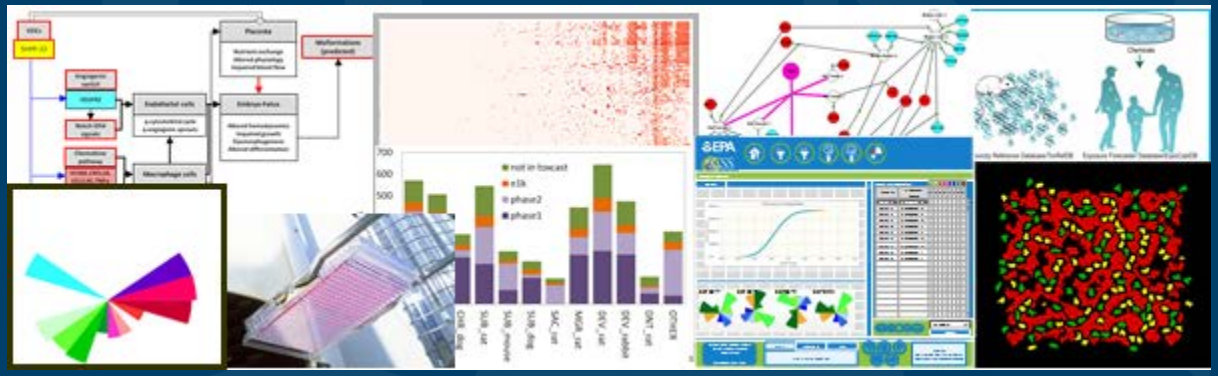
United States Environmental Protection Agency

Analysis of Transcriptomic Dose Response Data in the Context of Chemical Risk Assessment

My Journey Through the Toxicogenomics Bermuda Triangle



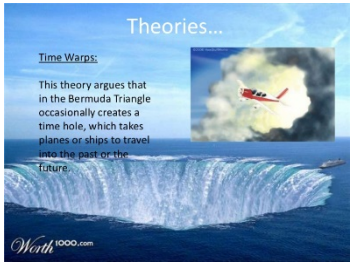
HESI-Health Canada-McGill Workshop
May 26, 2017

Russell Thomas
Director
National Center for Computational Toxicology

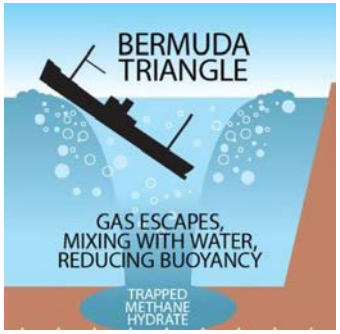
Myth of the Real Bermuda Triangle

http://onedio.co/content/new-mind-blowing-claim-about-the-bermuda-triangle-13519

http://www.crystalinks.com/bermuda_triangle.html

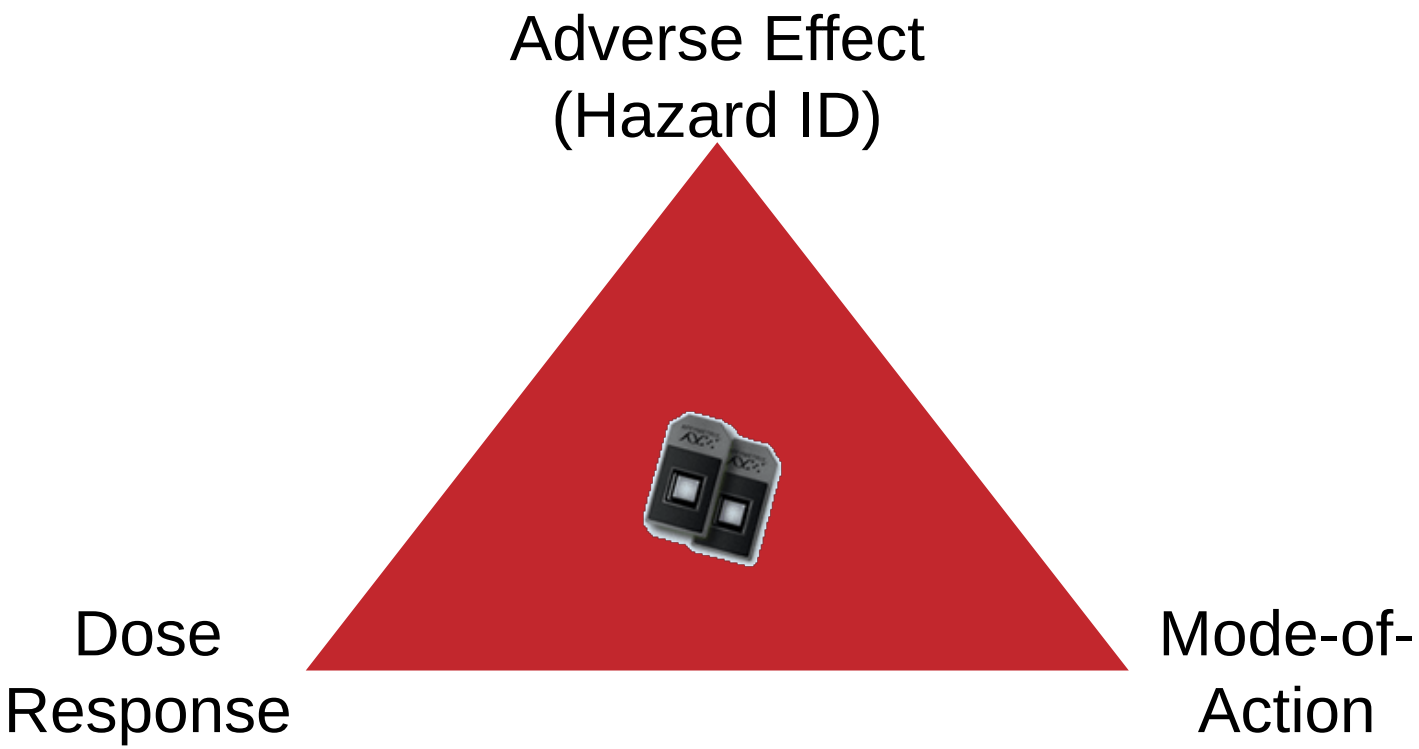


https://www.slideshare.net/jayakanthan75/bermuda-triangle-ppt

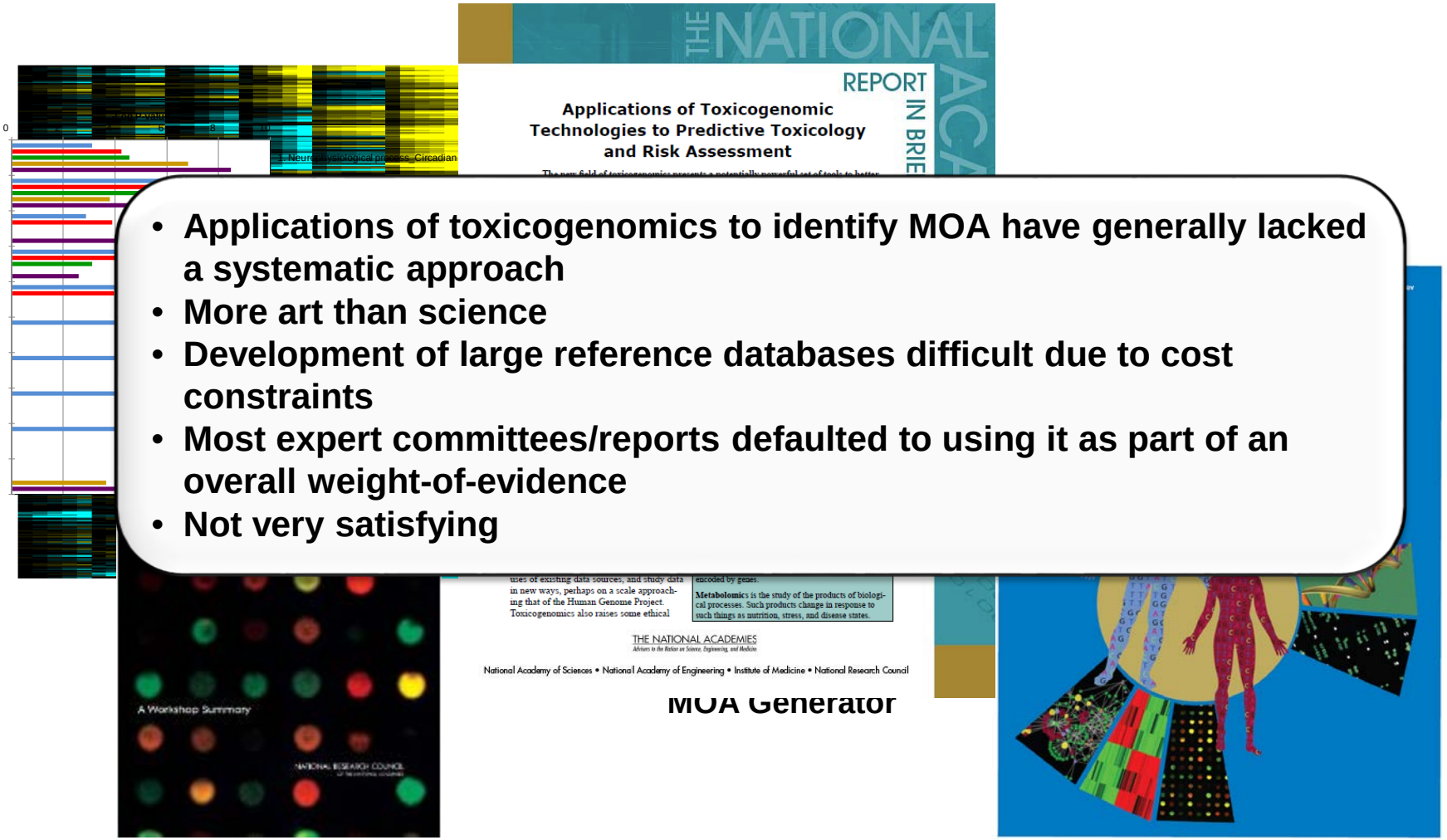


http://www.mensxp.com/special-features/today/29862-the-mystery-behind-the-disappearances-in-the-bermuda-triangle-may-just-have-been-solved.html

Bermuda Triangle of Toxicogenomics and Hazard Assessment

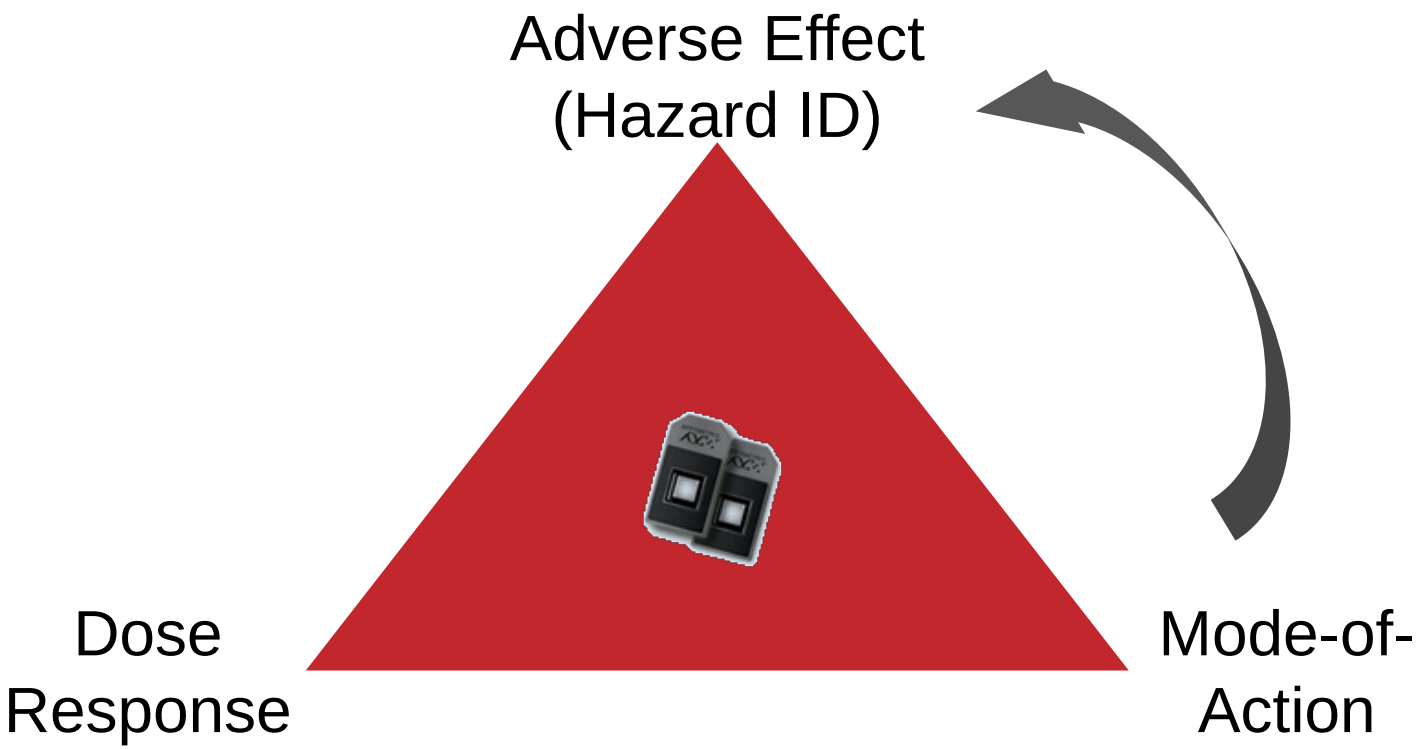


Initial Promise of Toxicogenomics Focused on Inferring MOA



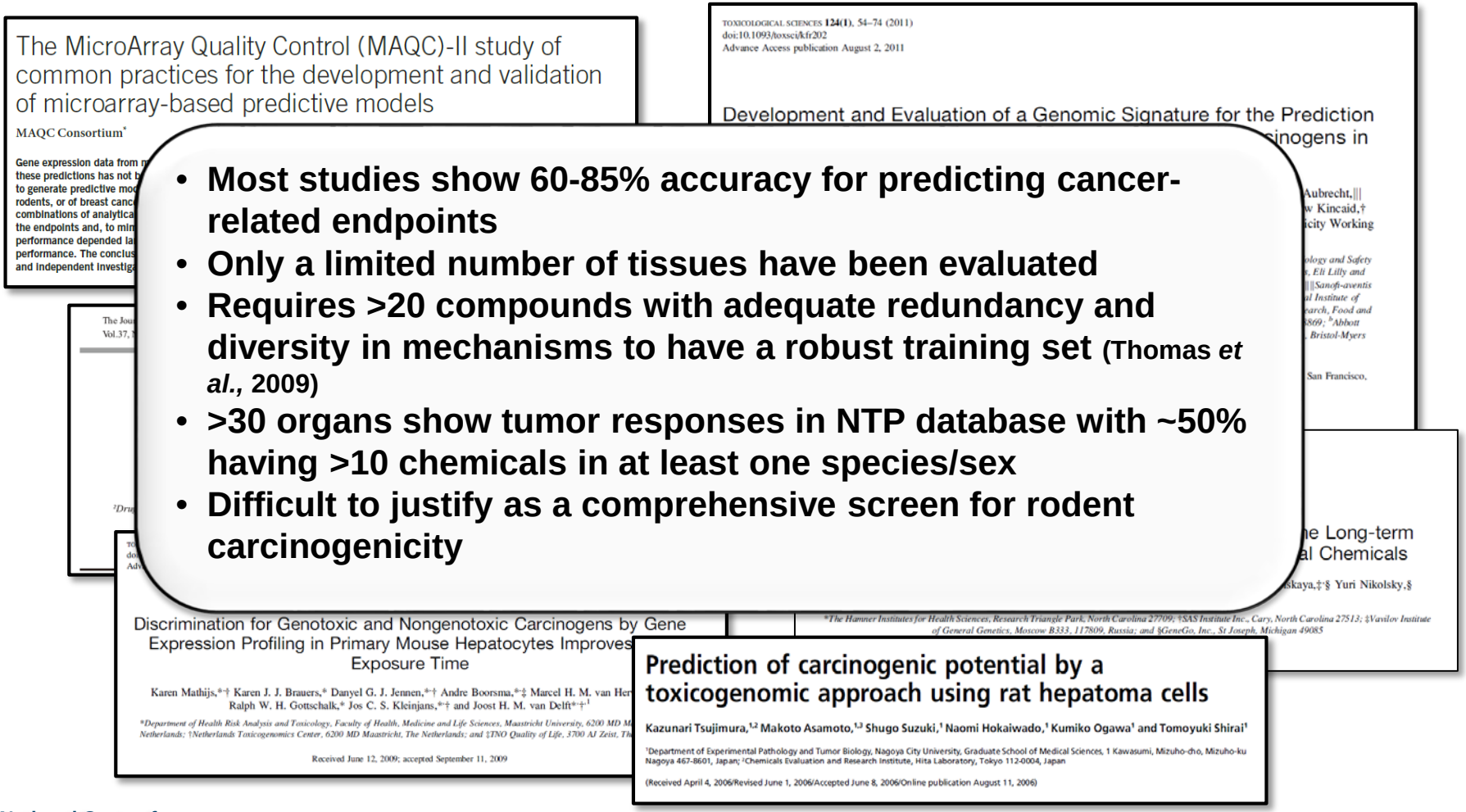
- Applications of toxicogenomics to identify MOA have generally lacked a systematic approach
- More art than science
- Development of large reference databases difficult due to cost constraints
- Most expert committees/reports defaulted to using it as part of an overall weight-of-evidence
- Not very satisfying

Bermuda Triangle of Toxicogenomics and Hazard Assessment

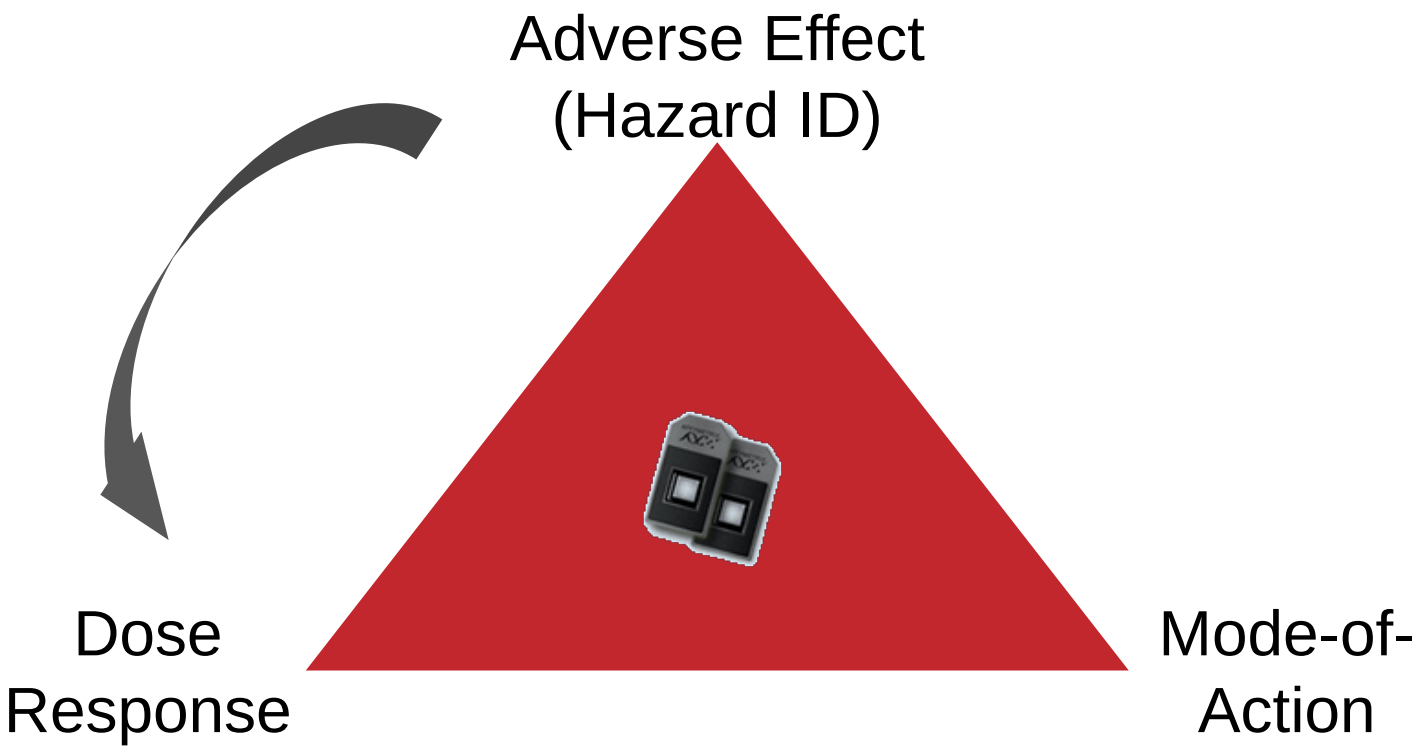


Developing Statistical Classification Models to Predict Hazard

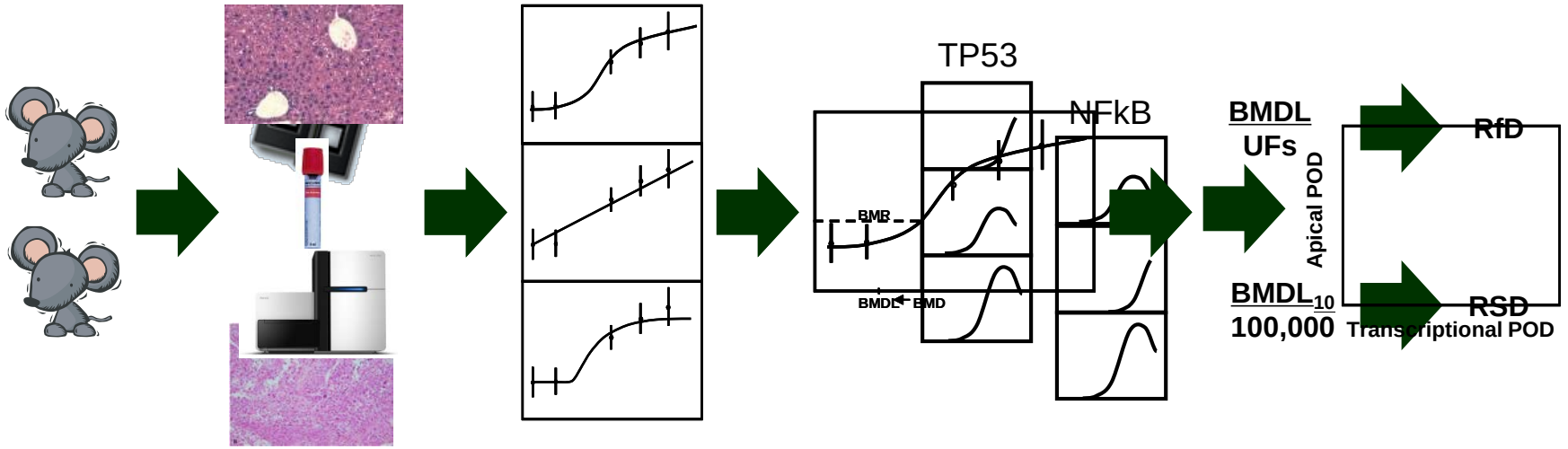
- **Most studies show 60-85% accuracy for predicting cancer-related endpoints**
- **Only a limited number of tissues have been evaluated**
- **Requires >20 compounds with adequate redundancy and diversity in mechanisms to have a robust training set (Thomas *et al.*, 2009)**
- **>30 organs show tumor responses in NTP database with ~50% having >10 chemicals in at least one species/sex**
- **Difficult to justify as a comprehensive screen for rodent carcinogenicity**



Bermuda Triangle of Toxicogenomics and Hazard Assessment



Using Transcriptomics to Define Dose Response



Short-term and Chronic Animal Studies

Transcriptional Most Sensitive Pathological Response Considered Adverse

Fit Data with Statistical Models

Identify Point of Departure (i.e., BMD)

Derive Safe Exposure Level

Short-term and Chronic Animal Studies

Transcriptional Most Sensitive Pathological Response Considered Adverse

Fit Data with Statistical Models

Identify Pathway Points of Departure (i.e., BMD)

Summarize Pathway and Calculate Summary Value (usually the median pathway BMD)

Derive Safe Exposure Level

Compare *In Vivo* Apical and Transcriptional PODs

Thomas *et al.*, *Tox Sci.*, 2011
Thomas *et al.*, *Mut Res.*, 2012
Thomas *et al.*, *Tox Sci.*, 2013

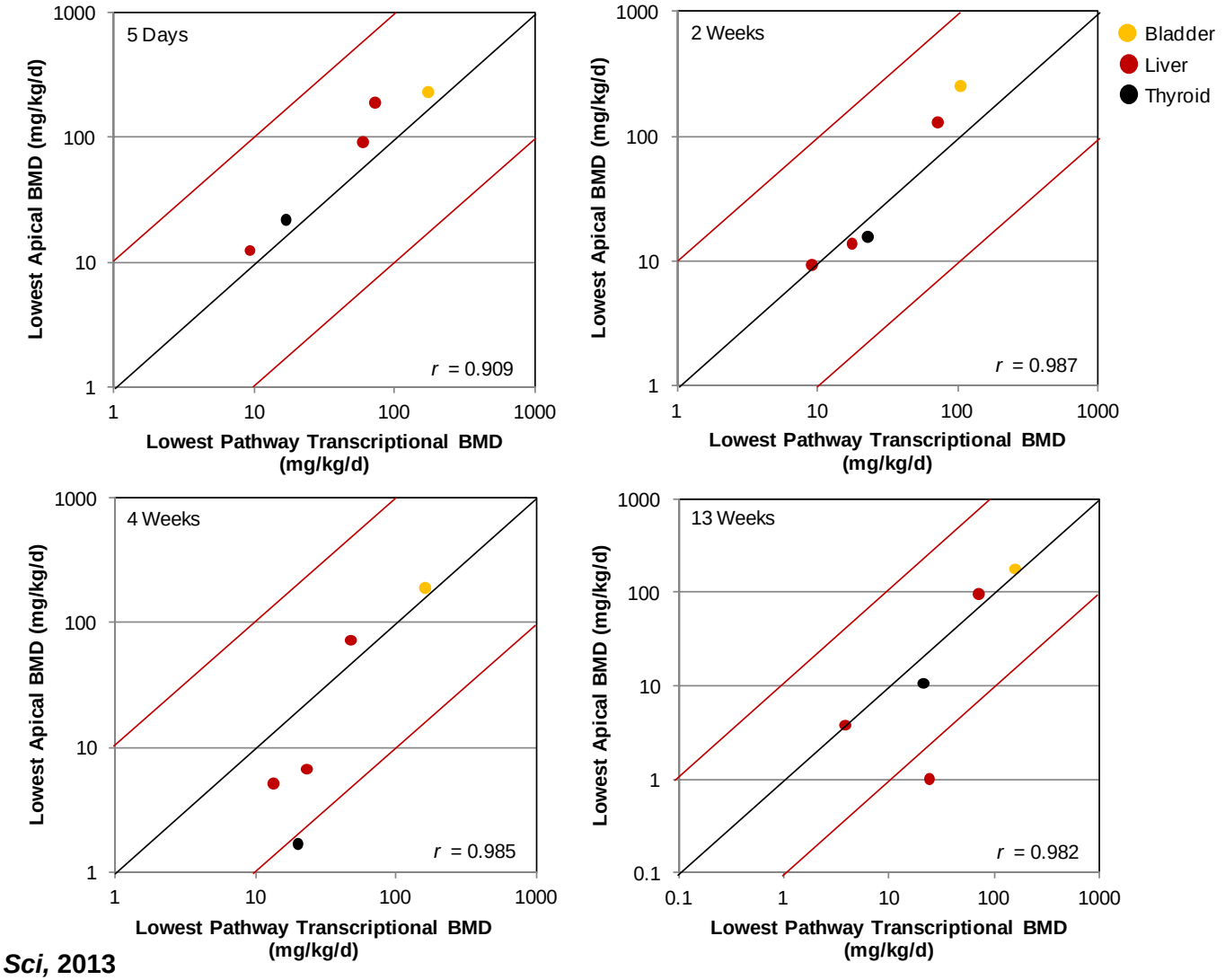
In Vivo Study to Assess Transcriptional and Apical Correlation

Chemical	Route	Doses[c]	Rodent Model	Time Point	Target Tissue
1,2,4-Tribromobenzene[a]	Gavage	2.5, 5, 10, 25, 75 mg/kg	Male Sprague Dawley rats	5 d, 2, 4, 13 wks	Liver
Bromobenzene[a]	Gavage	25, (50), 100, 200, 300, 400 mg/kg	Male F344 rats	5 d, 2, 4, 13 wks	Liver
2,3,4,6-Tetrachlorophenol[a]	Gavage	10, 25, 50, 100, 200 mg/kg	Male Sprague Dawley rats	5 d, 2, 4, 13 wks	Liver
4,4'-Methylenebis (N,N-dimethyl) benzenamine[b]	Feed	50, 200, 375, 500, 750 ppm	Male F344 rats	5 d, 2, 4, 13 wks	Thyroid[b]
N-Nitrosodiphenylamine[b]	Feed	250, 1000, 2000, 3000, 4000 ppm	Female F344 rats	5 d, 2, 4, 13 wks	Bladder[b]
Hyd					
1,4-					
Propylene glycol mono-t-butyl ether[b]	Inhalation	25, 75, 300, 800, 1200 ppm	Female B6C3F1 mice	13 wks	Liver
1,2,3-Trichloropropane[b]	Gavage	2, 6, 20, 40, 60 mg/kg	Female B6C3F1 mice	13 wks	Liver
Methylene Chloride[b]	Inhalation	100, 500, 2000, 3000, 4000 ppm	Female B6C3F1 mice	13 wks	Liver, Lung
Naphthalene[b]	Inhalation	0.5, 3, 10, 20, 30 ppm	Female B6C3F1 mice	13 wks	Lung
1,4-Dichlorobenzene[b]	Gavage	100, 300, 400, 500, 600 mg/kg	Female B6C3F1 mice	13 wks	Liver

Measured apical (histological and organ weight; n = 10) and gene expression changes (n = 5) at each dose and time point in the target tissue.

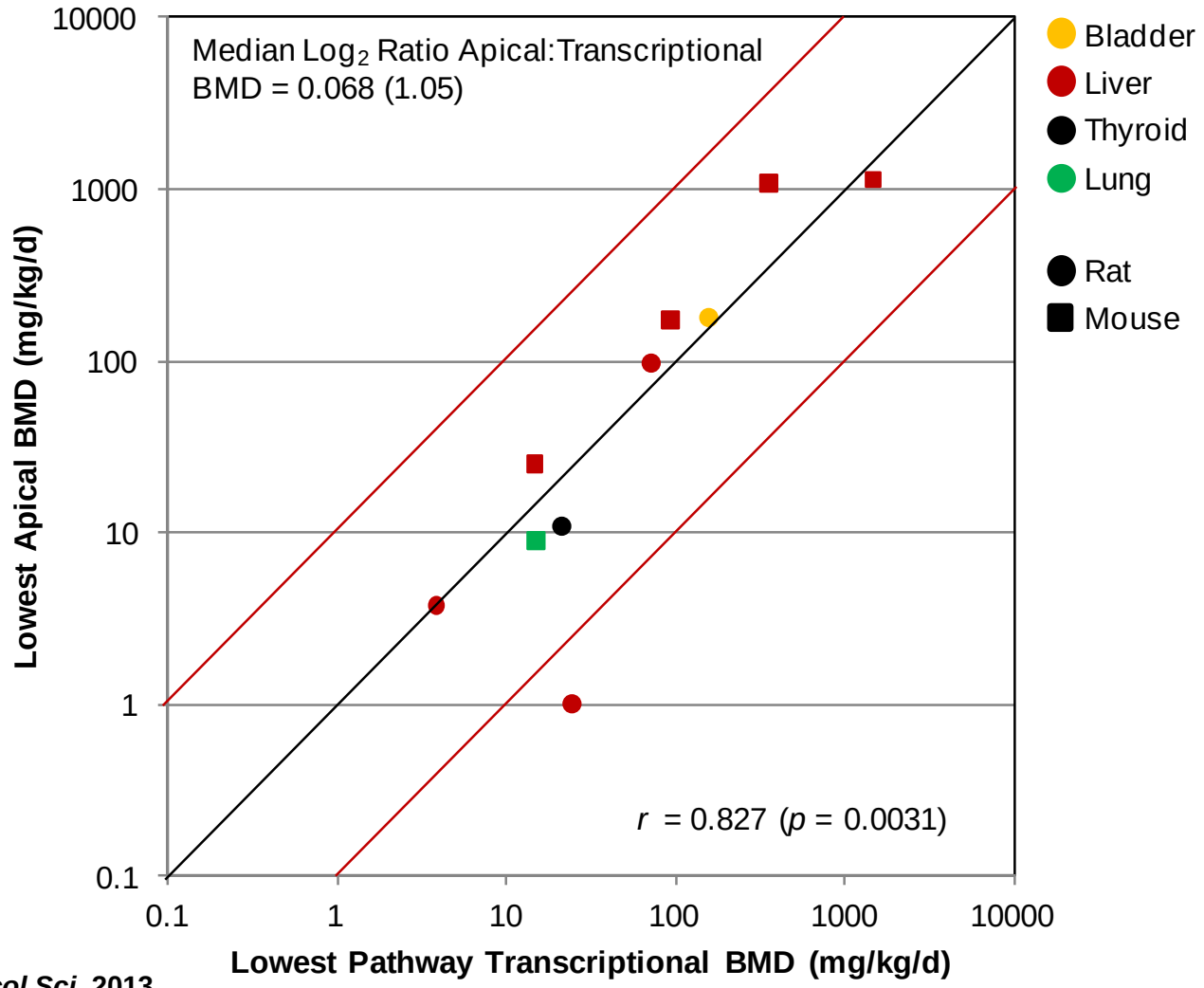
[a]Chemicals in IRIS database for non-cancer endpoints only
[b]Chemicals previously tested by the U.S. National Toxicology Program
[c]Underlined doses used in NTP two-year rodent bioassay or IRIS database

Temporal Changes Between Transcriptional and Non-Cancer PODs



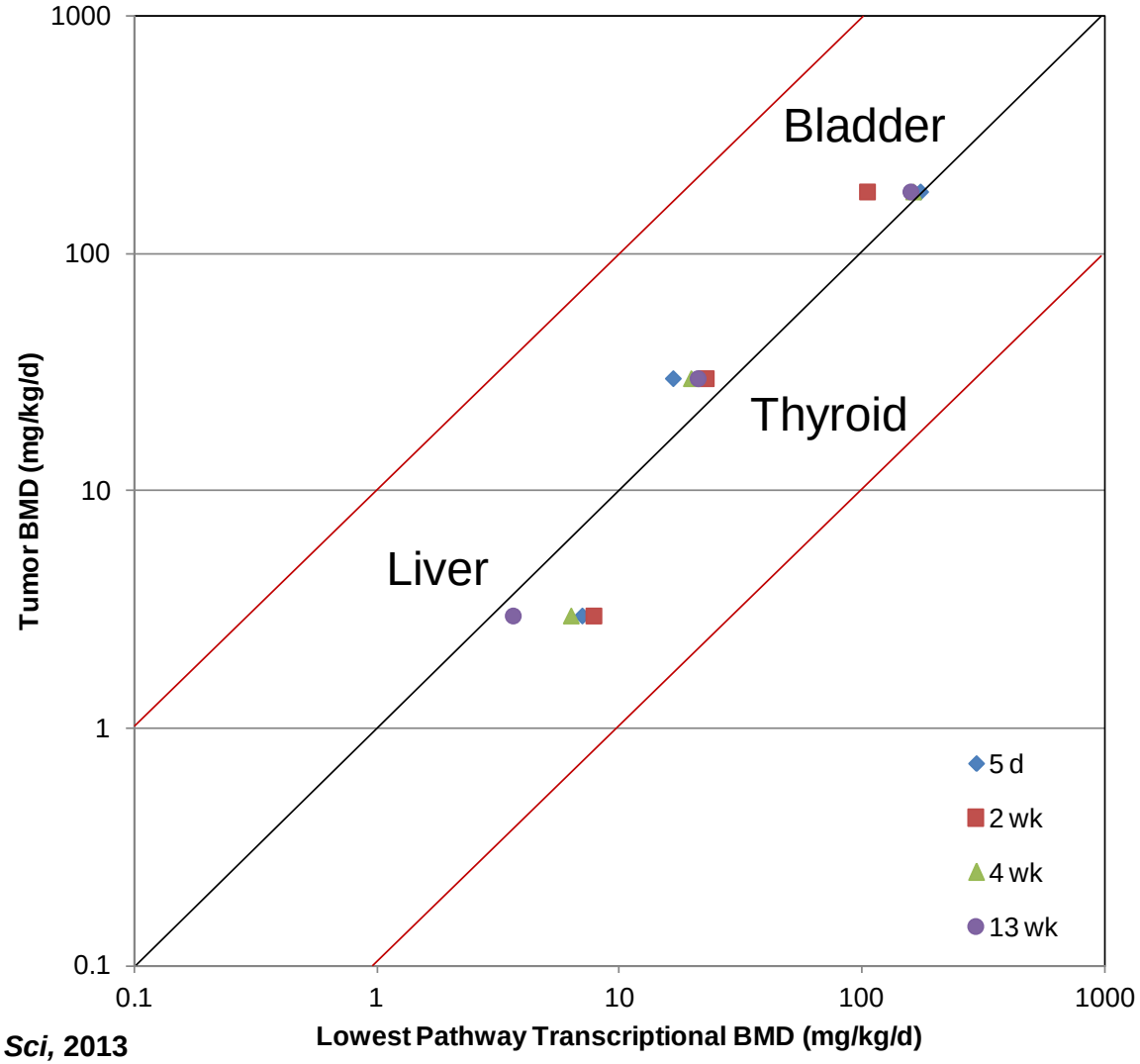
Thomas *et al.*, *Toxicol Sci*, 2013

Combined Correlation Between Non-Cancer and Transcriptional PODs



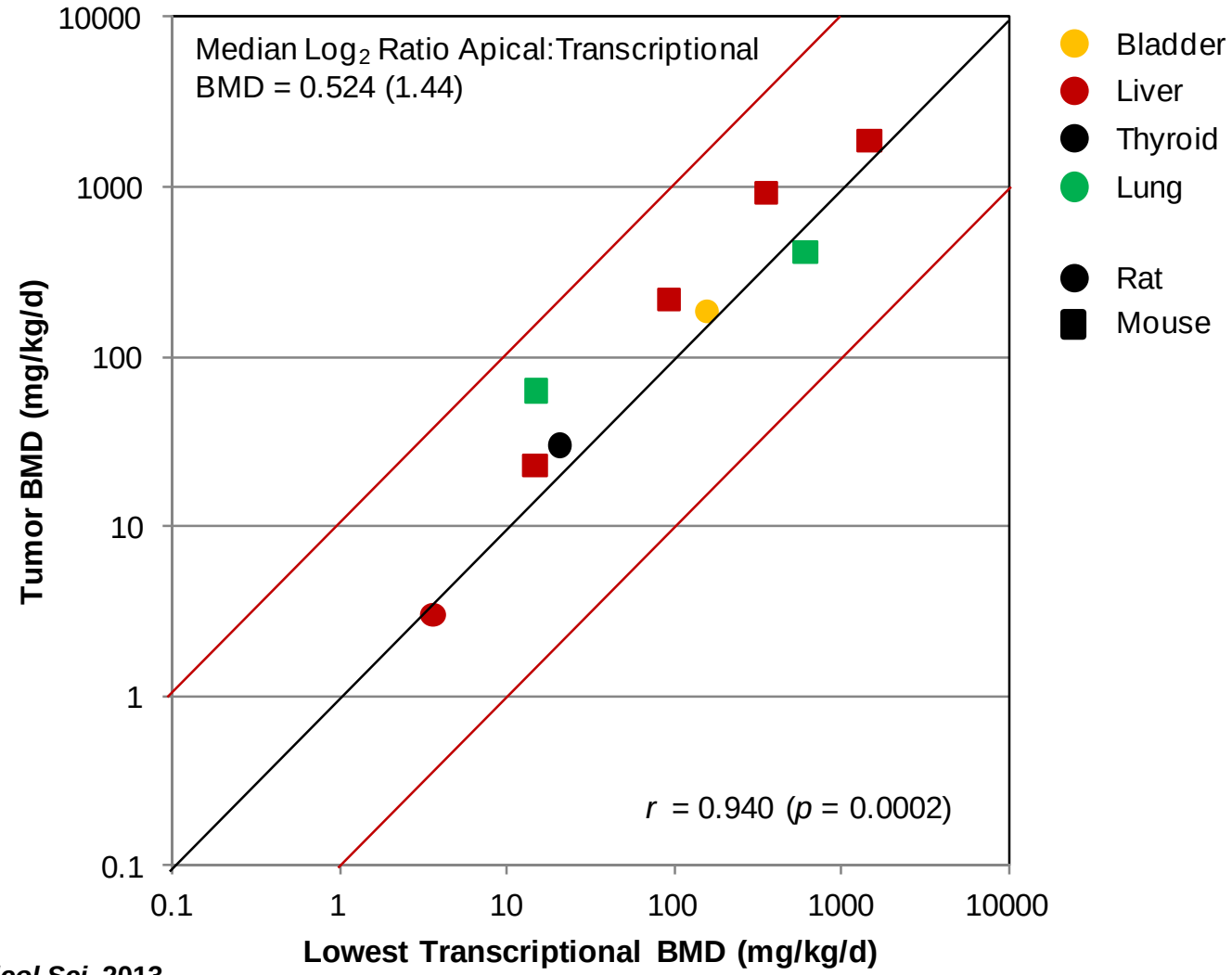
Thomas *et al.*, *Toxicol Sci*, 2013

Temporal Changes Between Transcriptional and Cancer PODs



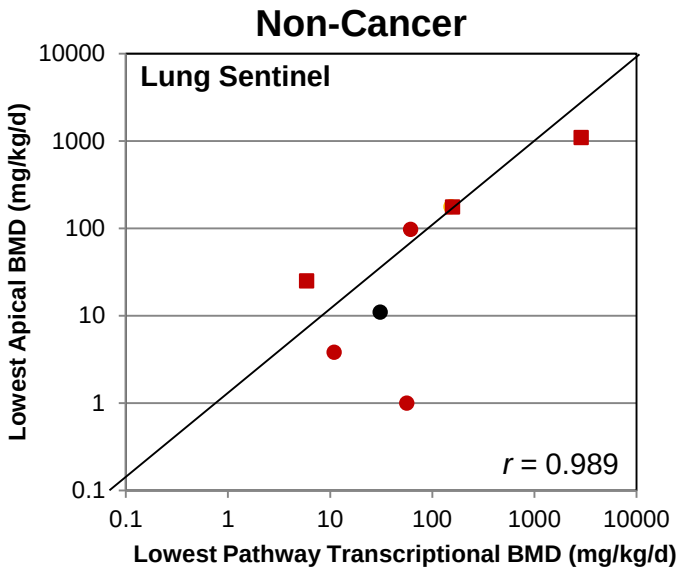
Thomas *et al., Toxicol Sci,* 2013

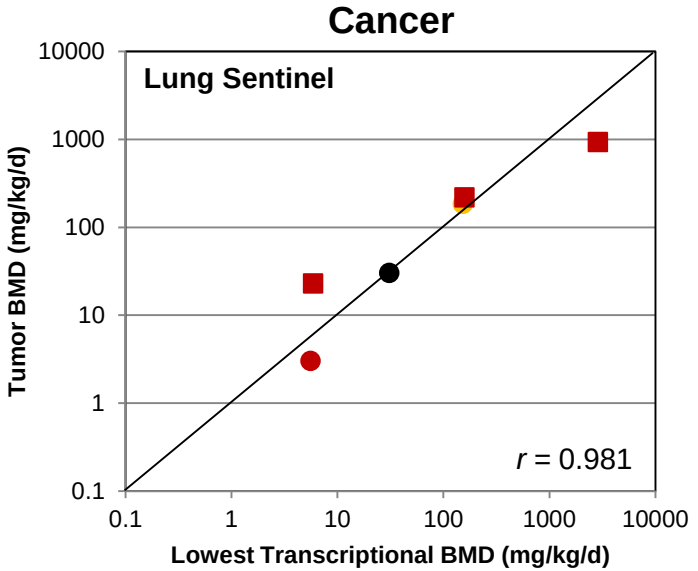
Combined Correlation Between Cancer and Transcriptional PODs

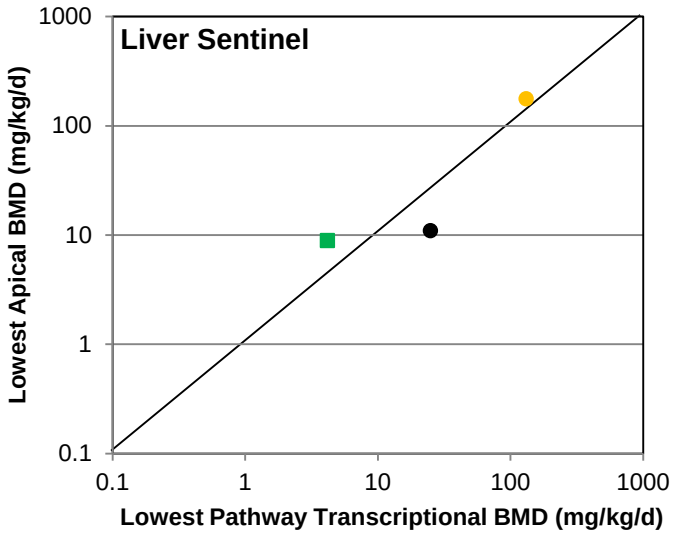


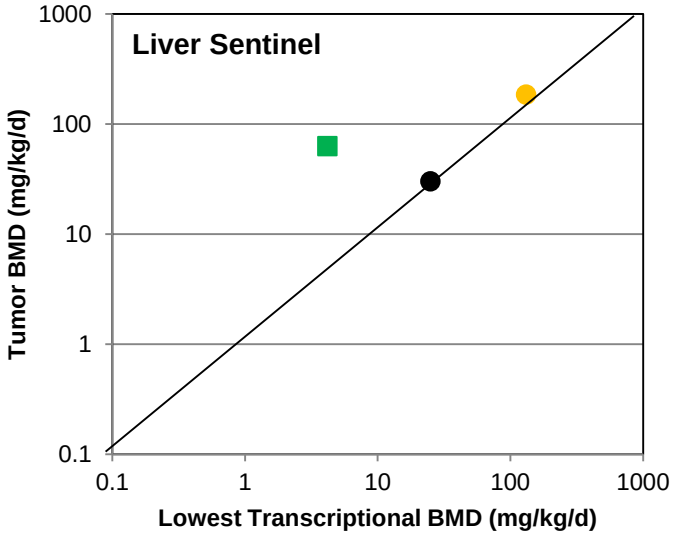
Thomas *et al., Toxicol Sci,* 2013

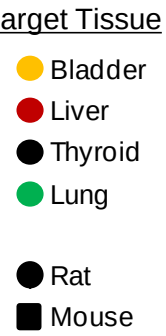
Correlation With Transcriptional PODs in Sentinel Tissue











Unpublished

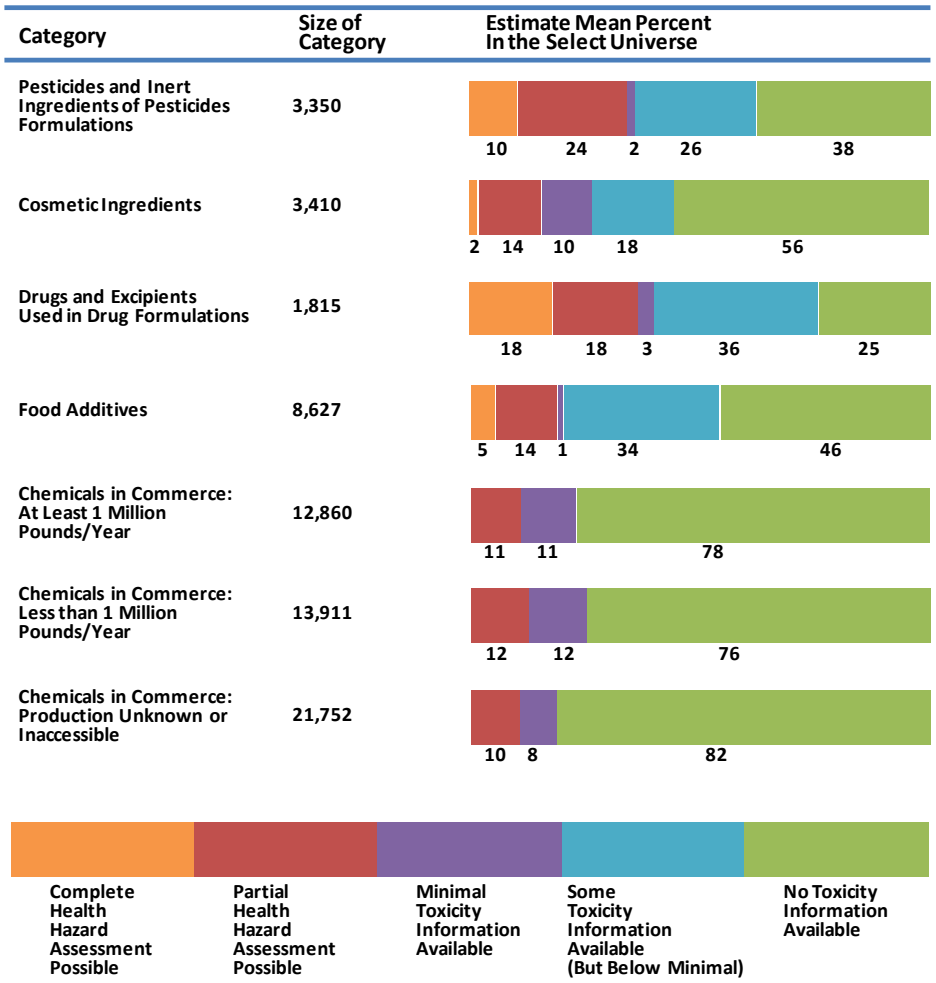
What Did These Studies Tell Us…

- Transcriptomic dose response alterations correlate with both noncancer- and cancer-related apical endpoints
- Correlation between apical responses and transcriptional no effect levels appear stable over time
- The average ratio apical and transcriptional points-of-departure for the most sensitive response was less than two-fold
- Transcriptional points-of-departure in sentinel tissues may provide reasonable surrogates for those in the target tissue

But, There are Other Challenges for EPA to Consider

- 1984 US NRC report
- Major challenge is too many chemicals and not enough data
- Total = 65,725
- No tox data = 46,000

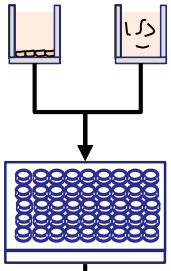
US National Research Council, 1984



Using High-Throughput Screening to Address Data Gap

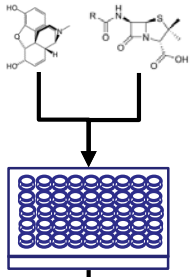
ToxCast
~600 assays

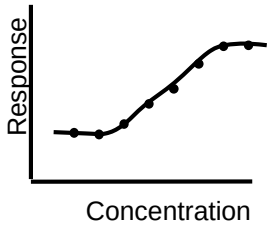
Tox21
~50 assays



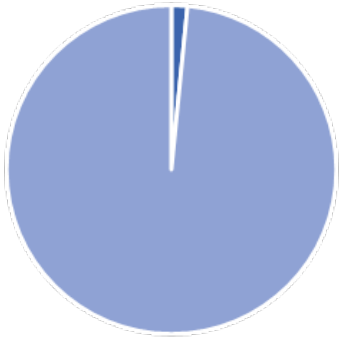
ToxCast
~1,000 chemicals

Tox21
~10,000 chemicals





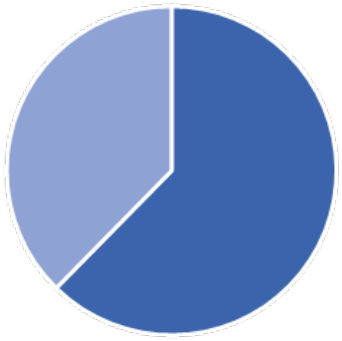
Gene Coverage



ToxCast

Not in ToxCast

Pathway Coverage*

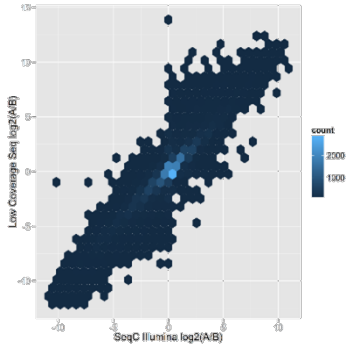


*At least one gene from pathway represented

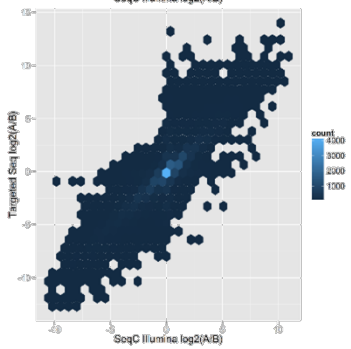
Searching for a High-Throughput Toxicogenomics Platform

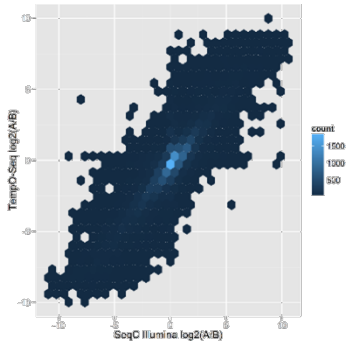
United States Environmental Protection Agency

Technical Comparison



TruSeq
r^2 0.74





Low Coverage
r^2 0.83

Functional Comparison

TruSeq
0/5 (0%)

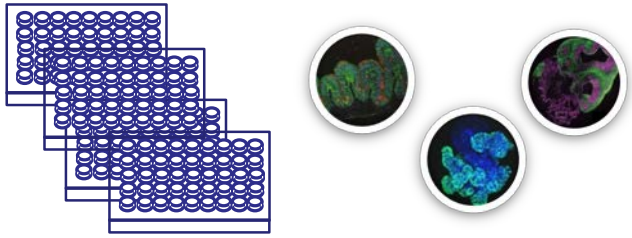
DSeq
30%)

Low Coverage
0/5 (0%)

Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

Developing a Portfolio of TGX Experimental and Analytical Tools



High-Throughput Toxicogenomics Screen

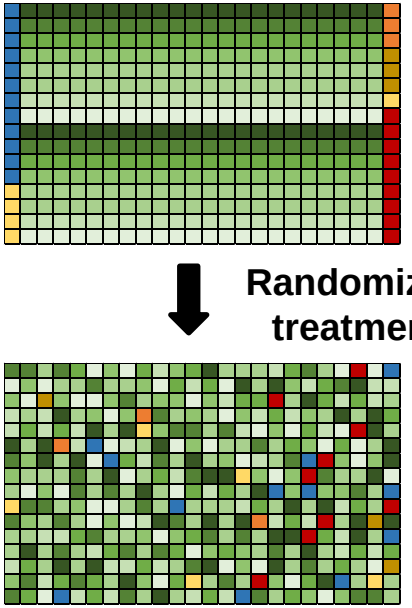
- Multiple cell types
- Thousands of chemicals
- Whole transcriptome (EPA)
- S1500+ (NTP)

Assay Design for Rigorous QC and Performance Validation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	MAQC-A (Us)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	non-treated
B	MAQC-A (Us)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	non-treated
C	MAQC-B (Us)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	non-treated
D	MAQC-B (Us)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	DMSO
E	Bulk Lysate (DMSO)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	DMSO
F	Bulk Lysate (DMSO)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	DMSO
G	Bulk Lysate (Trichostatin)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	DMSO [No Label]
H	Bulk Lysate (Trichostatin)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Trichostatin (1 µM)
I	Lysis Buffer (Us)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	Trichostatin (1 µM)
J	Lysis Buffer (Us)	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	Trichostatin (1 µM)
K	MAQC-A (Them)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	Genistein (10 µM)
L	MAQC-A (Them)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	Genistein (10 µM)
M	MAQC-B (Them)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Genistein (10 µM)
N	MAQC-B (Them)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	Sirolimus (0.1 µM)
O	Lysis Buffer (Them)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	Sirolimus (0.1 µM)
P	Lysis Buffer (Them)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	Sirolimus (0.1 µM)
	No Cells																							

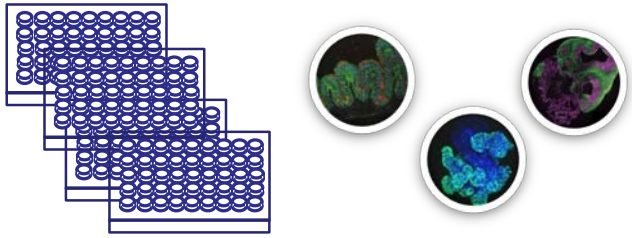
Screen Design

- Large bank of cytogenetically and functionally characterized cells
- 8 point concentration response
- Single time point
- Parallel HCI screen



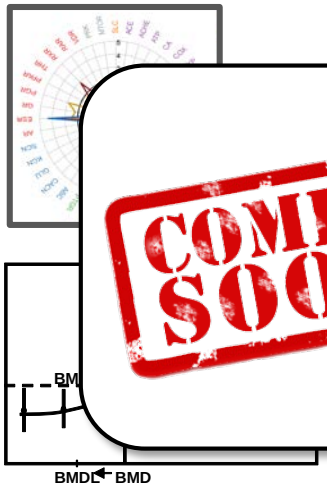
Josh Harrill, Unpublished

Developing a Portfolio of TGX Experimental and Analytical Tools



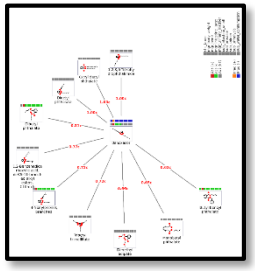
High-Throughput Toxicogenomics Screen
- Multiple cell types
- Thousands of chemicals
- Whole transcriptome (EPA)
- S1500+ (NTP)

Mode of Action/MIE



COMING SOON!

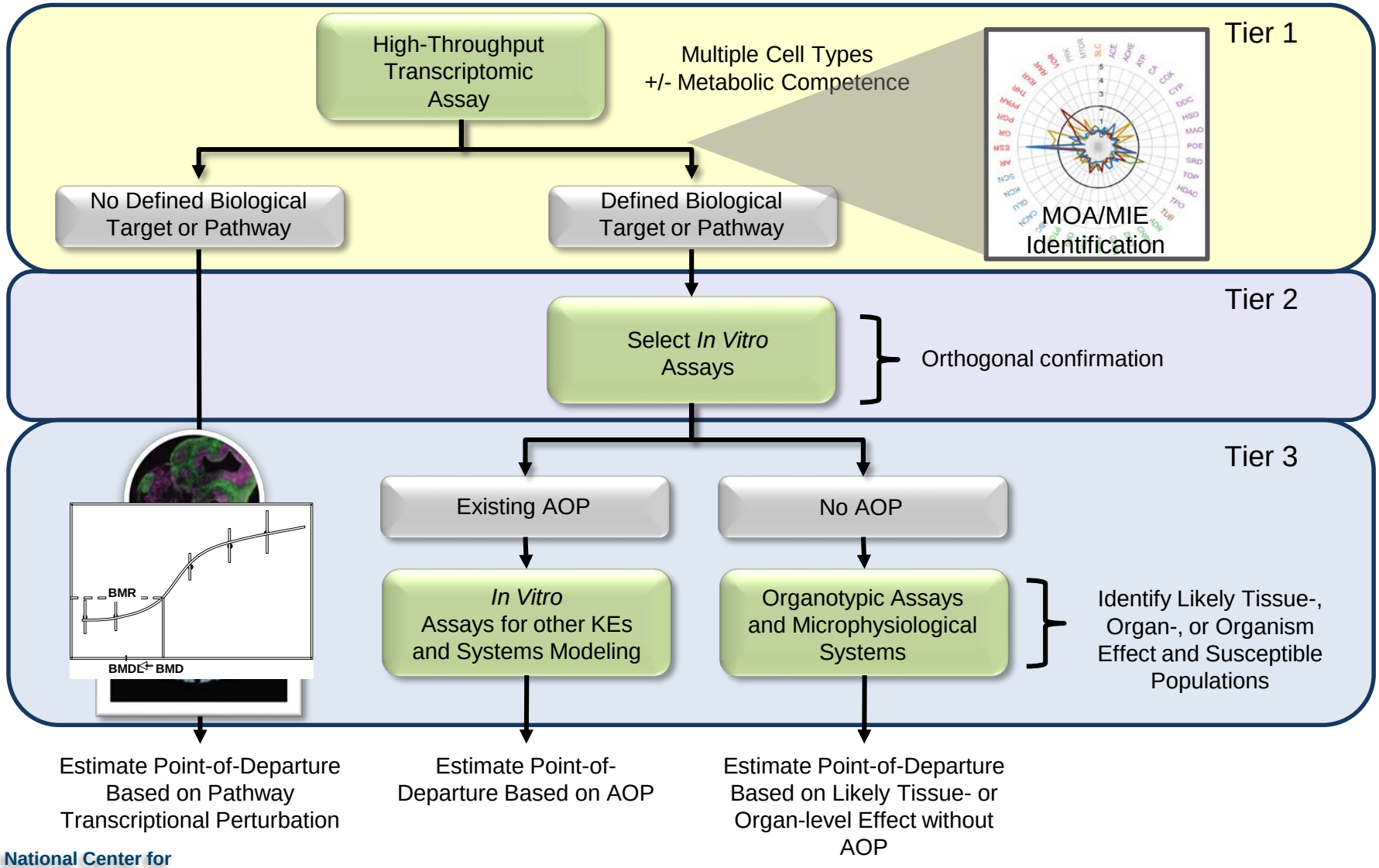
- Large scale screen of 1,000 chemicals (ToxCast I/II) in single cell type this summer
- Additional screens across multiple cell types/lines
- Additional reference chemicals and genetic perturbations (RNAi/CRISPR/cDNA)



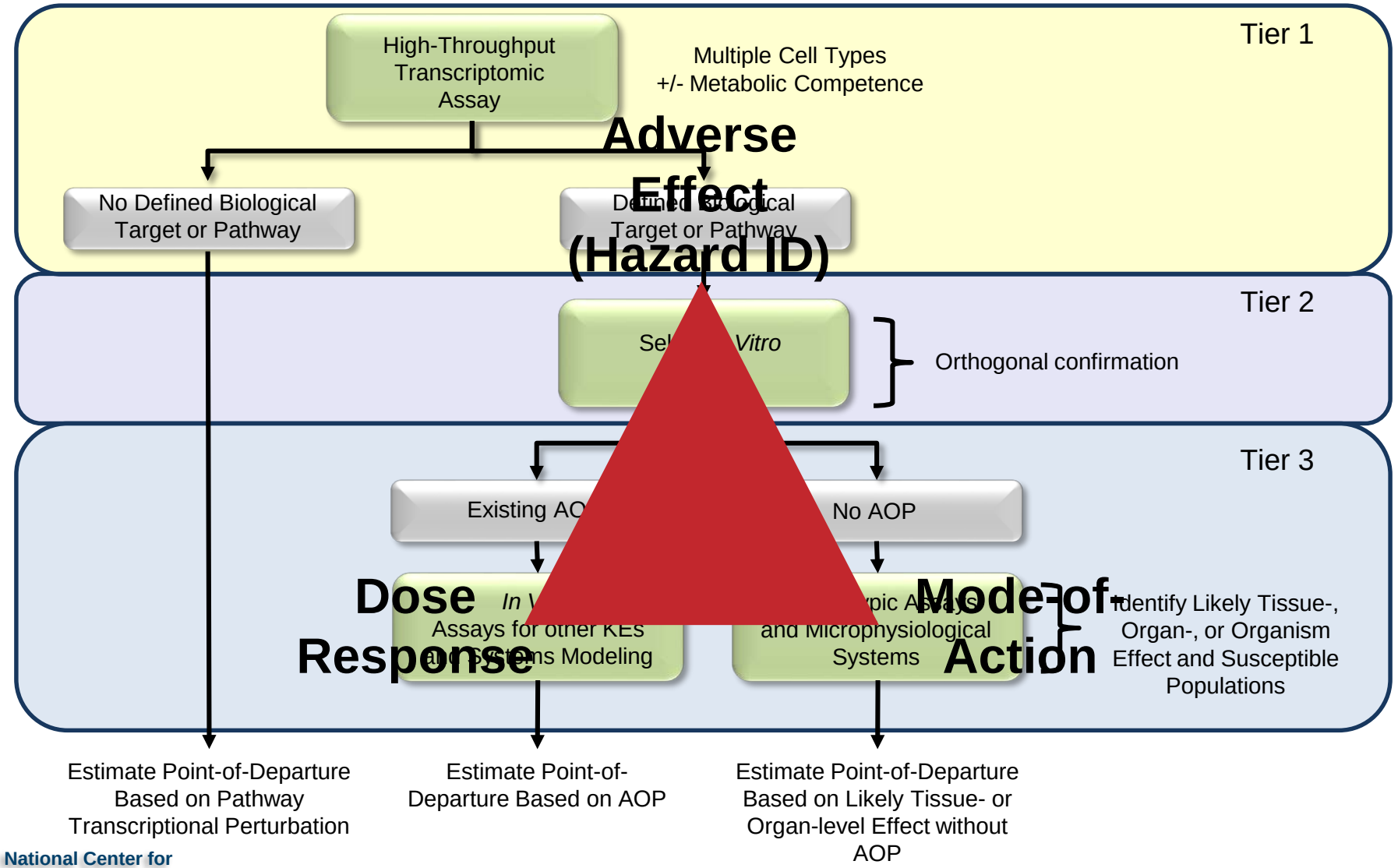
Shah et al., 2016

Read Across and Category Approach
- GenRA tool
- Chemical and Biological Read Across
- Quantitative estimates of uncertainty

Integrating Components Into a Tiered Testing and Assessment Strategy



Hitting All the Points of the Toxicogenomics Bermuda Triangle



Keys to Breaking Out of the Toxicogenomics Bermuda Triangle

- Develop best practices and data use recommendations that cover a range of regulatory decisions
- Systematize and convergence on analysis approaches
- Develop flexible validation approaches based on performance criteria and reference standards (e.g., MAQC) that adapt to evolution in technology
- Characterize qualitative and quantitative uncertainty in application of the technology to specific regulatory decisions while also doing this for legacy approaches
- Continue progress in the transition apical to molecular/pathway-based endpoints as a basis for safety-related decisions

Need evolutionary leap, not incremental advances

Thank You for Your Attention!

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

NCEA Colleagues:
- Scott Wesselkamper
- Nina Wang
- Jason Lambert
- Janet Hess-Wilson
- Dan Petersen
- Jay Zhao

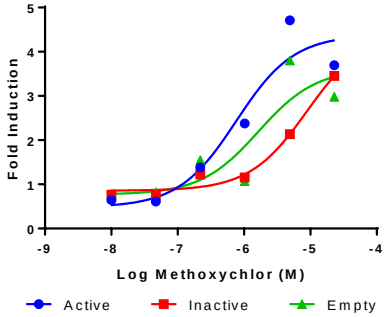
Hamner Colleagues:
- Barbara Wetmore
- Reetu Singh
- Bethany Parks
- Linda Pluta
- Michael Black
- Eric Healy
- Longlong Yang

EPA's National Center for Computational Toxicology

Beginning to Address Metabolic Competence

"Extracellular" Approach

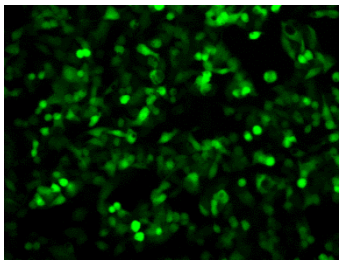
Chemicals metabolism in the media or buffer of cell-based and cell-free assays

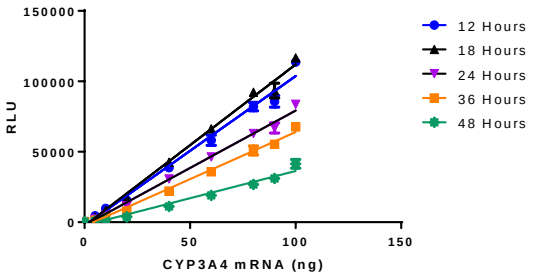


More closely models effects of hepatic metabolism and generation of circulating metabolites

"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

DeGroot and Simmons, Unpublished

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

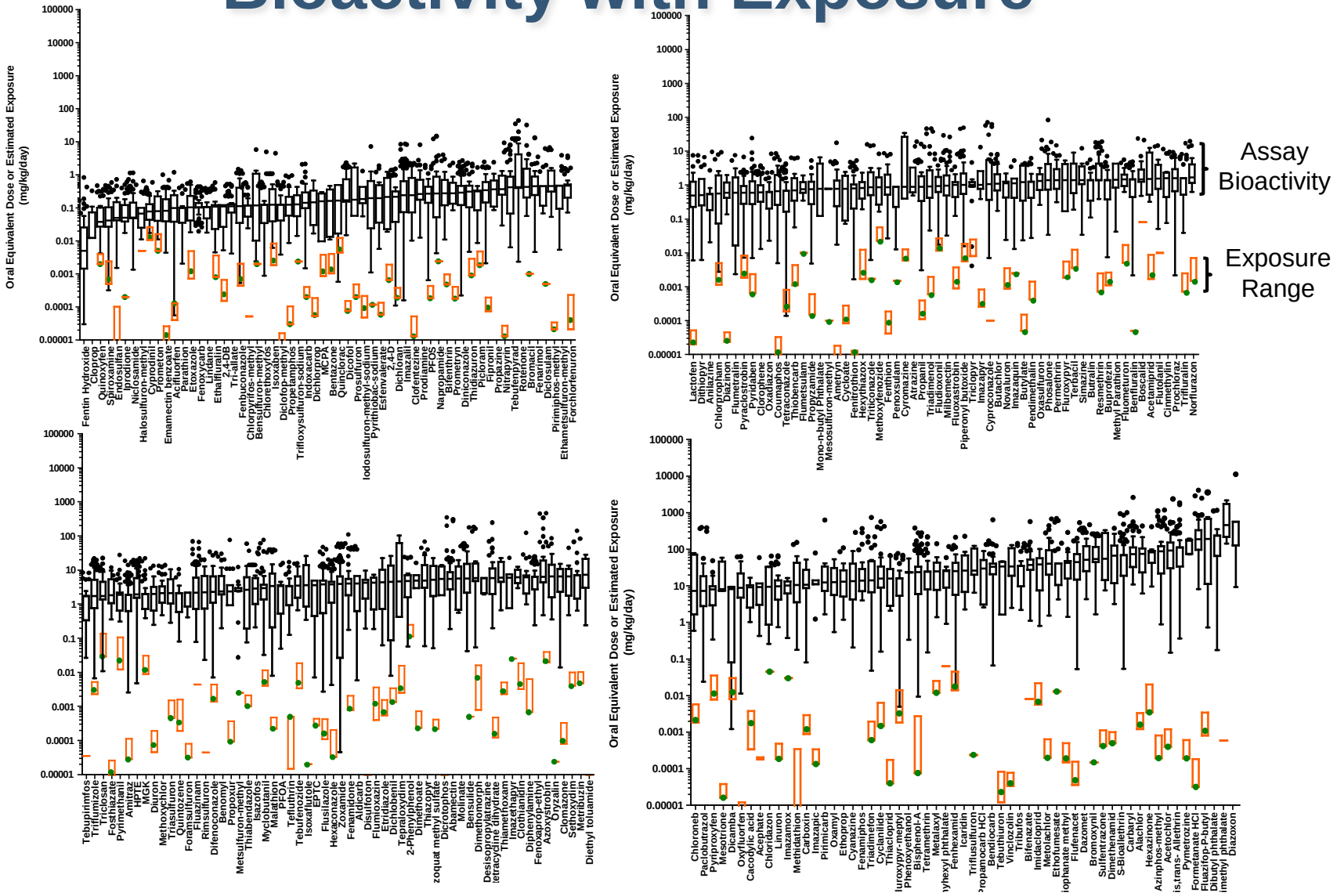
Reverse Dosimetry

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/)

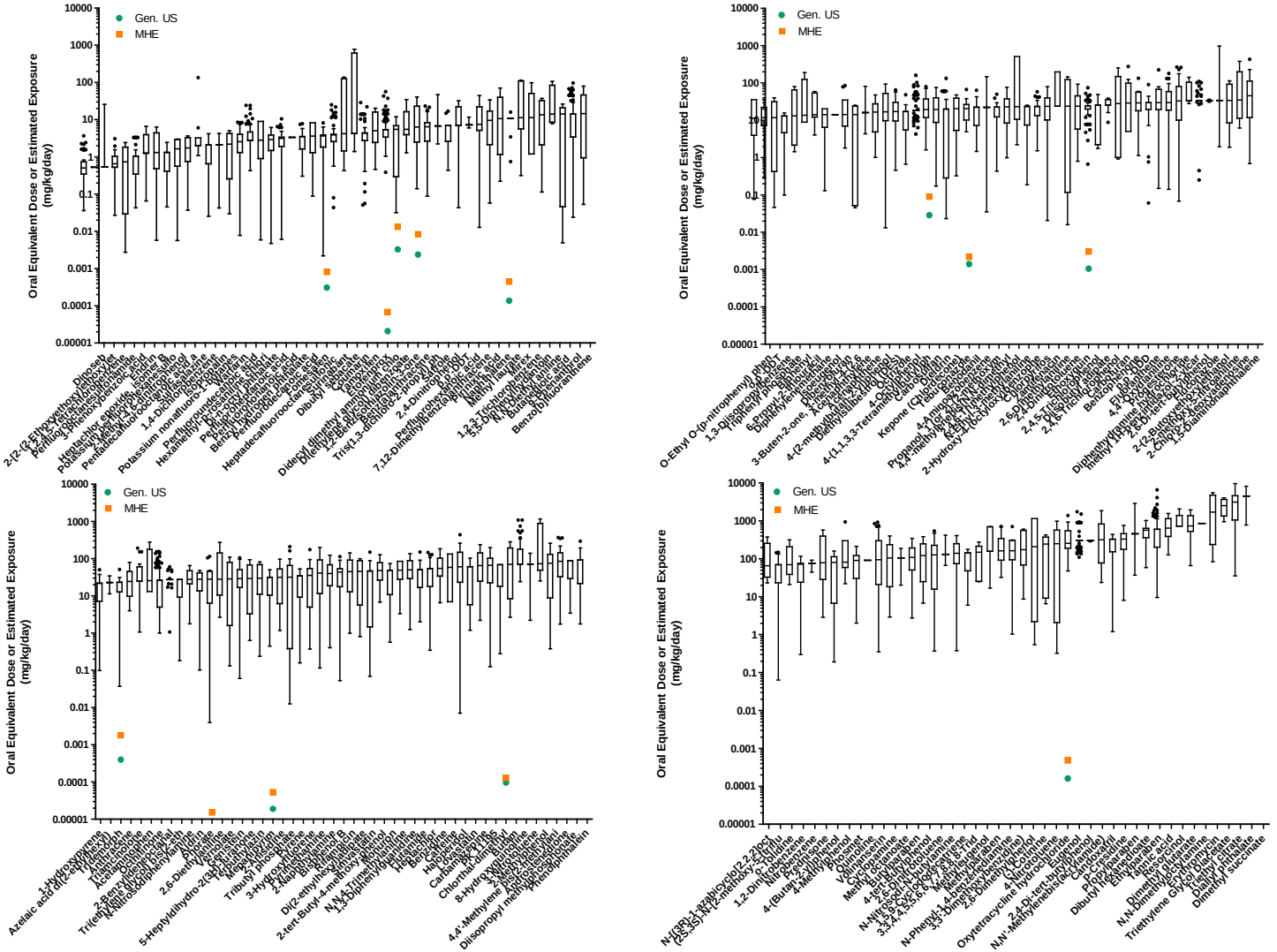
Rotroff *et al., Tox Sci.*, 2010
Wetmore *et al., Tox Sci.,* 2012

Comparing Dosimetry Adjusted Bioactivity with Exposure



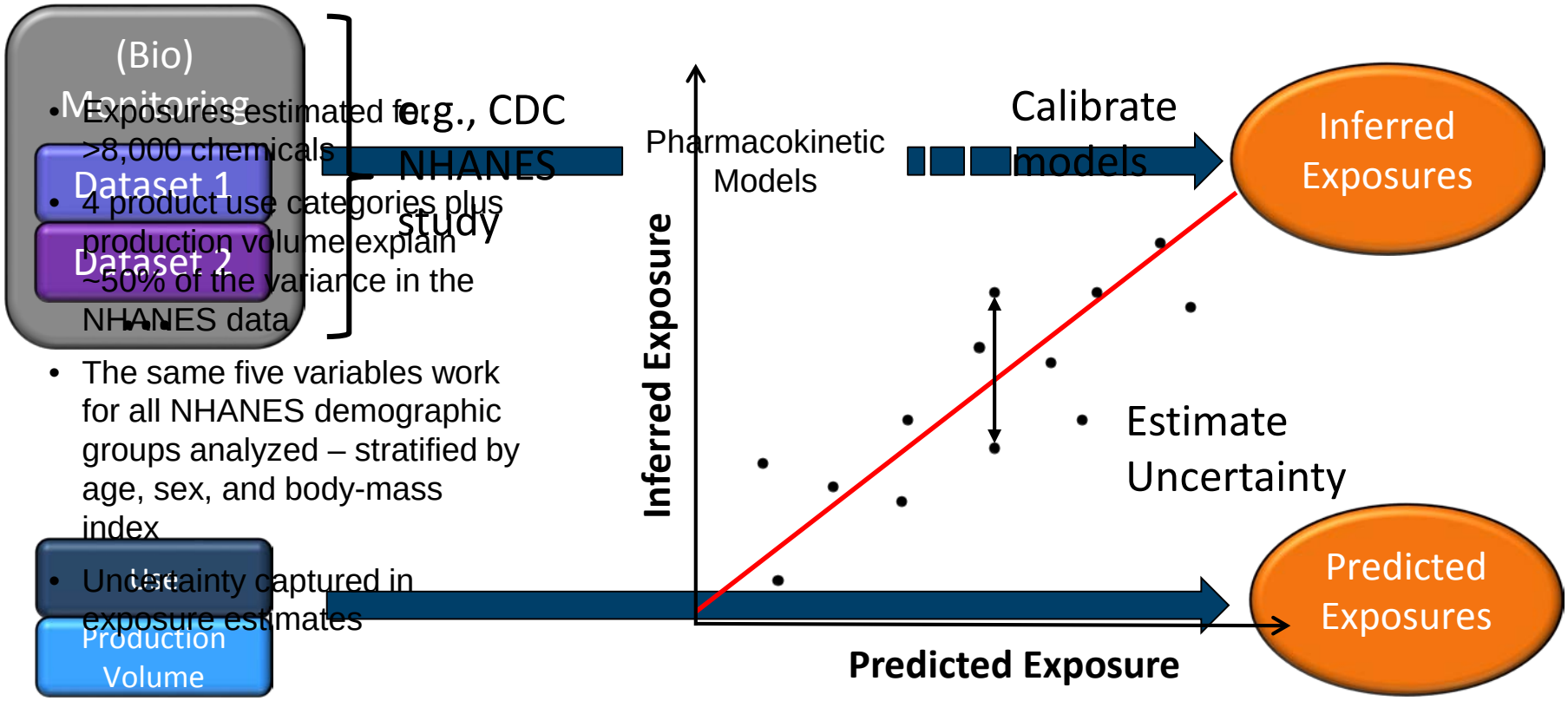
Wetmore *et al.*, *Tox Sci.*, 2012

But, Exposure Information is Lacking on Most Chemicals



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

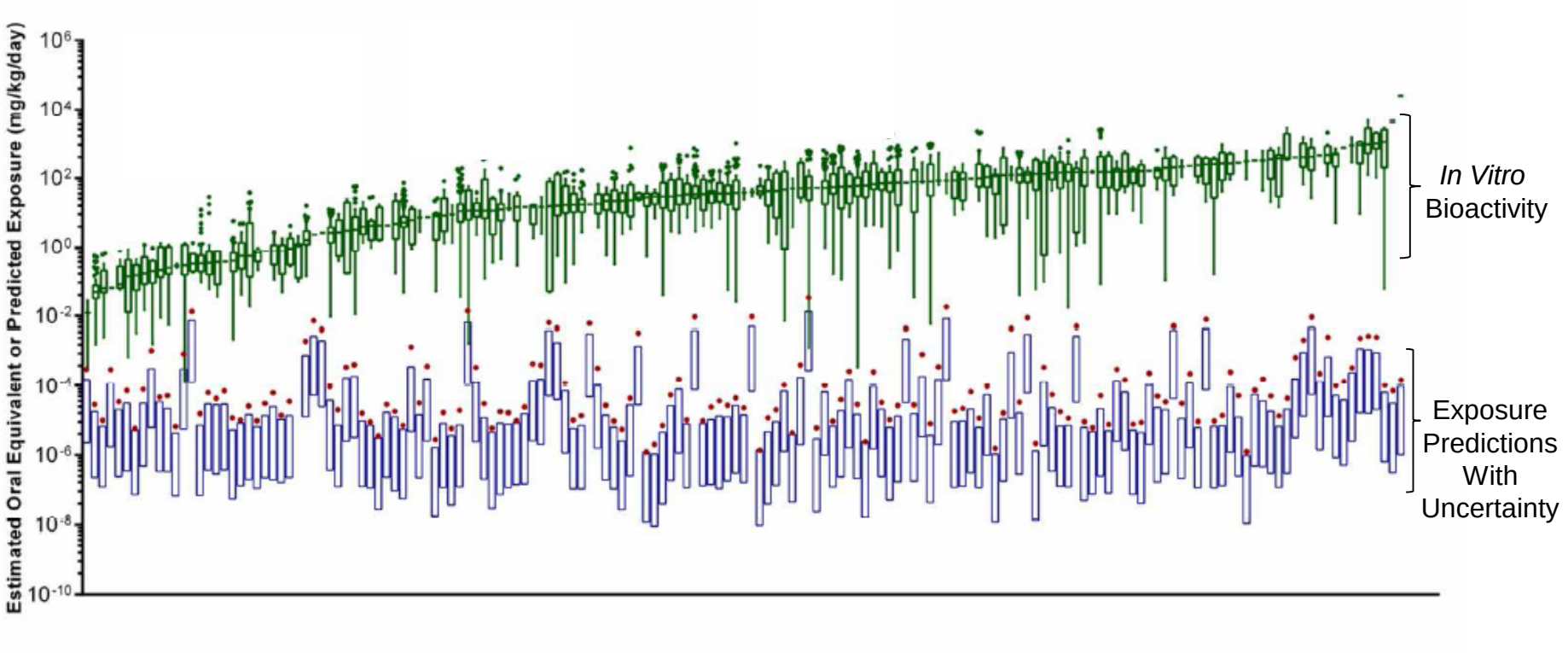
Adding the High-Throughput Exposure Component



- 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

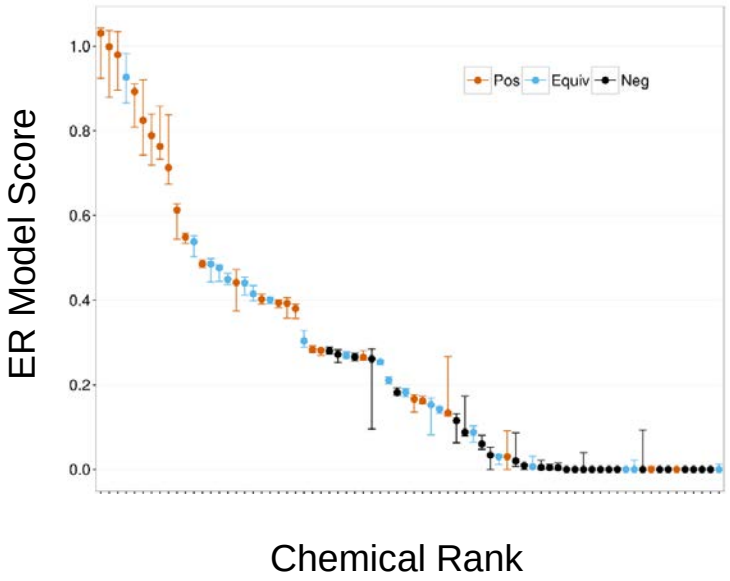
Comparing Bioactivity with Exposure Predictions for Risk Context



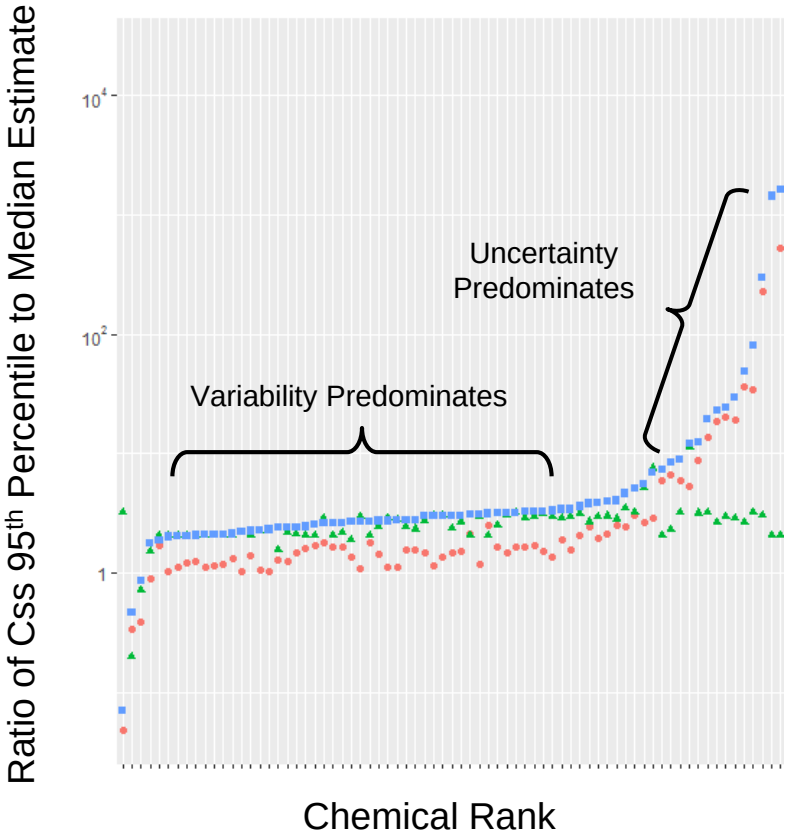
Wetmore *et al., Tox Sci.,* 2015

Adding in Uncertainty and Variability for PD and PK

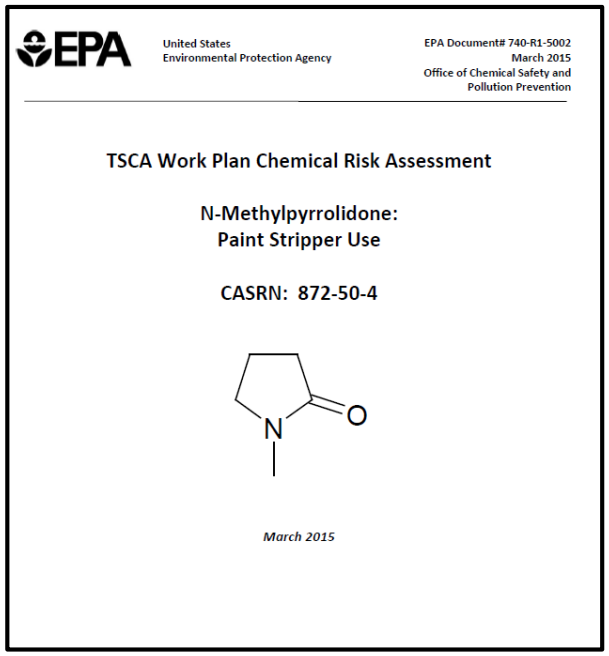
Propagation of Experimental Uncertainty in Models of ER Potency



Propagation of Experimental Uncertainty in High-Throughput Toxicokinetic Estimates



Covering All the Components of a 21st Century Risk Assessment



EPA United States Environmental Protection Agency

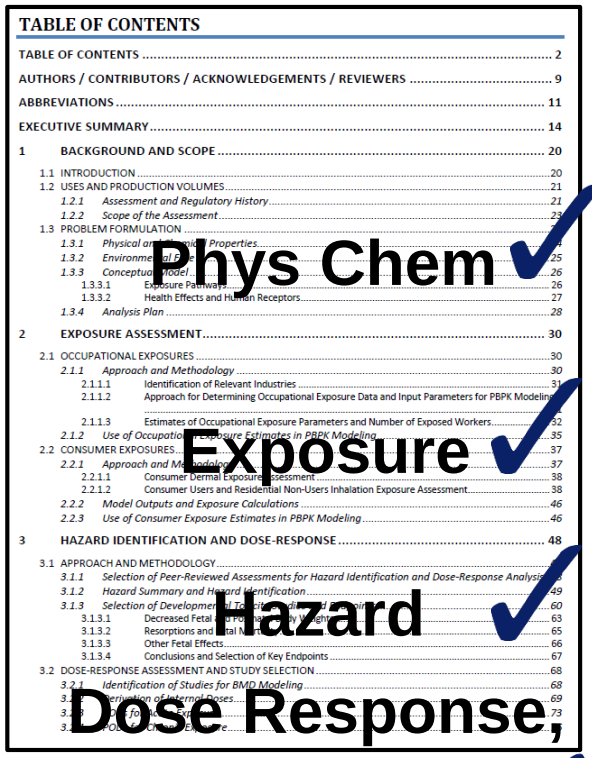
EPA Document# 740-R1-5002
March 2015
Office of Chemical Safety and Pollution Prevention

TSCA Work Plan Chemical Risk Assessment

N-Methylpyrrolidone: Paint Stripper Use

CASRN: 872-50-4

March 2015



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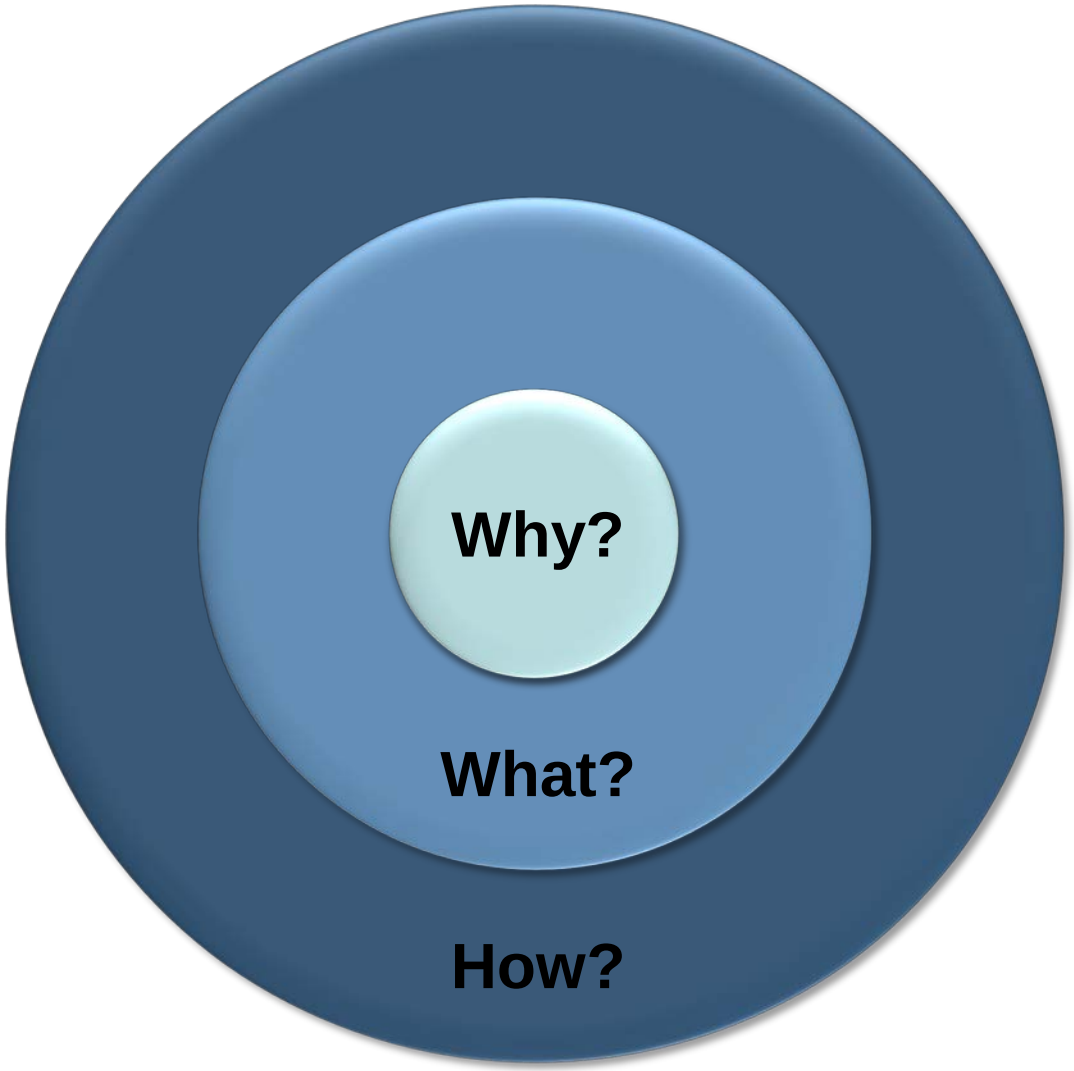
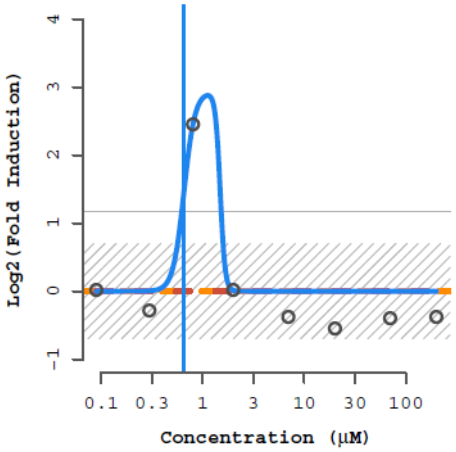
Risk Summary ✓

Uncertainty ✓

‘Golden Circle’ of 21st Century Risk Assessment



Regulatory Applications Require More Focus on Quality and Transparency

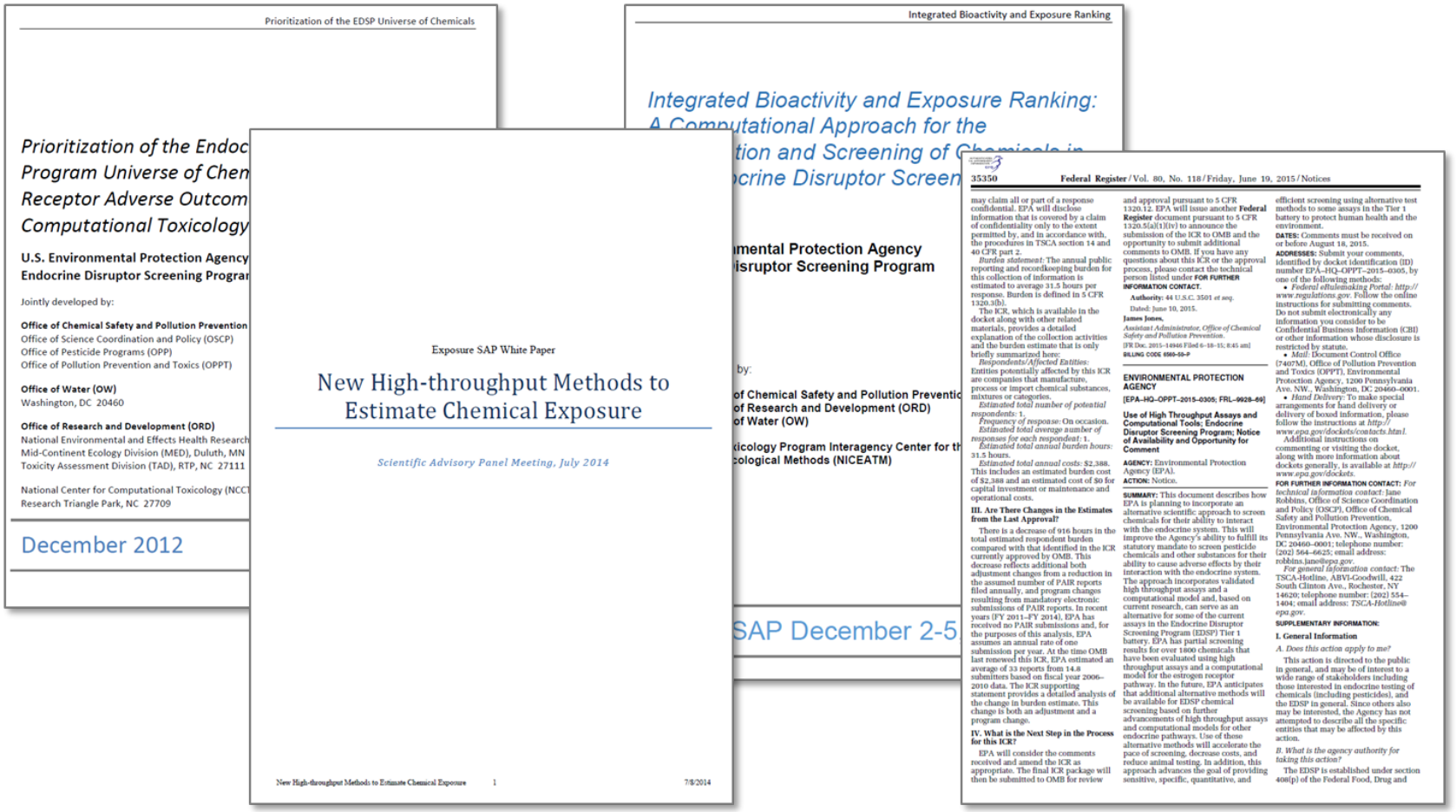



```
ASSAY:    AEID117 (ATG_ERa_TRANS)

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
```

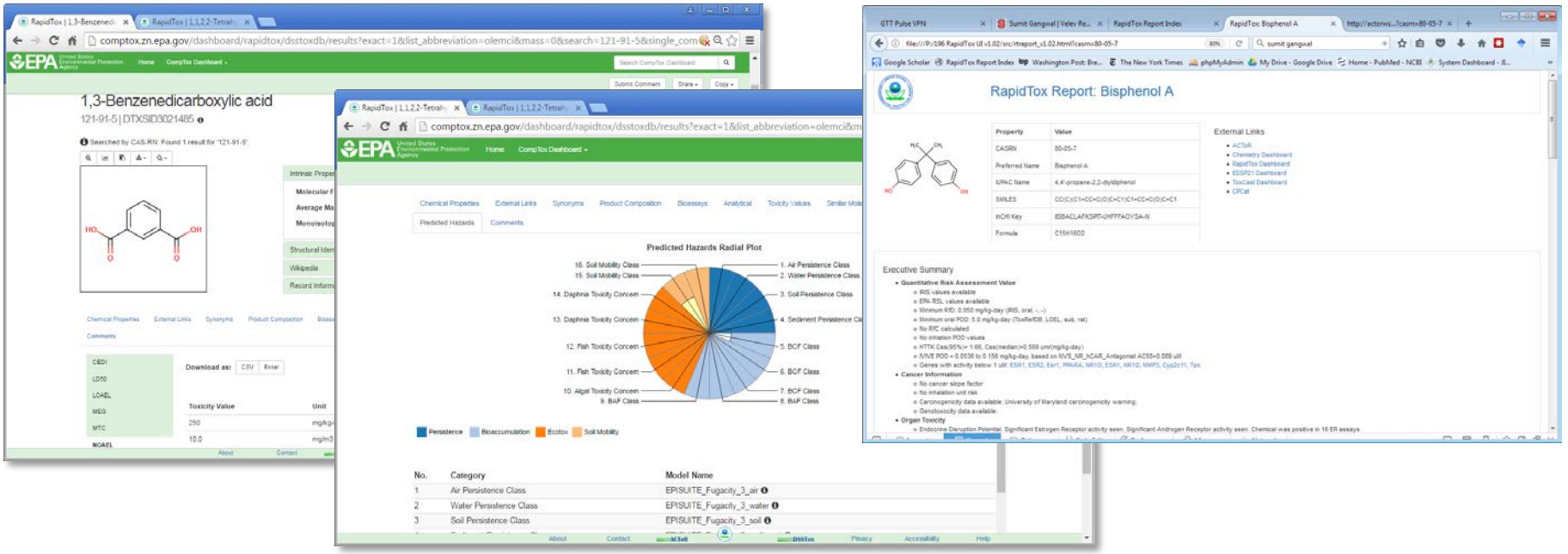
FLAGS:
Only one conc above baseline, active
Borderline active

- 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



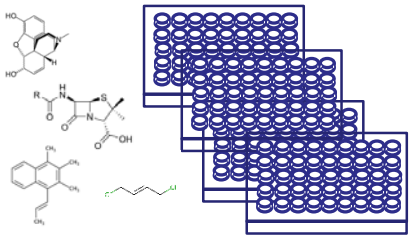
Beginning Application to Quantitative Risk Assessment Through New 'RapidTox' Dashboard

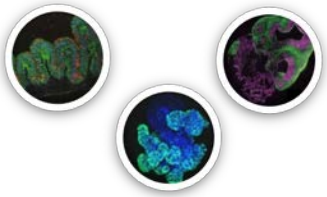


- Semi-automated decision support tool with dashboard interface for high-throughput risk assessments
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure
- Transparent and interactive enough to enable expert users to review the assumptions made and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

Incorporating HT Toxicogenomics to Address This Challenge

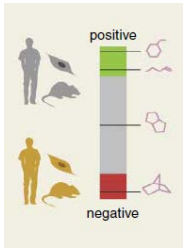
Thousands of Chemicals



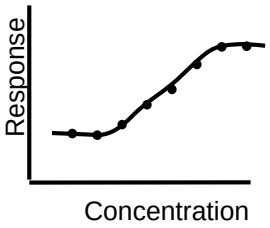


Multiple Cell Types

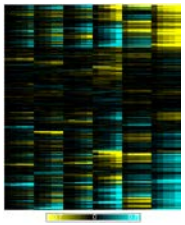
High-Throughput Toxicogenomics



Mode-of-Action



Dose Response



Grouping and Read Across