United States Environmental Protection Agency

The Future of Toxicity Testing: The NRC Vision and EPAs ToxCast Program

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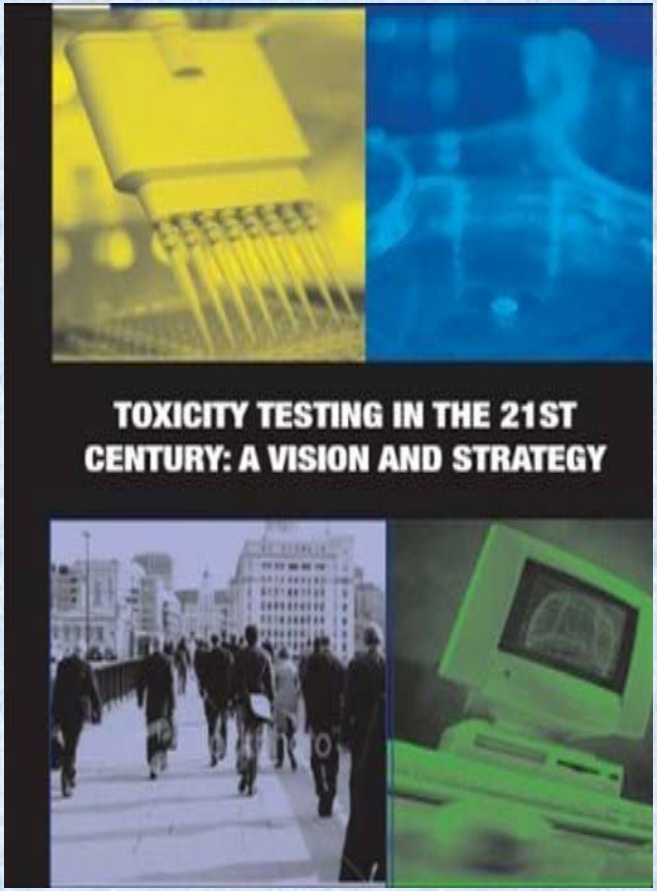
Office of Research and Development
National Center for Computational Toxicology

BTS/NBTS Annual Meeting, June 28 2009

Statement of Task: Final Report



- Assessment of key exposures (life stages) and toxicity outcomes (neurotoxicity)
- State-of-the-science testing and assessment procedures (genomics, bioinformatics, pharmacokinetics)
- Efficient experimental design and reduced use of laboratory animals
- New and alternative test methods
- Computational and molecular techniques in risk assessment

www.nas.edu

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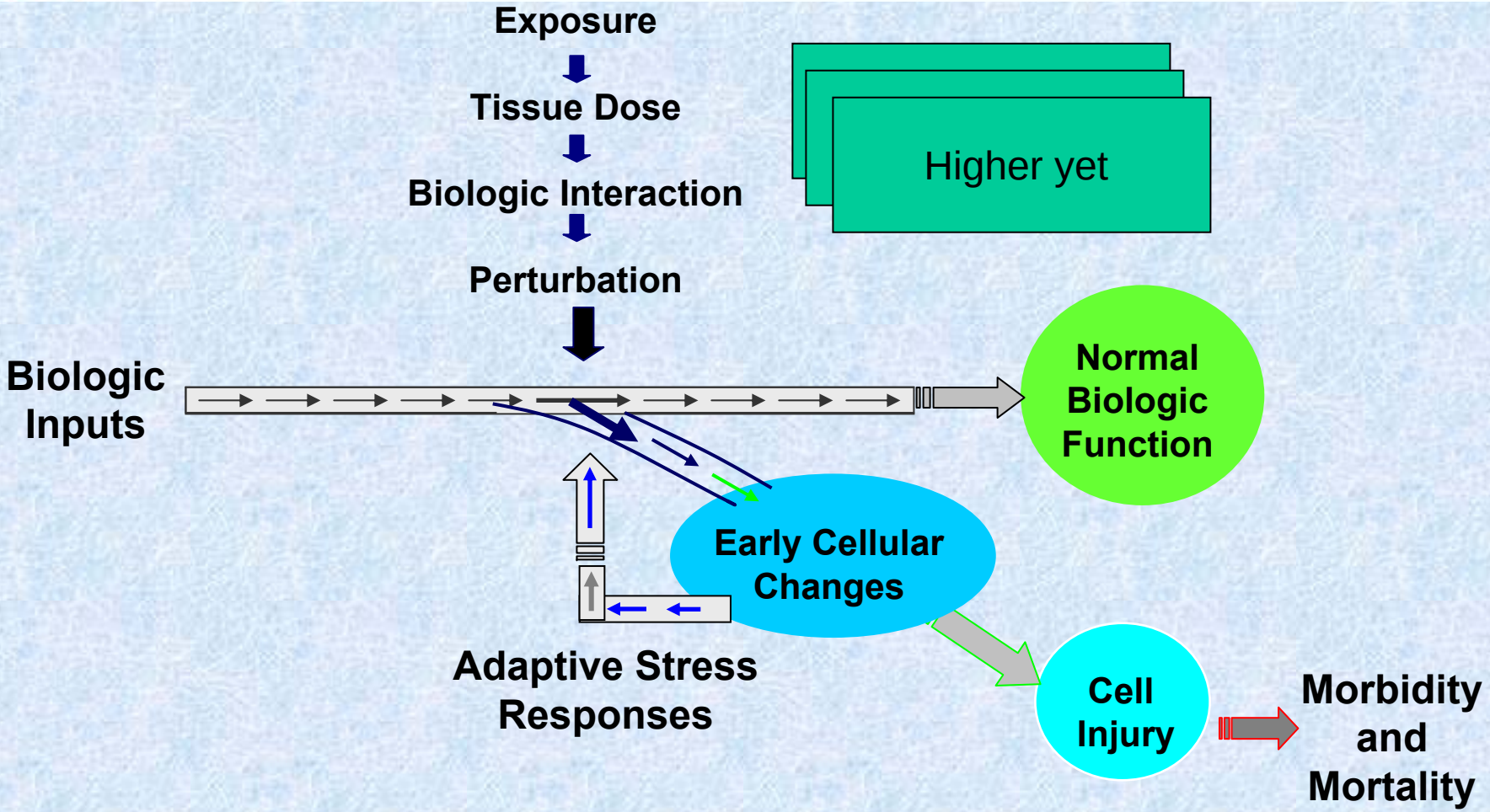
A New Paradigm: Activation of Toxicity Pathways



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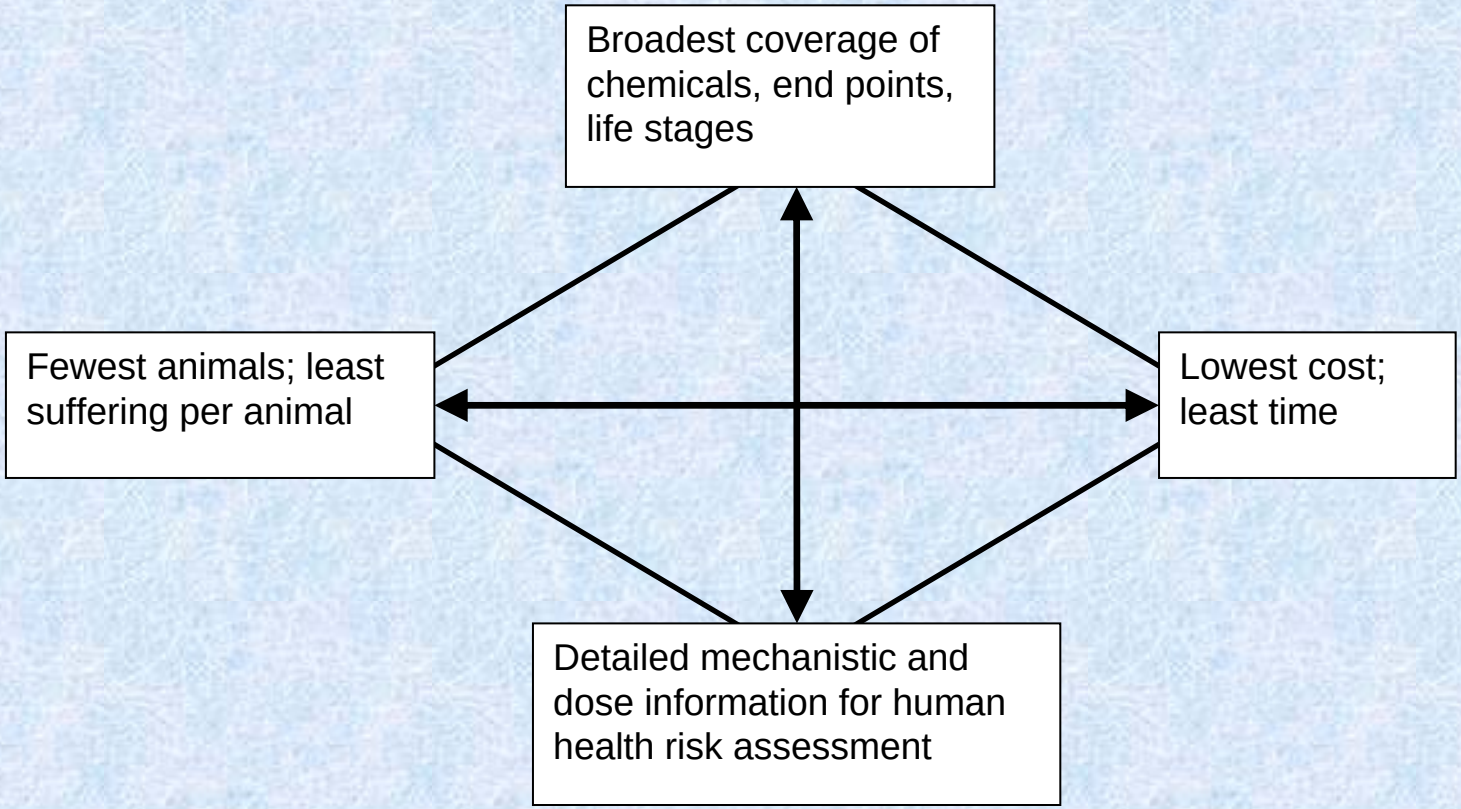
Design Criteria: Objectives of Toxicity Testing



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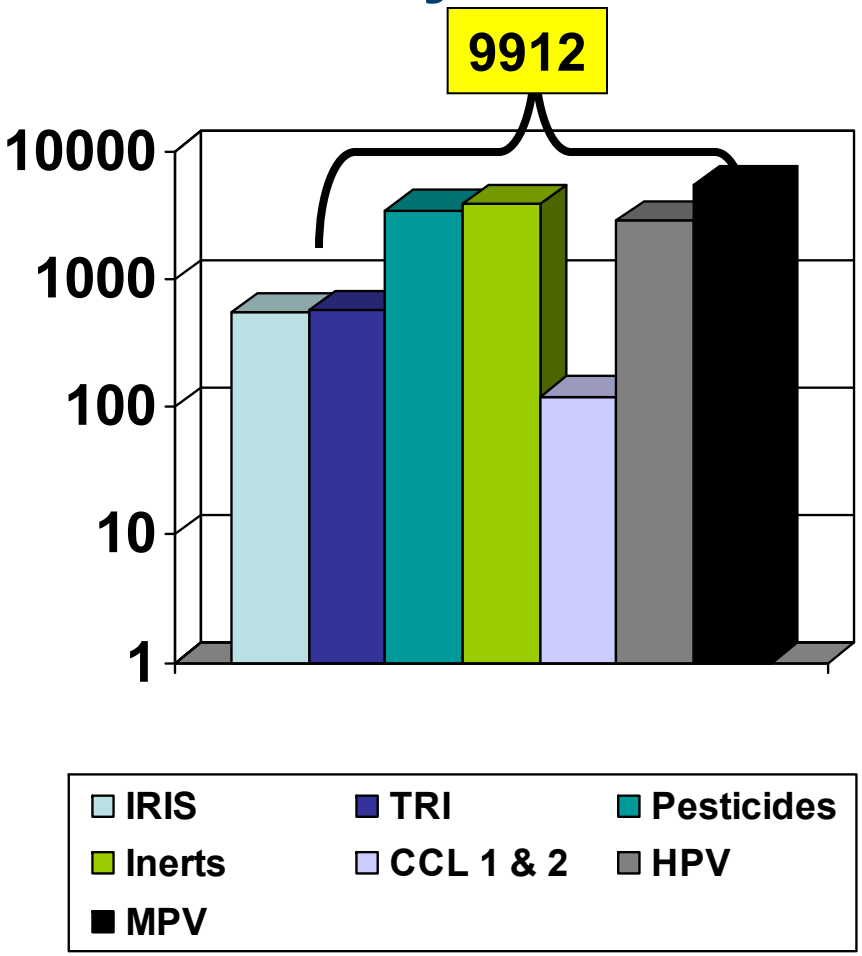
Options for Future Toxicity Testing Strategies

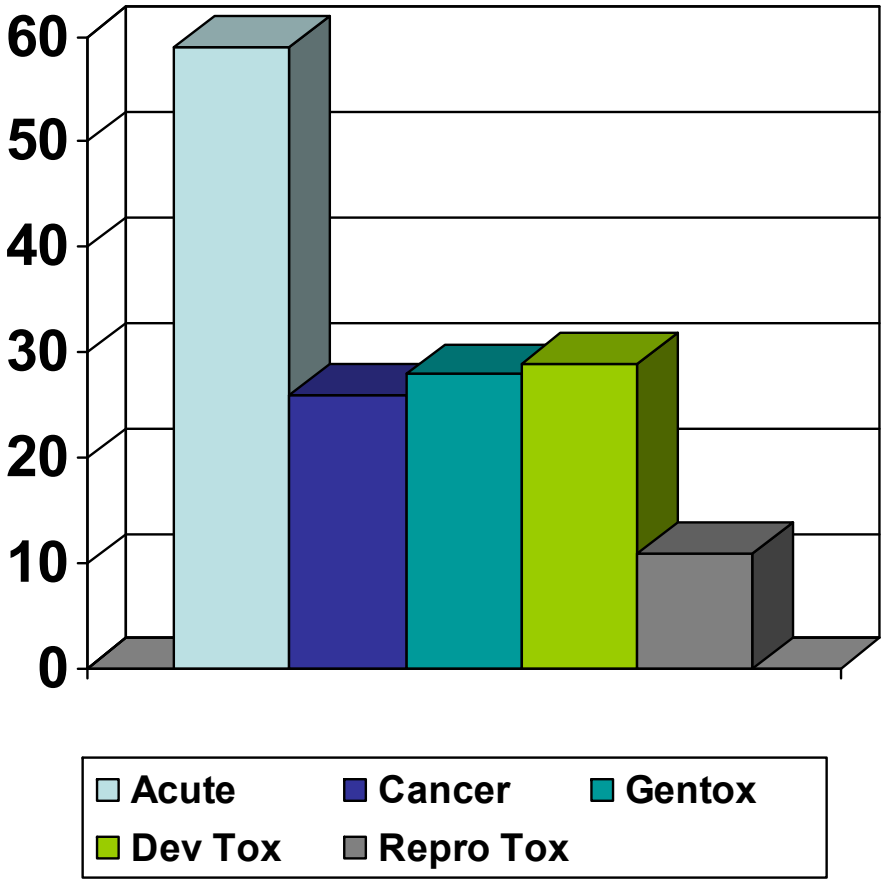
Option I **In Vivo**	**Option II** **Tiered In Vivo**	**Option III** **In Vitro/In Vivo**	**Option IV** **In vitro**
Animal biology	Animal biology	Primarily human biology	Primarily human biology
High doses	High doses	Broad range of doses	Broad range of doses
Low throughput	Improved throughput	High and medium throughput	High throughput
Expensive	Less expensive	Less expensive	Less expensive
Time consuming	Less time consuming	Less time consuming	Less time consuming
Relative large number of animals	Fewer animals	Substantially fewer animals	Virtually no animals
Apical endpoints	Apical endpoints	Perturbations of toxicity pathways	Perturbations of toxicity pathways
	Some *in silico* and *in vitro* screens	*In silico* screens possible	*In silico* screens

EPA's Need for Prioritization

Too Many Chemicals

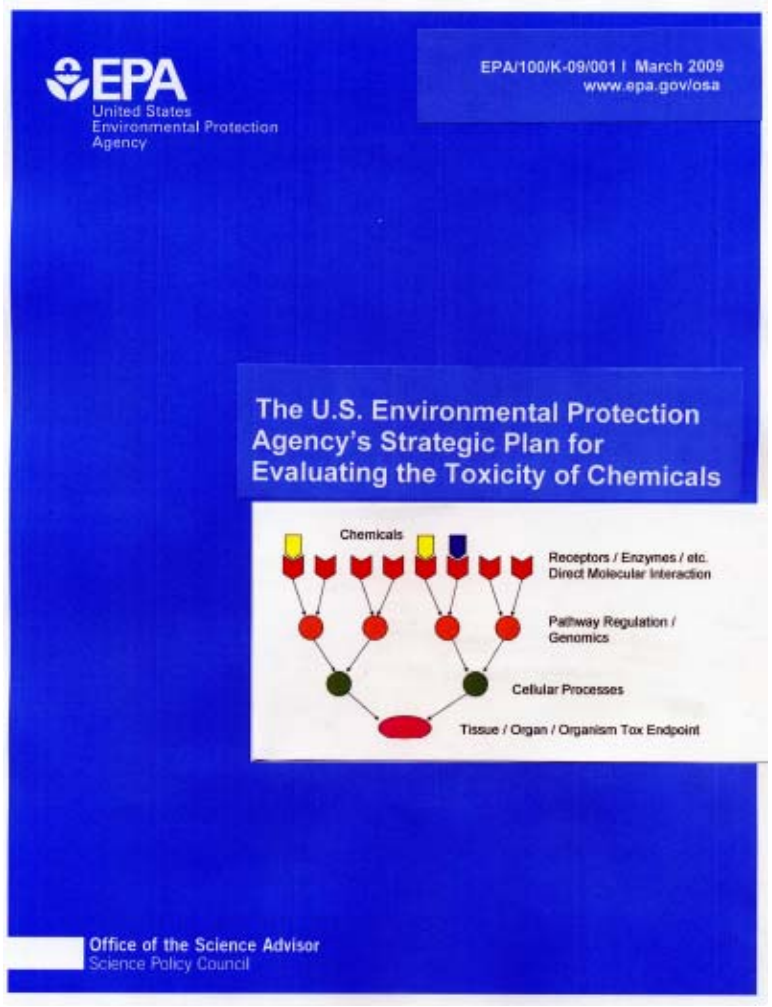


Too Little Data (%)



Judson, et al *EHP* (2009)

EPA Reacts to Challenge of the NRC on the Future of Toxicity Testing



Strategic Goals

- Toxicity Pathway ID and Screening
- Toxicity Based Risk Assessment
- Institutional Transition

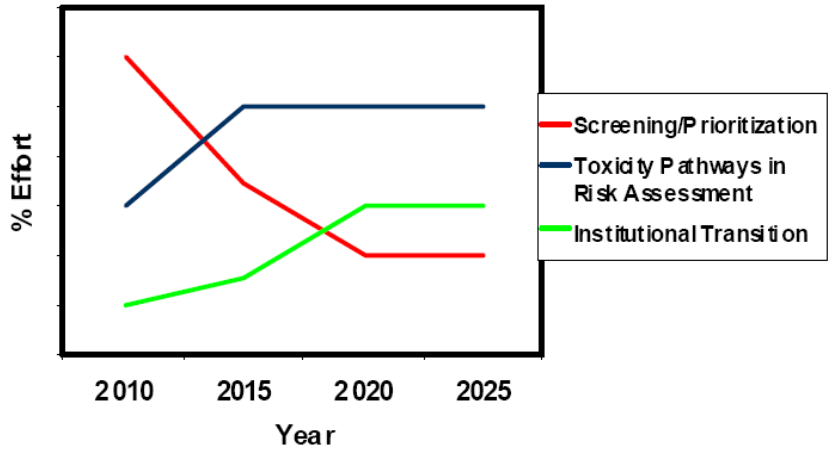
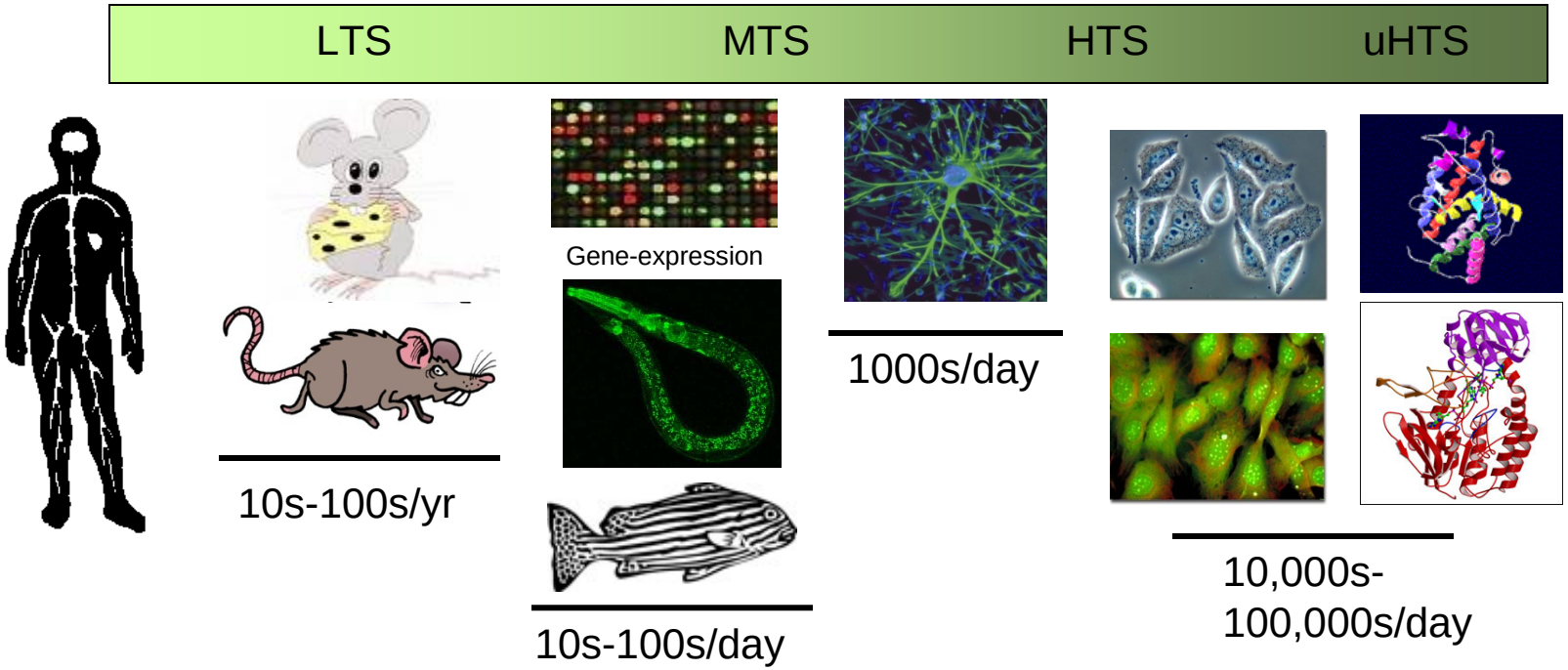


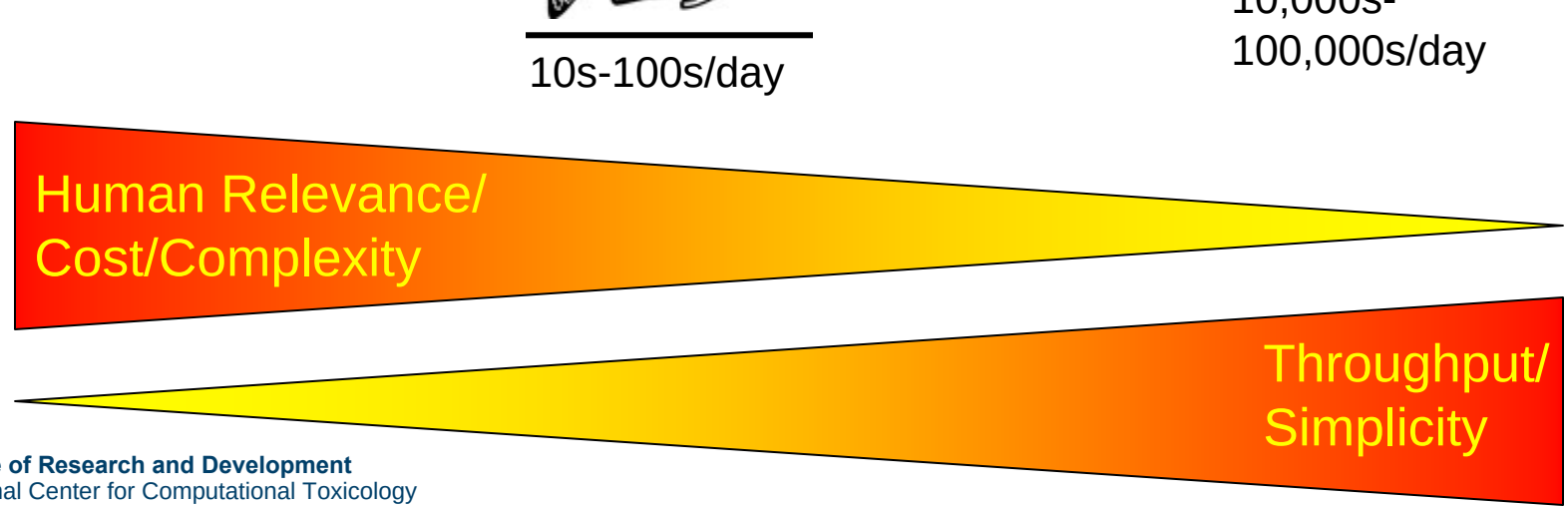
Figure 6. Relative (%) Emphasis of the Three Main Components of this Strategic Plan over its Expected 20-year Duration.

http://www.epa.gov/osa/spc/toxicitytesting/index.htm

High-Throughput Screening Assays

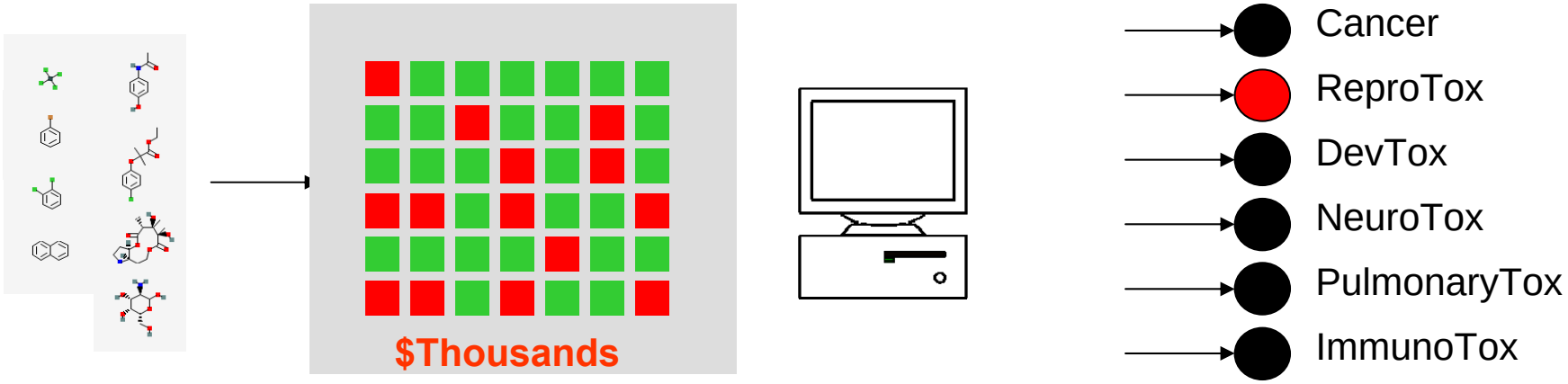
batch testing of chemicals for pharmacological/toxicological endpoints using automated liquid handling, detectors, and data acquisition





Future of Toxicity Testing

in vitro testing *in silico* analysis



HTS
-omics

Bioinformatics/
Machine Learning

The ToxCast_320

309 Unique Structures

Replicates for QC

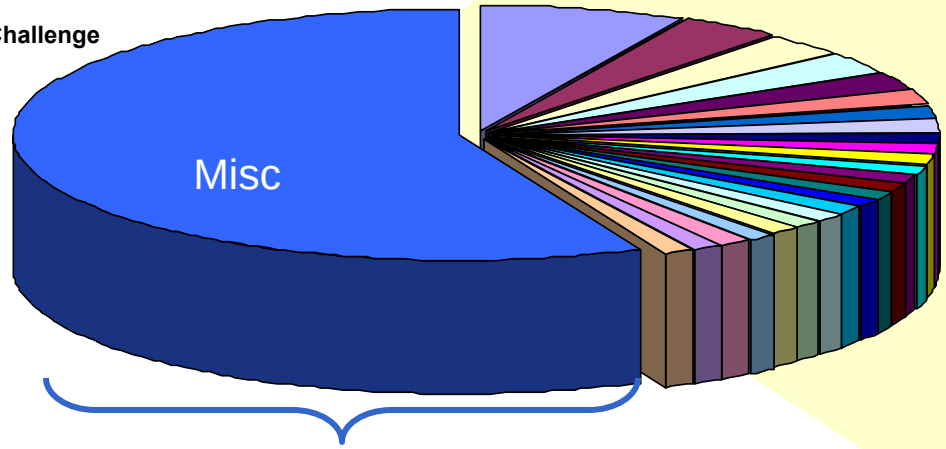
291 Pesticide Actives
9 Industrial Chemicals
8 Metabolites

56/73 Proposed Tier 1 EDSP

53 of 80 with DNTs

122 in IRIS

14 HPV
11 HPV Challenge



Misc MOA classes with
3 or fewer representatives

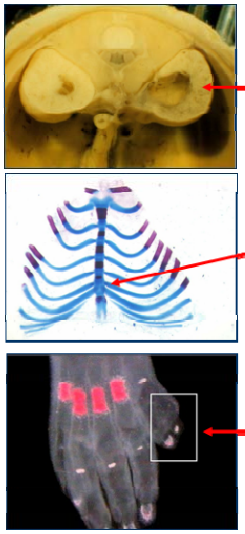
Classes with
> 3 chemicals

- Acetylcholine esterase inhibitors
- conazole fungicides
- Sodium channel modulators
- pyrethroid ester insecticides
- organothiophosphate acaricides
- dinitroaniline herbicides
- pyridine herbicides
- thiocarbamate herbicides
- imidazolinone herbicides
- organophosphate insecticides
- phenyl organothiophosphate insecticides
- aliphatic organothiophosphate insecticides
- amide herbicides
- aromatic fungicides
- chloroacetanilide herbicides
- chlorotriazine herbicides
- growth inhibitors
- organophosphate acaricides
- oxime carbamate insecticides
- phenylurea herbicides
- pyrethroid ester acaricides
- strobilurin fungicides
- unclassified acaricides
- unclassified herbicides

Classification based on OPPIN

Profiling developmental toxicity

***in vivo* endpoints** (target, description)
www.epa.gov/ncct/toxrefdb

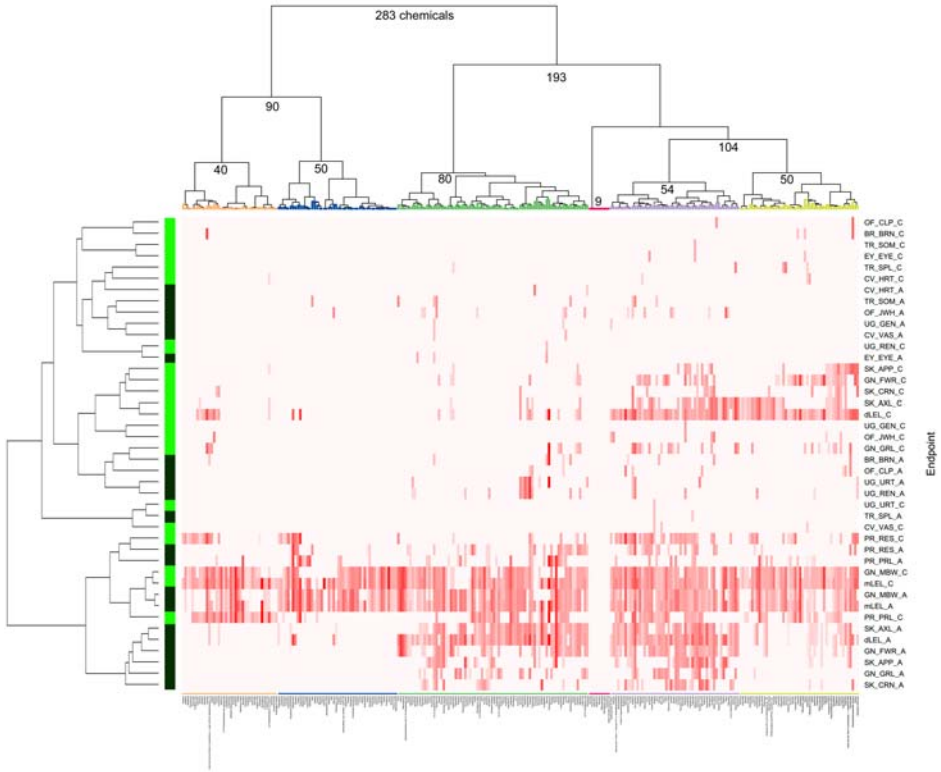


images from www.DevTox.org

target: kidney
description: absent renal papilla
code: UG_REN_3.1060.5013

target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130

target: hindpaw
description: polydactyly (digit I)
code: SK_APP_2.1051.5234

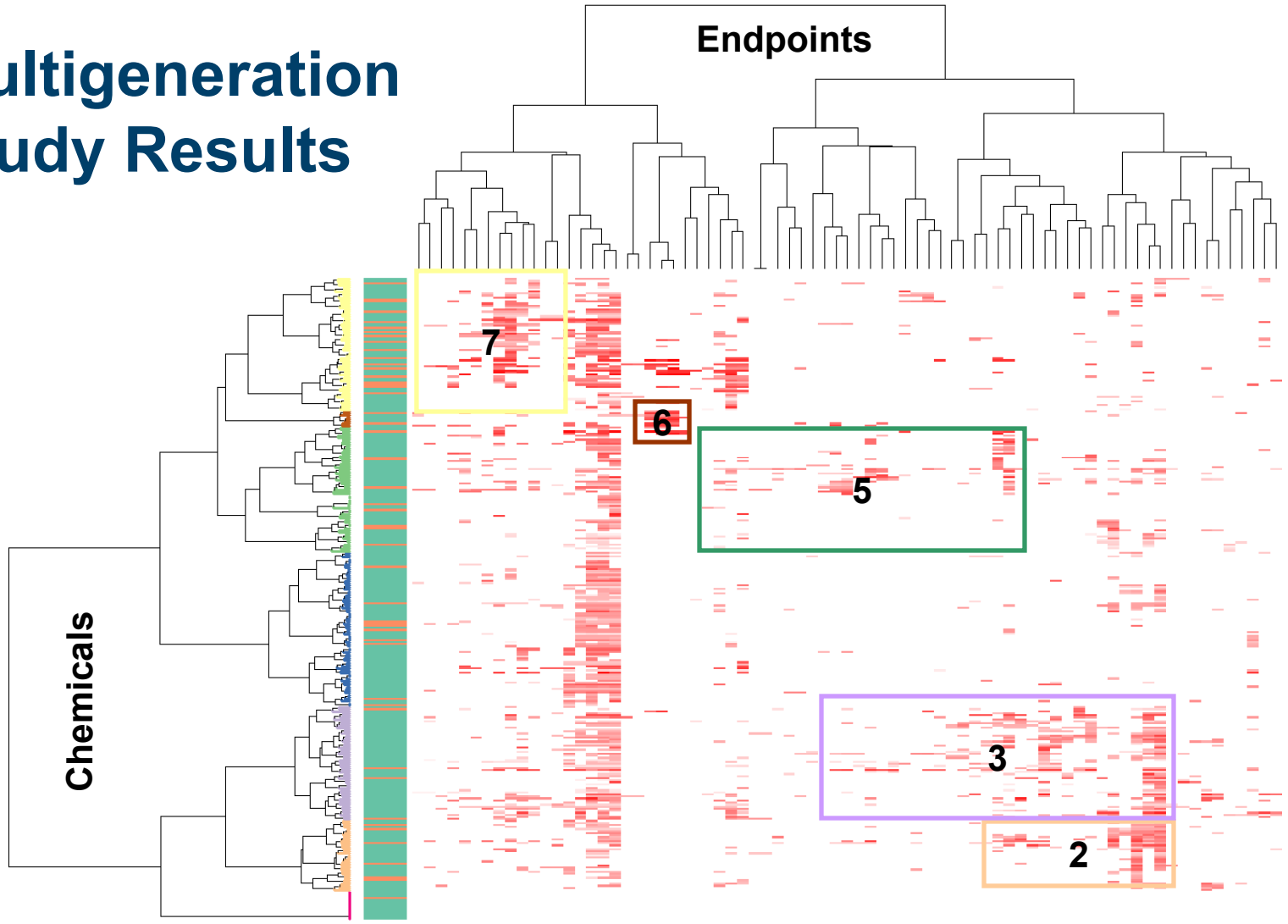


ToxRefDB 387 chemicals, 751 prenatal studies, 988 effects annotated (enhanced DevTox.org)

283 chemicals x 293 effects → 19 target systems from rat (■) and rabbit (■) studies

SOURCE: Knudsen et al. (2009) Reproductive Toxicology (in press) DOI 10.1016/j.reprotox.2009.03.016

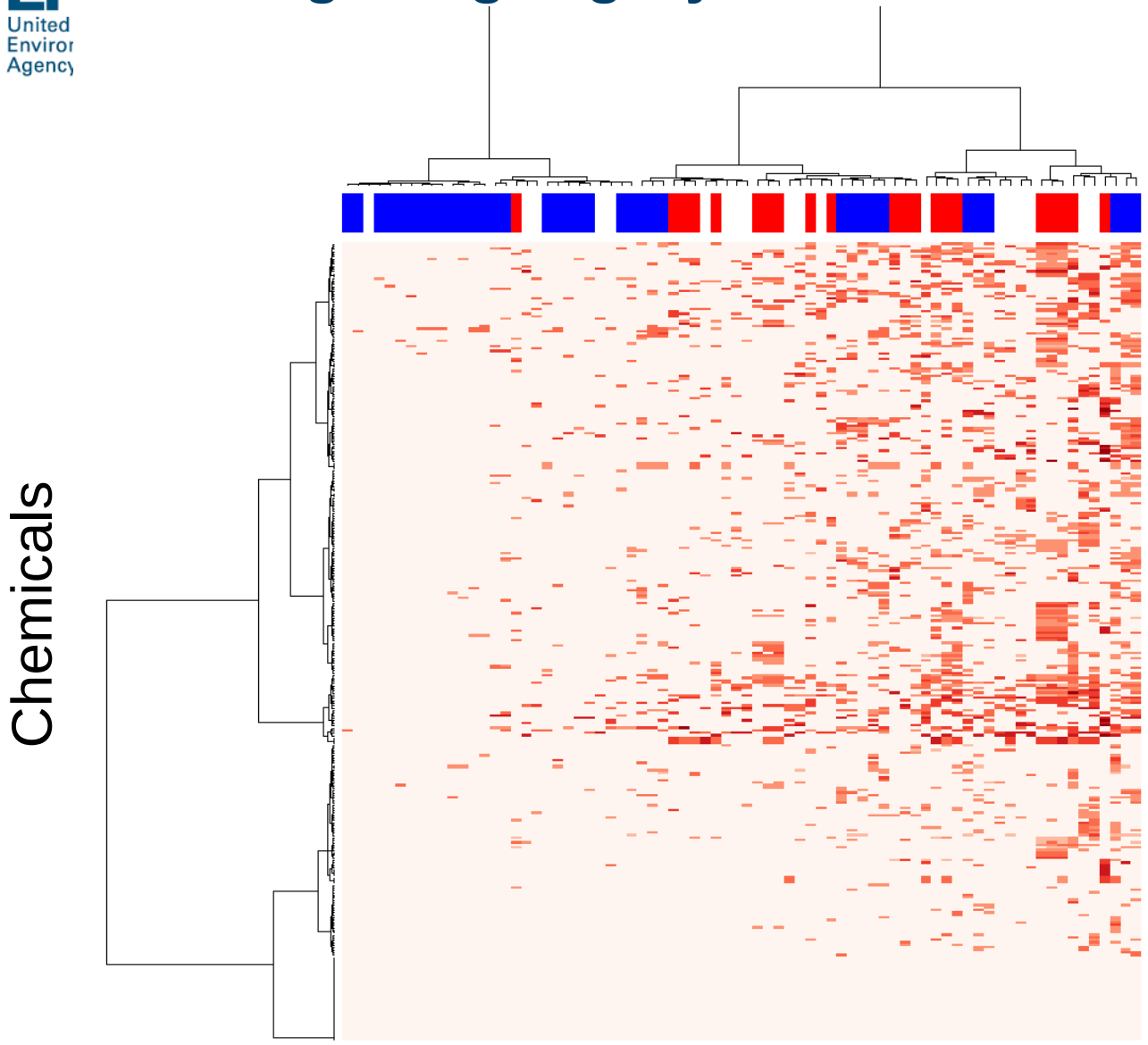
Multigeneration Study Results



CHEMICAL CLUSTER # (Top 10 Weighted Endpoints)						
1	2	3	4	5	6	7
	Adrenal Pathology	ALP		AGD	Birth Index	Clinical Signs
	AGD	Heart Weight		Bladder Pathology	ChE (Brain)	Eye Developmental
	Kidney Pathology	Lung Weight		Epididymal Pathology	ChE (Brain-regional)	Fetal Mortality
	Liver Pathology	Pituitary Weight		Epididymal Weight	ChE (Plasma)	Lactation Index
	Liver Pathology (g)	Sperm Morphology		HCT	ChE (RBC)	Litter Weight
	Liver Weight	Spleen Pathology		HGB	Epididymal Pathology	Live Birth Index
	Pituitary Weight	Spleen Pathology (g)		Prostate Weight	Lung Pathology	Lung Pathology
	Thyroid Pathology	Spleen Weight		Testis Pathology	Lung Pathology (g)	Lung Pathology (g)
	Thyroid Weight	Thymus Weight		Testis Pathology (g)	Prostate Pathology	Stomach Pathology (g)
	Uterine Weight	VO		Testis Weight	Water Consumption	Viability Index

- Martin, et al, ToxSci, in 2009

Digitizing Legacy *in Vivo* Data in ToxRefDB



Chronic/Cancer
Multigenation
Developmental

Martin et al 2009a,b
Knudsen et al 2009

30 years and more than $2B worth of data

ToxCast Assays

Biochemical Assays

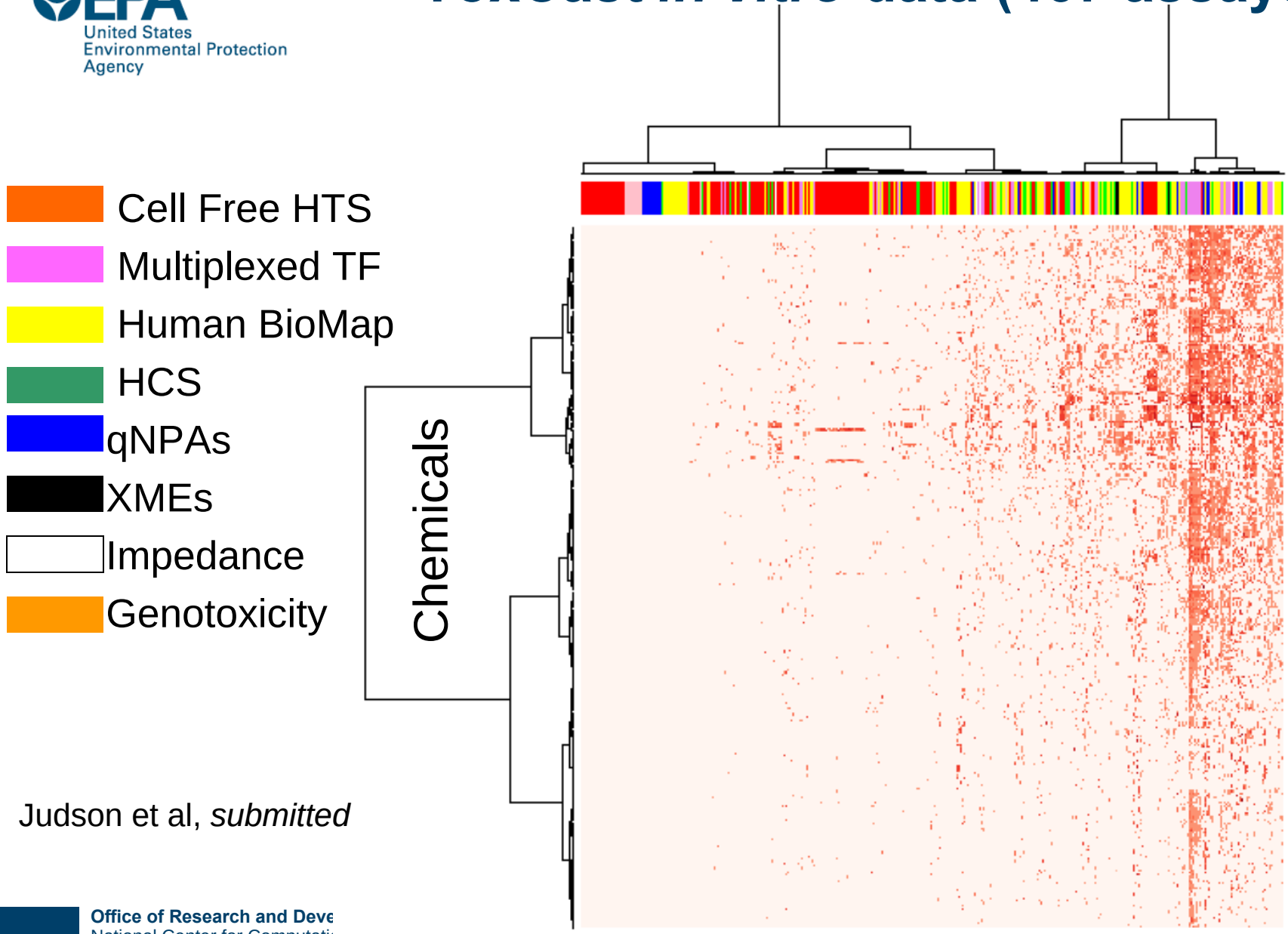
- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

Cellular Assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

ToxCast *in vitro* data (467 assays)

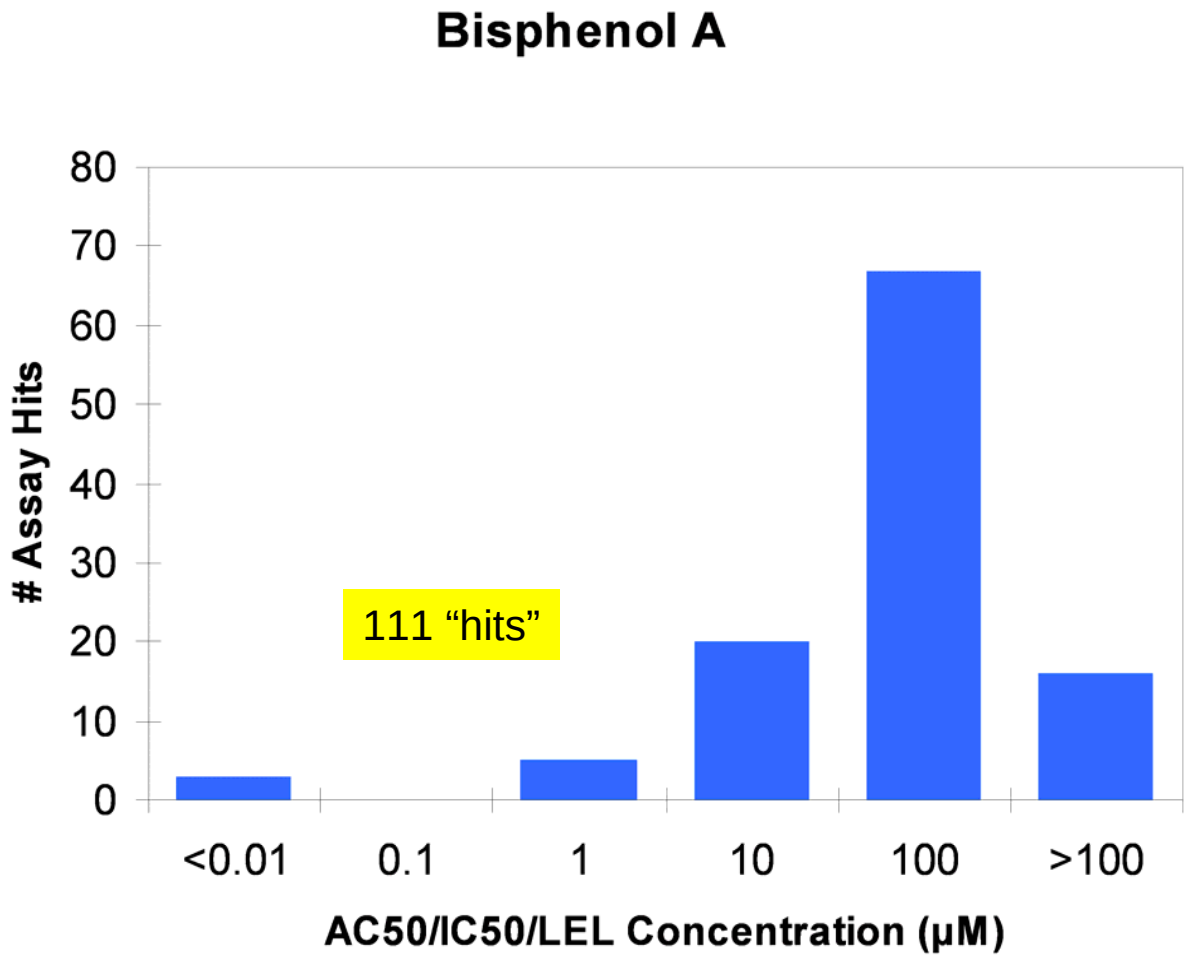
United States Environmental Protection Agency



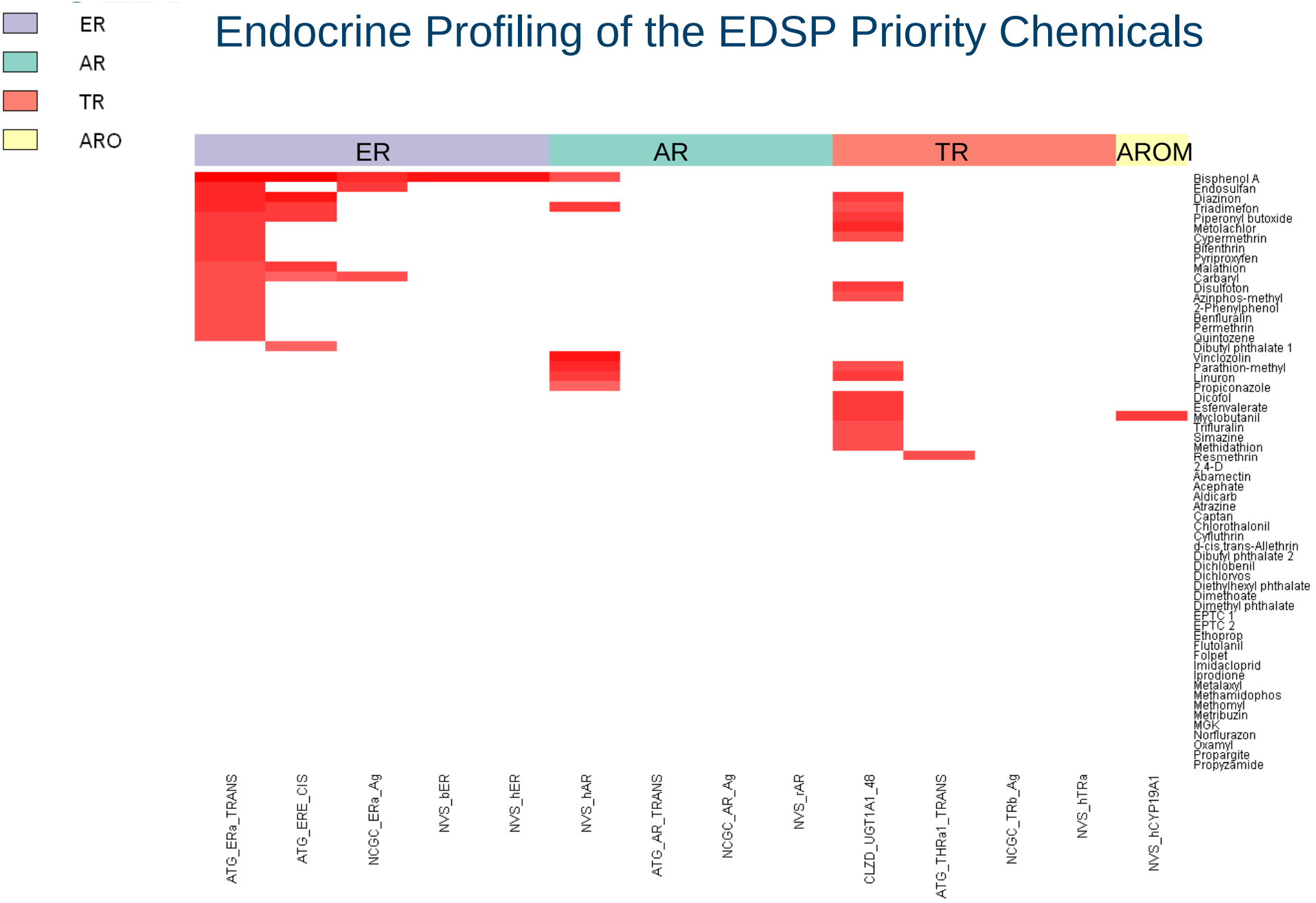
Judson et al, *submitted*

>200,000 dose response experiments

United States Environmental Protection Agency

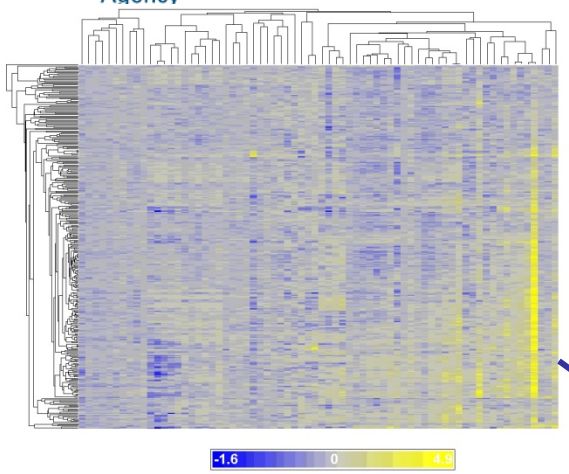


Endocrine Profiling of the EDSP Priority Chemicals

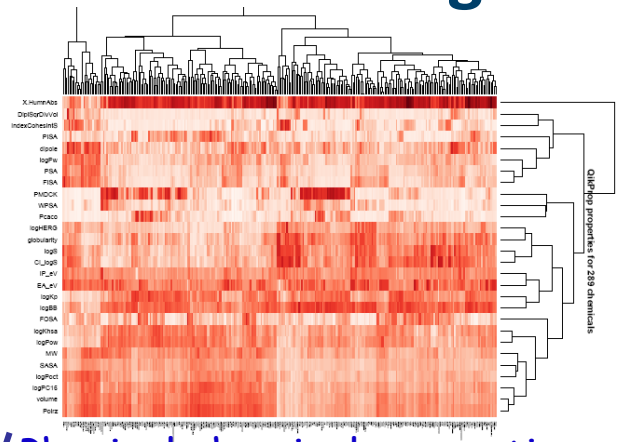


HTS Signature Matching

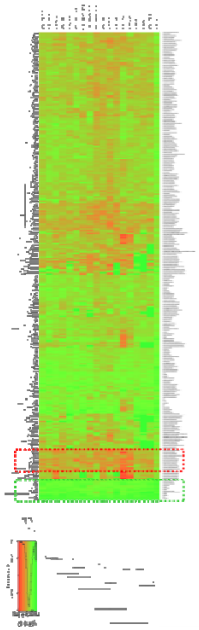
United States Environmental Protection Agency



Cellular Assays

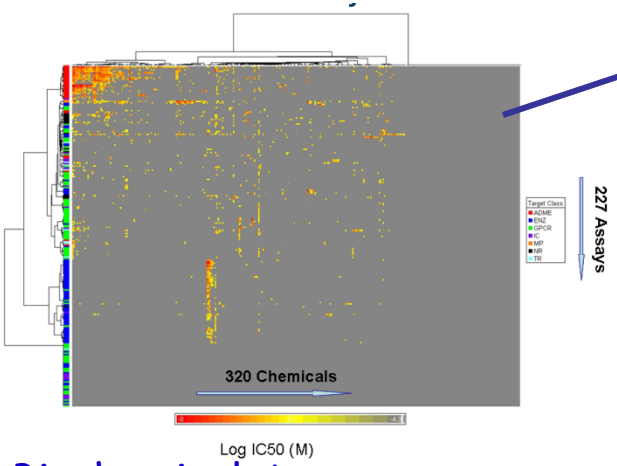


Physical chemical properties

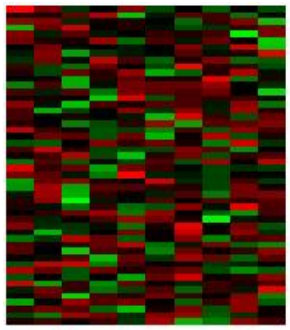


In silico Predictions

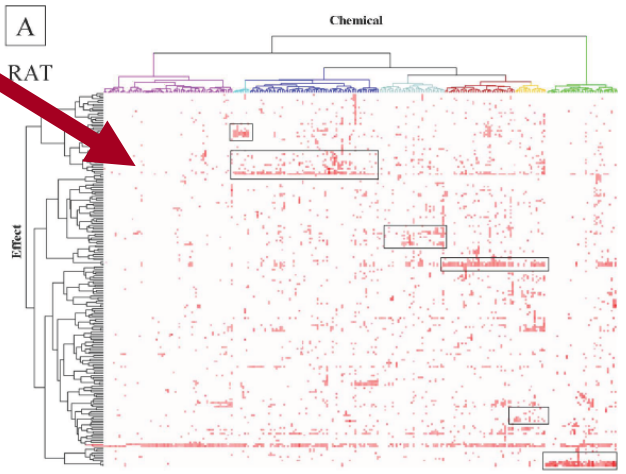
Profile Matching



Biochemical Assays

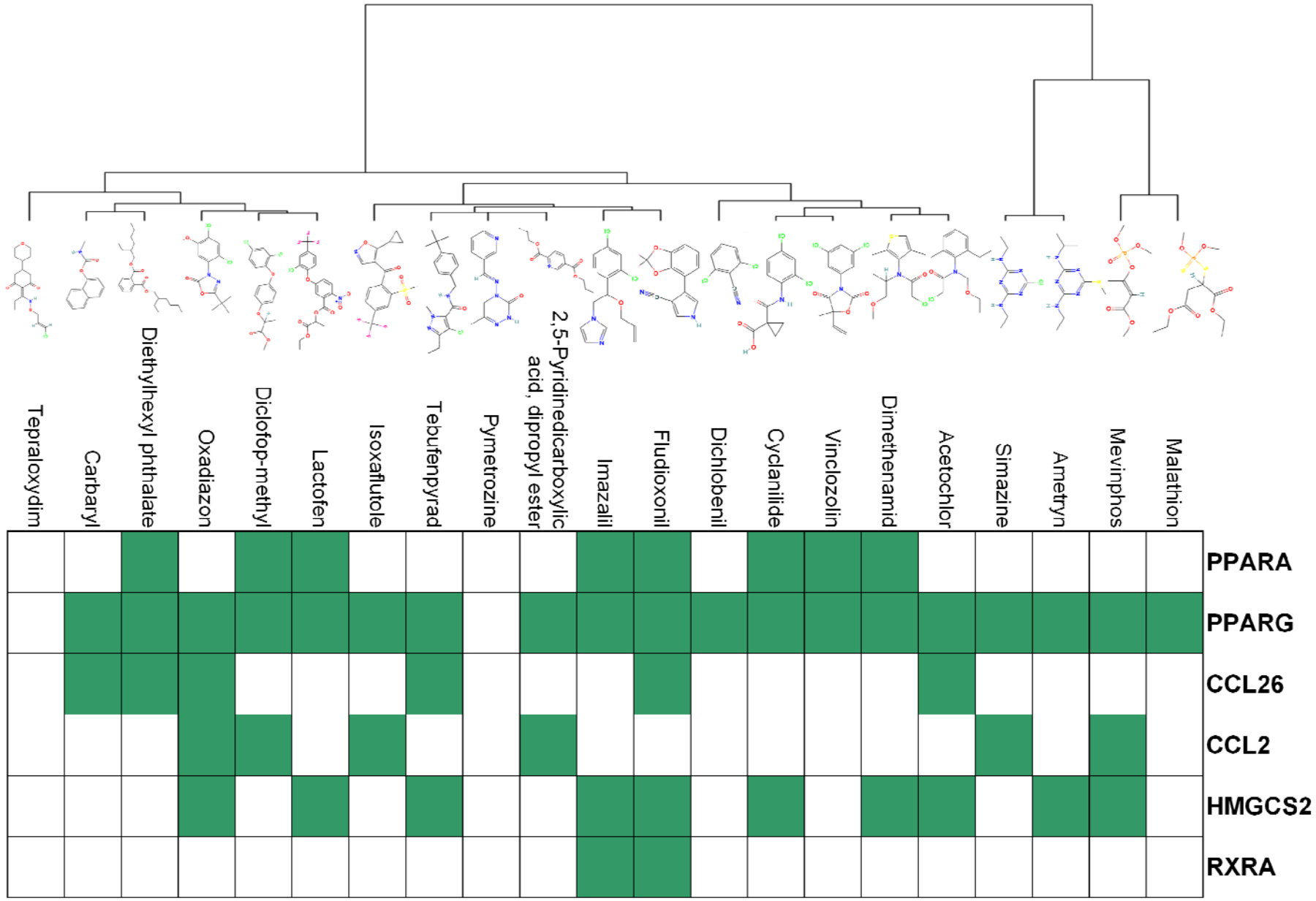


Genomic Signatures

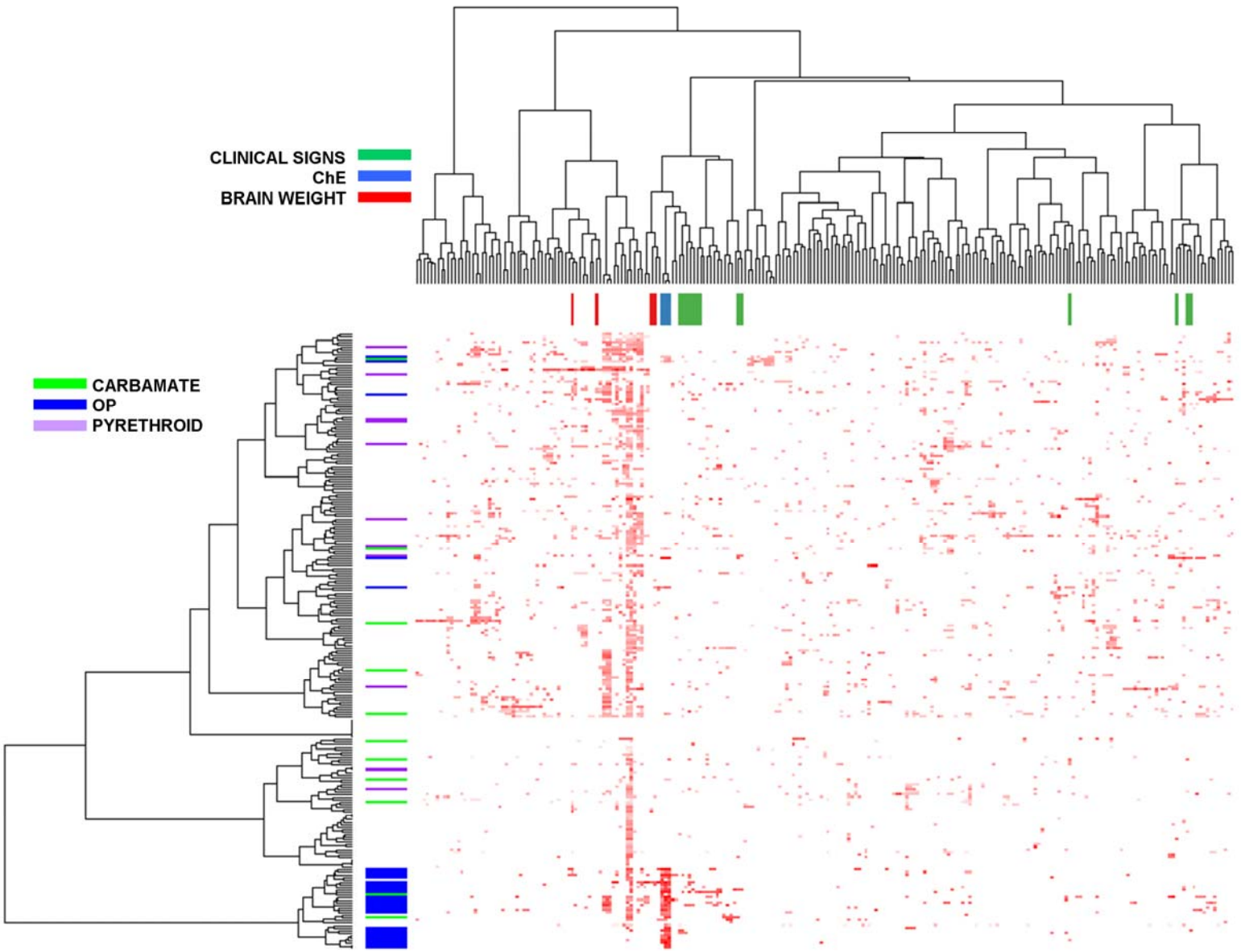


Toxicology Endpoints

Signature Derivation for Rat Liver Carcinogens



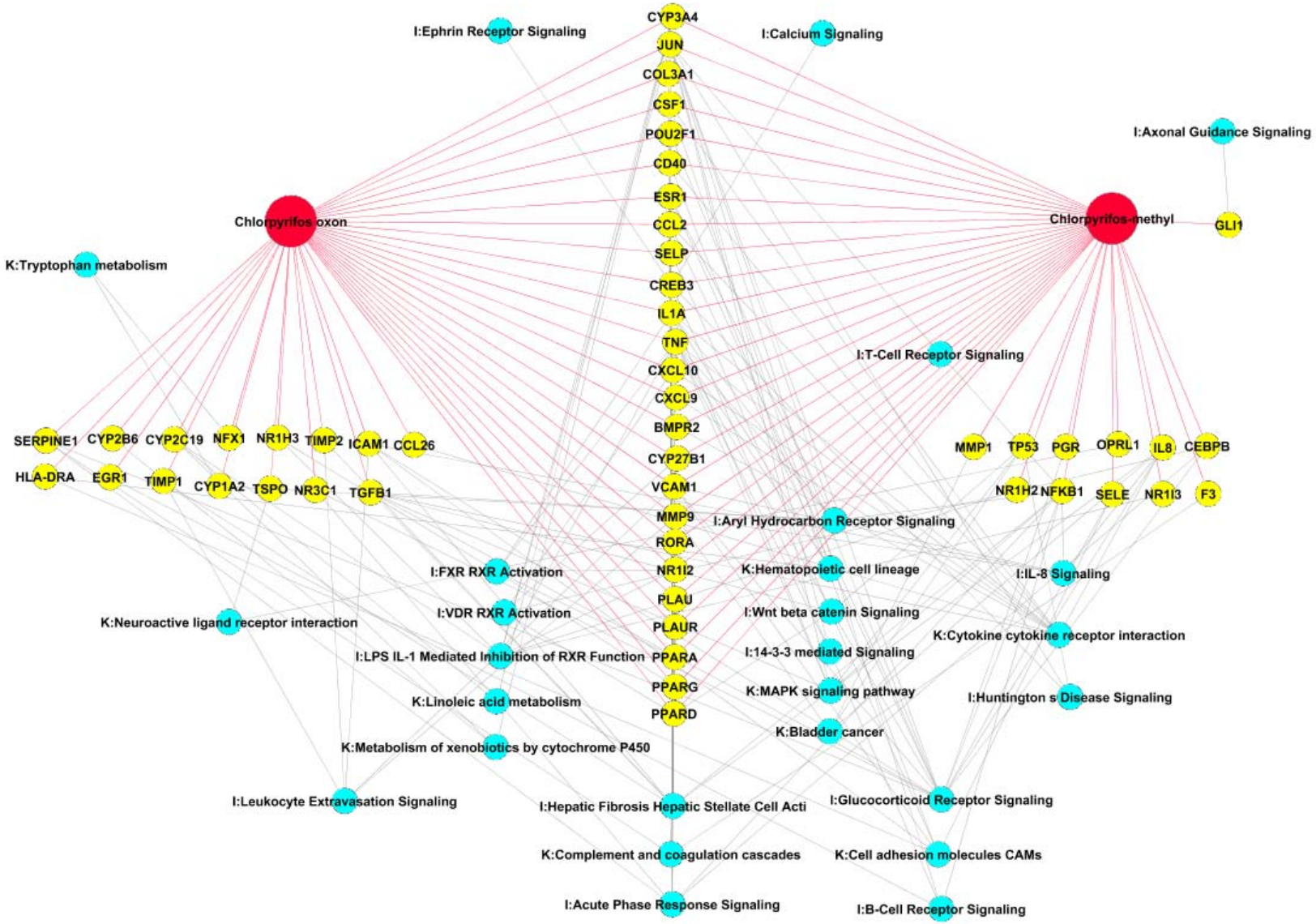
“Neuro” Endpoints in Chronic Rat Studies



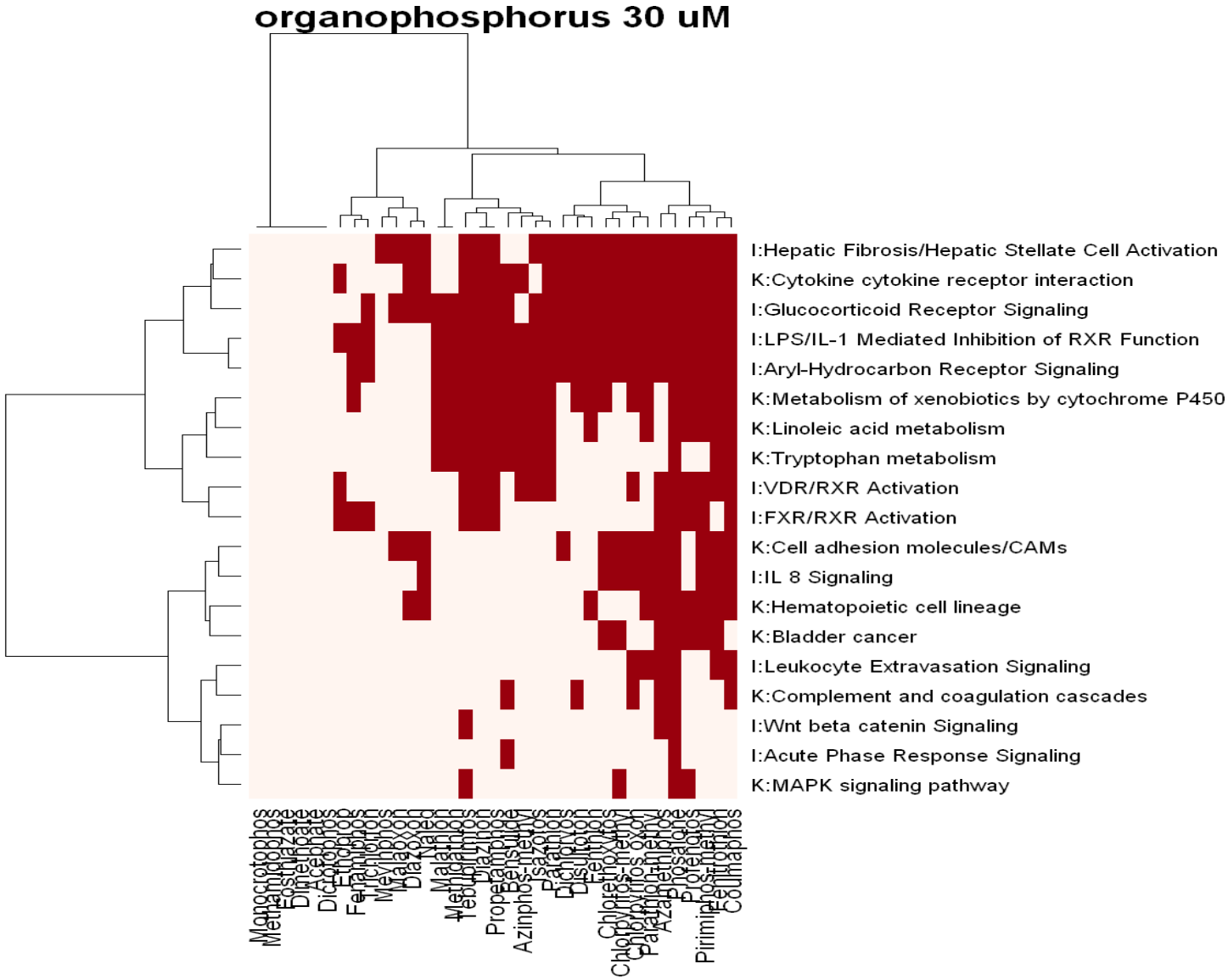
Brain Wt Decreases
- *Vinclozolin*
- *Acetochlor*
- *Simazine*
- *Flufenacet*
- *Fipronil*
- *Cyanamide*

Clinical Signs (>5 events)
- *Abnormal Gait*
- *Alopecia*
- *anogenital staining*
- *Defacation (diarrhea)*
- *Emaciation*
- *Fur (altered appearance*
- *hunched Posture*
- *hyperactivity*
- *Tremors*
- *Urine staining - perineum*
- *urine, discoloration*

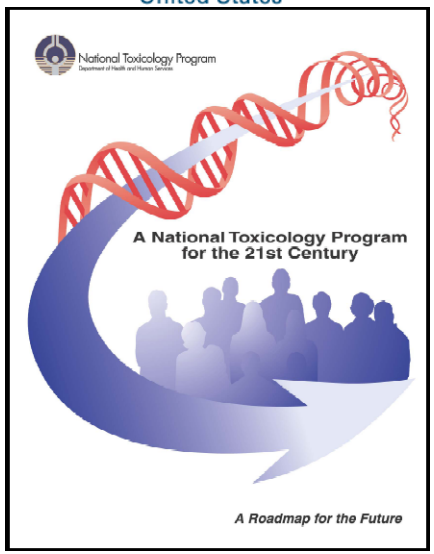
Pathway Targets for Chlorpyrifos

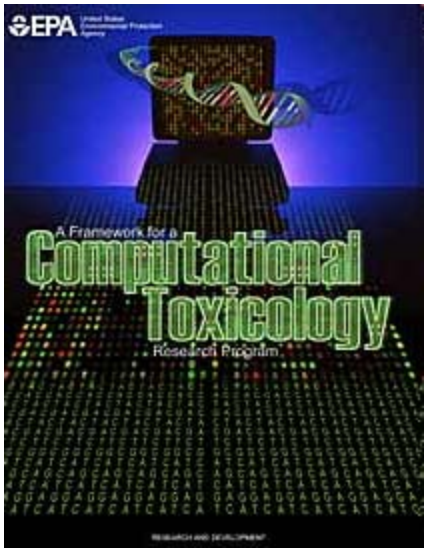


Pathway Hits for Organophosphates



Tox21 Community Development





2004 **1.4k Library** **2006** **2.8k Library** **2008** **10k Library** **2010**

2005

2007

2009

Tox21

5M data points to date

Tox21 Community

Activities	NTP	NCGC	EPA
Historical Toxicology Data	✓		✓
Experimental Toxicology	✓		✓
Ultra High-Throughput Testing		✓	
Mid- to High Throughput Systems			✓
Lower Organism Model System	✓ *C. elegans*		✓ Zebrafish
In Vitro 3-D Model Systems	✓		✓
Effect of Human/Rodent Genetic Background on Toxic Effects	✓	✓	
Computational Toxicology	✓	✓	✓
Validation Experience	✓ (NICEATM-ICCVAM)	✓	✓

Tox21 Working Groups

- **Pathways/Assays - K. Witt (NTP), K. Houck (EPA), M. Xia (NCGC)**
  - Identify key toxicity pathways/assays (with a focus on human cells) and prioritize assays for use
  - Identify assay gaps and consider methods for filling those gaps
  - Develop methods for incorporating hepatic metabolism into *in vitro* assays
  - Consider approaches for evaluating compound, pathway, and cell-to-cell interactions

- **Compounds - C. Smith (NTP), A. Richard (EPA), N. Southall (NCGC)**
  - Establish a library ~10,000 compounds with known structures for testing at the NCGC
  - Establish procedures for determining the identity, purity, and stability of each compound

- **Bioinformatics - K. Shockley (NTP), R. Judson (EPA), R. Huang (NCGC)**
  - Evaluate patterns of response and relationship to adverse health outcomes in experimental animals and humans
  - Evaluate consistency of response within assays and across related endpoints
  - *Make all data publicly accessible (PubChem, ACToR, CEBS)*

- **Targeted Testing - J. Bucher (NTP), S. Edwards (EPA), J. Inglese (NCGC)**
  - Prioritize substances for more complex testing, including the use of alternative assay platforms or species (e.g., *C. elegans*, zebrafish)

Tox21

Tox21 assays screened at NCGC to date

- Cell viability
  - ATP (Cell titer glo)
  - LDH
  - Protease release
- Caspases
  - 3/7
  - 8
  - 9
- Pathways
  - AP1
  - ARE
  - CRE
  - HRE
  - NFκB
- DNA damage
  - p53
  - Multiple repair gene-deficient cell lines
- Nuclear receptors
  - AR
  - ERα
  - FXR
  - GR
  - LXRβ
  - PPARα
  - PPARγ
  - PPARδ
  - PXR
  - RXR
  - TRβ
  - VDR
- Inter-individual variation in chemical response
  - 20 sets of identical twins

Limitations

- Not all *in vitro* assays are suitable for HTS and not all substances can be tested *in vitro* (volatiles, solvent requirement, practical concentration limitation)
- Responses are for the most part limited to cell-autonomous effects of parent compound
- Current *in vitro* assays do not take into consideration exposure (route, extent), ADME, or genetic heterogeneity relating to differences in sensitivity
- A gene is not a pathway, a pathway is not a cell, a cell is not an organ, an organ is not an animal, ……

Needs

- Develop comprehensive battery of pathway and phenotypic assays
- Determine which cell types are most useful for HTS
  - Human vs rodent, primary cells, stem cells
- Incorporate metabolism and genetic heterogeneity
- Orthogonal and higher order assays to confirm hits
  - (e.g., *in vitro* 3D organ models, zebrafish)
- Obtain existing *in vitro*, experimental animal, and human data
- User interfaces for data browsing
- Validate resulting testing strategies for reliability and relevance, and develop strategies for incorporation into regulatory decision-making

MATERIALS TRANSFER AGREEMENT

EPA:

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Pfizer:
Pfizer Inc, having a principal place of business at 235 East 42nd Street, New York, ("Pfizer") New York, 10017 and its Affiliates

WHEREAS the EPA wishes to obtain Pfizer Compounds to use in certain test assay panels, and whereas Pfizer wishes to have Pfizer Compounds evaluated on such test panels, the parties agree as follows:

"Affiliate" means any corporation, firm partnership or other entity which directly or indirectly controls, is controlled by, or is under common control with either of the parties.

1. EPA agrees to receive Pfizer's compounds, listed in Exhibit B, in any form or any of its intermediates and derivatives ("Pfizer Compound"), in order to perform the research activities, further described in Exhibit A, and known as the "ToxCast™ Program."

2. The Pfizer Compounds:
   a. are the property of Pfizer and all existing rights including, without limitation, patent rights in or to the Pfizer Compounds will remain the property of the Pfizer.
   b. will be used with caution and for research purposes only, and shall not be used for research involving human subjects.
   c. will be used only by the EPA in the ToxCast™ Program described below, under suitable containment conditions.
   d. will not be used for screening, production or sale, for which a commercialization license may be required.

Both Pfizer and EPA agree to comply with all applicable laws, rules, guidelines and regulations applicable to the use, storage, shipping and the handling of the Pfizer Compounds and ToxCast™ Program.

The Future State: Using Hazard and Exposure Information for Prioritizing Testing and Monitoring