EPA United States Environmental Protection Agency

The Accidental Toxicologist
A Career in the Science of Poisons

John Cowden
U.S. EPA, National Center for Computational Toxicology
Office of Research and Development



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.

What is Toxicology?

United States Environmental Protection Agency

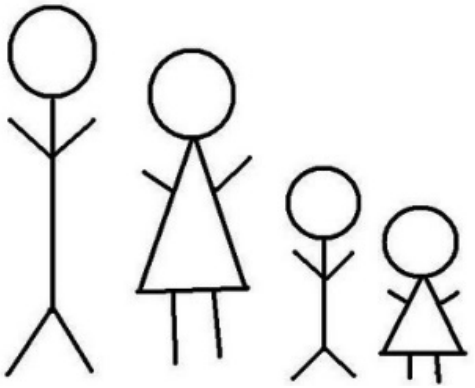
Study of the adverse *effects* of *chemicals* on living *organisms*

Exposure → Dose → Effects



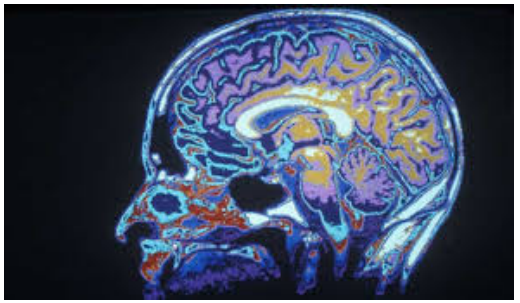










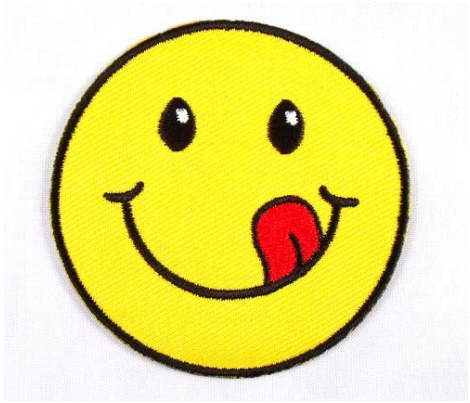






The *dose* makes the poison…





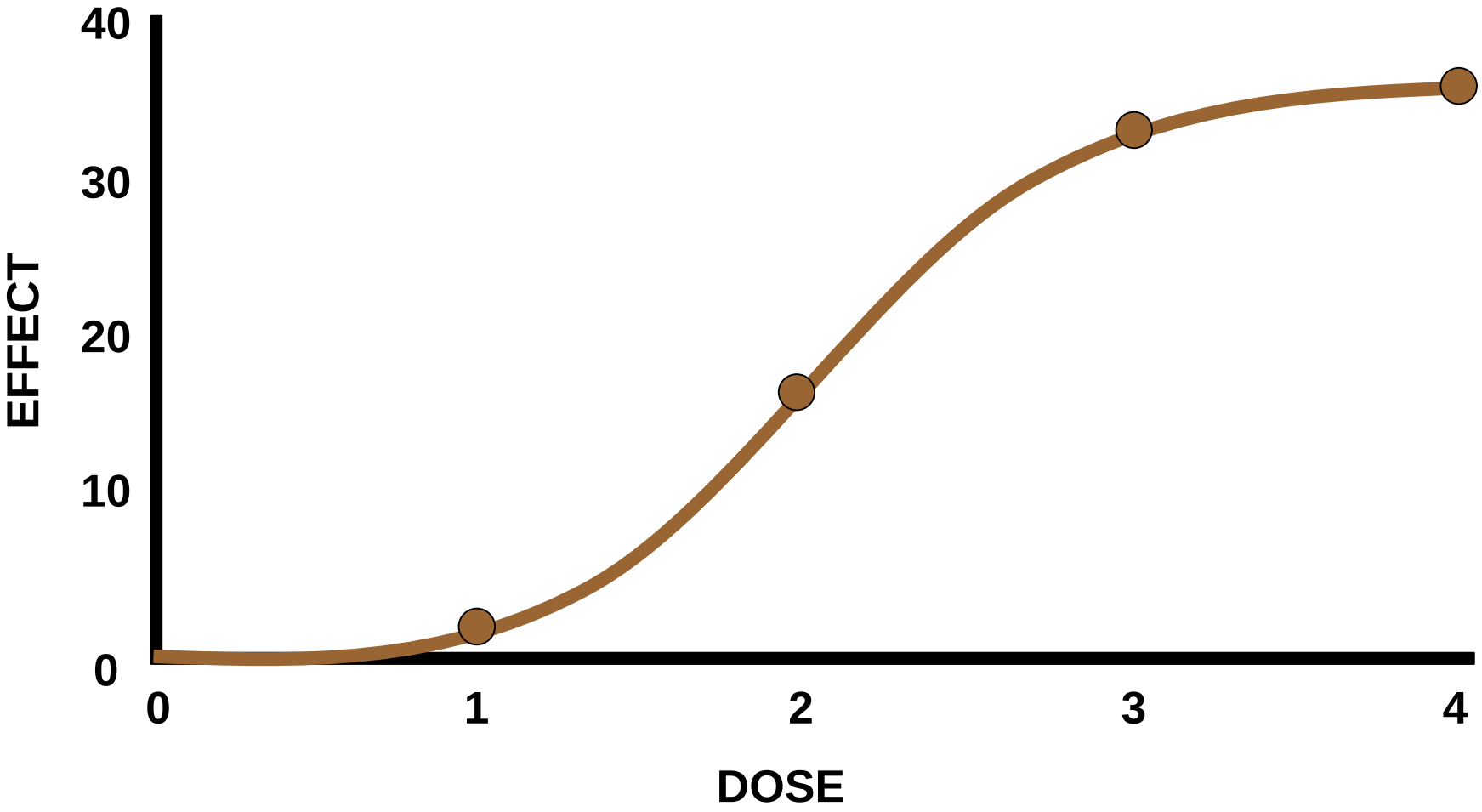




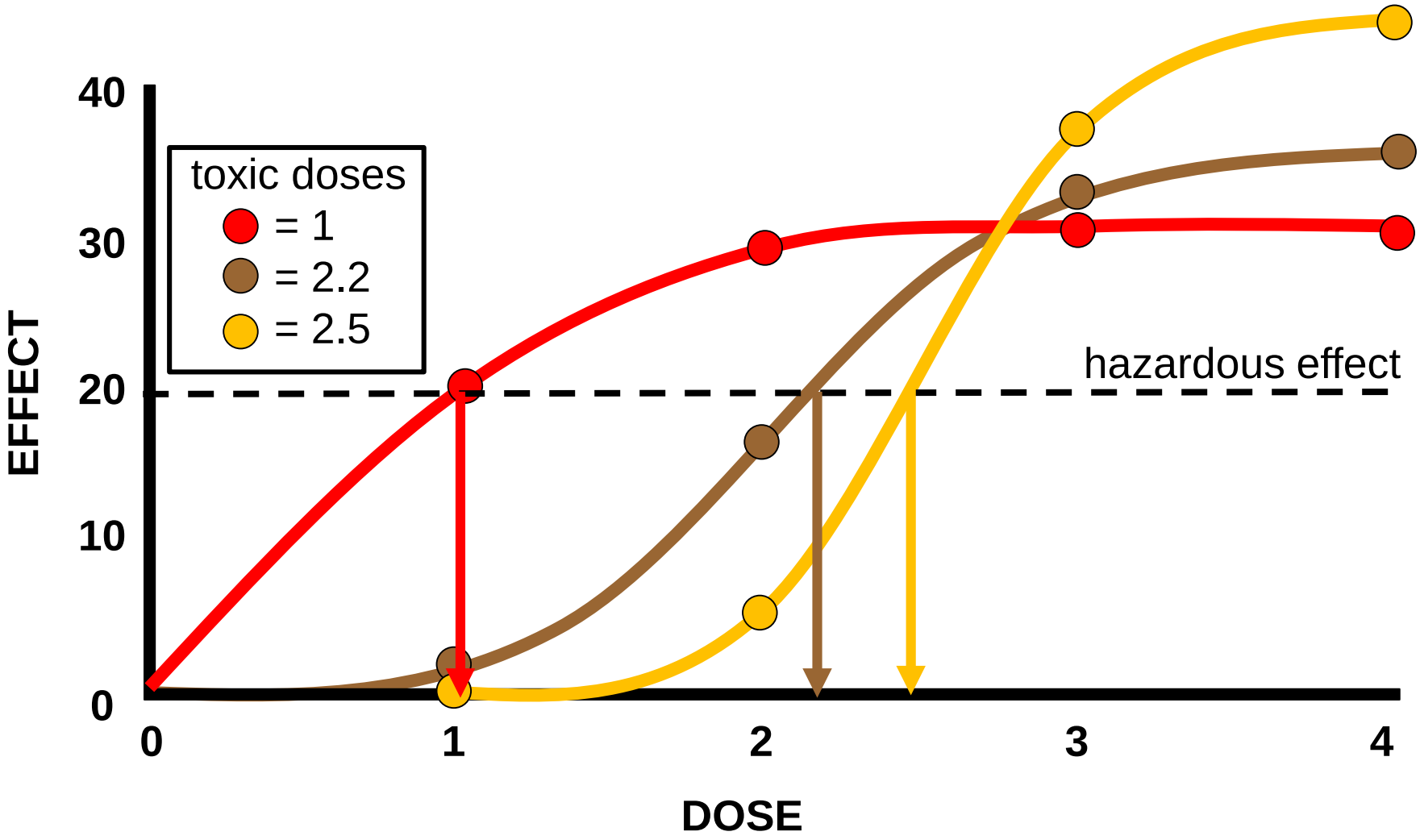
Office of Research and Development
National Center for Computational Toxicology

… but *what* dose?

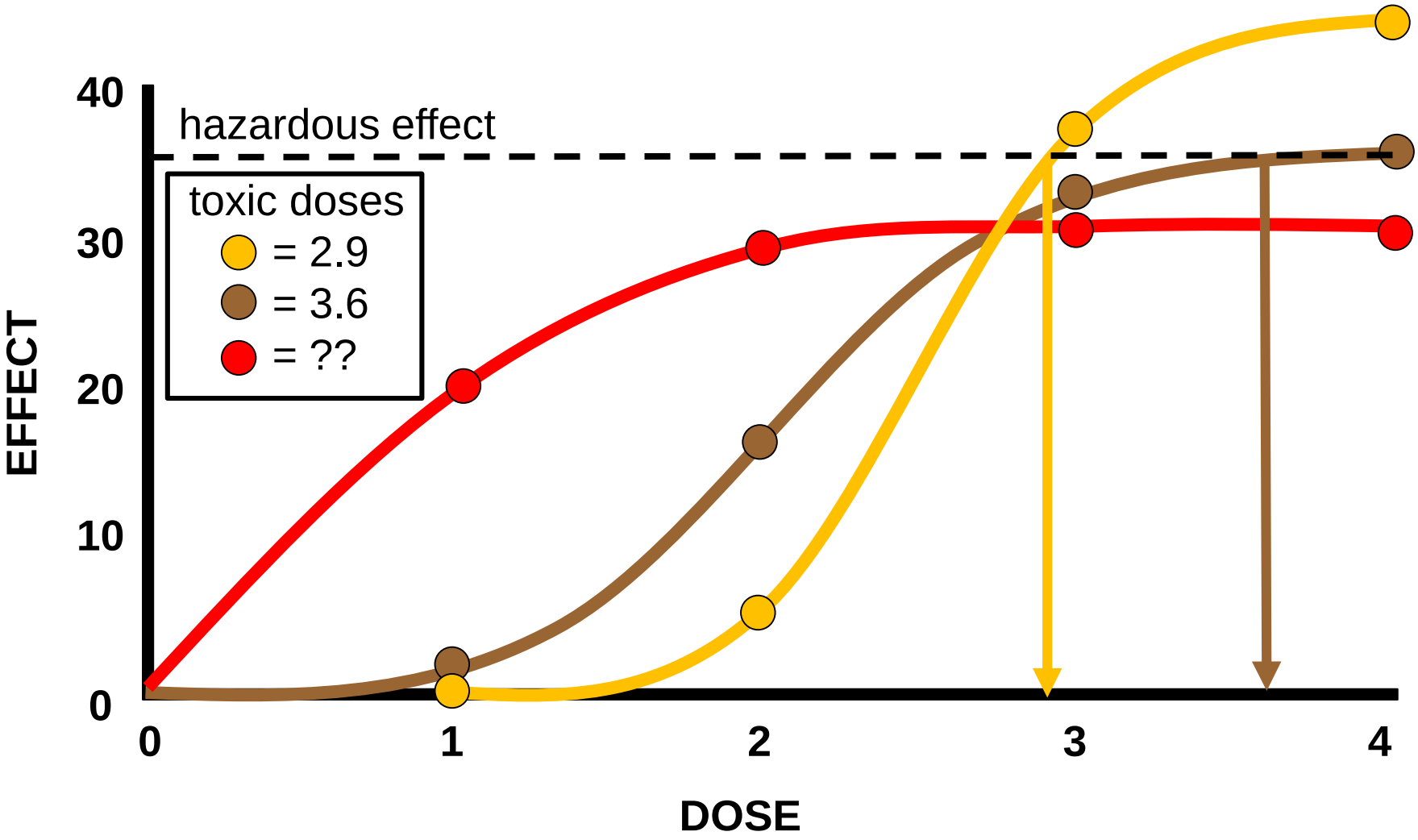
Dose Response Analysis



Determining Hazard



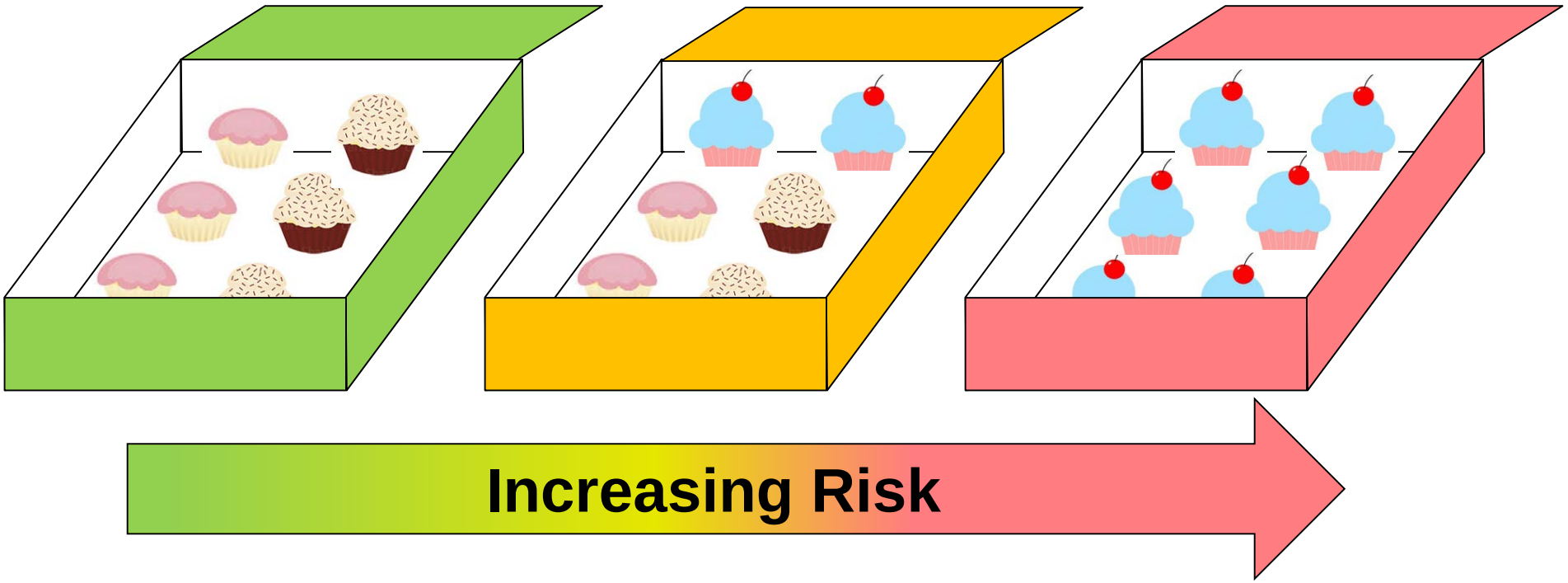
Determining Hazard



Determining Risk

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

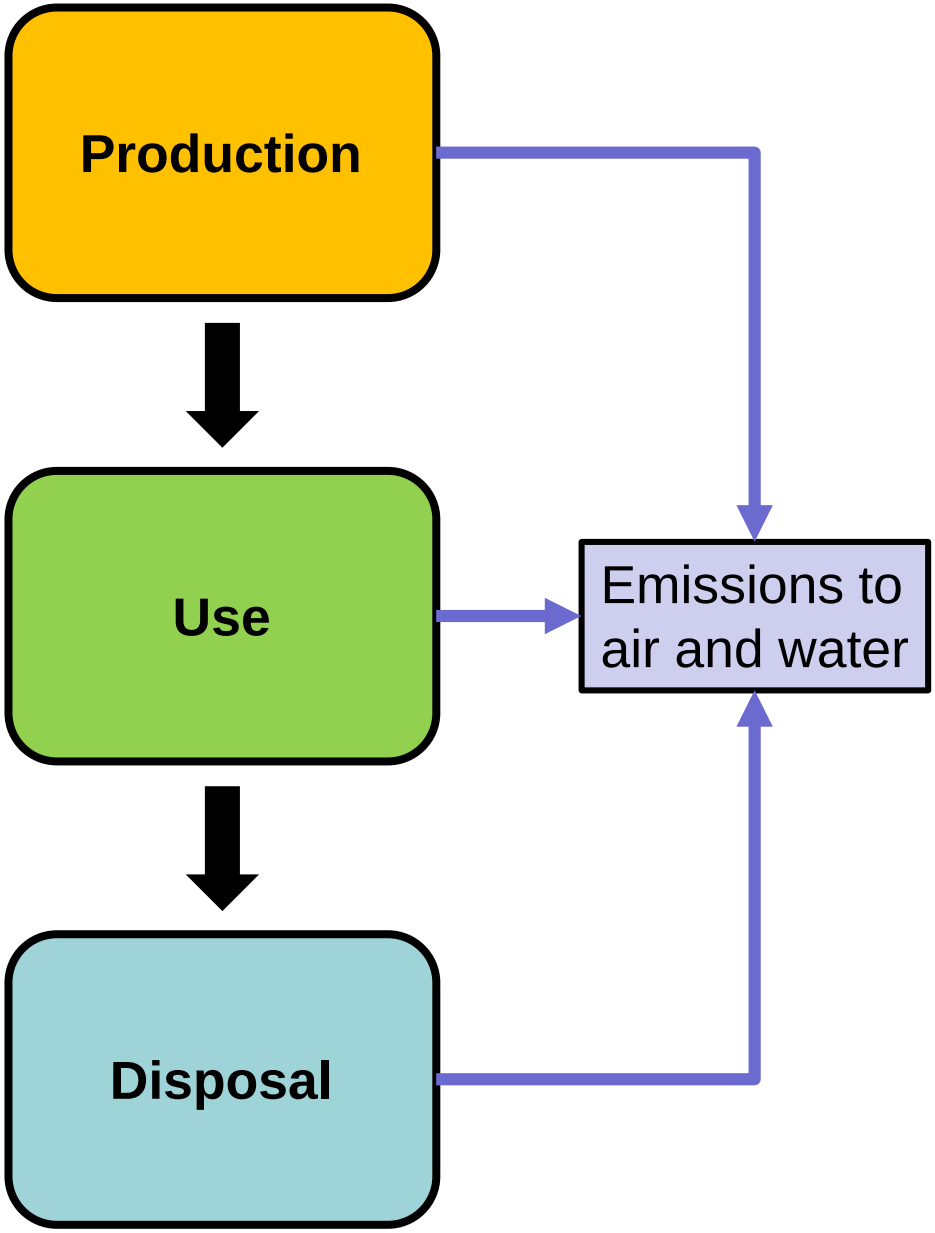
Risk = f (Hazard x Exposure)



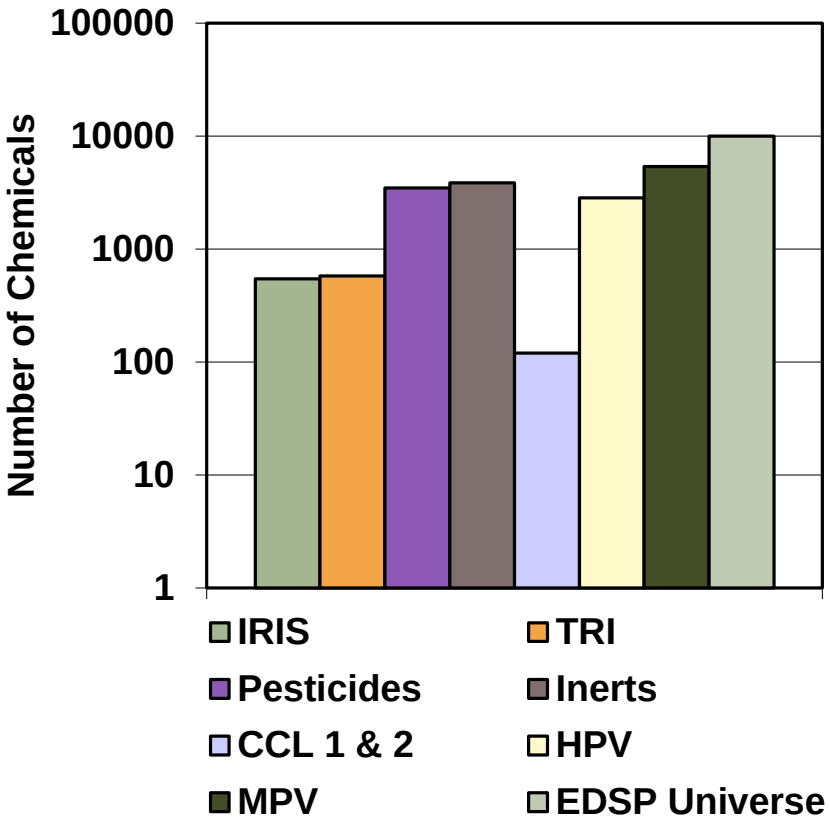
A challenge for regulatory toxicologists

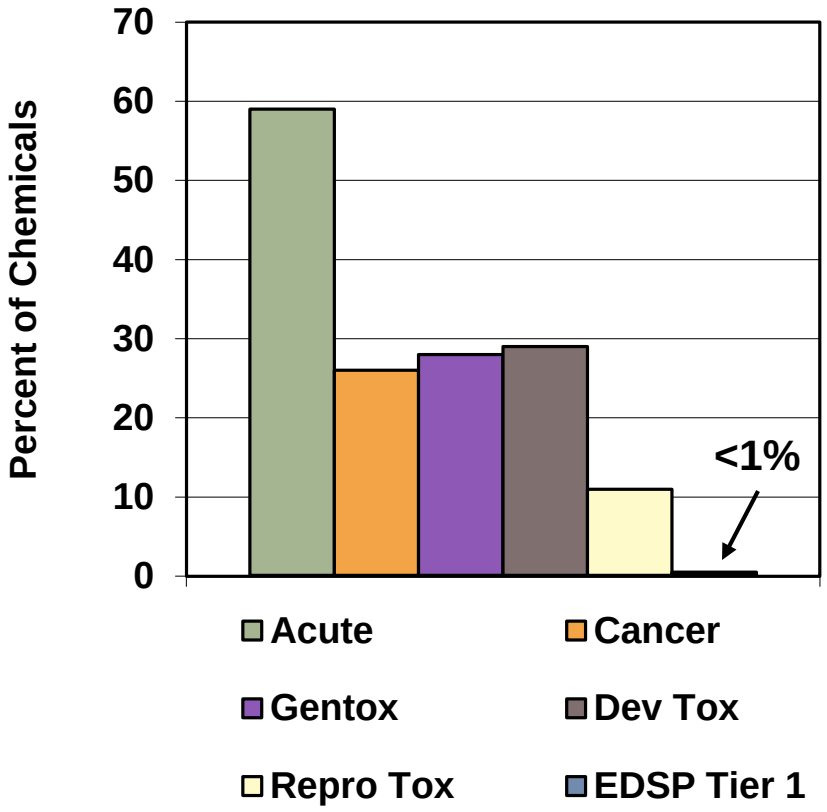
EPA United States Environmental Protection Agency





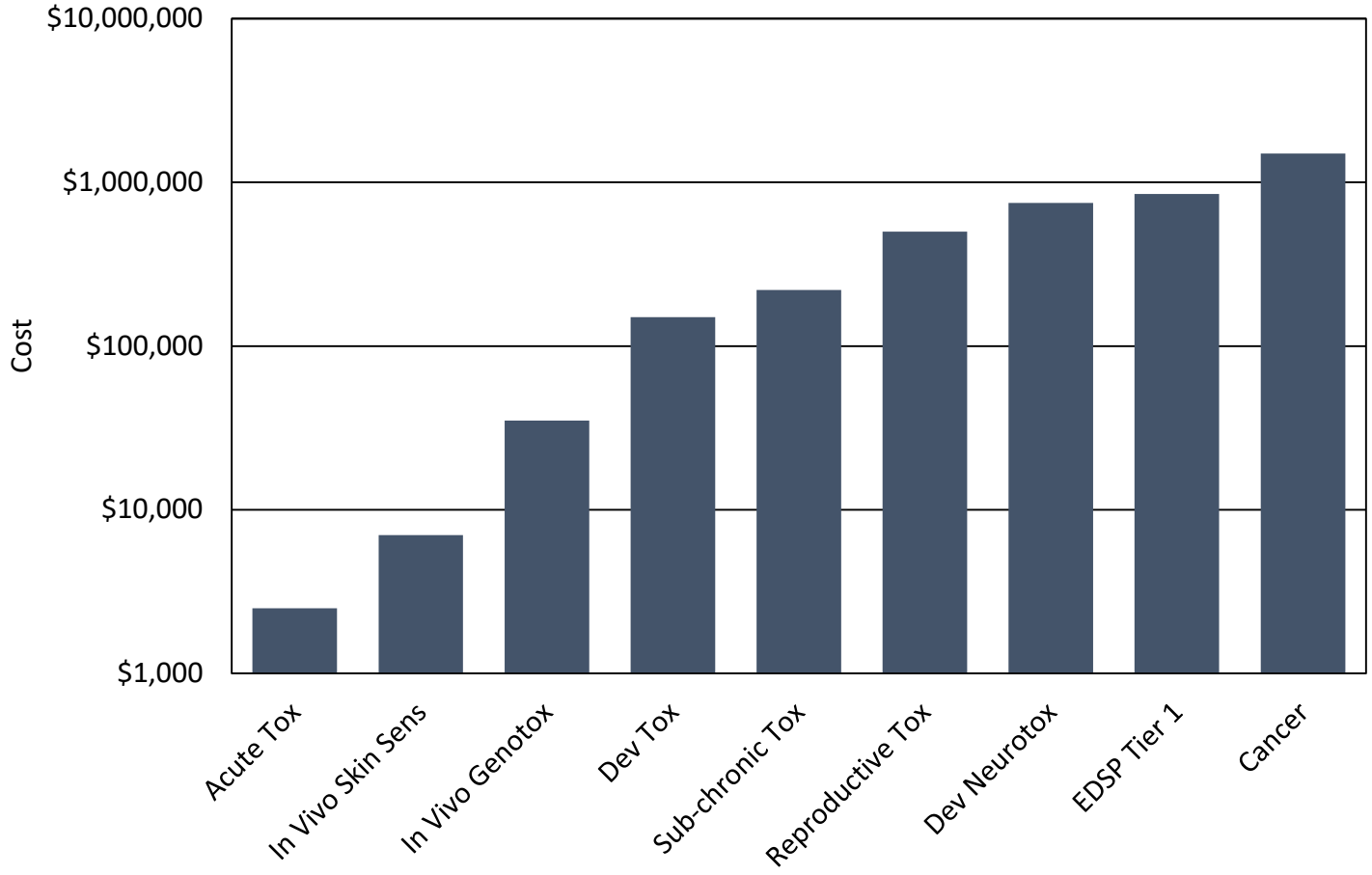
Too many chemicals, not enough data



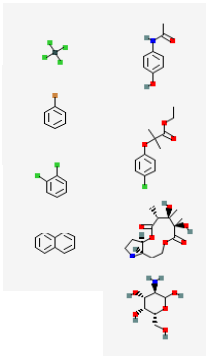


Modified from Judson, et al *EHP* (2010)

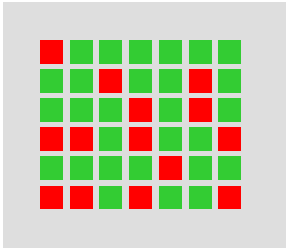
Economic cost of current test methods



Computational Toxicology Approaches



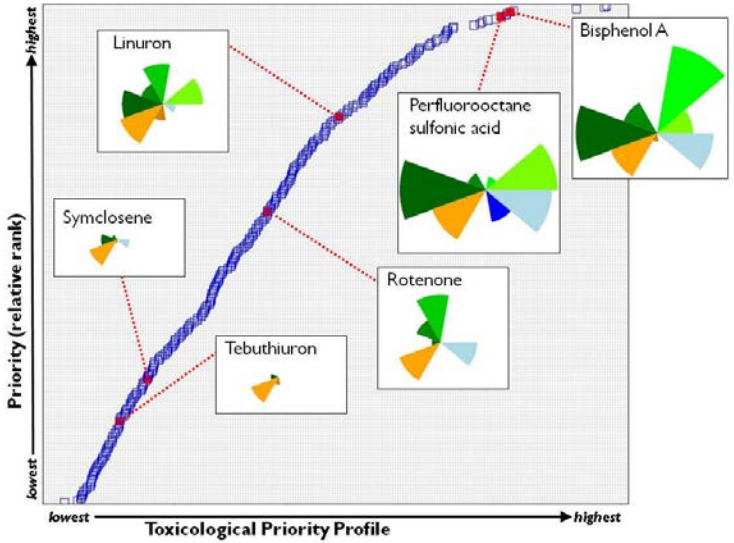
Thousands of chemicals



High throughput biology and chemistry



Bioinformatics/ machine Learning



Predictive toxicology

Benefits

- Less expensive
- More chemicals faster
- Fewer animals

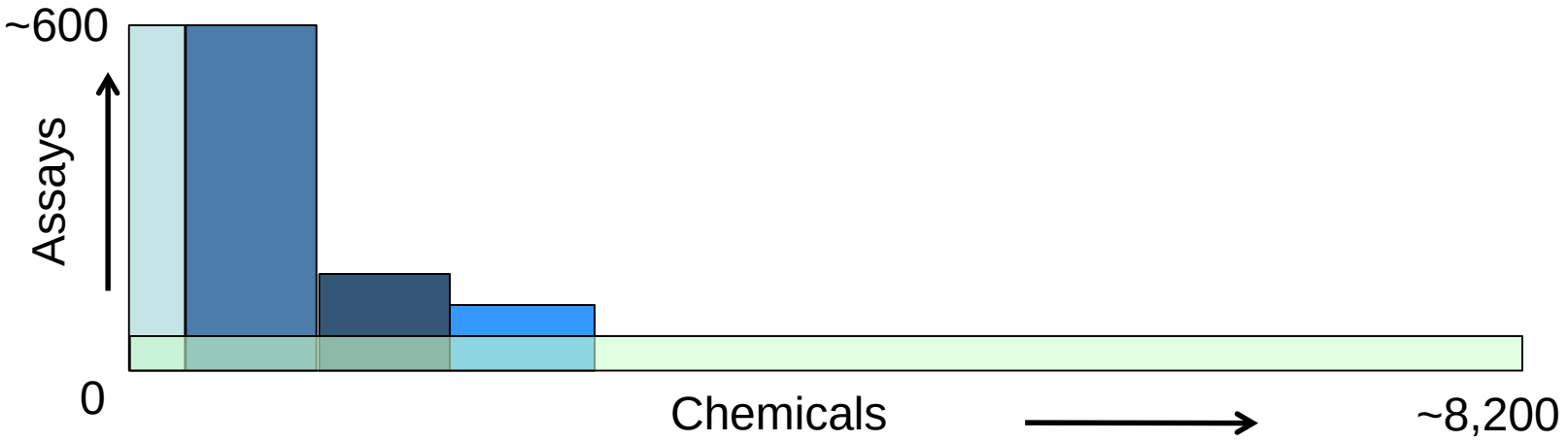
EPA United States Environmental Protection Agency

High-Throughput Screening for Toxicity Testing



Collaborative and Complementary Approaches to Chemical Screening

	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



High Throughput Exposure Predictions



= hazardous dose



= exposure estimate

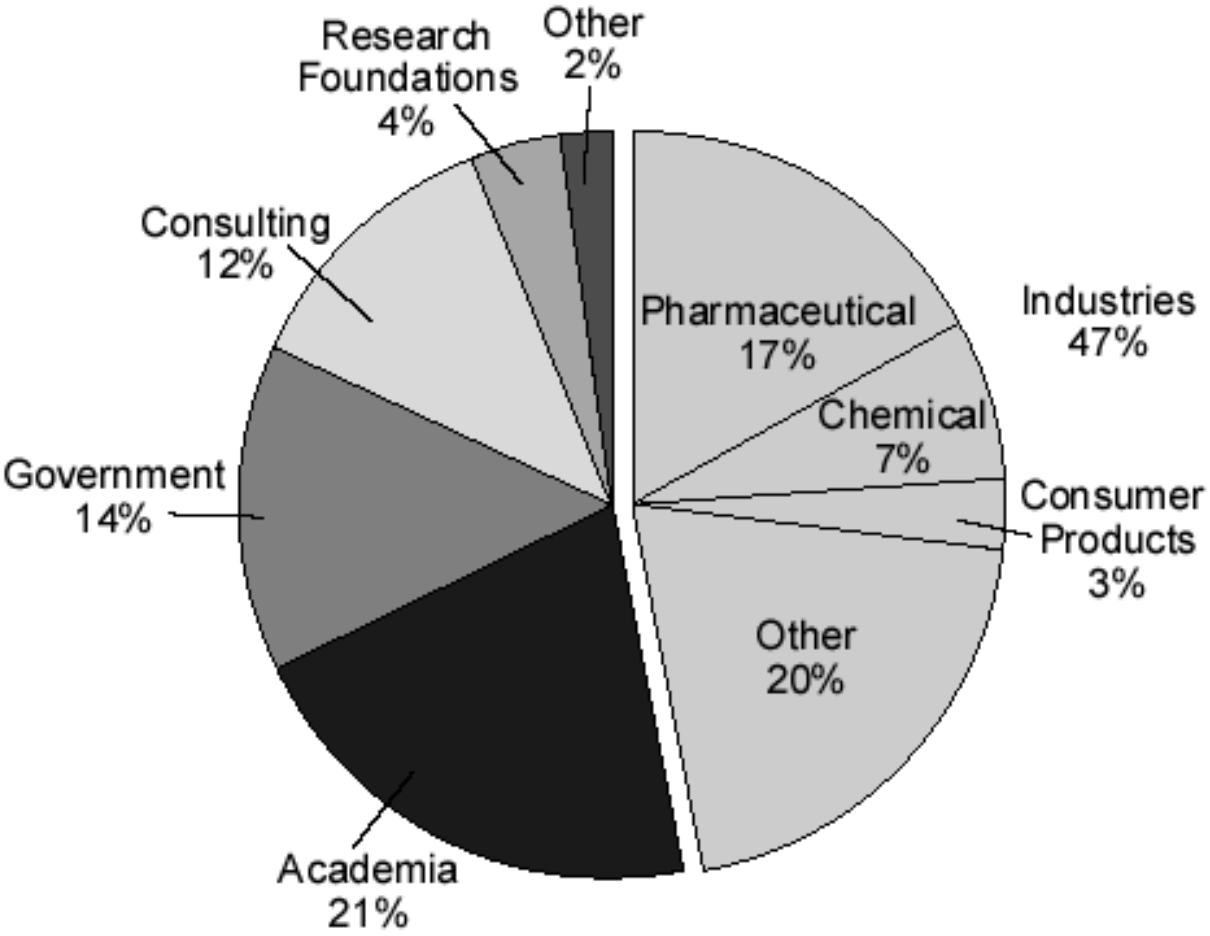
Wetmore *et al*. (2012)

Risk = f (Hazard x Exposure)

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.

Careers in Toxicology



www.toxicology.org

Preparing to become a Toxicologist

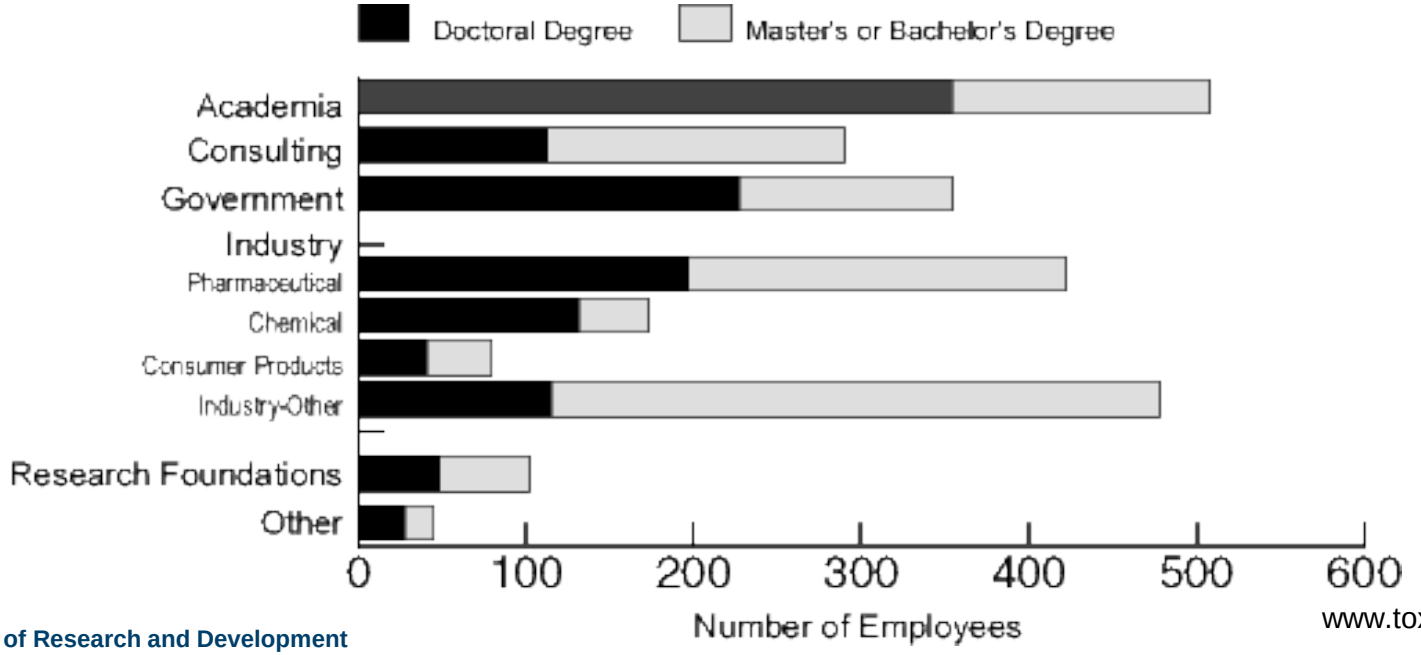
United States Environmental Protection Agency

Career Skills

- Strong communication and written
- Computer knowledge
- Good laboratory practice
- Project management
- Statistics experience

Education

- Computer science
- Math
- Biology
- Chemistry
- Toxicology
- Biochemistry
- Physics
- Statistics
- Pharmacology
- ***RESEARCH***



www.toxicology.org

Your career as a Toxicologist

Challenges for future Toxicologists

- Mixtures = Real world exposures
- Episodic exposures
- Biological plausibility and statistical significance
- Mechanisms of action
- Differential susceptibility
- Human relevance of non-animal models

Summary and Resources

What do toxicologists do?

- Determine the potential harmful effects of chemicals and the dose that will cause these effects.

Where do toxicologists work?

- Industry, academia, and government

How much more school?

- Post-baccalaureate degrees

Resources

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

EPA
United States
Environmental Protection
Agency

Questions?



United States Environmental Protection Agency

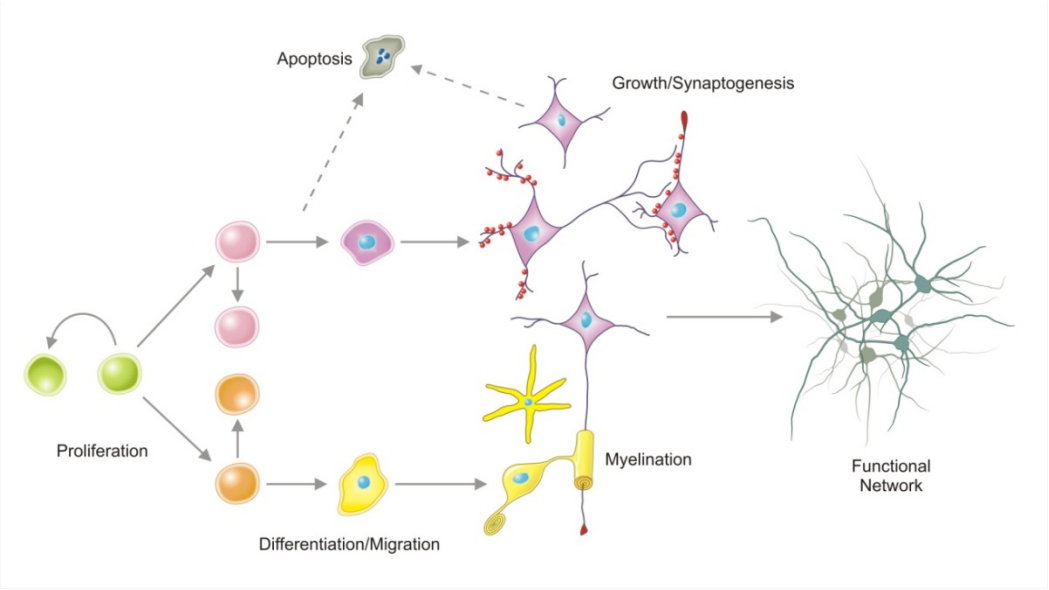
Extra slides

Application to 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)

Cell-Based Assays for Developmental Neurotoxicity

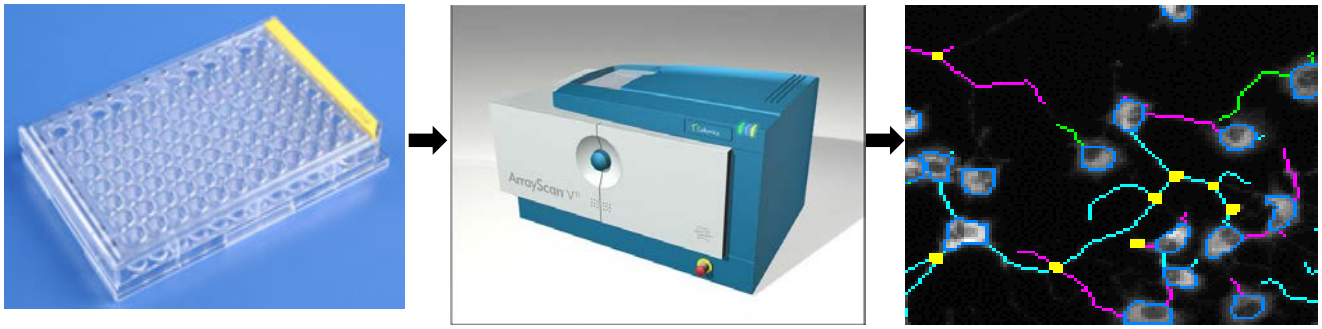


In Vitro Assays

- 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

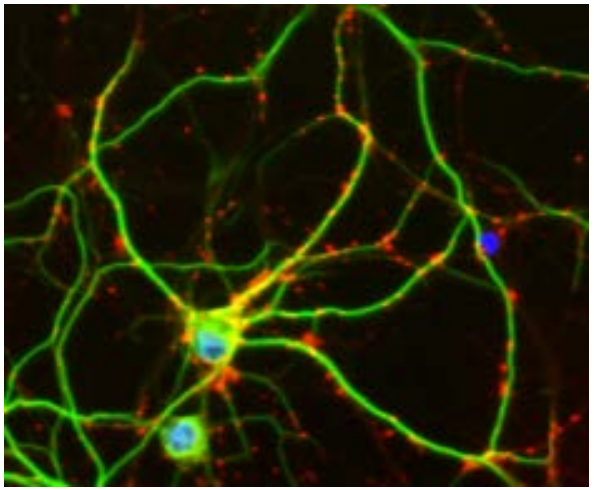


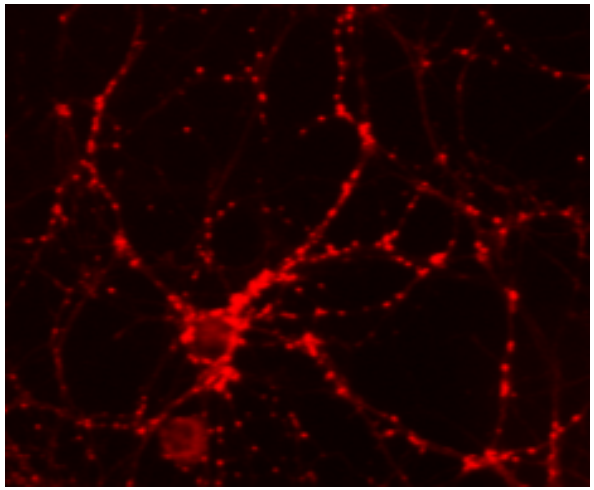
An Example with a Cell-Based Assay for Synaptogenesis

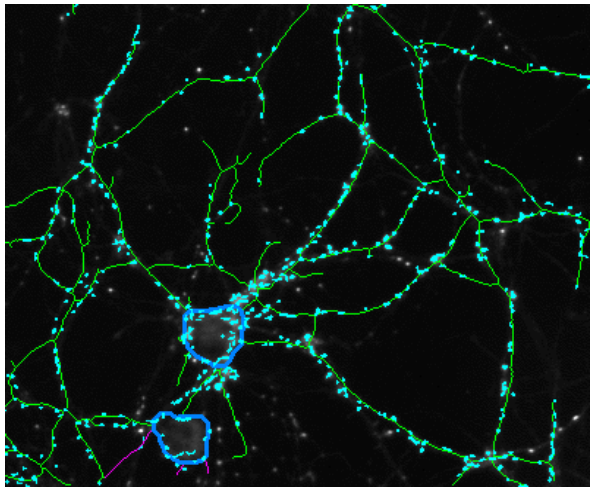
Synaptogenesis (formation of connections critical to a neural network)

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

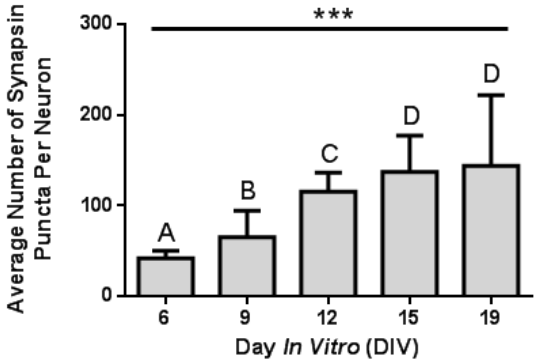
High Content Image showing identified neurites and synapses



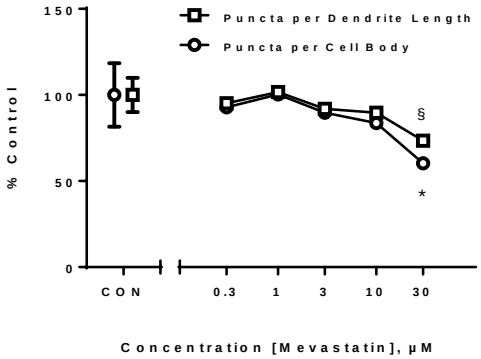




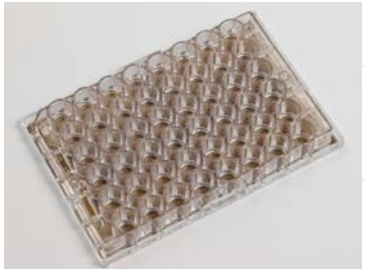
Synapses increase during development in vitro

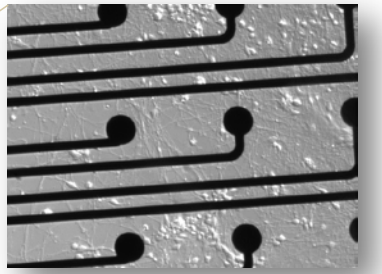


Chemical effect during critical period (DIV 9-15)



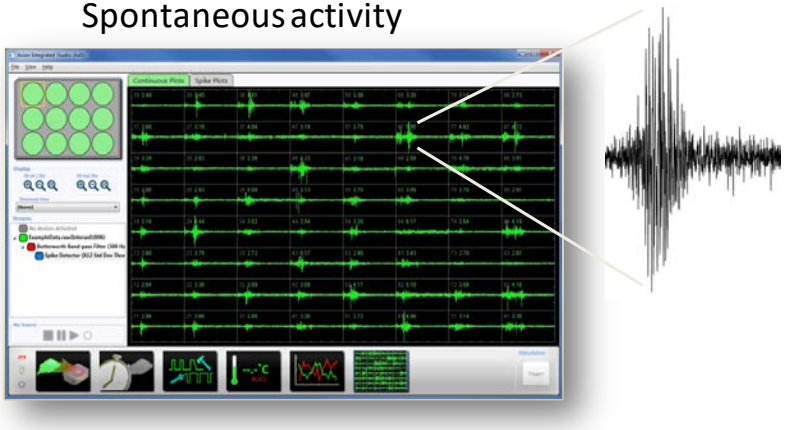
Developing a Cell-Based Assay for Neuronal Function



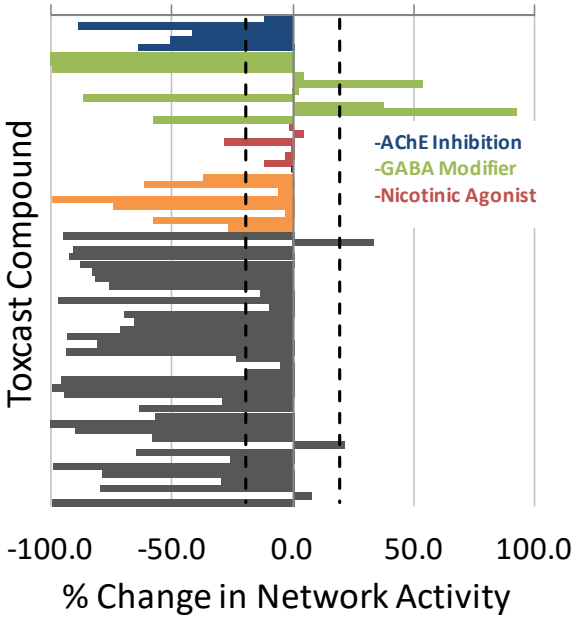


Primary cortical neurons are cultured in 48 well MEA plates

Spontaneous activity



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



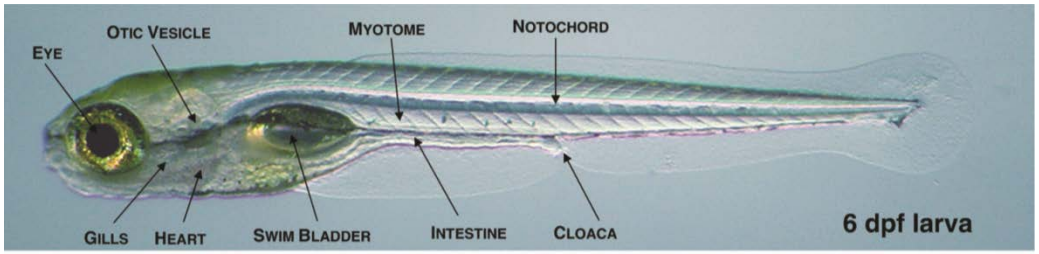
Zebrafish Model Development

Strengths

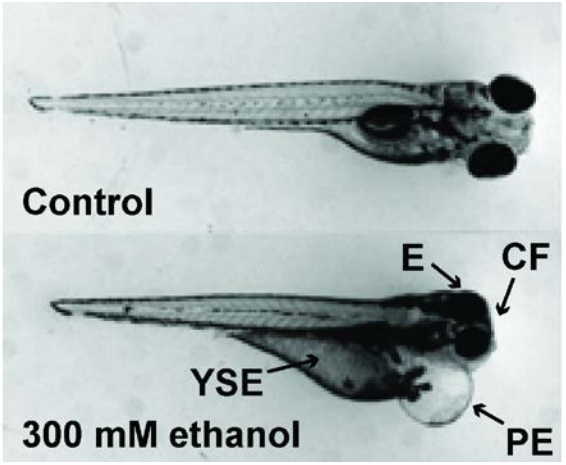
- Rapid development
- Transparent embryo
- Zebrafish have orthologs for 70% of human genes and 86% of 1318 human drug targets
- Genome is easy to manipulate
- Translational model for human- and eco- toxicology
- Apical endpoints, including functional assessments
- Metabolic capability
- Have tested >1000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

Weaknesses

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



Airhart *et al.* (2007)



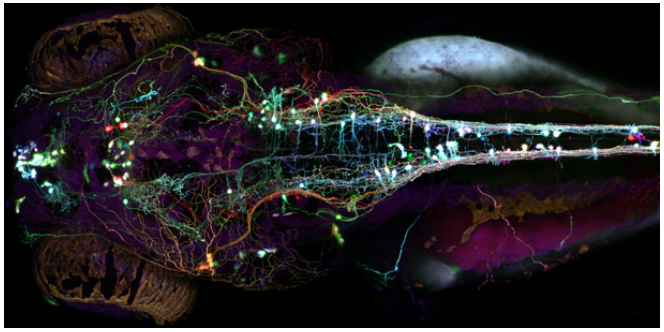
Tal *et al.* FASEB (2012)

Zebrafish Neurobehavioral Toxicity Assay

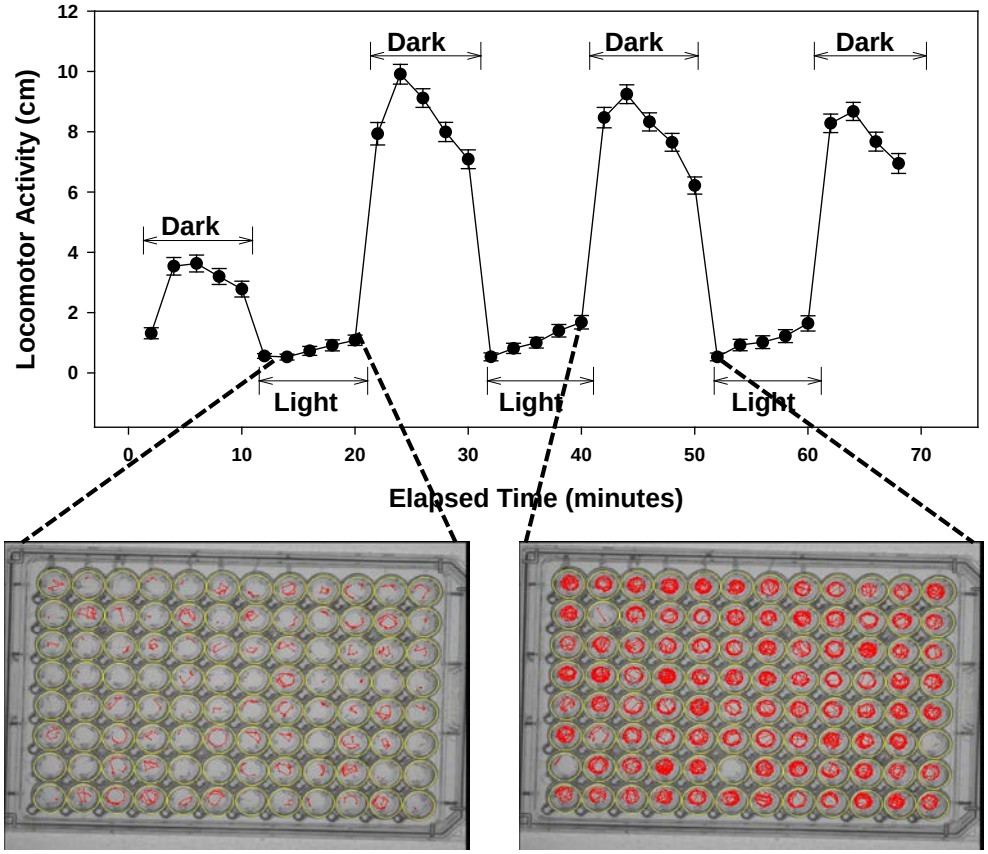
Spatial and temporal aspects of nervous system development include:

- 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)

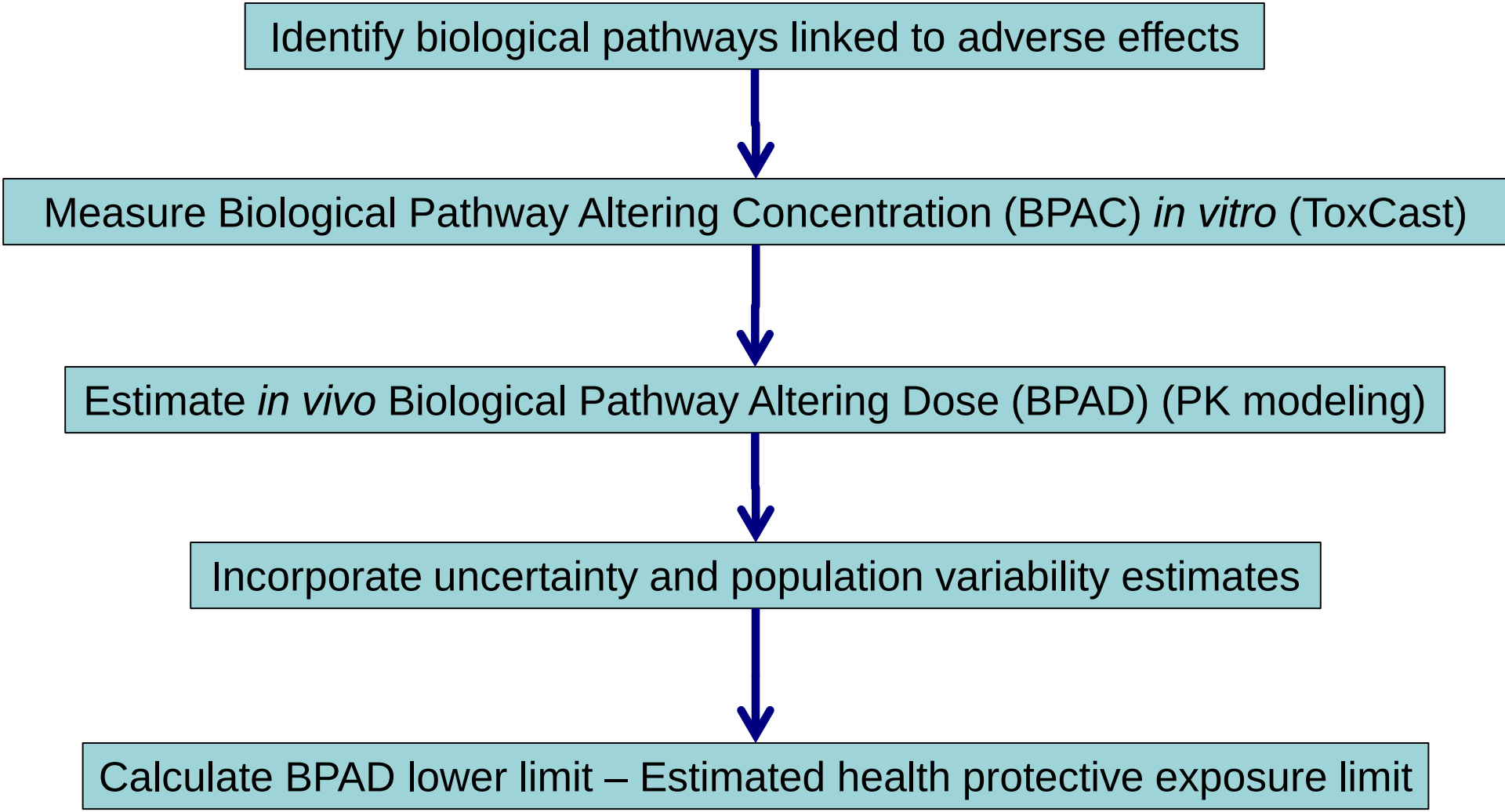
- Using HTS data for initial, rough risk assessment of data poor chemicals

- 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

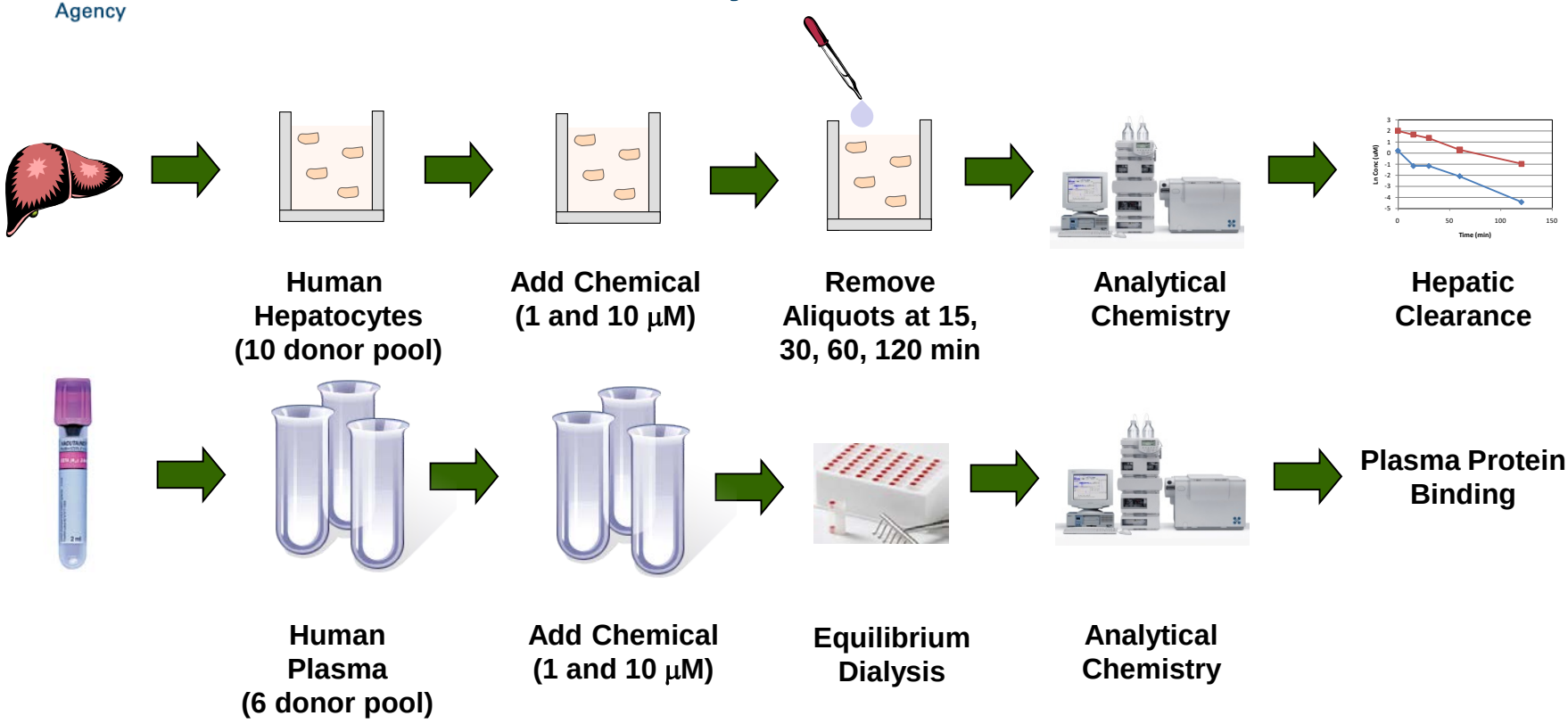
- Contributions of high-throughput methods
  - Focus on molecular pathways whose perturbation can lead to adversity
  - Screen 100s to 1000s of chemicals in HTS assays for those pathways
  - Estimate oral dose using High-Throughput pharmacokinetic modeling

- Incorporate population variability and uncertainty

HTRA Outline



Experimental Assays for Characterizing Steady-State Pharmacokinetics

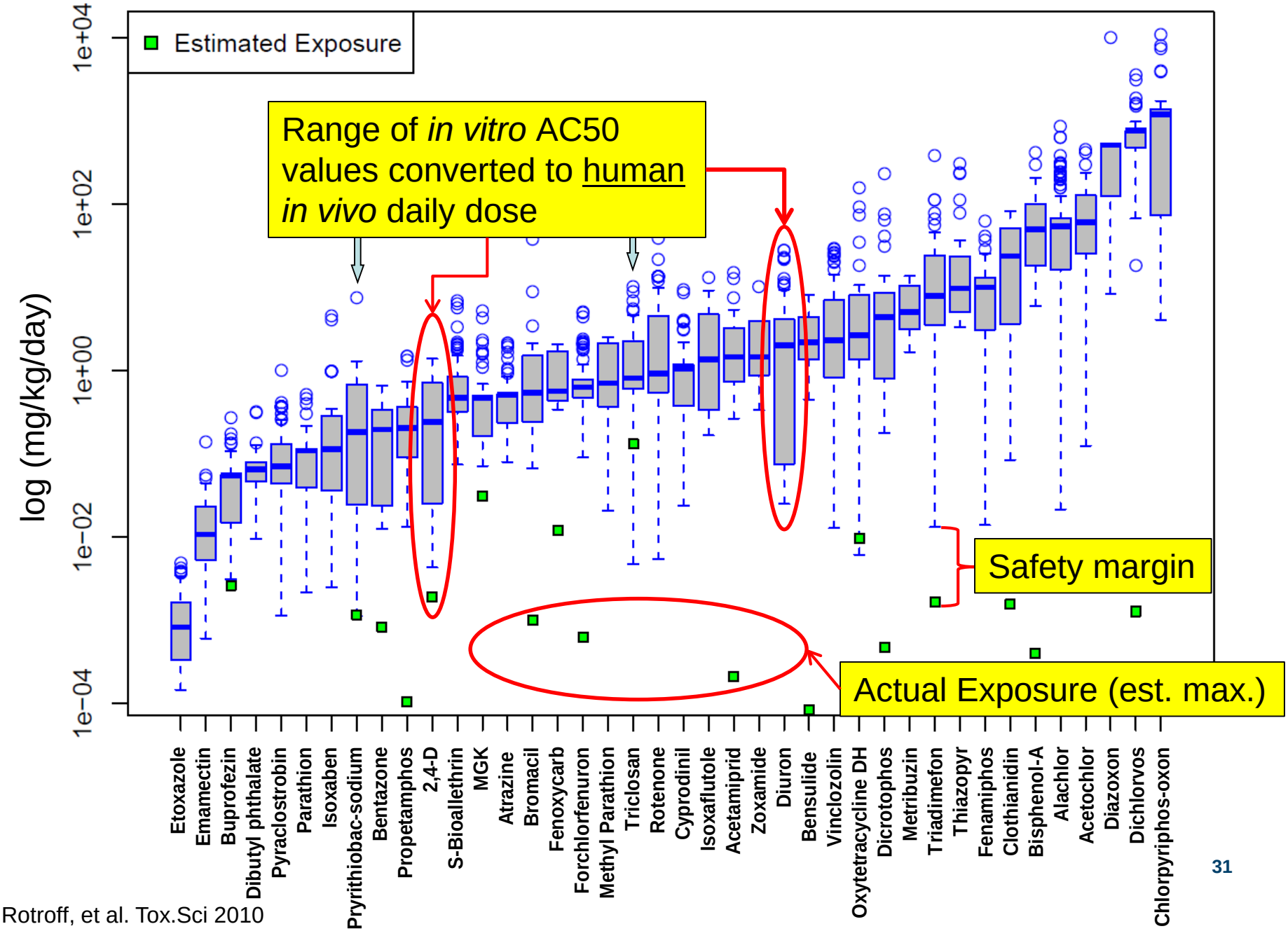


Combine experimental data with PK Model to estimate dose-to-concentration scaling

"Reverse Toxicokinetics"

In collaboration with Hamner Institutes / Rusty Thomas

Combining *in vitro* activity and dosimetry



Rotroff, et al. Tox.Sci 2010

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