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# The Accidental Toxicologist
## A Career in the Science of Poisons


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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.



Health Science Symposium
10/09/2015

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# What is Toxicology?

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

Exposure → Dose → Effects



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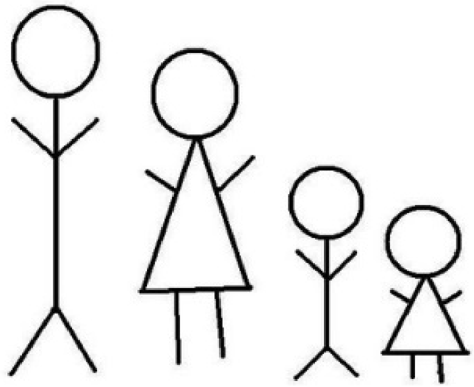
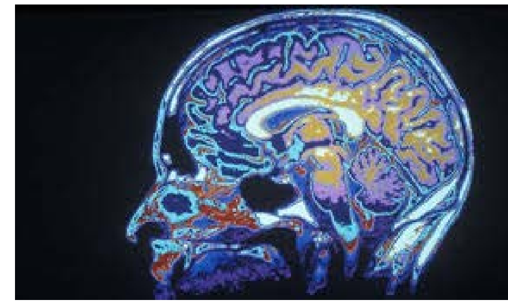
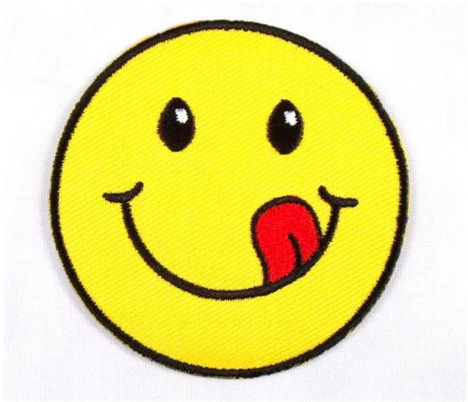
oxicology



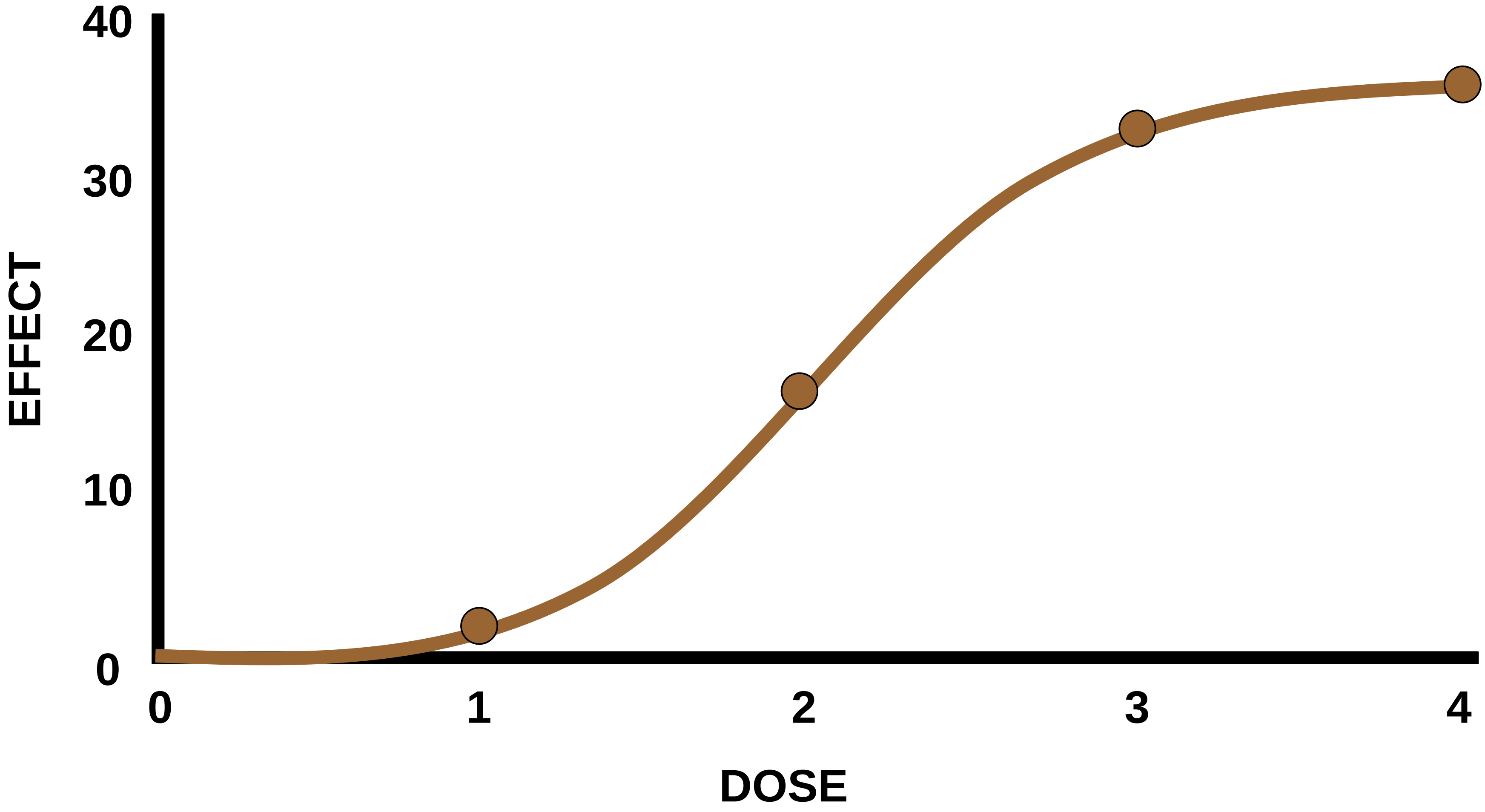




# The *dose* makes the poison…



## … but *what* dose?

# Dose Response Analysis



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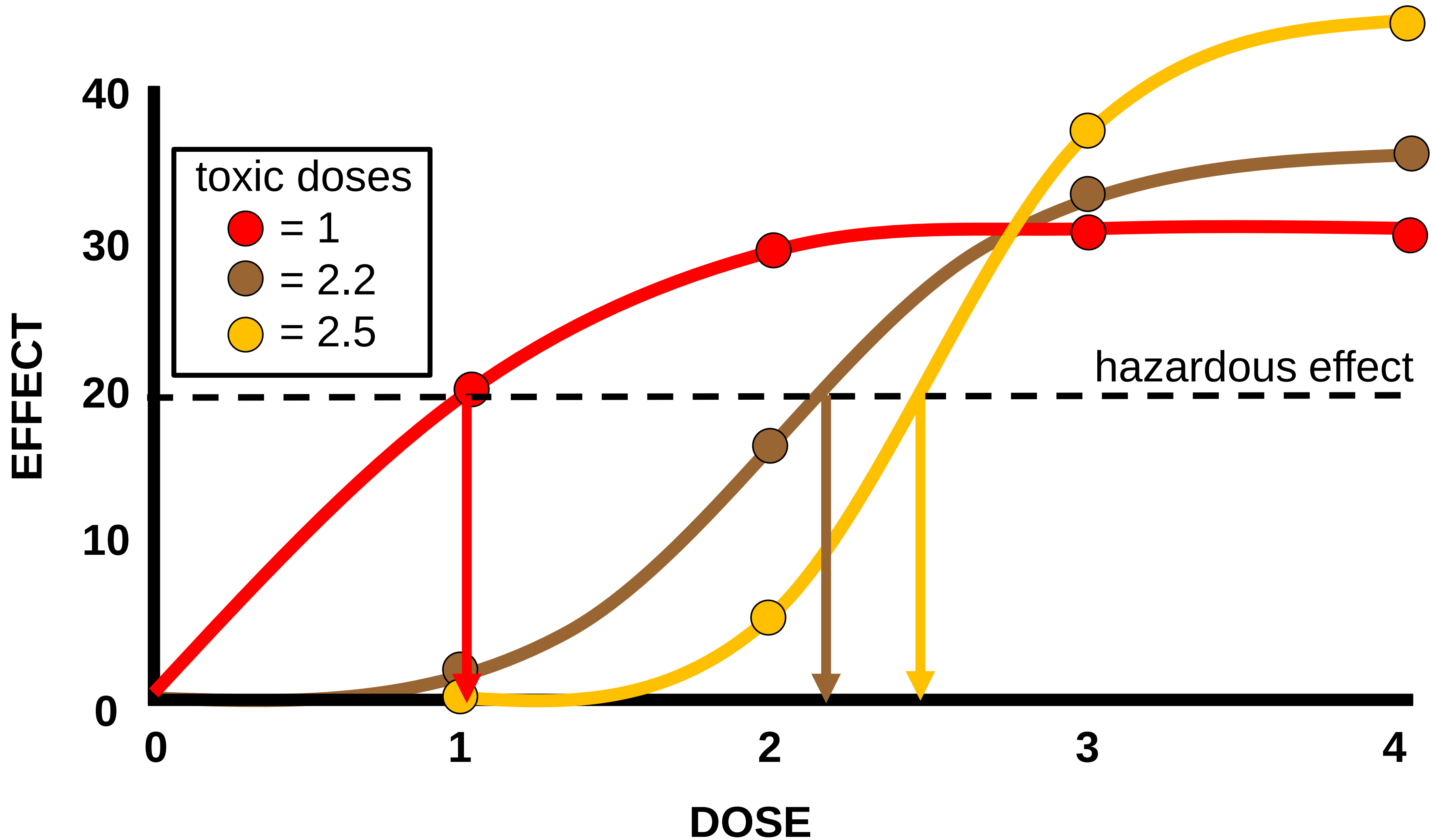
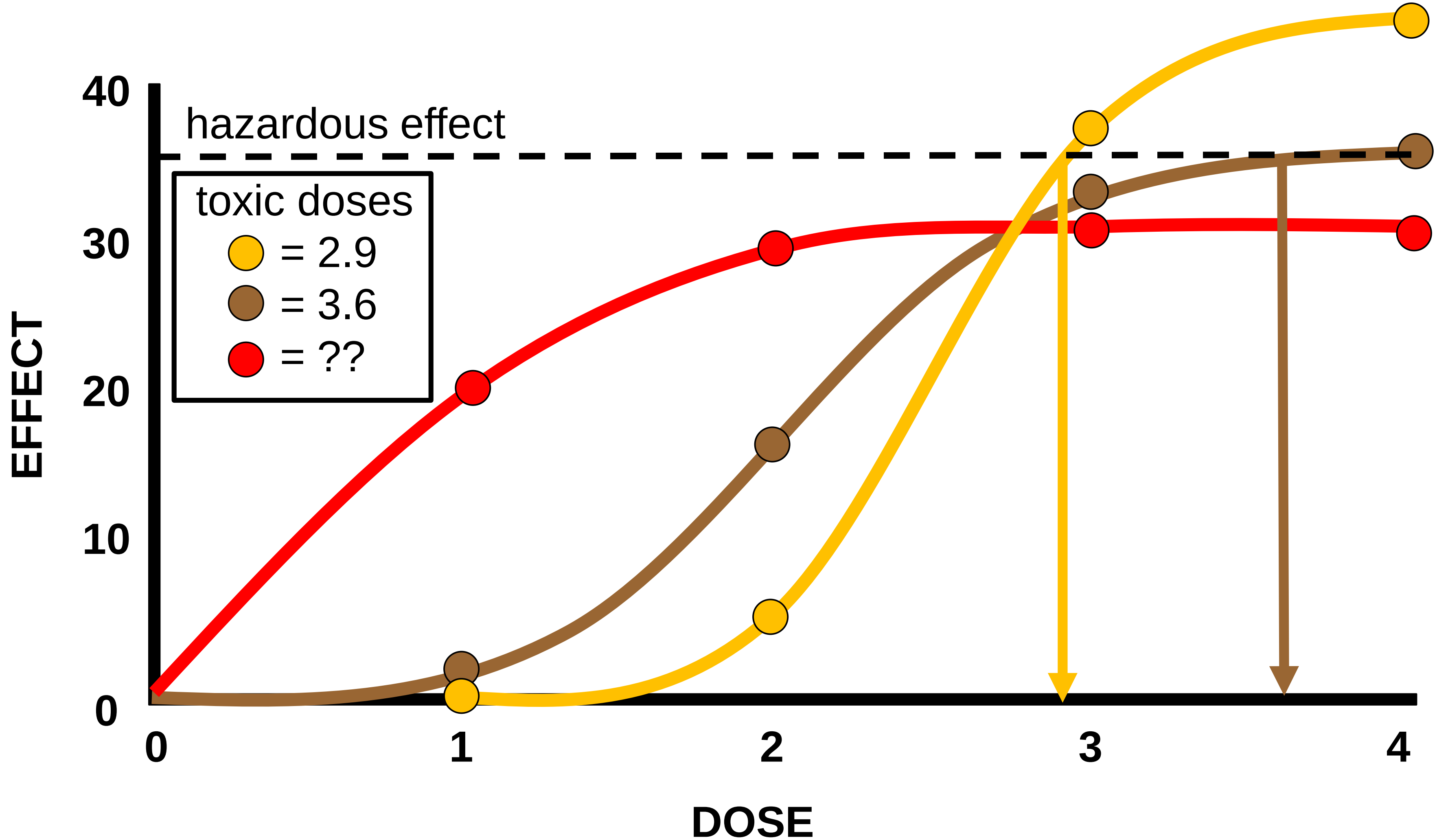
# Determining Hazard



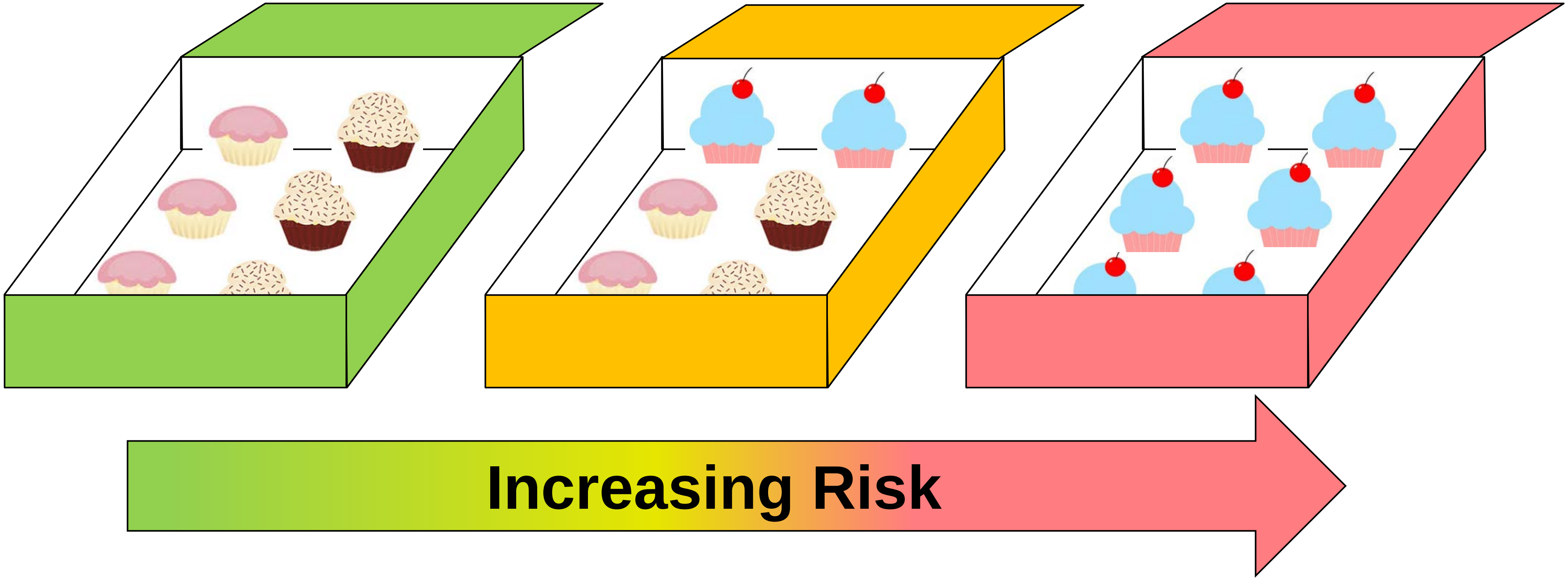
# Determining Hazard



# Determining Risk

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

Risk = f (Hazard x Exposure)



# A challenge for regulatory toxicologists


GIVE A DOG A PHONE
Technology for our furry friends

NewScientist

We've made 150,000 new chemicals

We touch them, we wear them, we eat them

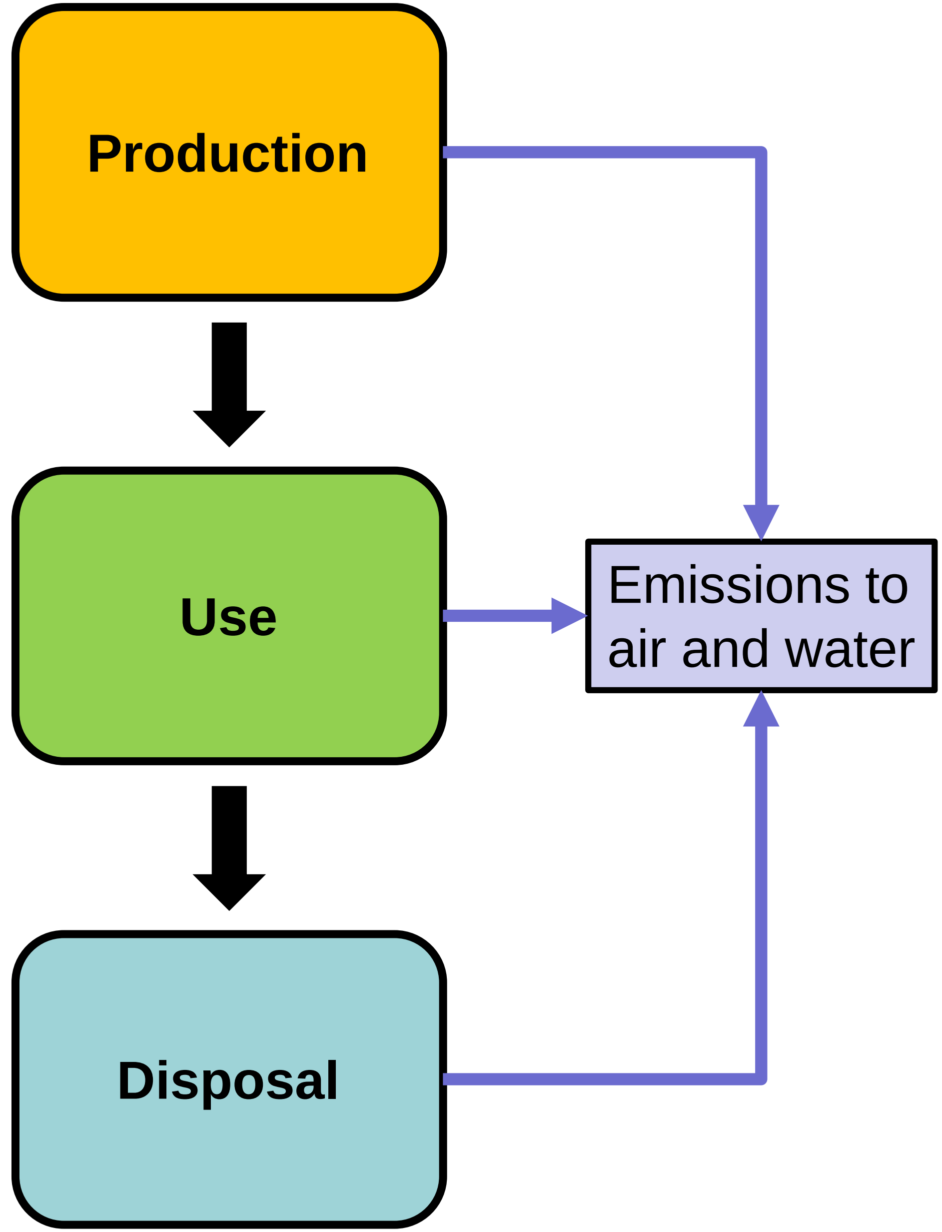
But which ones should we worry about?

SPECIAL REPORT, *page 34*

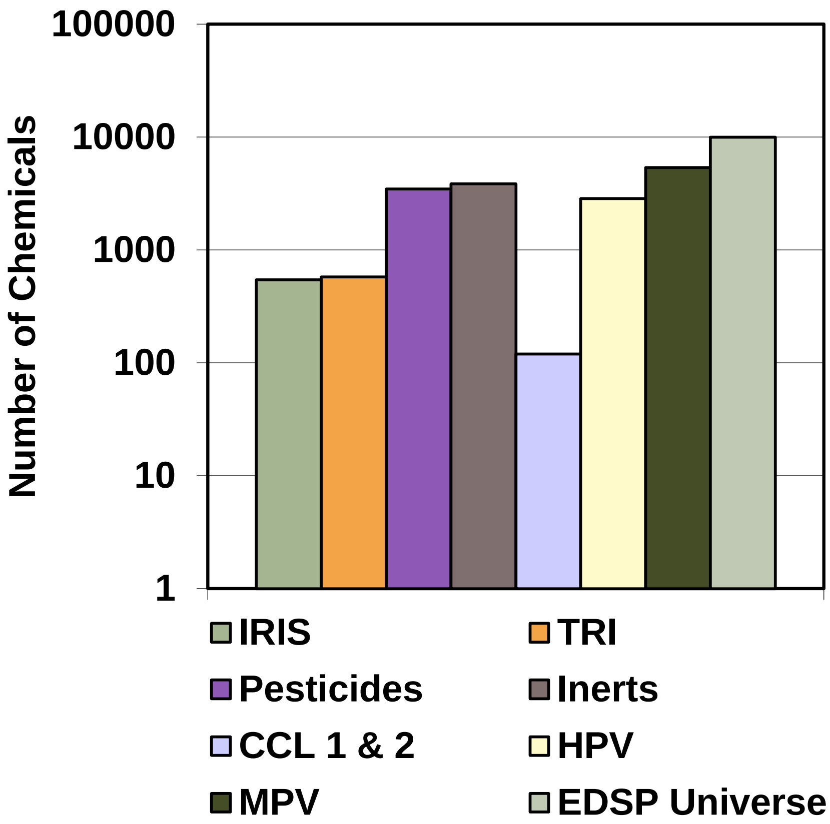
THE GOOD FIGHT
Most violence is also virtuous

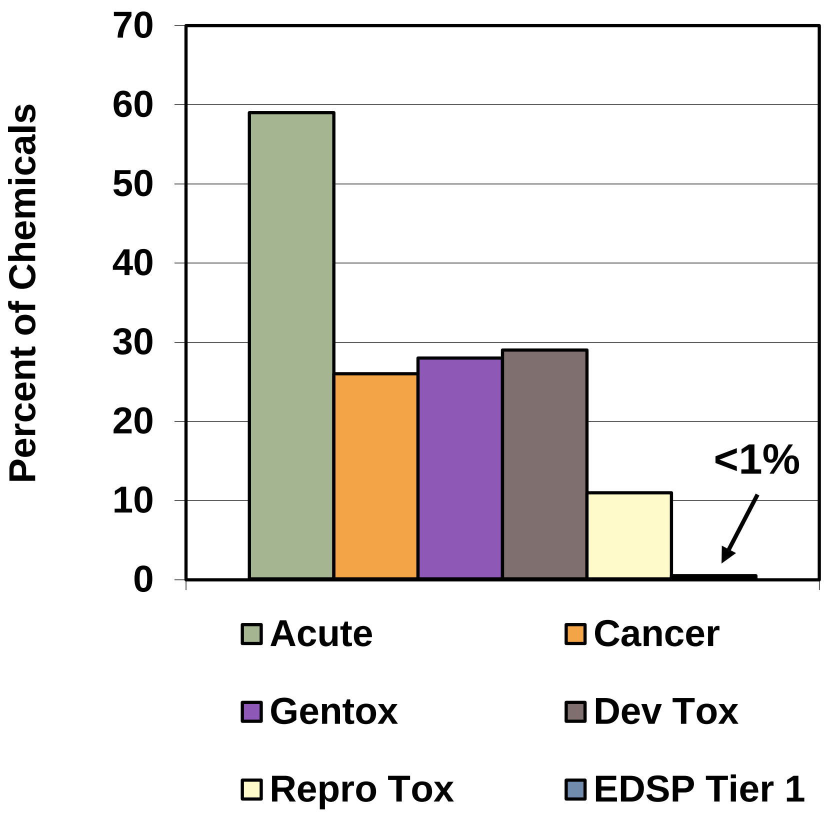
CHAMBER OF SECRETS
The greatest ever find of early human bones

IS IT ALIVE?
Artificial worm could be first digital animal



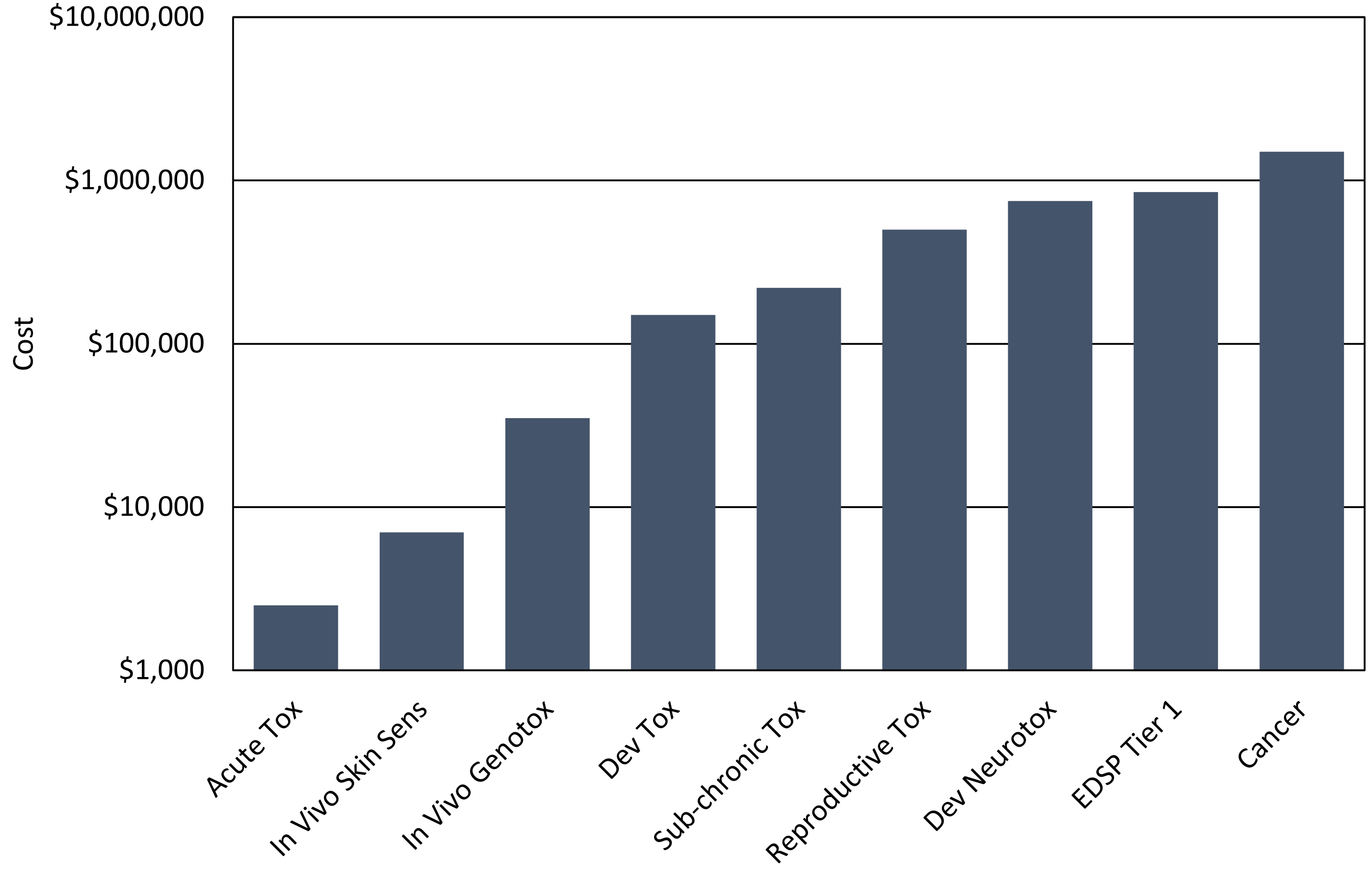
# Too many chemicals, not enough data




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

# Economic cost of current test methods



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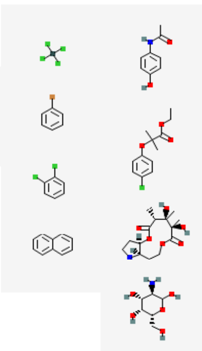
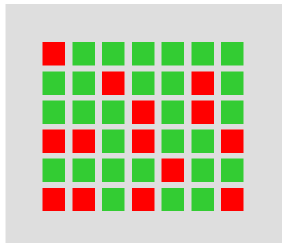
# Computational Toxicology Approaches

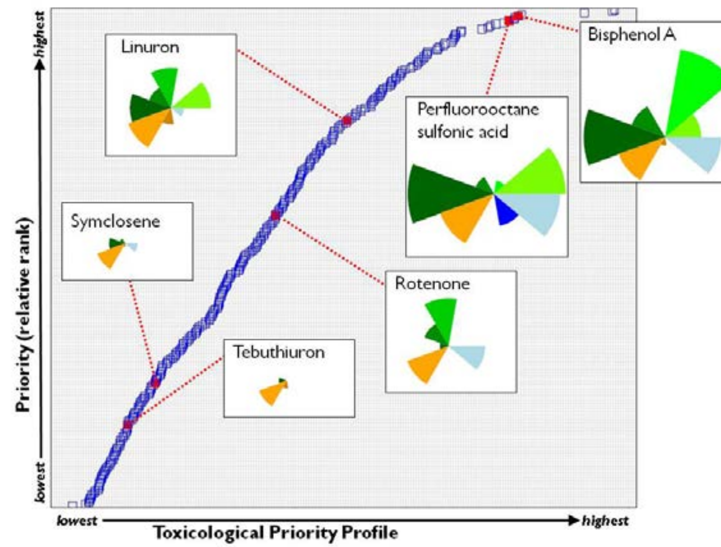


*Thousands of chemicals*



*High throughput biology and chemistry*

*Bioinformatics/ machine Learning*




*Predictive toxicology*

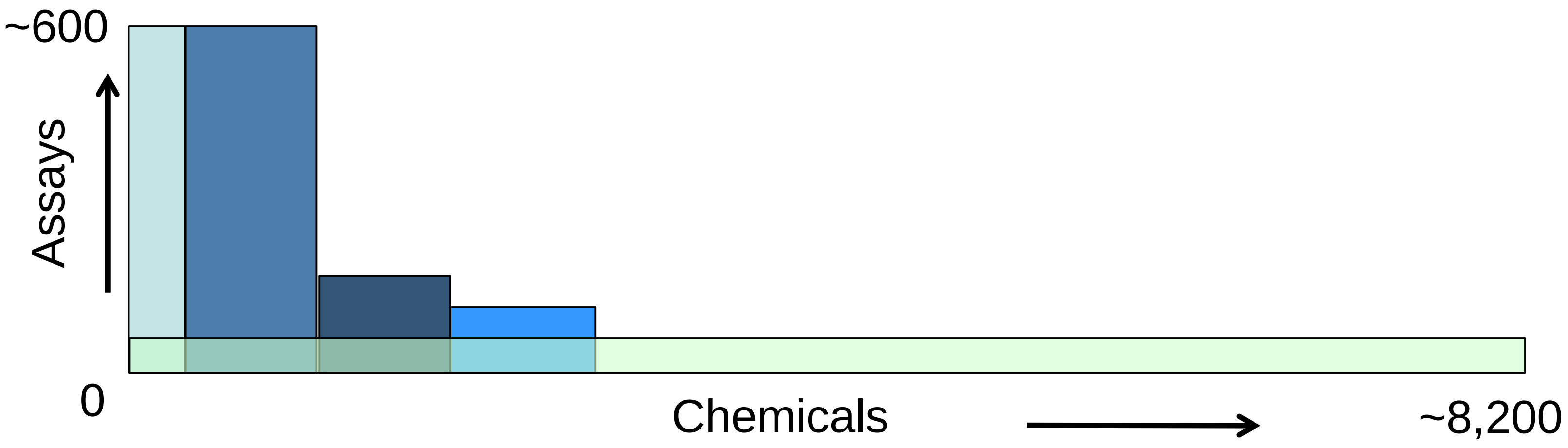
**Benefits**

- Less expensive
- More chemicals faster
- Fewer animals

# 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 |



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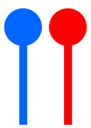
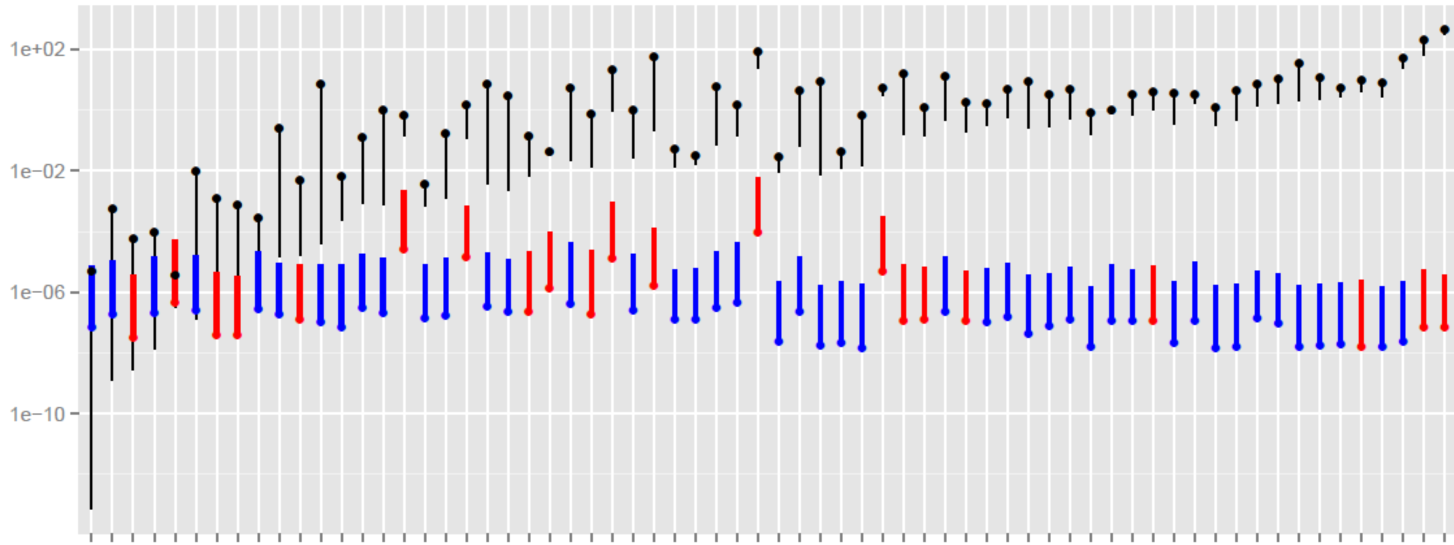
# High Throughput Exposure Predictions

= hazardous dose



= exposure estimate



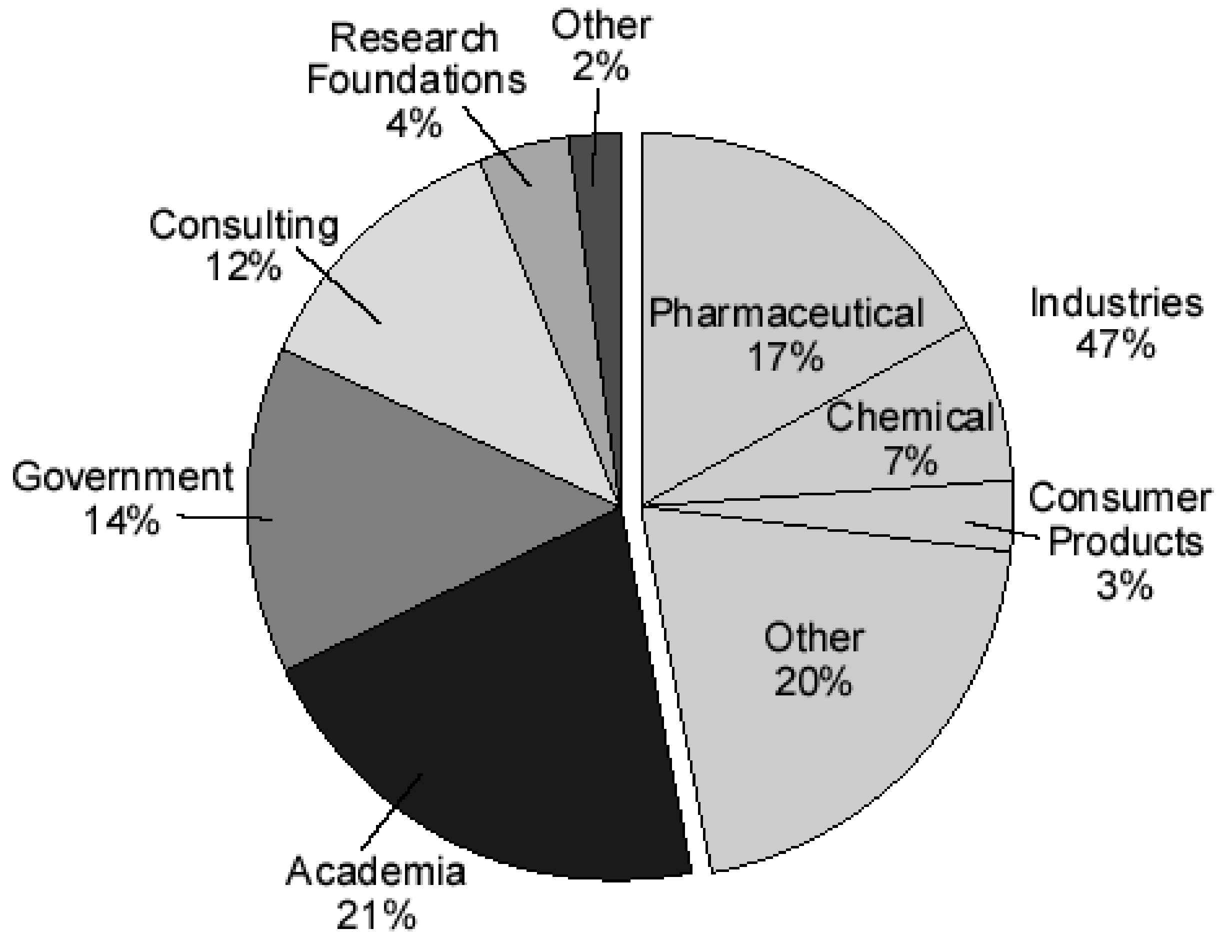

Wetmore *et al*. (2012)

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

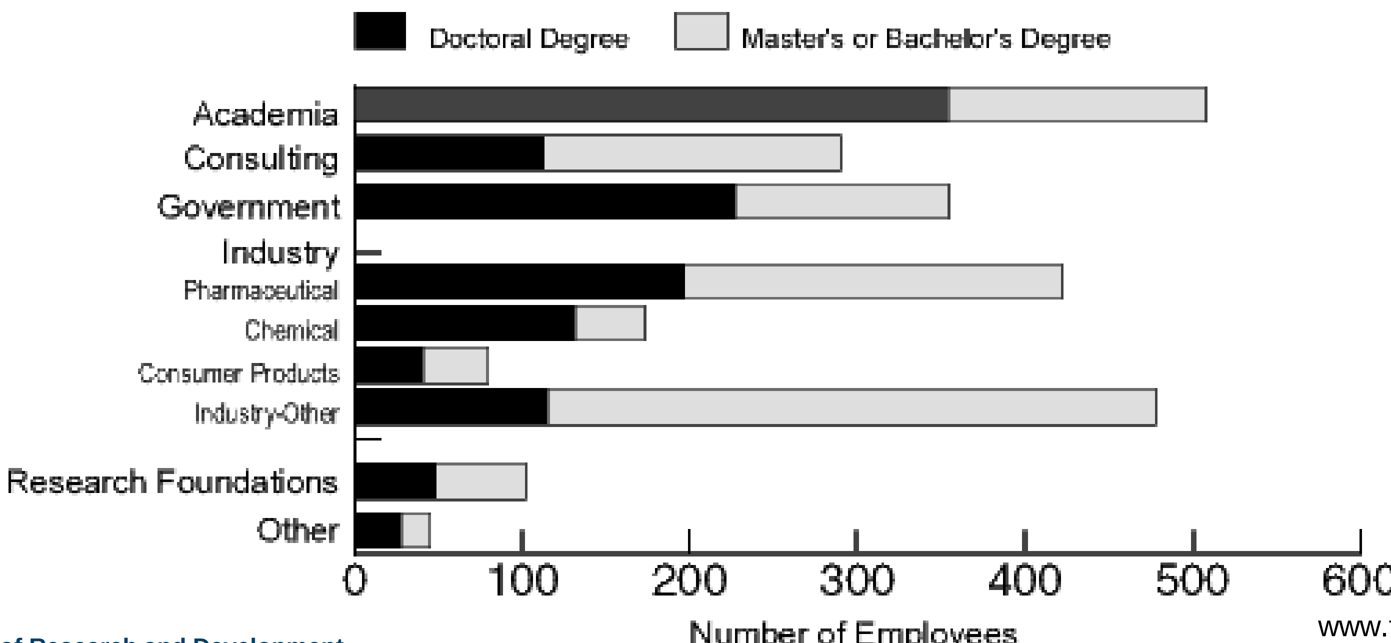
# Preparing to become a Toxicologist

## 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

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# 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)

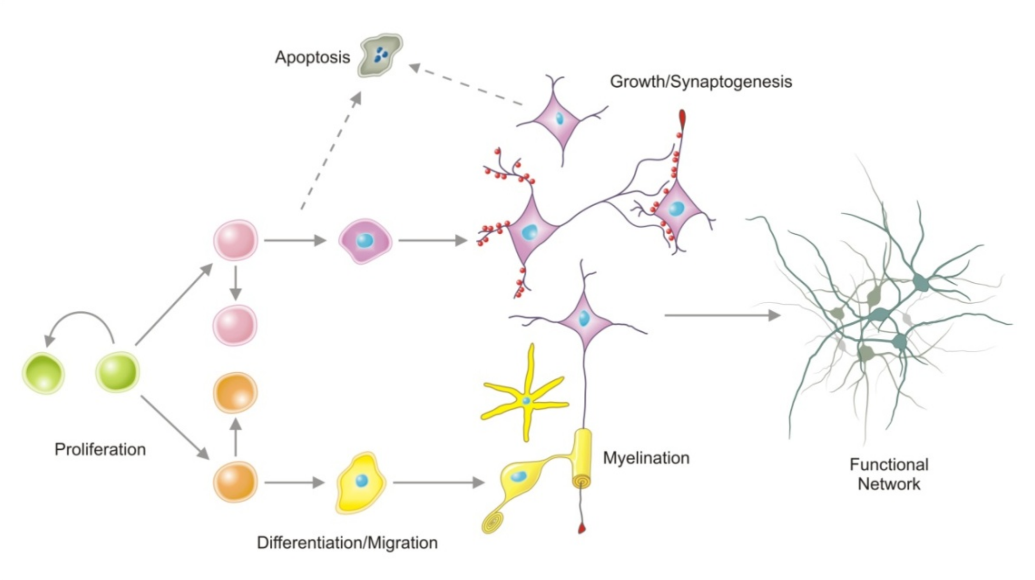
# Questions?

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*

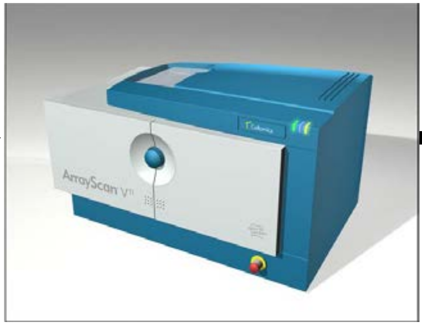


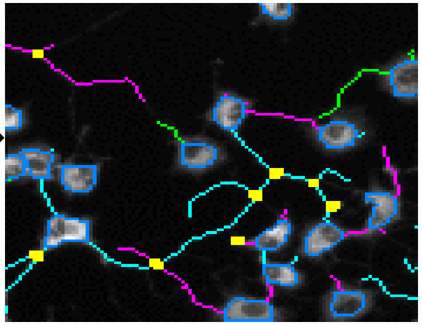

## In Vitro Assays

- 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



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# *An Example with a Cell-Based Assay for Synaptogenesis*

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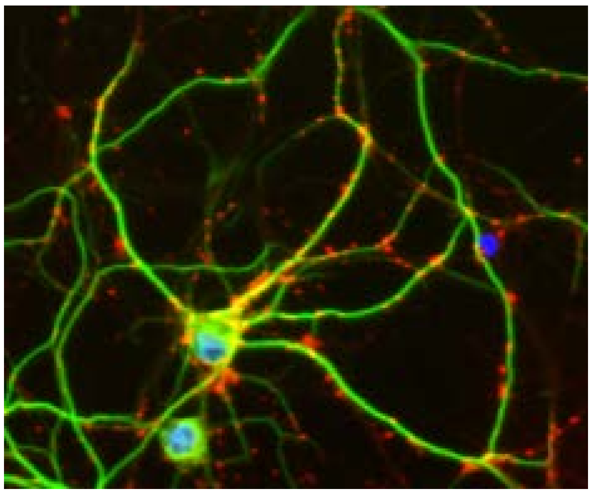
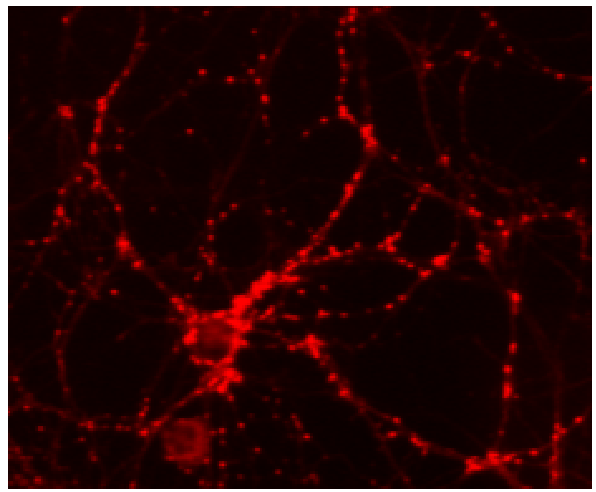
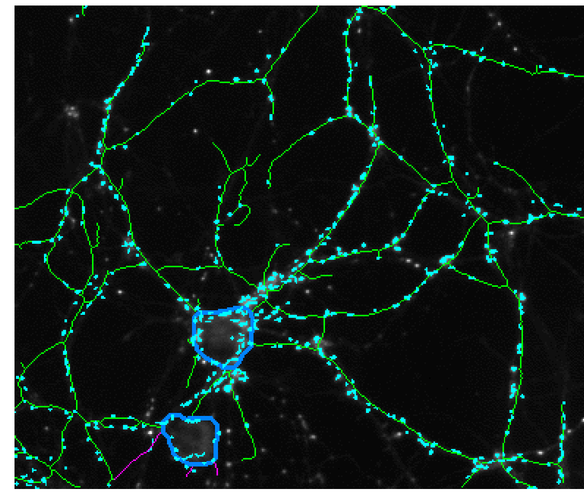
**Synaptogenesis (formation of connections critical to a neural network)**

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

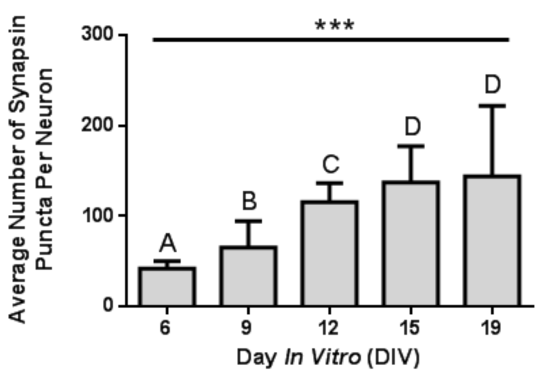
*High Content Image showing identified neurites and synapses*



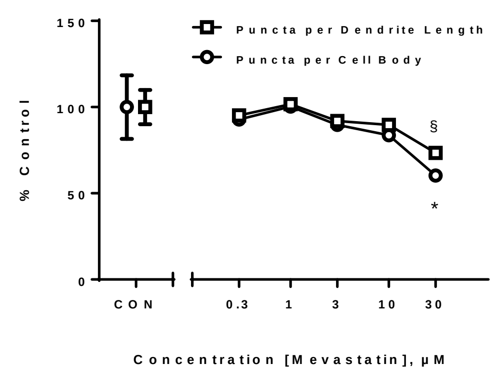




*Synapses increase during development in vitro*




*Chemical effect during critical period (DIV 9-15)*



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# Developing a Cell-Based Assay for Neuronal Function

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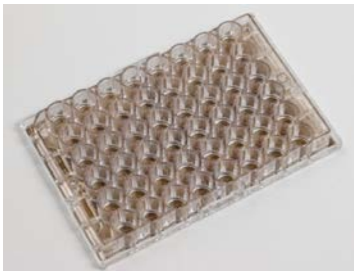
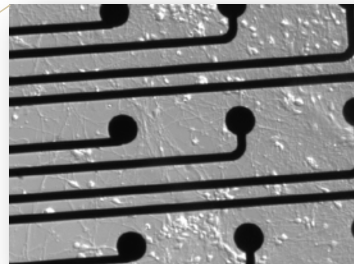
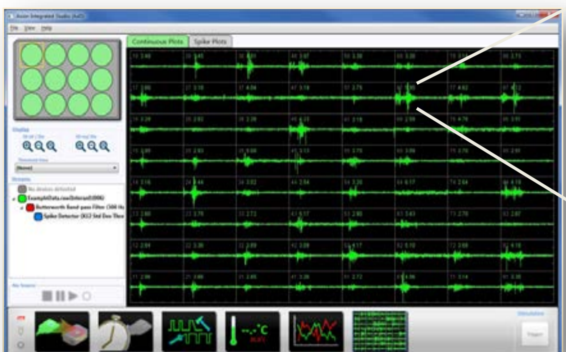
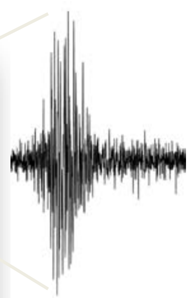


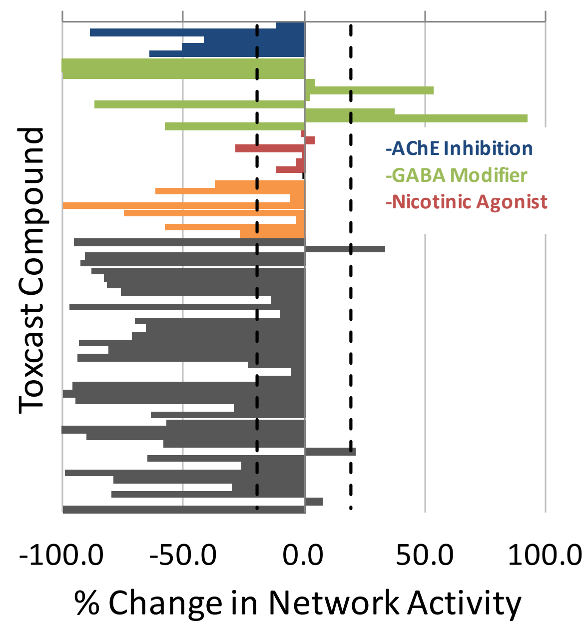


Primary cortical neurons are cultured in 48 well MEA plates

Spontaneous activity








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

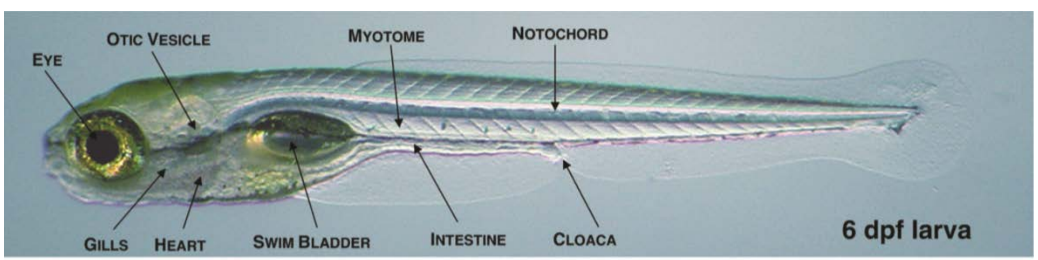
# Zebrafish Model Development

## Strengths

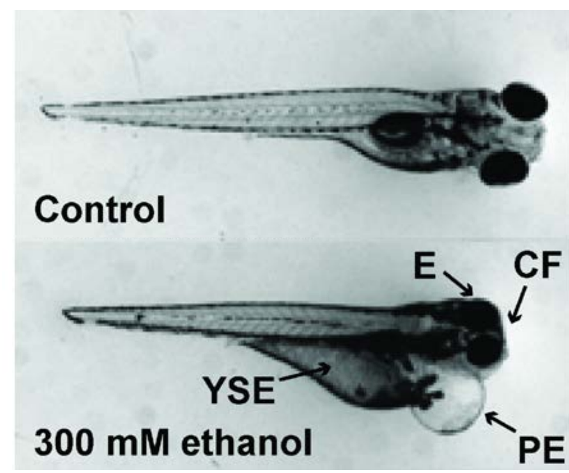
- Rapid development
- Transparent embryo
- Zebrafish have orthologs for 70% of human genes and 86% of 1318 human drug targets
- Genome is easy to manipulate
- Translational model for human- and eco- toxicology
- Apical endpoints, including functional assessments
- Metabolic capability
- Have tested >1000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

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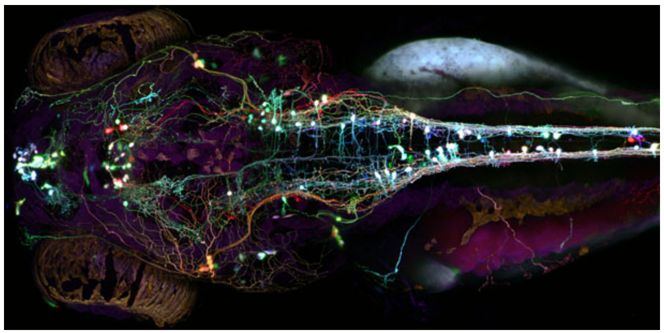
# Zebrafish Neurobehavioral Toxicity Assay

Spatial and temporal aspects of nervous system development include:

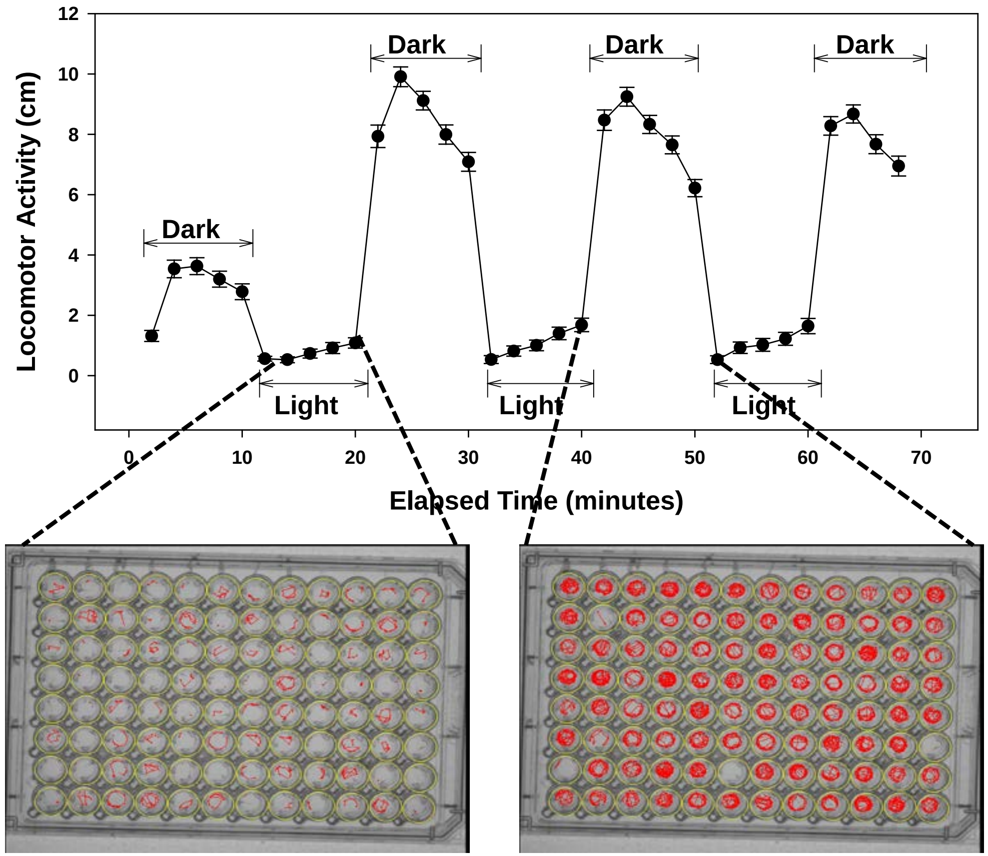
- Functional assessments
- Sensory assessments
- Learning and memory

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

1. Identify biological pathways linked to adverse effects
2. Measure Biological Pathway Altering Concentration (BPAC) *in vitro* (ToxCast)
3. Estimate *in vivo* Biological Pathway Altering Dose (BPAD) (PK modeling)
4. Incorporate uncertainty and population variability estimates
5. Calculate BPAD lower limit – Estimated health protective exposure limit

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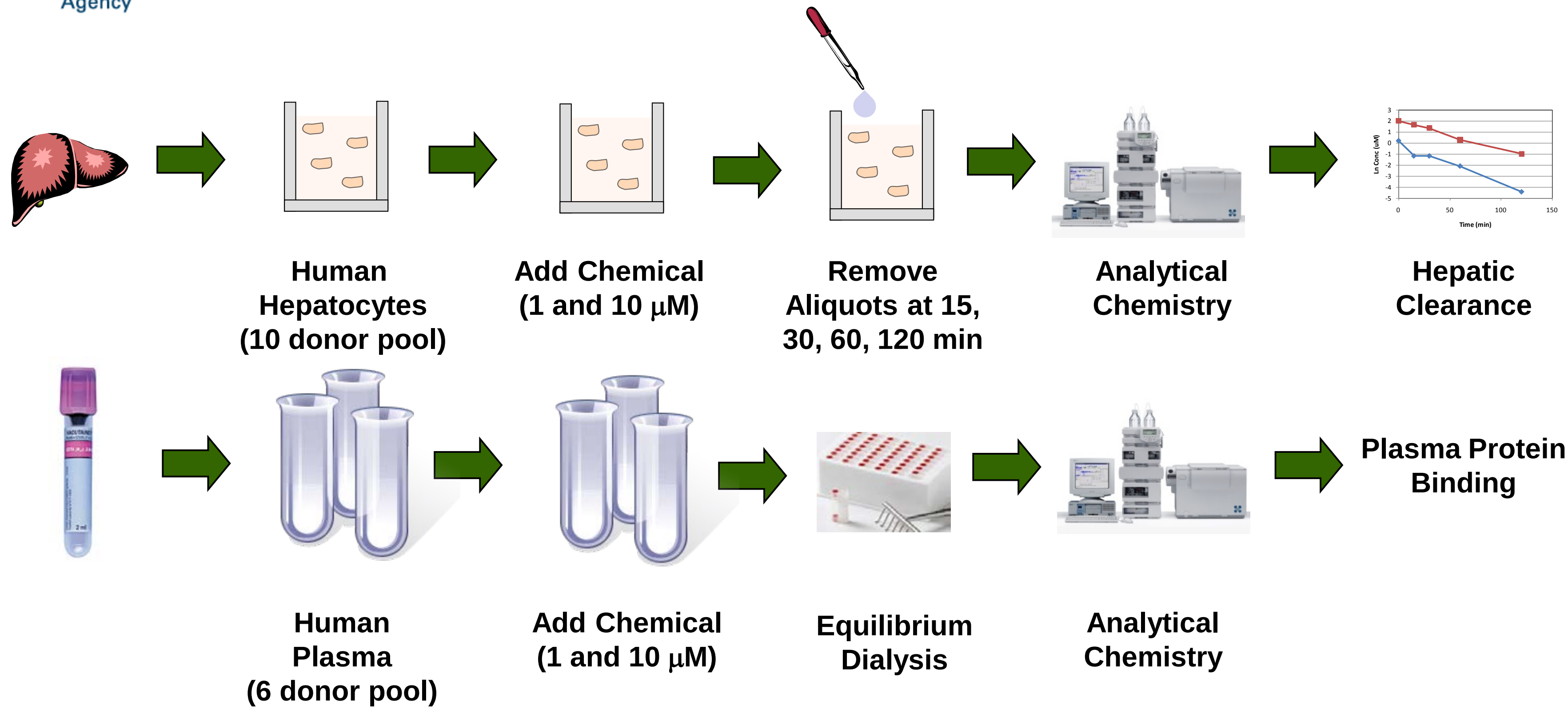
# Experimental Assays for Characterizing Steady-State Pharmacokinetics



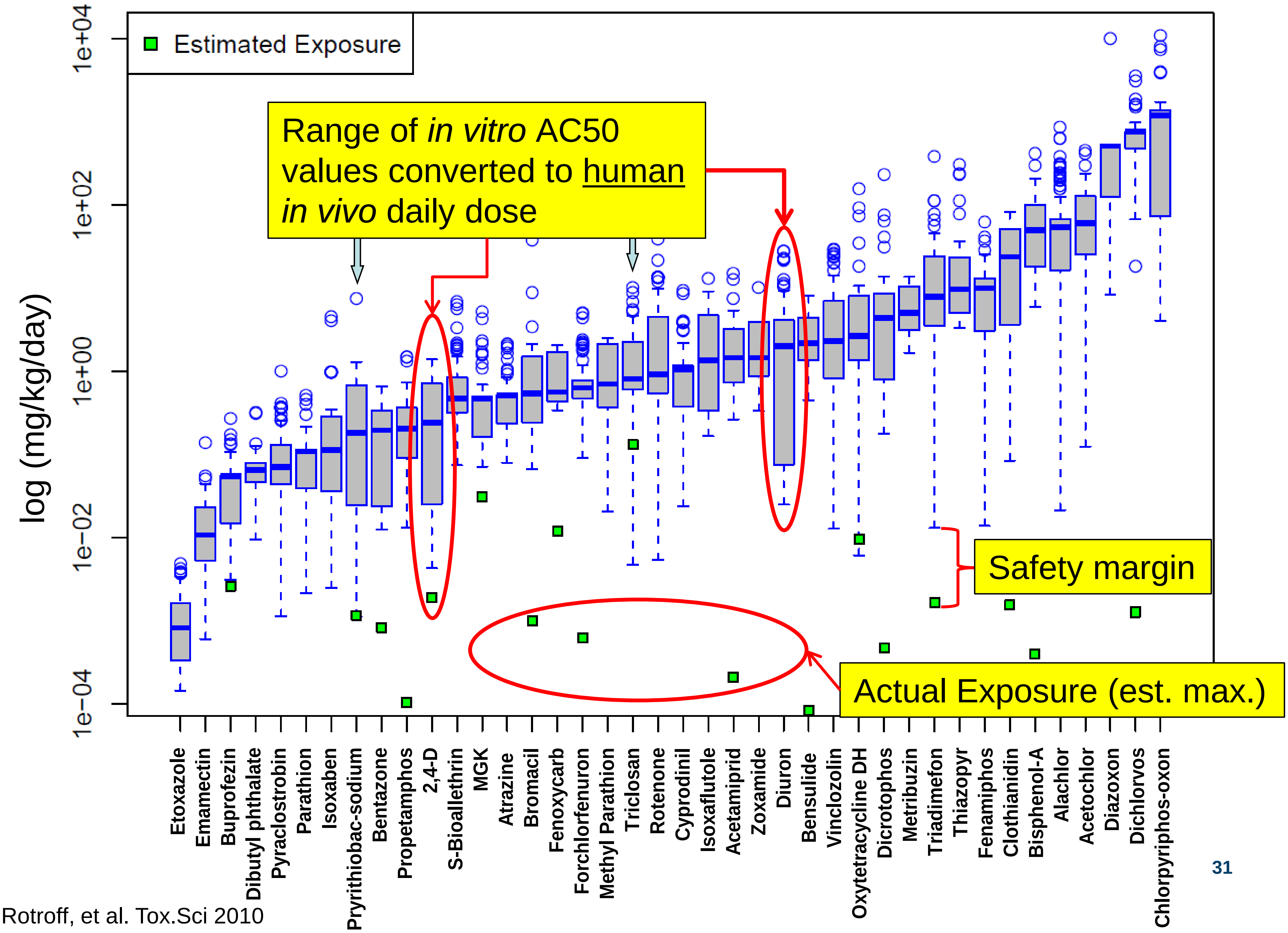

Combine experimental data with PK Model to estimate dose-to-concentration scaling

"Reverse Toxicokinetics"



In collaboration with Hamner Institutes / Rusty Thomas

# Combining *in vitro* activity and dosimetry




Rotroff, et al. Tox.Sci 2010

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# 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