Computational Toxicology and Risk Assessment

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The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

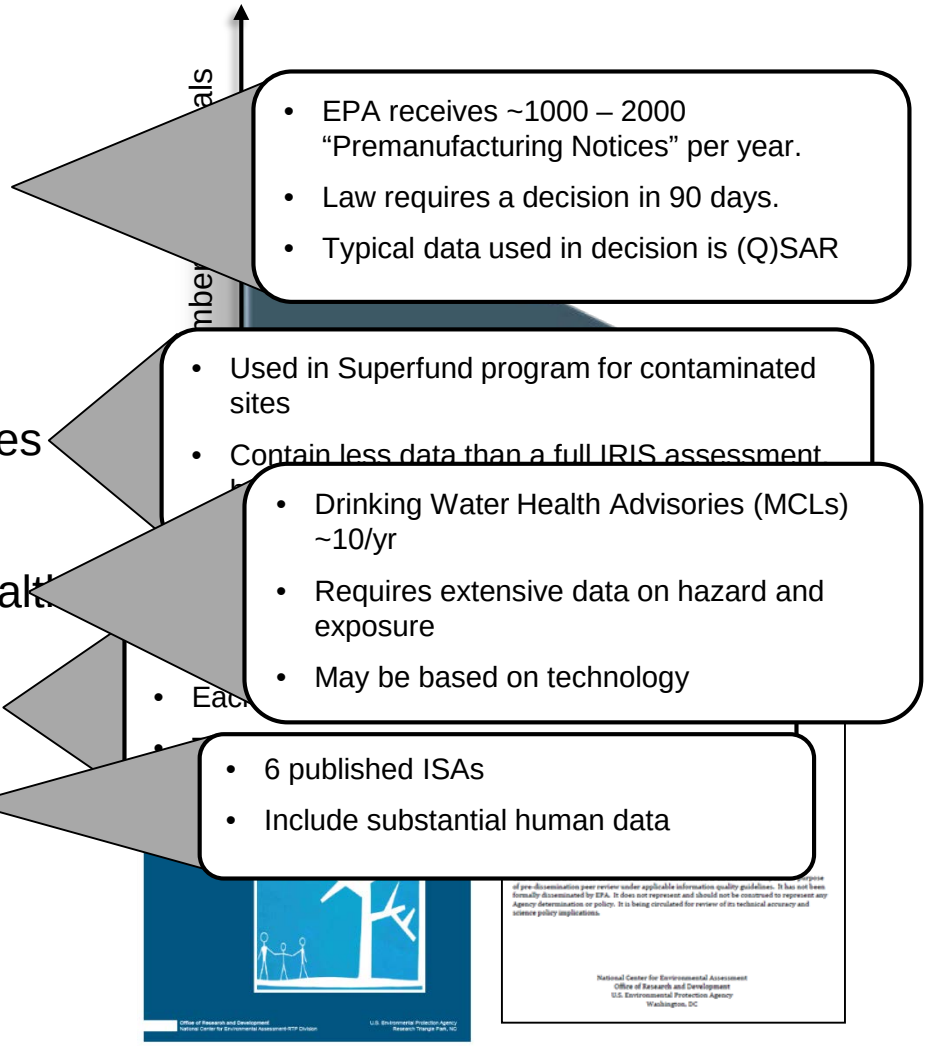
Risk Assessment in Regulatory Context

Regulatory Drivers	Regulators	Regulations
TSCA FIFRA Clean Air Clean Water Superfund Endangered Species Act Food Quality Protection Act	OSHA ATSDR IARC WHO EPA FDA ECHA State governments	Occupational Acute Chronic Susceptible populations Endpoint specific Food/pharmaceuticals Ecological

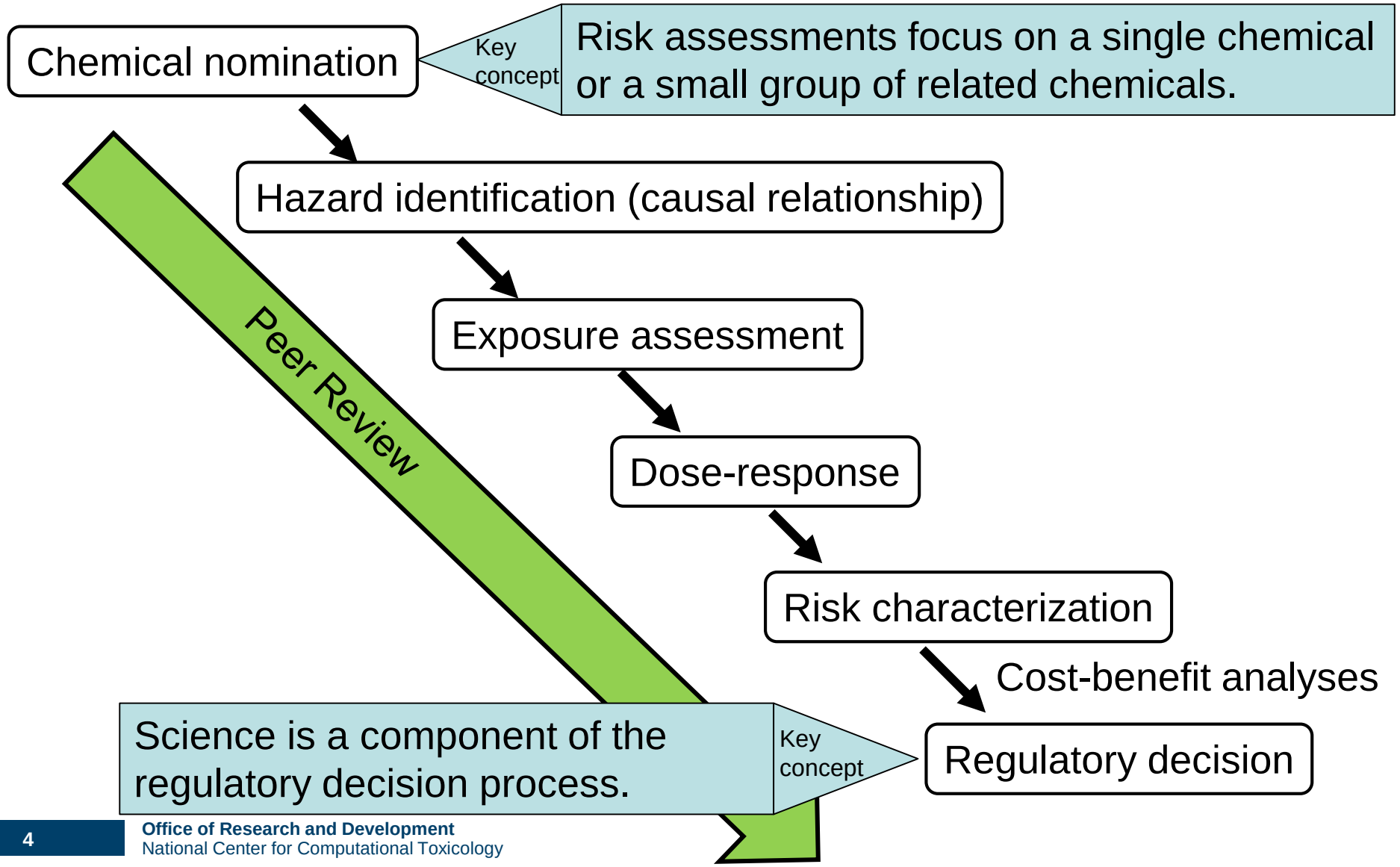
Key Concept: Regulatory decisions are context dependent; therefore, risk assessments are varied.

Regulatory Contexts at the EPA

- Chemical assessments are “fit-for-purpose”
  - Prioritization (e.g., EDSP, PMN, SNUR)
  - Screening-level values (eg., CCL, GreenChem)
  - Provisional Peer-Reviewed Toxicity Values (PPRTVs)
  - Pesticide Tolerances, Drinking Water Health Advisories, Integrated Risk Information System (IRIS),
  - Integrated Science Assessments (ISA)



Regulatory Risk Assessments



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Typical Data use in Human Health Assessments

Risk Assessment	Human data	In vivo mammalian tox data	In vitro or Alternative Species data
Hazard Identification	yes	yes	Mechanistic plausibility or susceptible populations
Exposure Assessment	yes	no	no
Dose-response	yes	yes	Informs uncertainty and shape of D-R curve
Risk Characterization	yes	no	no

Key concept: Epidemiology and in vivo toxicity data prioritized over in vitro data.

Challenge for regulatory toxicologists

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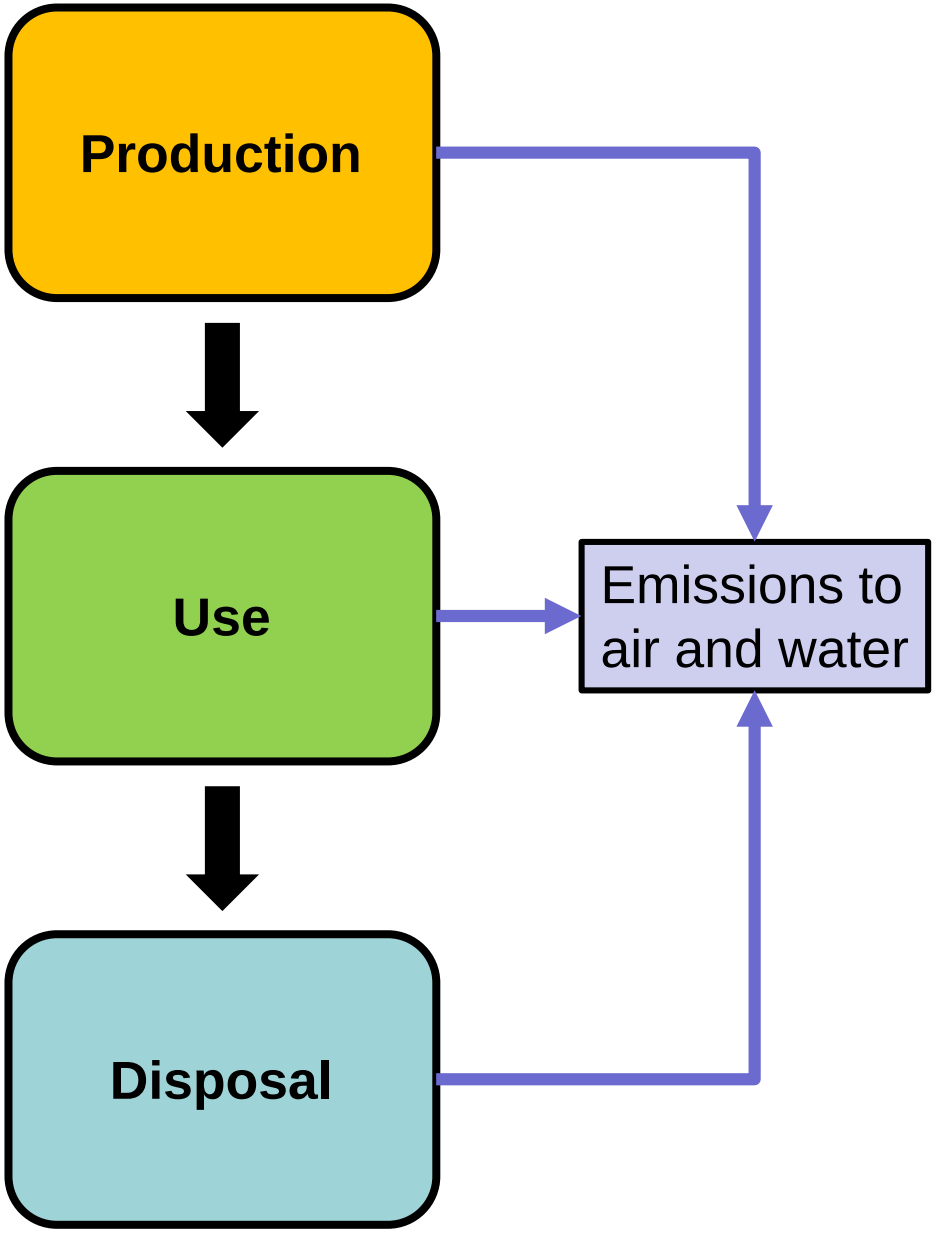
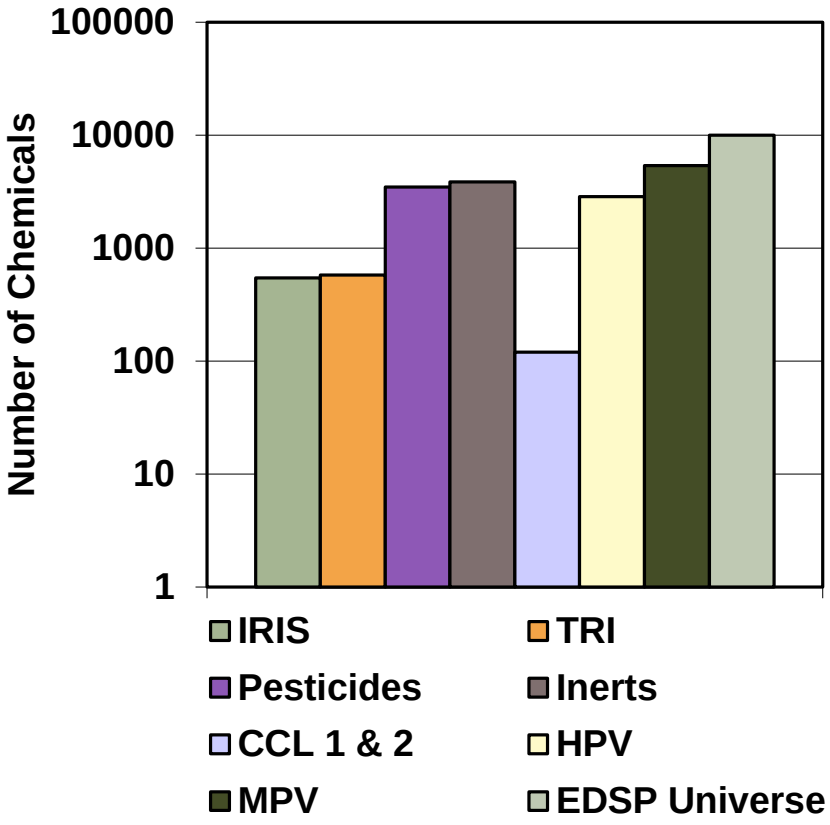
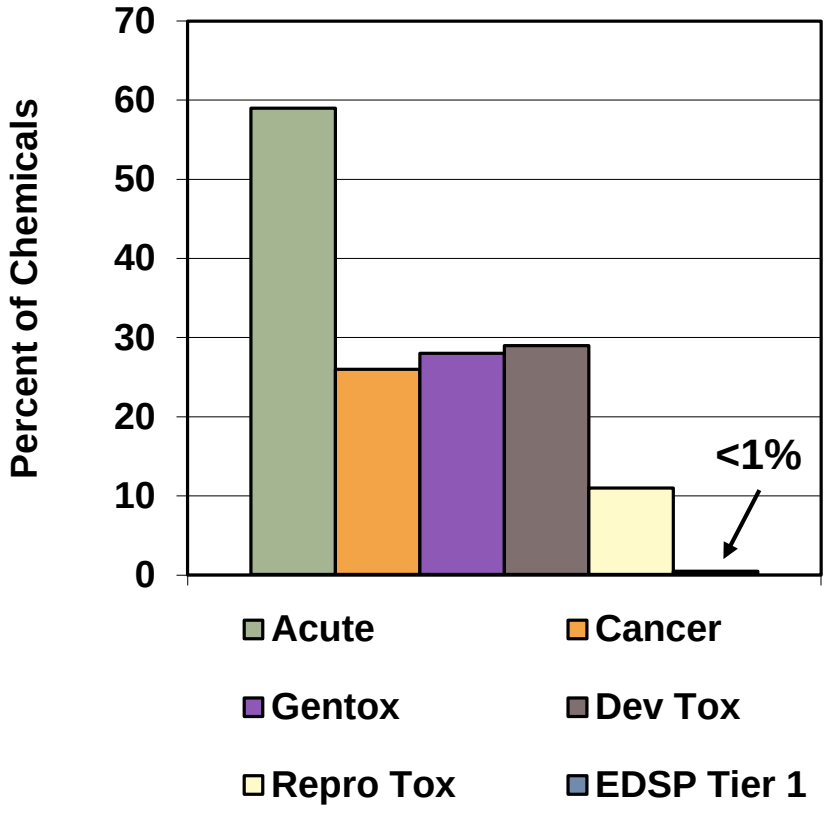


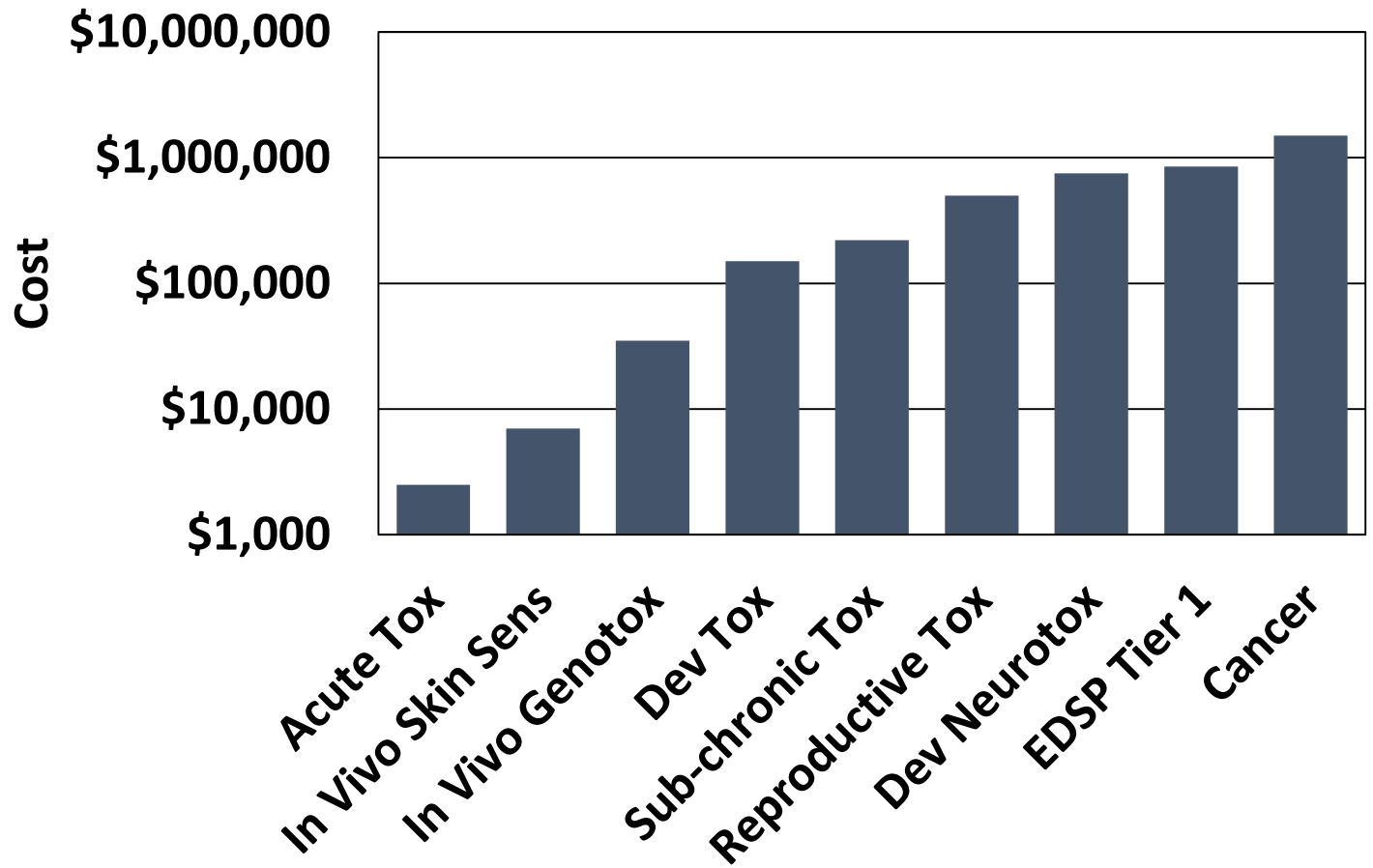
How much do we know? Not enough





Modified from Judson, et al *EHP* (2010)

Economic cost to generate data

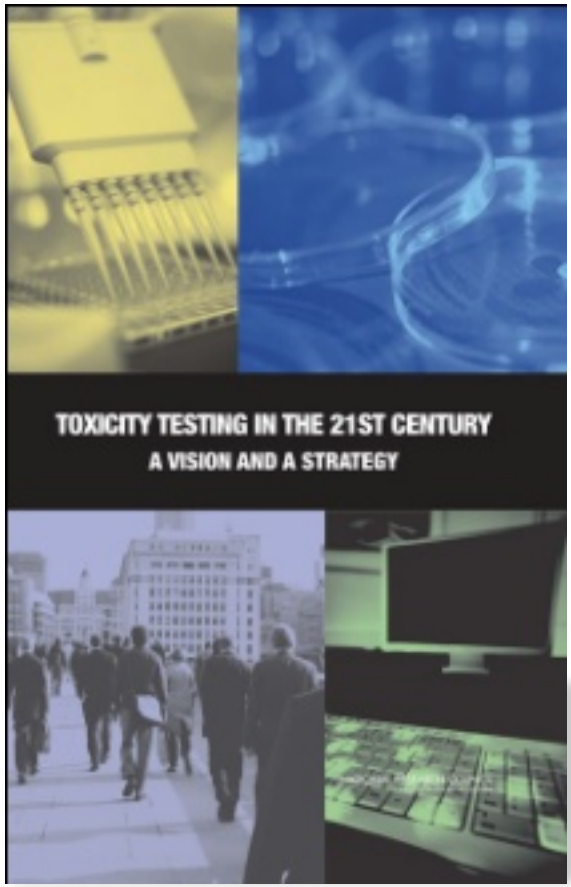


Key concept: Expensive and time consuming to collect data traditionally used for risk assessment.

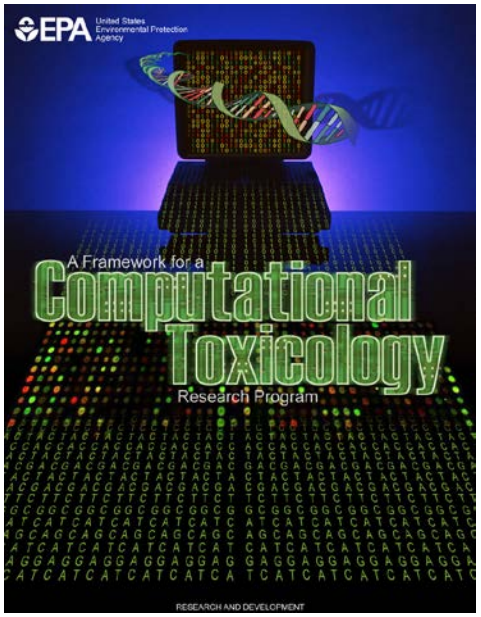
High-Throughput Approaches for Toxicology

Mandates for new technology

EPA United States Environmental Protection Agency

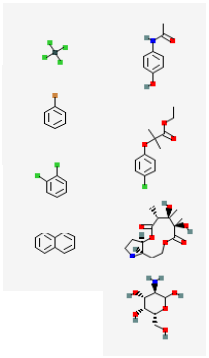


2007 NRC Report

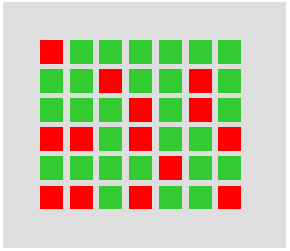


Registration Evaluation &
REACH
Authorisation of CHemicals

Computational Approaches to Hazard Identification

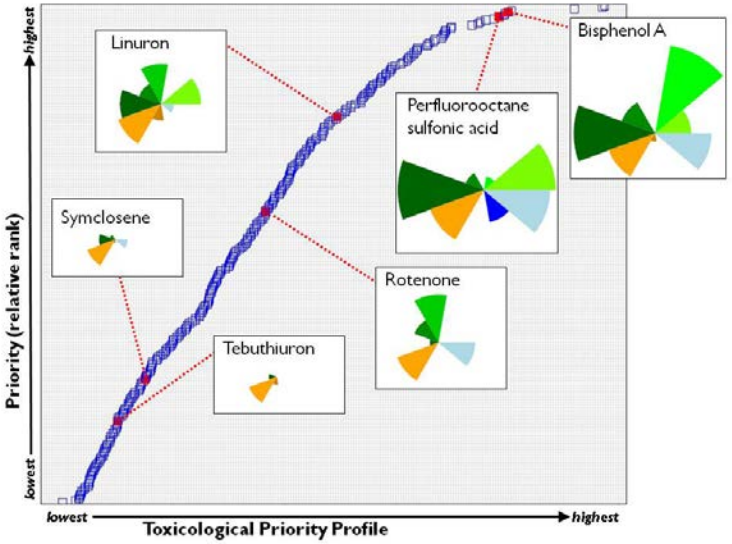


Thousands of chemicals



High throughput biology and chemistry

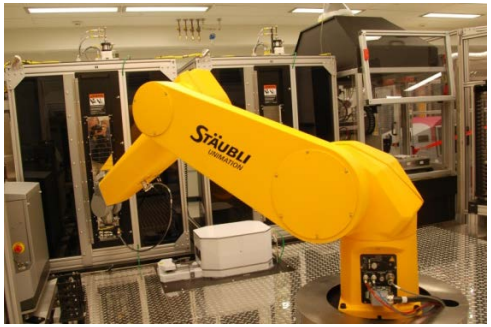
Bioinformatics/ machine Learning



Predictive toxicology

Key concept: Rapid collection of more data on more chemicals

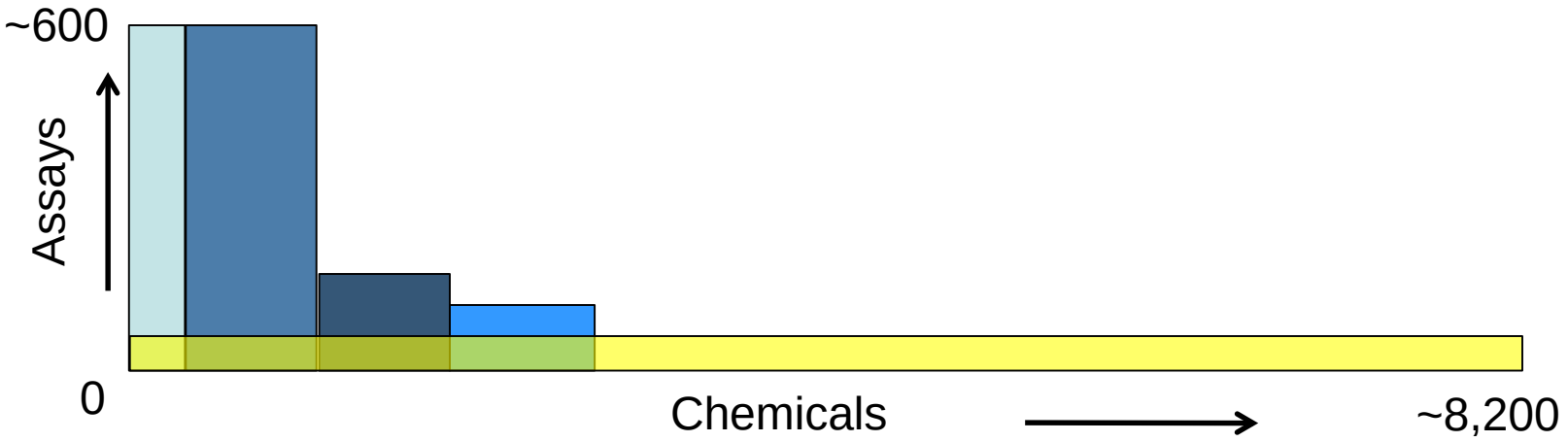
Responsiveness: Deepwater Horizon Accident



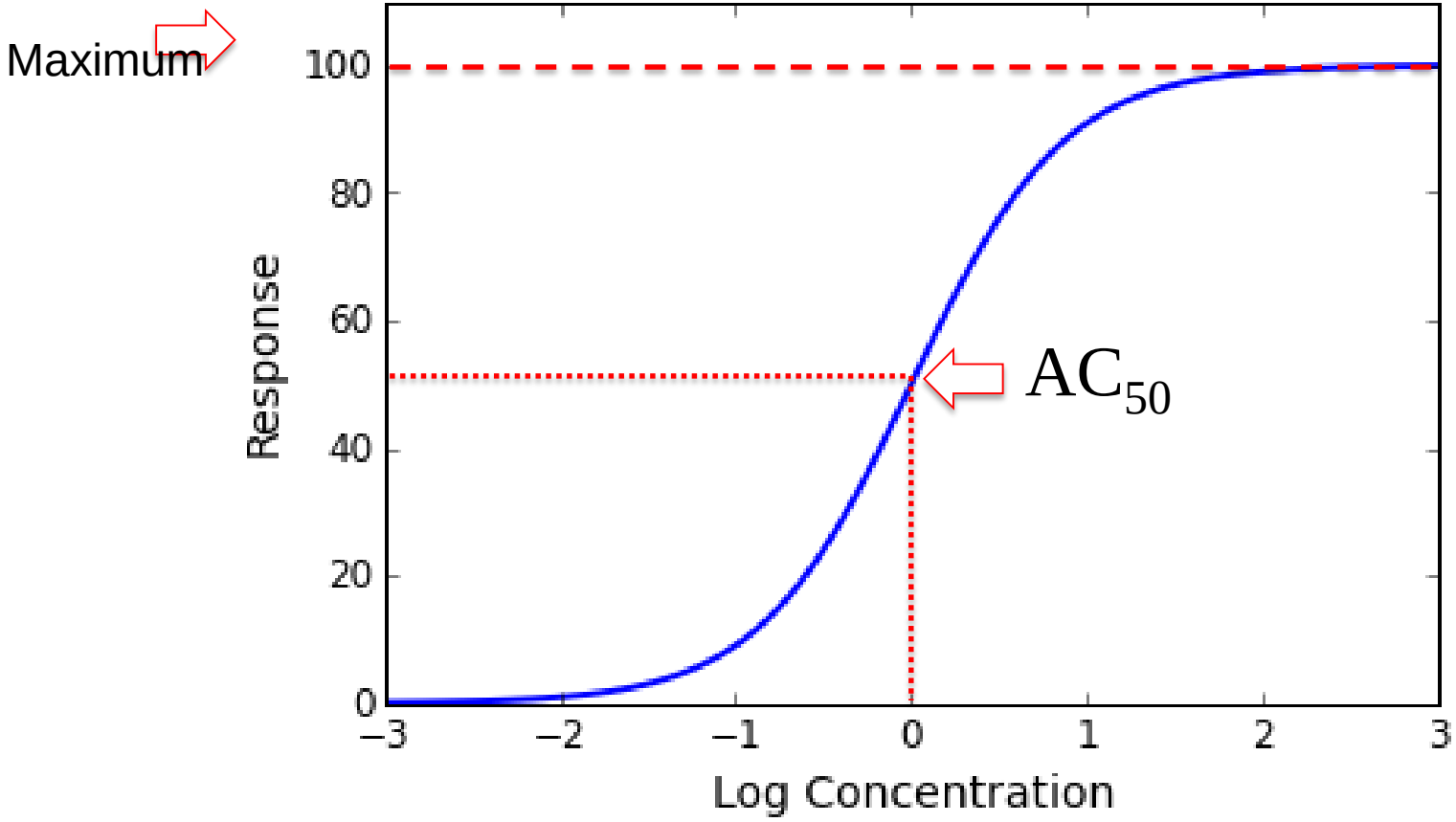
- Deepwater Horizon Oil Exploration Platform Explodes - estimated 4.9 million crude oil released
- 1.8 million gallons of dispersant used; EPA Administrator calls for less toxic alternative
- In ~ 6 weeks, dispersants tested for bioactivity (including endocrine activity and cytotoxicity)

Tox21 Consortium - Collaborative and Complementary Approaches

	Chemicals	Assays	Endpoints
ToxCast Phase I	293	~600	~1100
ToxCast Phase II	767	~600	~1100
ToxCast Phase IIIa	1001	~100	~100
E1K (endocrine)	880	~50	~120
Tox21	8,193	~25	~50



Assay Data and Bioactivity



Key concept: HT data provides *bioactivity* information, not *toxicity* data

HT Assay Endpoints and biological space

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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

Interpreting HT data for Hazard ID: Using AOPs

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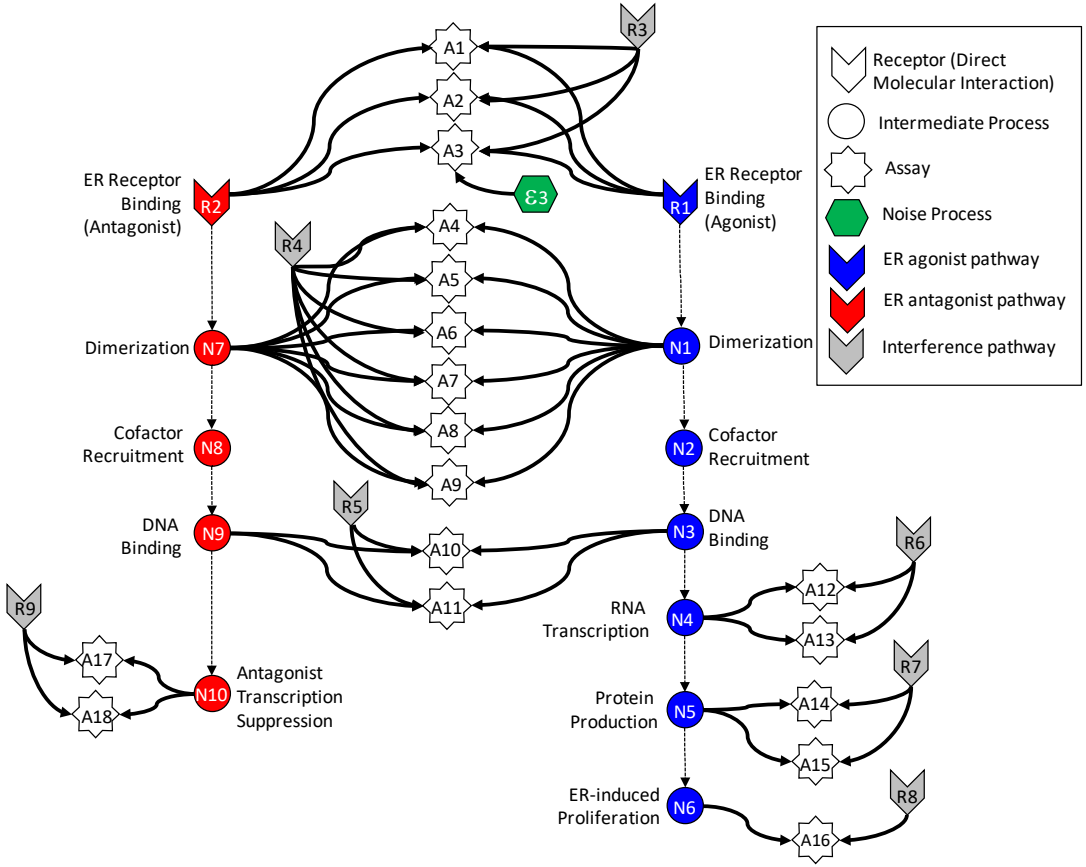
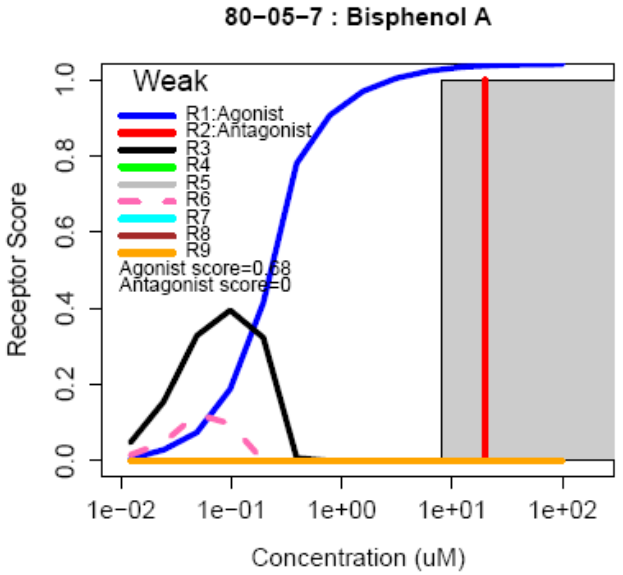
18 *In Vitro* Assays Measure ER-Related Activity



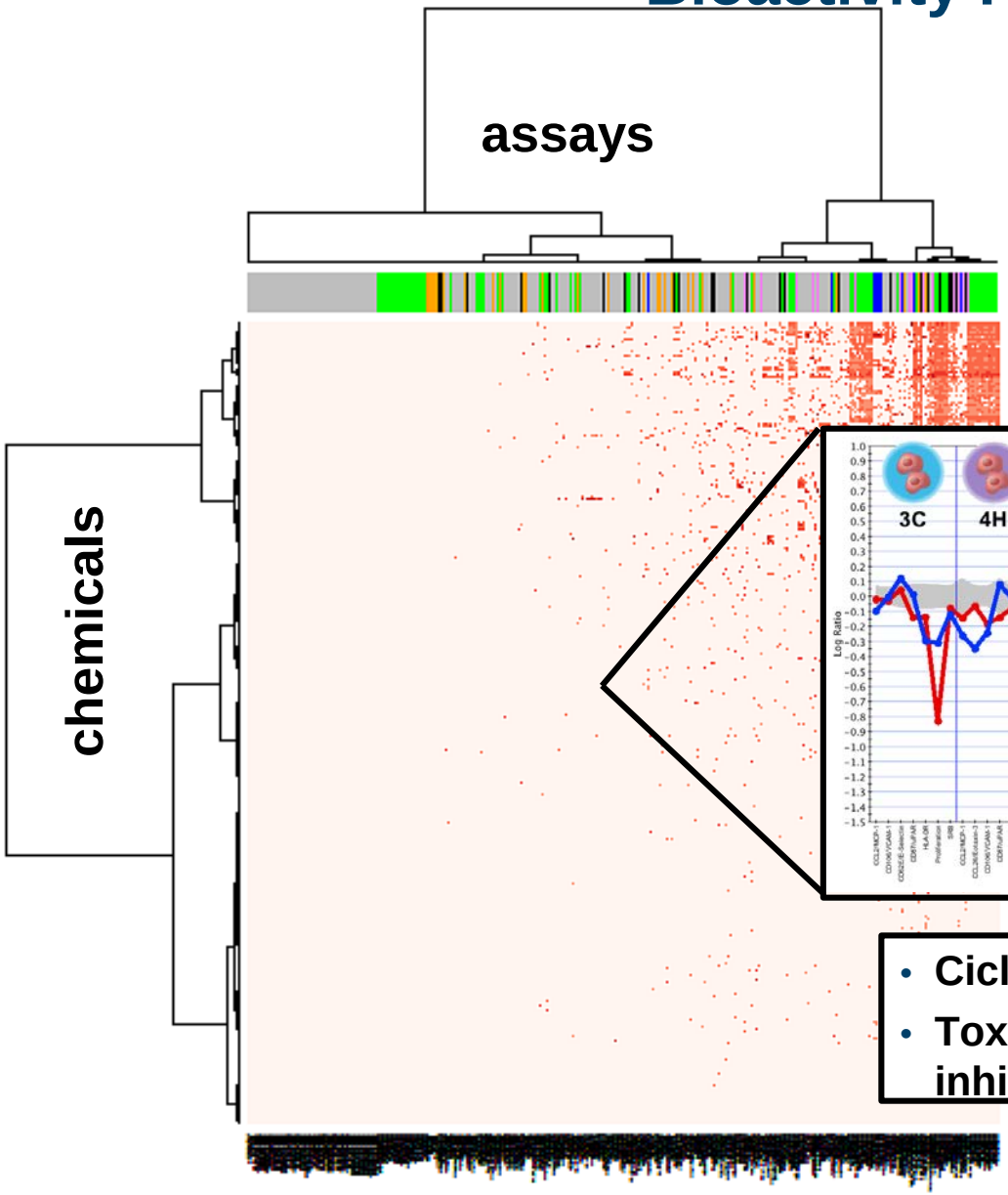


In Vitro Reference Chemicals

True Positive	26 (25)
True Negative	11 (11)
False Positive	1 (0)
False Negative	2 (2)
Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

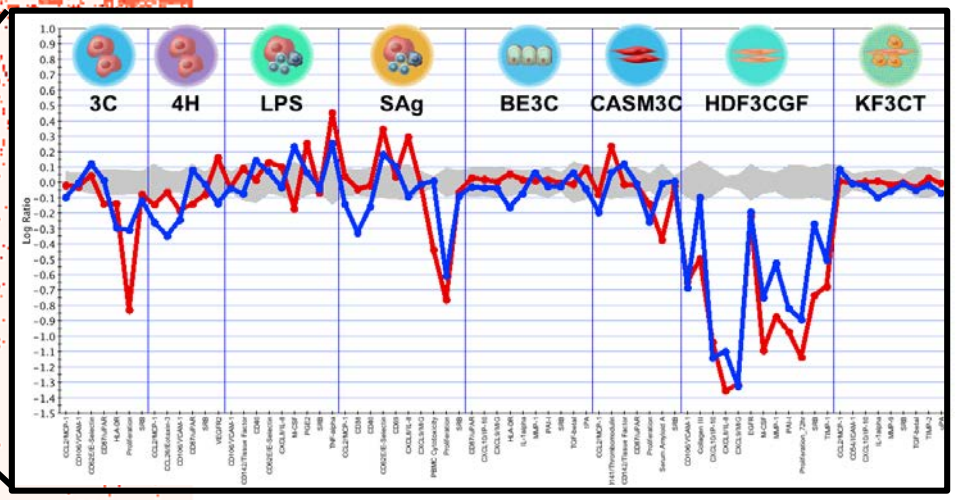
Judson *et al., Tox Sci.* , Browne *et al., ES&T.* 2015, Kleinstreuer *et al., EHP*

Interpreting HT data for Hazard ID: Using Bioactivity Profiles



NanoAg

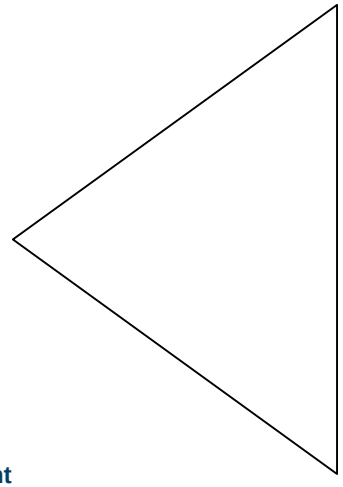
Ciclopirox



- Ciclopirox – inhibitor of Na+ K+ ATPase
- Toxicity of silver is associated with inhibition of Na+K+ATPase

Interpreting HT data for Hazard ID: Using Read Across

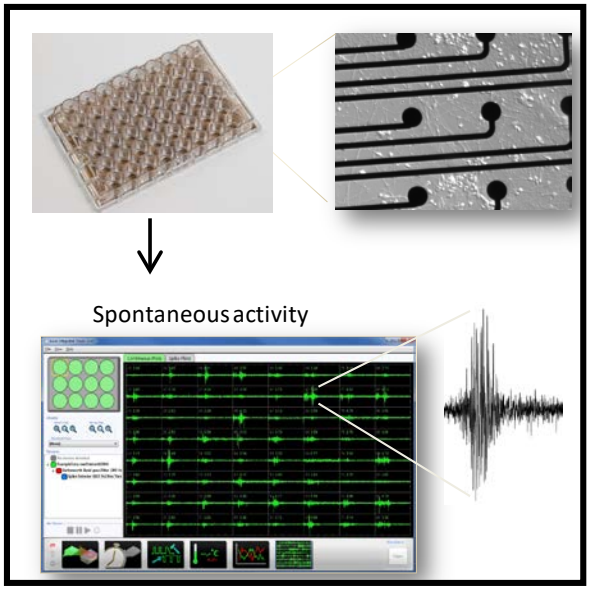
- Organize chemicals based upon chemical similarity
- Use to predict bioactivity in assays and/or adverse outcomes based on reference chemicals

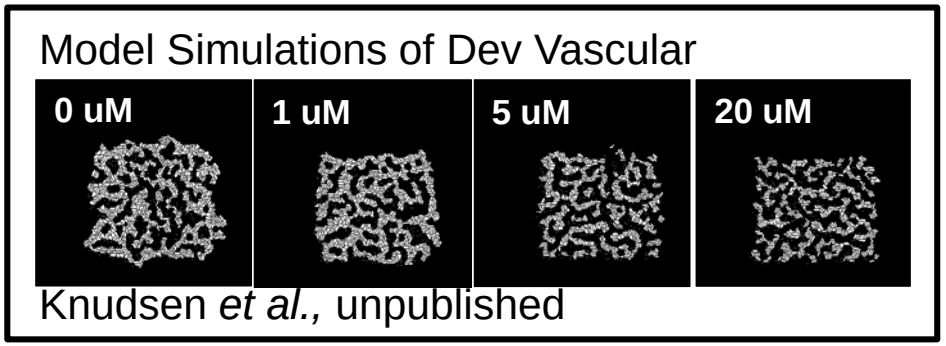


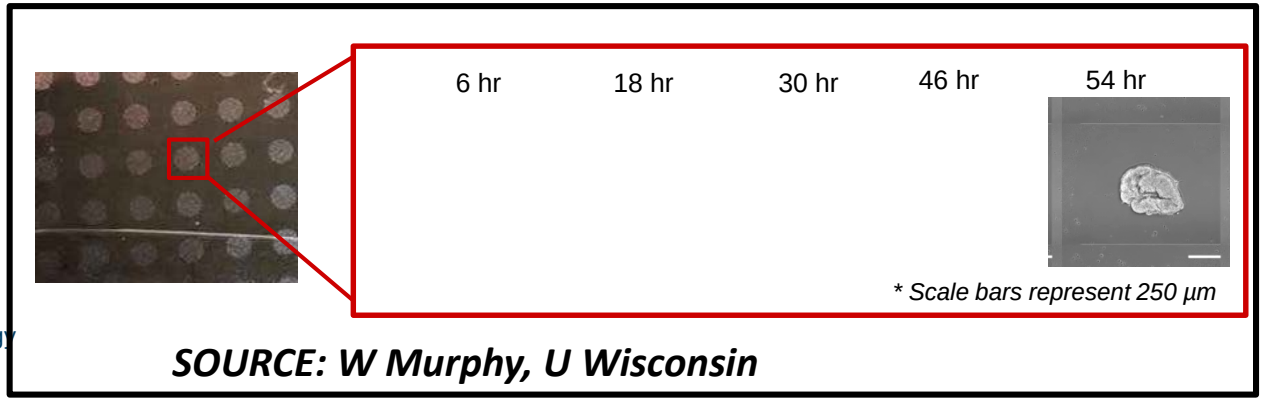
Near-term challenges for HT Toxicity Testing

United States Environmental Protection Agency

- Need to identify reference compounds and AOPs
- Volatile chemicals
- Metabolism
- Biological space – assay development
- Reproducing complex biology using reductionist approaches



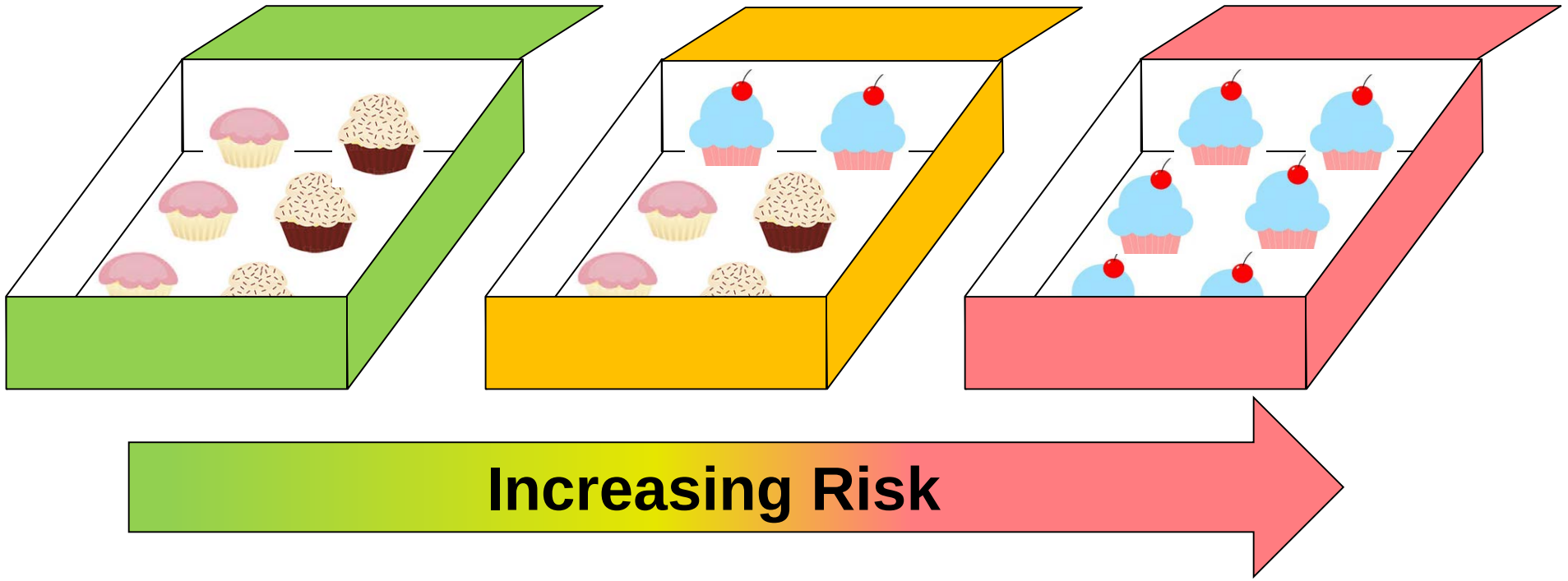




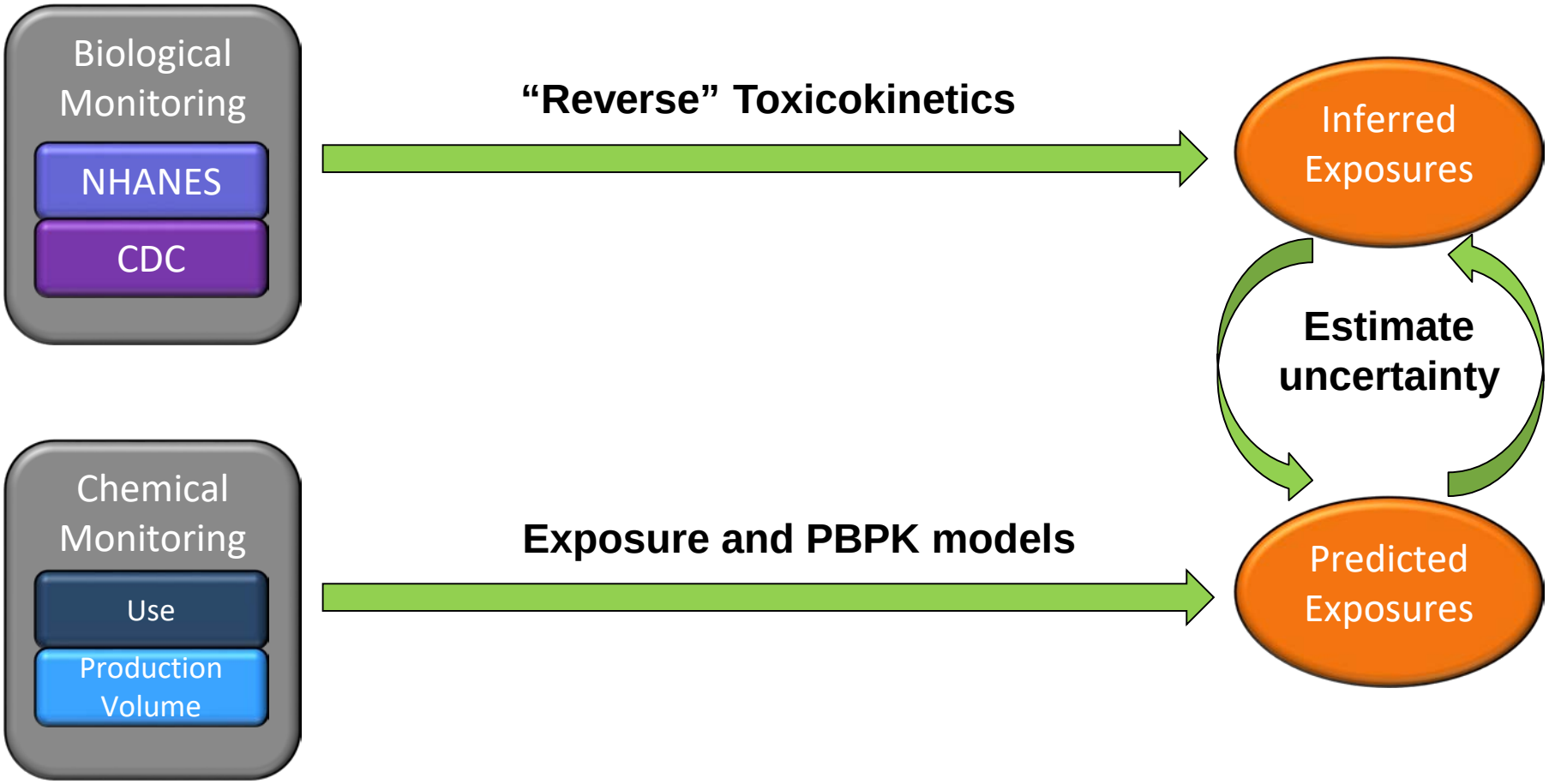
Hazard vs Risk

Risk = probability of effect from *hazard* under given *exposure*

Key Concept: Risk = f (Hazard x Exposure)



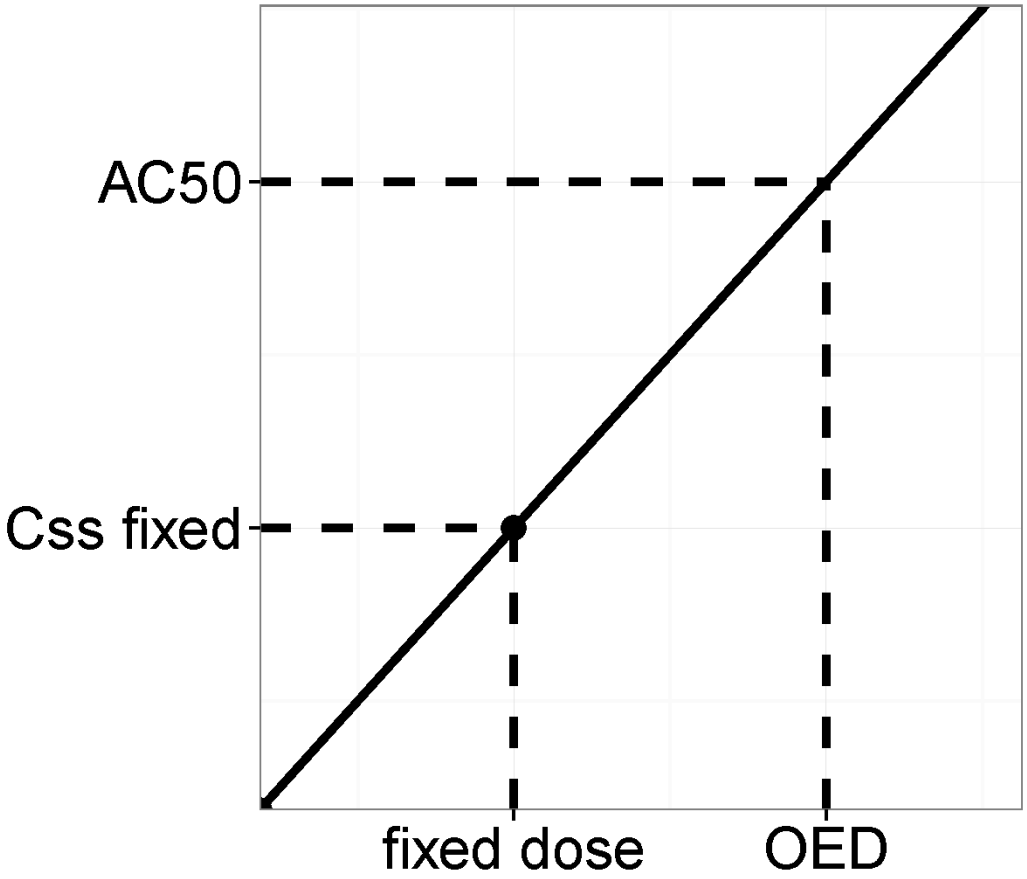
Computational Approaches to Predicting Chemical Exposure



Estimating Chemical Exposure - Reverse Toxicokinetics

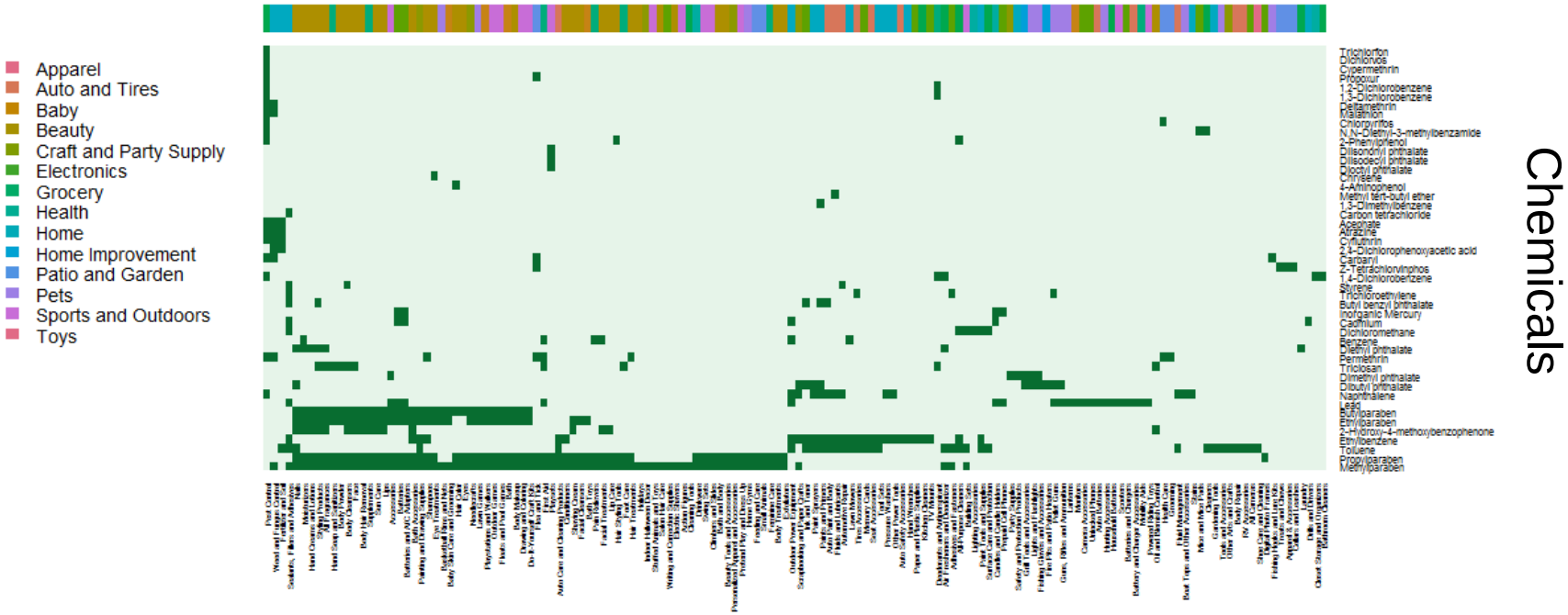
- Using biomonitoring data to estimate oral exposure
- Assume first-order metabolism
- Work with steady-state plasma concentration (C_{ss})

$$\text{Oral Equivalent Dose } (OED) = \text{Fixed dose} \times \frac{\text{AC}_{50}}{C_{ss} \text{ from fixed dose}}$$



Key concept: RTK assumes long-term, ambient exposures.

Estimating Chemical Exposure: Consumer Products and Use

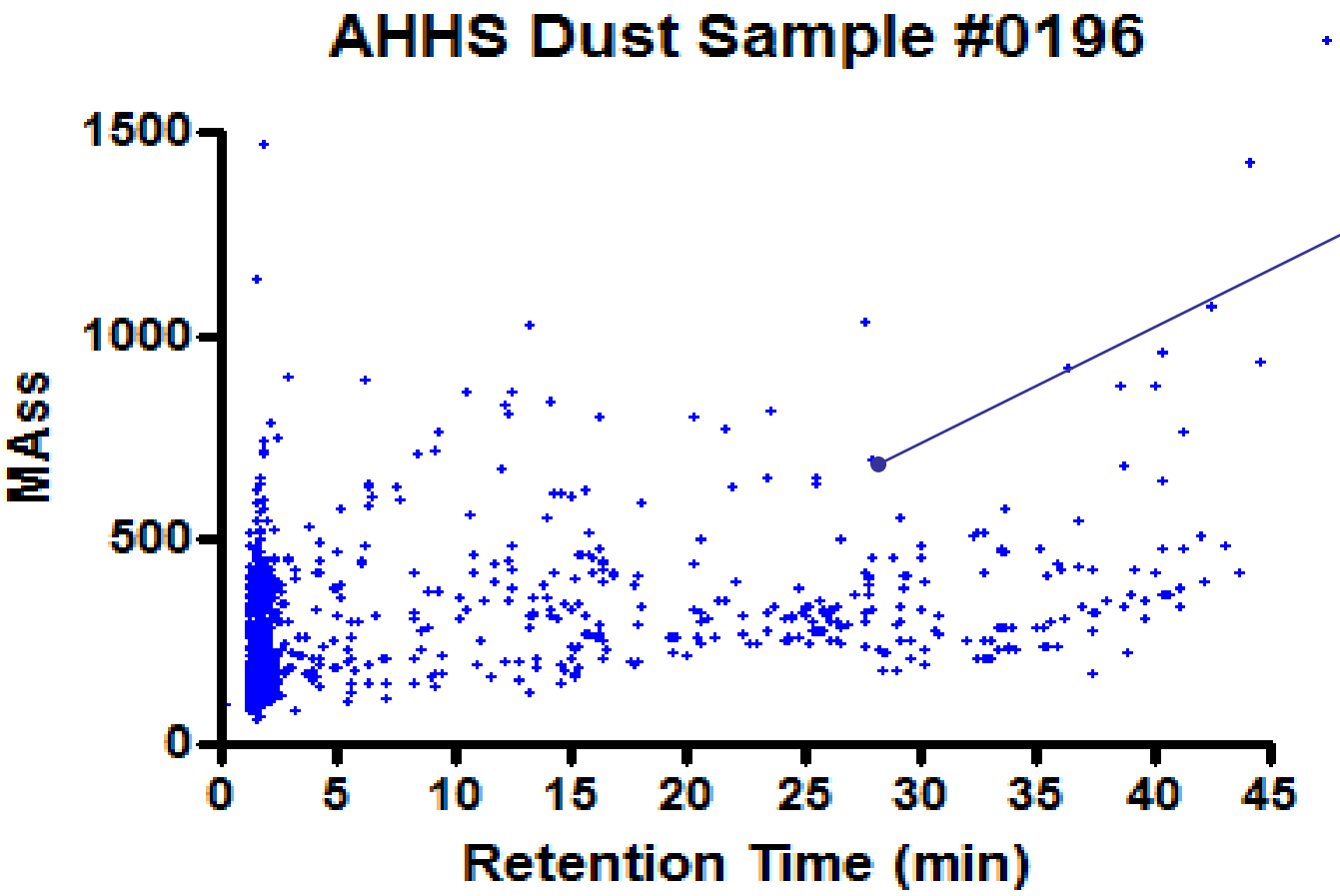


- Analyzed Materials Safety Data Sheets (MSDS) for ~20,000 products sold my a major U.S. retailer

Estimating Chemical Exposure through Consumer Product Use

Heuristic	Description	Number of Chemicals	
		Inferred NHANES Chemical Exposures (106)	Full Chemical Library (7784)
"Consumer use & Chemical/Industrial Process use"	Chemical substances in consumer products (*e.g.*, toys, personal care products, clothes, furniture, and home-care products) that are also used in industrial manufacturing processes. Does not include food or pharmaceuticals.	**37**	683
"Chemical/Industrial Process use with no Consumer use"	Chemical substances and products in industrial manufacturing processes that are not used in consumer products. Does not include food or pharmaceuticals	**14**	282
"Pesticide Inert use"	Secondary (*i.e.*, non-active) ingredients in a pesticide which serve a purpose other than repelling pests. Pesticide use of these ingredients is known due to more stringent reporting standards for pesticide ingredients, but many of these chemicals appear to be also used in consumer products	**16**	816
"Pesticide Active use"	Active ingredients in products designed to prevent, destroy, repel, or reduce pests (*e.g.*, insect repellants, weed killers, and disinfectants).	**76**	877
TSCA IUR 2006 Total Production Volume	Sum total (kg/year) of production of the chemical from all sites that produced the chemical in quantities of 25,000 pounds or more per year. If information for a chemical is not available, it is assumed to be produced at <25,000 pounds per year.	**106**	7784

Estimating Chemical Exposure: Non-targeted sampling

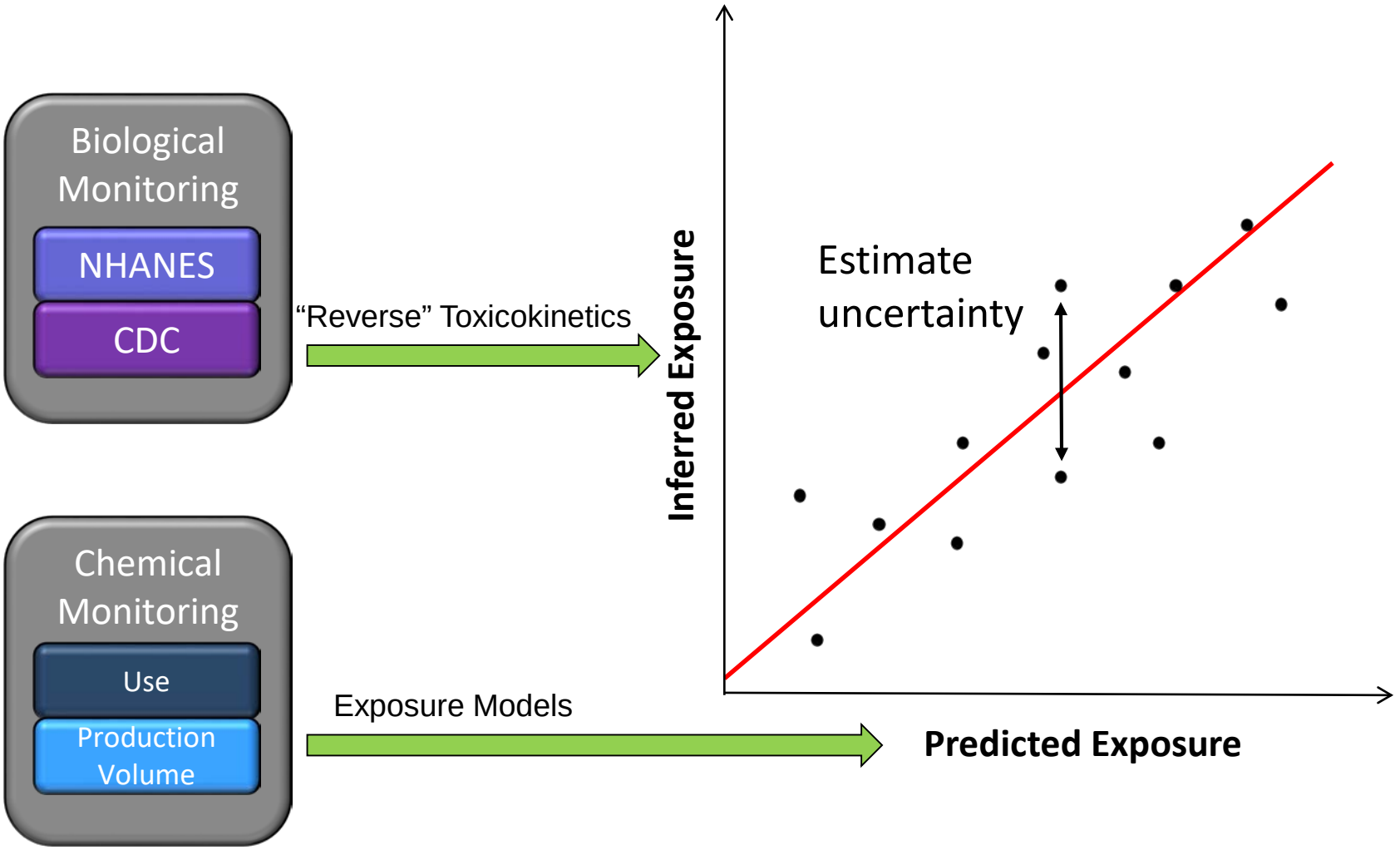


$C_{17}H_{19}NO_3$

Each peak corresponds to a chemical with an accurate mass and predicted formula:

Liang, Strynar, Sobus, Rager (NERL, EPA)

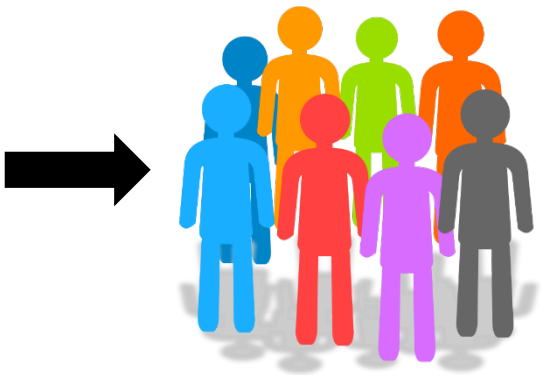
Uncertainty in Estimated Chemical Exposures

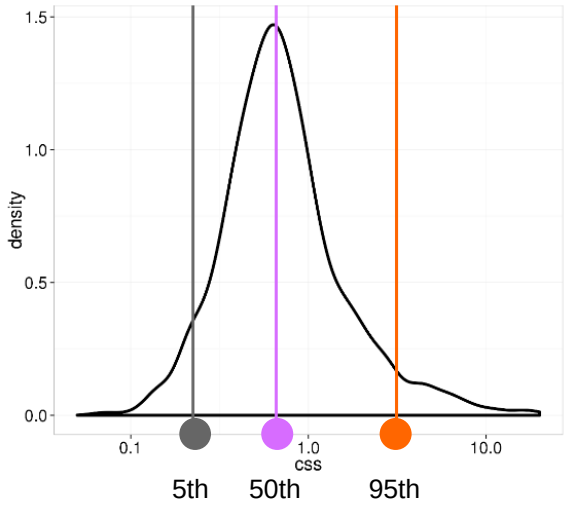


Near-term challenges for estimating chemical exposures

- Additional chemical use data, including:
  - key physical-chemical properties
  - chemical emissions from consumer products used indoors
  - chemical occurrence in products, environmental, and biological media
- Additional biomonitoring data, preferably using non-targeted approach
- Evaluating PBPK model for estimating chemical exposure
- Developing methods to address population variability in exposure estimates

Same chemical dose





Dose-response analysis – Selecting a POD

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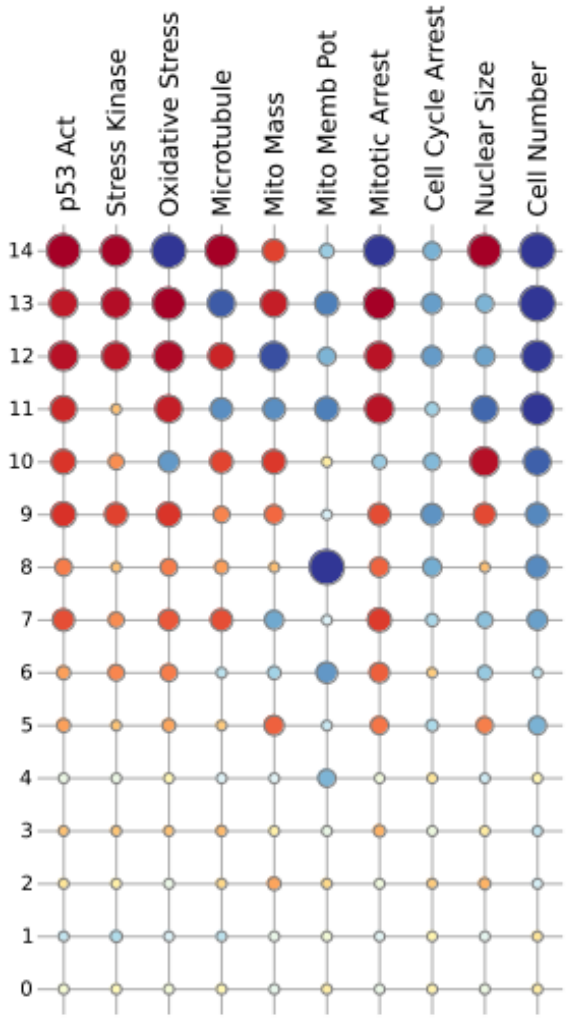
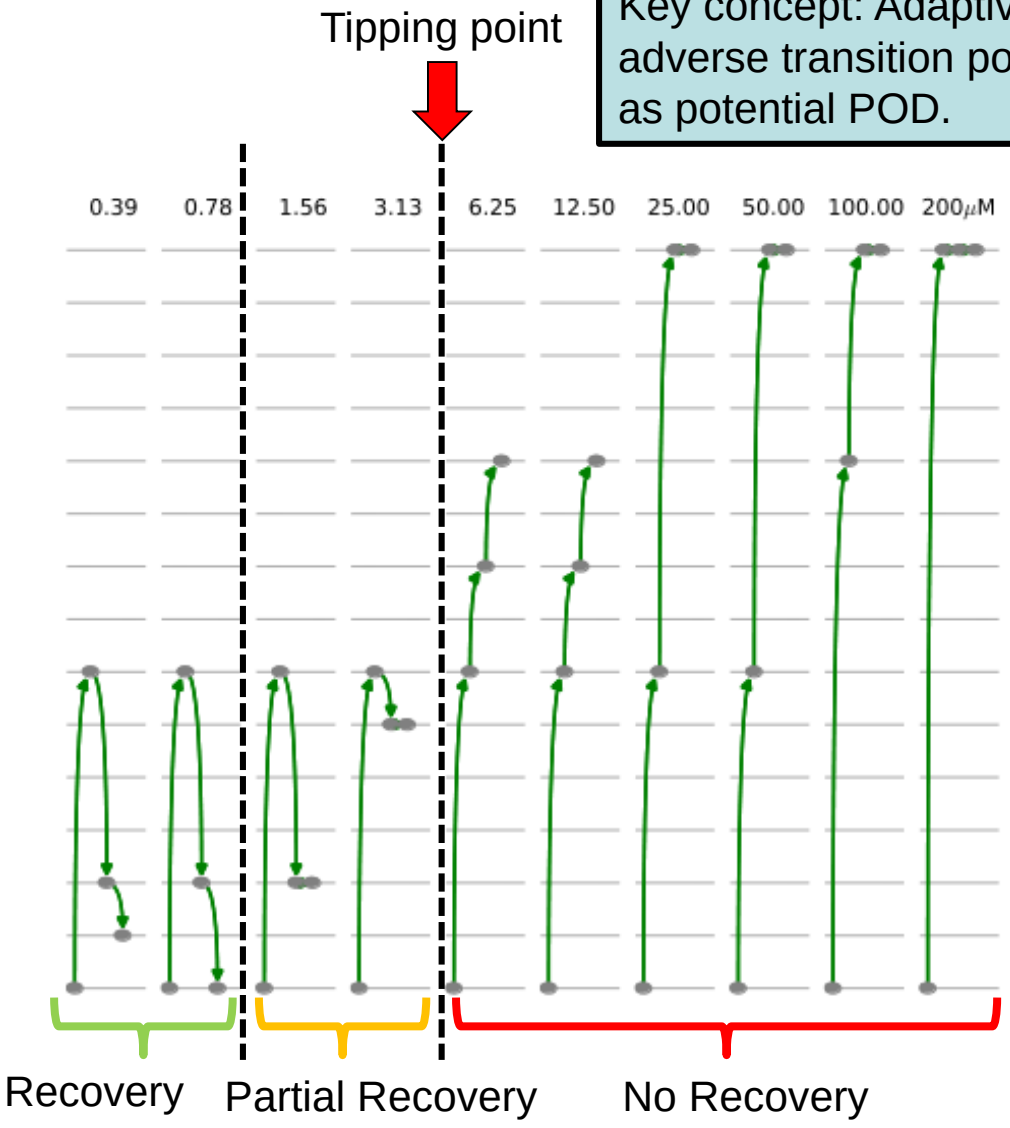


Assay Endpoints

Key concept: Adaptive vs adverse transition point as potential POD.



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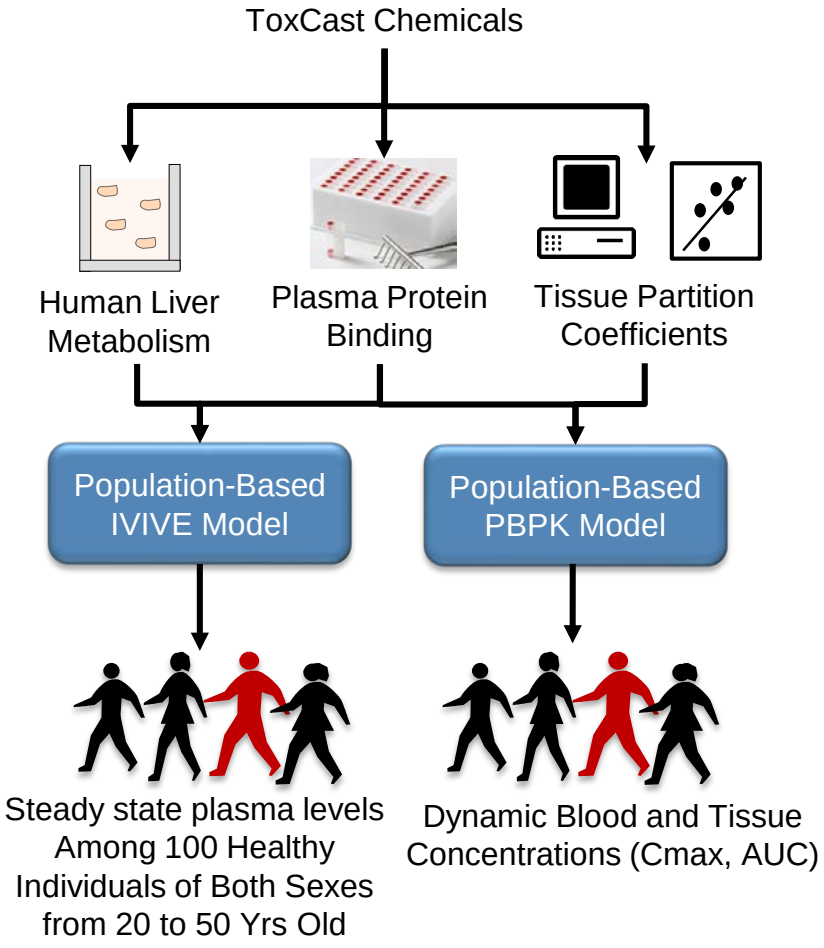
Dose-response analysis: In vitro to in vivo extrapolation (IVIVE)



- Steady-state IVIVE models for hundreds of chemicals based on limited high-throughput *in vitro* assays
- Structure-based methods to estimate tissue partitioning
- HT-Physiologically-Based Pharmacokinetic (HT-PBPK) models for hundreds of chemicals

Key concept: Methods to use in vitro concentrations to determine relevant in vivo doses.

Near-term challenges for dose-response

- Selecting PODs – do tipping points reflect biology/AOPs?
- Large dose-range – log scale data vs narrow dose-response range
- Characterizing uncertainty in IVIVE estimates – comparing in vitro and in vivo data

Risk characterization – Prioritizing chemicals using computational estimates

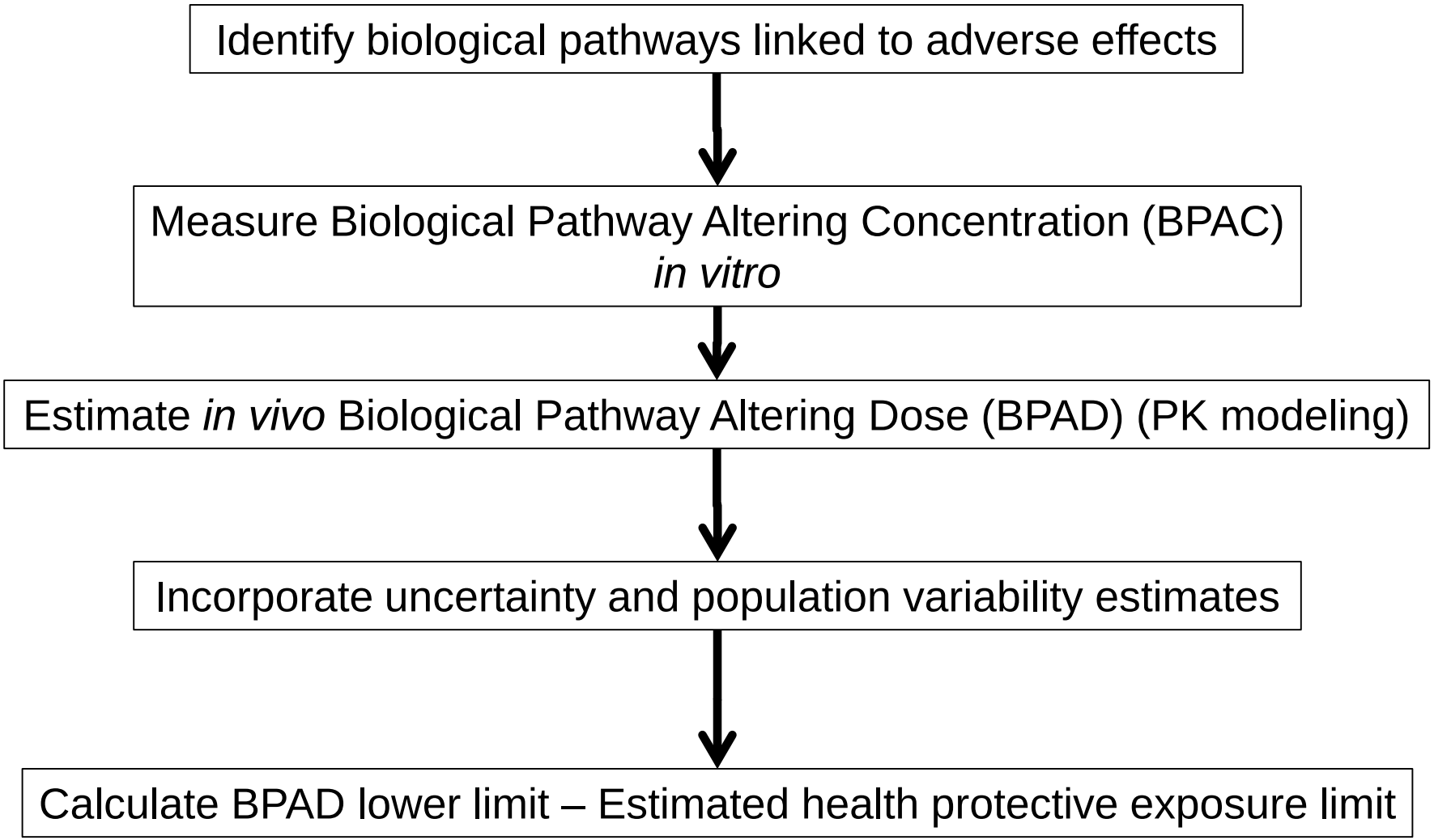
= hazardous dose

= exposure estimate

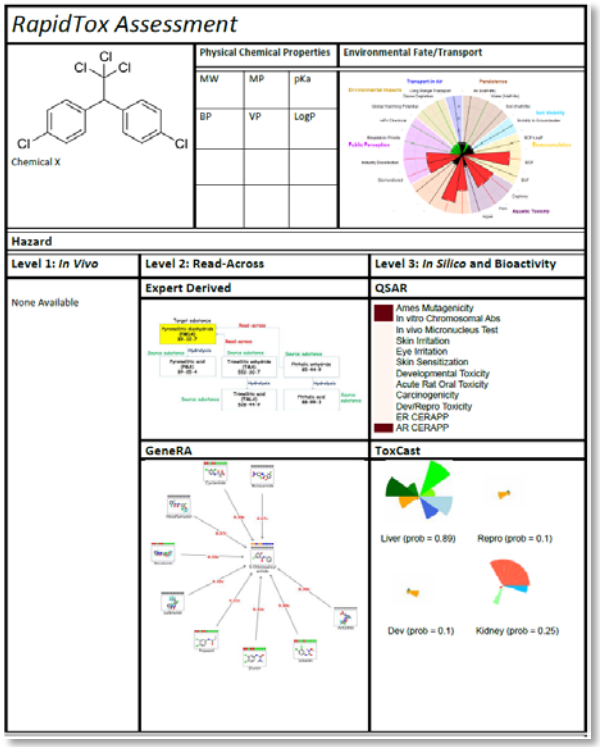
Wetmore *et al*. (2012)

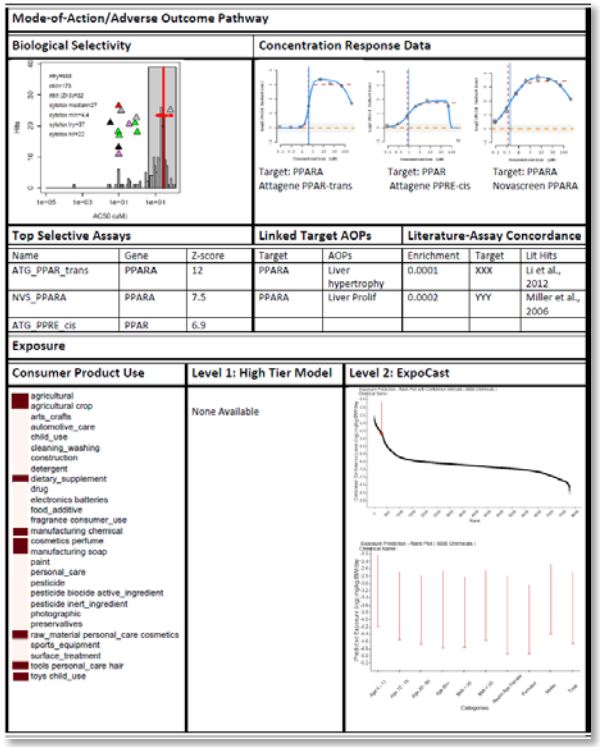
Risk = f (Hazard x Exposure)

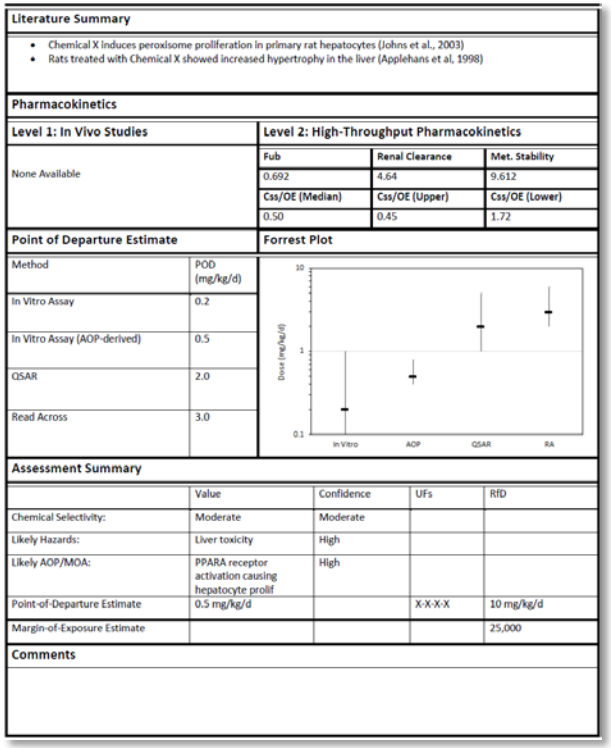
Risk characterization – Outline of HT data for risk assessment



Risk characterization – utility of HT approaches







Key point: modular and customizable given the decision context and needs of the program partner

Summary of Computational Toxicology approaches to Risk assessment

- Identify targets or pathways linked to toxicity (AOP focus)
- Develop high throughput assays for these targets or pathways
- Develop predictive systems models
  - *in vitro → in vivo*
  - *in vitro → in silico*
- Use predictive models (qualitative):
  - Prioritize chemicals for targeted testing
  - Suggest / distinguish possible AOP / MOA for chemicals
- High Throughput Exposure Predictions
- High Throughput Risk Assessments (quantitative)

Computational Toxicology – Future Challenges

- Mixtures
- Episodic exposures
- Biological plausibility and statistical significance
- Mechanisms of action and AOPs
- Differential susceptibility
- Human relevance of non-animal models
- Dose response analyses and quantifying uncertainty
- Regulatory acceptance

Thanks!

- US EPA National Center for Computational Toxicology (www.usepa.gov/ncct)
- Risk Bites “A New Way to Evaluate Chemical Safety – TOX21” (YouTube)
- cowden.john@epa.gov

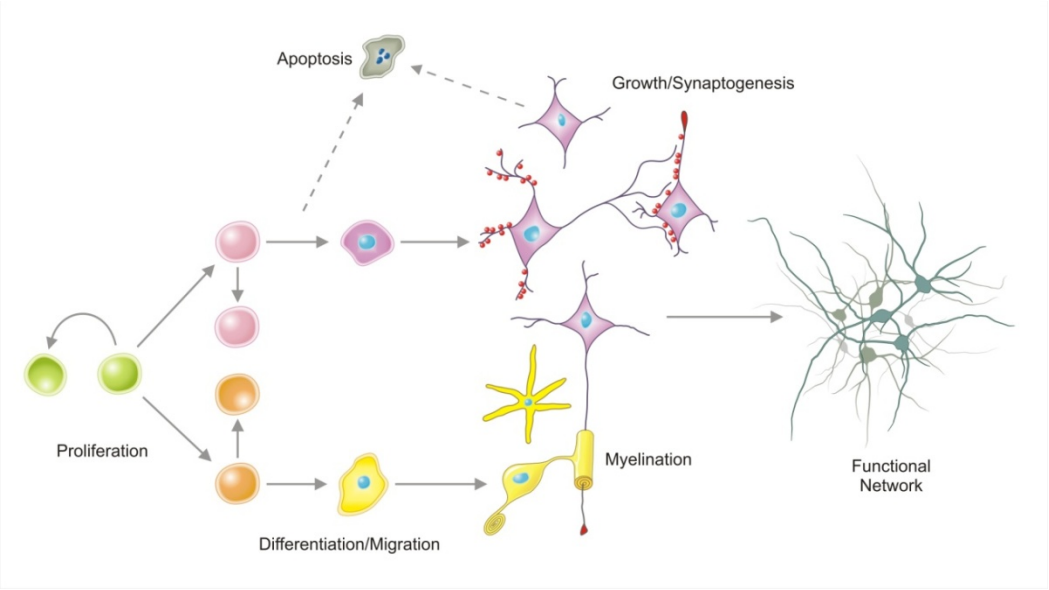
United States Environmental Protection Agency

Extra slides

Accomplishments

- Characterizing the biological activity of ~2000 chemicals in over 700 biochemical and cell-based assays.
- Additional assays being developed to fill data gaps in the high-throughput screens.
- Exposure estimates for over 7,000 chemicals based on production volume and chemical use
- Database of chemical-product categories (CPCat) that maps over 45,000 chemicals to ~8,000 product uses or functions
- Steady-state IVIVE models for hundreds of chemicals based on high-throughput in vitro assays
- Virtual tissue models are being constructed based on data collected from both high-throughput and “fit-for-purpose” assays and used to inform shape of the dose-response curve.

Cell-Based Assays for Developmental Neurotoxicity

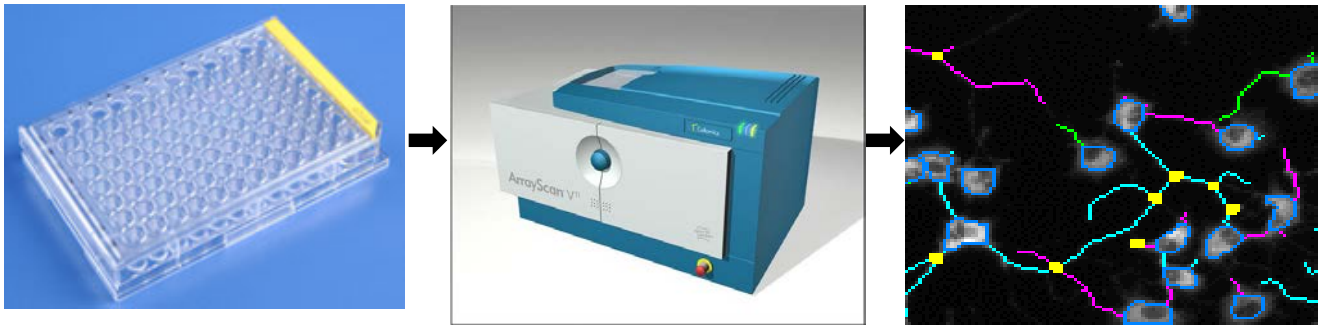


In Vitro Assays

- Use cell cultures including human neural stem cells
- Assess changes in key neurodevelopmental processes

High Content Imaging – automated microscopy provides data at level of individual cell

- High throughput: cells grown on multi-well plates
- High content: single image provides data on size/shape/location for 100's of cells

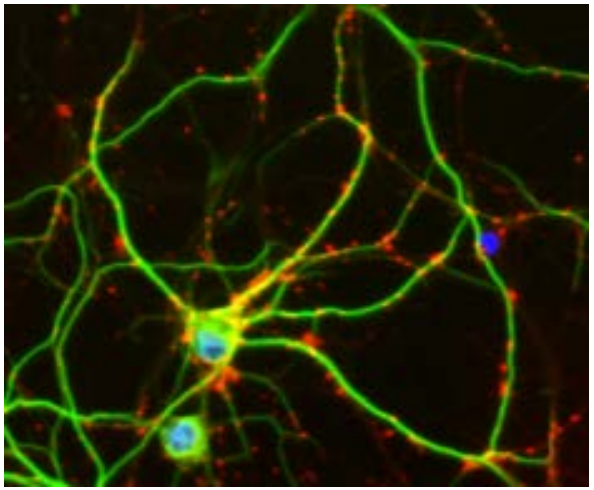


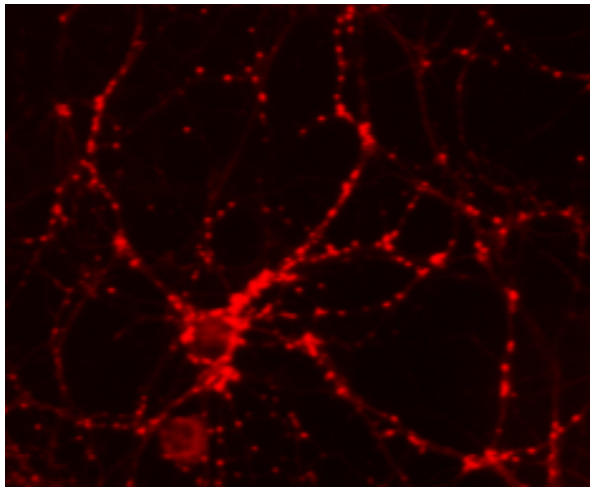
An Example with a Cell-Based Assay for Synaptogenesis

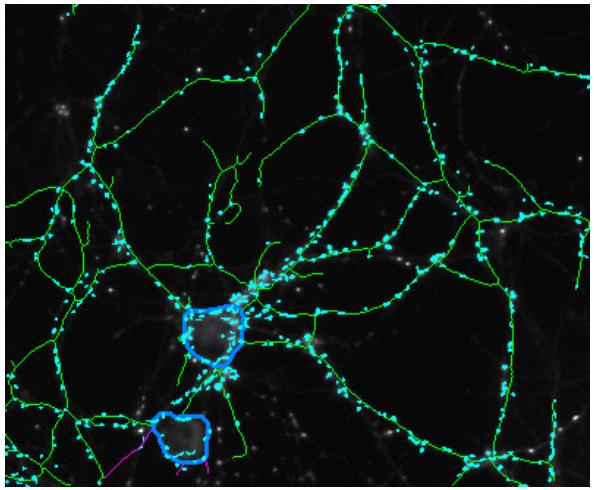
Synaptogenesis (formation of connections critical to a neural network)

- Primary neurons from rodent brain
- Stain for neurites (green) and synapses (red)

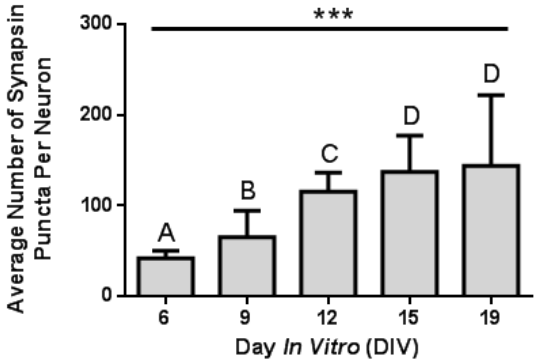
High Content Image showing identified neurites and synapses



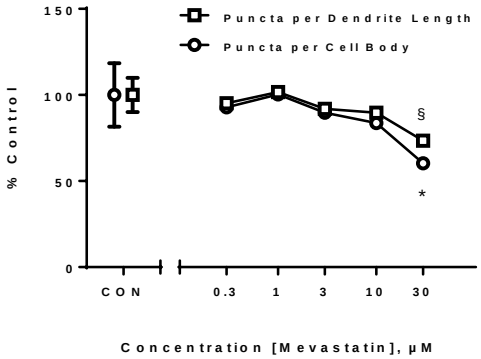




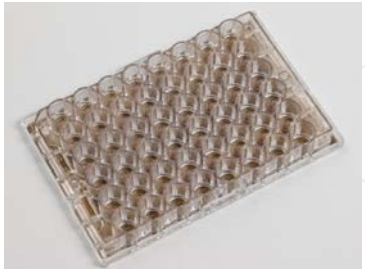
Synapses increase during development in vitro

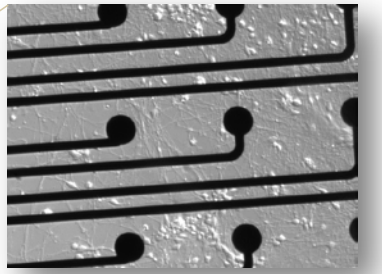


Chemical effect during critical period (DIV 9-15)



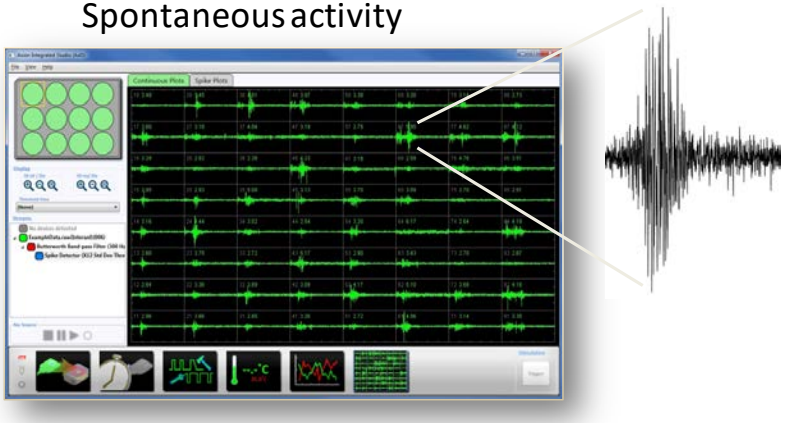
Developing a Cell-Based Assay for Neuronal Function



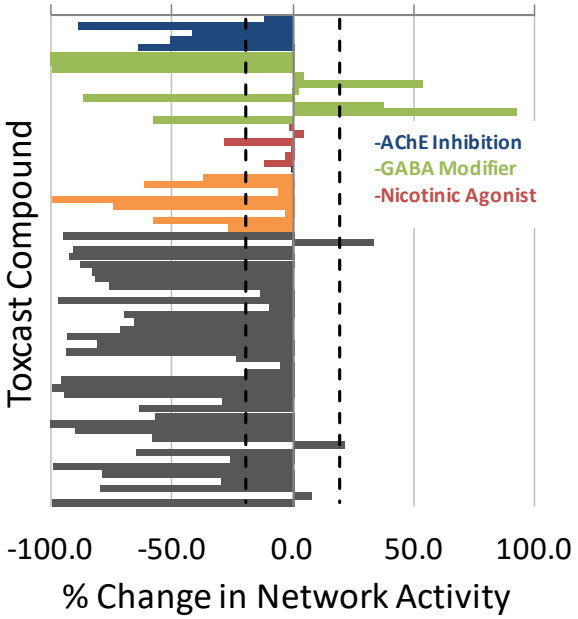


Primary cortical neurons are cultured in 48 well MEA plates

Spontaneous activity



Determine firing rate in each well:
60 min control and treated



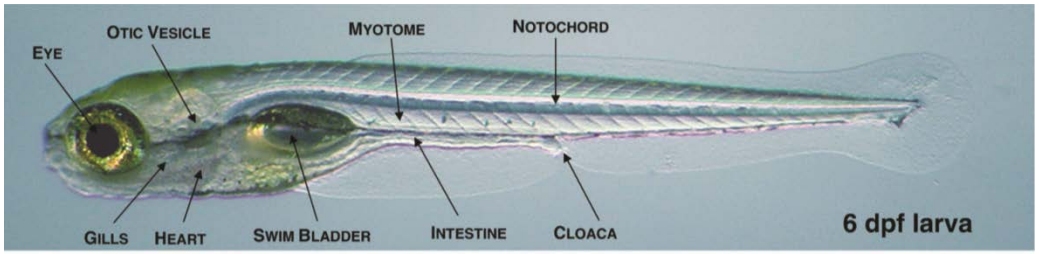
Zebrafish Model Development

Strengths

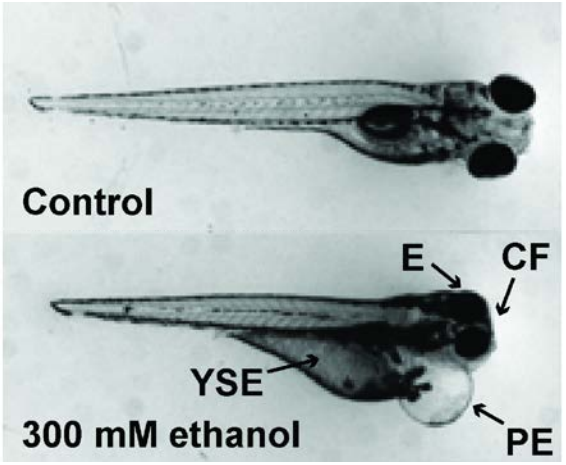
- Rapid development
- Transparent embryo
- Zebrafish have orthologs for 70% of human genes and 86% of 1318 human drug targets
- Genome is easy to manipulate
- Translational model for human- and eco- toxicology
- Apical endpoints, including functional assessments
- Metabolic capability
- Have tested >1000 chemicals

Weaknesses

- Difficult to assign causation without additional testing
- Internal dose of the chemical may not equal the waterborne dose



Airhart *et al.* (2007)



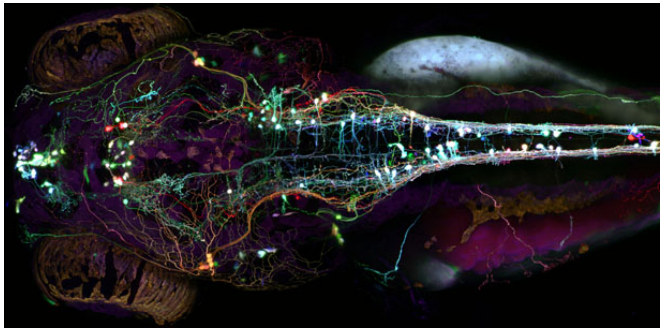
Tal *et al.* FASEB (2012)

Zebrafish Neurobehavioral Toxicity Assay

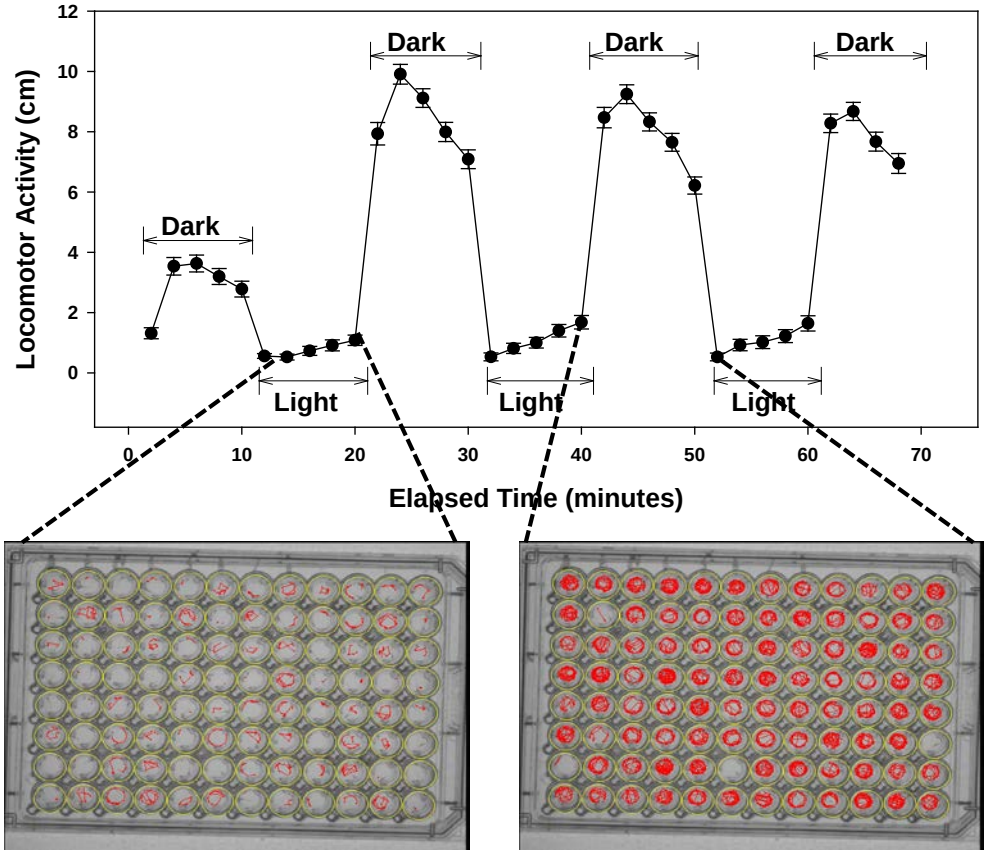
Spatial and temporal aspects of nervous system development include:

- Functional assessments
- Sensory assessments
- Learning and memory

} Behavior



"Brainbow" zebrafish



Using video tracking software, we measure the locomotion of 6 day old zebrafish larvae under different light and dark conditions. Zebrafish treated with neurotoxicants during development behave differently than control zebrafish.

Model ToxCast Application: High-Throughput Risk Assessment (HTRA)

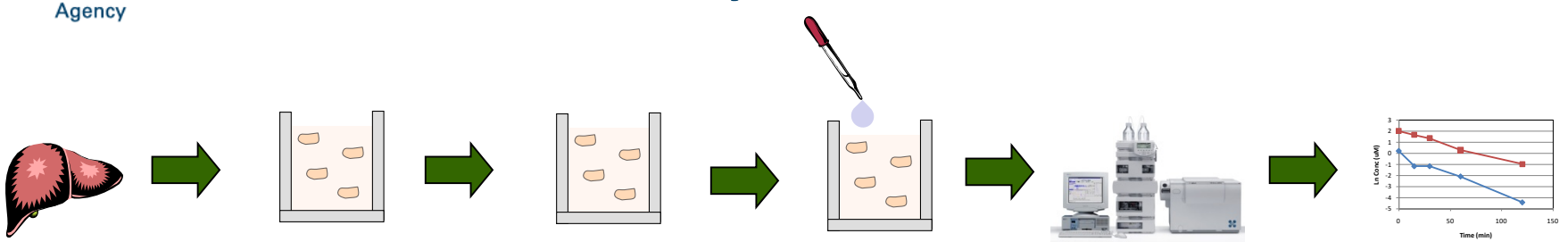
- Using HTS data for initial, rough risk assessment of data poor chemicals

- Risk assessment approach
  - Estimate upper dose that is still protective
  - In HTRA: BPAD (Biological Pathway Altering Dose)
  - Analogous to RfD, BMD
  - Compare to estimated steady state exposure levels

- Contributions of high-throughput methods
  - Focus on molecular pathways whose perturbation can lead to adversity
  - Screen 100s to 1000s of chemicals in HTS assays for those pathways
  - Estimate oral dose using High-Throughput pharmacokinetic modeling

- Incorporate population variability and uncertainty

Experimental Assays for Characterizing Steady-State Pharmacokinetics



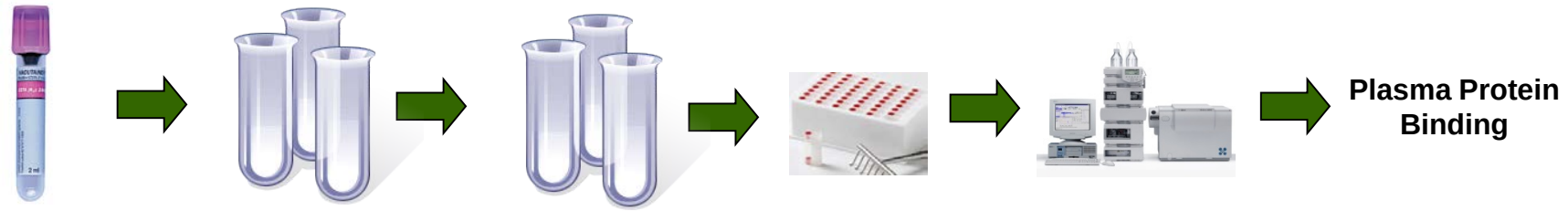
Human Hepatocytes (10 donor pool)

Add Chemical (1 and 10 μM)

Remove Aliquots at 15, 30, 60, 120 min

Analytical Chemistry

Hepatic Clearance



Human Plasma (6 donor pool)

Add Chemical (1 and 10 μM)

Equilibrium Dialysis

Analytical Chemistry

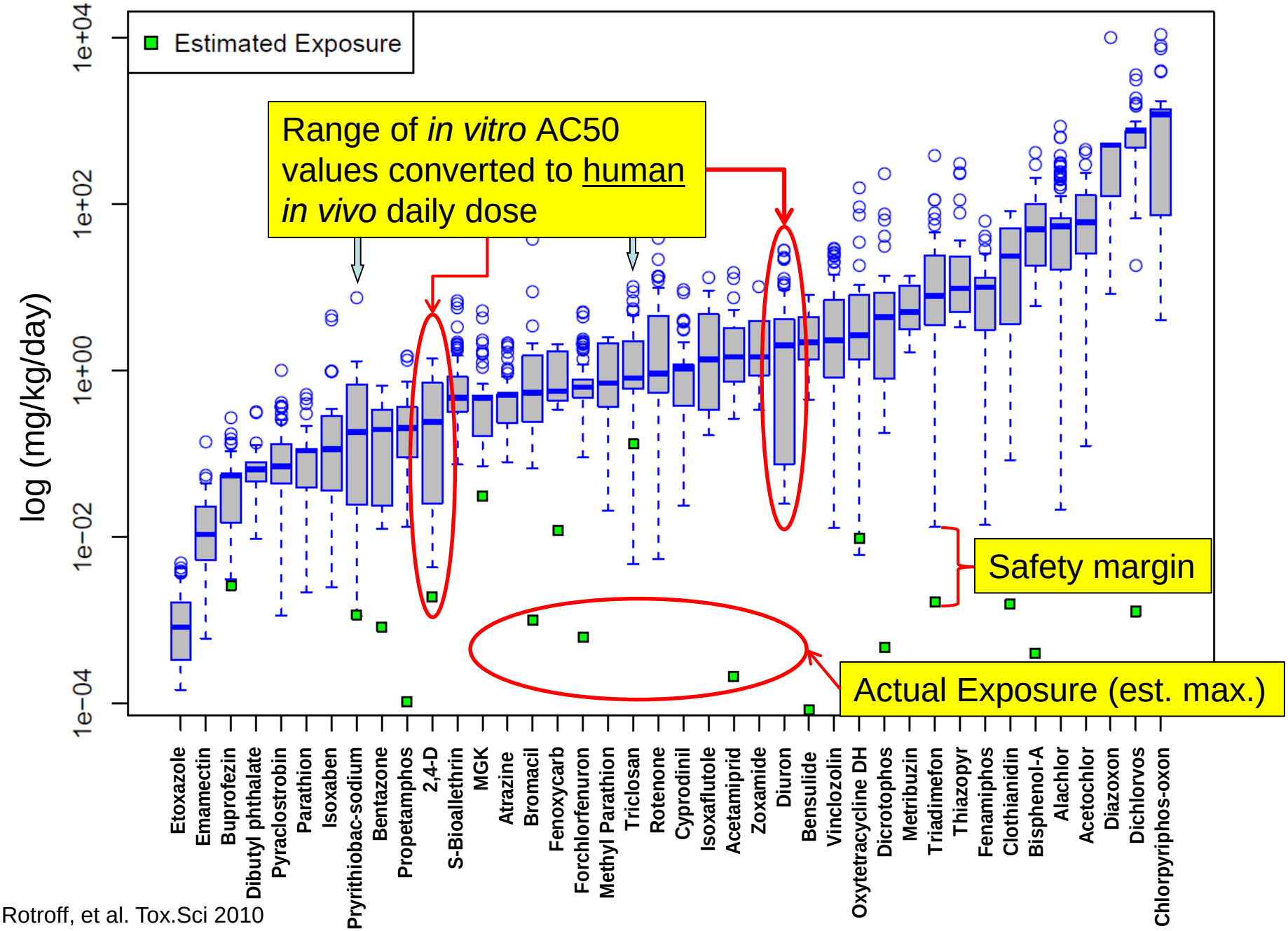
Plasma Protein Binding

Combine experimental data with PK Model to estimate dose-to-concentration scaling

"Reverse Toxicokinetics"

In collaboration with Hamner Institutes / Rusty Thomas

Combining *in vitro* activity and dosimetry



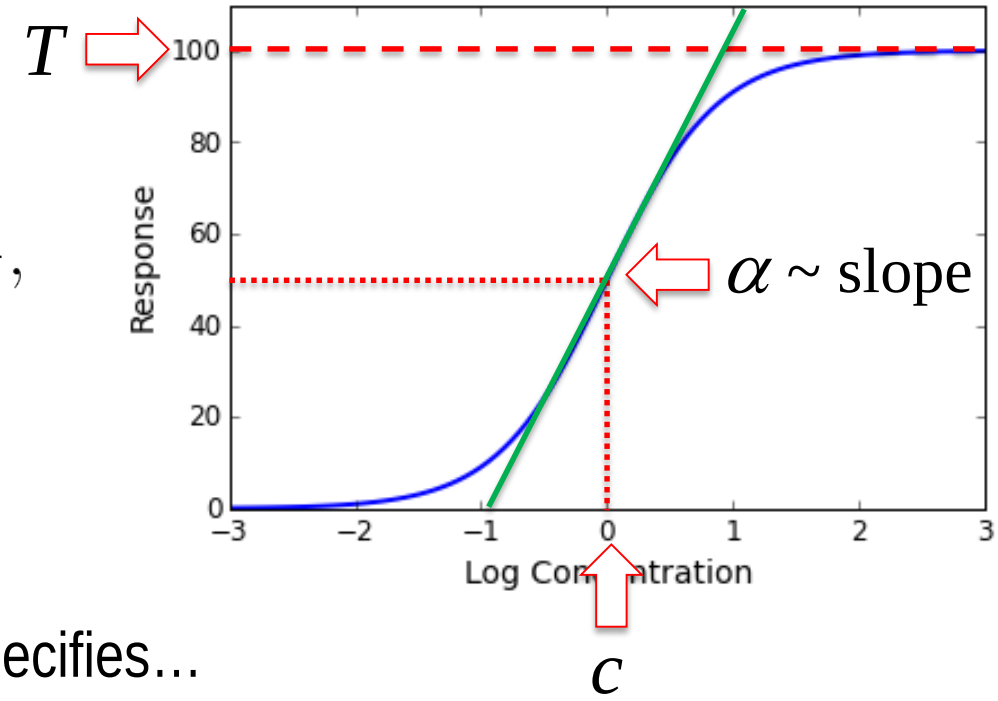
Rotroff, et al. Tox.Sci 2010

Hill Model Formulation

Response is given by

$$y = f(x; q) = \frac{T}{1 + 10^{\alpha(c-x)}},$$

where x is the log of the concentration considered.



Parameter vector $q = [T, c, \alpha]$ specifies…

- maximal response (T)
- half-maximal activity concentration (c)
- Hill slope (α)

HTRA Summary

1. Select toxicity-related pathways
2. Develop assays to probe them
3. Estimate concentration at which pathway is “altered” (PD)
4. Estimate *in vitro* to *in vivo* PK scaling
5. Estimate PK and PD uncertainty and variability
6. Combine to get BPAD distribution and health protective exposure limit estimate (BPADL)

- Many (better) variants can be developed for each step (1-6)
- Use for analysis and prioritization of data-poor chemicals

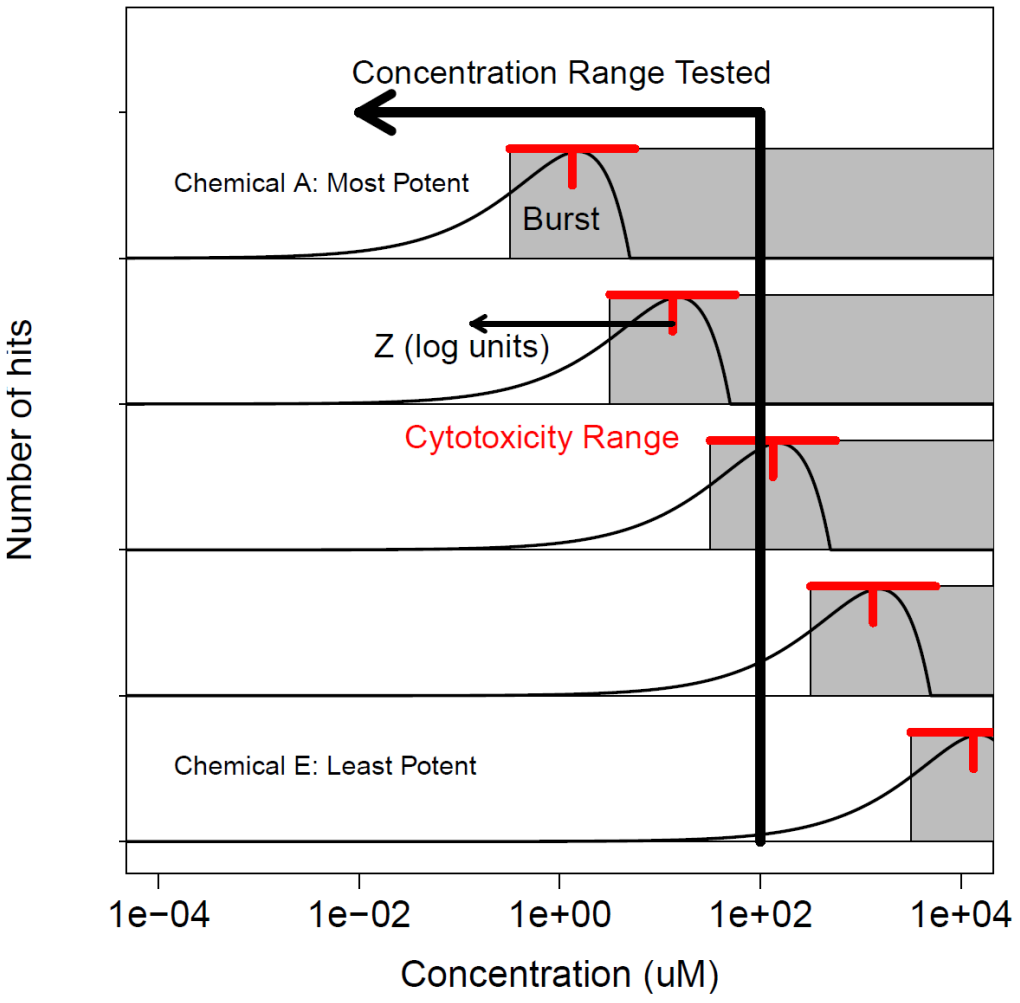
HTTK: High-throughput TK models

- Open-source R package `httk`, available on CRAN (Pearce *et al.*, submitted to *J Stat Soft*)
- General TK models can be parameterized for many chemicals using HT *in vitro* assays
  - At present, 554 chemicals
- General TK models:
  - 1-compartment
  - 3-compartment
  - PBTK (physiologically-based TK)
  - 3-compartment steady-state

HTTK parameters

Chemical-specific parameters	
Fraction unbound in plasma (Fub) Intrinsic clearance rate (CLint)	Measured in HT *in vitro* assays (Wetmore *et al.* 2012, 2014, 2015)
Tissue-plasma partition coefficients	Predicted from phys-chem properties; not included in 3-compartment steady-state model
Physiological parameters	
Body weight Tissue volumes & blood flows Glomerular filtration rate (GFR) Hematocrit Hepatocellularity	By default: “average” human values

Most chemicals display a “burst” of activity at same concentration as cytotoxicity

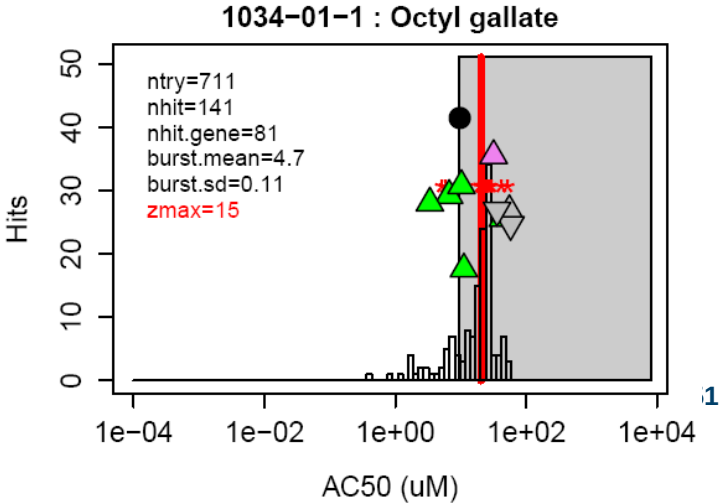


Most chemicals cause activity in many assays near the cytotoxicity threshold

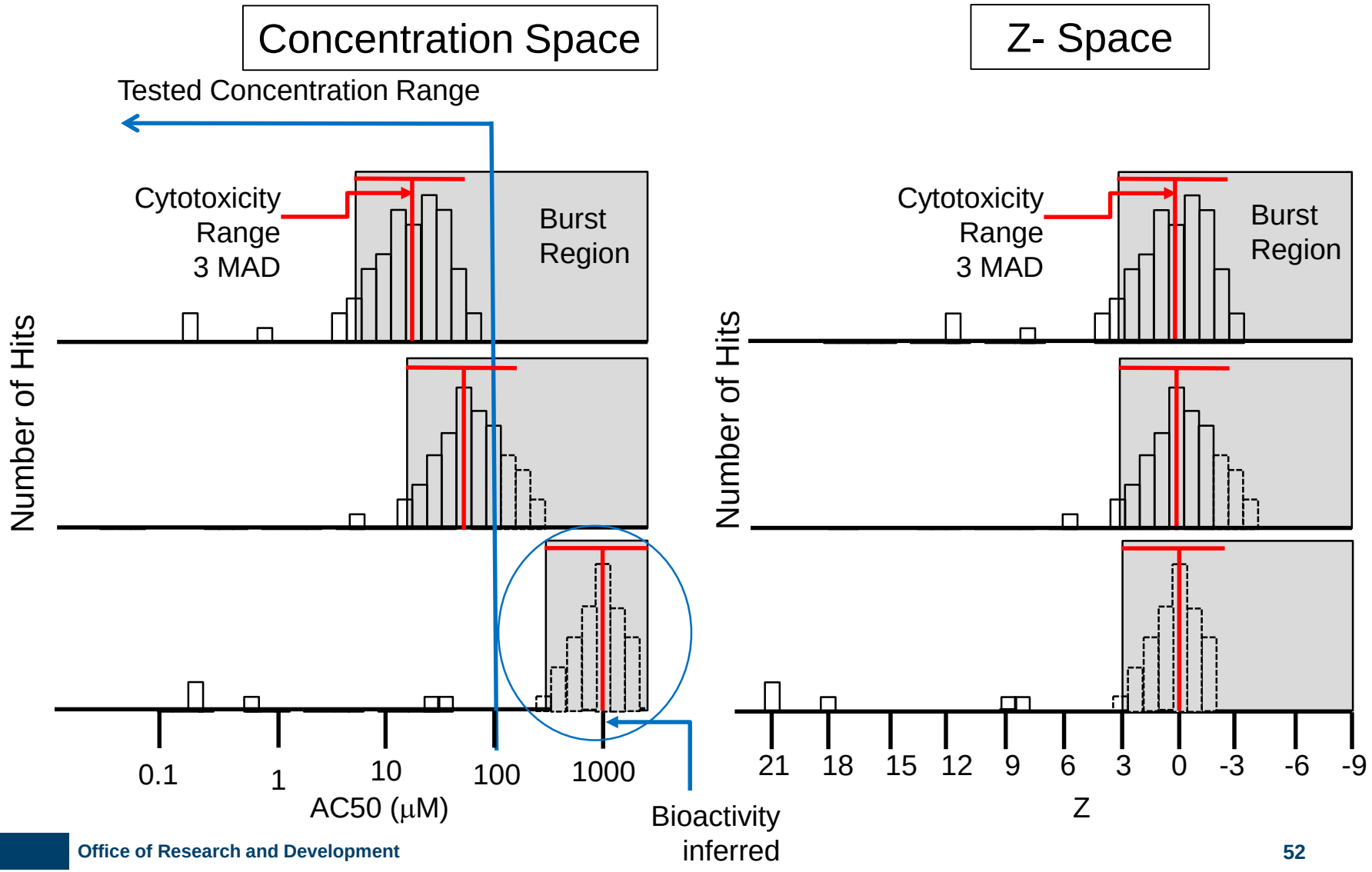
Cell-stress related assay interference

“Hit” (AC50) in burst region is less likely to result from specific activity (e.g. binding to receptor or enzyme)

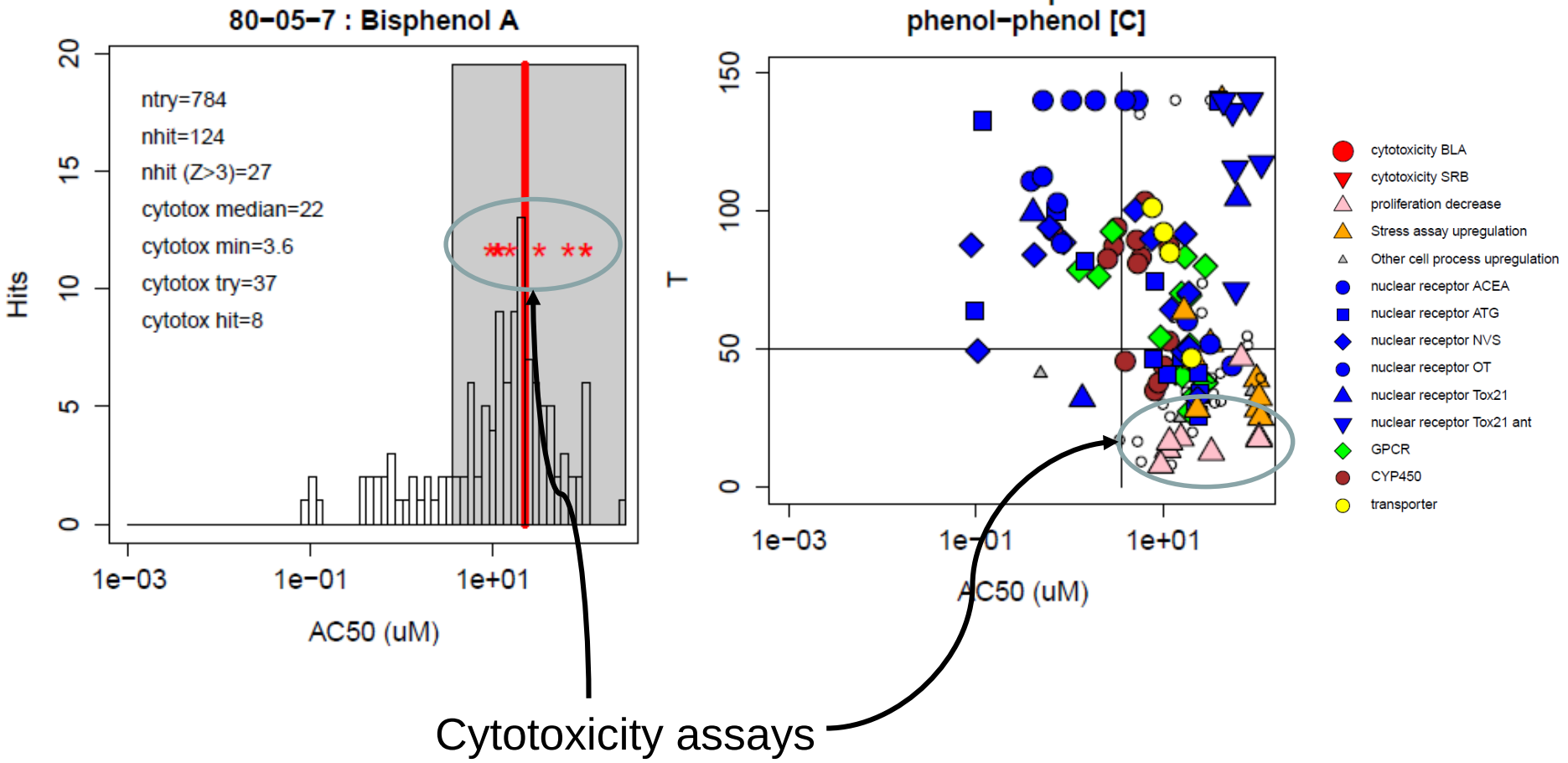
Z-score: # of SD from burst center
-High Z: more likely to be specific
-Low Z: less likely to be specific



Most chemicals display a “burst” of potentially non-selective bioactivity: Caused by cell-stress / cytotoxity



Example of burst bioactivity by chemical



Weight-of-Evidence (WOE) Approach

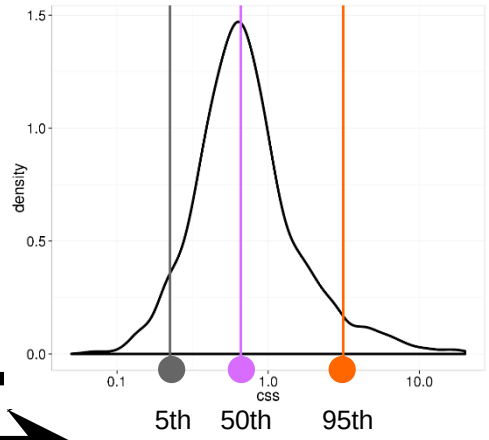
- All data is noisy
- All assays have false positives / negatives
- Using multiple assays can solve the positive / negative quandary
  - Qualitative uncertainty decreases
  - Quantitative (potency) uncertainty may increase

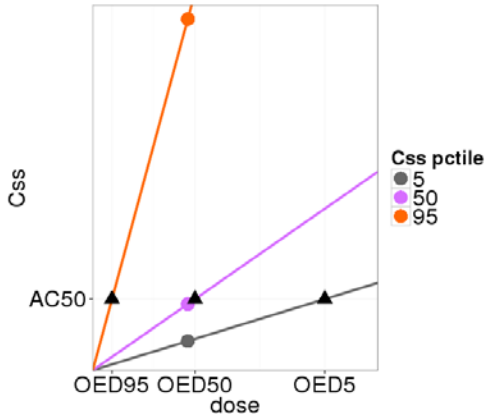
Estimating Variability in Chemical Exposure

Same dose of a given chemical

HTTK model parameters representing each individual

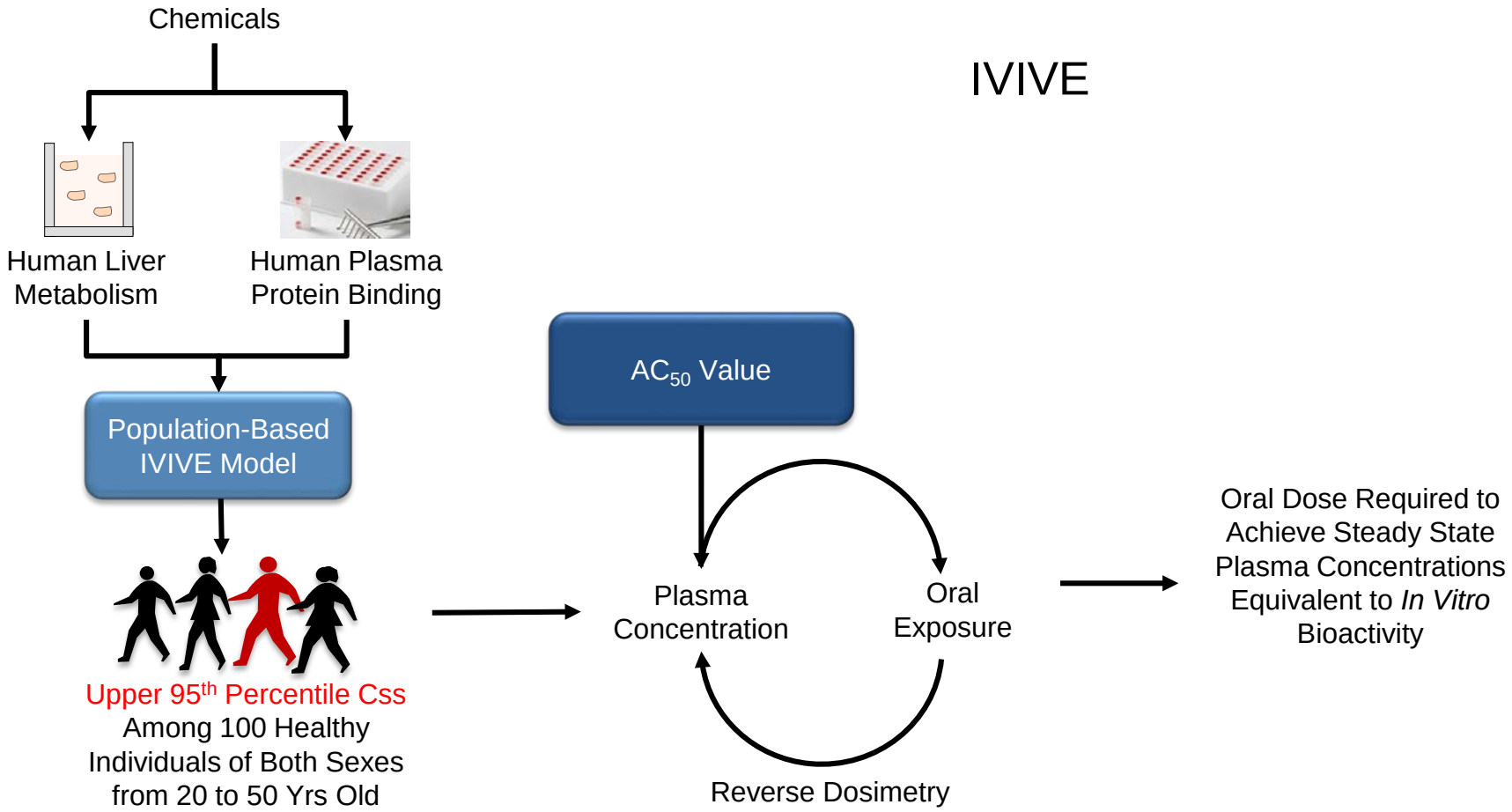
Varying C_{ss}





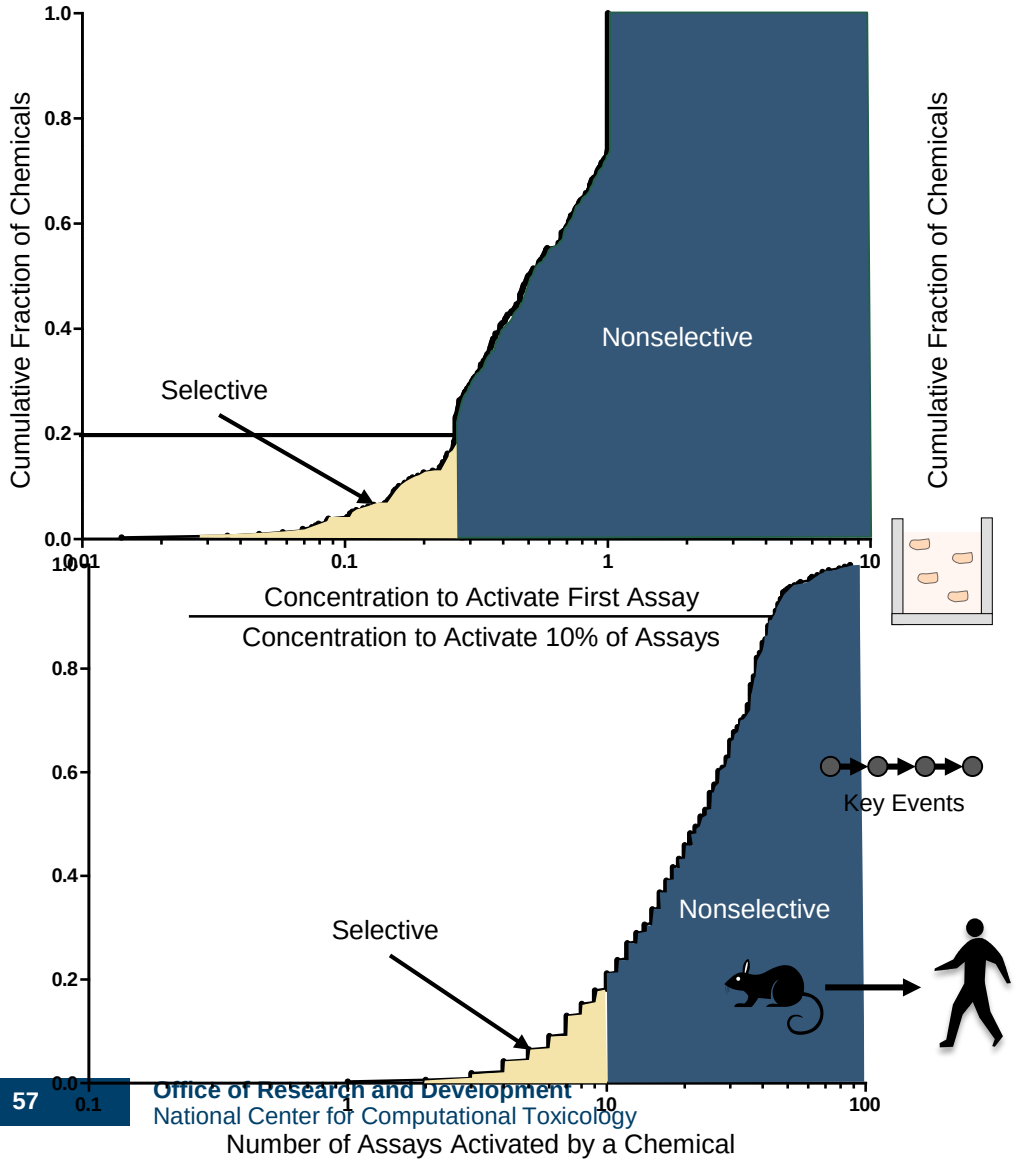
Css95 = conservative OED

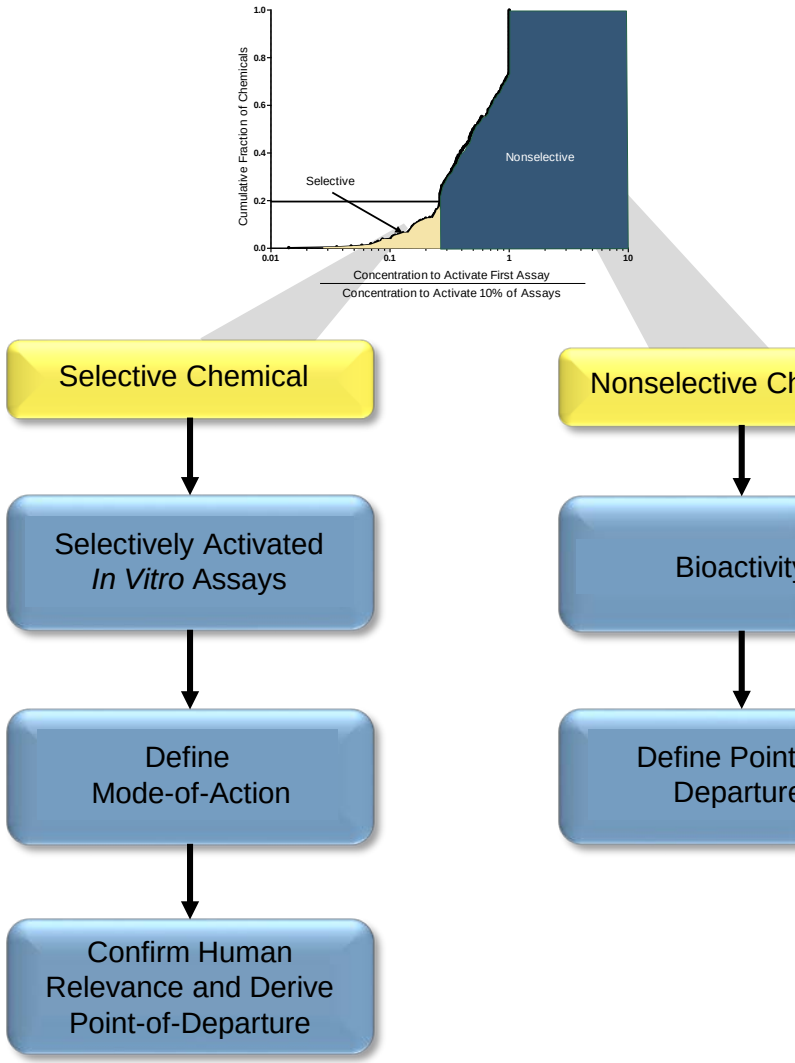
Dose-response: Extrapolating in vitro dose to in vivo analysis



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

Risk characterization – utility of HT approaches





Office of Research and Development
National Center for Computational Toxicology

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