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Applications of High Throughput Screening to Identify Patterns of Biological Activity

Robert Kavlock
Director, EPAs National Center for Computational Toxicology

Office of Research and Development
National Center for Computational Toxicology

20 th Anniversary of ZEBET
Berlin, Germany
October 27, 2009

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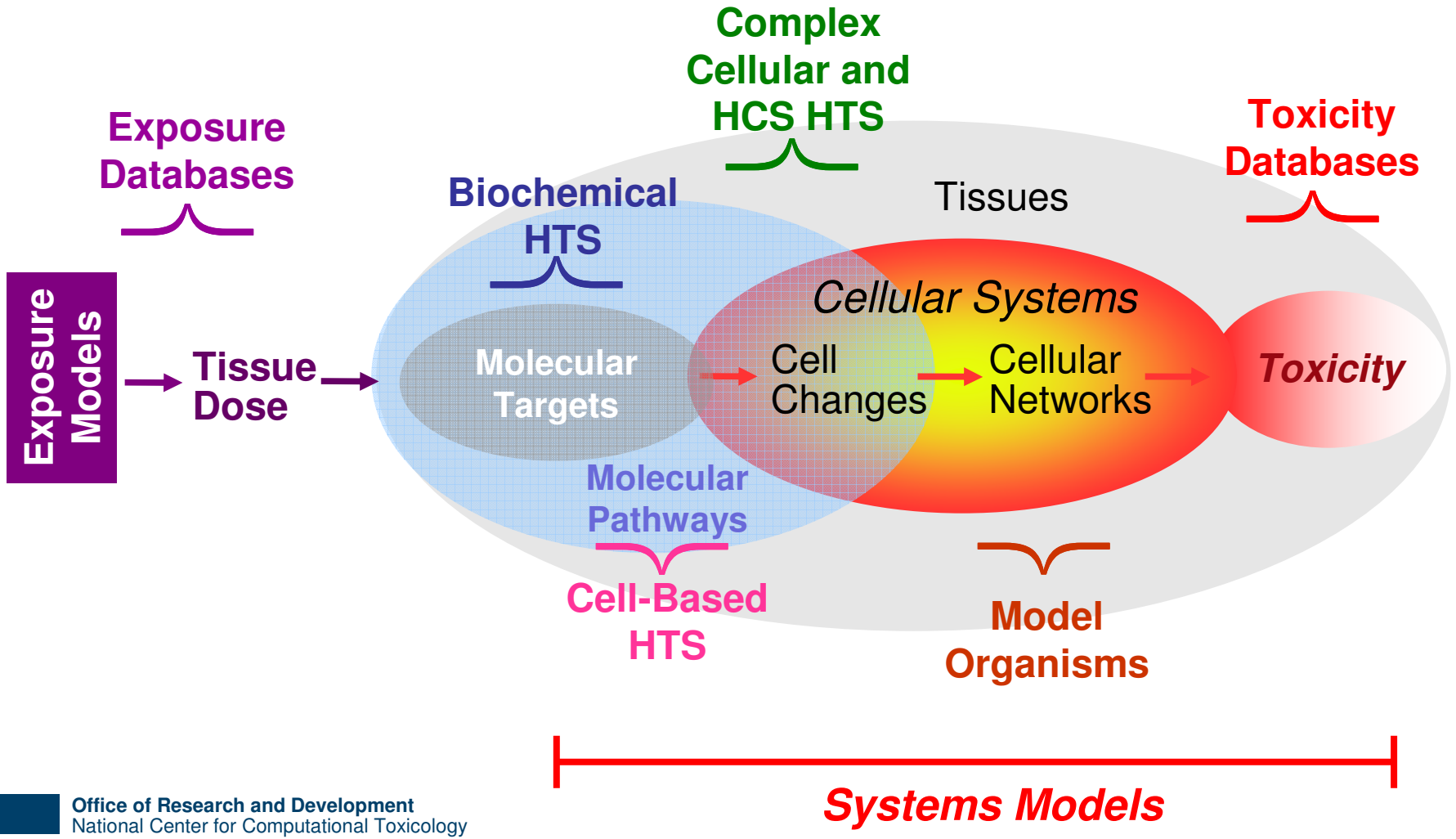
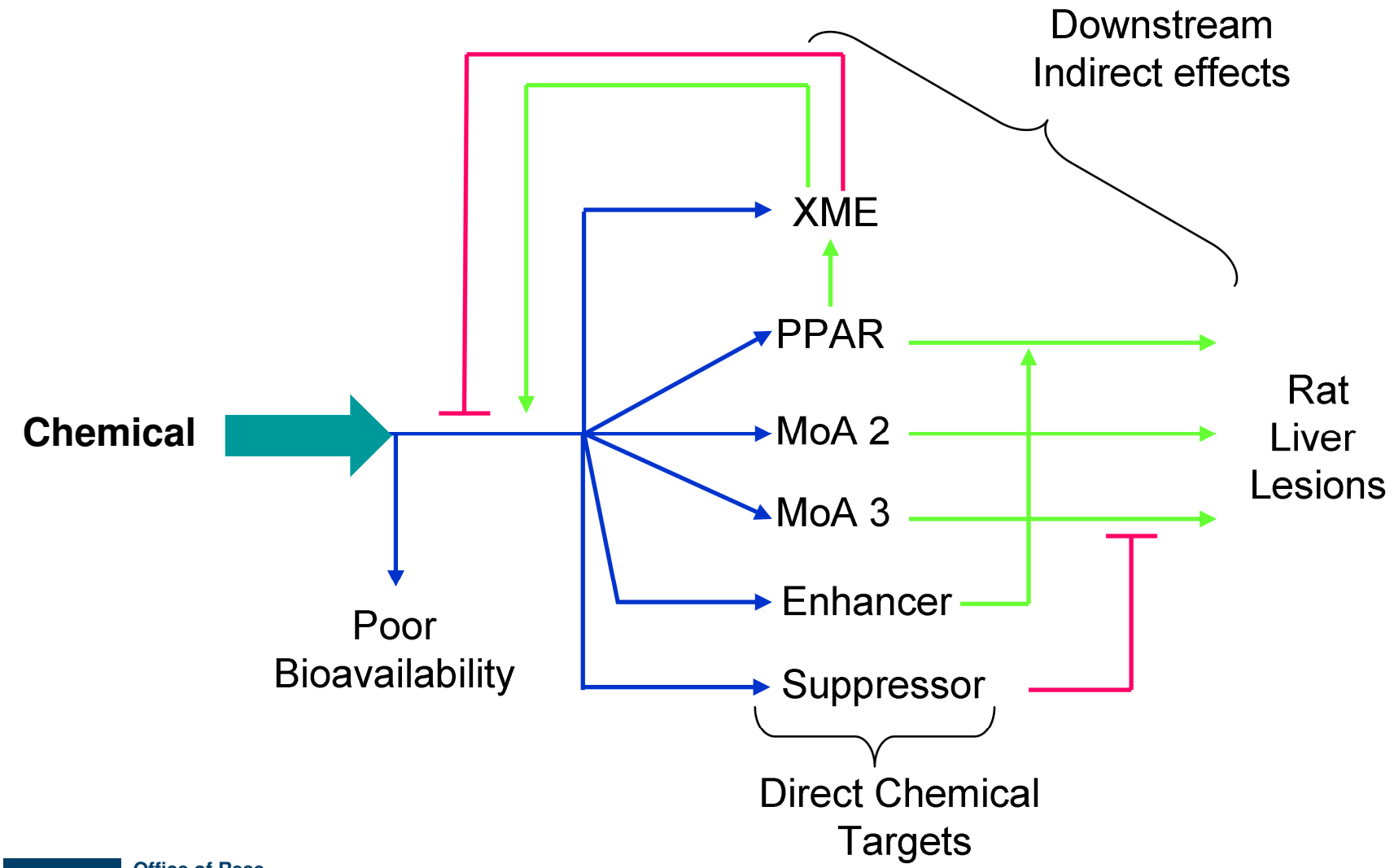
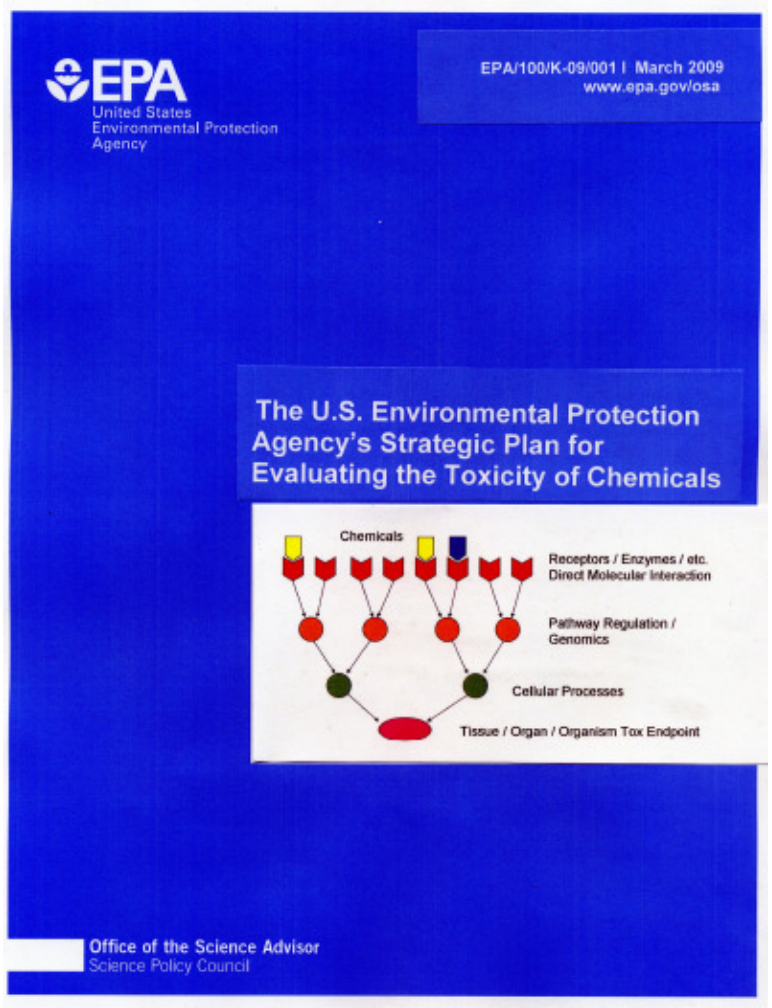
The Grand Challenge: Predicting Human Toxicity



Predicting Toxicity Will Not Be Easy



EPA Strategic Plan (2009)

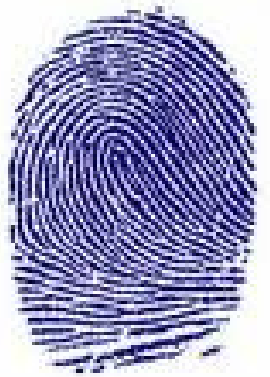


Strategic Goals
- **Toxicity Pathway ID and Screening**
- **Toxicity Based Risk Assessment**
- **Institutional Transition**

ToxCast™ Background

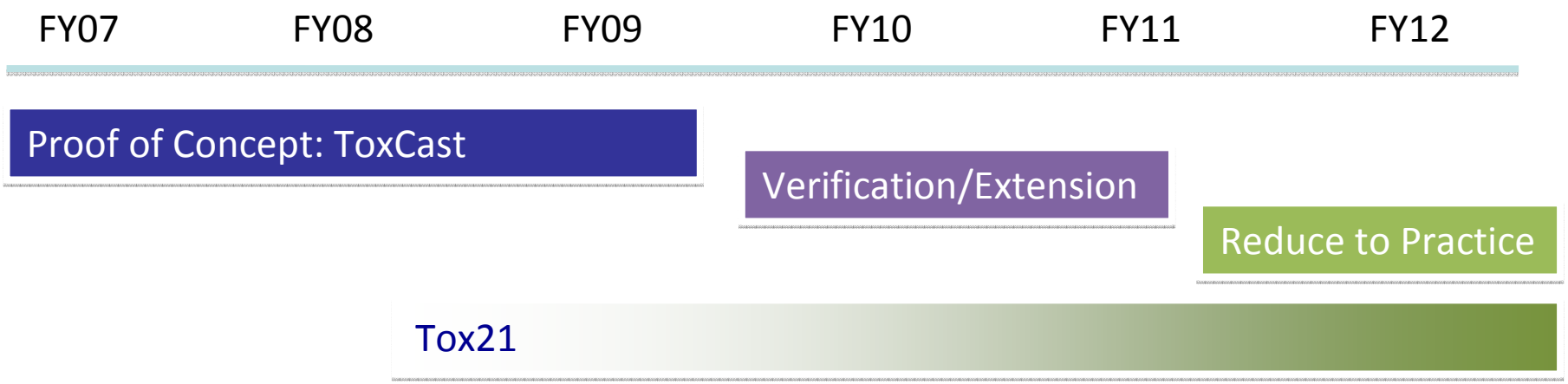
- Addresses chemical screening and prioritization needs for pesticidal inerts, anti-microbials, CCLs, HPVs and MPVs

- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures



- Committed to stakeholder involvement and transparency
  - Communities of Practice- Chemical Prioritization; Exposure
  - Release of all data upon peer review publication

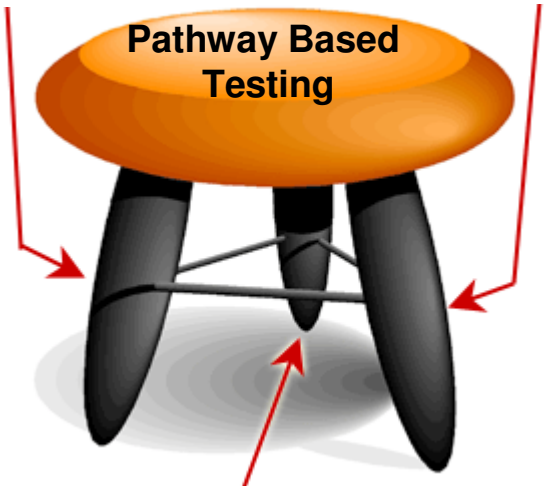
Prioritization Product Timeline



Component of the Transformation in Toxicology

High Throughput Screening

Information Technology and Management

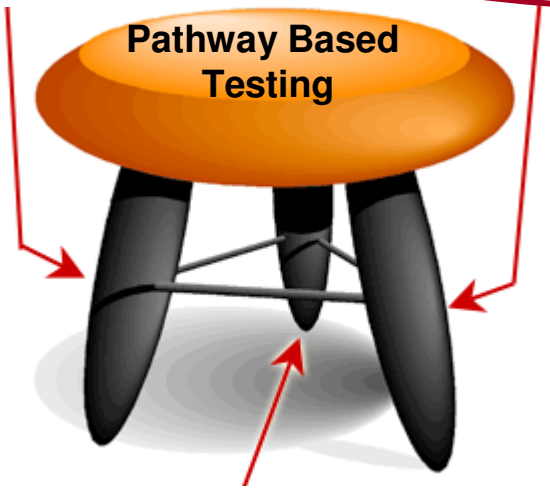


Molecular, Cellular and Systems Biology

Component of the Transformation in Toxicology

High Throughput Screening

Information Technology and Management



Molecular, Cellular and Systems Biology

ToxRefDB Endpoint Coverage

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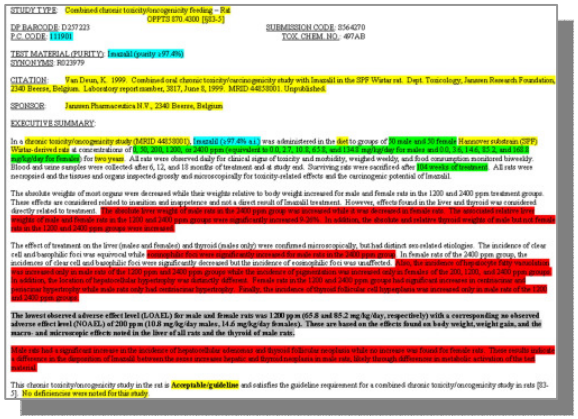
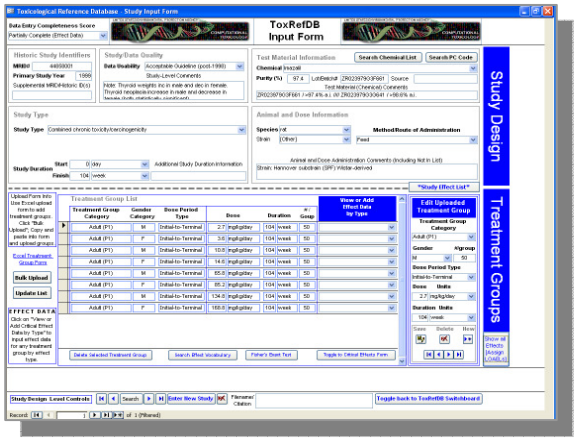
data evaluation records



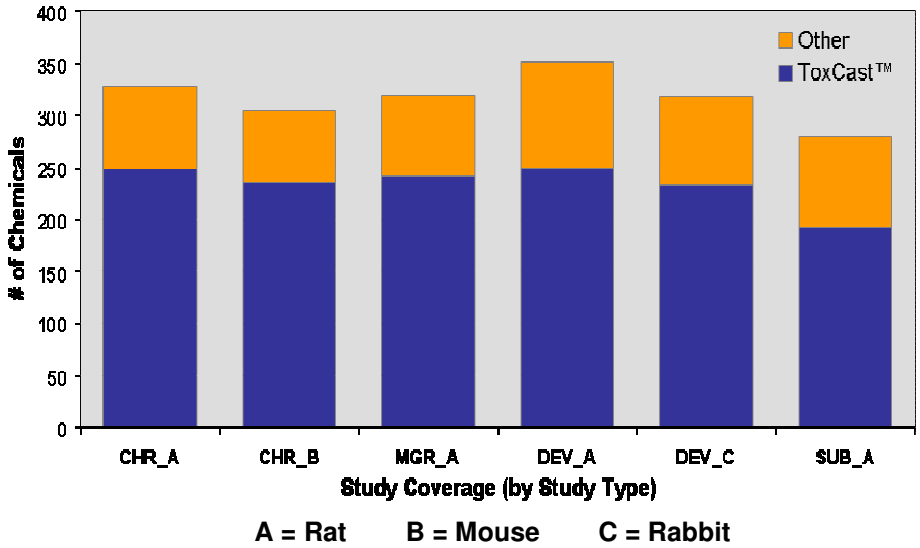
ToxRefDB



CHRONIC/CANCER (CHR)
Martin et al. (2008) Environ Hlth Persp
doi:10.1289/ehp.0800074

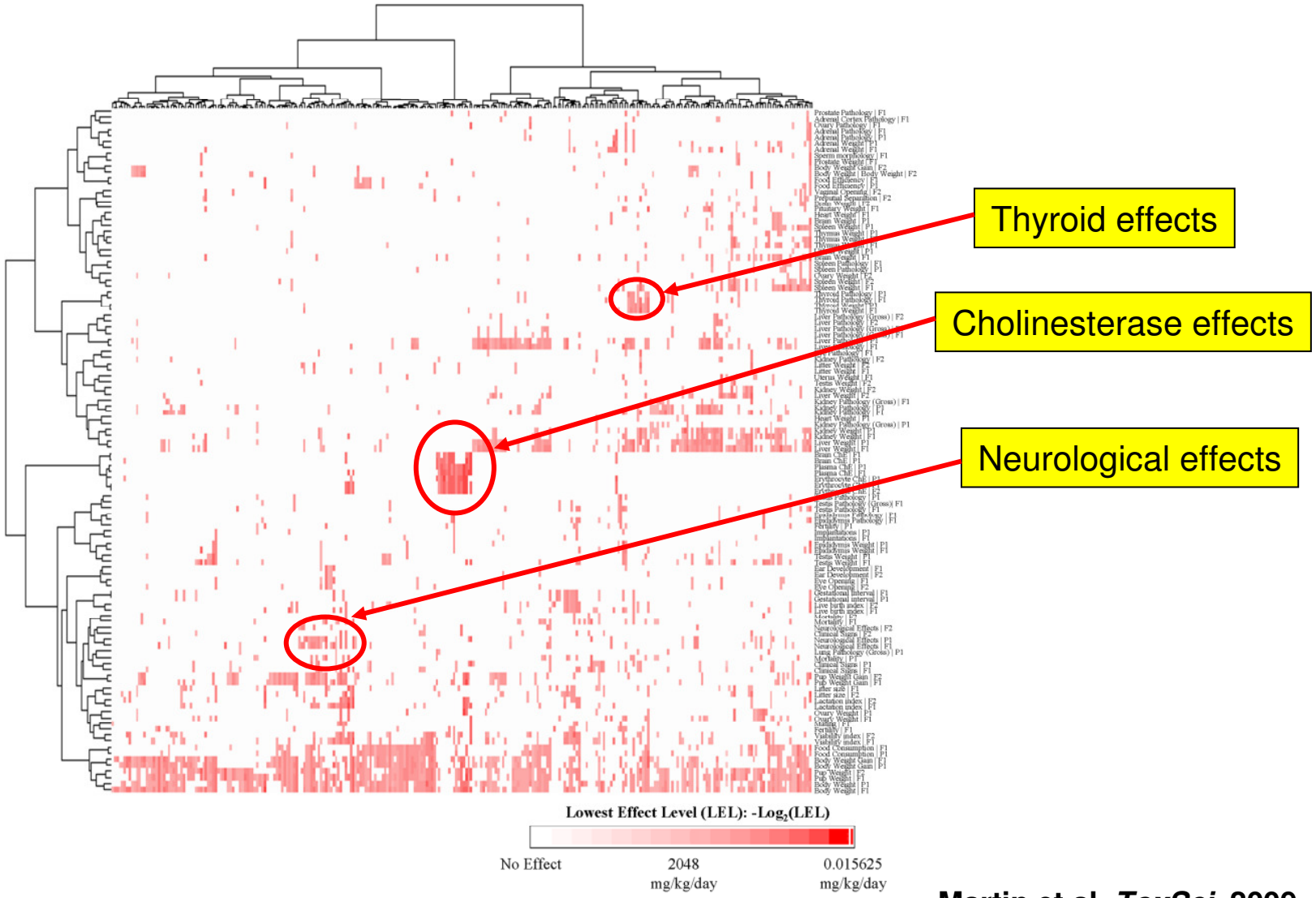
MULTIGENERATION REPRODUCTIVE (MGR)
Martin et al. (2009) Toxicol Sci
doi: 10.1093/toxsci/kfp080

PRENATAL DEVELOPMENTAL (DEV)
Knudsen et al. (2009) Reprod Toxicol
doi: 10.1016/j.reprotox.2009.03.016



SOURCE: Matt Martin, NCCT, 2009

ToxRefDB Multigeneration Studies



Martin et al, *ToxSci*, 2009

ToxRefDB Multigeneration Studies

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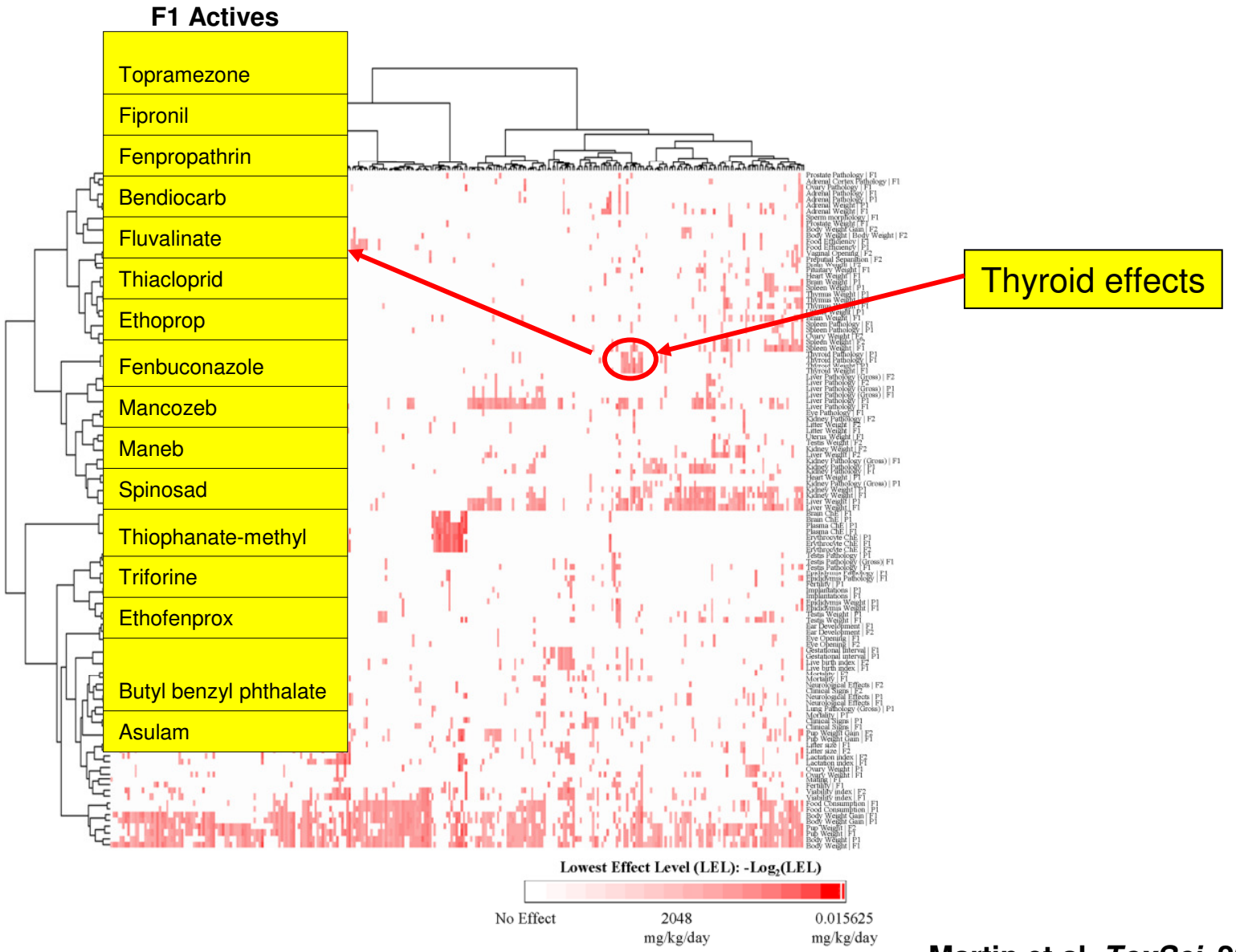
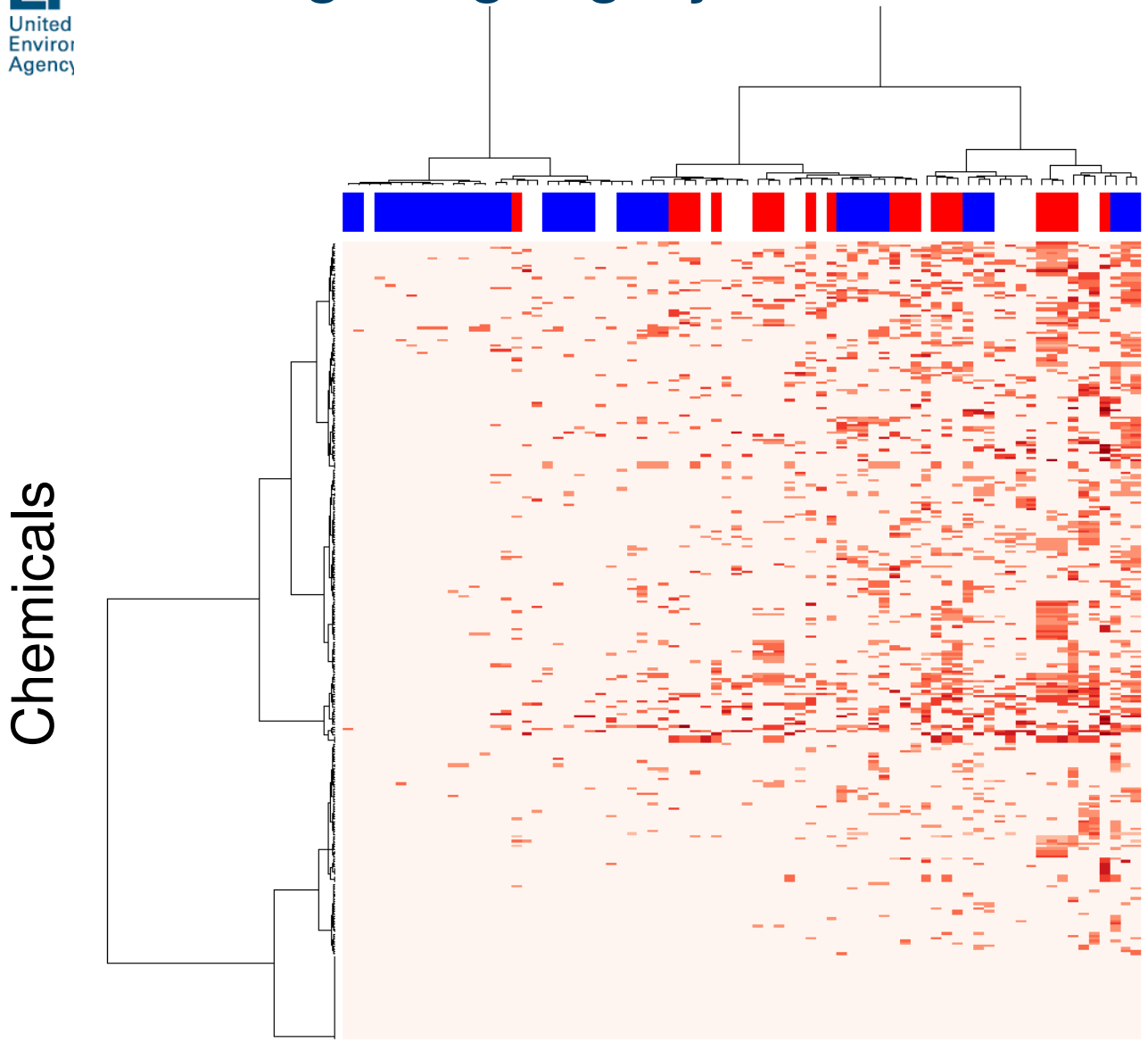


Martin et al, *ToxSci*, 2009

Digitizing Legacy *in Vivo* Data in ToxRefDB

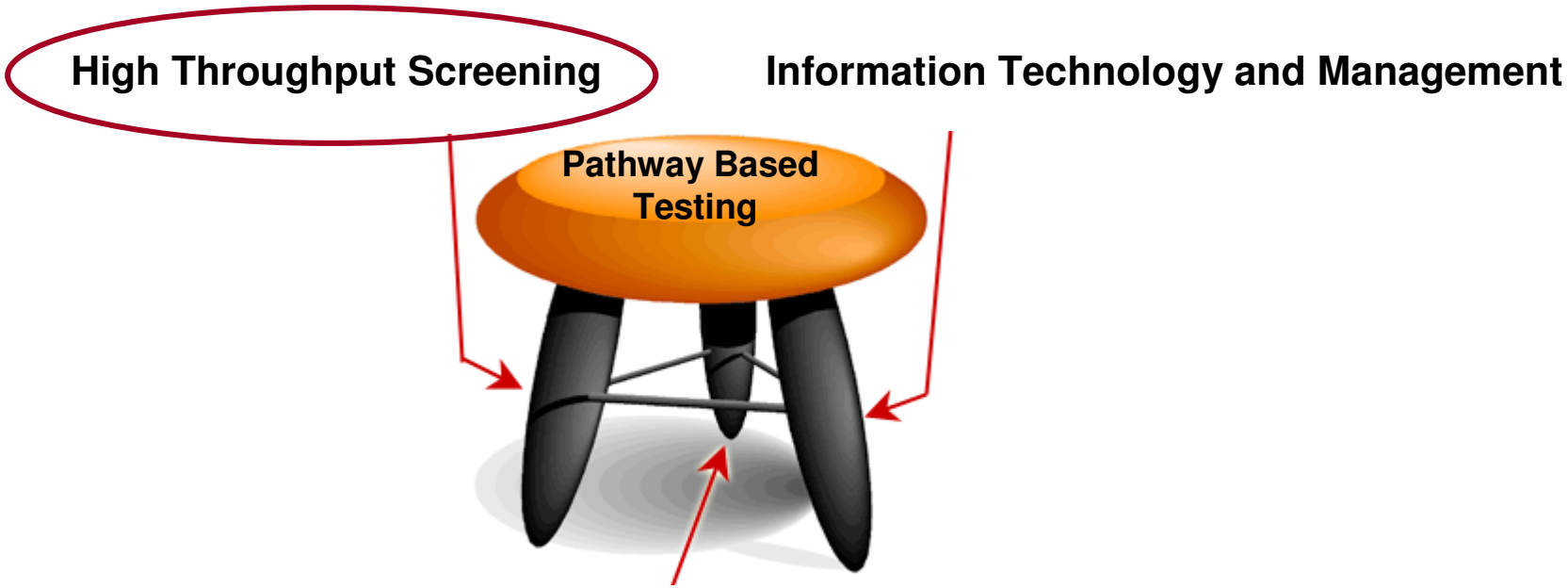


Chronic/Cancer
Multigenation
Developmental

Martin et al 2009a,b
Knudsen et al 2009

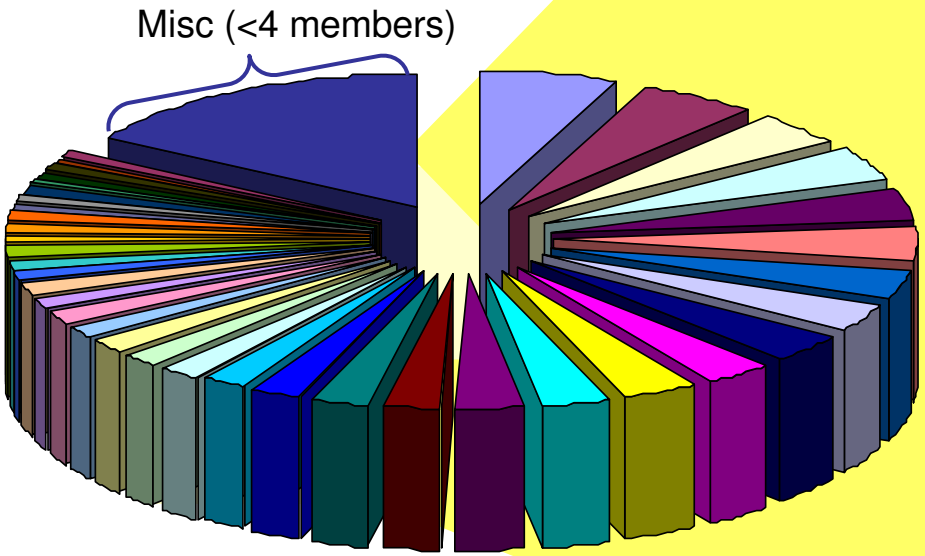
30 years and more than $2B worth of data

Component of the Transformation in Toxicology



Chemical Classes in ToxCast_320 (Phase I)

- 309 Unique Structures
- Replicates for QC
- 291 Pesticide Actives
- 14 HPVs
- 54/67 Proposed Tier 1 Endocrine Disruption Screening Program



- CHLORINE
- ORGANOPHOSPHORUS
- AMIDE
- ESTER
- ETHER
- PYRIDINE
- FLUORINE
- CARBOXYLIC ACID
- PHENOXY
- KETONE
- TRIAZINE
- CARBAMATE
- PHOSPHOROTHIOATE
- PYRIMIDINE
- BENZENE
- ORGANOCHLORINE
- AMINE
- PYRETHROID
- SULFONYLUREA
- TRIAZOLE
- UREA
- IMIDAZOLE
- NITRILE
- ALCOHOL
- CYCLO
- PHOSPHORODITHIOATE
- THIOCARBAMATE
- ANILINE
- THIAZOLE
- DINITROANILINE
- OXAZOLE
- PHOSPHATE
- IMINE
- NITRO
- PHENOL
- PHTHALIMIDE
- PYRAZOLE
- SULFONAMIDE

ToxCast *In vitro* HTS Assays

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Biochemical Assays

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

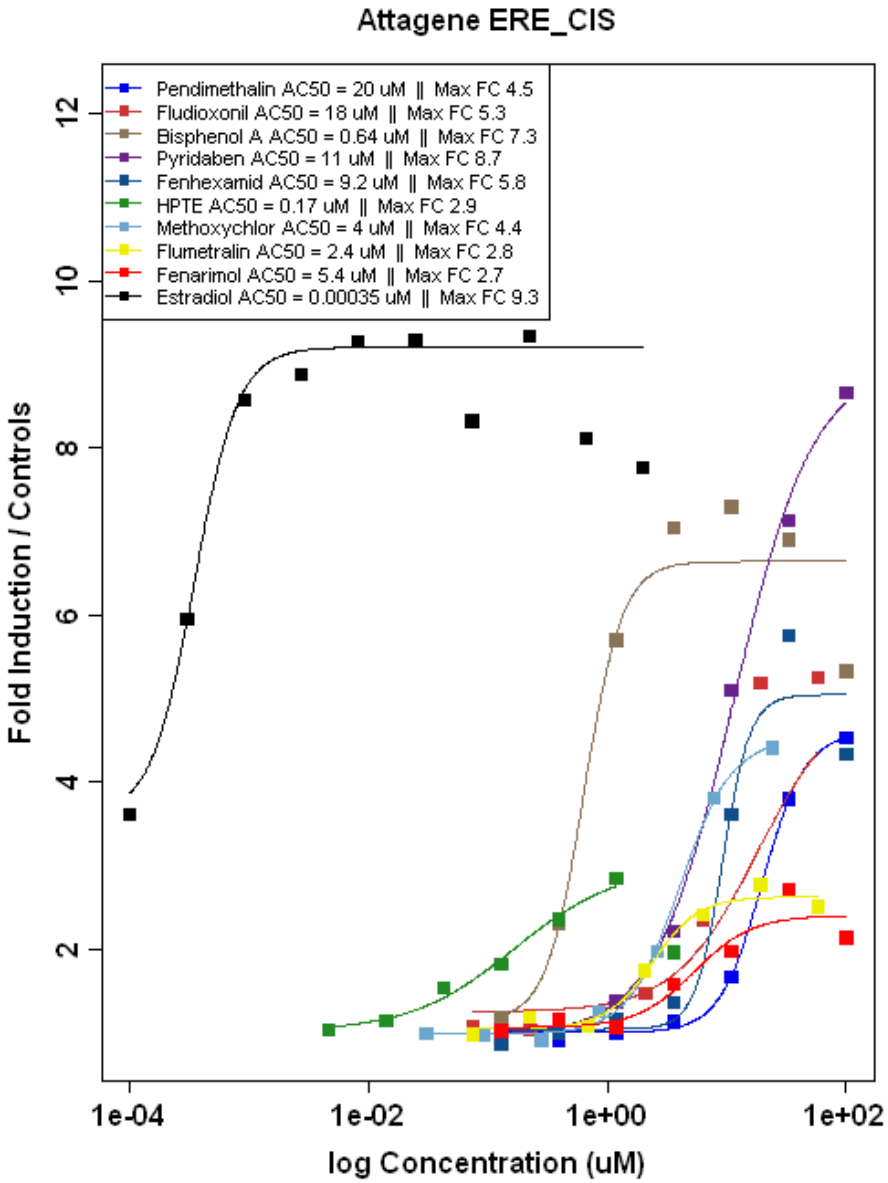
467 Endpoints

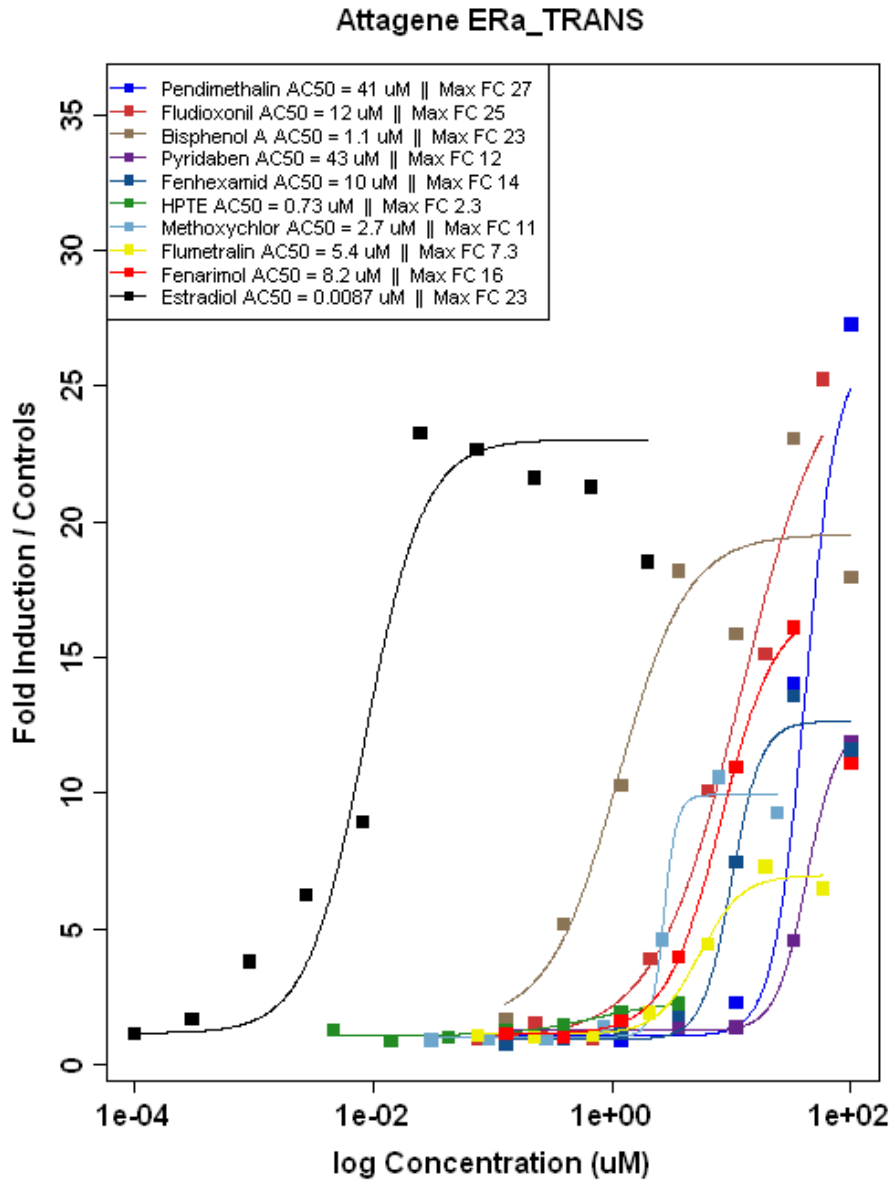
Cellular Assays

- **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

Data Analysis:

What is a hit?





ToxCast Phase I Assay Results

(n=624 measurements)

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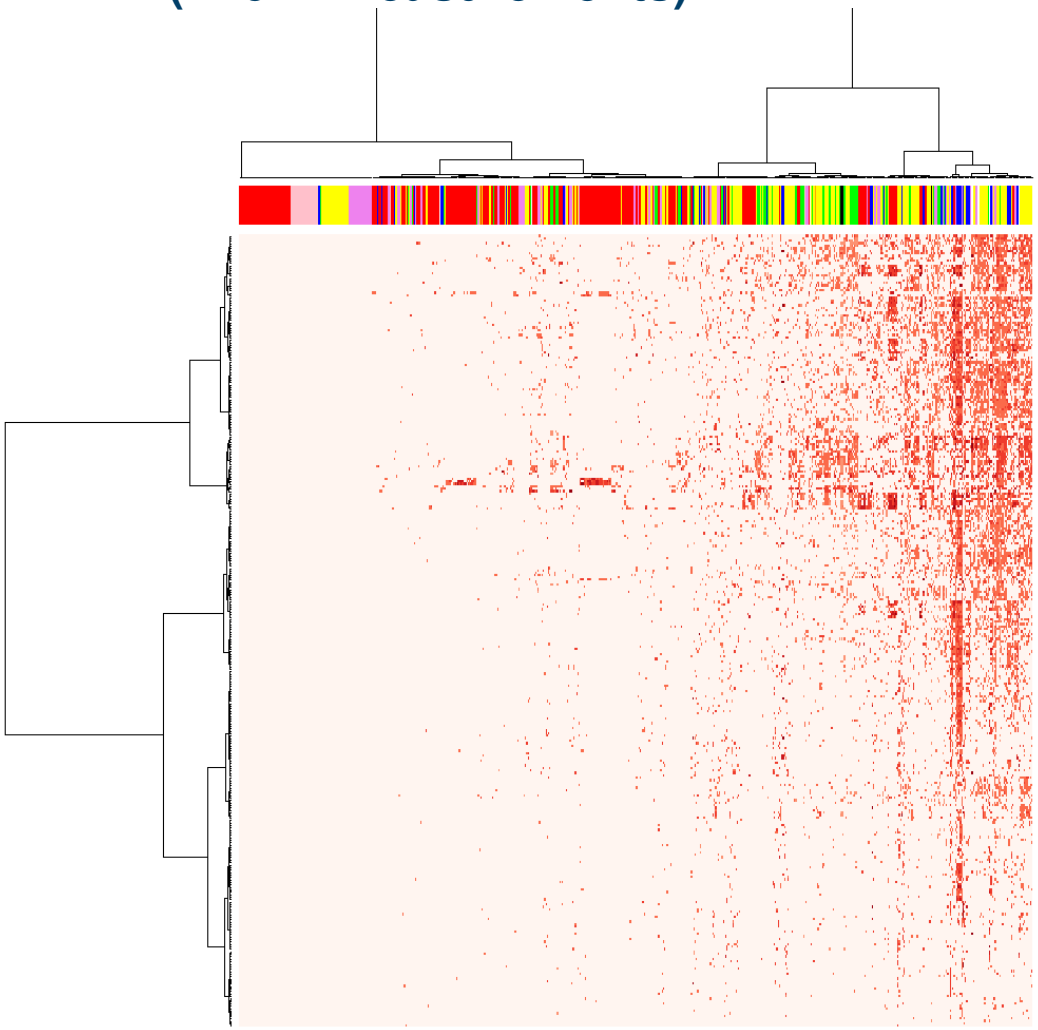
- Cell Free HTS
- Multiplexed TF
- Human BioMap
- HCS
- qNPAs
- XMEs
- Impedance
- Genotoxicity



>64,000 dose-response curves

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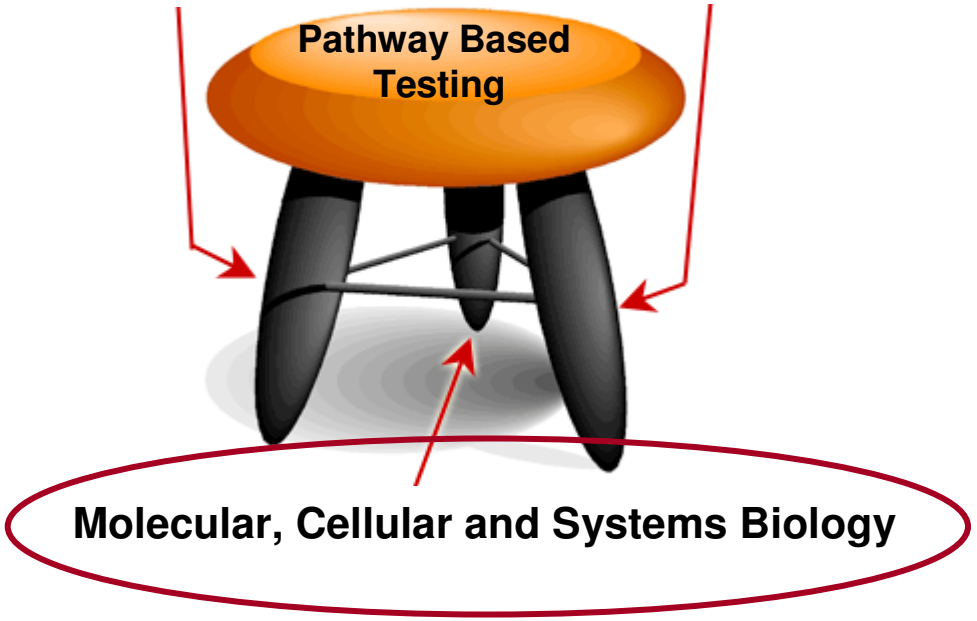
National Center for Computational Toxicology

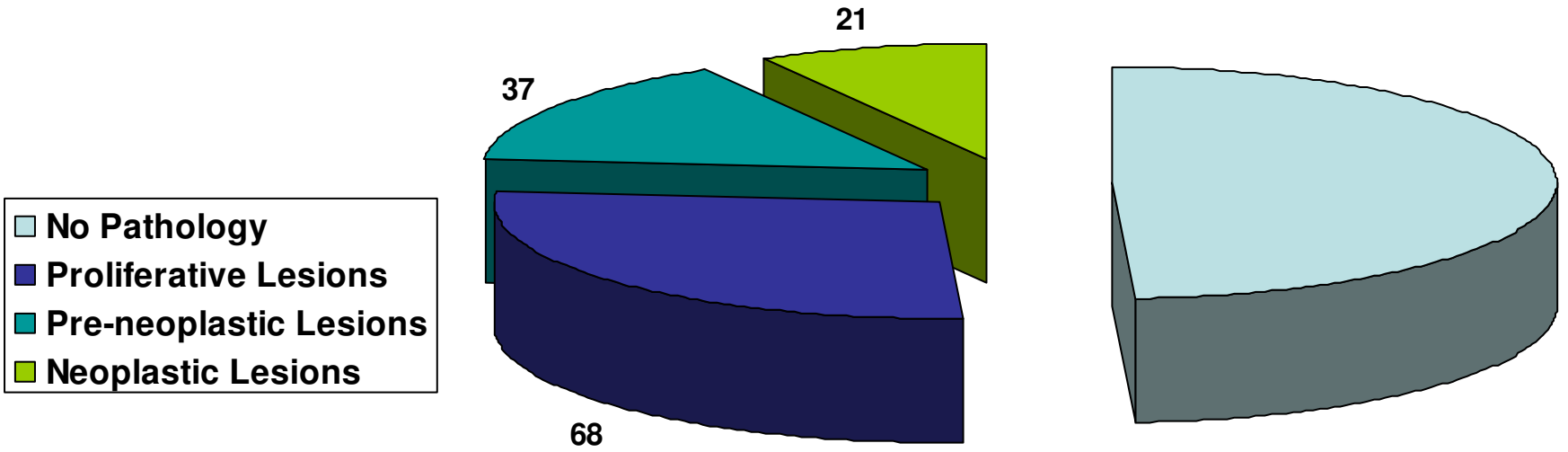
Judson et al, EHP, submitted

Component of the Transformation in Toxicology

High Throughput Screening **Information Technology and Management**



Rat Liver Histopathology from Chronic Bioassays



N = 248 Chemicals

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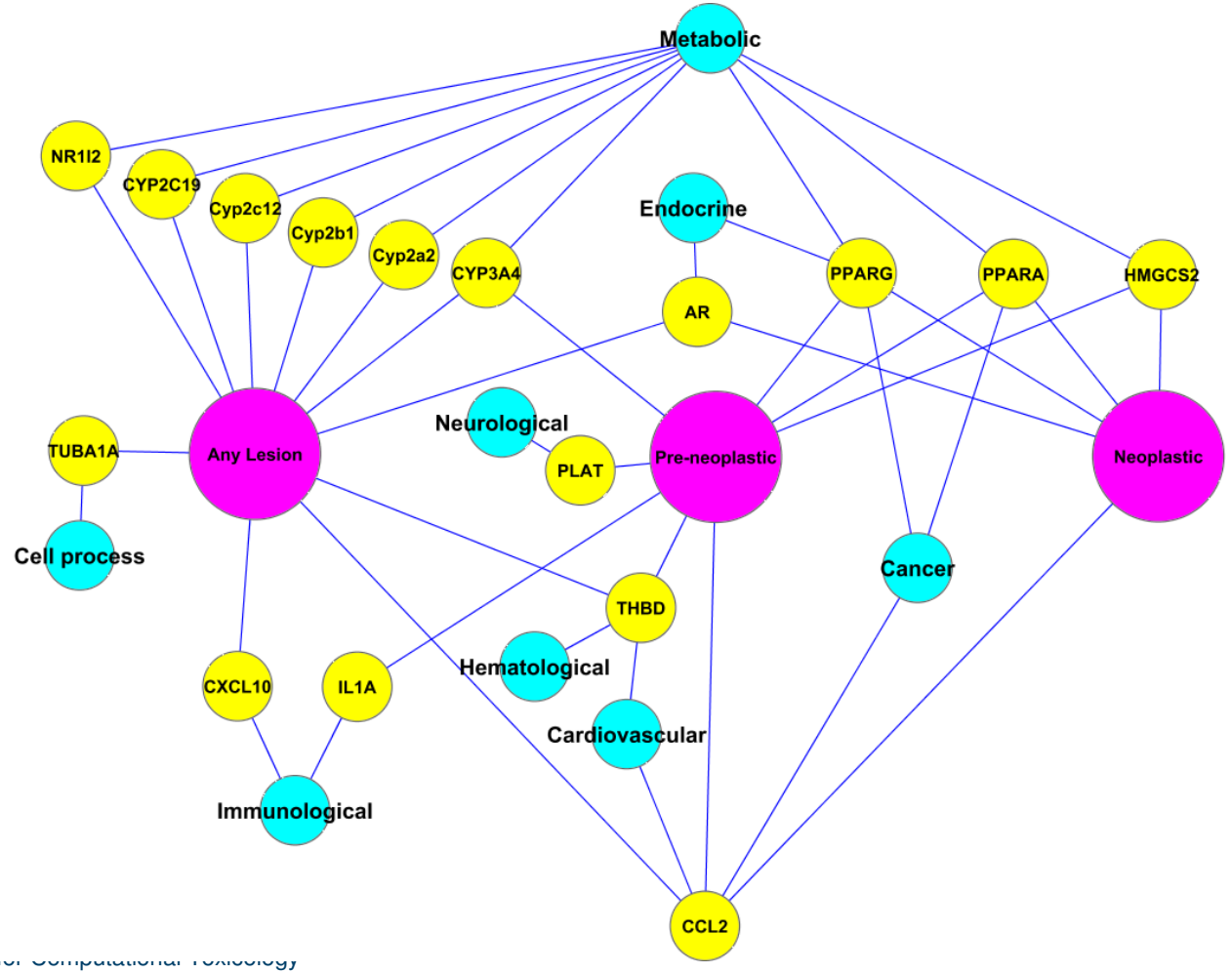
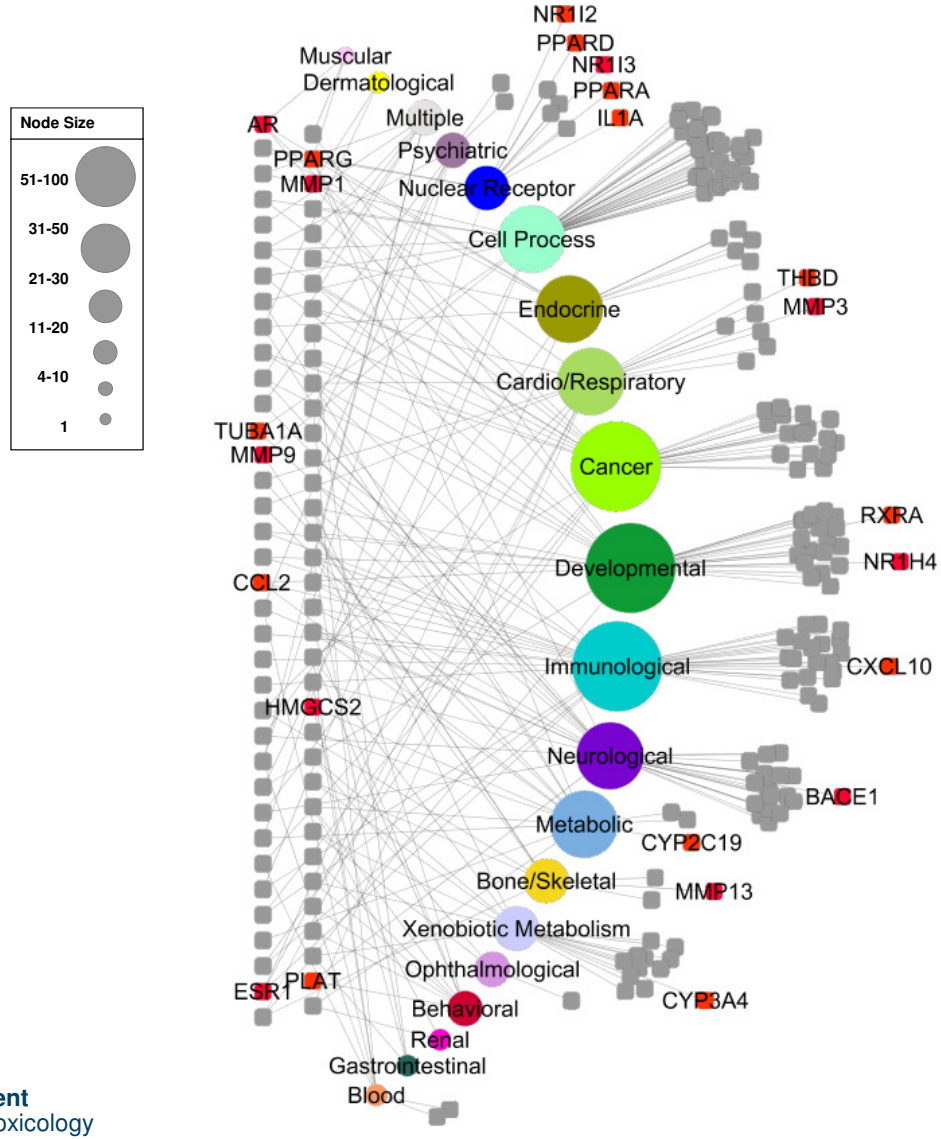
Source: Judson et al, *submitted*

Gene Networks Associated with Progression of Rat Liver Tumor Endpoints



Judson et al, submitted

ToxCast Assays Mapped to Disease Pathways



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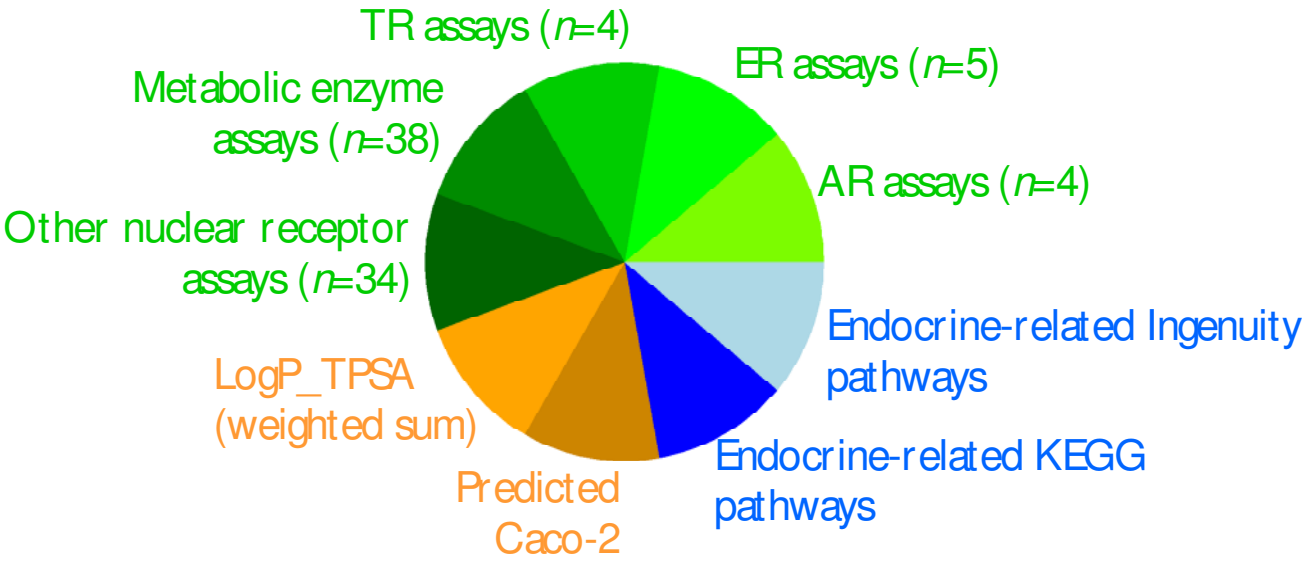
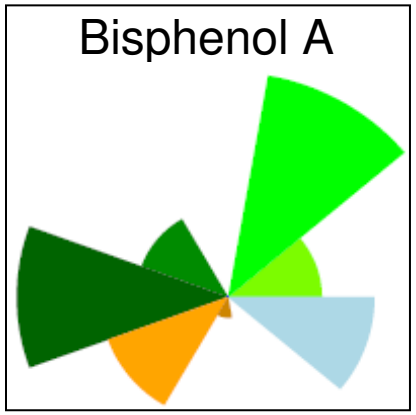
Potential Application of ToxCast:

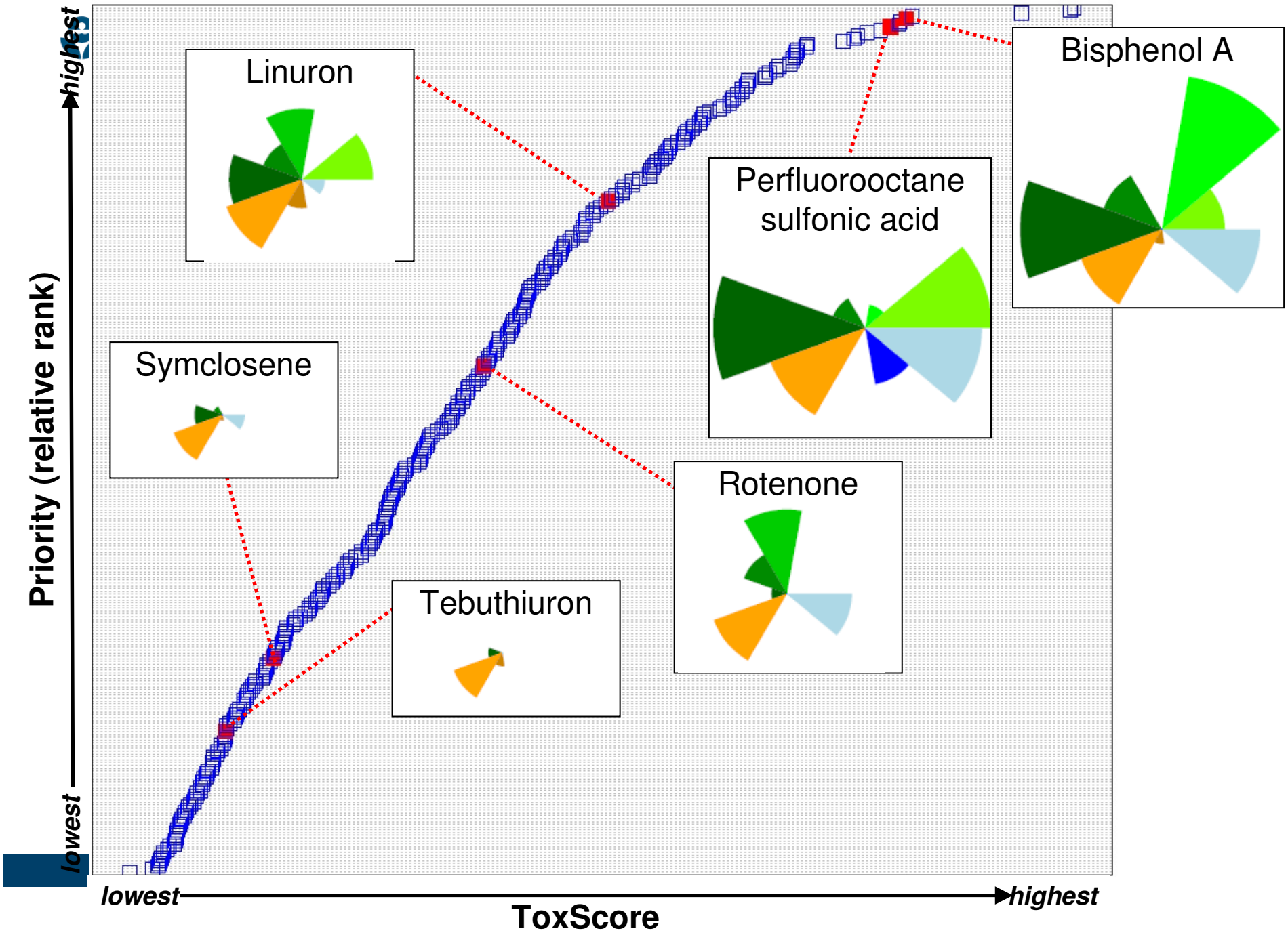
Endocrine Profiling & Prioritization





ToxScore = f(In vitro assays + Chemical properties + Pathways)

$$\text{ToxScore} = \sum_{1}^{I} w_i * assay_i + \sum_{1}^{C} w_c * chemProp_c + \sum_{1}^{P} w_p * pathway_p$$


highest
Priority (relative rank)
lowest
Linuron
Bisphenol A
Perfluorooctane sulfonic acid
Symclosene
Rotenone
Tebuthiuron
lowest
highest
ToxScore

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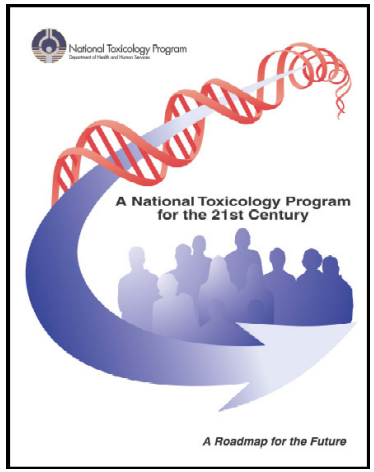
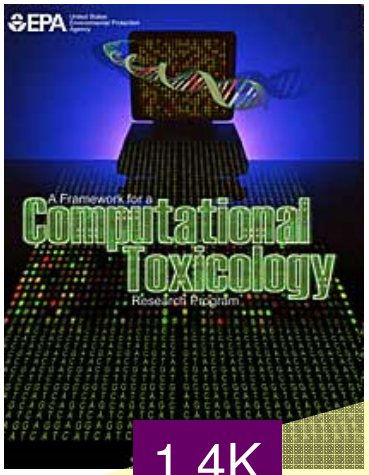
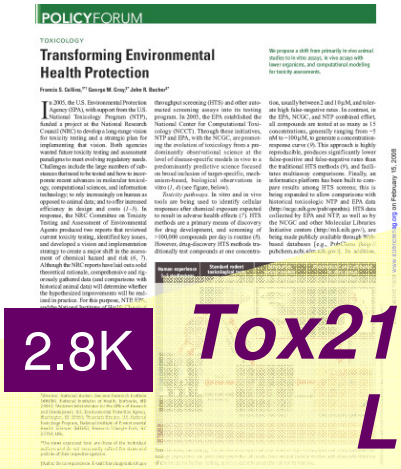
Tox21 Community





1.4K



2.8K

10K

Tox21 Chemical Library

2004 2005 2006 2007 2008 2009 2010

FDA
EU JRC

Tox21

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Acknowledgements

Virtual Embryo

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Nicole Kleinstreuer
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NHEERL

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Kelly Chandler
Stephanie Padilla
Chris Lau
John Rogers

Virtual Liver

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John Wambaugh
John Jack

NHEERL

Chris Corton

ToxCast

David Dix
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Matt Martin
Richard Judson
Ann Richard
David Reif
Woody Setzer
Holly Mortenson

NHEERL +
NTP

Ray Tice+

NCGC

Chris Austin +