United States Environmental Protection Agency

Chemical & Biological Profiling Approaches for exploring Mutagenicity & Carcinogenicity of EPA ToxCast Chemicals

EMS, St. Louis, MO, October 26, 2009

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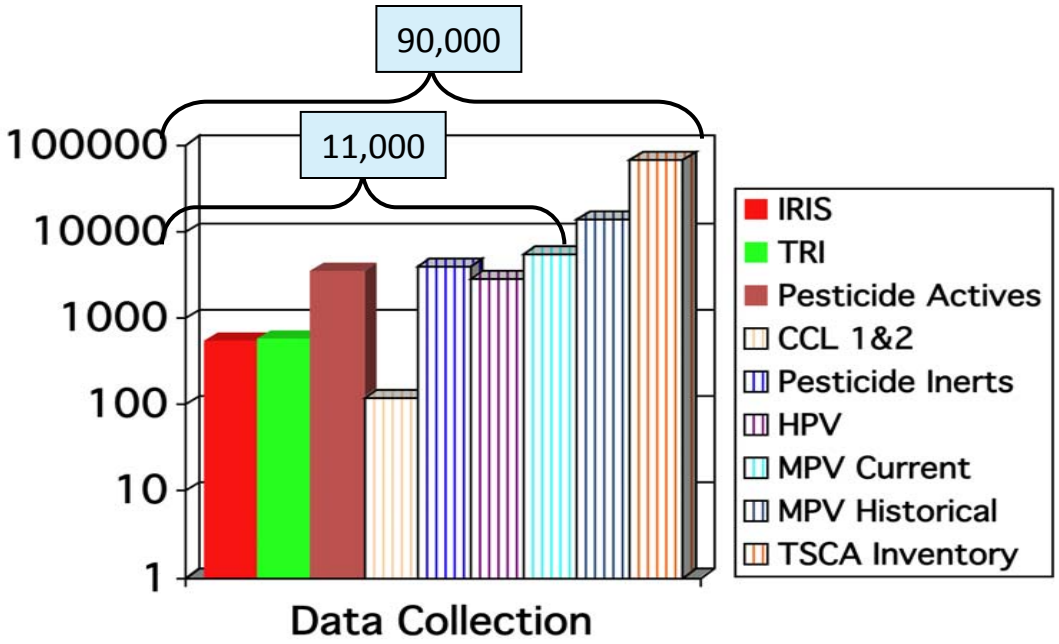
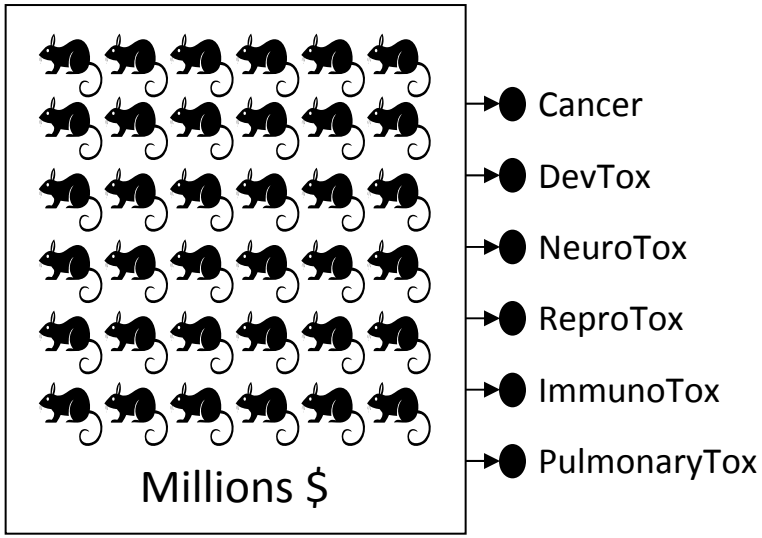
Office of Research and Development
National Center for Computational Toxicology

Change Needed Because …..

Too Many Chemicals



Too High a Cost

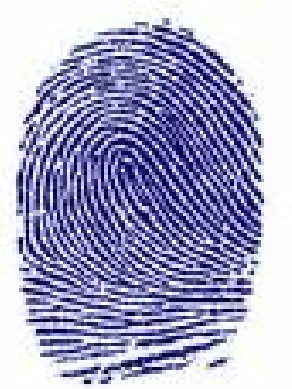


…and not enough data.

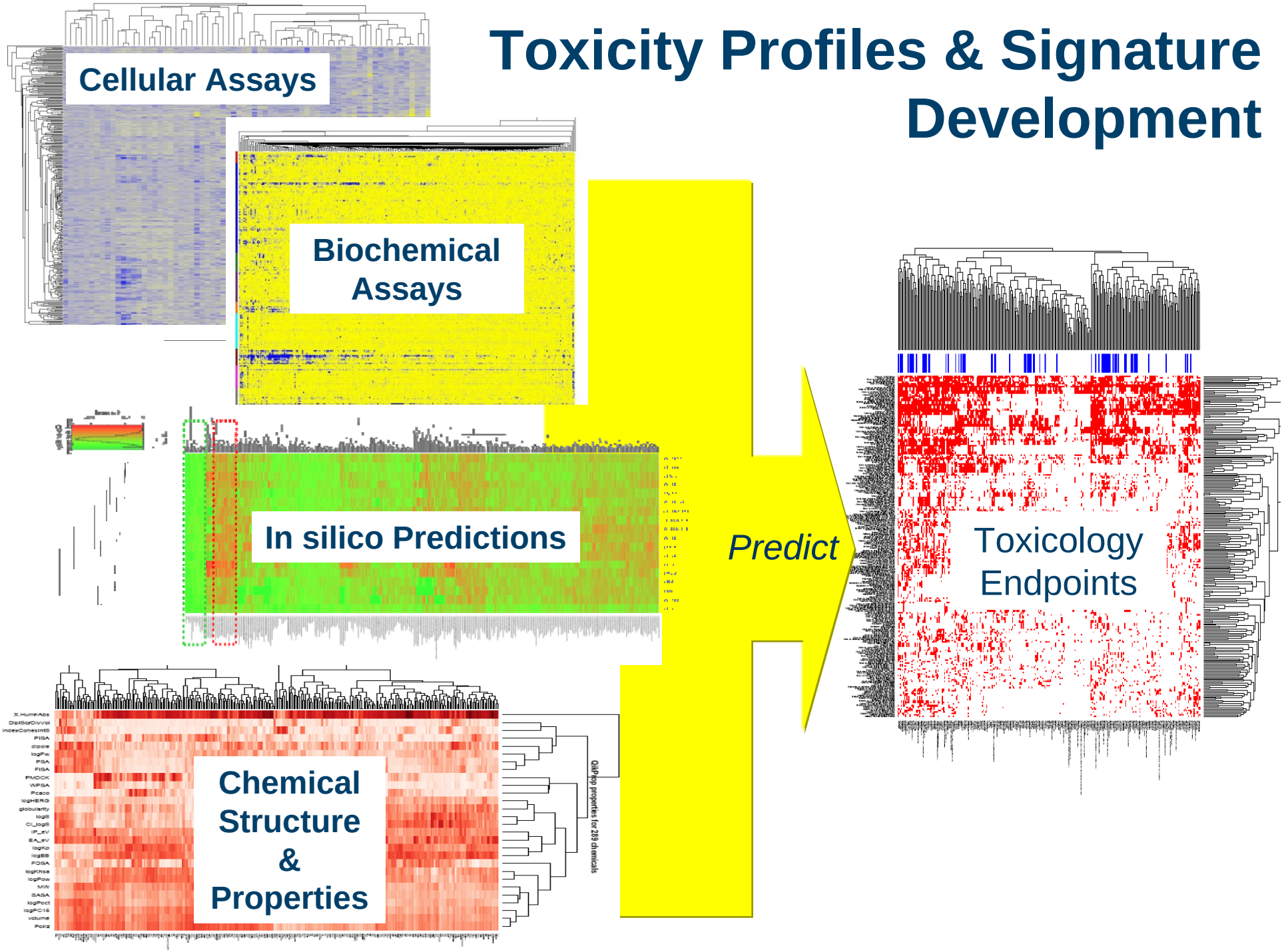
Judson, et al *EHP, 2008*

ToxCast™ Background

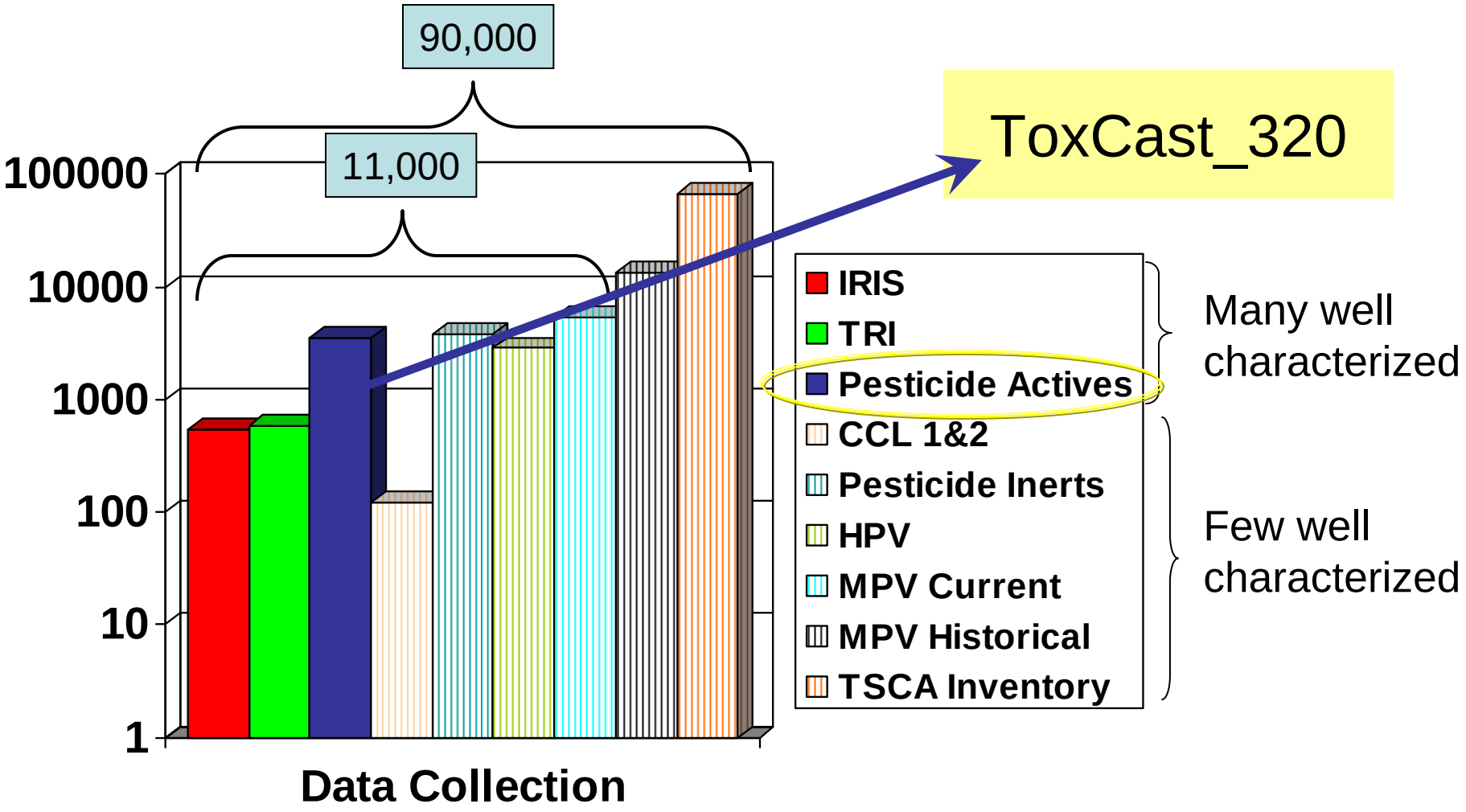
- Goal to address chemical screening and prioritization needs for high priority EPA chemical inventories:
  - *pesticidal inerts, anti-microbials, CCLs, HPVs, MPVs*
- Coordinated with NTP and NIH/NCGC via Tox21
- Committed to stakeholder involvement and public release of data & tools
  - Communities of Practice- Chemical Prioritization & Exposure
  - NCCT website http://www.epa.gov/ncct/toxcast
    - *ACToR http://www.epa.gov/actor/*
    - *ToxRef DB http://www.epa.gov/ncct/toxrefdb/*
    - *DSSTox (PubChem) http://www.epa.gov/ncct/dsstox/*



Toxicity Profiles & Signature Development

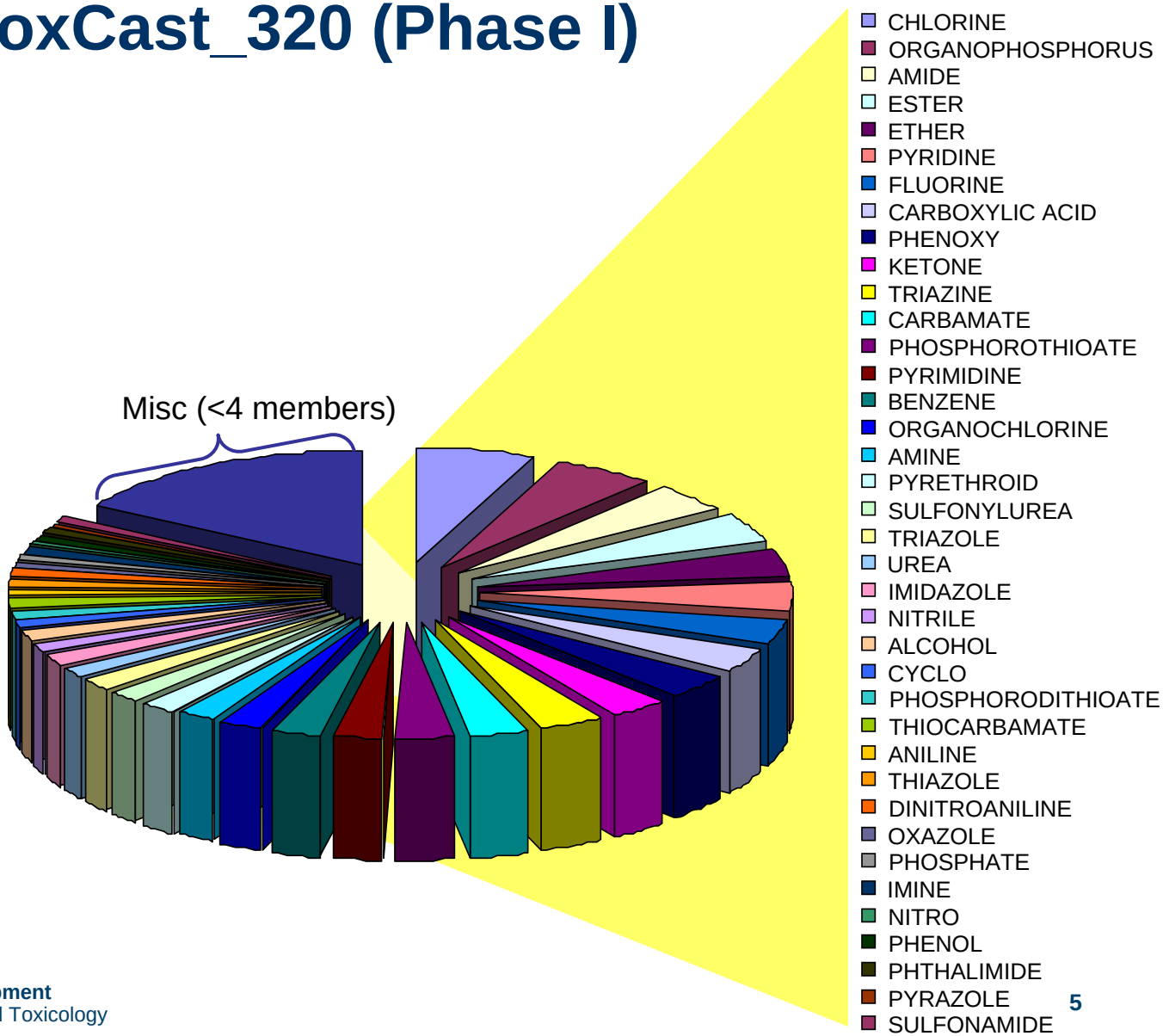


ToxCast Phase I Chemicals

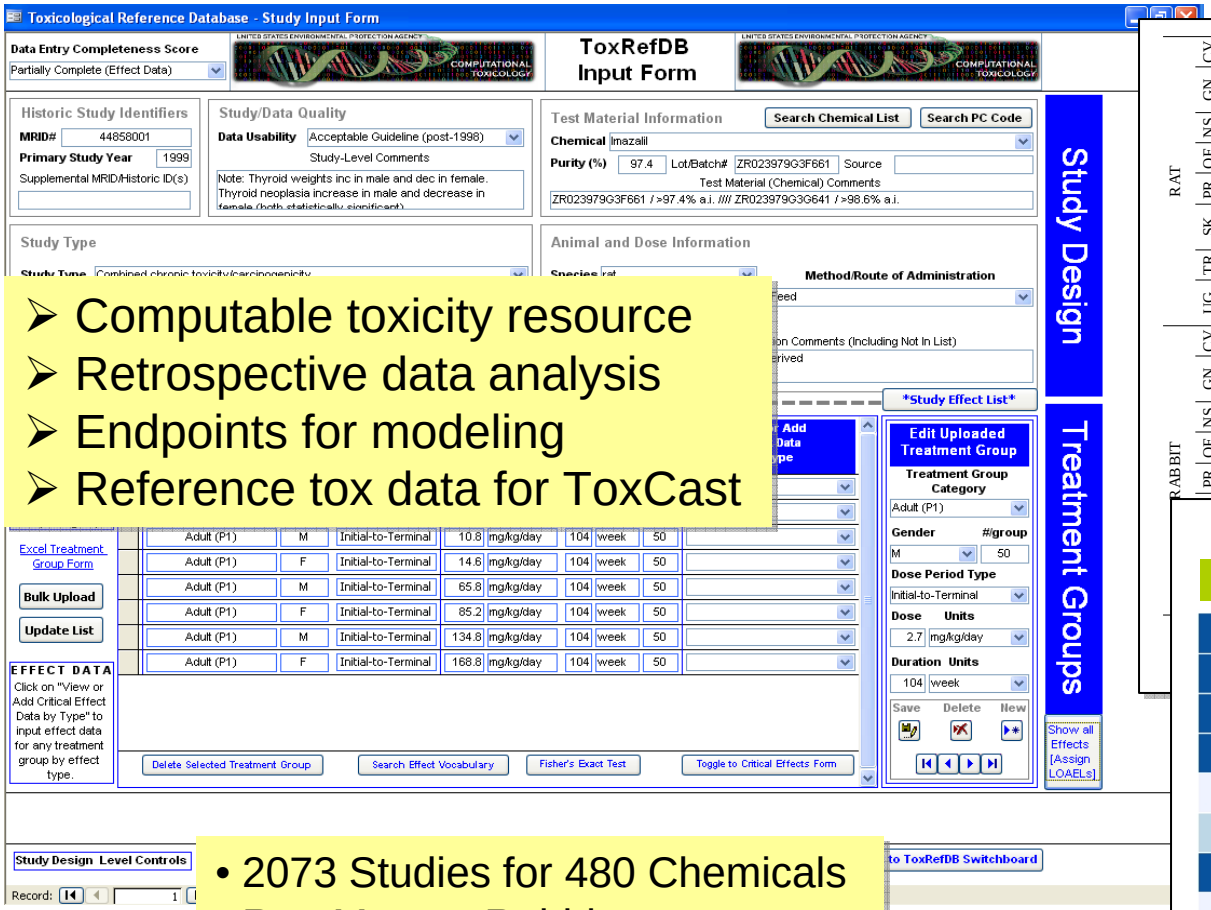


Chemical Classes in ToxCast_320 (Phase I)

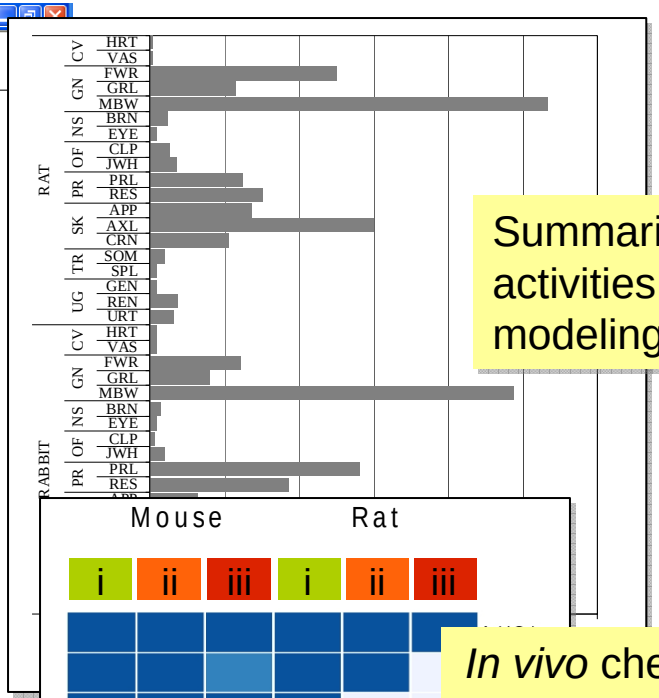
- 309 Unique Structures
- Replicates for QC
- 291 Pesticide Actives
- 9 Industrial Chemicals
- 13 Parent/Metabolite pairs
- 56/73 Proposed Tier 1 Endocrine Disruption Screening Program
- 14 High Production Volume Chemicals
- 11 HPV Challenge



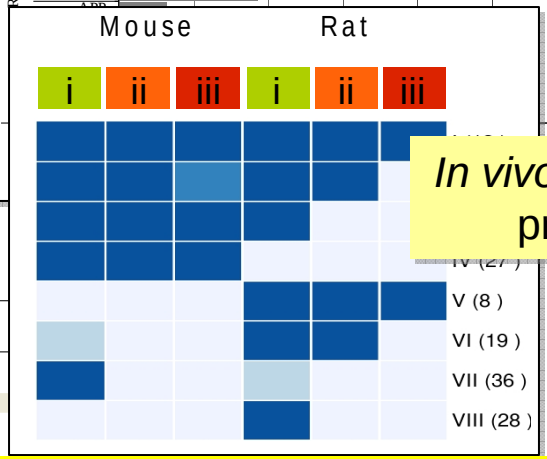
ToxRefDB: EPA Pesticide DERs



- Computable toxicity resource
- Retrospective data analysis
- Endpoints for modeling
- Reference tox data for ToxCast



Summarized activities for modeling



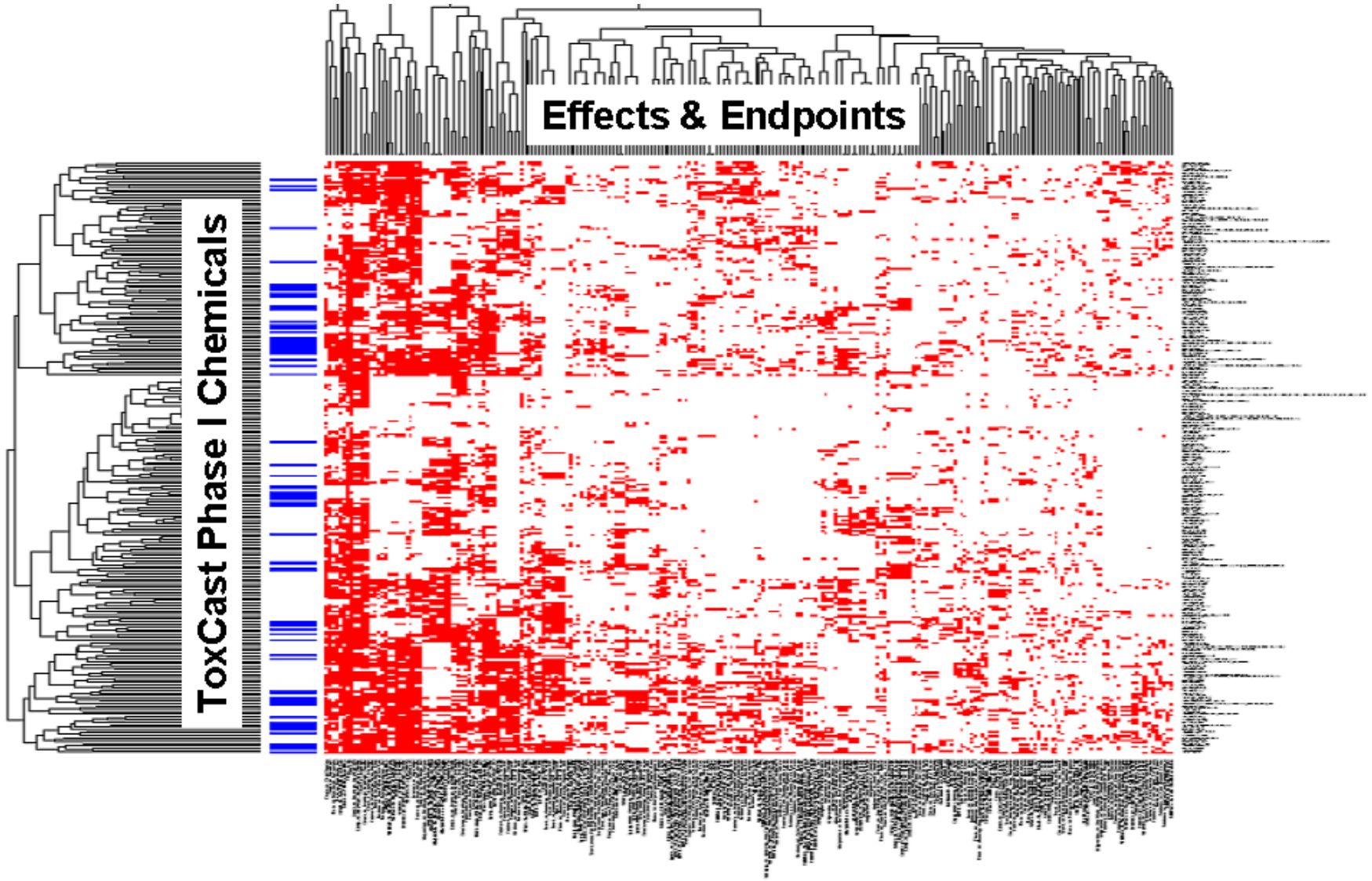
In vivo chemical profiles

- 2073 Studies for 480 Chemicals
- Rat, Mouse, Rabbit
- Chronic, Repro, Developmental

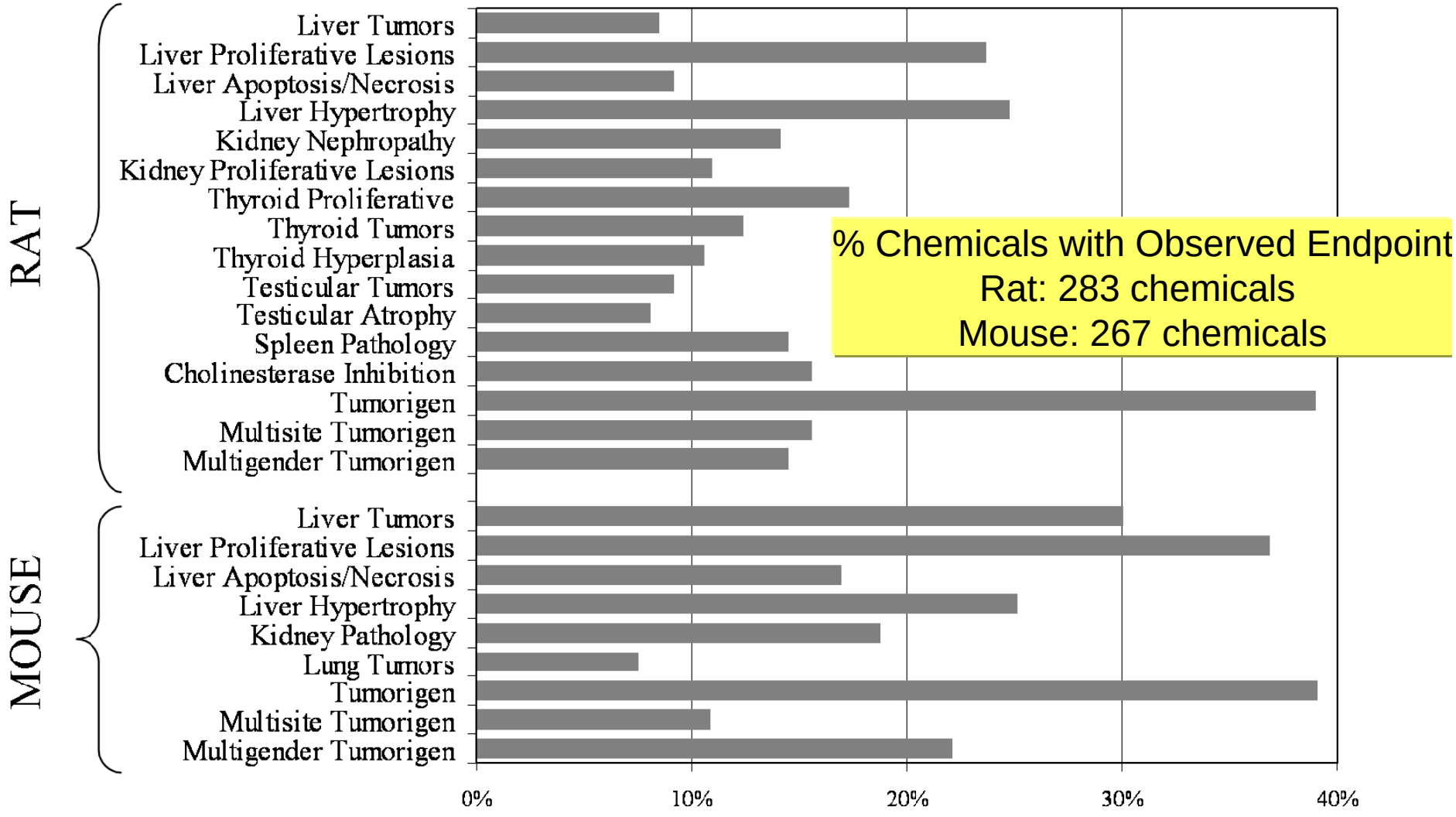
http://www.epa.gov/ncct/toxrefdb/

Martin et al. (2009) Environ Health Perspec 117:392-399

>$1B *In Vivo* Chronic/Cancer Bioassay Effects and Endpoints



Initial Chronic Rat & Mouse Endpoints for Predictive Modeling



% Chemicals with Observed Endpoint
Rat: 283 chemicals
Mouse: 267 chemicals

Martin et al. (2009) Environ Health Perspec 117:392-399

ToxCast *In vitro* HTS Assays

Biochemical Assays

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

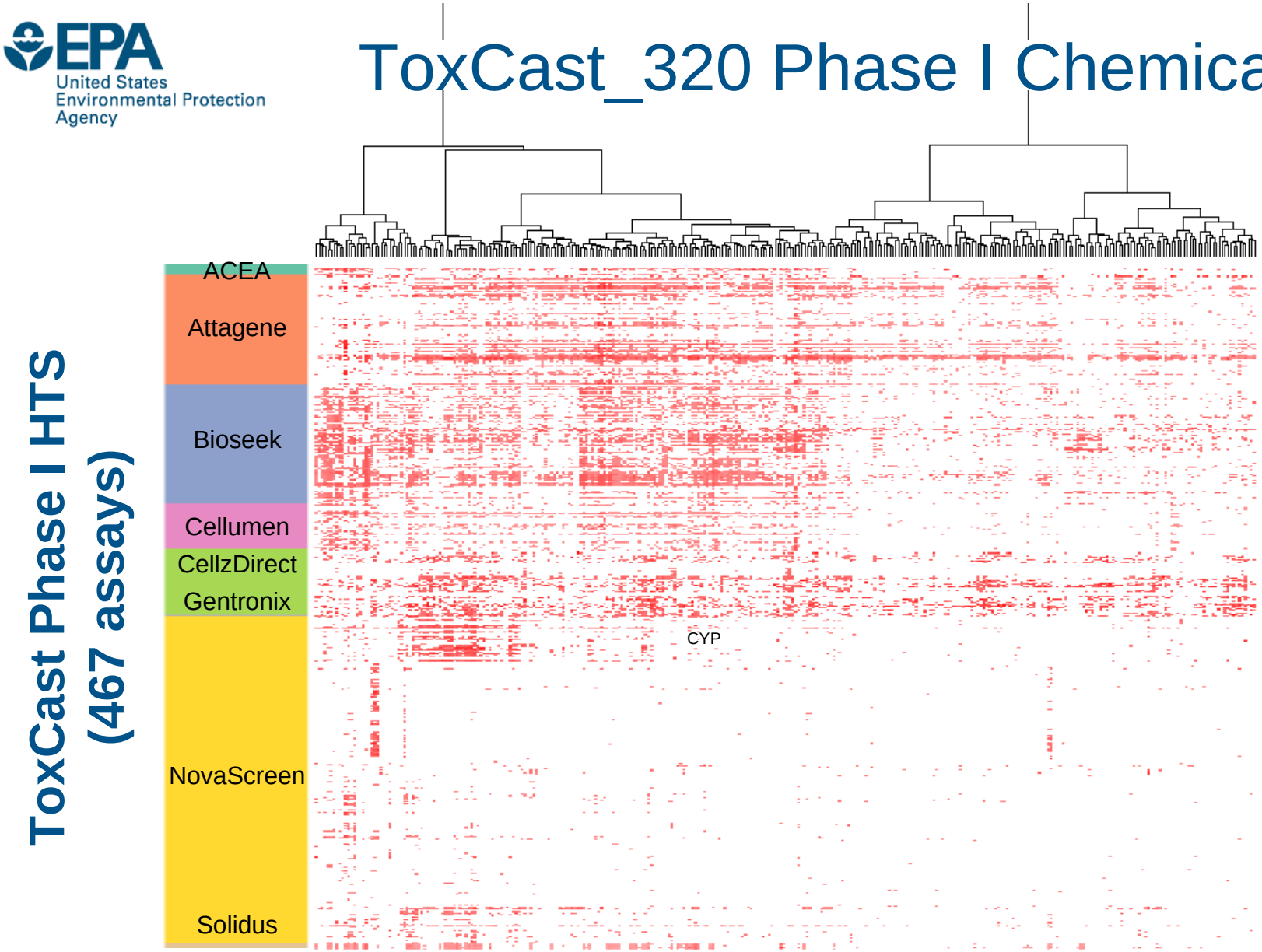
467 Endpoints

Cellular Assays

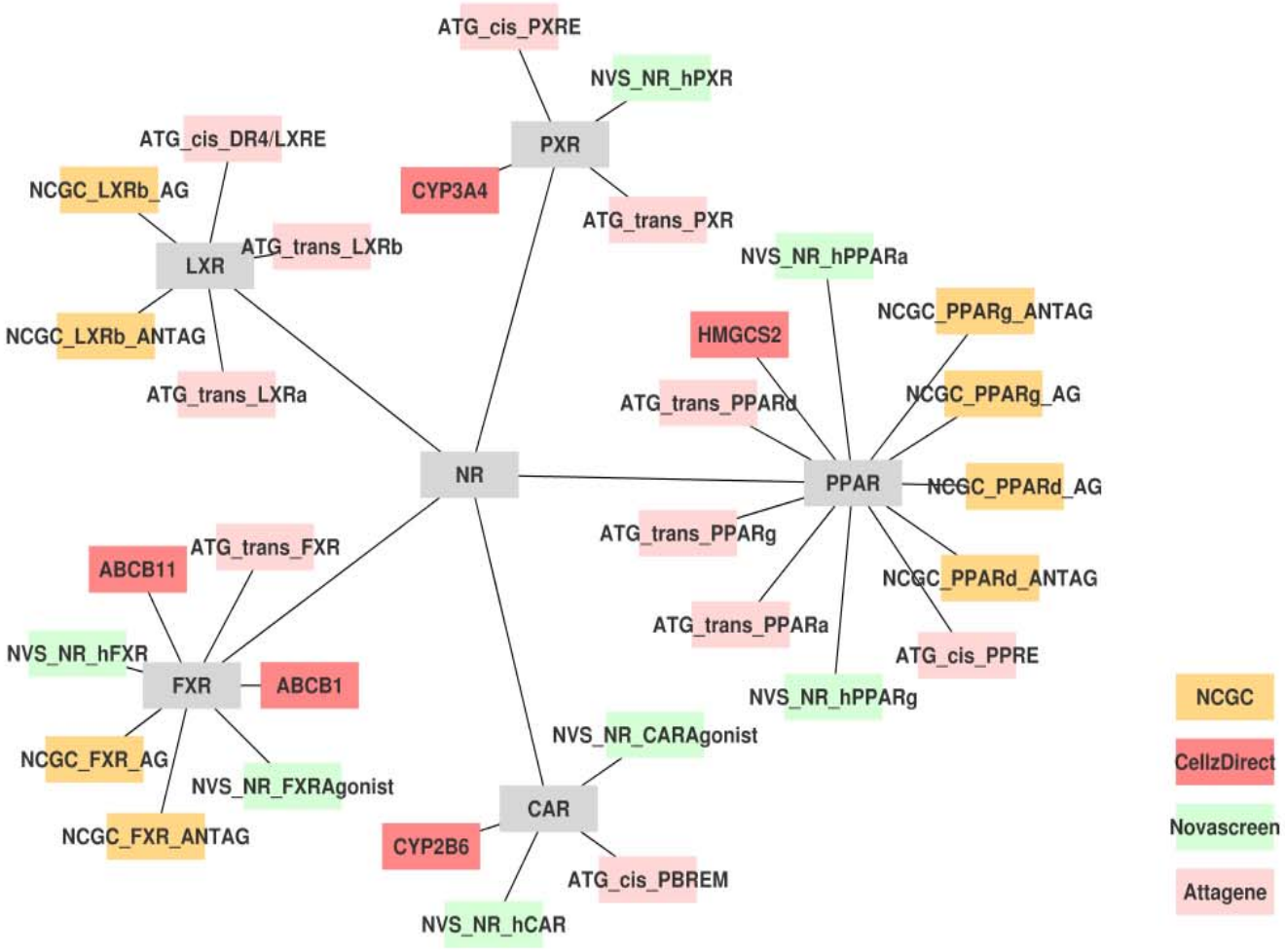
- **Cell lines**
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
- **Primary cells**
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells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

United States Environmental Protection Agency

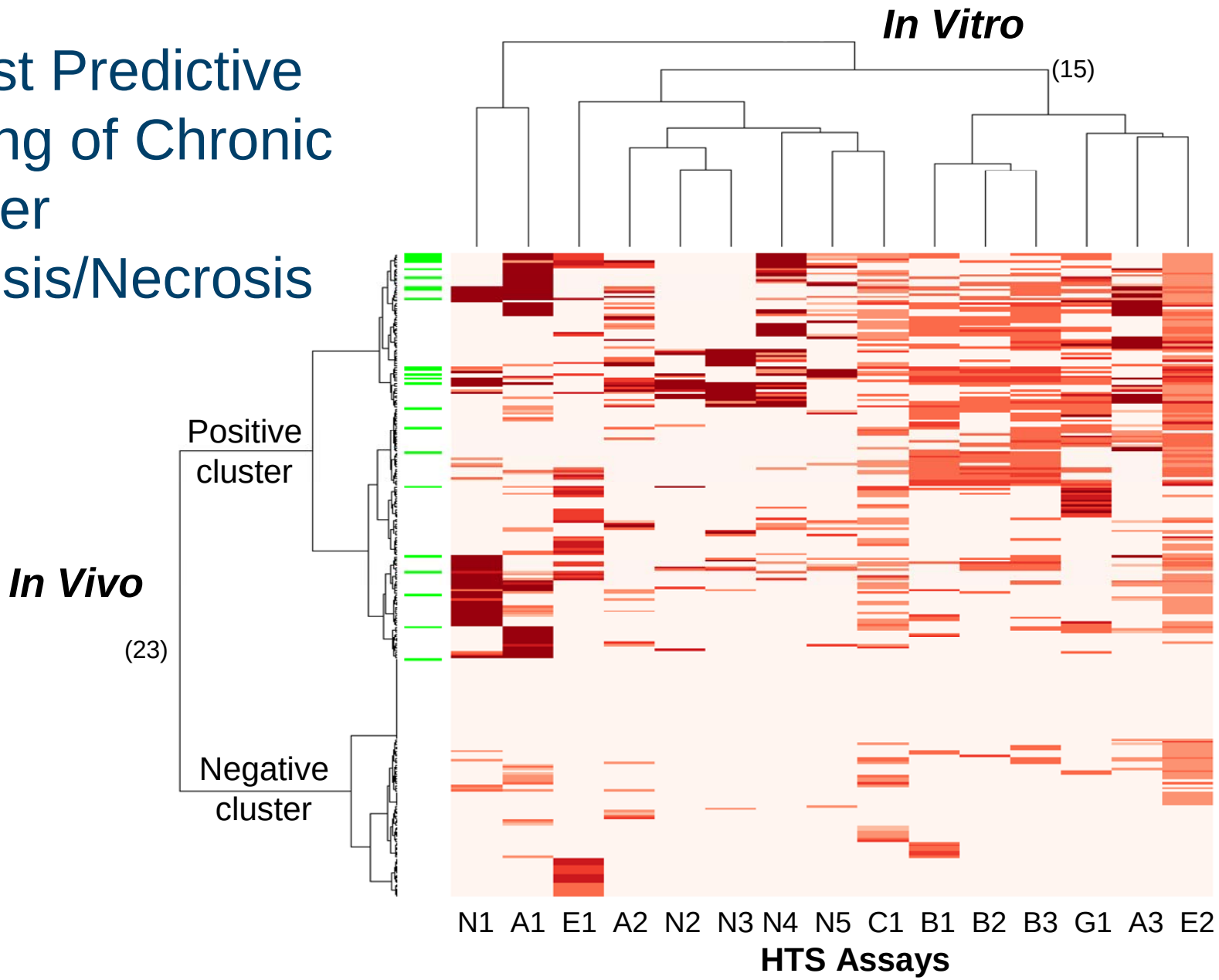
ToxCast_320 Phase I Chemicals



Multiple Assays per Endpoint

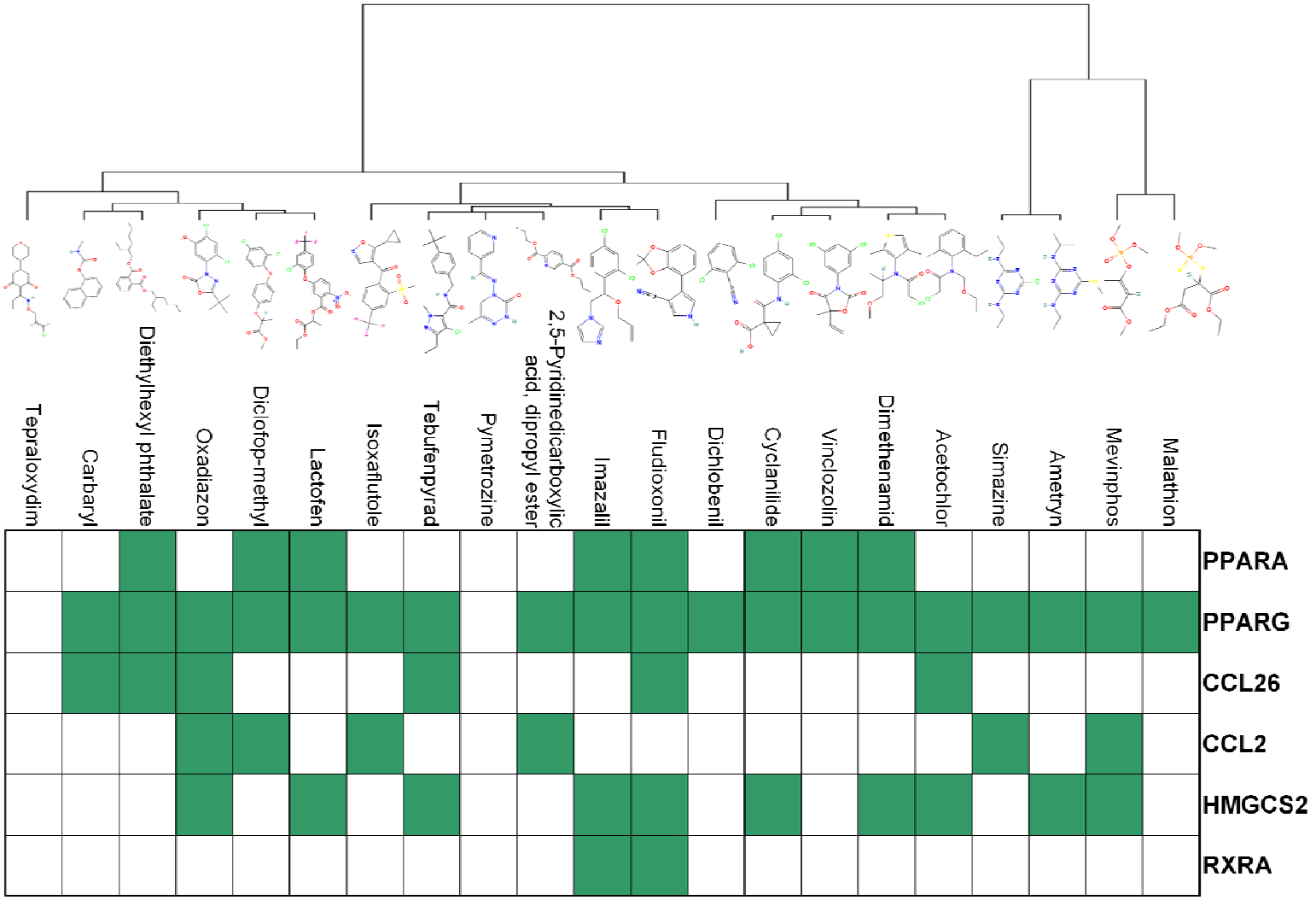


ToxCast Predictive Modeling of Chronic Rat Liver Apoptosis/Necrosis



Judson et al (2008) BMC Bioinformatics 9:241

Rat Liver Tumorigens are diverse in chemical structure and *in vitro* Signature



Judson et al (2008) BMC Bioinformatics 9:241

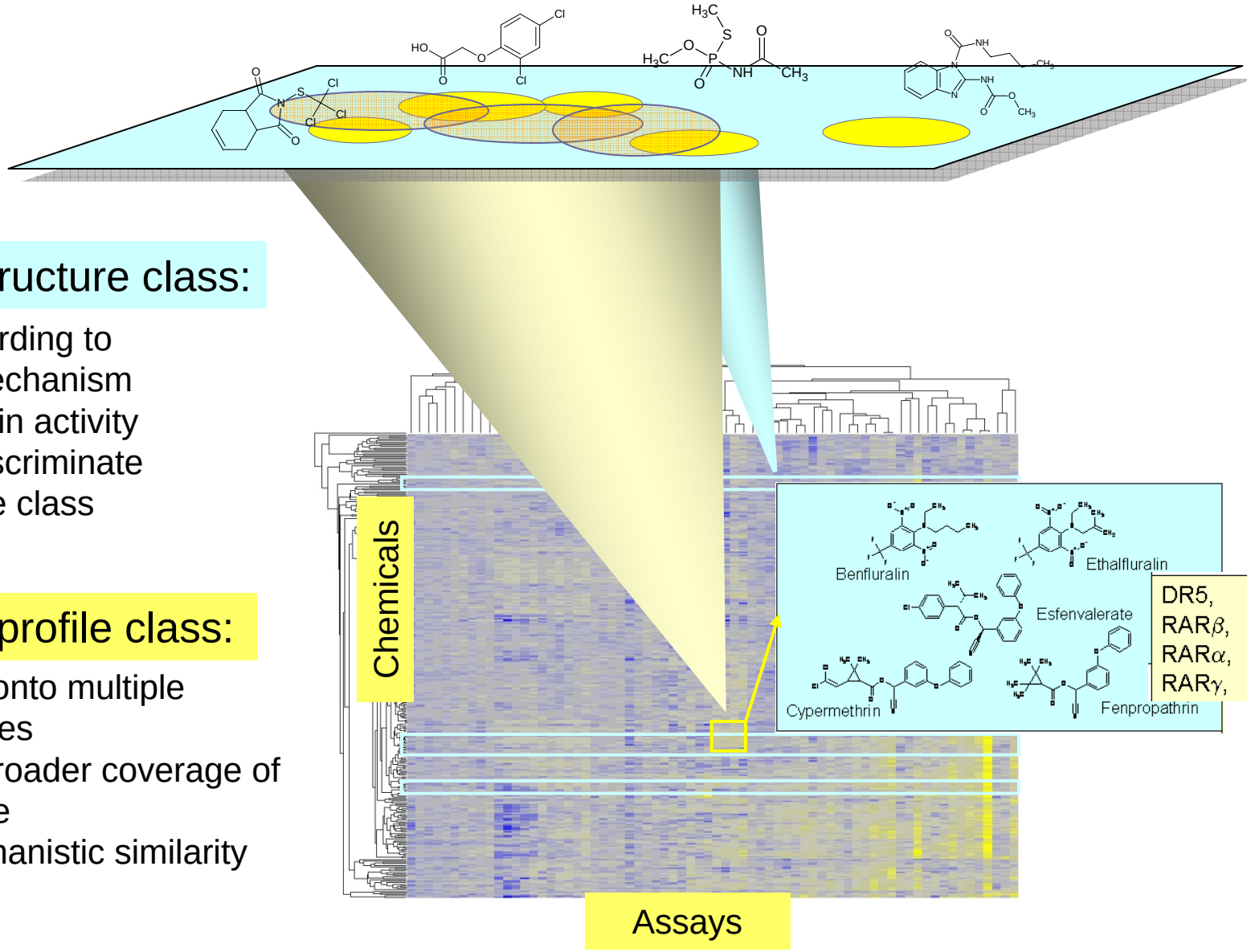
Structure Class vs Bioactivity Class

Chemical structure class:

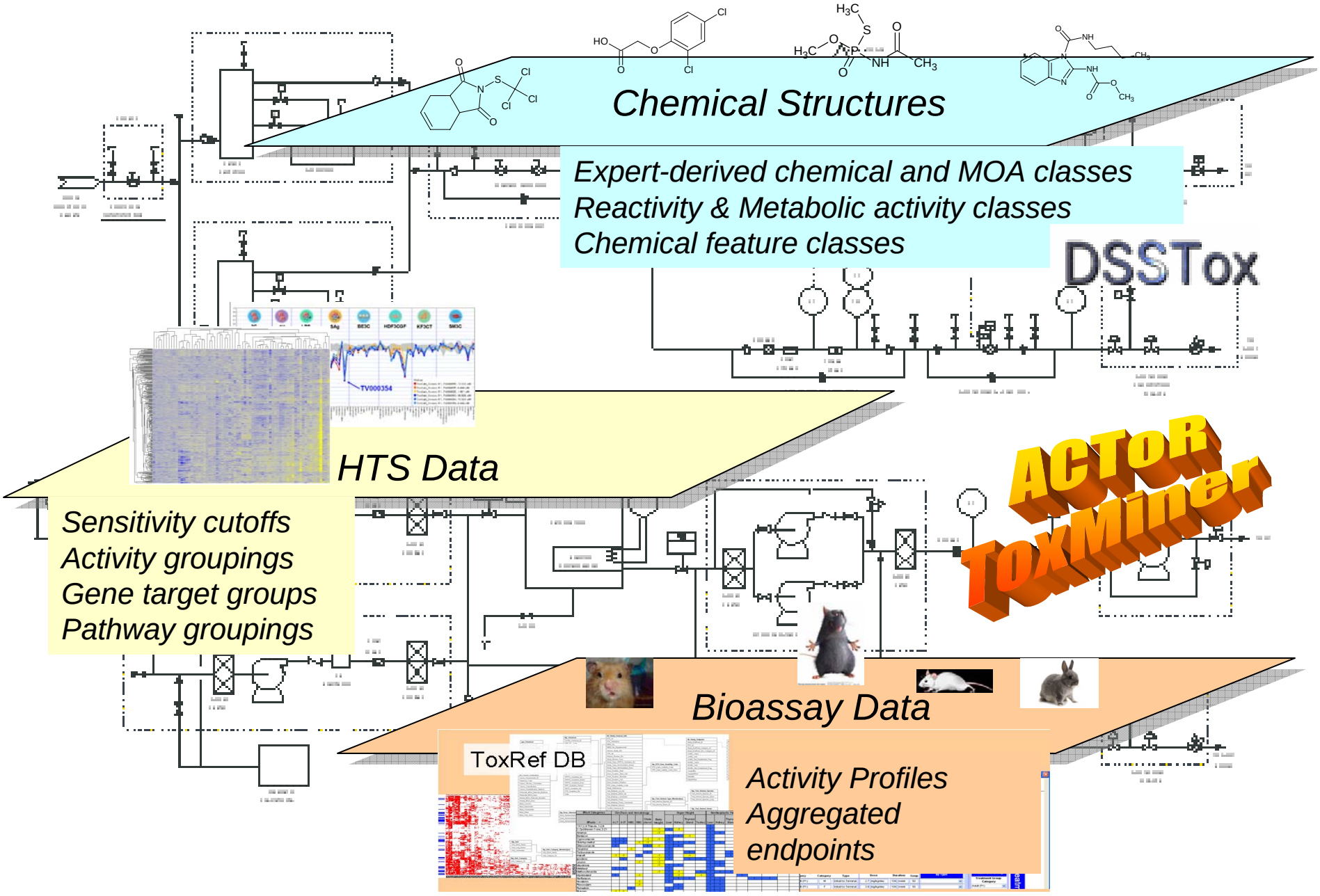
- Cluster according to activity and mechanism
- Differences in activity profiles can discriminate within structure class

Bioactivity profile class:

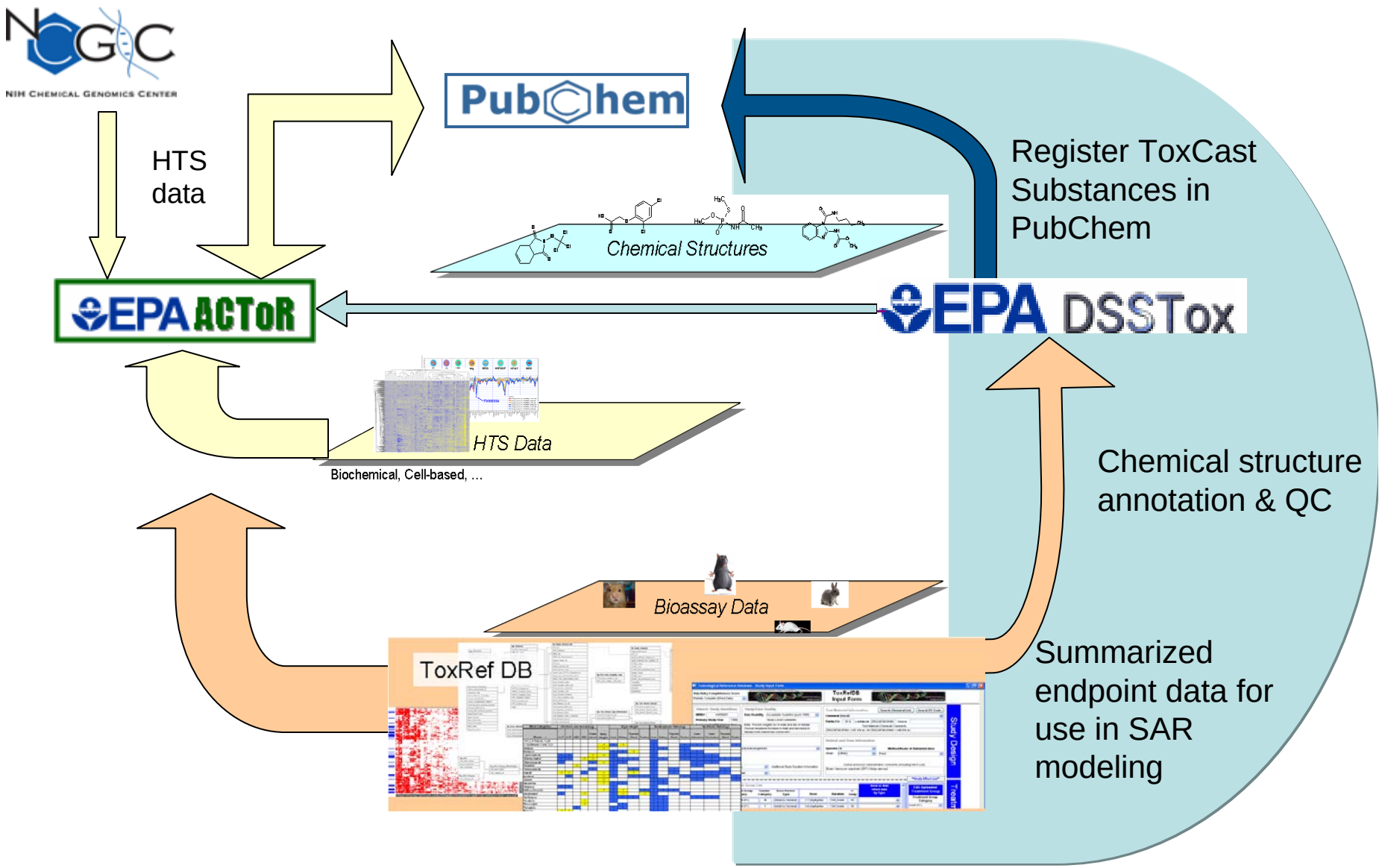
- Can project onto multiple chemical classes
- Potentially broader coverage of chemical space
- Implies mechanistic similarity



ToxCast: High-Multi-Dimensional Data



ToxCast: Data Publication & Exploration



ToxCast™ Data Analysis Summit, May 14-15, 2009

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ToxCast™ Program

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

ToxCast™ Data Analysis Summit

Transforming Toxicity Testing From In Vivo to In Vitro: A Computational Toxicology Challenge

The First ToxCast™ Data Analysis Summit
Hosted by U.S. EPA"s National Center for Computational Toxicology
EPA Campus, Research Triangle Park NC

ToxCast™ Navigation
Introduction
Chemicals
Assays
Information Management
Partnerships
Contractors
Presentations

- Pre-release of Phase 1 ToxCast data to analysis partners
- Over 200 registered attendees, 60 presenters
- Wide range of statistical, machine learning approaches
- Wide variety of prediction schemes (HTS+SAR)

ToxCast™ Data Analysis Summit, May 14-15, 2009

Impressions, Conclusions, Lessons…

- Global associations (*in vitro* to *in vivo*) not apparent
  - → *local models possible chemical feature/biology space*
- Statistical approaches for highly sparse, unbalanced data needed
  - → *new methods proposed*
- Chemical descriptors alone better than HTS alone
  - → HTS+chemical descriptors give best QSAR models
- Existing SAR carcinogenicity prediction models (LAZAR, ToxTree, PASS) built on public data performed poorly
  - → *point to lack of coverage of non-genotoxic mechanisms*
- Public data availability and transparency successful in engaging wide range of researchers and capabilities in analysis

New Structural Alerts for Carcinogenicity Derived from a Pesticide Data Set

Susanne A. Stalford, Carol A. Marchant and Richard V. Williams
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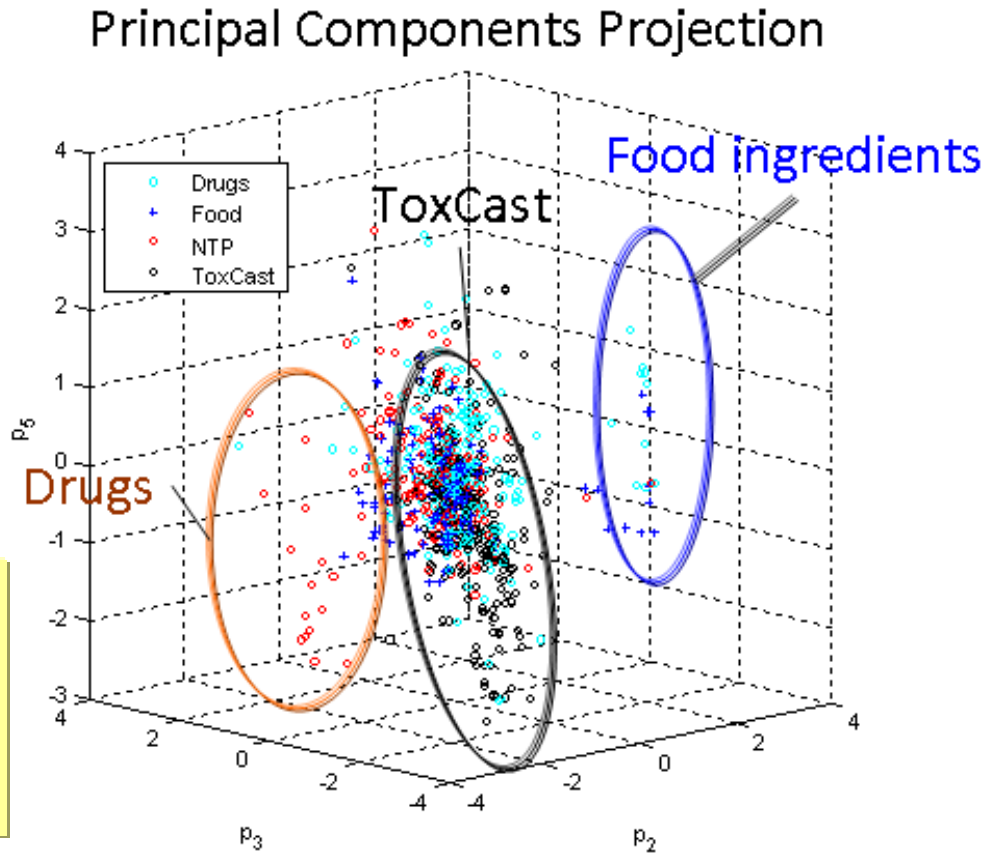
susanne.stalford@lhasalimited.org

ToxCast Data Landscape: Implications for Genetox

- 309 ToxCast Phase I chemicals
  - *Mostly pesticides*
  - *Unique chemical space*
- High proportion of non-genotoxic carcinogens
- No genetox data provided

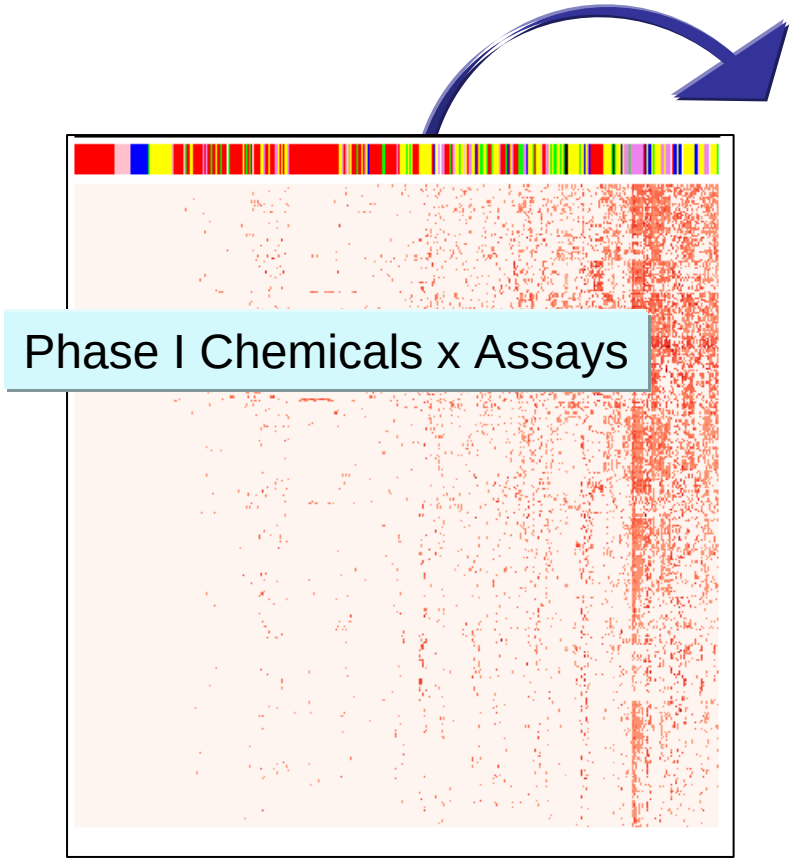


SAL data collected from public sources for approx 108 ToxCast chemicals to assess genetox-related assays *

Knight et. al, Reg Tox Pharmacol, 2009, 55:188-199.

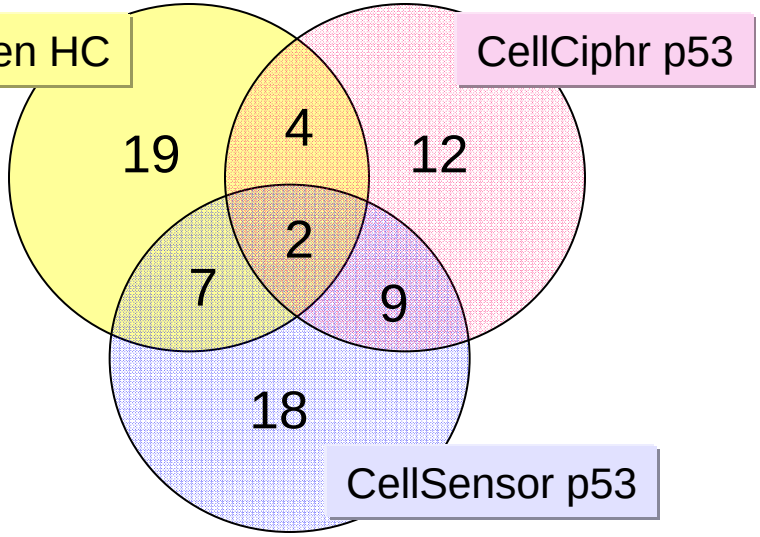
Slide results courtesy of ChihaeYang, FDA CFSAN, ToxCast Data Analysis Summit, May 2009

ToxCast/Tox21: Genetox Assays



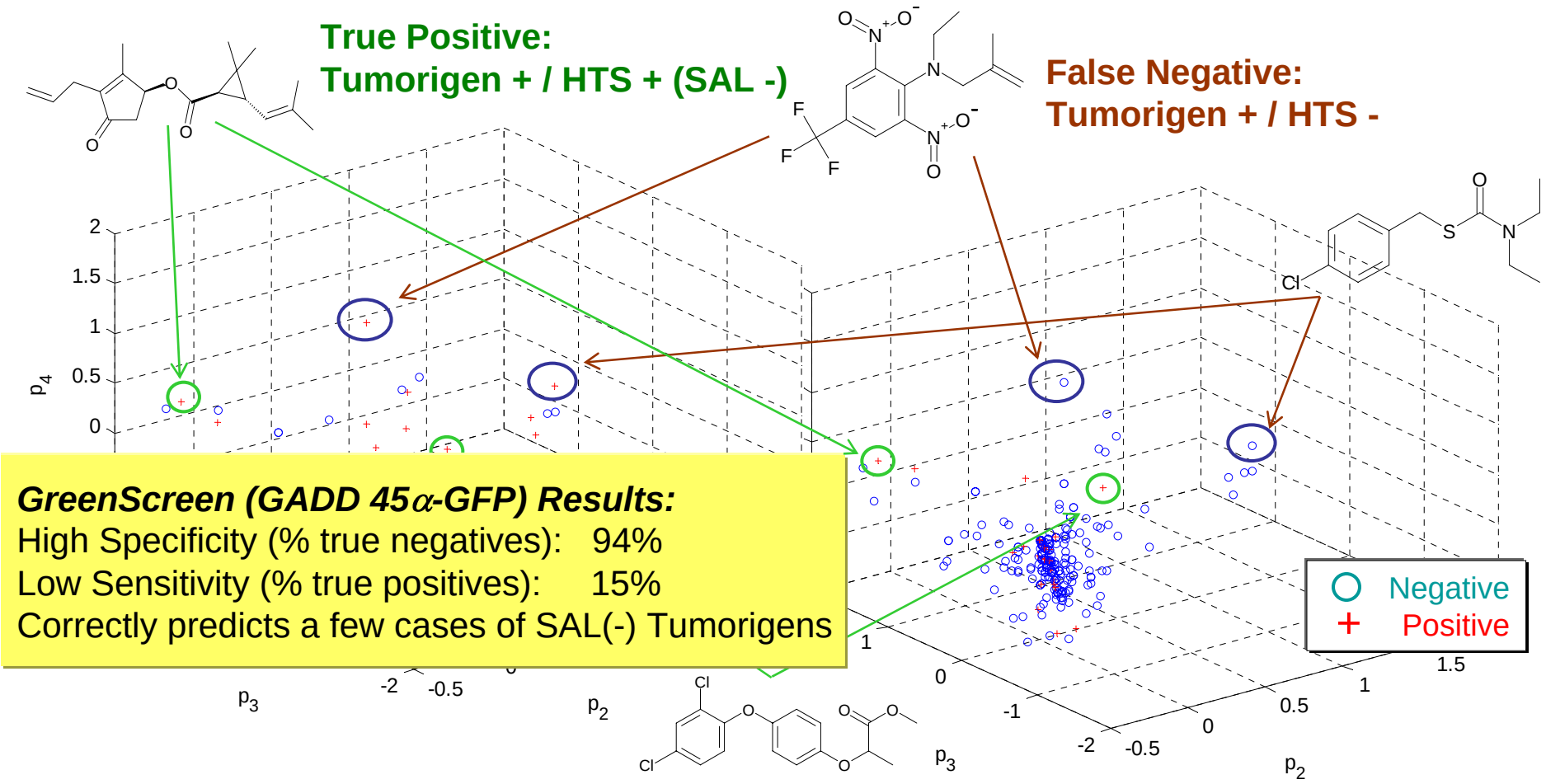
- GreenScreen HC GADD45α-GFP Reporter Assay (p53 competent) *(Gentronix, Ltd.)*
- CellCiphr p53 *(Cellumen Inc.)*
- CellSensor p53RE-bla *(Invitrogen Corp., provided by NCGC)*

Overlap of positive results



Knight et. al, Reg Tox Pharmacol, 2009, 55:188-199.

A rodent bioassay vs. an HTS genetox assay

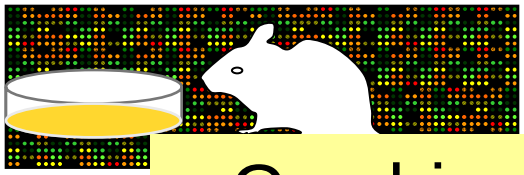


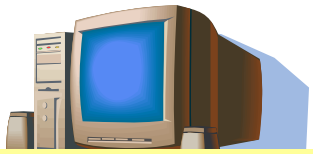
GreenScreen (GADD 45α-GFP) Results:
High Specificity (% true negatives): 94%
Low Sensitivity (% true positives): 15%
Correctly predicts a few cases of SAL(-) Tumorigens

Knight et. al, Reg Tox Pharmacol, 2009, 55:188-199.

Slide results courtesy of ChihaeYang, FDA CFSAN, ToxCast Data Analysis Summit, May 2009

Tox21 Collaboration





- Combined HTS plates (7x1408) high interest chemicals
- Joint assay development
- NTP Analytical QC
- Use of NCGC HTS testing capabilities
- EPA informatics (ACToR/DSSTox)
- *FDA preclinical toxicity data*

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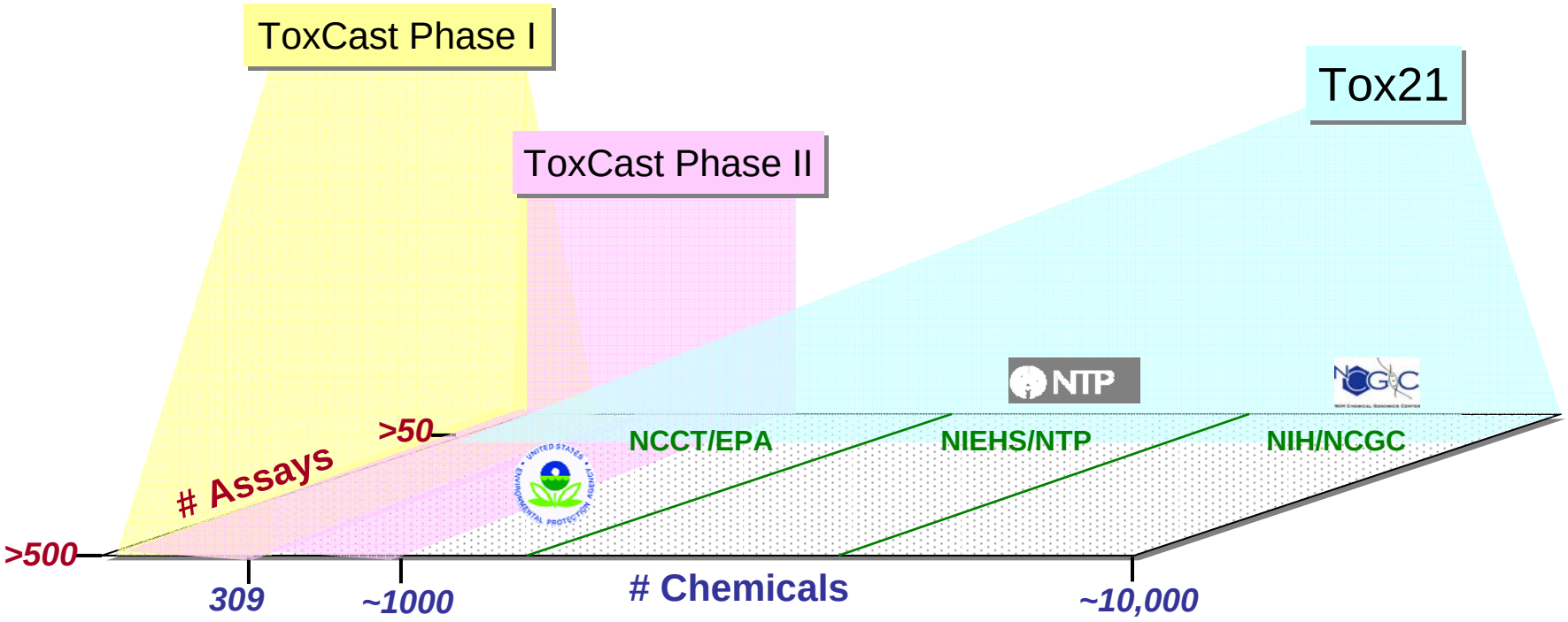
CFSAN / CDER

NIH Chemical Genomics Center

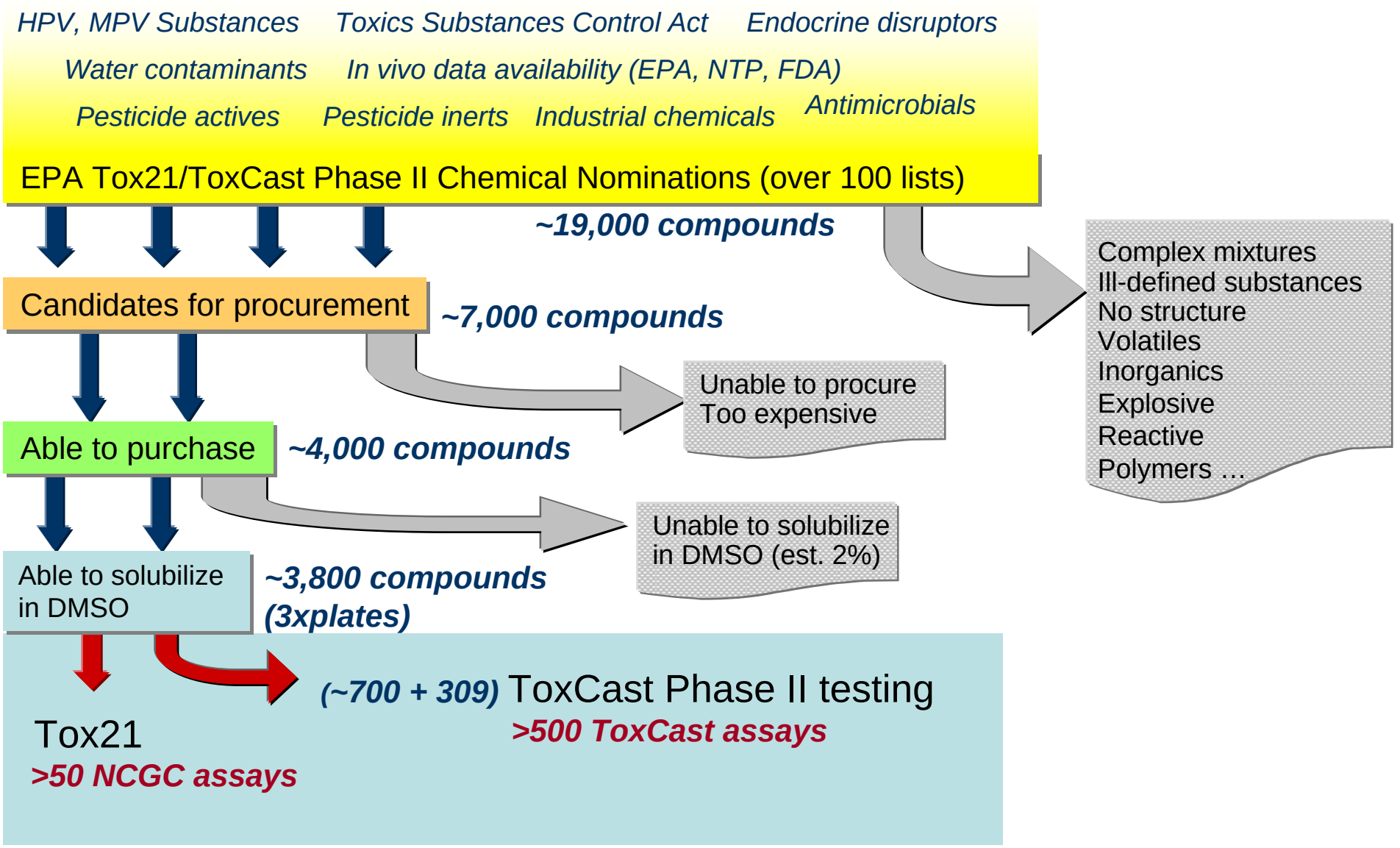
ToxCast/Tox21 Testing Landscape



ToxCast Phase II

- Add approx 700 new chemicals to 309 Phase I set
- Pfizer: ~ 100 failed drugs with pre-clinical/clinical tox data
- Glaxo: liver toxicity data for approx 150 drugs
- L'Oreal: sponsoring 10 chemicals for Phase II
- FDA CFSAN/CDER data rich chemicals to be included
- Model organisms: *c. elegans* (NTP), whole embryo zebrafish (EPA)
- Expanded toxicity data models and databases to include:
  - *Developmental Neurotox, Immunotox, Genetox*

ToxCast/Tox21 Testing Landscape



Challenges for Tox21/ToxCast

United States Environmental Protection Agency

Assays

- ❖ Reproducibility
- ❖ Sensitivity
- ❖ Biological relevance

- ✓ Plate replicates
- ✓ Dose response
- ✓ Assay replicates
- ✓ Positive controls

Chemicals

- ❖ Purity, Identity
- ❖ Stability
- ❖ Solubility
- ❖ Accuracy of representation

- ✓ Chemical filters & selection process
- ✓ Analytical QC
- ✓ Structure QC

Metabolism, ADME

- ❖ Many assays do not have metabolic capability
- ❖ ADME missing in *in vitro*

- ✓ Assays with metabolic capability
- ✓ Include known metabolites
- ✓ Active metabolite features represented
- ✓ Metabolic prediction models

ToxCast/Tox21: GeneTox

- Available HTS GeneTox tests under consideration:
  - GreenScreen Human Cell Assay (GADD45α –GFP reporter) ±S9
  - Ames II assay
  - *In vitro* Micronucleus assay
  - *In vitro* Comet assay
  - *In vitro* Caspase 3/7 Cytotoxicity assay
- ToxRefDB Genetox data from DERs for pesticides
- ToxML efforts to capture Genetox data for public and FDA chemicals
- Availability of hundreds of chemicals having rich profile of both in vivo chronic (cancer) data & genetox data
  - EPA, NTP, FDA CFSAN, FDA CDER

SAR - SAL Mutagenicity Prediction

Hansen et al (2009) JCIM, 49:2077-2081

- Created large public Benchmark dataset of SAL results:
  - 6512 compounds (3503 positive, 3009 negative)
  - Conflicting results removed, est. 10-15% experimental error
- 3
- 4
- C

erform worse than machine learning methods

- On large diverse set of >6000 chemicals, SAR methods reliably predict correct SAL outcome 86% of the time
- Experimental reproducibility of SAL experiment estimated between at 85-90%

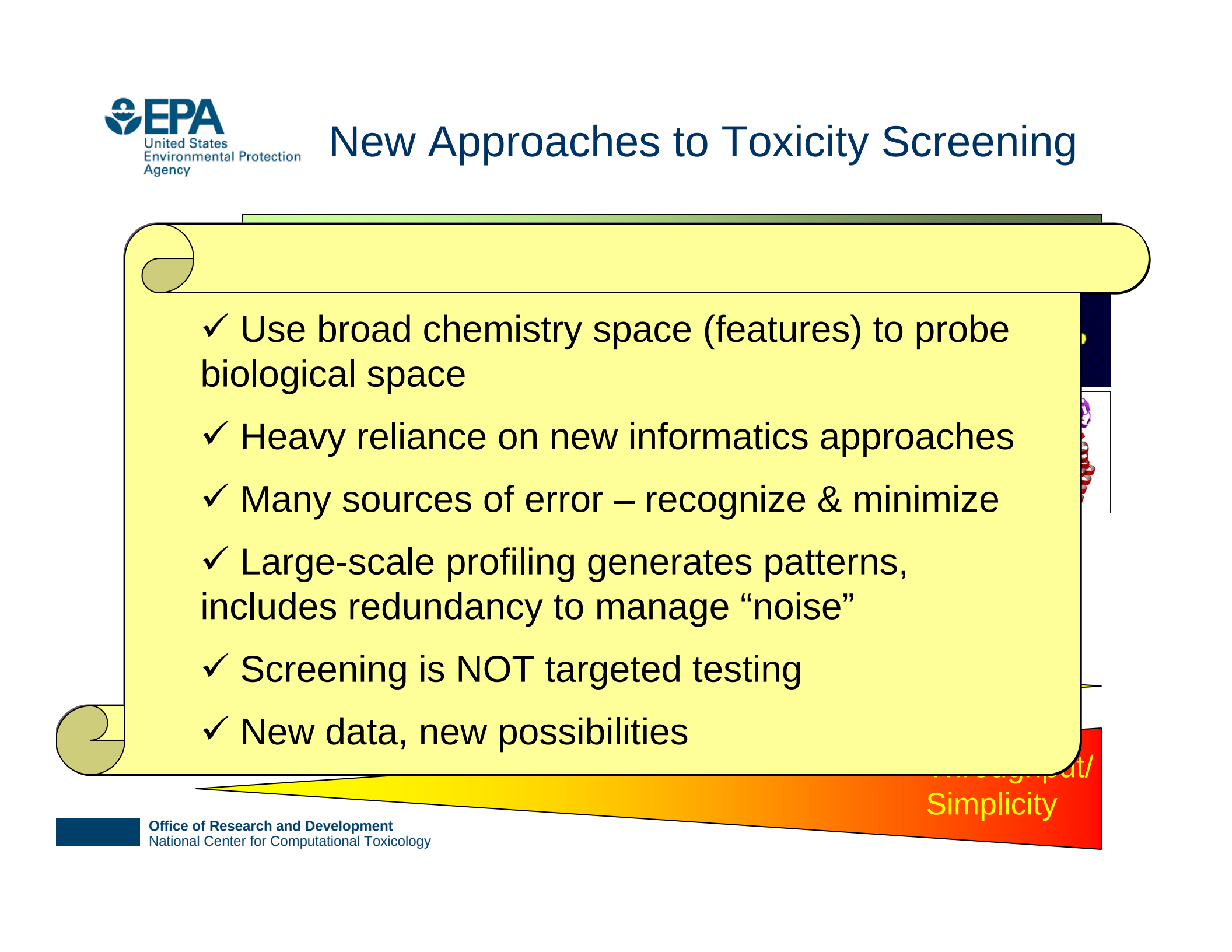
What regions of chemical space are best predicted? Most poorly predicted?

Questions for Genetox Screening

- What endpoint are of most interest?
  - Genetox or cancer?
  - In rodents or humans?
- Is SAL mutagenicity the best target for modeling?
- Are SAR models for SAL mutagenicity good enough?
  - In some areas of chemical (MOA) space, perhaps yes
  - In other areas of chemical space, may need to augment with HTS results
- Can we make better use of SAR & HTS tailored to regions of chemical feature/mechanism space?

New Approaches to Toxicity Screening

- ✓ Use broad chemistry space (features) to probe biological space
- ✓ Heavy reliance on new informatics approaches
- ✓ Many sources of error – recognize & minimize
- ✓ Large-scale profiling generates patterns, includes redundancy to manage “noise”
- ✓ Screening is NOT targeted testing
- ✓ New data, new possibilities



Acknowledgements:

- **EPA NCCT ToxCast Team:**
  Robert Kavlock (Director, NCCT)
  David Dix (ToxRefDB, HTS, Genomics)
  Keith Houck (HTS)
  Matt Martin (ToxRefDB)
  Richard Judson (ACToR, ToxMiner)
  Thomas Knudsen (ToxRefDB, v-Embryo)
  David Reif (ToxMiner)
  Stephen Little (Genetox)
- **EPA NCCT DSSTox Team:**
  Maritja Wolf, Tom Transue – Lockheed Martin, Contractors to the US EPA
- **External Collaborators:**
  Chihae Yang, FDA/CFSAN
  Alex Tropsha, UNC-Chapel Hill
  Andrew Knight & colleagues, Gentronix Ltd.
  Chris Austin & colleagues, NCGC/NIH
  Ray Tice & colleagues, NTP/NIEHS

This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.