United States Environmental Protection Agency

HIGH THROUGHPUT SCREENING FOR HAZARD & RISK OF ENVIRONMENTAL CONTAMINANTS

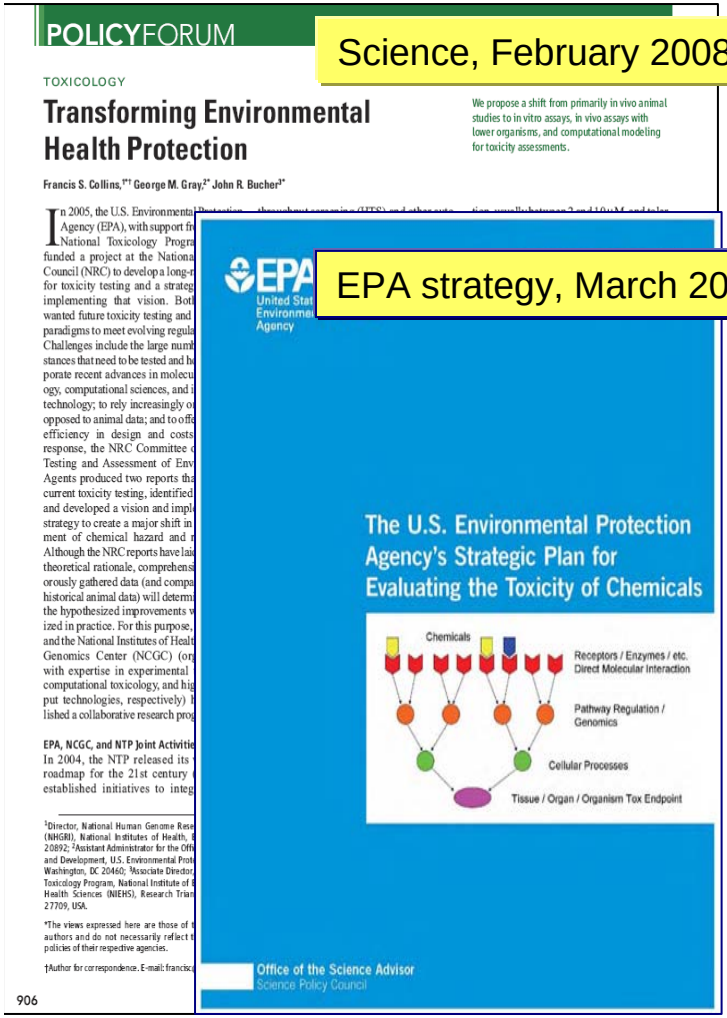
David Dix

Office of Research and Development
National Center for Computational Toxicology

SOT 2010

Future of Toxicity Testing and Ultimately, Environmental Risk Assessments

United States Environmental Protection Agency



POLICYFORUM

TOXICOLOGY

Transforming Environmental Health Protection

We propose a shift from primarily in vivo animal studies to in vitro assays, in vivo assays with lower organisms, and computational modeling for toxicity assessments.

Francis S. Collins,*† George M. Gray,2* John R. Bucher3*

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the National Toxicology Program, funded a project at the National Research Council (NRC) to develop a long-range strategy for toxicity testing and a strategy for implementing that vision. Both EPA and NRC wanted future toxicity testing and risk assessment paradigms to meet evolving regulatory needs. Challenges include the large number of chemical substances that need to be tested and how to incorporate recent advances in molecular biology, computational sciences, and information technology; to rely increasingly on human cell-based data as opposed to animal data; and to offer greater efficiency in design and costs. In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that critiqued current toxicity testing, identified key issues, and developed a vision and implementation strategy to create a major shift in the assessment of chemical hazard and risk. Although the NRC reports have laid out the theoretical rationale, comprehensive, mechanistically gathered data (and comparisons to historical animal data) will determine the hypothesized improvements will be realized in practice. For this purpose, the U.S. Environmental Protection Agency and the National Institutes of Health National Human Genome Research Institute Genomics Center (NCGC) (organizations with expertise in experimental toxicology, computational toxicology, and high-throughput technologies, respectively) have established a collaborative research program.

EPA, NCGC, and NTP Joint Activities

In 2004, the NTP released its roadmap for the 21st century, which established initiatives to integrate

*The views expressed here are those of the authors and do not necessarily reflect the policies of their respective agencies.

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The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals

Chemicals

Receptors / Enzymes / etc. Direct Molecular Interaction

Pathway Regulation / Genomics

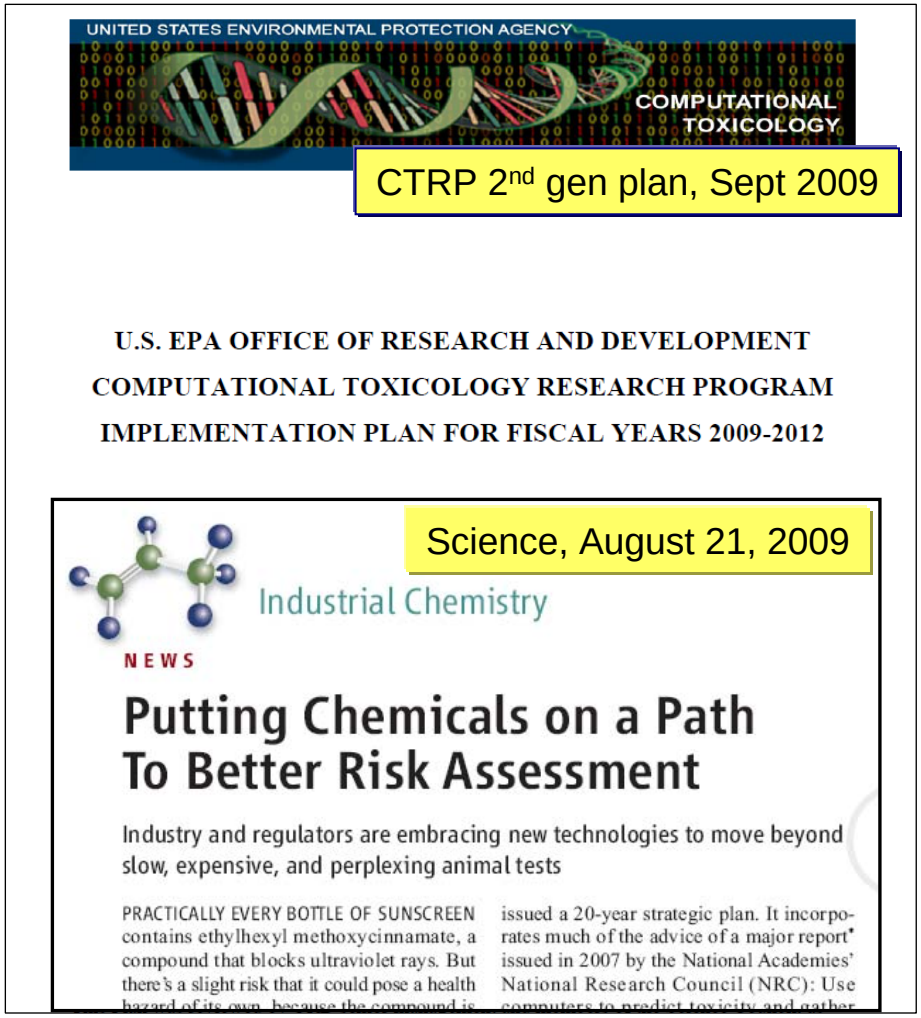
Cellular Processes

Tissue / Organ / Organism Tox Endpoint

Office of the Science Advisor
Science Policy Council

Science, February 2008

EPA strategy, March 2009



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

COMPUTATIONAL TOXICOLOGY

CTRP 2nd gen plan, Sept 2009

U.S. EPA OFFICE OF RESEARCH AND DEVELOPMENT

COMPUTATIONAL TOXICOLOGY RESEARCH PROGRAM

IMPLEMENTATION PLAN FOR FISCAL YEARS 2009-2012

Science, August 21, 2009

Industrial Chemistry

NEWS

Putting Chemicals on a Path To Better Risk Assessment

Industry and regulators are embracing new technologies to move beyond slow, expensive, and perplexing animal tests

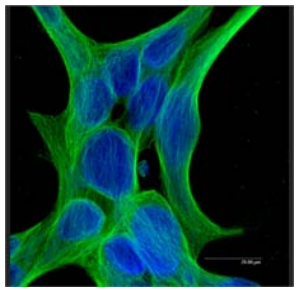
PRACTICALLY EVERY BOTTLE OF SUNSCREEN contains ethylhexyl methoxycinnamate, a compound that blocks ultraviolet rays. But there's a slight risk that it could pose a health hazard of its own, because the compound is

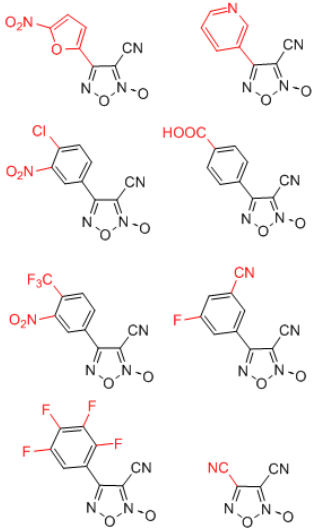
issued a 20-year strategic plan. It incorporates much of the advice of a major report* issued in 2007 by the National Academies' National Research Council (NRC): Use computers to predict toxicity and gather

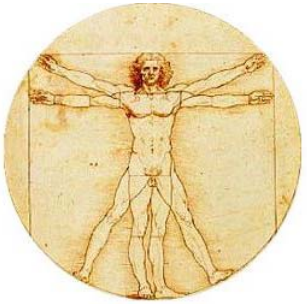
United States Environmental Protection Agency

HTS in Drug Development

Identify target, pathway, or cellular phenotype → Create testing system (aka, "assay") → HTS tests >100,000 chemicals with *no known activity* for effect on target → Make modifications to most active chemicals to make suitable for in vivo testing → Test in animals for safety, effectiveness → Test in humans for safety, effectiveness







HTS in Toxicology

Chemicals with known or suspected toxicity/ activity

Identify toxicity pathways, cellular phenotypes

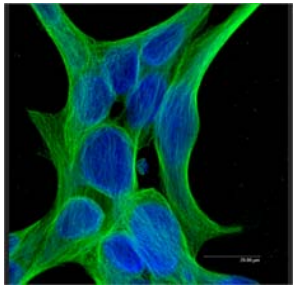
Obtain or create testing systems ("assays")

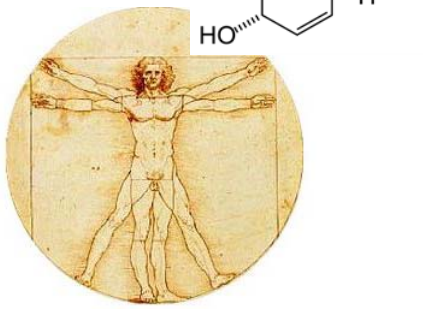
HTS tests chemicals for effect on assays

Computational analysis & Synthesis of HTS results

Test prioritized chemicals in animals

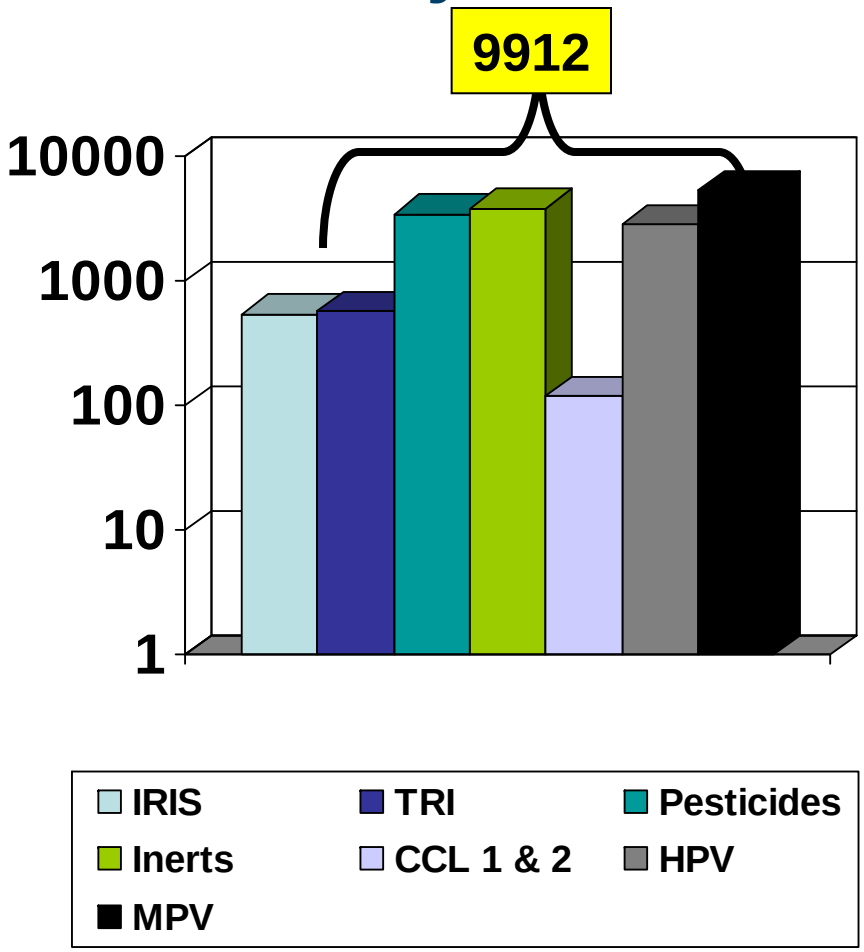
Categorize as inactive subject to further testing



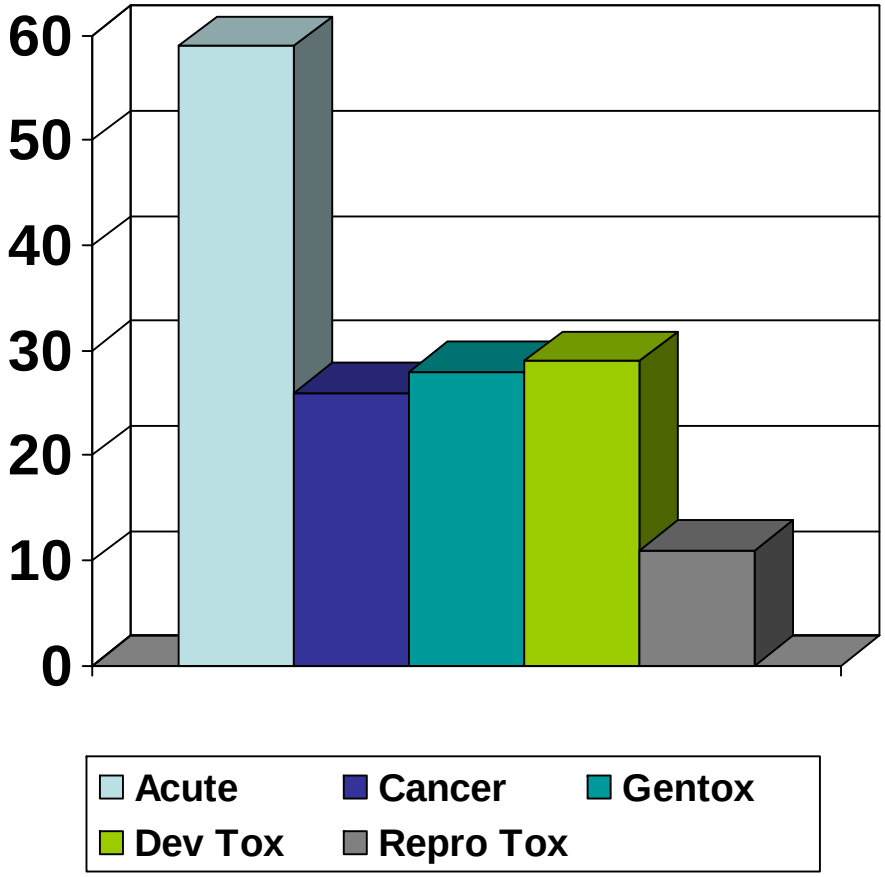


The Need

Too Many Chemicals

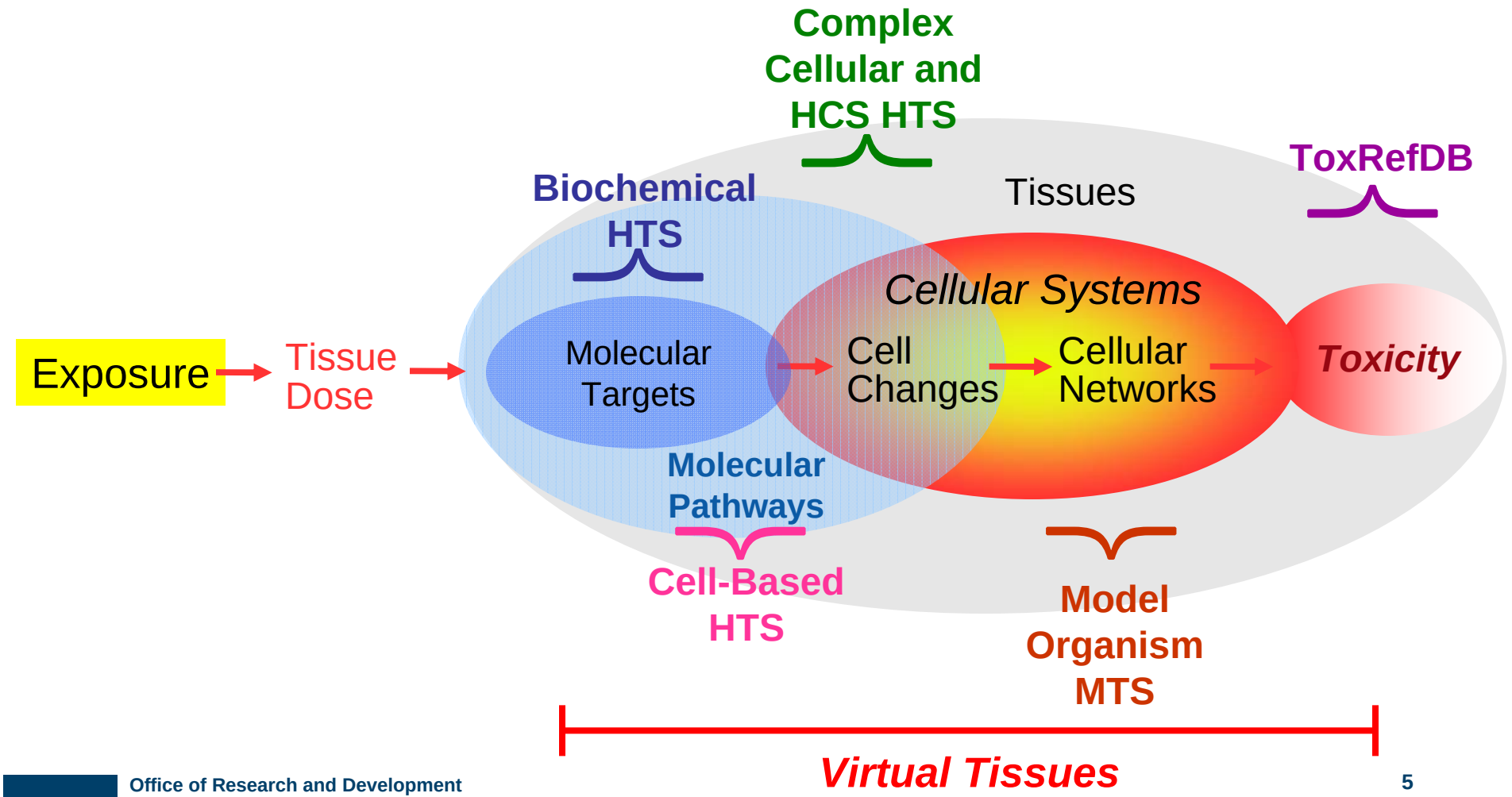


Too Little Data (%)

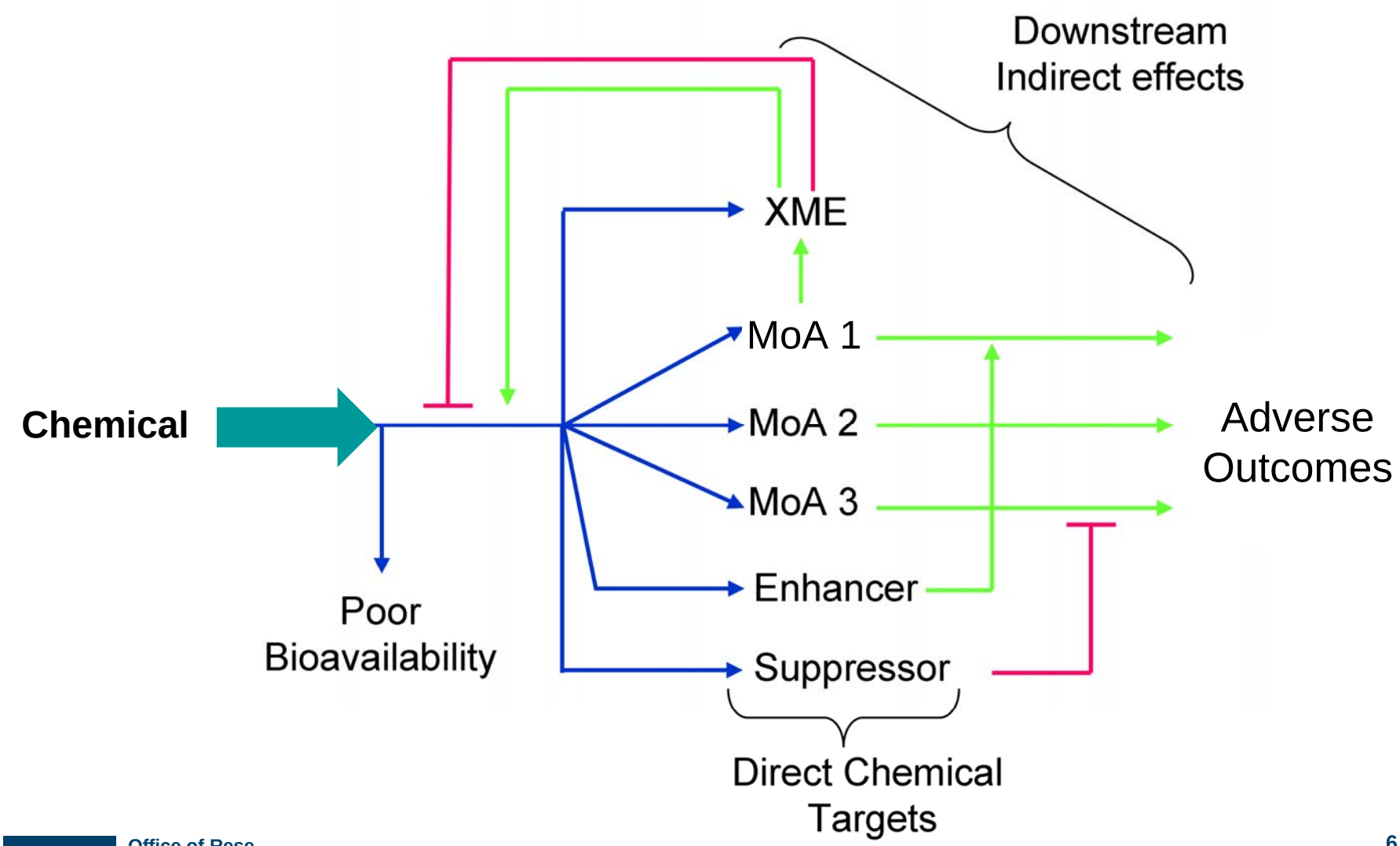


Judson, et al *EHP* (2009)

Grand Challenge for Computational Toxicology: Predicting Human Toxicity



Predicting Toxicity Will Not Be Easy



ToxCast™
HTS to Profile Chemical Bioactivity

- 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

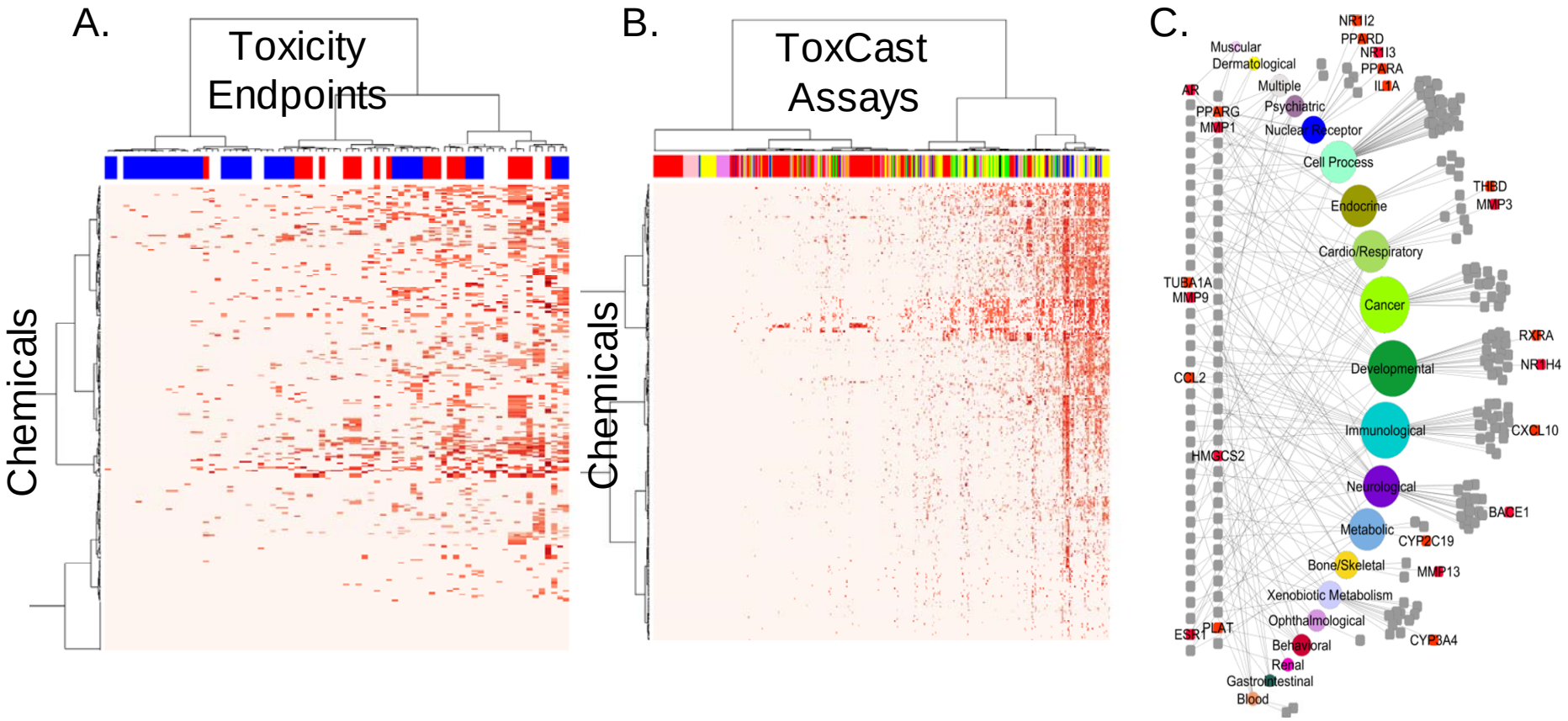
- Committed to stakeholder involvement and transparency
  - Communities of Practice- Chemical Prioritization; Exposure
  - Release of all data upon peer review publication

Goals of ToxCast

- Identify Toxicity Pathways
- Obtain HTS Assays for Pathways
- Screen a Large Chemical Library
- Initially link Results to In Vivo Adversity
  - Toxicity signatures
- Eventually identify points of departure from HTS data
  - Toxicity pathways

United States Environmental Protection Agency

ToxRefDB ← ToxCast → Human Disease



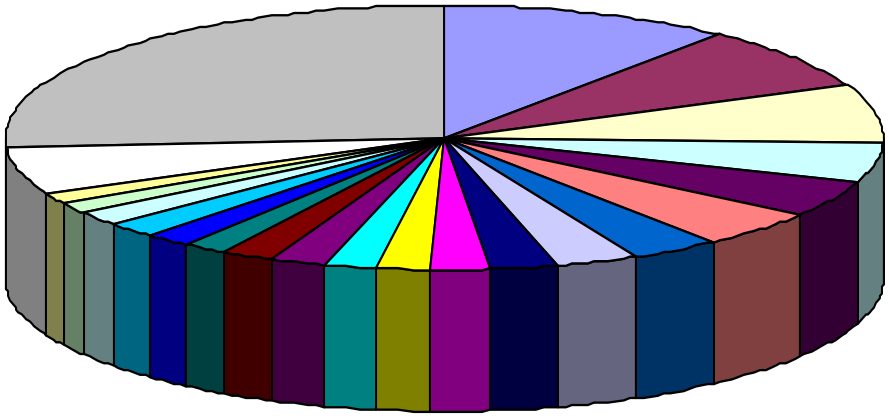
Phase I ToxCast_320

309 Unique Chemicals

- **3** Triplicates
- **5** Duplicates

- **276** Conventional Actives
- **16** Antimicrobials
- **9** Industrial Chemicals
- **8** Metabolites

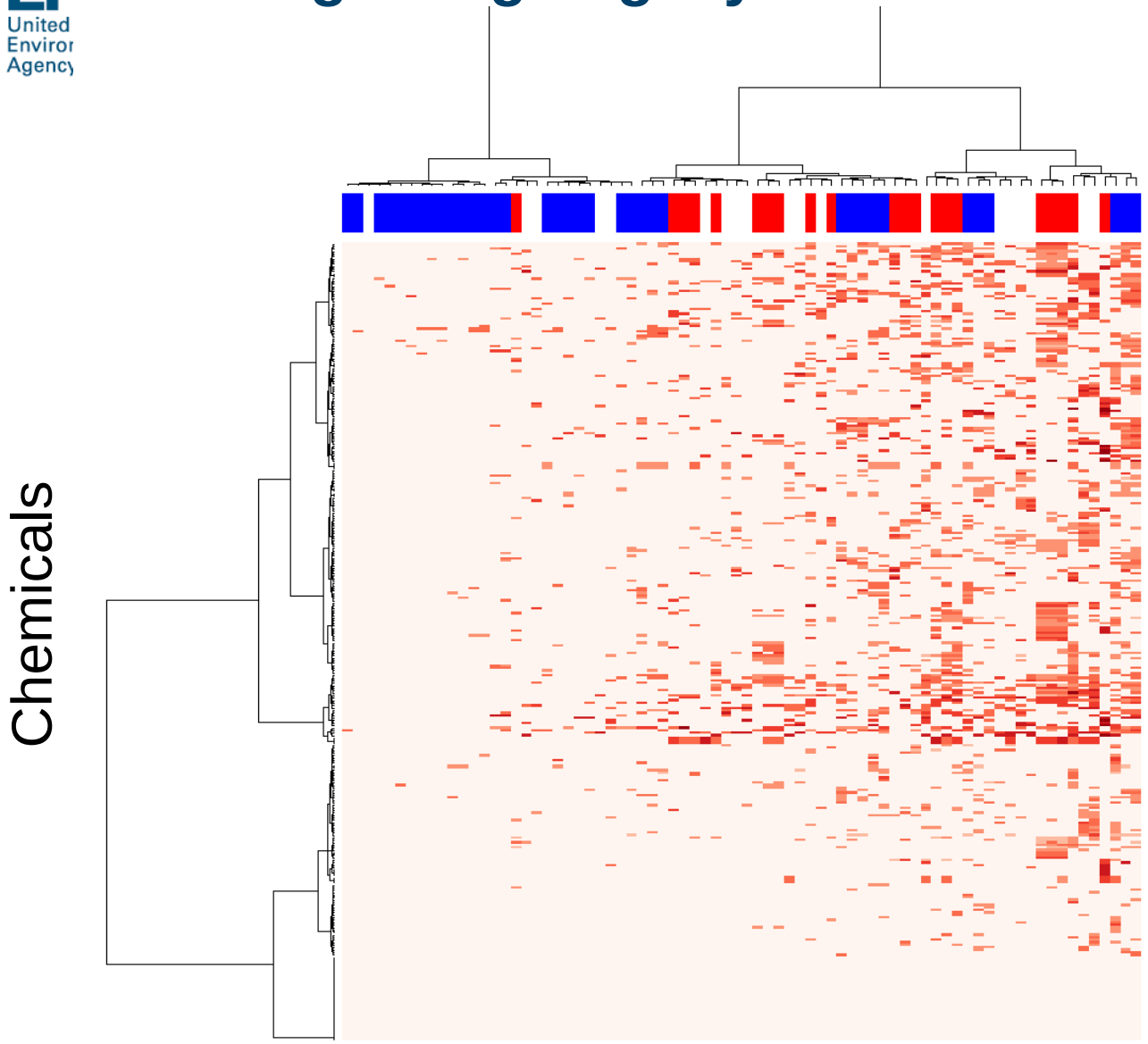
- **75** Chemical Classes



Chemical Class Distribution
(≥5/Class)

- Organophosphorus (39)
- Amide (26)
- Urea (26)
- Conazole (18)
- Carbamate (16)
- Phenoxy (15)
- Pyrethroid (12)
- Pyridine (11)
- Triazine (9)
- Dicarboximide (8)
- Phthalate (7)
- Dinitroaniline (7)
- Antibiotic (7)
- Thiocarbamate (7)
- Pyrazole (6)
- Nicotinoid (6)
- Dithiocarbamate (6)
- Aromatic Acid (6)
- Insect Growth Regulators (5)
- Imidazolinone (5)
- Unclassified (21)
- Other (93)

Digitizing Legacy *in Vivo* Data in ToxRefDB



Chronic/Cancer
Multigenation
Developmental

Martin et al 2009a,b
Knudsen et al 2009

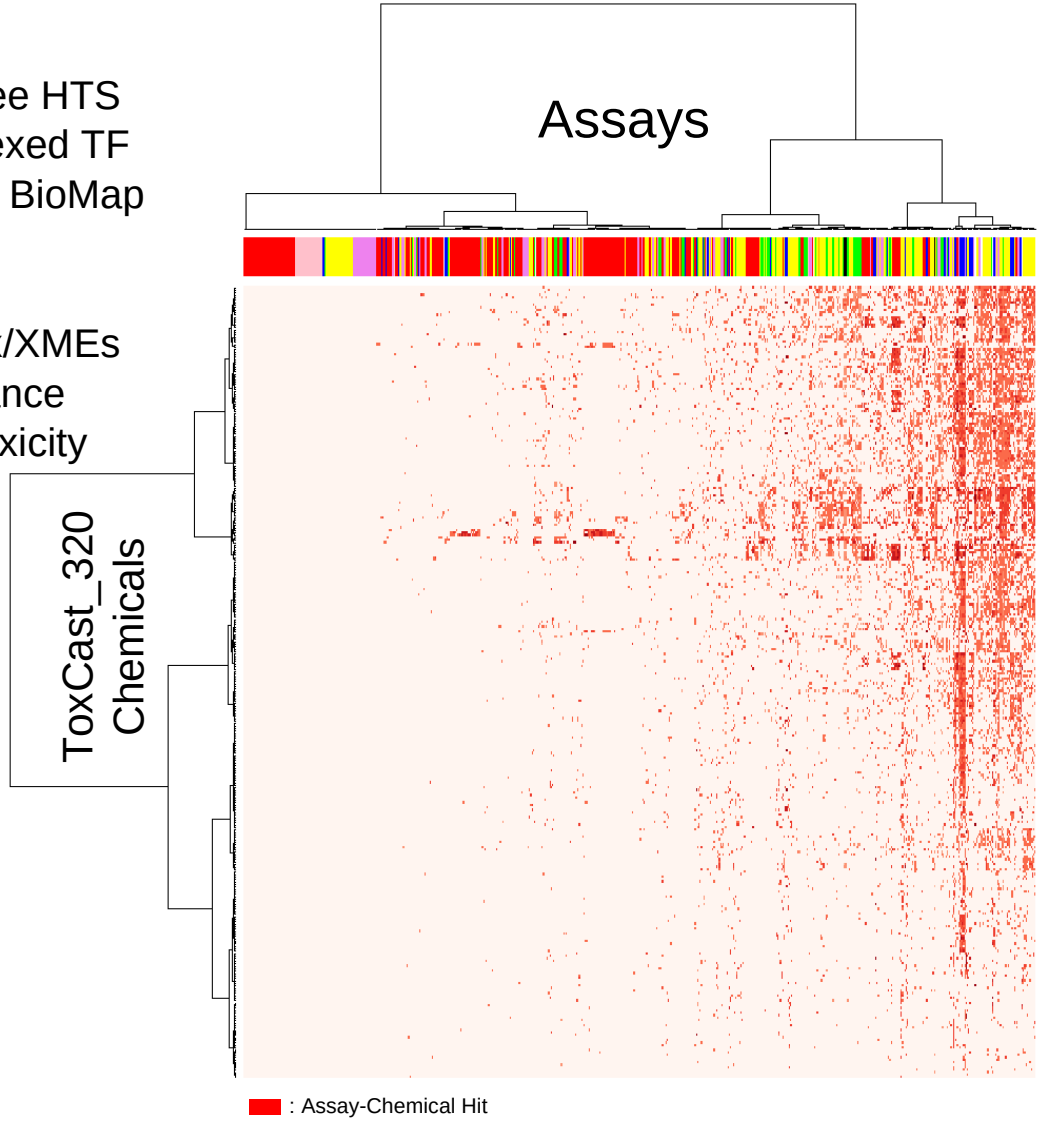
30 years and more than $2B worth of data

Phase I ToxCast In Vitro Bioactivity

United States Environmental Protection Agency

Novascreen (Knudsen et al, submitted)
Attagene (Martin et al, submitted)
Bioseek (Houck et al, JBS, 2009)
Cellumen (Houck et al, In prep)
CellzDirect (Rotroff et al, submitted)
Solidus (Ryan et al, In prep)
ACEA (Judson et al, In prep)
Gentronix (Knight et al, RT, 2009)

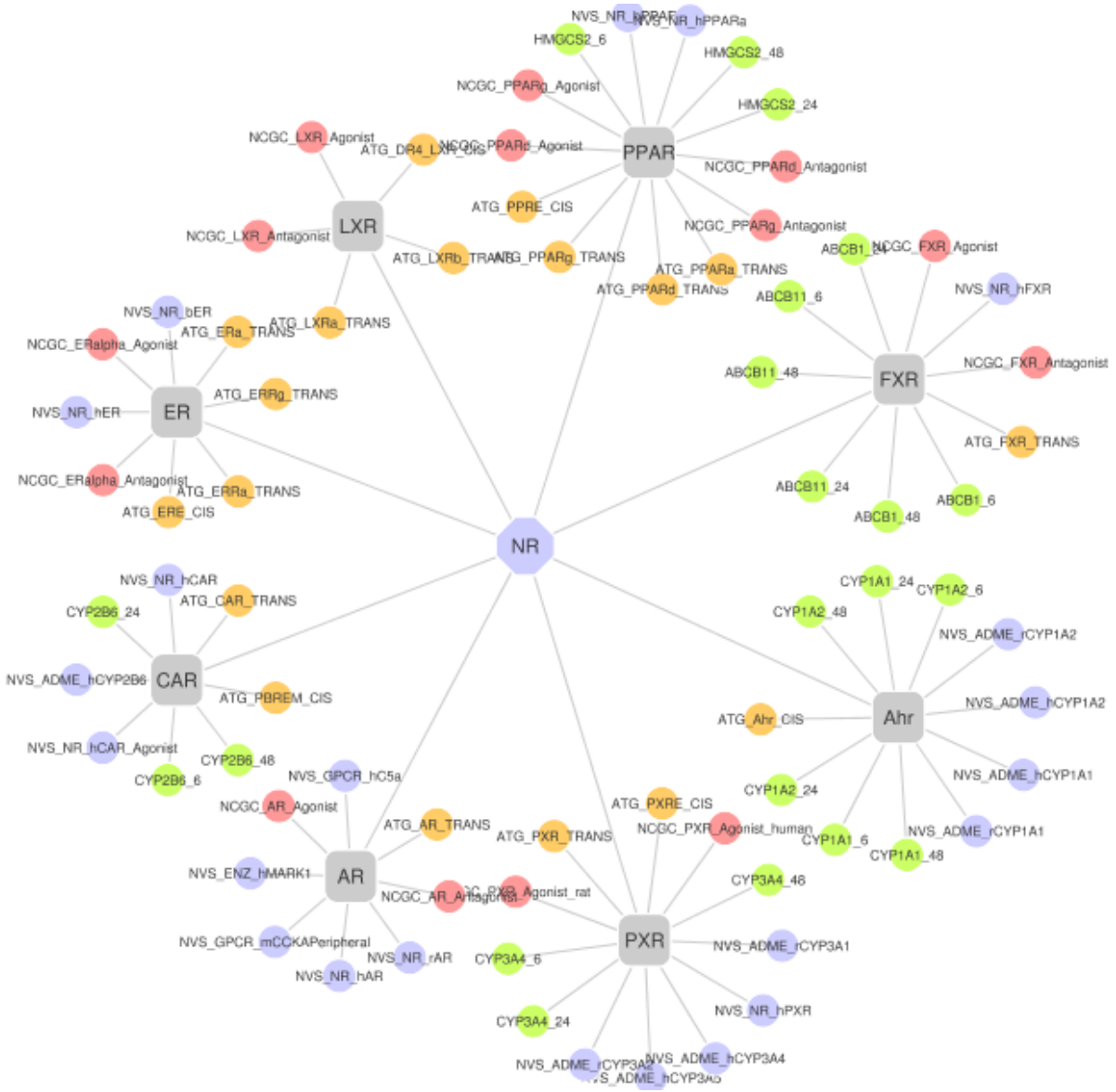
- Cell Free HTS
- Multiplexed TF
- Human BioMap
- HCS
- qNPAs
- Cytotox/XMEs
- Impedance
- Genotoxicity



828 of 199,680 Assay-Chemical Pairs had AC50s of less than 1μM

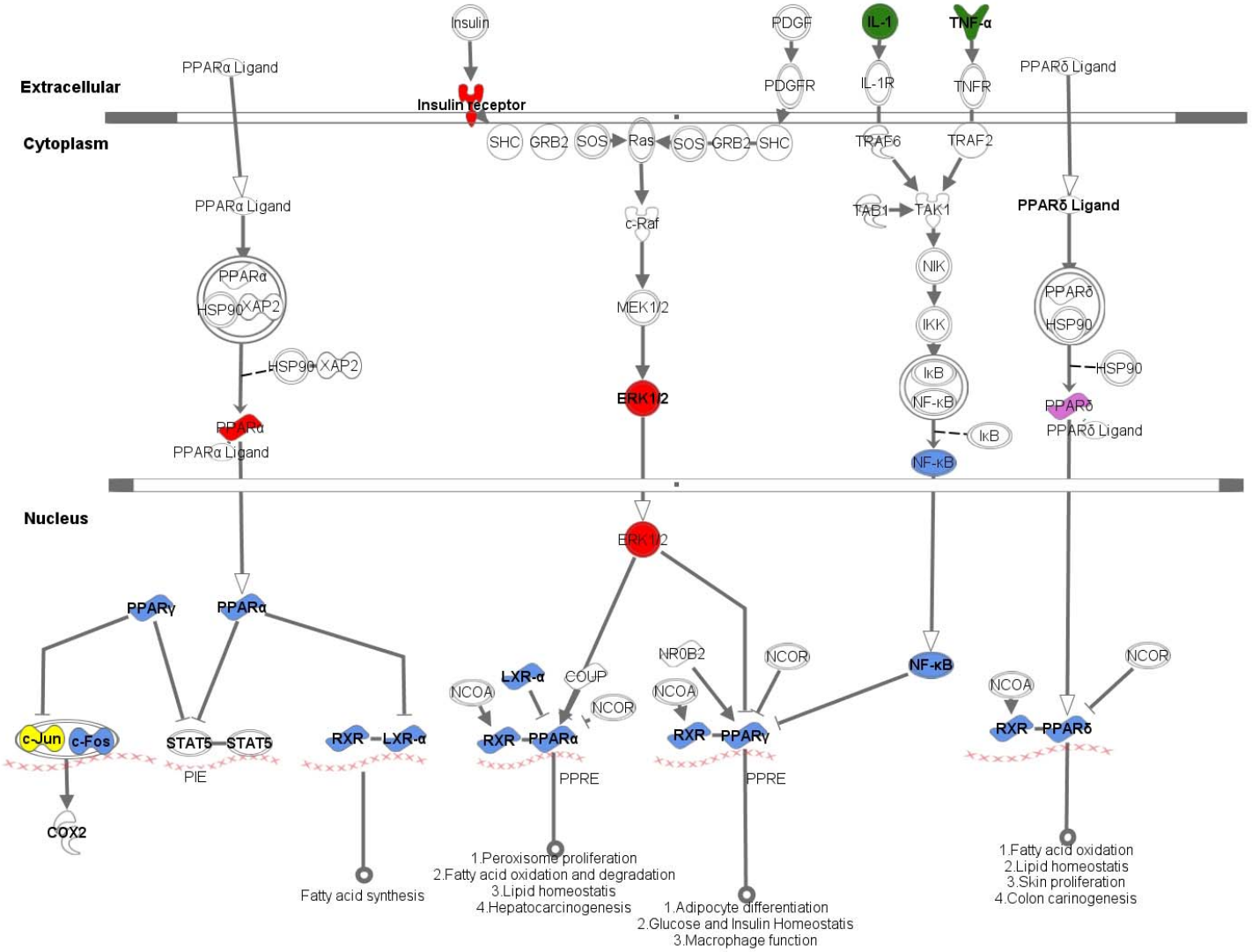
Judson et al, submitted

ToxCast: Multiple Assays and Technologies per Target

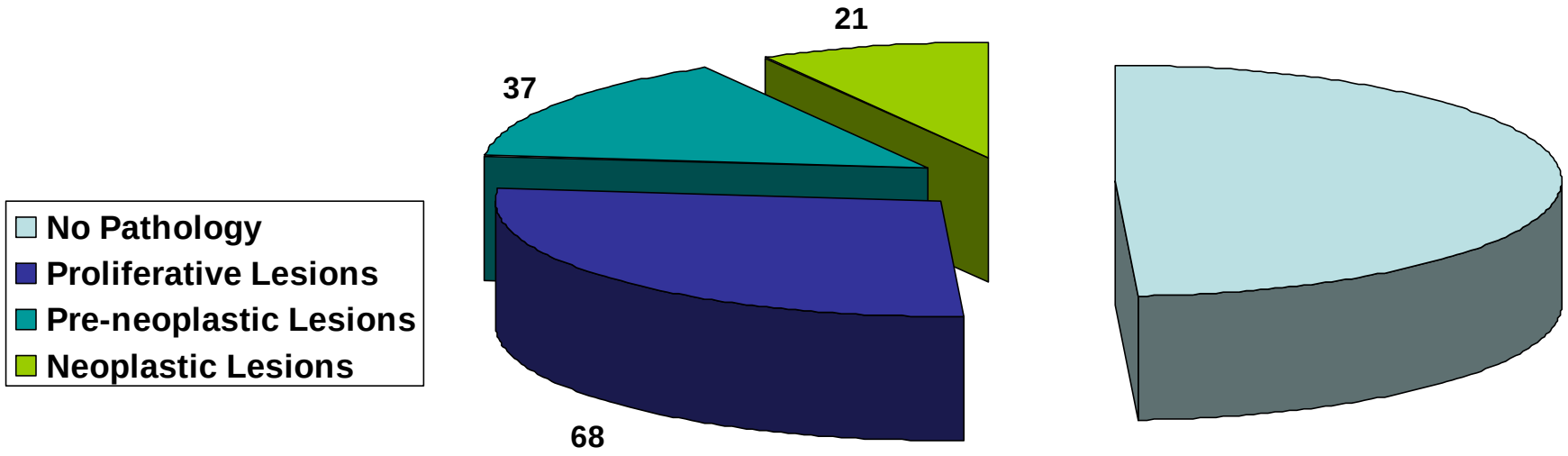


ToxCast: Multiple Targets per Pathway

- **Biologically Multiplexed Activity Profiling (BioMAP)**
- **Multiplex Transcription Reporter Assay**
- **Cell-based HTS Assays**
- **Cell-free HTS Assays**
- **High Content Cell Imaging Assays**

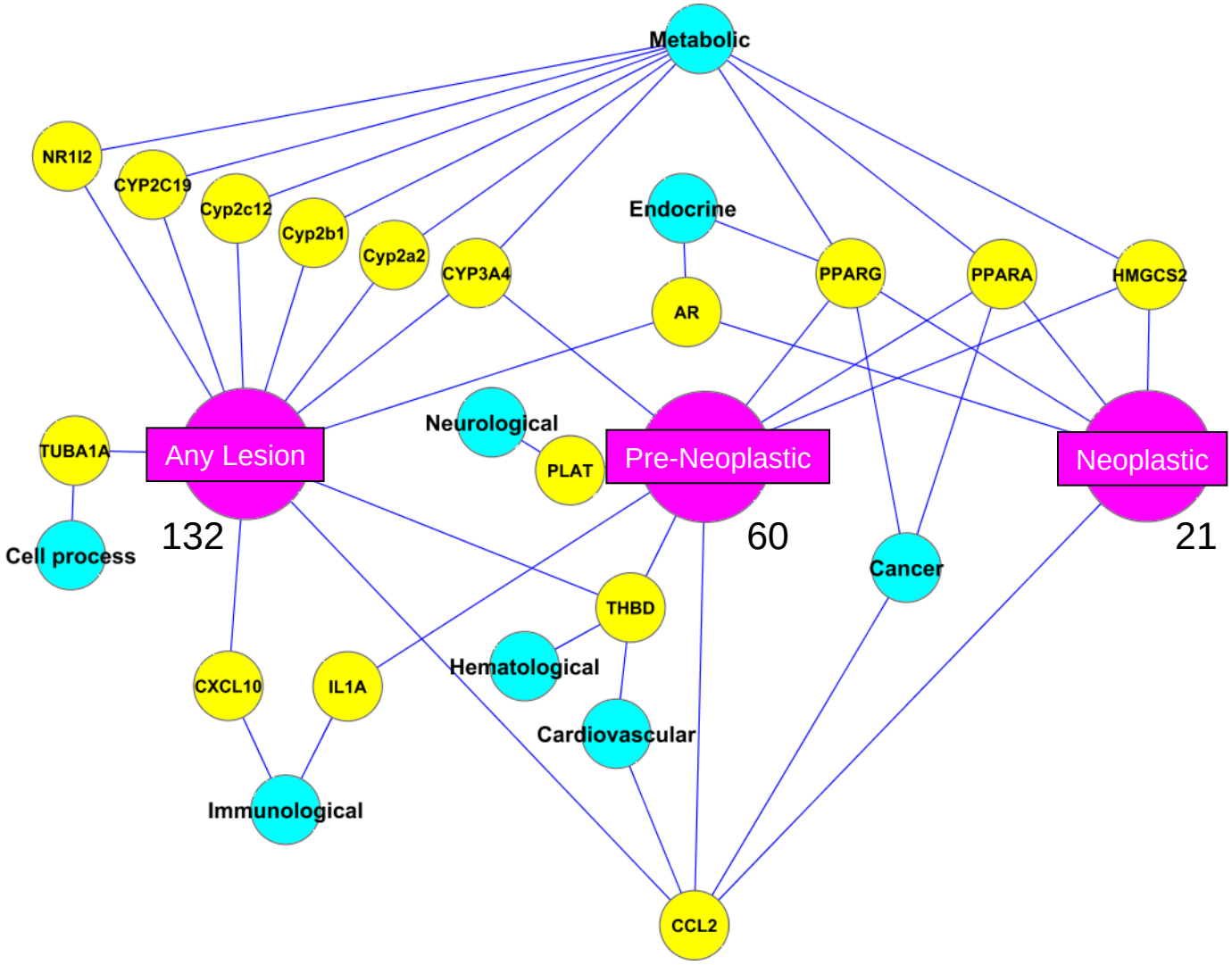


Building Toxicity Signatures for Rat Liver Histopathology from Chronic Bioassays



N = 248 Chemicals in ToxRefDB

ToxCast Identied Genes Associated with Progression of Rat Liver Disease

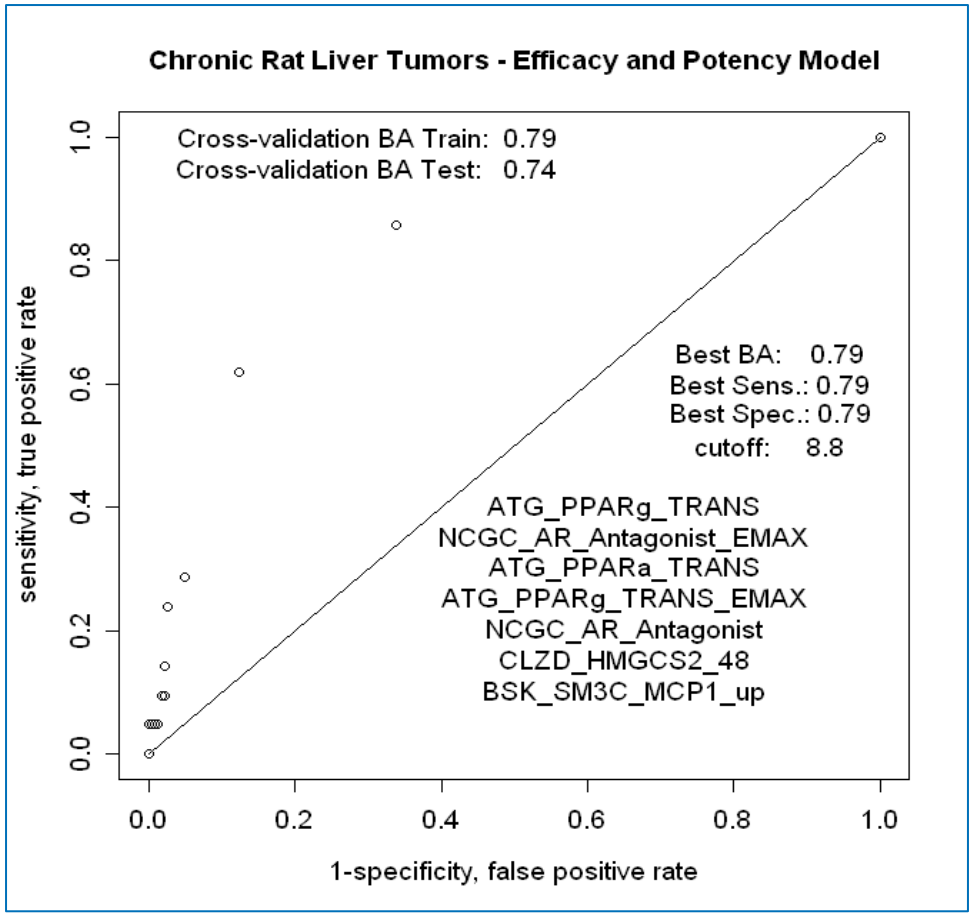


Liver Disease Progression

Judson et al, EHP (2010)

Rat Liver Tumor Signature Using Both Efficacy and Potency HTS Data from ToxCast

Beam et al
Abstract #98
Monday AM Poster



Model performance improves (balanced accuracy in test) when efficacy data is added.

Predictive Signatures from ToxCast for Chronic, Developmental and Reproductive Toxicity

Judson et al

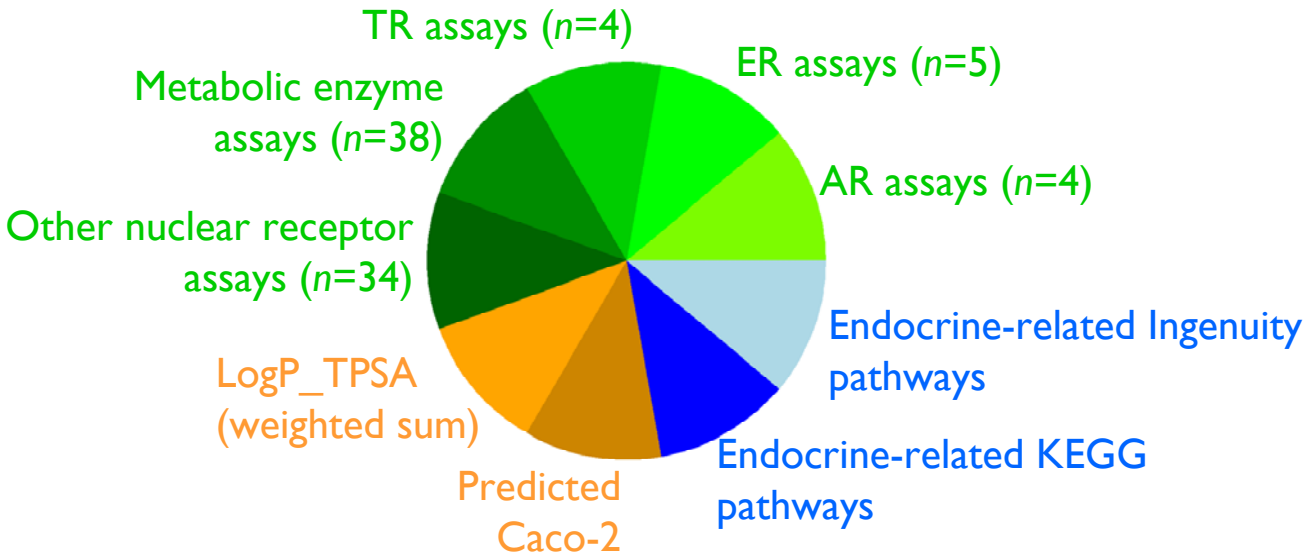
Abstract #96

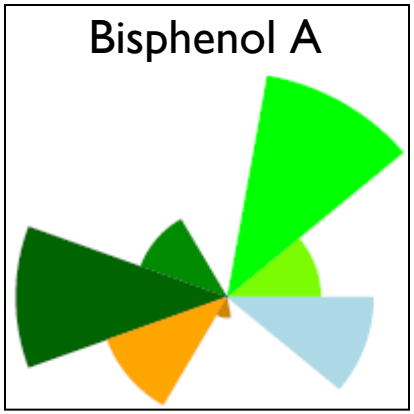
Monday AM Poster

- Chronic/Cancer: Rat Liver Tumor
- Developmental: Rat and Rabbit Cleft Palate
- Reproductive: Rat Reproductive Performance

Pathway/MOA Based Application of ToxCast:

Endocrine Profiling & Prioritization



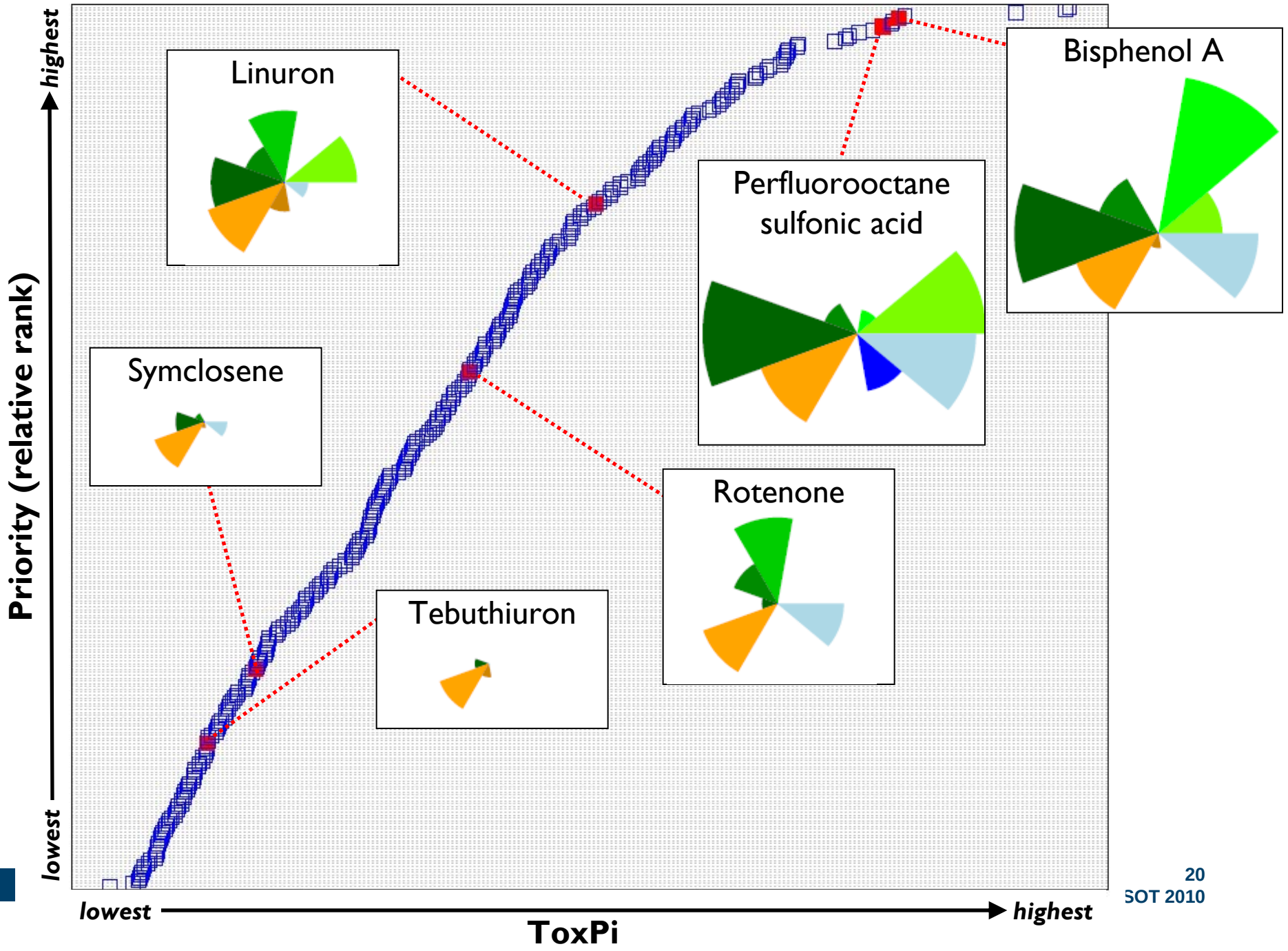


Toxicological Prioritization Index = ToxPi™

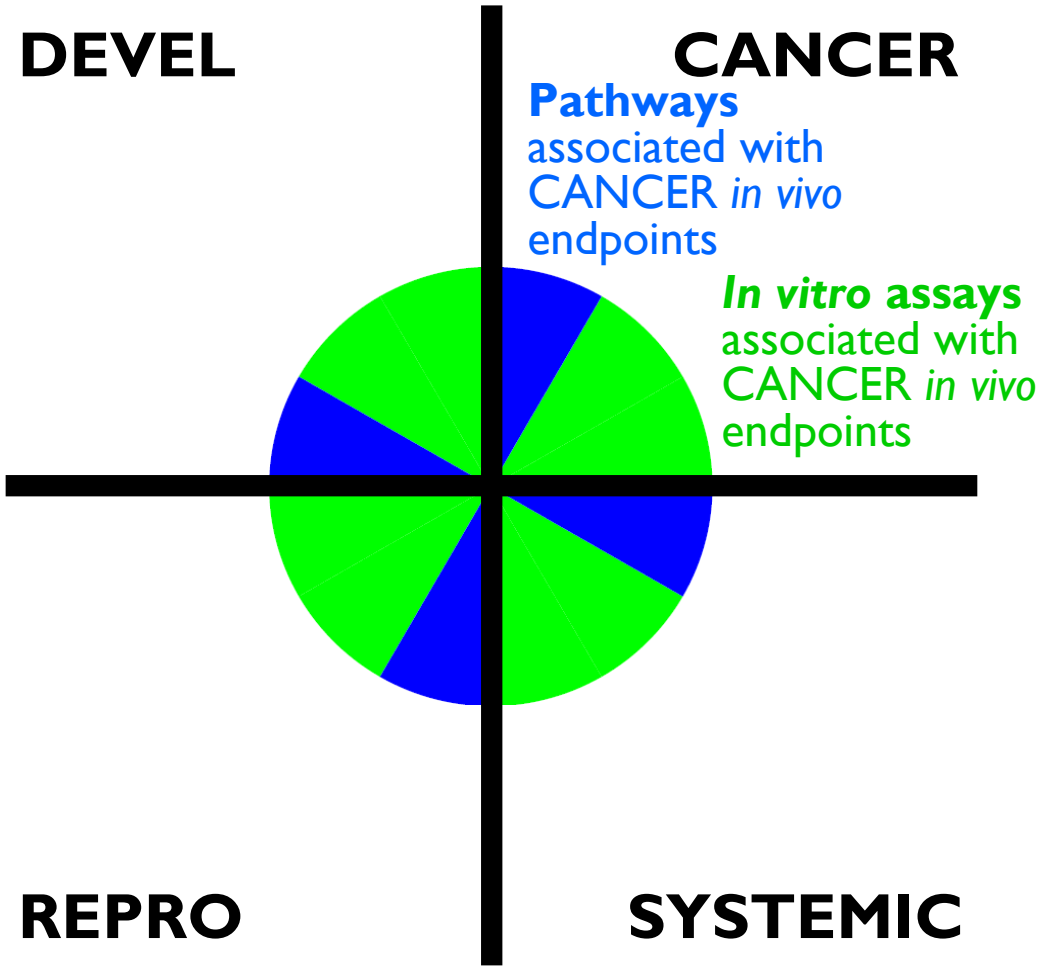
ToxPi = f(In vitro assays + Chemical properties + Pathways)

$$\text{ToxPi} = \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{P} w_p * \text{pathway}_p$$

Reif, et al, submitted


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

Expanding the ToxPi Approach for Prioritization of Toxicity Testing Based on ToxCast Chemical Profiling



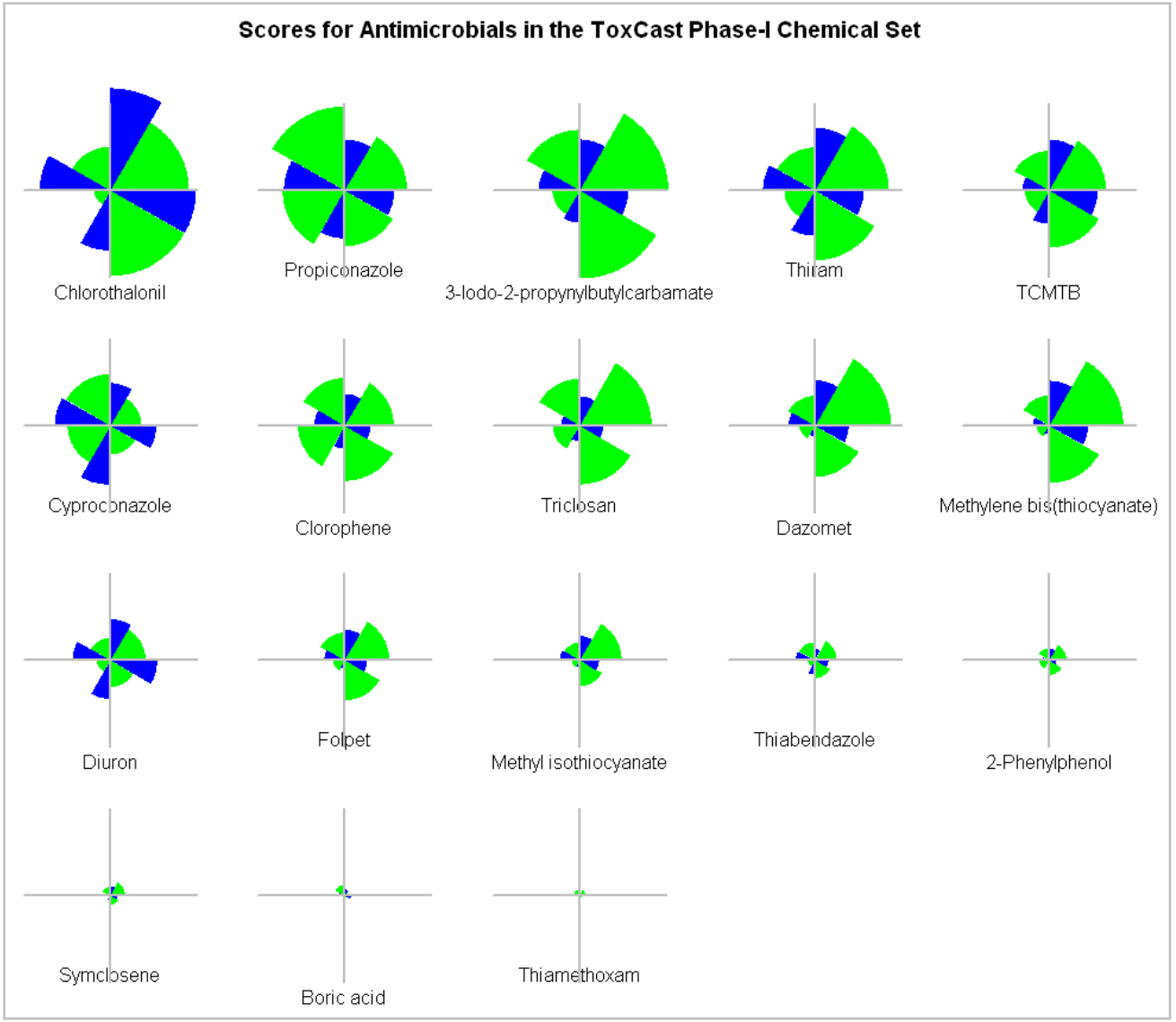
Identify *in vitro* assays, targets, genes and pathways associated with multiple sectors of *in vivo* toxicity.

Reif et al
Abstract #198
Monday AM Poster

ToxPi Scores for Antimicrobial Pesticide Actives

One of the methods be developed to use ToxCast data for classifying and prioritizing antimicrobials and inerts.

Martin et al
Abstract #1901
Wednesday PM Poster

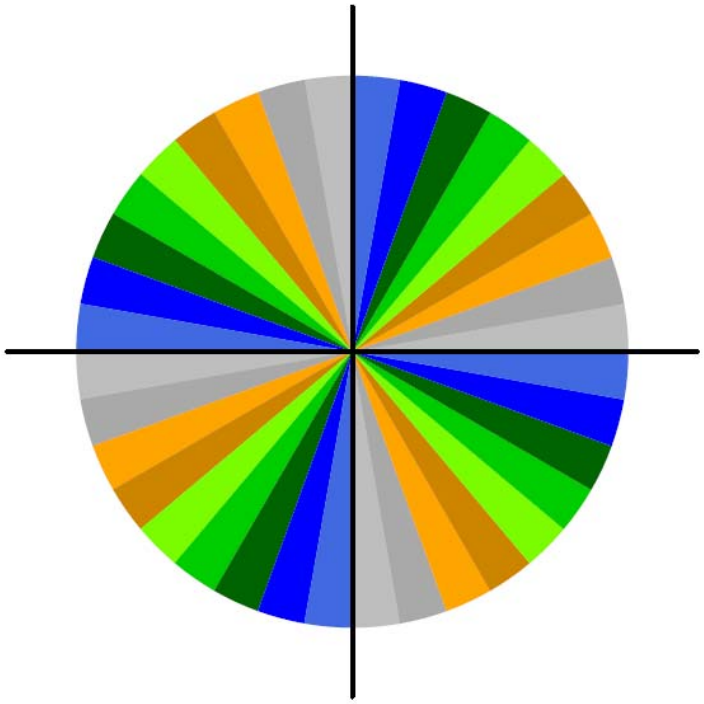


Future ToxPi (Toxicological Prioritization Index)

ToxPi = f(Exposure + Chemical properties + In vitro assays + Pathways)

Incorporate additional components (slices) from other domains:

- Exposure
- Chemical properties
- QSAR



ToxCast Phase I Publications & Data Release

U.S. ENVIRONMENTAL PROTECTION AGENCY

National Center for Computational Toxicology

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- Home
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  - DSSTox
  - ExpoCast™
  - ToxCast™
  - ToxRefDB
  - v-Liver™
  - v-Embryo™
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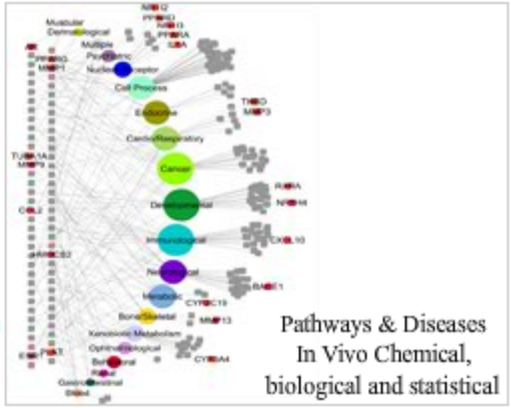
ToxCast™ Program

Predicting Hazard, Characterizing Toxicity Pathways, and Prioritizing the Toxicity Testing of Environmental Chemicals

In 2007, EPA launched ToxCast™ to develop a cost-effective approach for efficiently prioritizing the toxicity testing of thousands of chemicals.



Pathways & Diseases
In Vivo Chemical, biological and statistical

- Uses data from state-of-the-art high throughput screening (HTS) bioassays.
- Builds computational models to forecast potential chemical toxicity in humans.
- Provides EPA regulatory programs with science-based information helpful in prioritizing chemicals for more detailed toxicological evaluations and more efficient use of animal testing.
- Phase I profiled over 300 well-characterized chemicals (primarily pesticides) in over 400 HTS endpoints. Endpoints include biochemical assays of protein function, cell-based transcriptional reporter and gene expression, cell line and primary cell functional, and developmental endpoints in zebrafish embryos and embryonic stem cells.
- Phase 1 chemicals have already been tested using traditional toxicology methods including developmental toxicity, multi-generation reproductive studies, and sub-chronic and chronic rodent bioassays. ToxRefDB is the relational database storing this information- nearly $2 billion worth of animal toxicity studies.
- Phase II of ToxCast will screen additional chemical compounds representing broader chemical structure and use classes to evaluate the predict
signatures developed in Phase I.
- Toxicity signatures from ToxCast will be defined and evaluated by how well they predict outcomes from mammalian toxicity tests and identify toxicity pathways

ToxCast™ Navigation

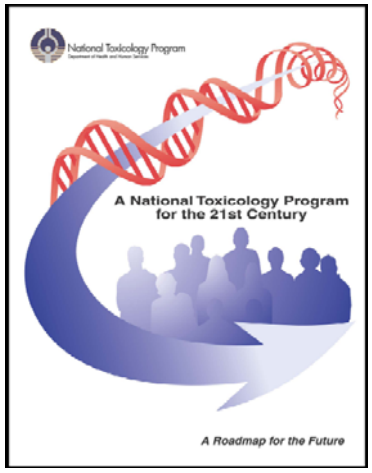
- Introduction
- Chemicals
- Assays
- Information Management
- Partnerships
- News

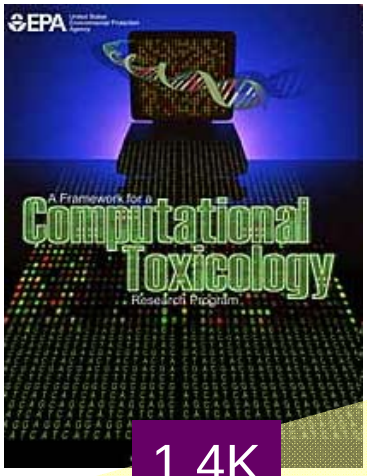
http://epa.gov/ncct/toxcast/

ToxCast Phase II Plans
(700 additional chemicals)

- Chemical Diversity
  - More data rich food use active pesticides
  - Pesticidal antimicrobials & inerts
  - Food use chemicals (FDA/CFSAN)
  - Failed pharmaceuticals (Pfizer, GSK, Sanofi, Merck)
  - “Green” chemicals
  - HPV Categories (4)
  - Liver toxicants (GSK)
  - OECD Molecular Screening Group nominations
- Re-Evaluation of Phase I Assays
- Additional assays via competitive procurements, collaborative partners…

Tox21 Community





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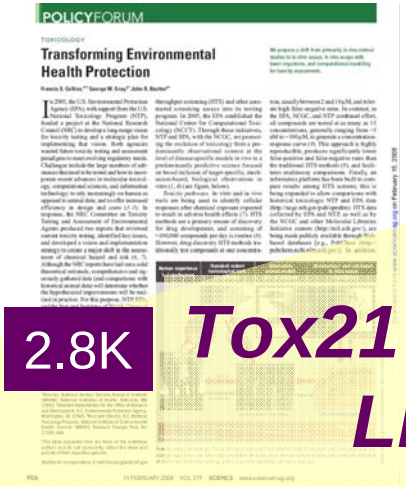


2.8K

10K

Tox21 Chemical Library

2004 2006 2008 2010

2005

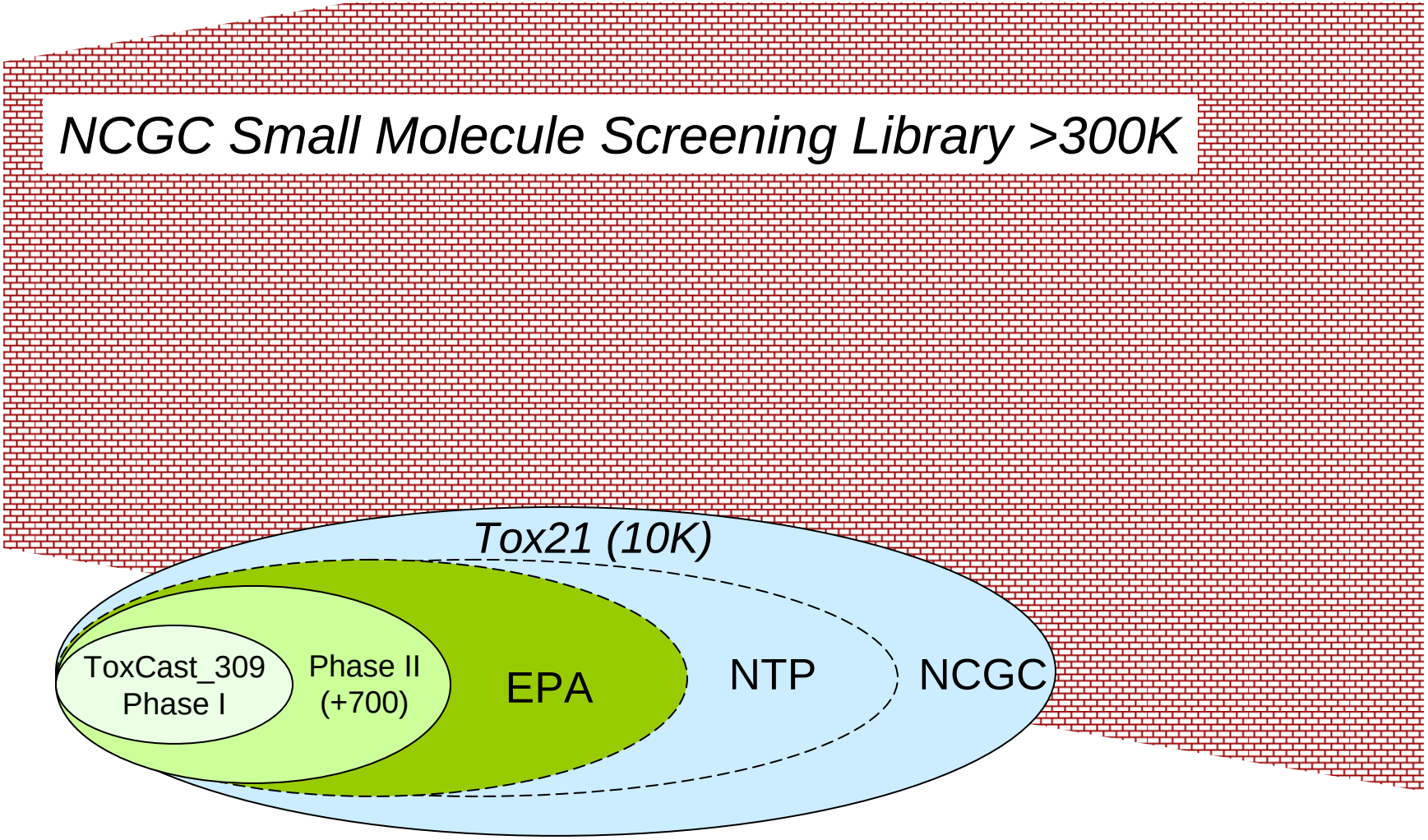
2007

2009

FDA
EU JRC

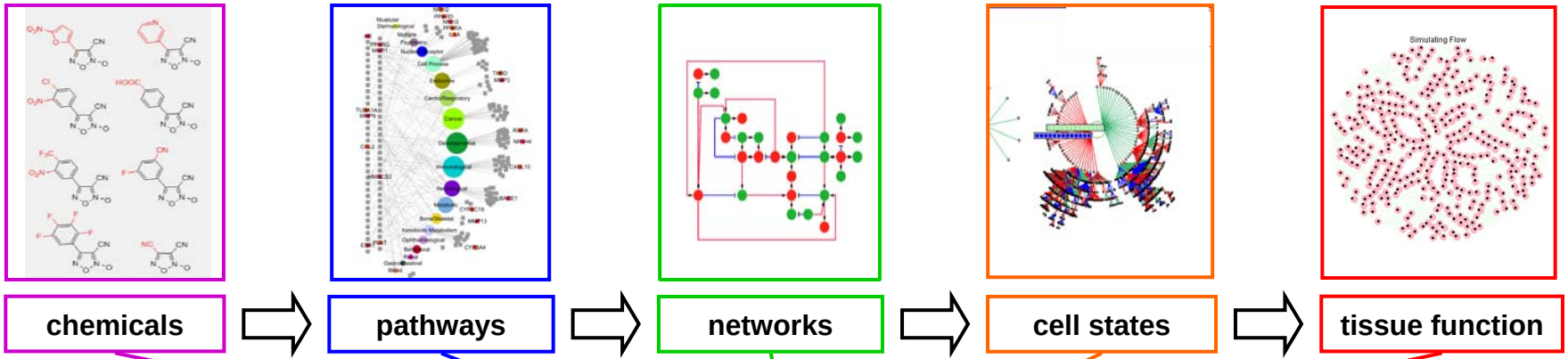
Tox21

Tox21 Expansion of the ToxCast Effort: Exploring Common Biology with Small Molecules



Slide courtesy of Ann Richard (EPA)

Systems Approaches to Modeling Toxicity: From Pathways to Virtual Tissues



Moving beyond empirical models, to multi-scale models of complex biological systems.

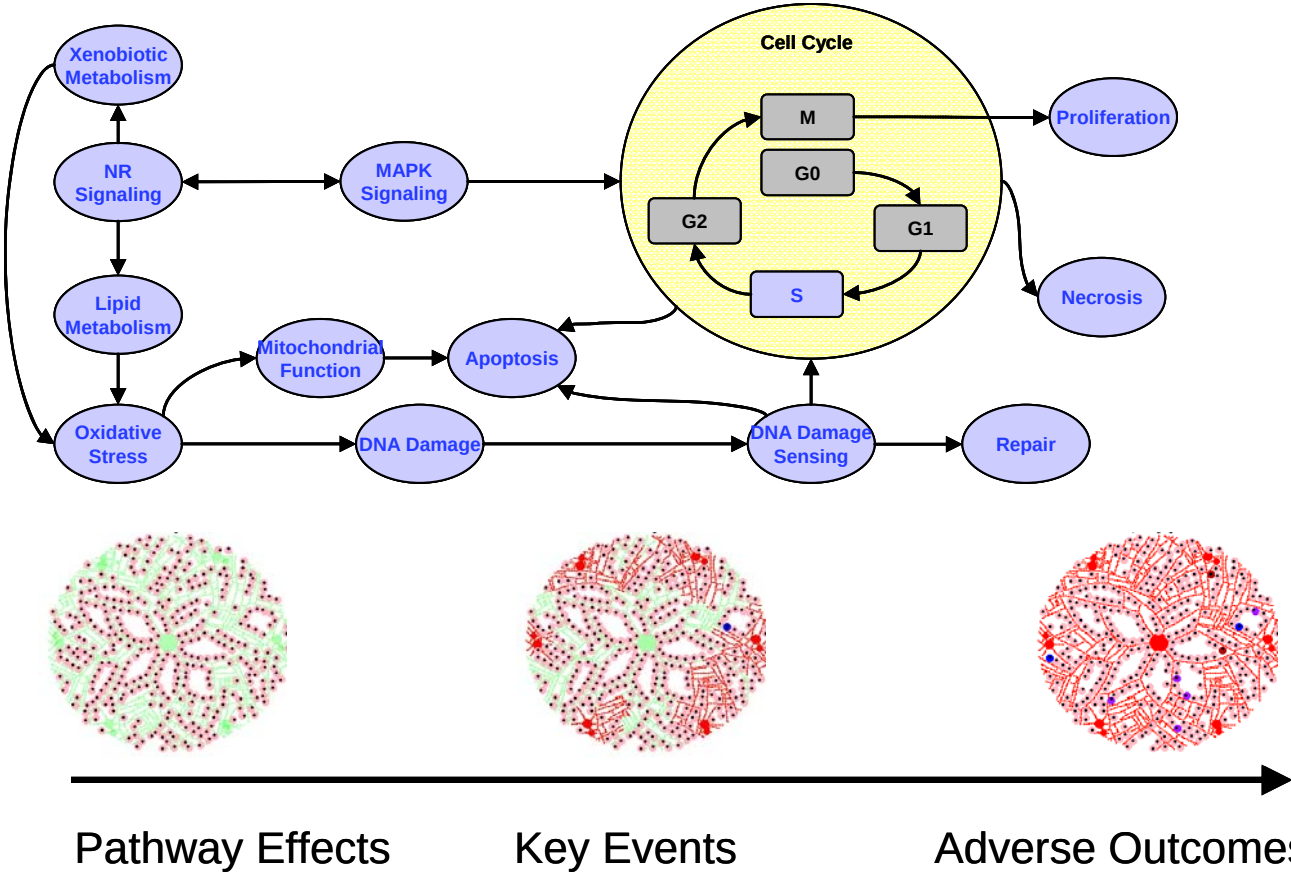
Identify Key Targets and Pathways For Prioritization

Quantitative Dose-Response Models

Next Generation Risk assessments

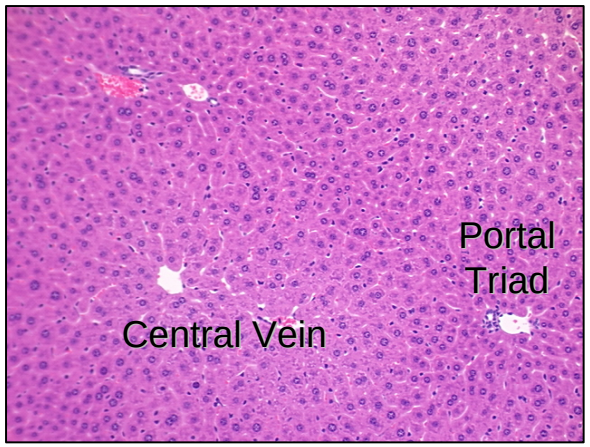
Incorporating Metabolism and Dosimetry to Better Model From Exposure to Effects

V-Liver Modeling of Metabolism, Dosimetry and Toxicity

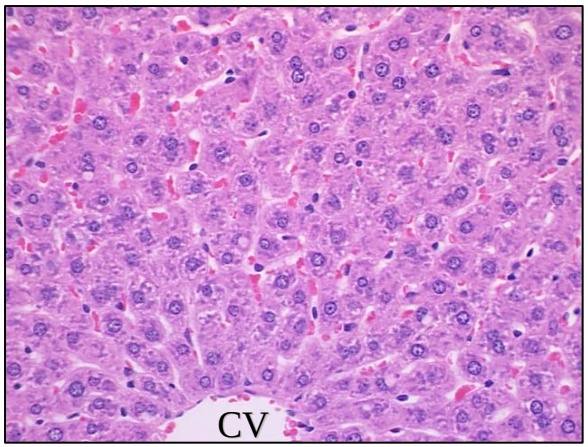


Slide courtesy of Imran Shah (EPA)

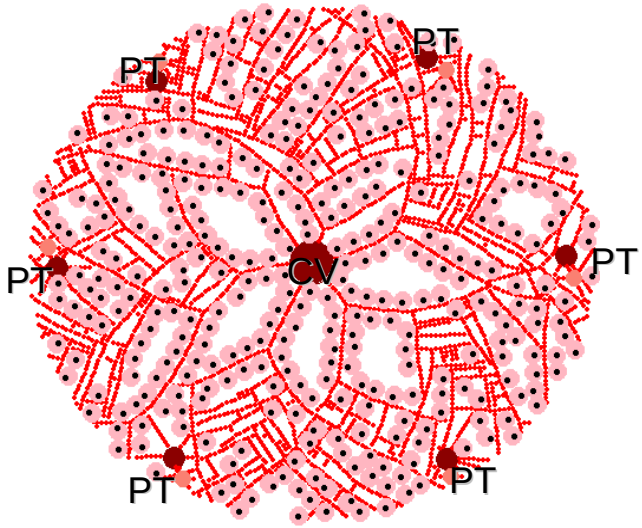
Virtual Liver: Dose-Response for a Virtual Lobule



The classic lobule consists of a single central vein fed venous and arterial blood via multiple portal triads



Blood flows through a network of sinusoids, supplying and exposing hepatocytes



Synthetic lobule sufficiently complex to determine that approach would work with actual lobule morphology (e.g. Drasdo et al.)

Slide courtesy of John Wambaugh (EPA)

Virtual Embryo Workflow:

From ToxCast and ToxRefDB to Systems Models

ToxCast™ HTS data (AC50s)

	(+)ve	(-)ve
(+)ve	TP	FP
(-)ve	FN	TN

ToxRefDB™ DEV data (cLELs)

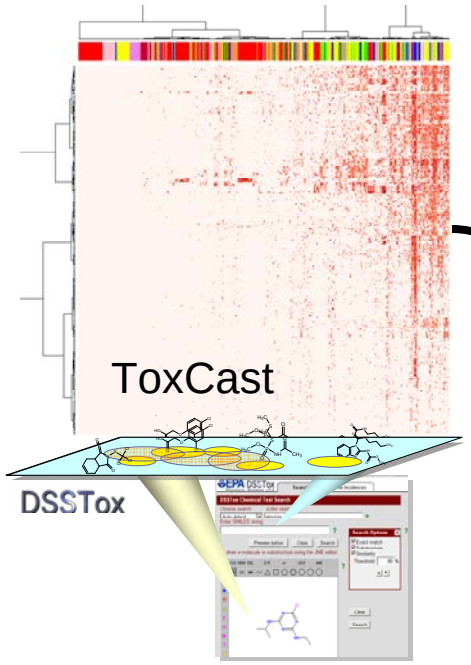
UNIVARIATE ASSOCIATIONS
- *t-test (continuous assay data)*
- *classifier (2x2 contingency table)*
- *Fisher's exact test (dichotomous data)*

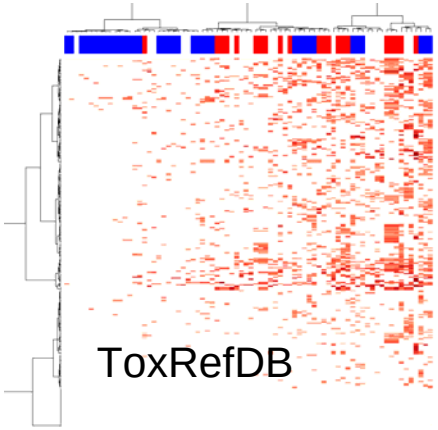
MULTIVARIATE MODELS
- *machine learning, 80/20 split*
- *5- or 10-fold cross-validation*
- *sensitivity analysis of performance*

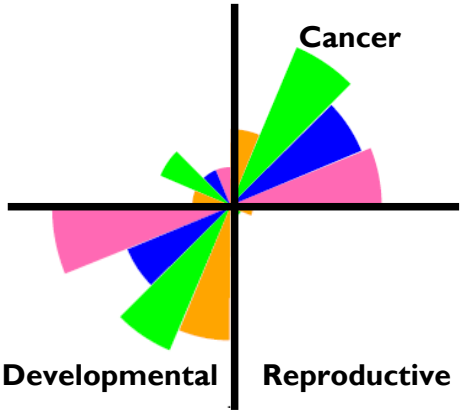
MULTICELLULAR SYSTEMS
- *knowledge-base (VT-KB)*
- *multicellular models (VT-SE)*
- *biological inferences / plausibility*

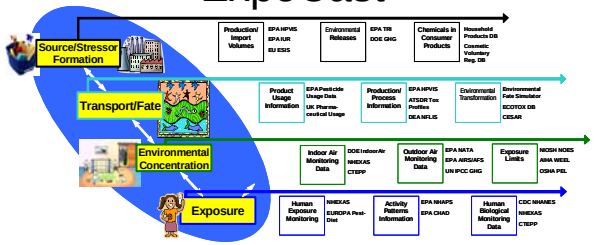
Slide courtesy of Tom Knudsen (EPA)

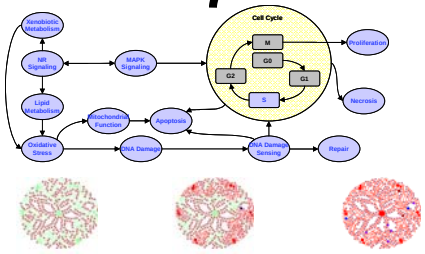
Future Chemical Prioritizations and Assessments Based on Risk Inferred from High Throughput Data

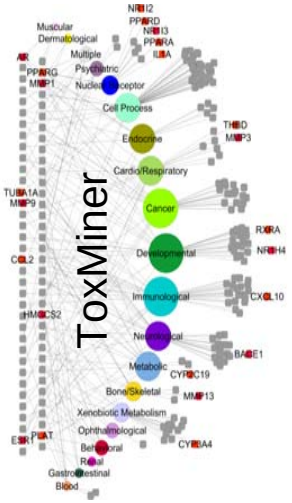




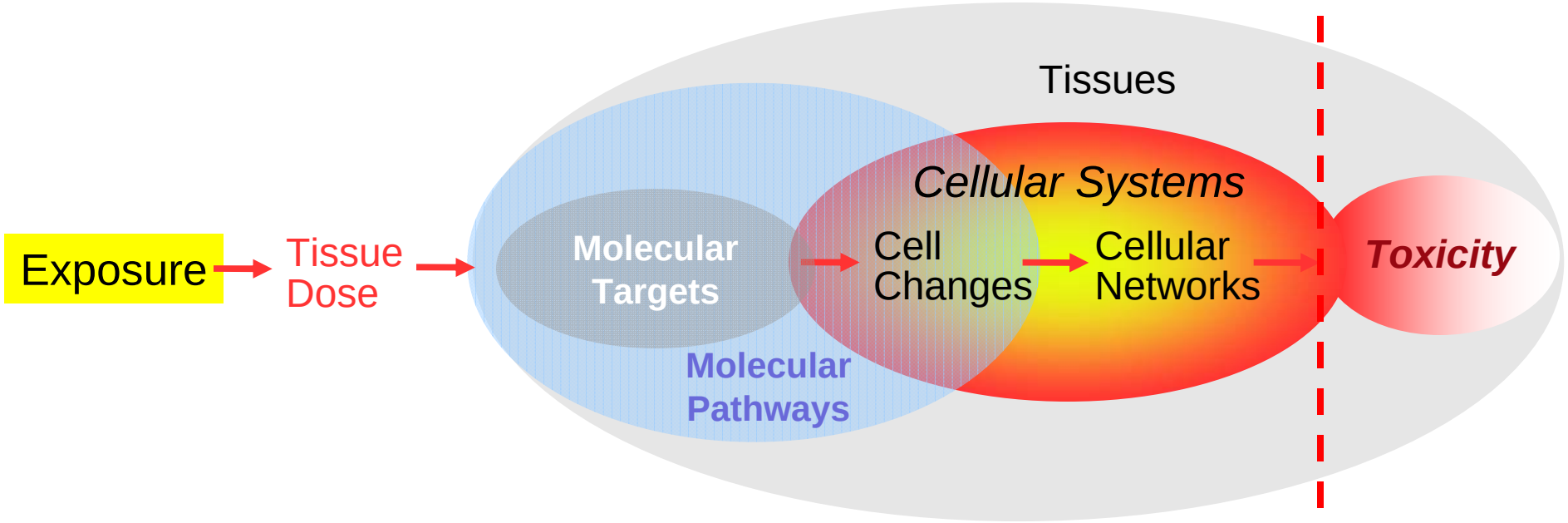








HIGH THROUGHPUT SCREENING and CHARACTERIZATION OF HAZARD & RISK



IN VITRO: concentration response

Human IN SILICO: exposure dose response

Animal IN VIVO: exposure dose response

Acknowledgements

Robert Kavlock
Keith Houck
Matt Martin
Richard Judson
Ann Richard
David Reif
Daniel Rotroff
Woody Setzer
Holly Mortenson
Andrew Beam

Tom Knudsen
Imran Shah
John Wambaugh

Ray Tice
Chris Austin

http://www.epa.gov/ncct/