Translating HTS Bioassay Results to Risk Estimates

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Direct Potable Reuse in California
Specialty Seminar
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Office of Research and Development
National Center for Computational Toxicology

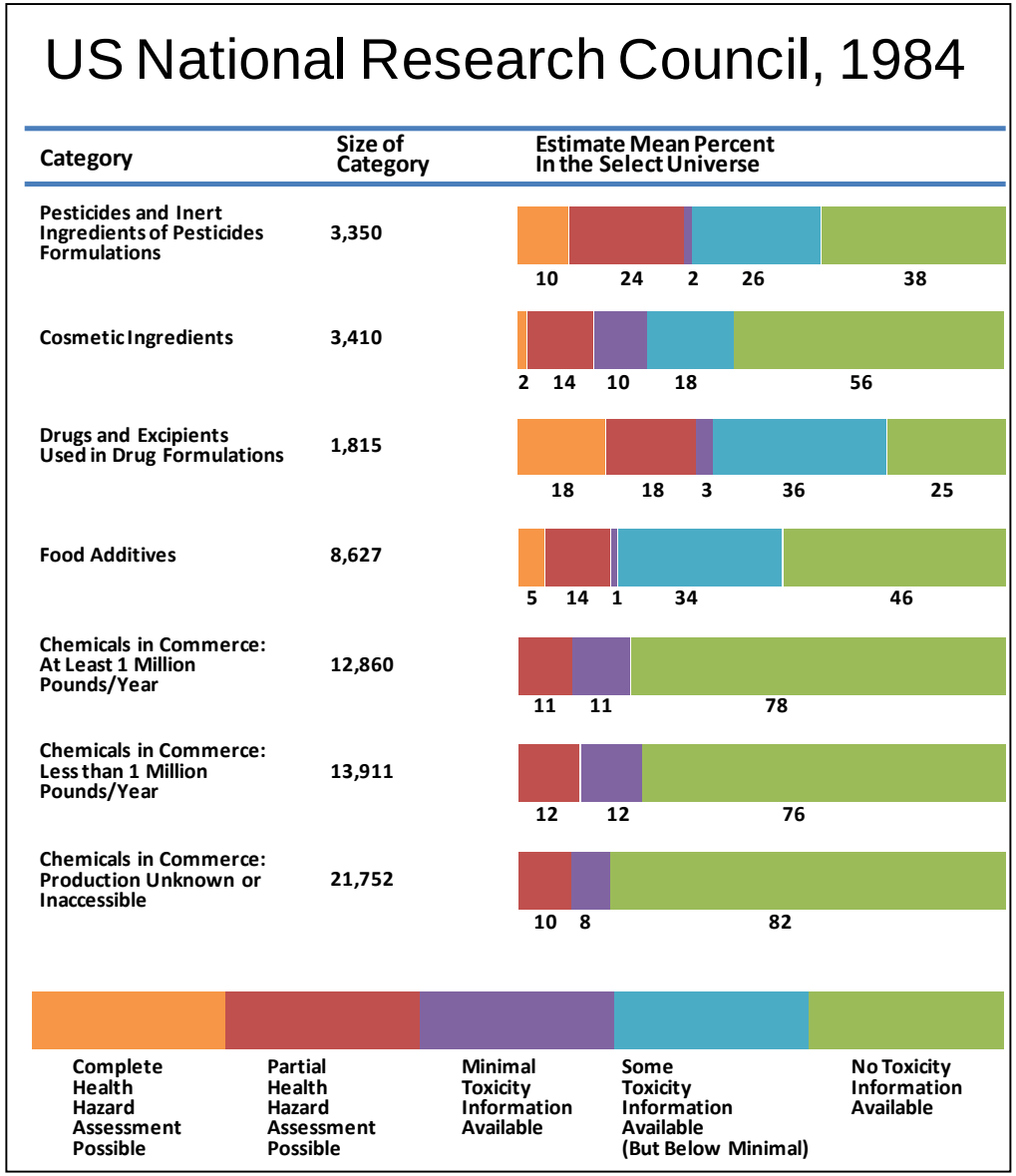
Outline

- **The Problem**
  - Thousands and thousands of chemicals with no hazard info
- **Addressing the Problem**
  - Part 1 – Chemicals – How many? Which ones?
  - Part 2 – ToxCast & Tox21 - Hazard predictions
    - Developing data – high-throughput in vitro and QSAR
    - Data interpretation - Consensus model development
  - Part 3 – ExpoCast
    - Dosimetry – estimating daily dose
    - High-throughput exposure predictions
  - Part 4 – Putting it all together
    - Cost efficient and rapid prioritization
- **Example of ToxCast and Real World Water Samples**
- **Caveats and Uncertainties**

Risk Assessment and the Chemical Universe
A Long-Term Problem

1974 US NRC report

- Major challenge is too many chemicals and not enough data
- Estimated number of chemicals = 65,725
- Number of chemical with no toxicity data of any kind = 46,000



Chemical Universe

- *Since 1984 some progress has been made*

- *Other estimates of the chemical universe*
  - *Chemical Abstract Registry - >100 million*
  - *TSCA Inventory = ~85,000*
  - *REACH Inventory = ~150,000*
  - *US & Canadian estimates of ~30-40k substances in active commercial use*

How to visualize the problem?
60,000 Chemicals
Black dot = no data, Red dot = data*

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

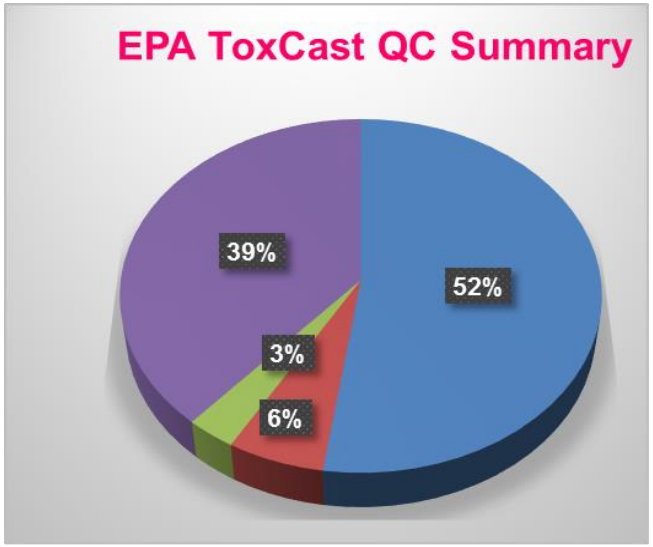
Part 1

Chemical Libraries

Environmental Chemical Libraries

Critical needs for high-throughput bioactivity screening

1. **Must have a highly curated chemical structure library**
   - DSSTOX –chemicals database
     - 150k structure with highly curated structures and CAS numbers
     - ~600k chemicals with CAS numbers, structures for about 70%
2. ToxCast/Tox21 Chemical Repository
   - repository for about 8500 chemicals
   - QA and QC metrics (e.g., analytical chemistry)
   - allows platting and shipping of 96 & 384 well-plates for testing



Pass = 92% Fail = 6%

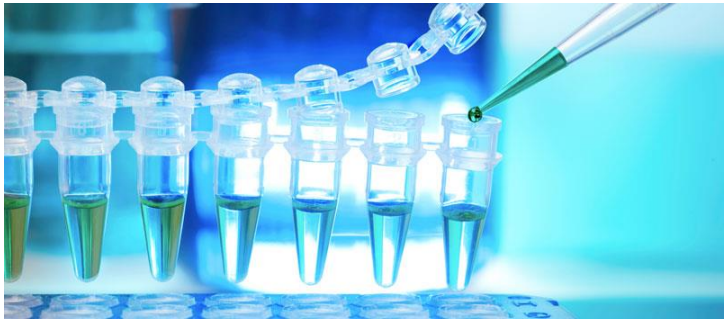
- Information Sources
  - DSSTOX http://www.epa.gov/ncct/dsstox
  - Chemical Library – White paper on chemicals management

http://epa.gov/ncct/toxcast/files/ToxCast%20Chemicals/ToxCast_Chemicals_QA_QC_Management_%20141204.pdf

Part 2

Hazard Predictions for Prioritization

ToxCast and Tox21

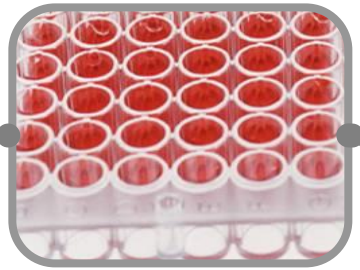


- **ToxCast – EPA program**
  - Multi-year research program started in 2007
  - Use automated in vitro chemical screening technologies to expose living cells or isolated proteins to chemicals where changes in biological activity may suggest potential toxic effects
  - Chemical library
    - ~3500 environmentally relevant chemicals http://www.epa.gov/ncct/toxcast/

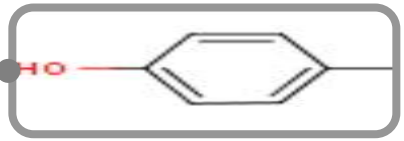
- **Tox21 – Collaborative effort of US EPA, National Institutes of Health and Food and Drug Administration**
  - aimed at developing better toxicity assessment methods using HTS.
  - Chemical library
    - ~10,000 environmental chemicals, food additives and pharmaceuticals http://www.ncats.nih.gov/research/reengineering/tox21/tox21.html

High-Throughput Screening (HTS)

Robots

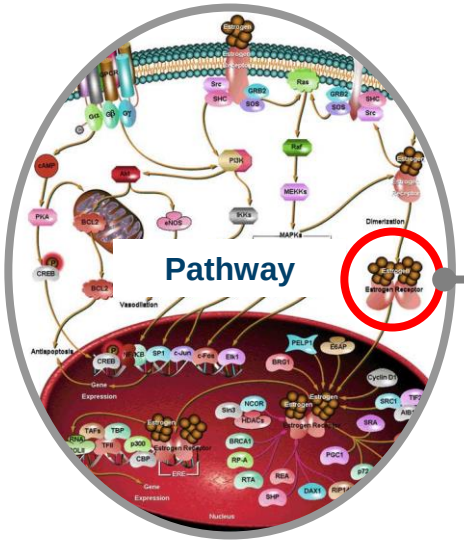


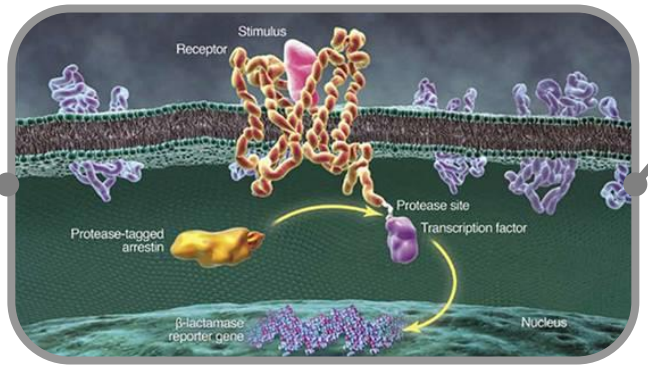
96-, 384-, 1536 Well Plates



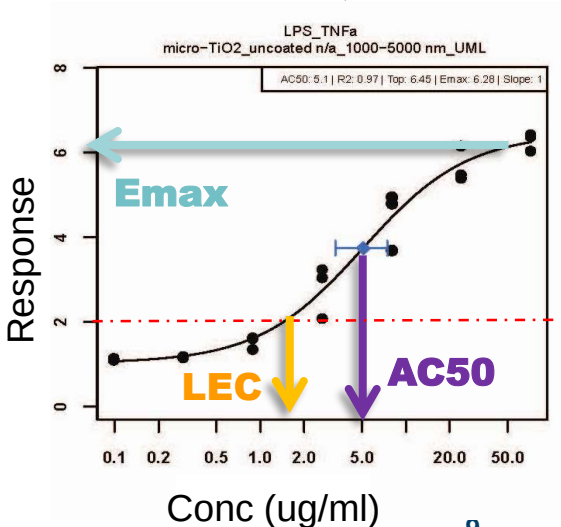
Chemical Exposure

Cell Population

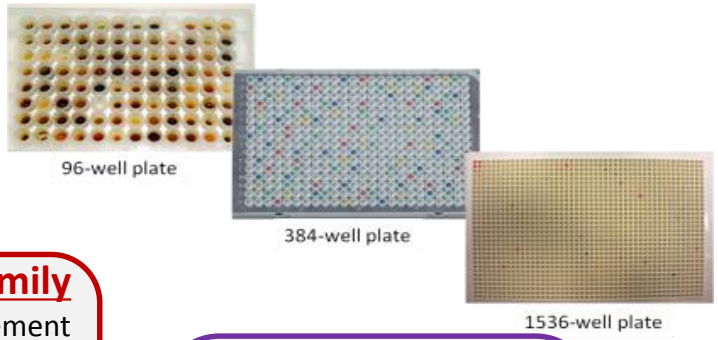




Target Biology (e.g., Estrogen Receptor)



ToxCast In Vitro Assays (>700 assay 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

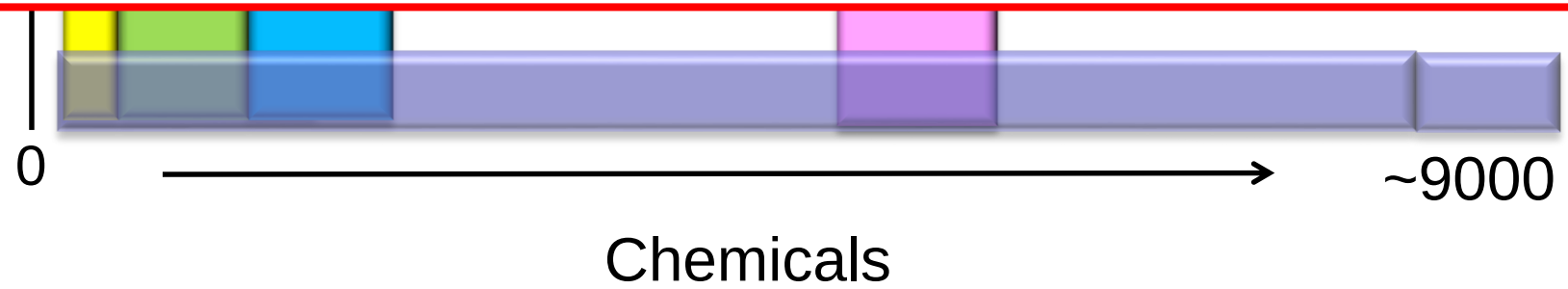
Tissue Source
Lung
Liver
Skin
Cervix
Uterus
Intestinal
Bladder
Pancreas
Inflammatory
Breast
Vascular
Kidney
Testis
Brain
Spleen
Ovary
Prostate
Bone

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

EPA and NCCT policy is to make all data, models, code publically available
http://www.epa.gov/comptox/

New web-based application for easier access
http://actor.epa.gov/dashboard2/



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.*

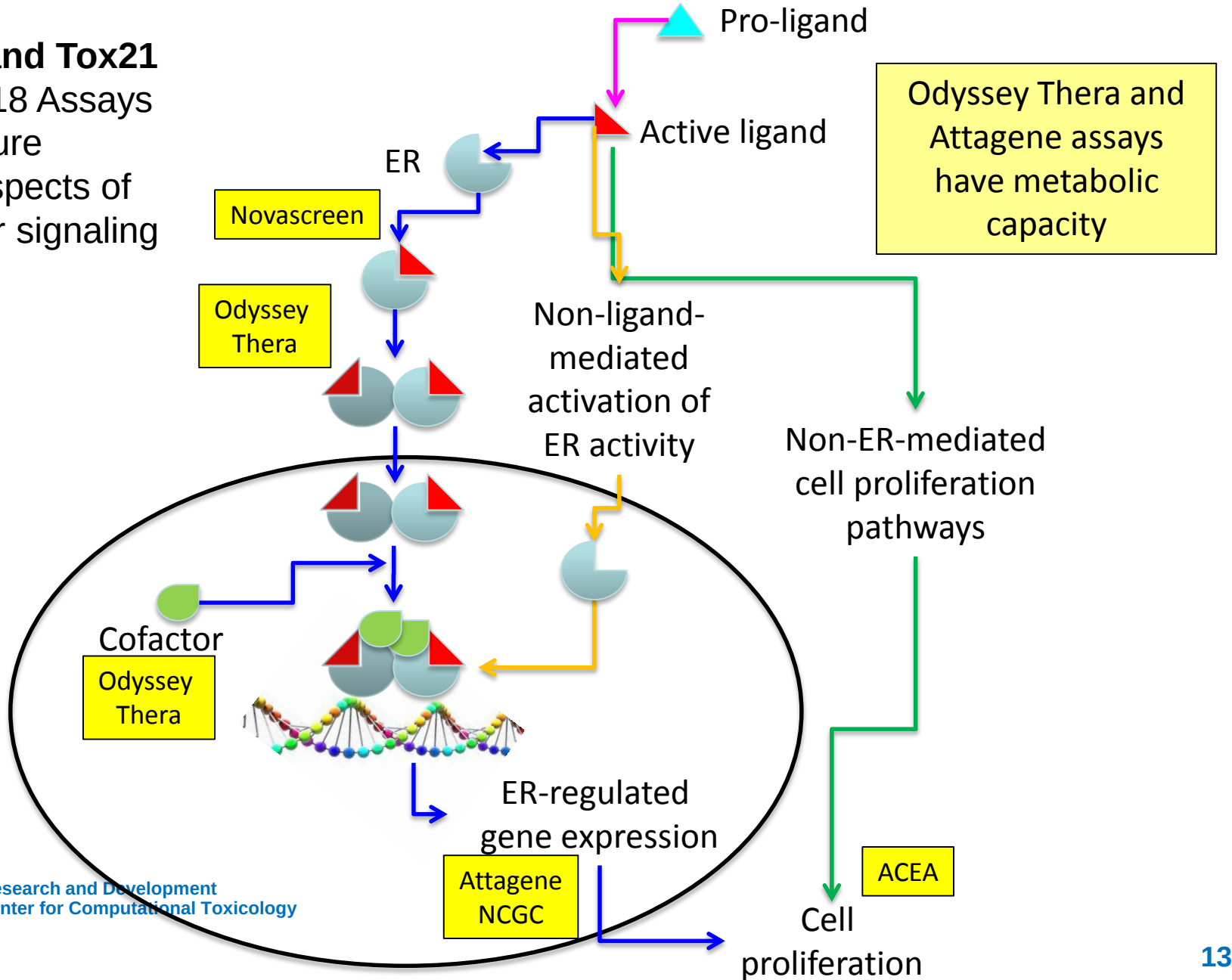
- Example: ToxCast currently has 18 assays that have readouts for different parts of ER signaling pathways

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

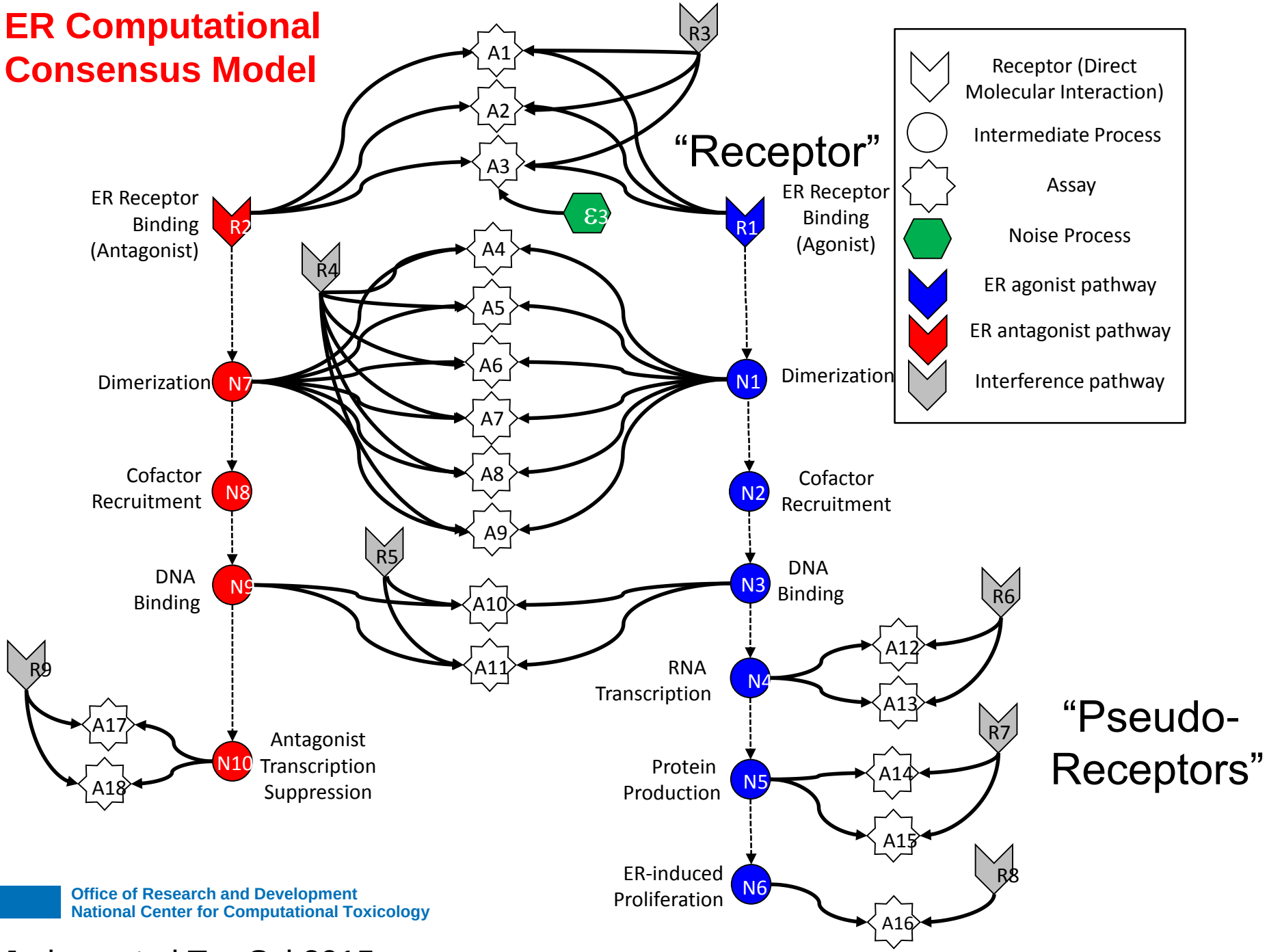
* Apologies to A Lincoln & B Dylan

Using Multiple Lines of Evidence to Predict ER Activity

ToxCast and Tox21
Currently 18 Assays that measure multiple aspects of ER cellular signaling



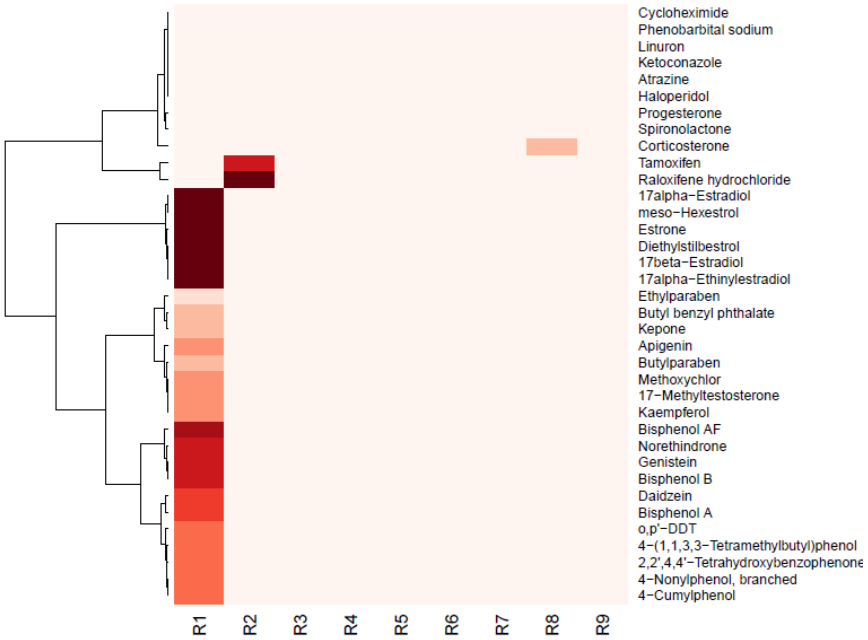
ER Computational Consensus Model



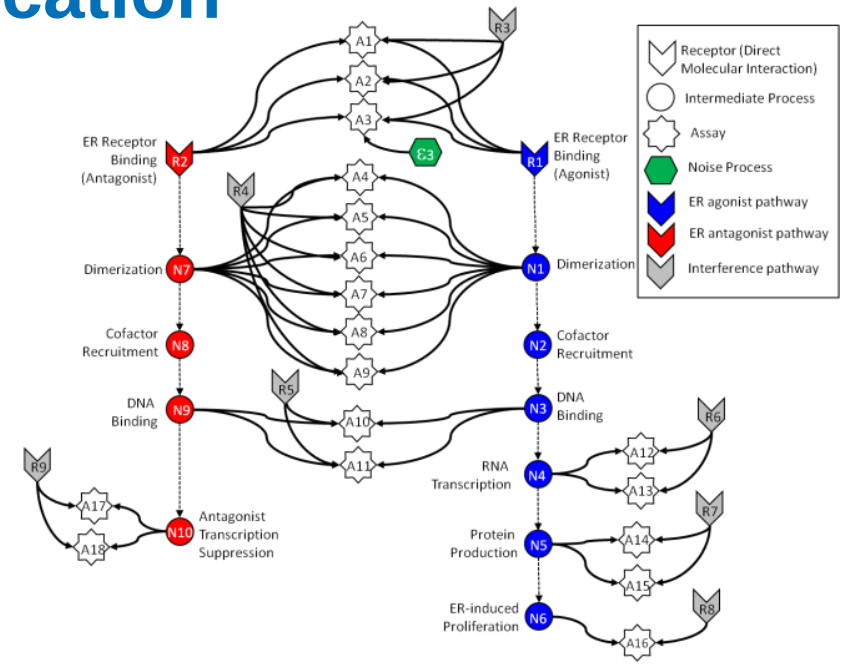
Judson et al Tox Sci 2015

Reference Chemical Classification

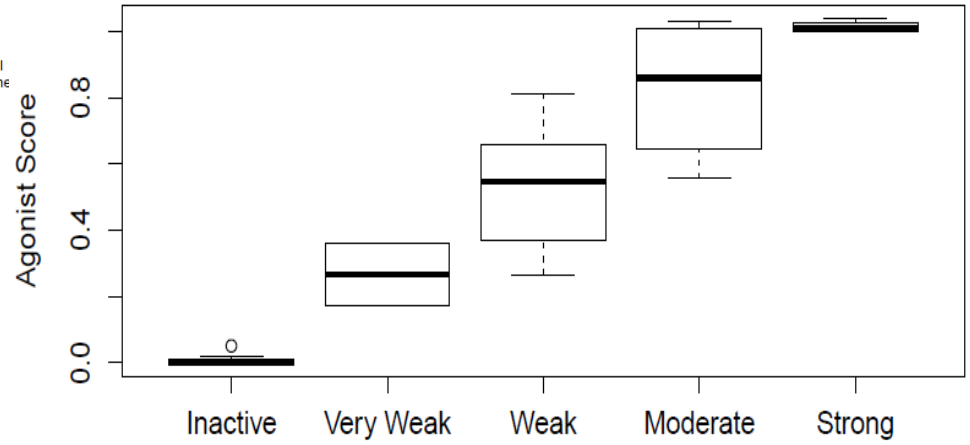
- 36 chemicals reviewed by ORD scientists
  - Inactive vs Active
  - Active –very weak, weak, moderate, or strong



Consensus Model “Receptors’



Model Agonist Score and Expert Calls



In Vivo Activity Class

Demonstrates the ability to predict in vivo outcome (uterotrophic assay)

Judson et al. Tox Sci 2015

Part 3

ExpoCast

Estimating Exposure Dose From in vitro Experiments

Reverse Toxicokinetics

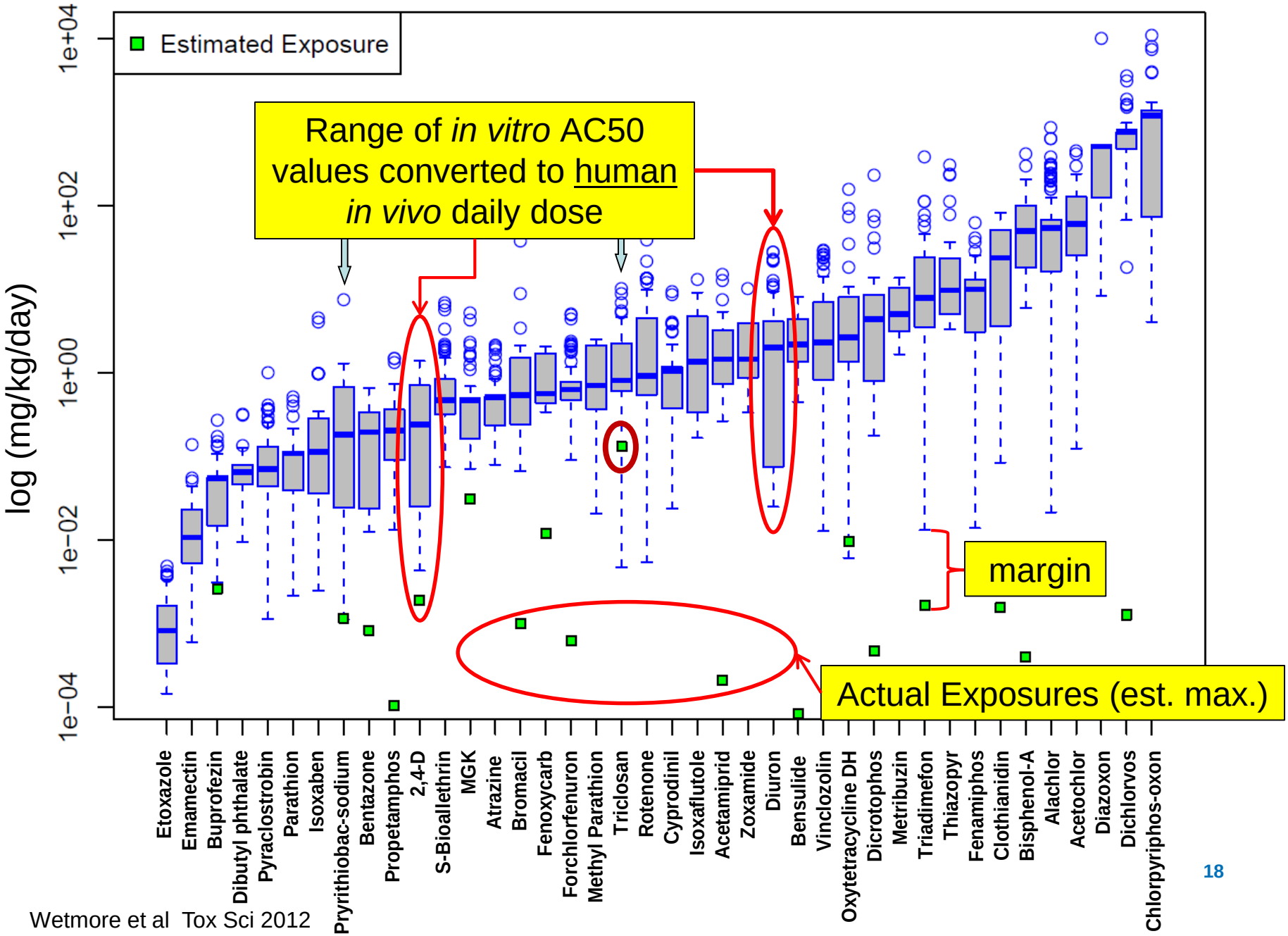
Reverse Toxicokinetics (In Vitro Dosimetry)

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

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

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

Combining *in vitro* activity and dosimetry



Wetmore et al Tox Sci 2012

Part 3

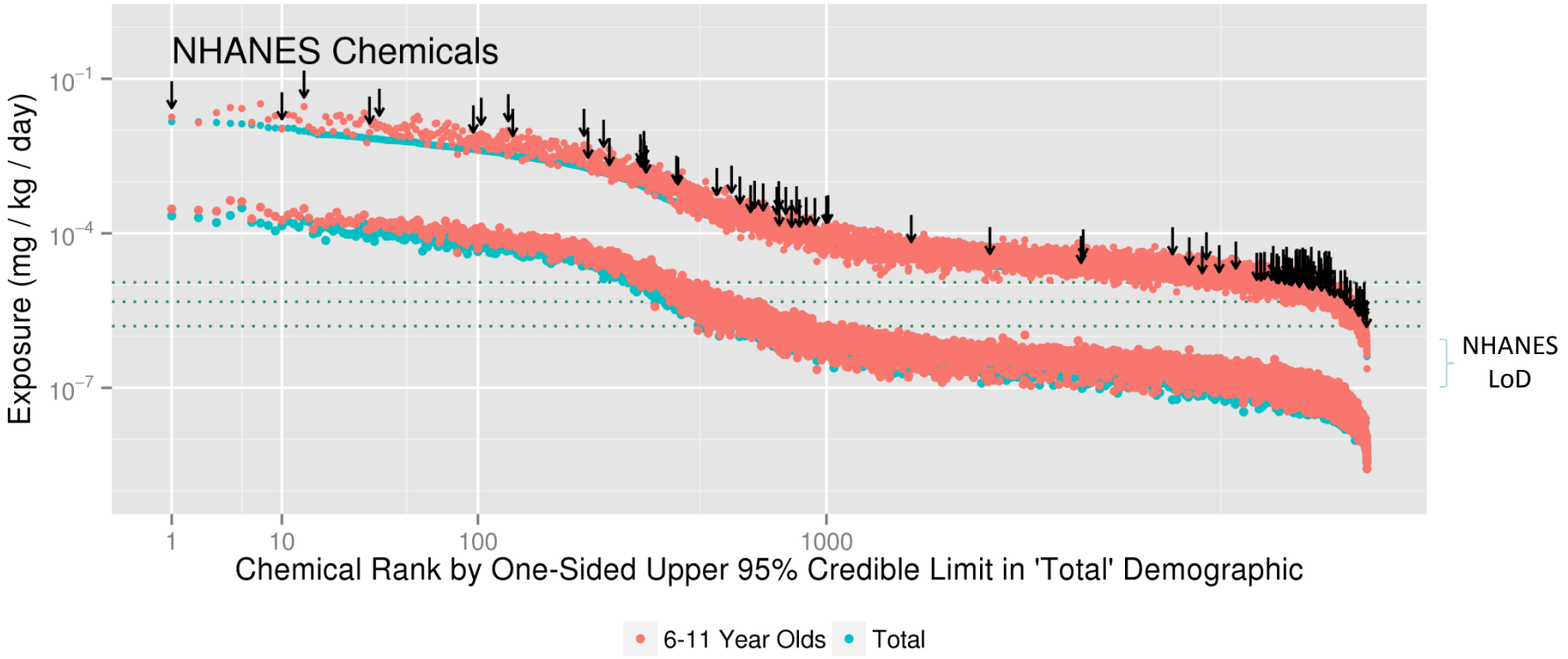
ExpoCast

High-Throughput Exposure Predictions

ExpoCast
HTP Exposure Predictions

- **Current exposure modeling is no the answer**
  - Most models require extensive information on production, use, fate and transport and rely on empirical data *(no measurement = no exposure?)*
- **ExpoCast Expsoure Models**
  - Exposure predictions are based on:
    - pChem properties
    - production values
    - fate and transport
    - product use categories (e.g., industrial, pesticide use, consumer personal care)
  - Yields exposure estimates and Baysian confidence intervals

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

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

Part 4

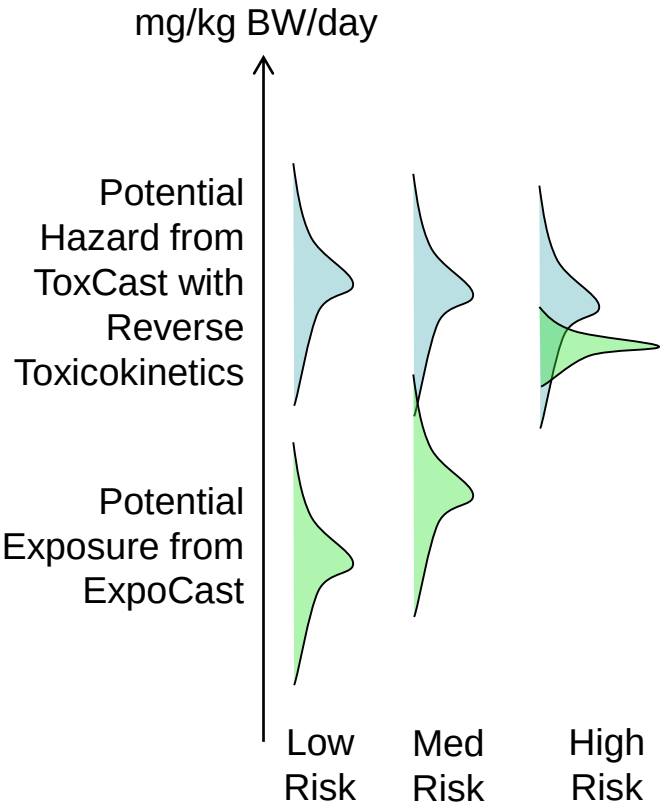
Putting It All Together

For Rapid Prioritization

Putting It All Together
HT Prioritization

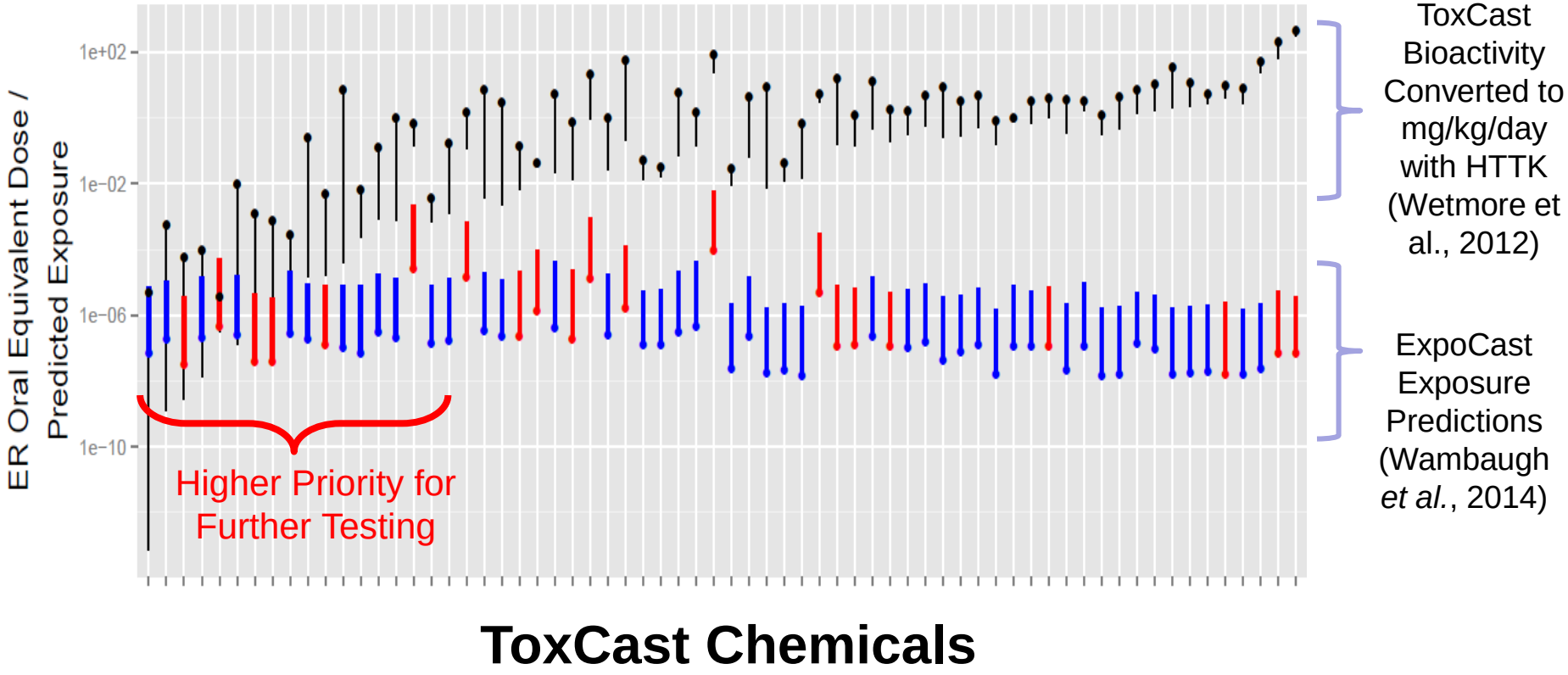
Risk is the product of hazard and exposure

- 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)
- High throughput exposure estimates are not available for thousands of chemicals



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

Combining Bioactivity and Exposure For Estrogen Active Chemicals



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

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

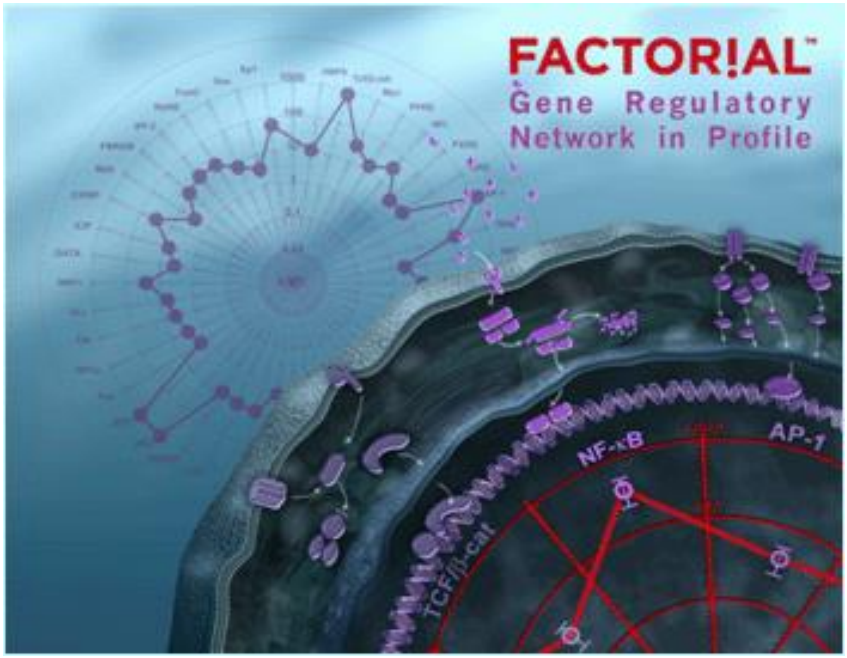
Progress!

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

- Fed Reg 80(118):3530, June 19, 2015
  - *Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment*

Using HTS Assays as 'Biosensors"
Ex: Surface water samples

- Attagene Inc. (Morrisville, NC)
  Assay Battery
- Factorial cellular biosensor system (HepG2 cell line) with multiplexed transcription factor reporter constructs
- Covers most human nuclear receptors
- Tested surface water extracts from St. Louis River, Duluth MN at mulitple locations downstream from paper pulp mill

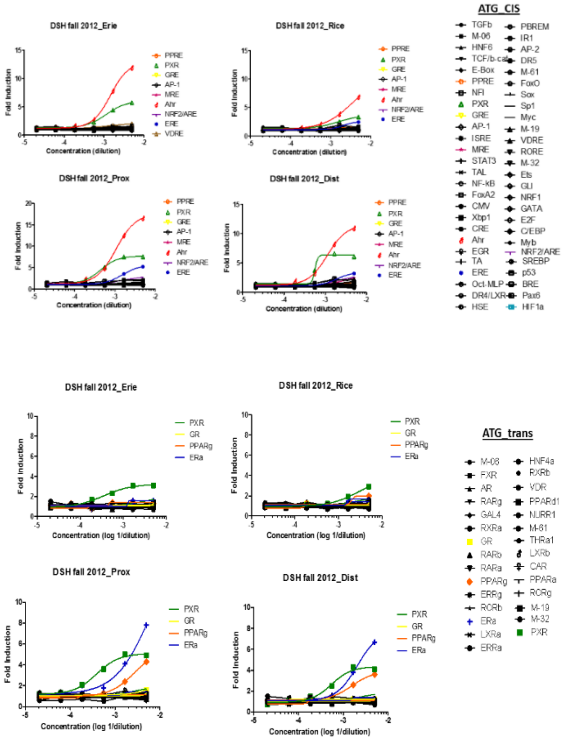


Slide courtesy of Dan Villeneuve

Bio-effects Surveillance

What bioactivity is associated with known and unknown contaminants present at a site?

Results are consistent with analytical data



Transcription Factors	Genes	St. Louis River AOC Sites – Fall 2012			
		Erie Pier	Rice's Point	WLSSD Prox	WLSSD Distal
Aryl hydrocarbon receptor (AhR) / Xenobiotic Response	AHR	38.8	48.9	18.2	21.5
Pregnane X receptor (PXR), Xenobiotic Pathway	PXRE	32.2	36.9	8.2	15.5
Pregnane X receptor	PXR	9.2	65.7	7.0	14.3
Estrogen Receptor (ER) pathway	ERE		85.8	42.7	62.4
Estrogen receptor-α	ERα			53.9	59.9
Estrogen receptor-β	ERβ			63.6	80.0
Vitamin D receptor (VDR) / vitamin D pathway	VDRE	33.3		28.9	22.2
Antioxidant Response Pathway	NRF2			52.4	52.1
Hypoxia-inducible factor-1a (HIF1a) / hypoxia pathway	HIF1a			7.6	9.4
Peroxisome proliferator-activated receptor-d	PPARg			67.4	63.2
Metal Response Pathway (MTF-1)	MRE				78.9
Phenobarbital responsive enhancer module /constitutive androstane receptor (CAR) pathway	PBREM				35.9
Retinoic acid receptor -related orphan receptor proteins (ROR) a,b,g	RORE				63.1

All concentrations reported in µM

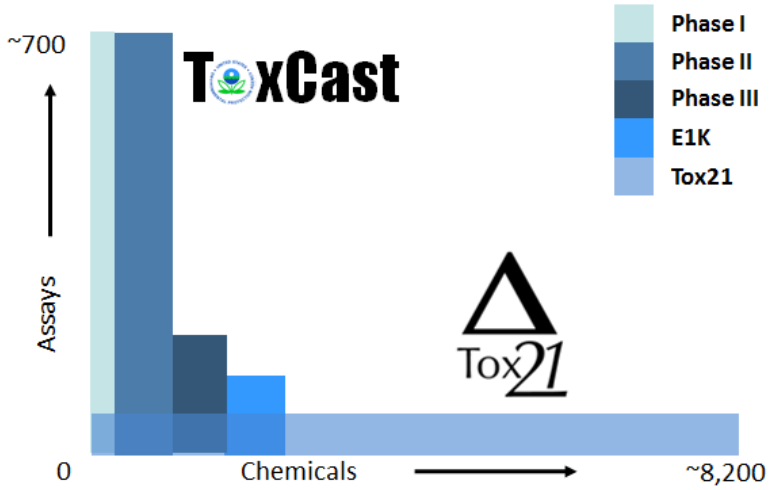
Slide courtesy of Dan Villeneuve

Caveats and Uncertainties

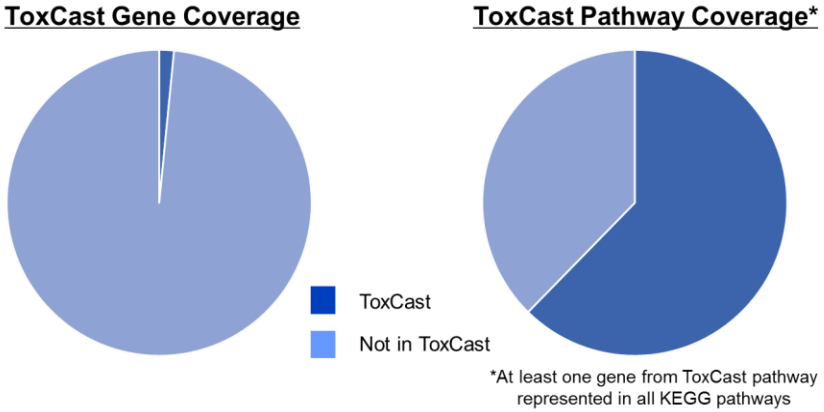
Uncertainties and Caveats in Use of HTS Data

- Lack of metabolic capability of most cells and cell lines
- Acute exposures
- Volatile chemicals are difficult to test
- These assays are not instantaneous (days, weeks)
- Many are based on proprietary technology (e.g., Attagene)
- Coverage of chemical and biological space is incomplete
  - Some targets = multiple orthogonal assays (e.g., estrogen receptor)
  - Some targets = one assay *or none….*

Chemistry



Biology



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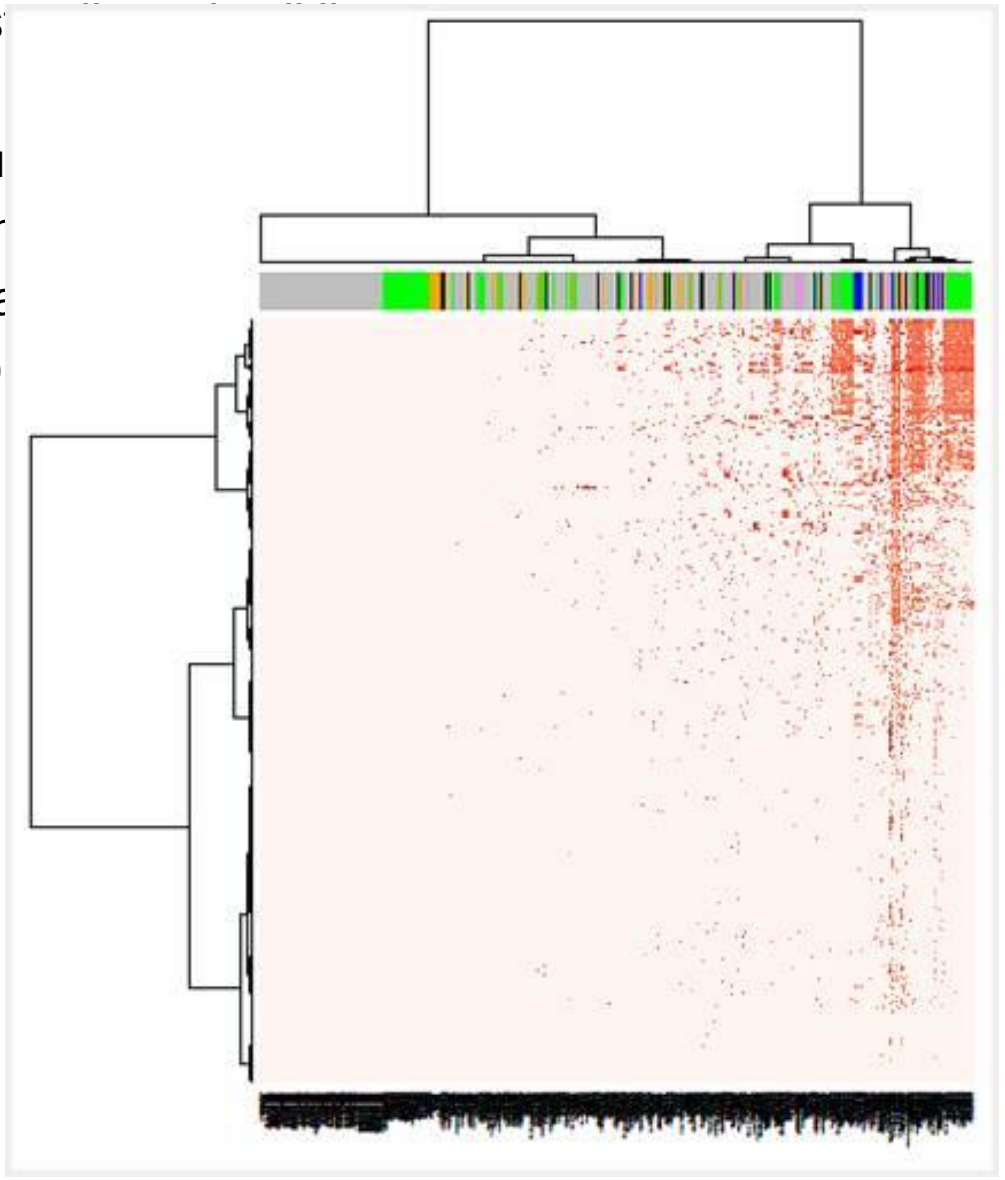
- BUT
  - All assays are not targets for all chemicals
  - Heatmap for bioactivity of 1800 chemicals and 700 assay endpoints
  - Red = activity



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- Many are based on proprietary technology (e.g., Attagene)
- Coverage of chemical and biological space is incomplete
  - Some targets = multiple orthogonal assays (e.g., estrogen receptor)
  - Some targets = one assay (e.g., thyroid receptor) or none….

- BUT
  - **If you know the biological target(s) – you can build HTS screens**
    - e.g., ER assays downstream from French pharma plant with wastewater "problem"
  - **Developing cheminformatics platforms to expand into unknown chem-space**
  - **New biotechnologies promise better biological coverage**
    - Currently testing new 'global' genomics technologies that promise ability to tests 20-30k genes at ~$25/sample
  - **Future – patterns across multiple assays at relevant concentrations will increase confidence in use for more than prioritization "risk" decisions**


Thanks for Listening