Developing Data and Computational Models to Enhance Risk Decisions

An Update on ToxCast and ExpoCast

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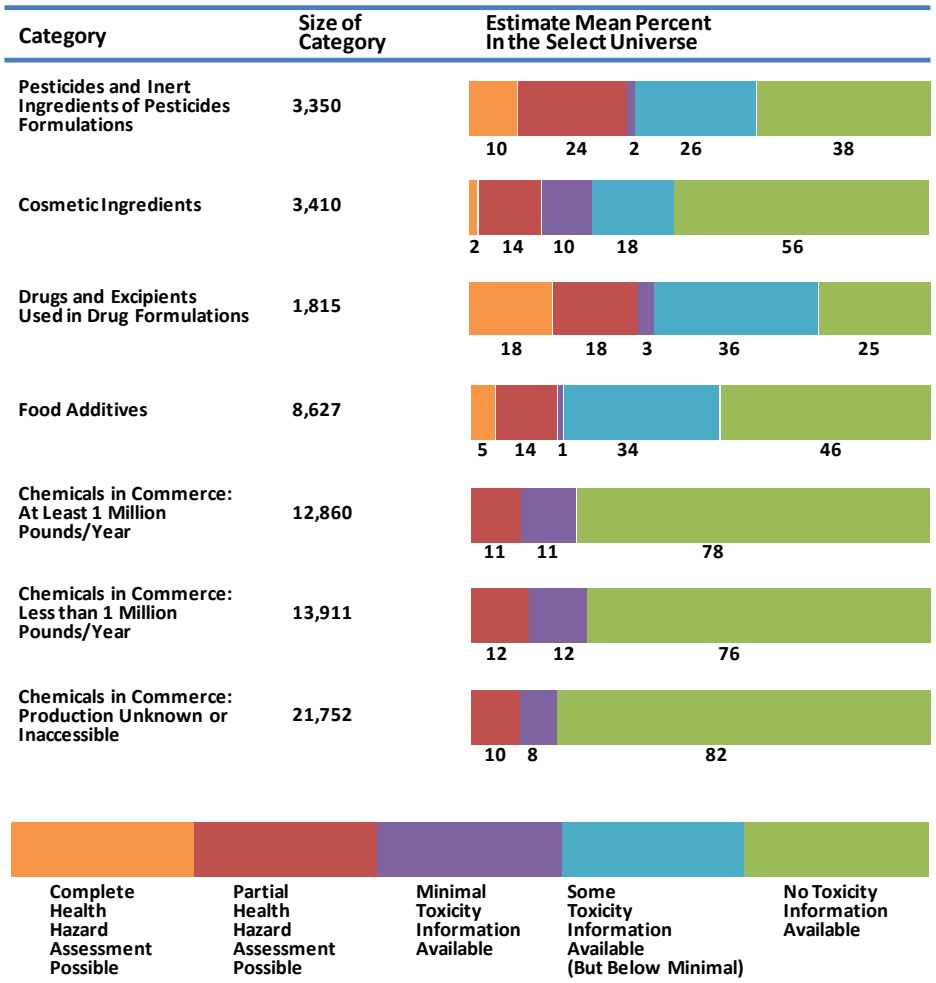
Outline

- **The Problem**
  - There are thousands and thousands of chemcials that have never been tested for hazardous effects
- **Addressing the Problem**
  - Part 1 – Chemicals
  - Part 2 – ToxCast & Tox21 - Hazard estimates
    - Developing data – high-throughput in vitro and QSAR
    - Data interpretation - Consensus model development
  - Part 3 – ExpoCast
    - Dosimetry – estimating daily dose
    - High-throughput exposure predictions
- **Putting it all together**
  - Cost efficient and rapid prioritization

Risk Assessment and the Chemical Universe
A Long-Term Problem

- 1974 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



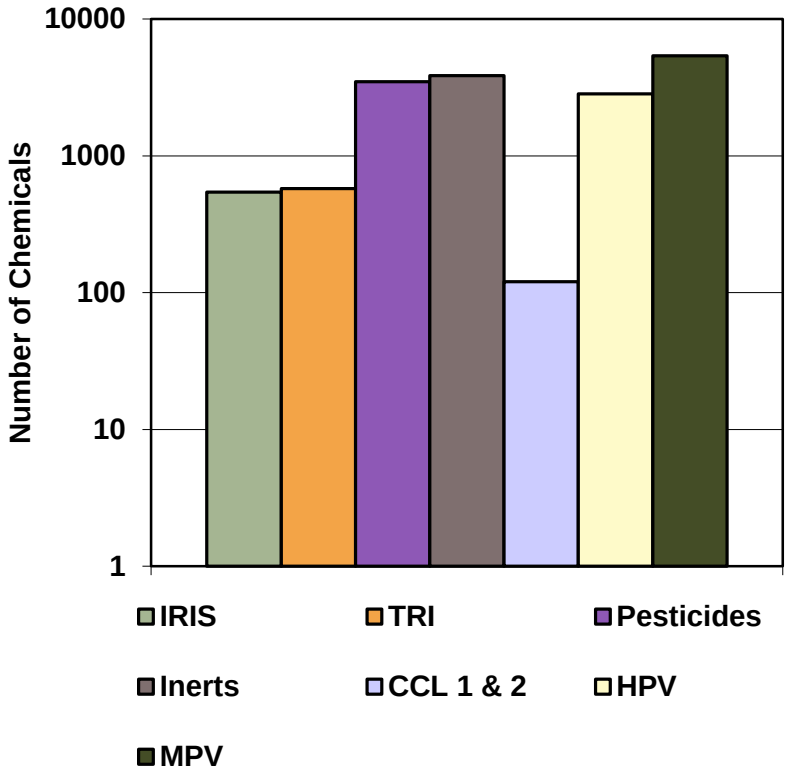
60,000 Chemicals
Black dot = no data, Red dot = data*

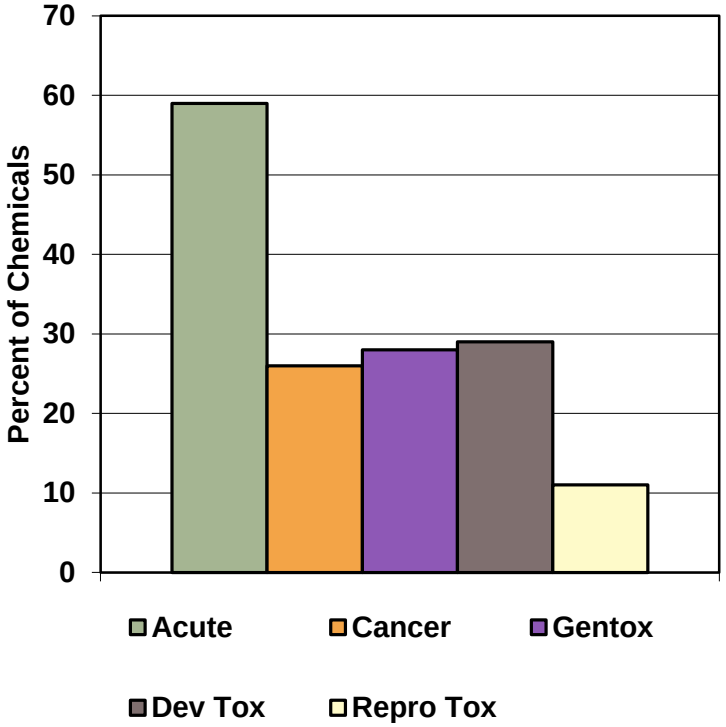
* ~5-6% with adequate info for hazard assessment

Risk Assessment and the Chemical Universe

- Since 1984 some progress has been made
- Refined chemical universe
  - TSCA Inventory = 75,000
  - REACH Inventory = 150,000
  - US & Canadian estimates of about 30 thousand substances in active commercial use
- Accelerated chemical testing
  - ToxCast program
  - US Tox21 testing program

High Priority Chemical Universe and Available Data 2010 Update





Judson, et al *EHP* (2010)

Part 1

Hazard Predictions for Prioritization

Chemicals

Two critical aspects to High-throughput bioactivity screening

1. Must have a highly curated chemical structure library
   - DSSTOX –chemicals database
     - 750k chemicals with CAS numbers
     - contains over structures for about 70%

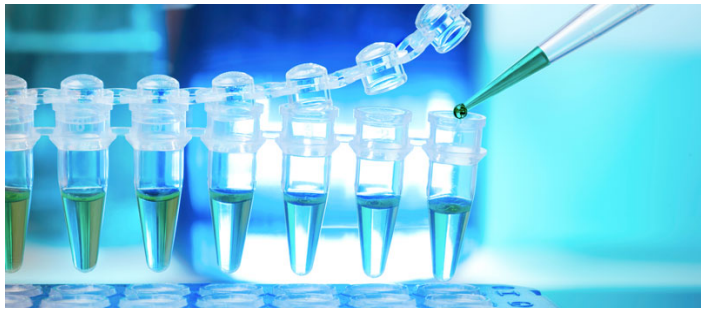
2. Chemical Repository
   - Developed a chemical repository for about 4500 chemicals
   - Includes QA and QC metrics (e.g., analytical chemistry)
   - Allows for platting and shipping of 96 and 384 well-plates for testing

- Information Sources
  - DSSTOX http://www.epa.gov/ncct/dsstox
  - Chemical Library – White paper on chemicals management
    http://epa.gov/ncct/toxcast/files/ToxCast%20Chemicals/ToxCast_Chemicals_QA_QC_Management_%20141204.pdf

Part 2

Hazard Predictions for Prioritization

ToxCast and Tox21

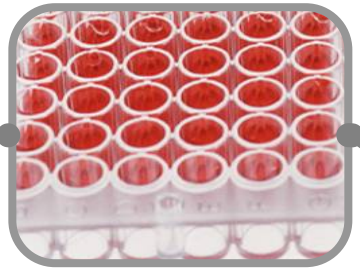


- ToxCast – EPA program
  - Multi-year research program started in 2007
  - Use automated in vitro chemical screening technologies to expose living cells or isolated proteins to chemicals where changes in biological activity may suggest potential toxic effects
  - Chemical library
    - ~3000 environmentally relevant chemicals

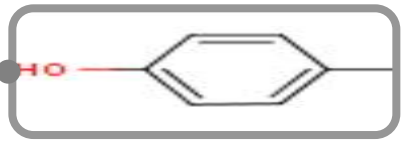
      http://www.epa.gov/ncct/toxcast/
- Tox21 – Collaborative effort of US EPA, National Institutes of Health and Food and Drug Administration
  - aimed at developing better toxicity assessment methods using HTS.
  - Chemical library
    - ~10,000 chemicals, including environmental chemicals, food additives and pharmaceuticals http://www.ncats.nih.gov/research/reengineering/tox21/tox21.html

High-Throughput Screening (HTS)

Robots

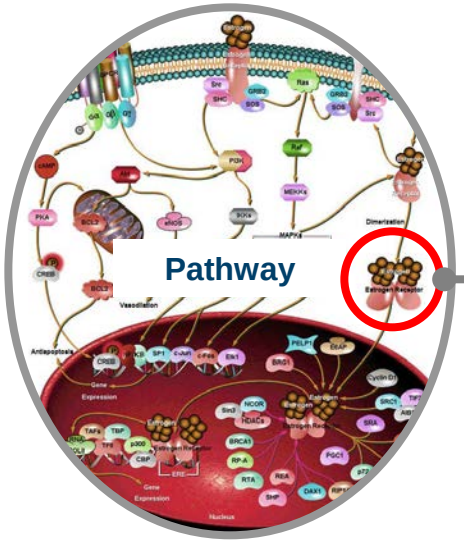


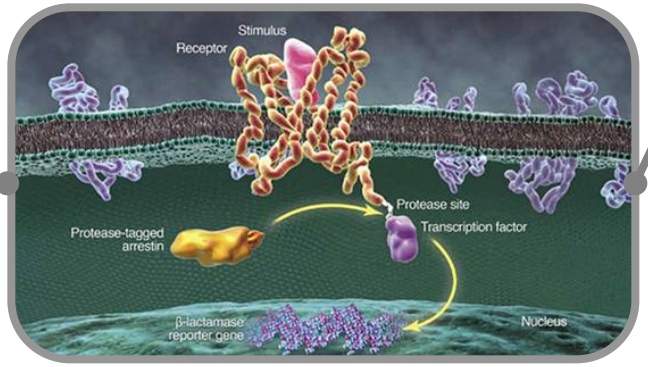
96-, 384-, 1536 Well Plates



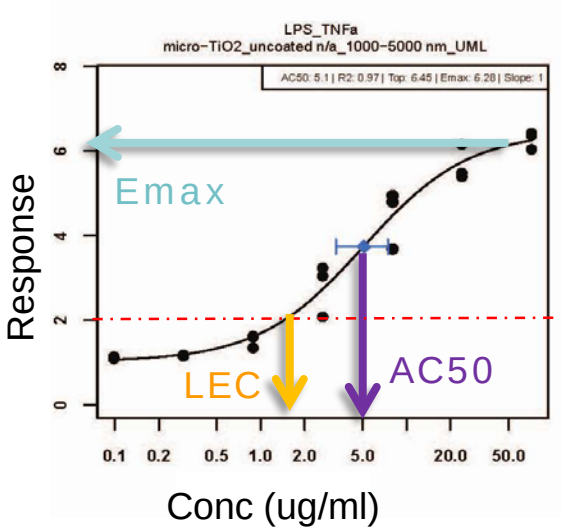
Chemical Exposure

Cell Population

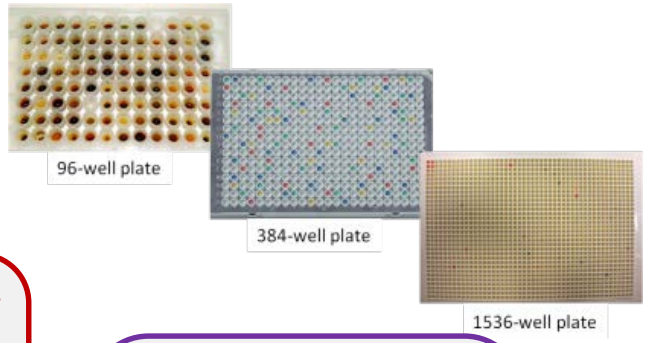




Target Biology (e.g., Estrogen Receptor)



ToxCast In Vitro Assays (>700 endpoints)



Assay Provider
ACEA
Apredica
Attagene
BioReliance
BioSeek
CeeTox
CellzDirect
Tox21/NCATS
NHEERL MESC
NHEERL Zebrafish
NovaScreen (Perkin Elmer)
Odyssey Thera
Vala Sciences

Biological Response
cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor binding
receptor activity
steroidogenesis

Target Family
response Element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases
XME metabolism
GPCRs
ion channels

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type
single
multiplexed
multiparametric

Cell Format
cell free
cell lines
primary cells
complex cultures
free embryos

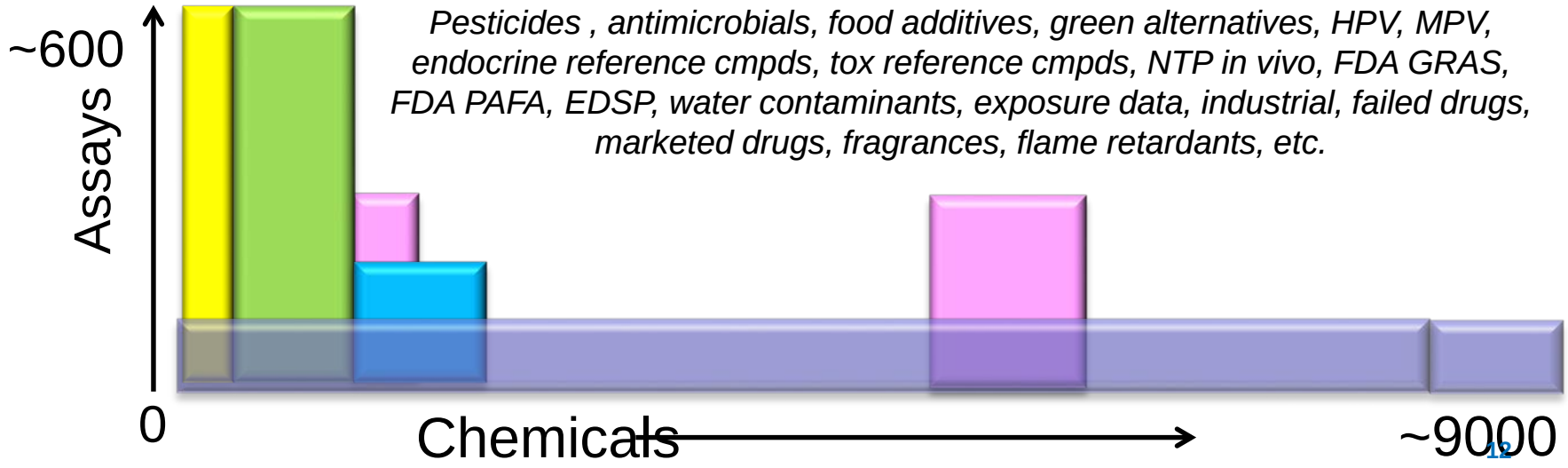
Species
human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source
Lung
Liver
Skin
Cervix
Uterus
Intestinal
Bladder
Pancreas
Inflammatory
Breast
Vascular
Kidney
Testis
Brain
Spleen
Ovary
Prostate
Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

ToxCast & Tox21: Chemicals, Data and Release Timelines

Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~700	2011	Now
ToxCast Phase II	767	~600	~700	03/2013	Now
ToxCast E1K	800	~50	~120	03/2013	Now
Tox21	~9000	~80	~150	Ongoing	Ongoing
ToxCast Phase III	~900	~300	~300	Ongoing	2014-2015



Pesticides , antimicrobials, food additives, green alternatives, HPV, MPV, endocrine reference cmpds, tox reference cmpds, NTP in vivo, FDA GRAS, FDA PAFA, EDSP, water contaminants, exposure data, industrial, failed drugs, marketed drugs, fragrances, flame retardants, etc.

High Throughput In Vitro Test Methods

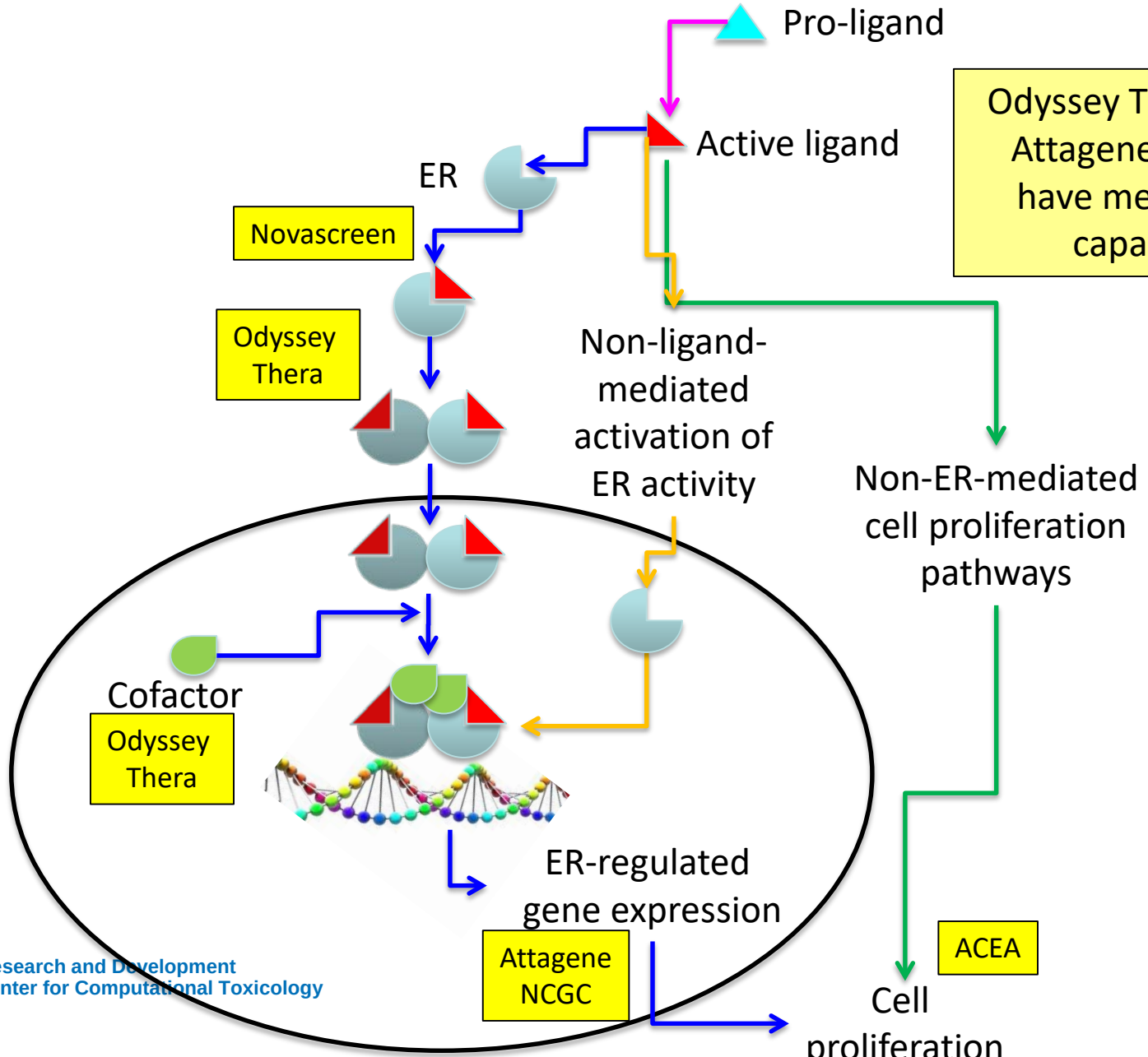
- Half the assays can be part right all of the time,
  And some of the assays can be all right part of the time
  But all the assays can't be all right all of the time.*

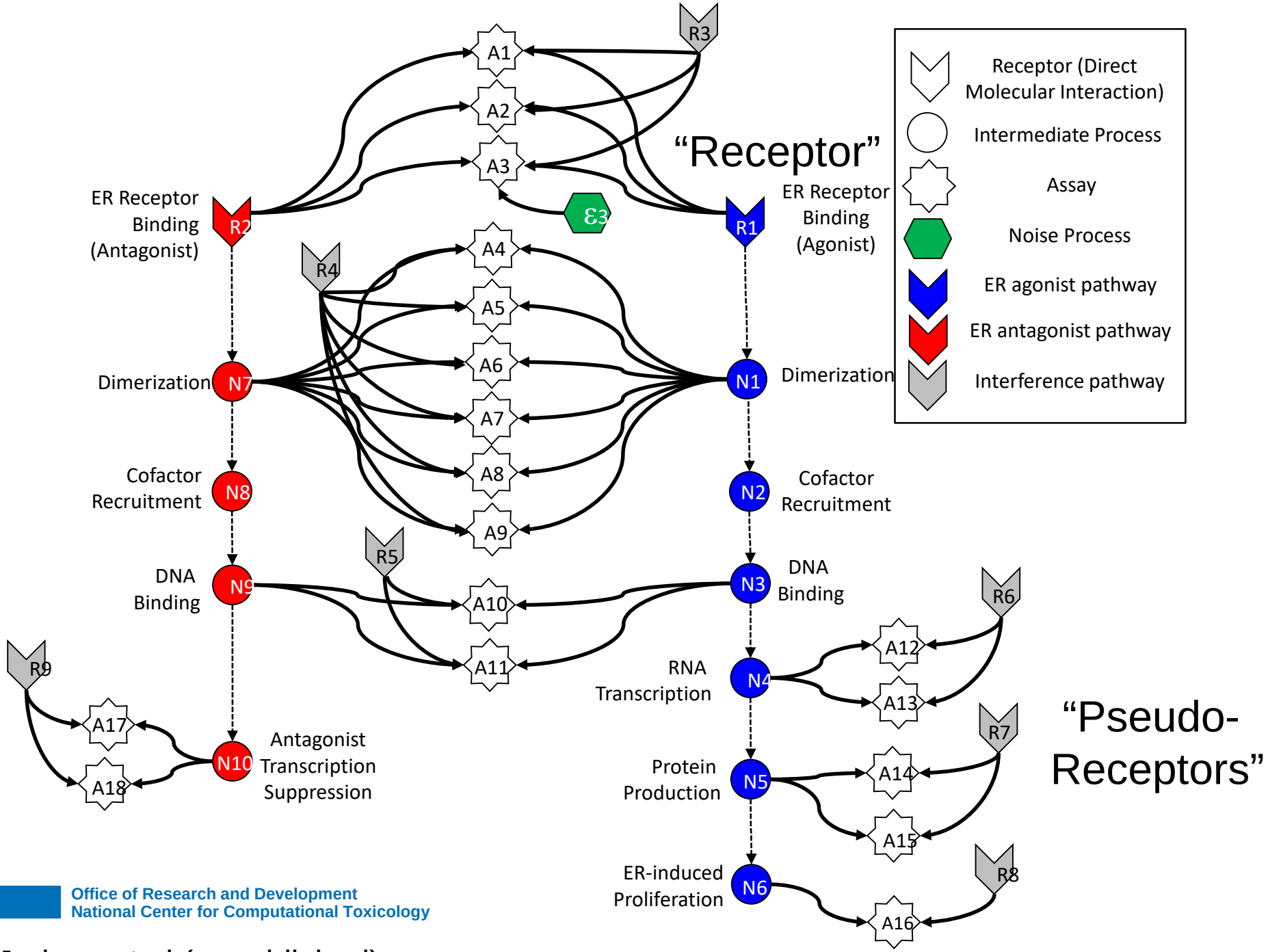
- Example: ToxCast currently has 18 assays that have readouts for different parts of ER signaling pathways

- Idea: Combine these using a pathways approach and develop a probabilistic predictive model based on all of the data, not just one assays

* Apologies to A Lincoln & B Dylan

Using Multiple Lines of Evidence to Predict ER Activity

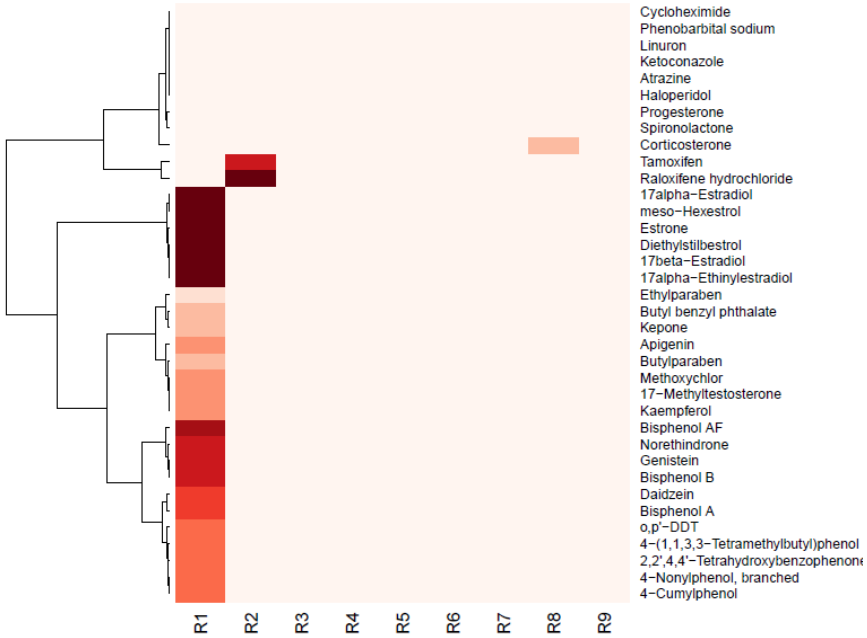




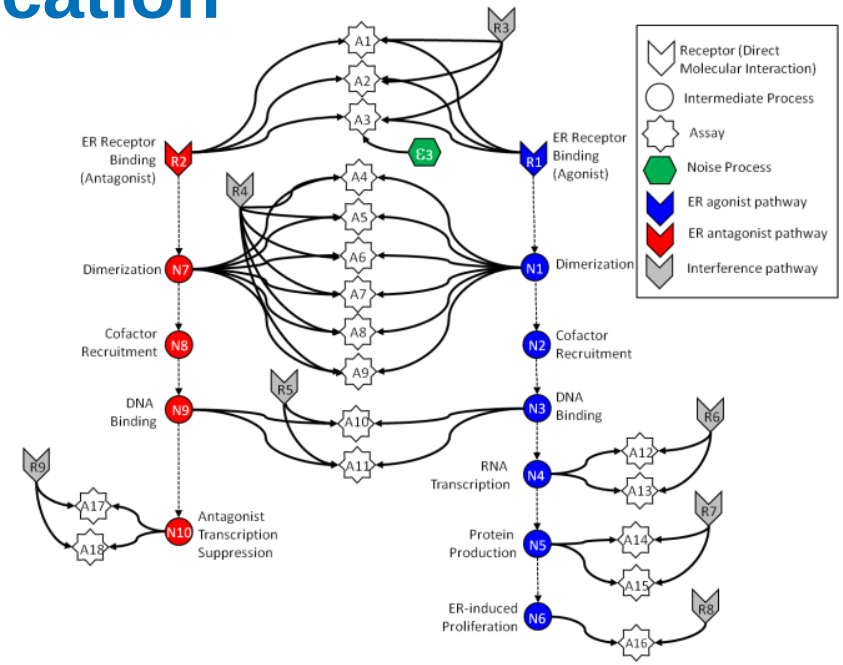
Judson et al (unpublished)

Reference Chemical Classification

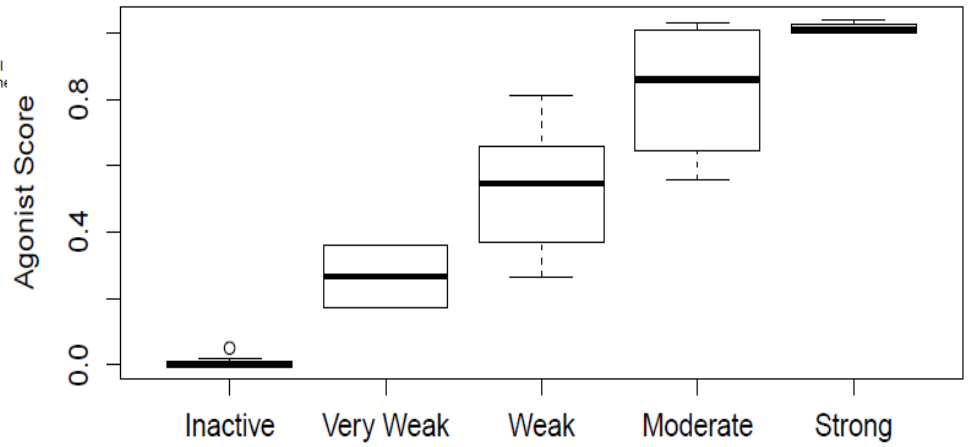
- 36 chemicals reviewed by ORD scientists
  - Inactive vs Active
  - Active –very weak, weak, moderate, or strong



Consensus Model “Receptor’



Model Agonist Score and Expert Calls



In Vivo Activity Class

Demonstrates the ability to predict in vivo outcome

Judson et al (unpublished)

Addition of ER Structure-Based models

- Problem:
  - ToxCast data for only about 1800 chemicals for 18 assays and 8500 chemicals for ER and AR assays
  - ER Expert System covers up to 70-80% of highest-priority 5000 EDSP chemicals

- Augment HTS with Expanded QSAR and docking models.
  - Allows for large universe of chemicals to be evaluated
  - Provides another way to evaluate HTS data

- Cooperative Estrogen Receptor Activity Prediction Project (CERAPP)
  - Collaborate with 10 expert QSAR and docking groups to generate consensus ER predictions for EDSP universe *and beyond*
  - Currently working to develop a curated list of ~30k structures

Part 3

Estimating Exposure Dose From in vitro Experiments

Reverse Toxicokinetics

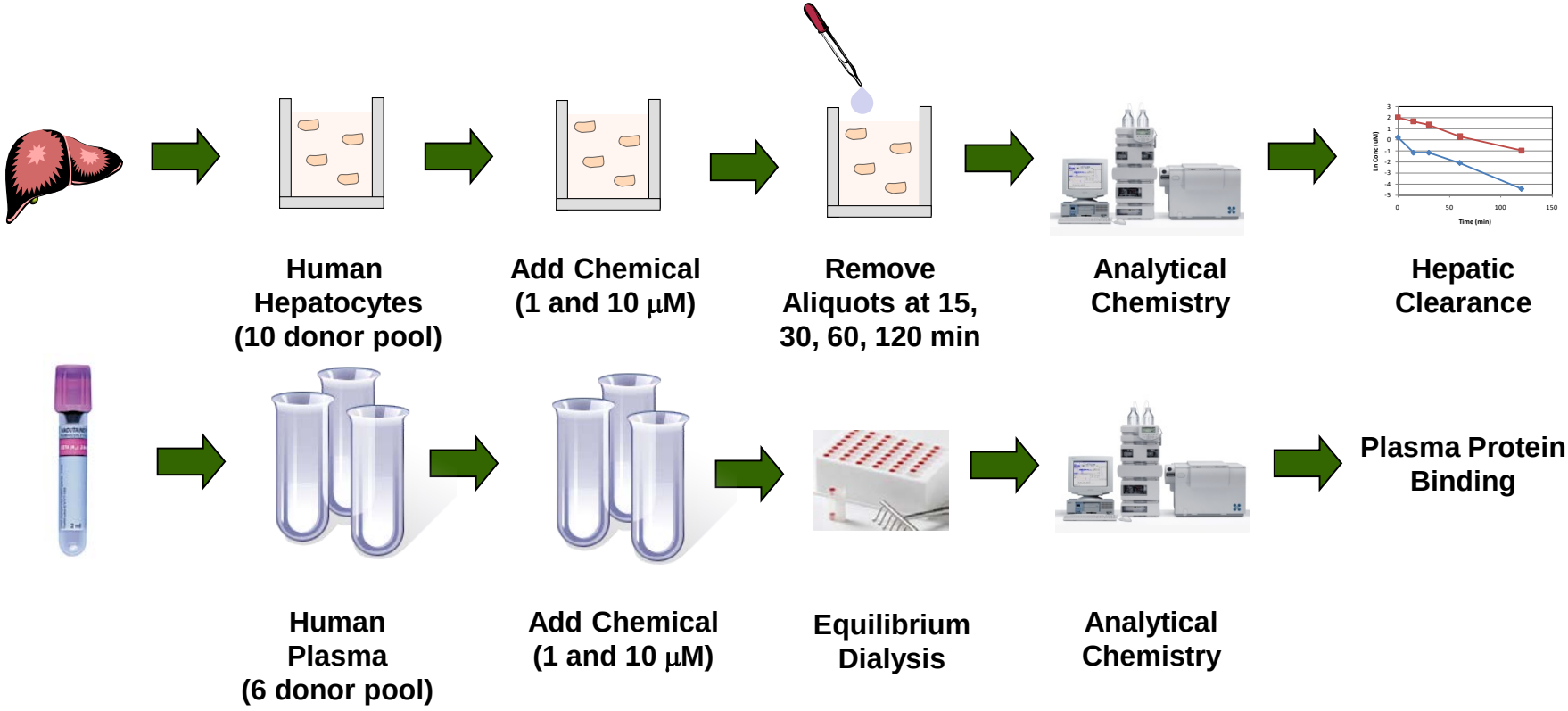
Reverse Toxicokinetics (In Vitro Dosimetry)

- **Problem**: How to estimate daily exposure dose from in vitro media concentration

- Use Reverse Toxicokinetics (RTK)
  - very simple 2 parameter PK models
  - in vitro measurements of disappearance of parent compound and serum binding values

- Provides scaling from concentration in which there is in vitro biological activity to in vivo activity dose (mg/kg/day)

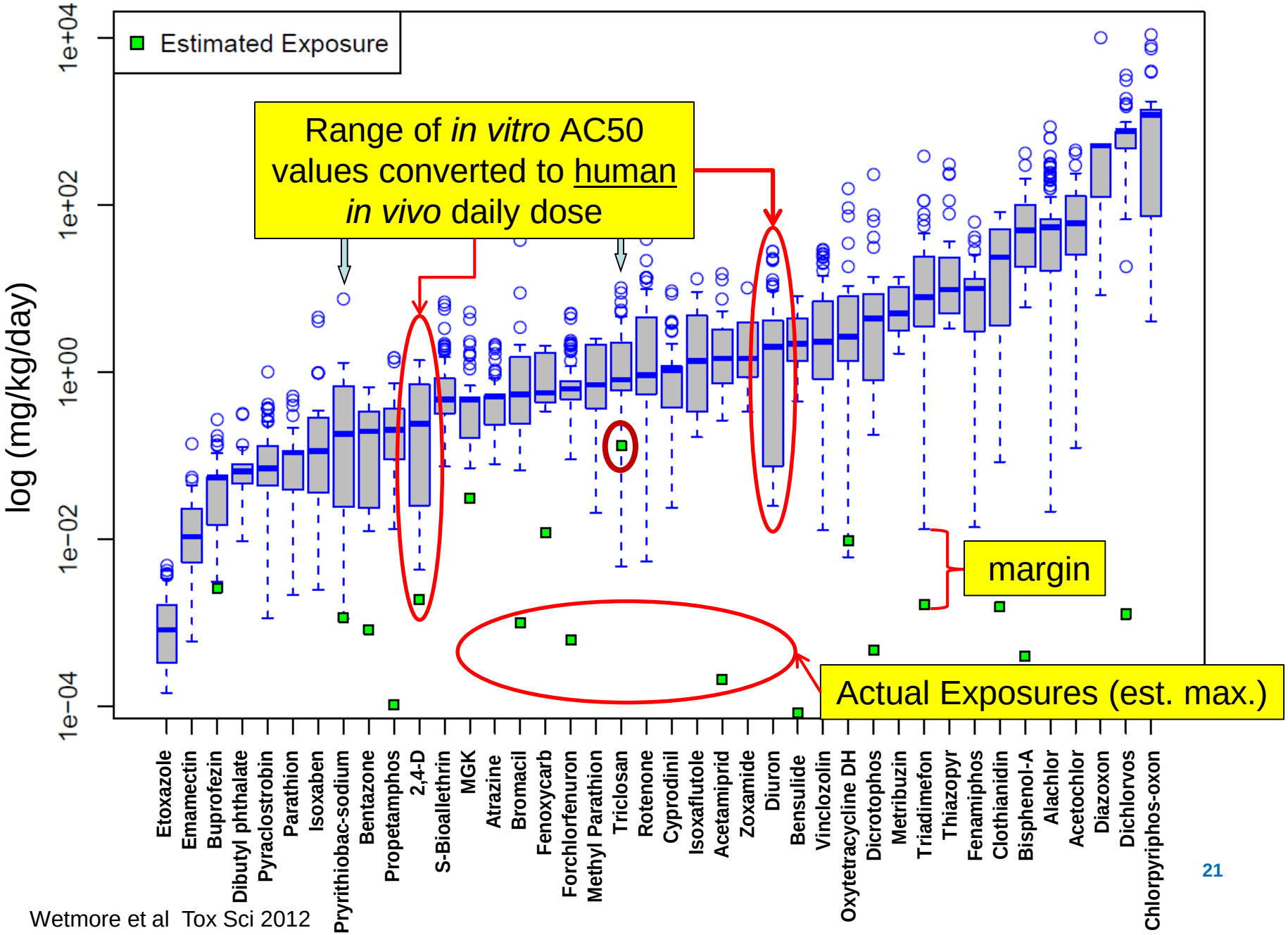
Adding Pharmacokinetics
Reverse ToxicoKinetics (rTK)



- Combine experimental data w/ PK Model to estimate dose / concentration scaling
- RatCast: Same experiment, but with rat hepatocytes and plasma

(Rotroff et al, ToxSci 2010, Wetmore et al, ToxSci 2012)

Combining *in vitro* activity and dosimetry



Wetmore et al Tox Sci 2012

Part 4

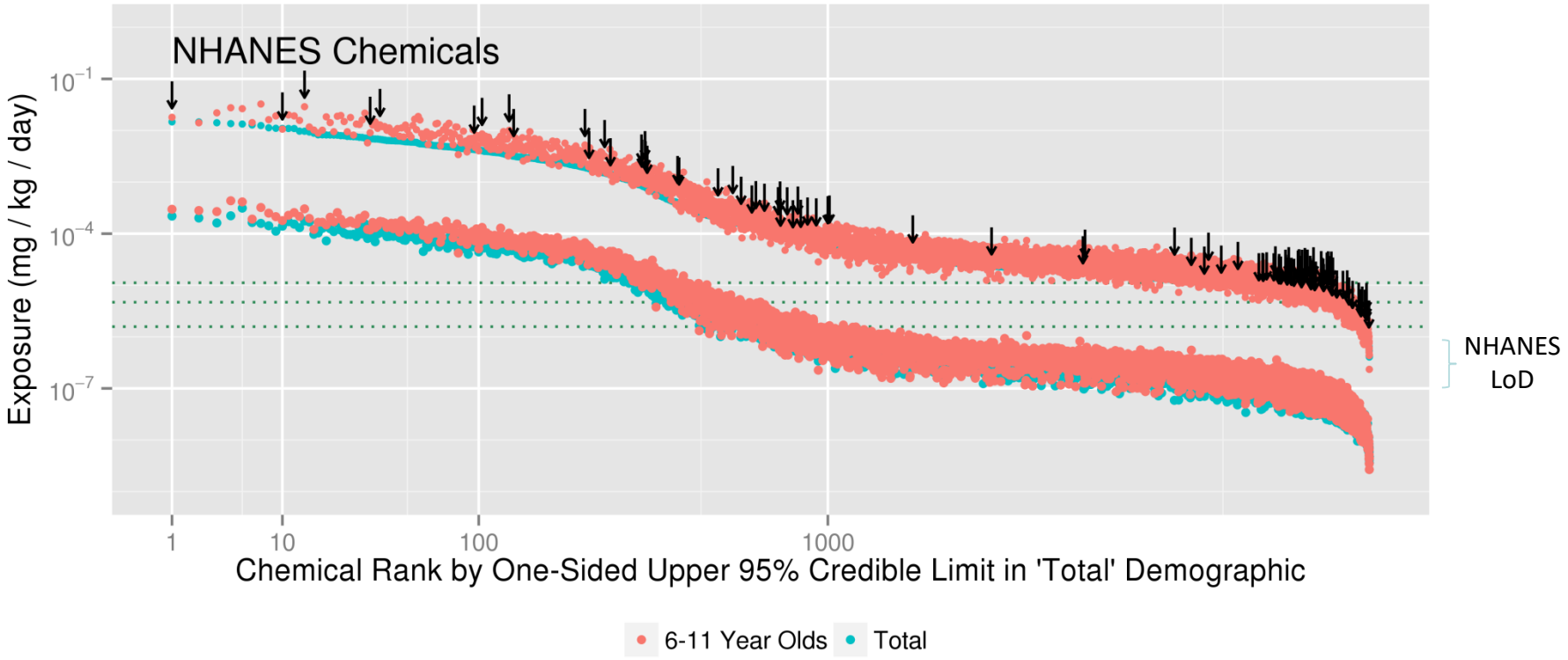
High-Throughput Exposure Predictions

ExpoCast
HTP Exposure Predictions

- **Exposure science lags behind**
  - Most models require extensive information on production, use, fate and transport and rely on empirical data *(no measurement = no exposure?)*

- **ExpoCast**
  - Exposure predictions based on pChem, production values, fate and transport, and product use categories (e.g., industrial, pesticide use, consumer personal care)
  - Industrial vs consumer use
  - Yields exposure estimates and Baysian confidence

Exposure Predictions for 7968 Chemicals & Comparison to NHANES



- NHANES – US National Study – measures exposures in human serum and urine
- Chemicals currently monitored by NHANES are distributed throughput the predictions
- Shows accuracy of the prediction model

Environ. Sci. Technol., **2014**, *48* (21), pp 12760–12767

Putting It All Together
For Rapid Prioritization

Putting It All Together
HT Prioritization

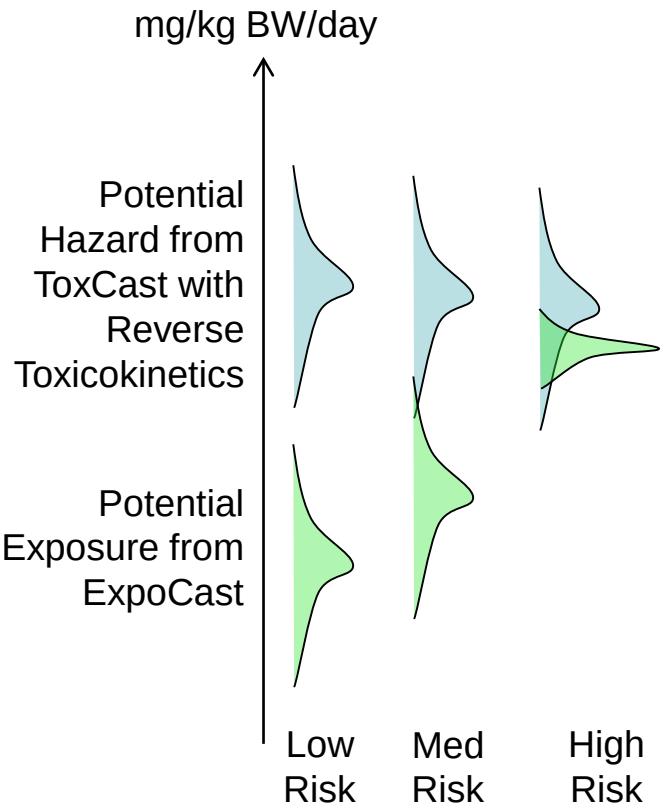
Risk is the product of hazard and exposure

There are thousands of chemicals in commerce, most without enough data for risk evaluation

High throughput *in vitro* methods beginning to bear fruit on potential hazard for many of these chemicals

Methods exist for approximately converting these *in vitro* results to daily doses needed to produce similar levels in a human (IVIVE)

What can we say about exposure with the limited data we have?



Judson *et al.*, (2011)
Chemical Research in Toxicology

Putting It All Together
HT Prioritization

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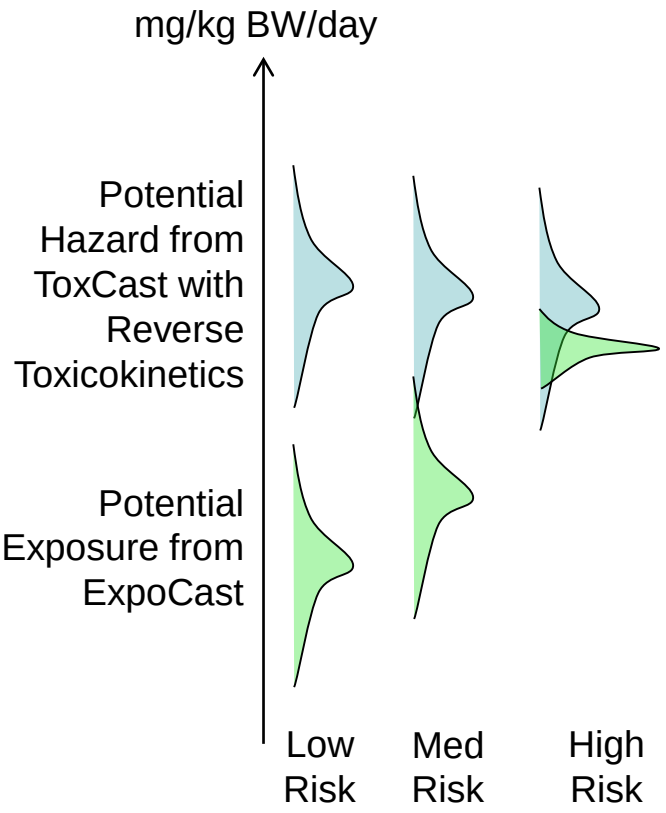
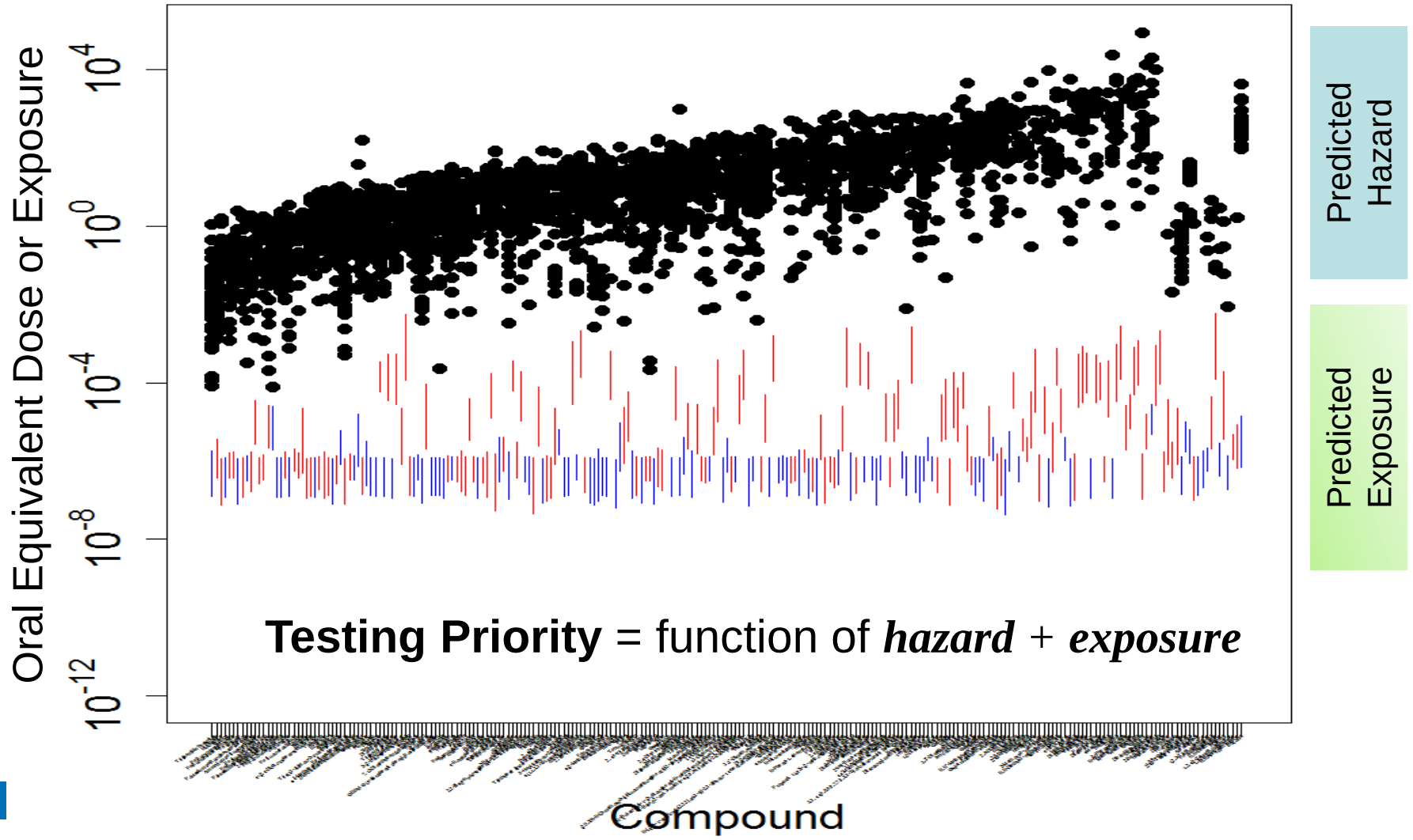


Judson *et al.*, (2011)
Chemical Research in Toxicology

Combining 2nd Generation ExpoCast Exposure Preditions with Predicted Hazard



Combining Bioactivity and Exposure For Estrogen Active Chemicals

Higher Priority for Further Testing

ToxCast Bioactivity Converted to mg/kg/day with HTTK (Wetmore et al., 2012)

ExpoCast Exposure Predictions (Wambaugh *et al.*, 2014)

ToxCast Chemicals

Prioritization = test the chemicals that might be the worst, first!

60,000 Chemicals
Black dot = no data, Red dot = data*

Progress!

- ToxCast Tox21 and ExpoCast have produced bioactivity and exposure estimates for ~8500 chemicals
- Currently being used for prioritization of endocrine disrupting chemicals

Some of the Reactions We Get

- You don’t include metabolism in you in vitro assays
- 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)
- You can’t possibly do RTK modeling with only 2 parameters!
- You can’t do HT Exposure predictions based on simple use models!

- If not this, then what? What other methods could we use for prioritization of thousands of chemicals?

Alternative Approaches to Solving the Problem of How to Prioritize Thousands of Chemicals for Further Testing


Thanks for Listening