GLBTS Teleconference Summary
June 23, 2009

Attendees: No roll call was taken due to the large number of expected participants on the call.

Welcoming Remarks, Introductions

Ted Smith, United States Environmental Protection Agency (US EPA) Great Lakes National Program Office, welcomed everyone to the Great Lakes Binational Toxics Strategy (GLBTS) teleconference and reviewed the agenda. Alan Waffle, Environment Canada (EC), also welcomed participants and reported that the Parties (US EPA and EC) are working on an internal framework for the Great Lakes that relates to the GLBTS as well as to the Great Lakes Water Quality Agreement (GLWQA) and Canada-Ontario Agreement (COA). Alan also reported that the Parties are working toward the September GLBTS meetings and intend to present new information and next steps for the GLBTS at those meetings.

Great Lakes Water Quality Agreement Revision – Status Update

Linda Klaamas, EC, presented details of a June 13, 2009, announcement of plans for the U.S. and Canada to enter into talks to renegotiate the GLWQA. The U.S. Secretary of State, Hillary Clinton, announced plans for the two countries to modernize the agreement to address emerging chemical threats to the Great Lakes and to address climate change and invasive species. The U.S. and Canada are currently establishing a negotiation process between US EPA and Canada. An initial meeting is expected to be held in July, although no date has been set. This initial meeting would determine the negotiation process and outline substantive areas of agreement and disagreement between the two countries. Barring unanticipated interruptions, the process is expected to take a year and conclude with a revised GLWQA.

Ted Smith explained that emerging chemicals of concern will be one issue included in a revised GLWQA and the GLBTS will be used as part of the negotiation process.

Comments/Questions

1. There has already been extensive input into revising the GLWQA. How do you plan to incorporate stakeholder input into the GLWQA negotiation process? (Response) Canada has a proposal to include stakeholder input in the process, but the two countries need to agree on a joint process for including stakeholder input. We recognize the valuable input that GLBTS stakeholders have provided to date, and stakeholders will be engaged accordingly once the formal public consultation mechanism for the GLWQA renegotiation process is established. Public meetings are likely to be held as well.
2. Will stakeholders have an opportunity to discuss the negotiation process with the governments before the process is decided? (Response) It is possible but uncertain whether stakeholders will be able to comment on the negotiation process. This can be

clarified after the initial meeting is held in July. However, the Canadian minister is keen on including stakeholder input.

3. When will the initial meeting happen? (Response) It is hoped that the meeting can be held in July, within a month of the June 13th announcement, or at least sometime over the summer. However, many U.S. political appointees have yet to be put in place, which may delay the process.

UNEP POPs Fourth Meeting of the Conference of the Parties (COP4) Outcomes (meeting held in Geneva, May 2009)

Karrisa Kovner, US EPA, Office of Pollution Prevention and Toxics, presented an update, from a U.S. perspective, of a United Nations Environmental Program (UNEP) Persistent Organic Pollutants (POPs) fourth meeting of the Conference of the Parties (COP4) to the Stockholm Convention. She explained the scope of issues involved and what was discussed during the sessions. On May 9, 2009, the Stockholm Convention was doubled in scope following the addition of 9 new substances to the original 12 "dirty dozen" POPs. The nine substances added to the Convention have been through the POP review committee (POPRC) process, which considers chemical identity, long-range transport, persistence, bioaccumulation, and adverse effects. Annex A of the Convention lists chemicals identified for elimination. Annex B identifies chemicals to be restricted, with a goal of elimination. Annex C lists pollutants formed and released unintentionally from anthropogenic sources. New pollutants listed under Annex A included: alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH, or lindane), chlordecone, hexabromobiphenyl, hexa- and hepta-bromodiphenyl ether, and tetra- and pentabromodiphenyl ether. PFOS was added under Annex B, and pentachlorobenzene was added under both Annex A and Annex C.

The U.S. is not yet a party to the agreement (the U.S. has not ratified the Convention). Karrisa is promoting ratification on Capitol Hill and expects the Obama Administration to ratify it.

Nav Khera, EC Chemicals Management Division, presented a Canadian perspective of the UNEP POPs Conference and explained other key issues that were discussed at COP4. The Convention's allowance for continued use of DDT for disease vector control is being evaluated in light of a proposal to eliminate this use. The World Health Organization (WHO) purports that DDT is needed for emergencies and should remain available for use. Current exemptions for chemicals listed in Annexes A and B will not be allowed after May 17, 2009, except those for PCBs. There were no new exemptions requested.

The COP4 meeting included a discussion on PCBs. A PCB elimination network is being formed, and representatives are being invited to join.

The COP4 meeting also included a discussion of revisions to a Best Available Technology (BAT)/Best Environmental Practices (BEP) document on measures to reduce or eliminate releases from unintentional production. There was support for a proposal to issue a revised BAT/BEP document at the next Conference of Parties meeting in 2011.

COP4 included a discussion of the ongoing review and updating of a Toolkit for Identification and Quantification of Dioxin and Furan Releases. The toolkit, which most Parties are using, is currently open for comments and may be revised, based on comments received.

The issue of POPs in waste was discussed at the COP4 meeting. Some developing countries are concerned about low-level POPs in waste, and guidelines for waste are being developed. The issue was reverted back to the Basel Convention for discussion.

The Parties to the Stockholm Convention have attempted to evaluate the effectiveness of the Convention. A first evaluation was adopted by the Conference of Parties as a baseline to assess the effectiveness of the Convention as a whole. The evaluation did not receive good representation; therefore, an ad-hoc group was formed to improve reporting for the next evaluation, which is scheduled to occur every 6 years.

The issue of noncompliance was raised at the COP4 meeting. There is no mechanism for enforcing compliance under the Convention. No consensus on the issue was reached, and it was deferred to the next Conference of Parties meeting in 2011.

Nav then presented an update on the Long-Range Transboundary Air Pollution Convention (LRTAP). Similarities between LRTAP and the Stockholm Convention were discussed. LRTAP is more regionally based and is a forum that provides history and inspiration to the Stockholm Convention. In December 2007, the Executive Body of the LRATAP Conference of Parties decided to negotiate amendments to the POPs Protocol. Seven substances were adopted as POPs in 2006: PFOS, pentabromodiphenyl ether, octbromodiphenyl ether, short-chain chlorinated paraffins (SCCP), polychlorinated naphthalenes (PCNs), pentachlorobenzene (PeCB) and hexachlorobutadiene (HCBD). The protocol has had three negotiation sessions to date, and consensus has been reached on most issues, with the exceptions of PFOS, Penta-BDE, Octa-BDE, SCCP and revisions to the emission limit value (ELV) and best available techniques (BAT) annexes. In December 2008, five new substances were proposed for addition to the protocol. The LRTAP Task Force has conducted a technical review of the proposed substances and will be presenting the recommendations at the 45th Session of the Working Group in September. Final endorsement will occur in December 2009 at a meeting of the Executive Body.

Details of COP4 can be found at http://www.iisd.ca/chemical/pops/cop4/ and http://chm.pops.int/.

More information on the LRTAP Task Force is available at http://www.unece.org/env/lrtap/welcome.html.

Questions/Comments

1. Will Congress ratify the Stockholm Convention? (Response) US EPA has the lead and is looking at POPs and LRTAP as part of a package. However, US EPA has struggled to assemble a package in the last 8 years that the Senate would pass. There is significant interest in the U.S. (on Capitol Hill and in the White House) to sign onto the Convention.

2. Would FIFRA and TSCA need to be modified to conform to the Stockholm Convention? (Response) Yes, separate committees for each (FIFRA and TSCA) would be formed to revise FIFRA and TSCA as needed. We believe that FIFRA must be revised to include the ability to ban exports.

3. Do you believe that TSCA legislation will move forward before ratification of the Stockholm Convention? (Response) TSCA reform is a huge undertaking. POPs is more manageable within a reasonable timeframe. Pieces of a revised TSCA could easily be used in new legislation for the Stockholm treaty. Experts are currently following both issues.

US EPA Strategic Plan for Evaluating Toxicity of Chemicals

Keith Houck, Ph.D., US EPA, National Center for Computational Toxicology, presented an overview and update on the US EPA's ToxCast Chemical Prioritization Project. The reason for the ToxCast program is that there is a plethora or chemicals in existence, with little known about their toxicity and relevancy to human health protection. ToxCast is a research effort planned to address chemical screening and prioritization needs for pesticidal inerts, anti-microbials, drinking water contaminants, and other chemicals. ToxCast will aim to derive "signatures" from *in vitro* and *in silico* assays to predict *in vivo* endpoints, with the ultimate goal of predicting human toxicity. Phase I of the project examined 320 chemicals of various structural classes. US EPA is organizing the data collected and making it available via PubChem. US EPA is evaluating the data to determine whether there are significant associations with rodent liver tumors. US EPA is also analyzing pathways of disease progression. A ToxCast Data Analysis Summit was held in May 2009 to discuss the results, conclusions, and lessons learned from Phase I of ToxCast. US EPA has begun Phase II, and a third phase is planned in order to analyze thousands of potentially toxic chemicals.

Slides from the presentations are included in Appendix A.

Comments/Questions

1. There is a wide diversity of chemicals present in the environment. Is there currently any discussion of possibly monitoring chemicals by chemical group? For example, one could look at estrogenic chemicals in wastewater effluent and test mixtures for estrogenicity. (Response) There has been some pilot screening work with mixtures to see if the data are useful. There is definitely potential for screening wastewater effluents or other mixtures.

2. How is the relationship between dose and response considered? (Response) All ToxCast results are generated in concentration/response format. We are not trying to relate to exposure at this point, only to correlate to *in vitro* toxicity information (generate signatures), with an emphasis on prioritization.

3. How do you isolate substances for more intensive testing? (Response) We accomplish this through prioritization.

4. If ToxCast will be useful for human health risk assessment, we need to verify animal gene expression in humans. How will you quantitatively to do that? (Response) We compare data, looking for similarities. The next phase of ToxCast will include human clinical testing results that might be useful for drawing analogies between humans and animals.

5. Is the information on slide 26 related to well-characterized pesticides? Are you suggesting an approximately 50% likelihood of predicting lesions in well-evaluated materials? (Response) Yes, with well-evaluated materials, we predict the likelihood of lesions as approximately 50%.

IJC Contaminants of Emerging Concern Nearshore Workgroup Recommendations

Alan Waffle, EC, presented a brief overview of the International Joint Commission (IJC) Work Group addressing chemicals of emerging concern. The IJC has formed a multi-board work group to address emerging chemicals, and a list is being generated to identify such chemicals. The work group has been charged with assessing current scientific and policy information to identify gaps and new approaches that could be applied to existing policy frameworks. The work group is co-chaired by Ted Smith of US EPA and Gary Klecka of Dow Chemical. The work group has prepared a report of its findings which will be released for comment over the summer and then be revised and presented to the IJC. The work group also created a database of reported concentrations and compared them against currently available regulatory standards, guidelines, or criteria. Maps were also created to display results. Analysis of human exposure was beyond the scope of the work group's charge.

The work group focused on the following list of emerging chemicals: synthetic musks, fluorinated surfactants, brominated diphenyl ethers, other flame retardants, alkylphenol ethoxylates, chlorinated paraffins, pharmaceuticals and personal care products, and current use pesticides.

Alan presented the results of the work group's literature search and policy analysis. Wastewater treatment plants were identified as an important source of contaminants to surface waters. The work group also identified several recommendations, including a recommendation for the U.S. to ratify the LRTAP and Stockholm Conventions.

Slides from the presentations are included in Appendix A.

Comments/Questions

1. Wastewater treatment plants are not sources of contaminants but pathways. Technologies can be used to control sources of contaminants to wastewater treatment plants. Combined sewer overflows (CSOs) to the Great Lakes will obviate treatment technologies. Sources of contaminants to treatment plants are important to address. (Response) Two-thirds of the budget proposed for the Great Lakes Regional Collaboration (GLRC) was aimed at water infrastructure.

2. GLRC funding will be used to address CSOs and microbial concerns rather than contaminants of emerging concern. A policy report for this effort proposed going upstream to sources, which is a much more cost-effective option than allowing contaminants to enter the treatment system. (Response) The IJC work group decided that both control and upstream approaches were needed.

3. Biological impacts approaches are sufficient; however, ultimately, future policy making will require a chemical approach (or suite of chemicals) for industry to take action. (Response) The work group recommended supplementing the chemical approach. After the biological impacts approach, we would drill down to specific chemicals. The work group's final report contains more details on this subject.

Great Lakes Restoration Initiative – Status Update on Toxics

Ted Smith discussed progress to date on the Great Lakes Restoration Initiative. Ted presented background information on the initiative's development, founding, and funding, including the initiative's five focus areas: 1) toxic substances and areas of concern, 2) invasive species, 3) nearshore health and nonpoint source pollution, 4) habitat and wildlife protection and restoration, and 5) accountability, monitoring, evaluation, communication, and partnerships. The initiative identified $475 million to implement the recommendations of the GLRC. Of the $475 million, $147 million is targeted for toxics and areas of concern; this is a significant investment compared to past funding amounts. Other projects were outlined for reducing the use and release of toxic substances, as well as measuring progress and assessing new toxic threats. US EPA is developing a 5-year strategy for 2010 to 2014, and public meetings will be held beginning in July to discuss the 5-year plan. The plan is due to US EPA's Office of Management and Budget by September 1, 2009.

Slides of the presentation are included in Appendix A.

Comments/Questions

1. Will all agencies agree to the 5-year plan? (Response) Yes, it is an interagency plan, not solely US EPA's.

Next Steps for GLBTS

A Substance/Sector Workgroup meeting and Integration Workgroup meeting are scheduled for September 22-23, 2009, in Chicago.

Comments/Questions

1. The information presented during this call provides a good background. For what purpose has this information been presented, and where are we headed? (Response) With

the amount of information provided with respect to new substances, it is difficult to determine next steps for the GLBTS and priorities within EC and US EPA. Administrative changes in the U.S. are delaying policy decisions. For example, US EPA's Chemical Assessment and Management Program (ChAMP) has been put on hold as plans for a new and revised program are developed. A proposal for the future direction of the GLBTS will be presented at the September meetings. The U.S. focus for the GLBTS is developing priorities for the Great Lakes Basin. Canada is also in the process of establishing priorities for the basin.

2. How can stakeholders help, or are we observers? (Response) We hope that stakeholders are involved. We anticipate the GLBTS stakeholders playing an active role in addressing priorities in the basin, and engaging in the GLWQA renewal process.

3. The GLBTS could start with the nine chemicals recently added to the Stockholm Convention as a starting point for stakeholders to begin analyzing new substances of concern, similar to the work done under the GLBTS in analyzing the Level 1 substances using the four-step process (e.g., assessing sources, emissions, and reduction options). The nine substances appear to be a relatively well-accepted group of chemicals, particularly if the U.S. is expected to ratify the Stockholm Convention.

4. In terms of invasive species, what actions are in mind for prevention in the short term? (Response) There are many efforts being undertaken with respect to ballast water, a pathway for invasive species introduction. US EPA has issued a general permit, and states are required to certify ballast tanks through the Section 401 program. A potential new Coast Guard ballast water rule is pending. Some legal challenges exist as well.

Closing Remarks

At the previous GLBTS Substance/Sector Workgroup meeting, US EPA and EC asked for comments on the substance selection process. The governments are drafting a response to the comments. Much is going on with changes occurring in both countries. There is an opportunity for the GLBTS to use the information being generated on substances of concern and effectively utilize resources for maximum benefit to the Great Lakes Basin. The challenge is to clearly identify priorities. The governments hope to obtain feedback from stakeholders in September on the path forward for the GLBTS.

Appendix A:
June 23, 2009, GLBTS Teleconference Presentations

EPA
United States
Environmental Protection
Agency

Overview and Update on EPA's ToxCast Chemical Prioritization Project

NICEATM-ICCVAM 5YPISC and RDWG, June 22, 2009

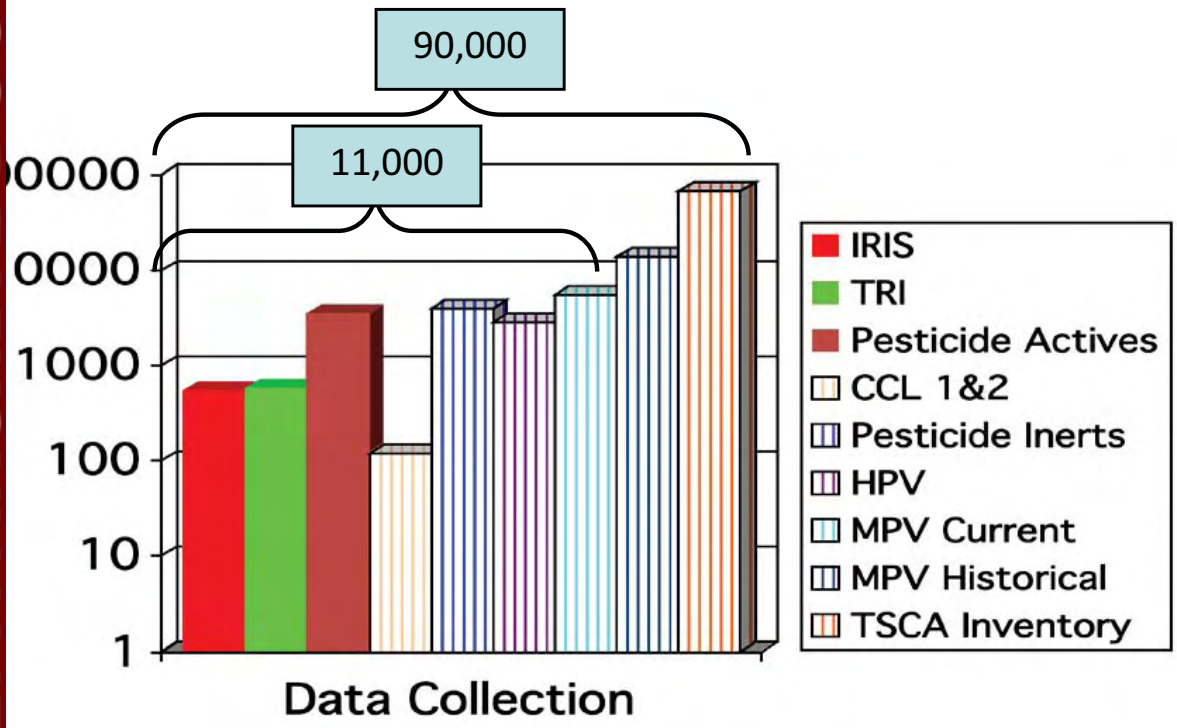
Keith Houck
houck.keith@epa.gov

Office of Research and Development
National Center for Computational Toxicology

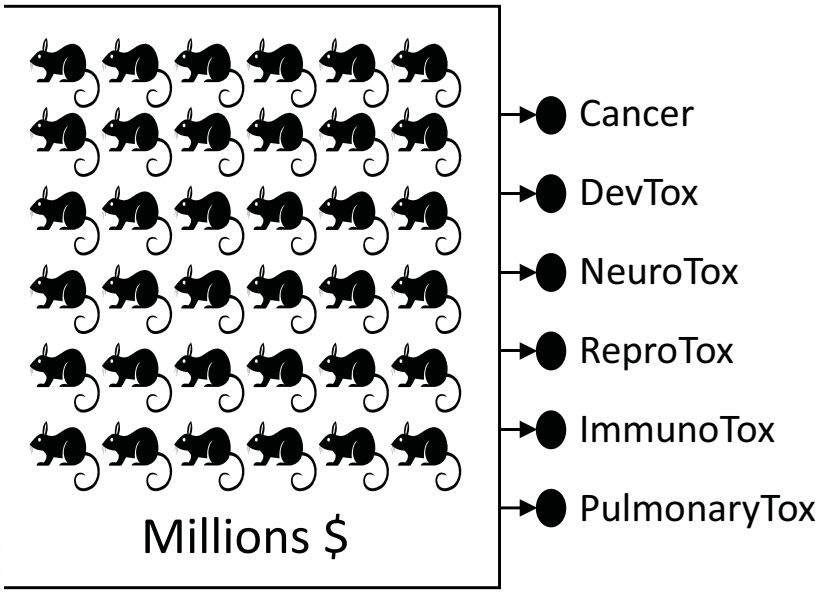
United States Environmental Protection Agency

Change Needed Because …..

Too Many Chemicals



Too High a Cost



…and not enough data.

Judson, et al *EHP, 2008*

US EPA ARCHIVE DOCUMENT

ational Academy of Sciences Report (2007)

oxicity Testing in the Twenty-first Century: A Vision and a Strategy

NAS PANEL SEEKS MAJOR SHIFT IN HOW EPA ASSESSES CHEMICALS' TOXICITY

Date: June 22, 2007 -

Inside EPA

Online access provided by InsideEPA.com

National Academy of Sciences (NAS) panel is calling for a major shift in how EPA assesses hemicals' toxicity, recommending that the agency base its toxicological research and regulatory rocesses on how substances affect biological pathways -- which send information within and etween cells -- rather than so-called health endpoints, such as cancer.

POLICYFORUM

Science: Feb 15, 2008

TOXICOLOGY

Transforming Environmental Health Protection

Francis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

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.

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. throughput screening (HTS) and other automated screening assays into its testing tion, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in

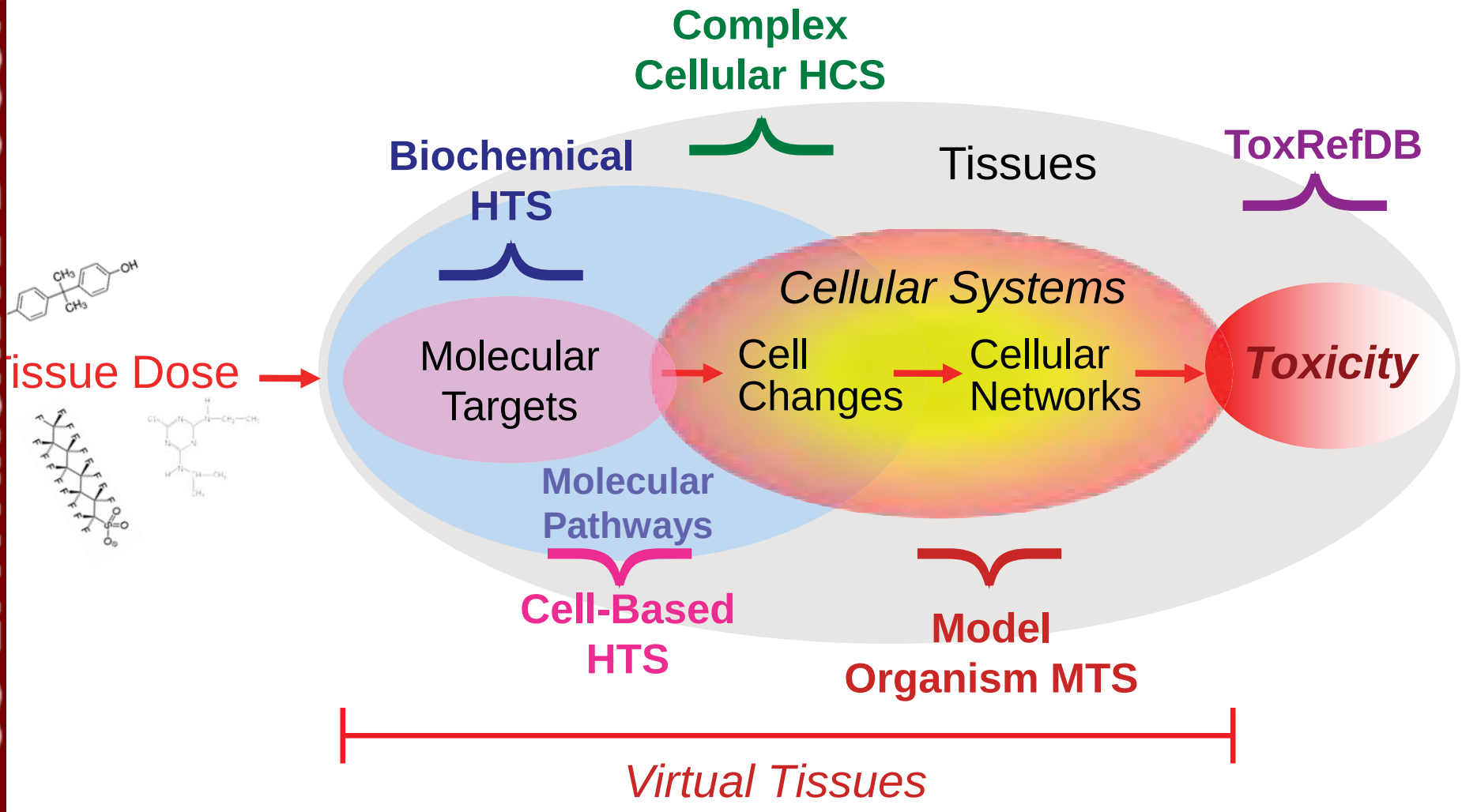
United States Environmental Protection Agency

ToxCast Background

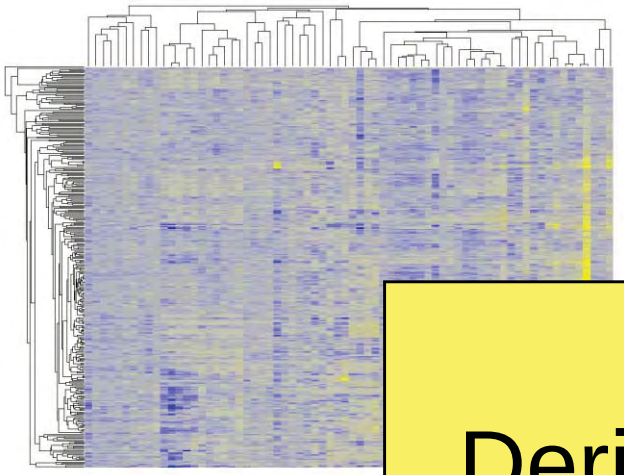
- Research program of EPA's National Center for Computational Toxicology (NCCT)
- Addresses chemical **screening** and **prioritization** needs for pesticidal inerts, anti-microbials, CCLs, HPVs and MPVs
- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures
- Coordinated with NTP and NHGRI/NCGC via Tox21
- Committed to stakeholder involvement and public release of data
  - 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/*

EPA United States Environmental Protection Agency

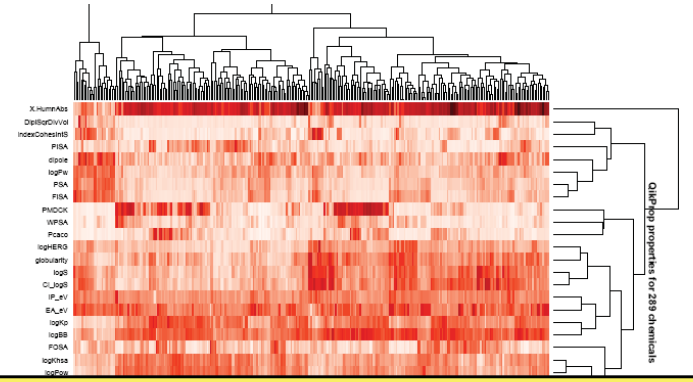
Ultimate Goal of ToxCast: Predicting Human Toxicity

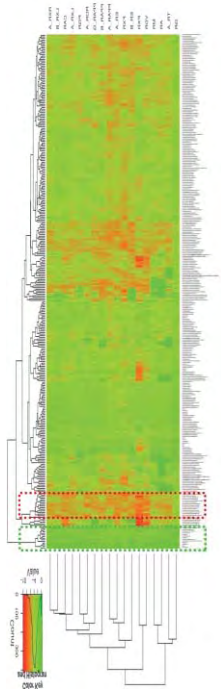


Correlating Domain Outputs



Cellular Assays

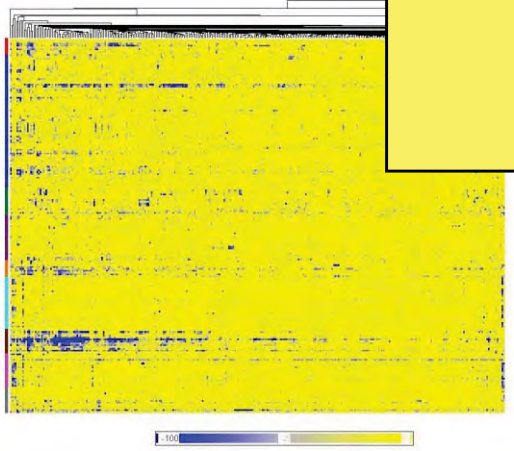




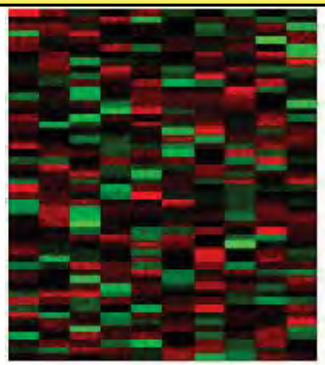
silico Predictions

EPA ToxCast Goal:

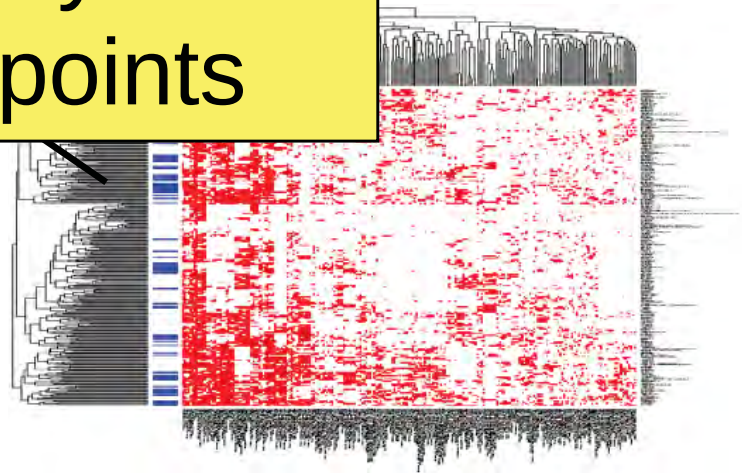
Derive "Signatures" from *in vitro* & *in silico* assays to predict *in vivo* endpoints



Biochemical Assays



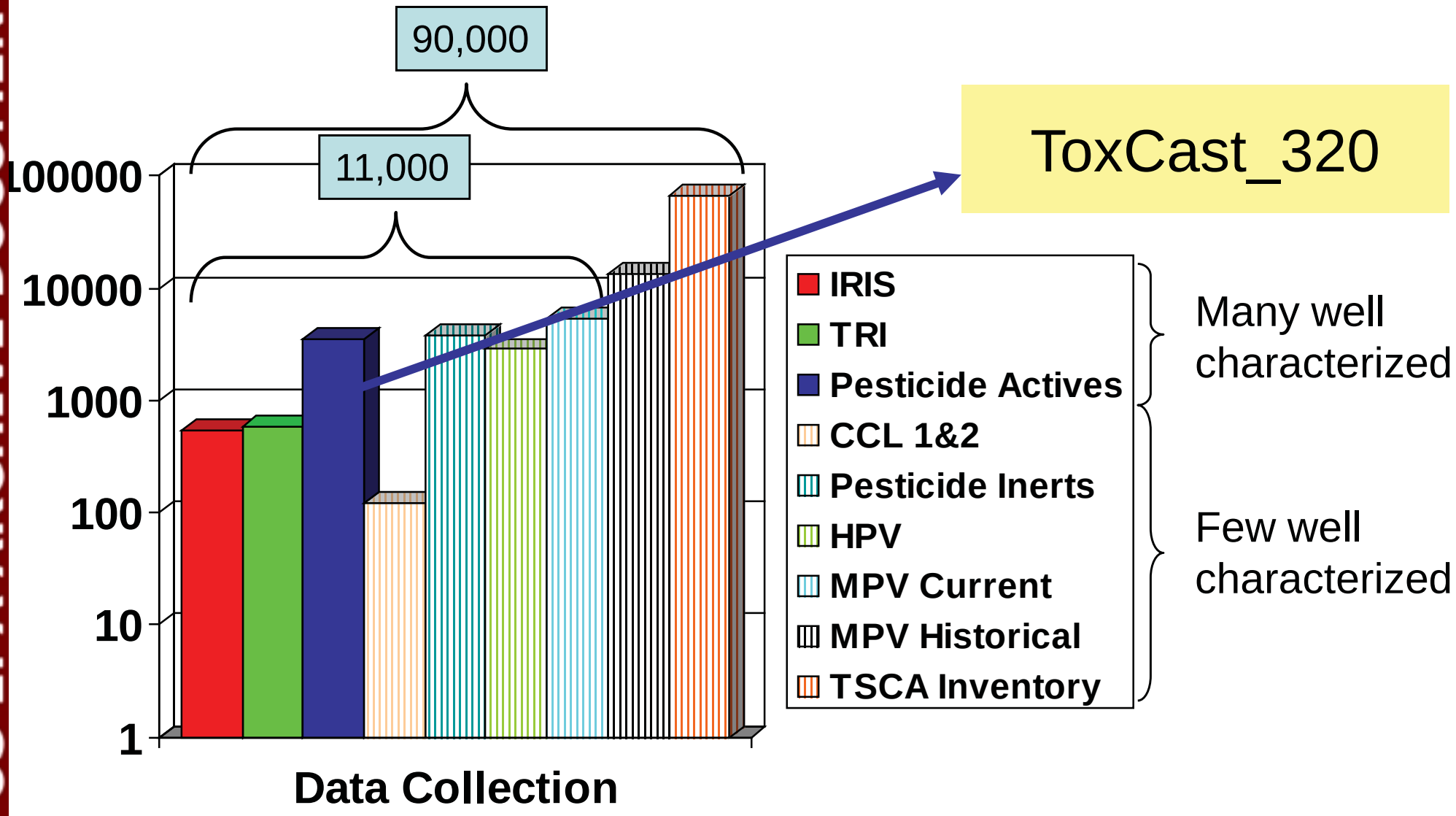
Genomic Signatures



Toxicology Endpoints

United States Environmental Protection Agency

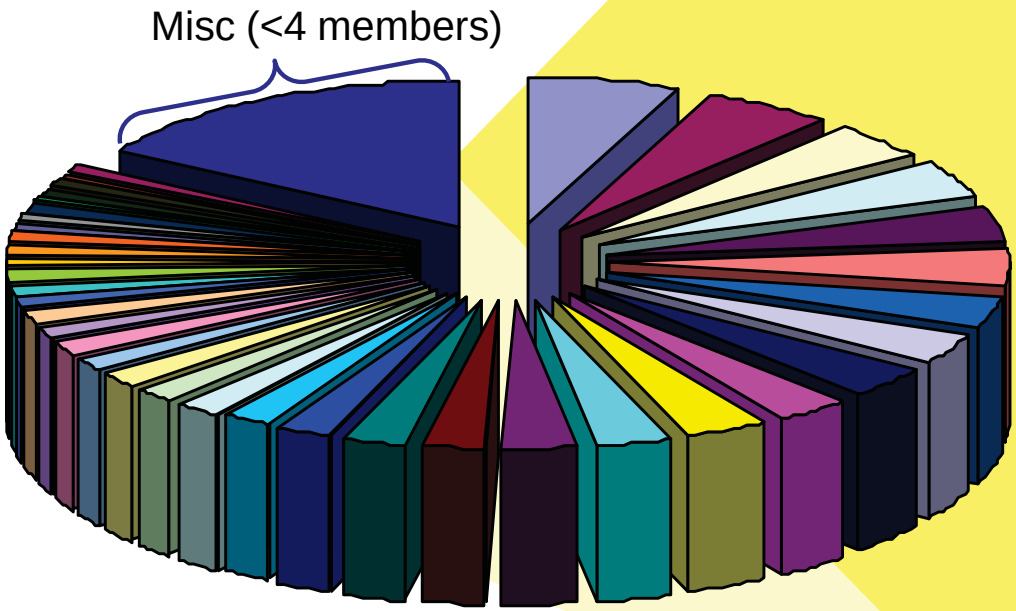
ToxCast Phase I Chemicals



United States Environmental Protection Agency

Chemical Classes in ToxCast_320 (Phase I)

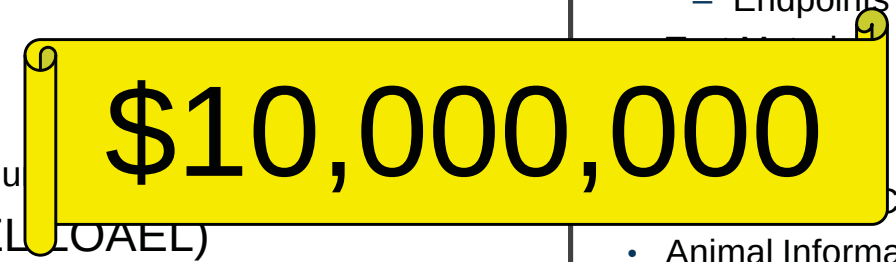
- 309 Unique Structures
- Replicates for QC
- 291 Pesticide Actives
- 9 Industrial Chemicals
- 13 Parent/Metablolite pairs
- 56/73 Proposed Tier 1 Endocrine Disruption Screening Program
- 14 High Production Volume Chemicals
- 11 HPV Challenge



- 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

EPA Pesticide Programs: Data Evaluation Records (DERs)

- Used for hazard identification and characterization
- Study Types
  - Chronic
  - Cancer
  - Subchronic
  - Multigeneration
  - Developmental
  - Others: DNT, Neurotox, Immu
- Derive Endpoints (NOAEL/LOAEL)
  - Systemic
  - Parental
  - Offspring
  - Reproductive
  - Maternal
  - Developmental
- Critical Effects for Endpoints

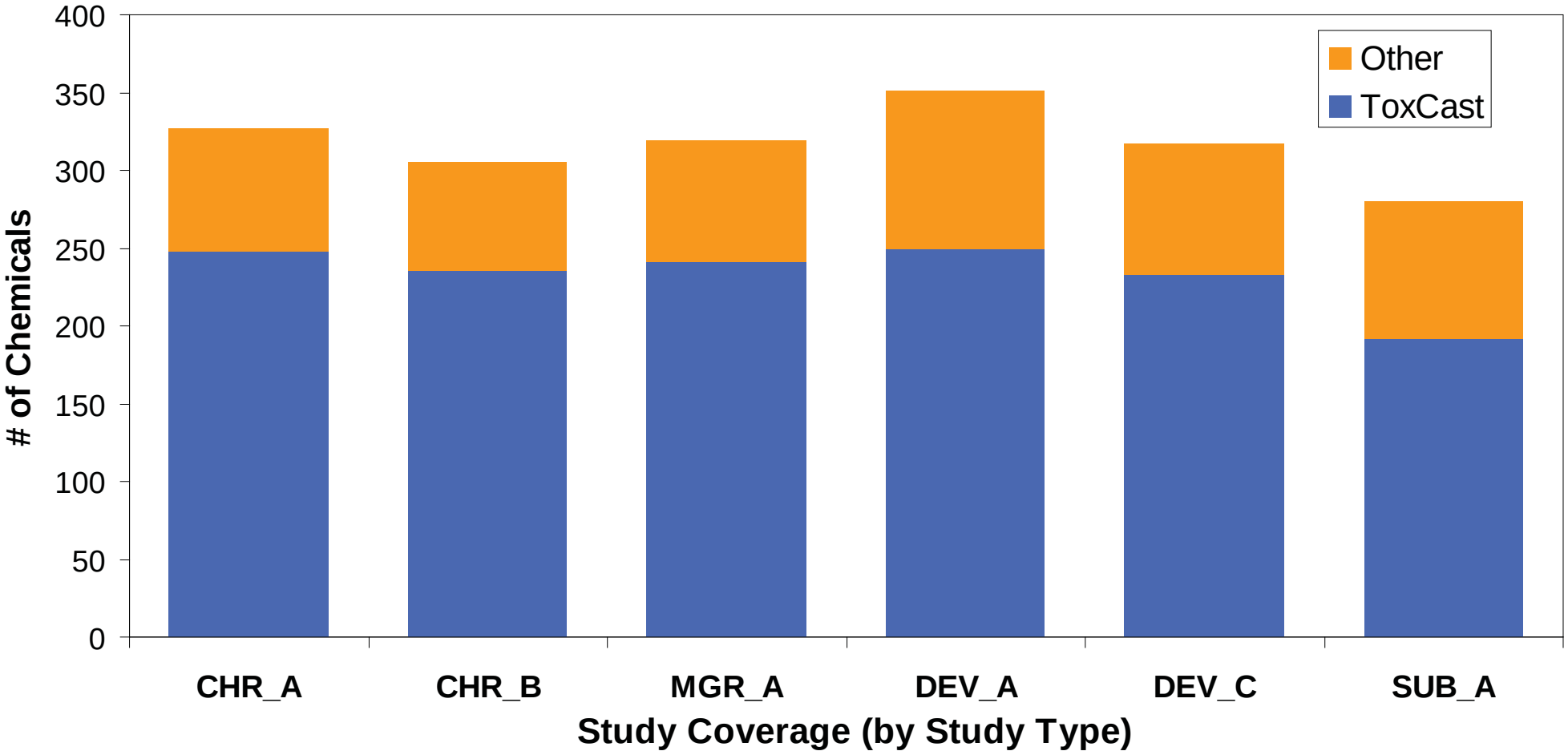


DER Format

- Study Identifiers
  - Tested Chemical Information
    - IDs
    - Name
    - Purity
  - Study Type IDs
  - Reviewer Information
- Citation(s)
- Executive Summary
  - Summary Study Design
  - Summary Effects
  - Endpoints (NOAEL/LOAEL)
- Chemical Properties
- Animal Information
  - Species
  - Strain
  - Husbandry
- Results (full dose-response)
  - Clinical signs
  - Body weight
  - Clinical Chemistry/ Hematology
  - Gross Pathology
  - Non-neoplastic Pathology
  - Neoplastic Pathology
  - Parental vs. Offspring
  - Maternal vs. Fetal

United States Environmental Protection Agency

2073 Studies Entered For 480 Chemicals

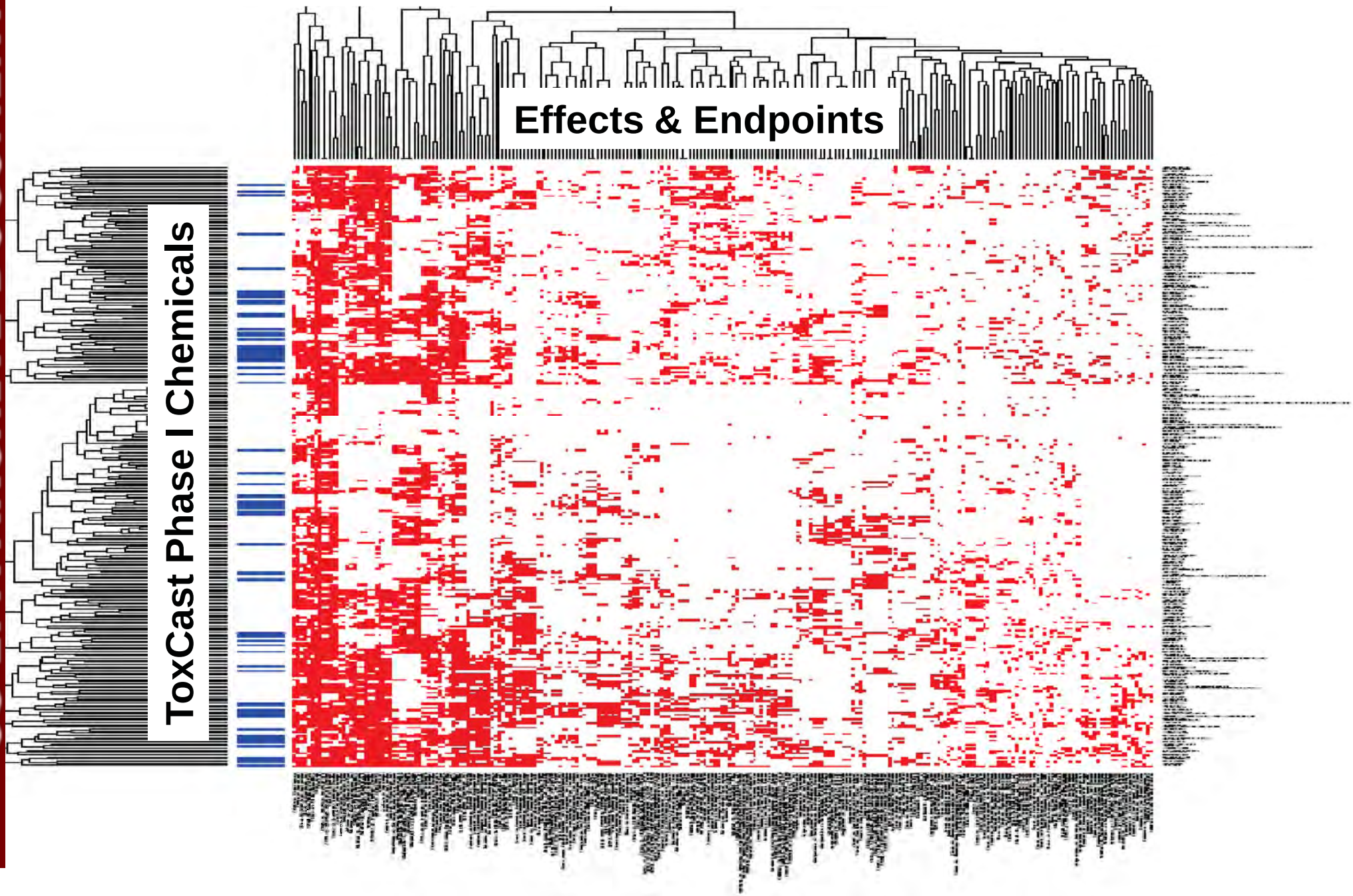


CHR = Chronic/Cancer
MGR = Multigeneration Reproductive
DEV = Prenatal Developmental

A = Rat
B = Mouse
C = Rabbit

United States Environmental Protection Agency

>$1Billion Million Dollars Worth of *In Vivo* Chronic/Cancer Bioassay Effects and Endpoints



EPA United States Environmental Protection Agency

ToxCast Data Sources

Compound Focus, Inc.
a subsidiary of BioFocus DPI
A Galápagos Company

CELLUMEN

attagene
The Home of TFomics™

CellzDirect™
invitrogen corporation

NOVASCREEN
a Caliper Life Sciences company

BioSeek

L'ORÉAL

SOLIDUS

ACEA Biosciences, Inc.

gentronix

NCGC
NIH

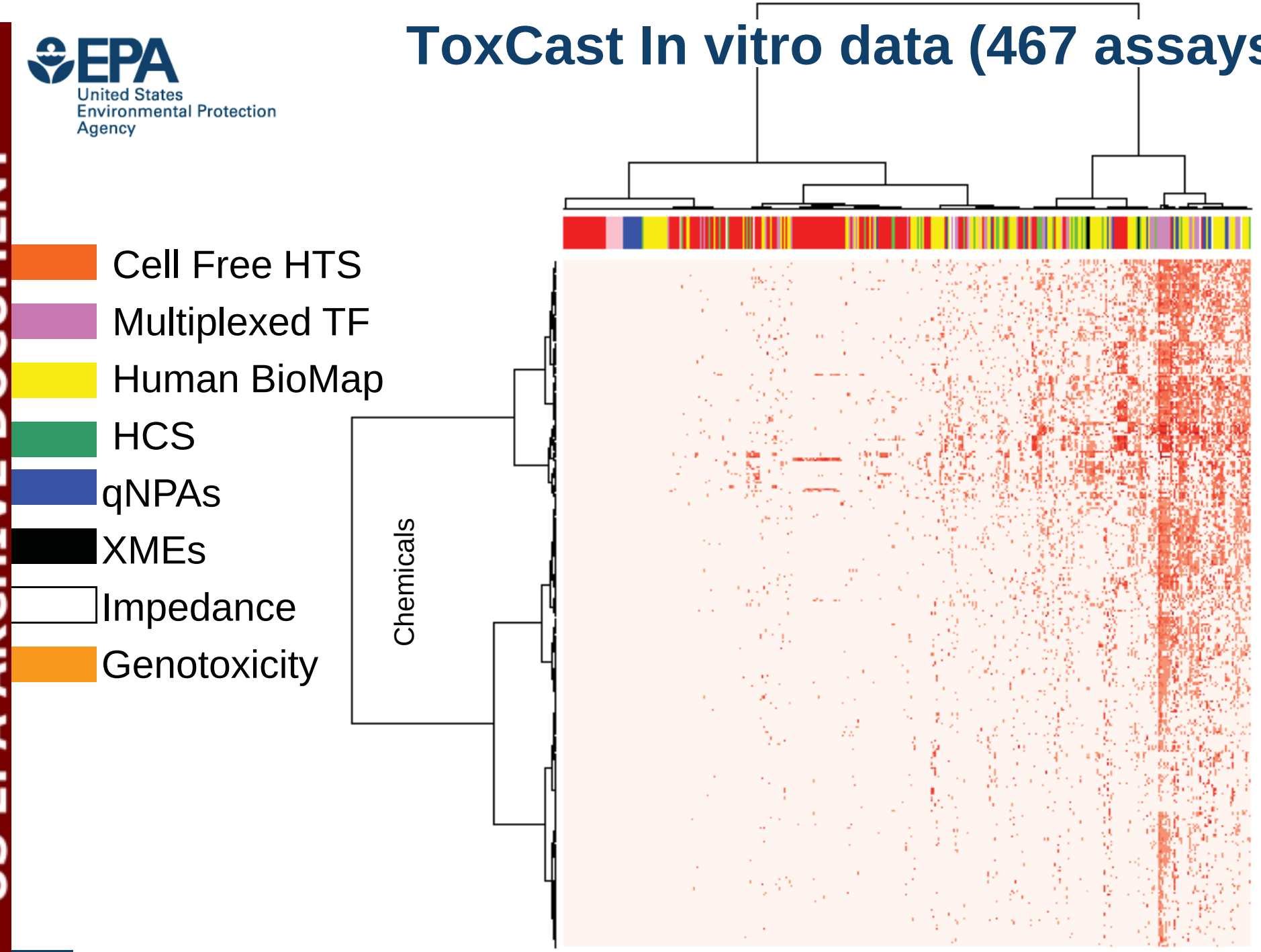
Tox21

National Toxicology Program
U.S. Department of Health and Human Services

6 contracts, 4 collaborations
467 assays, 534 endpoints

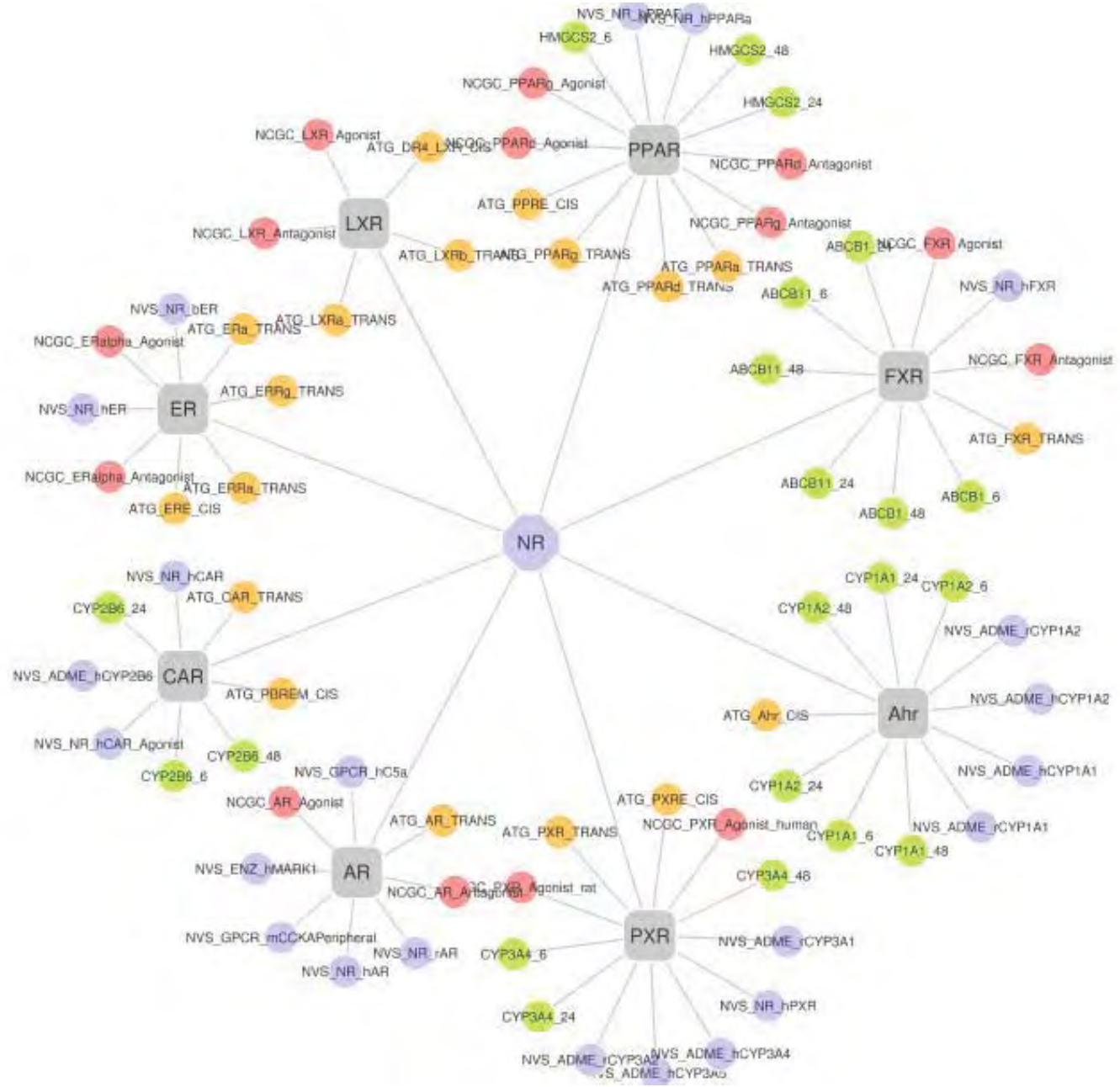
United States Environmental Protection Agency

ToxCast In vitro data (467 assays)

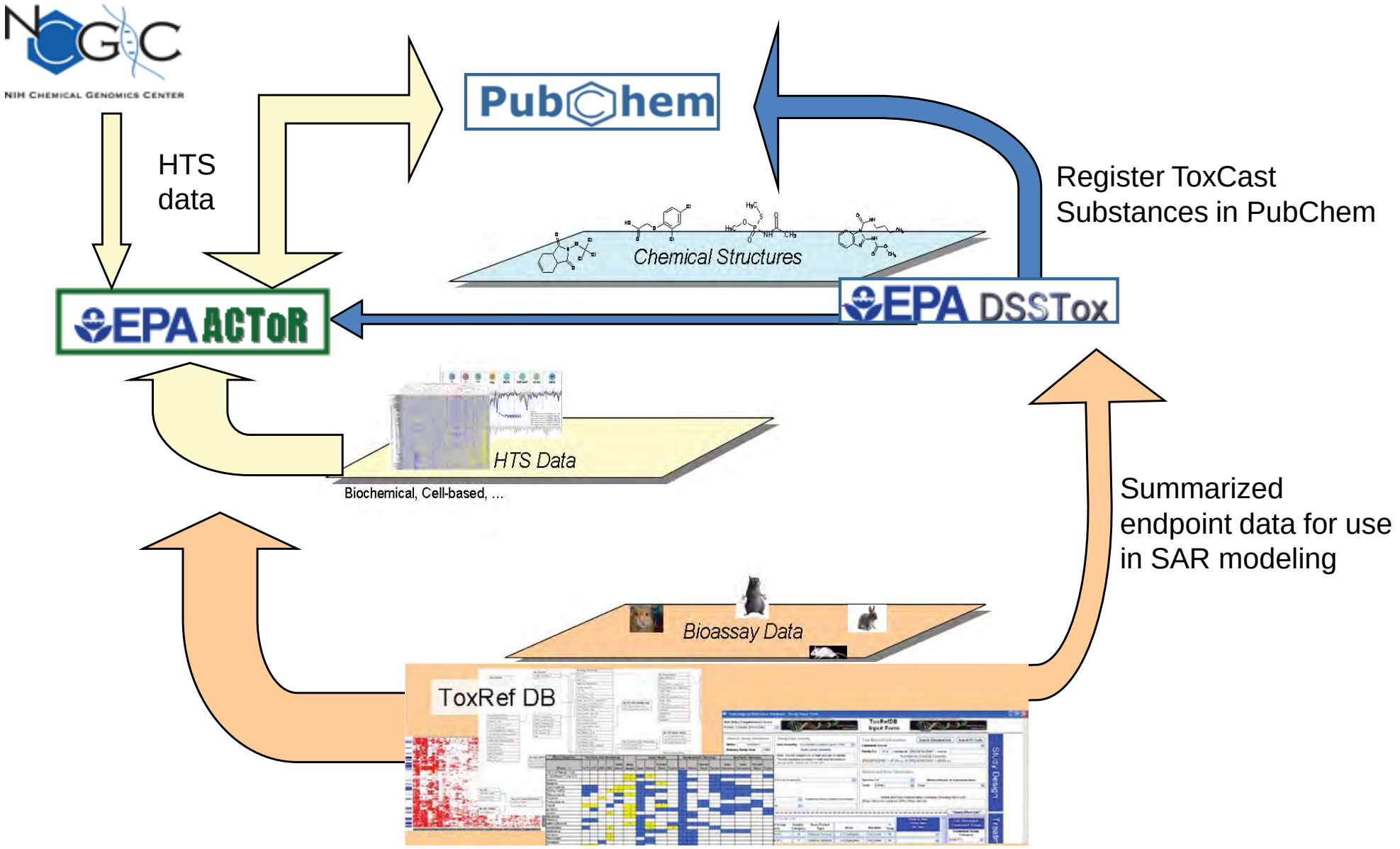


EPA United States Environmental Protection Agency

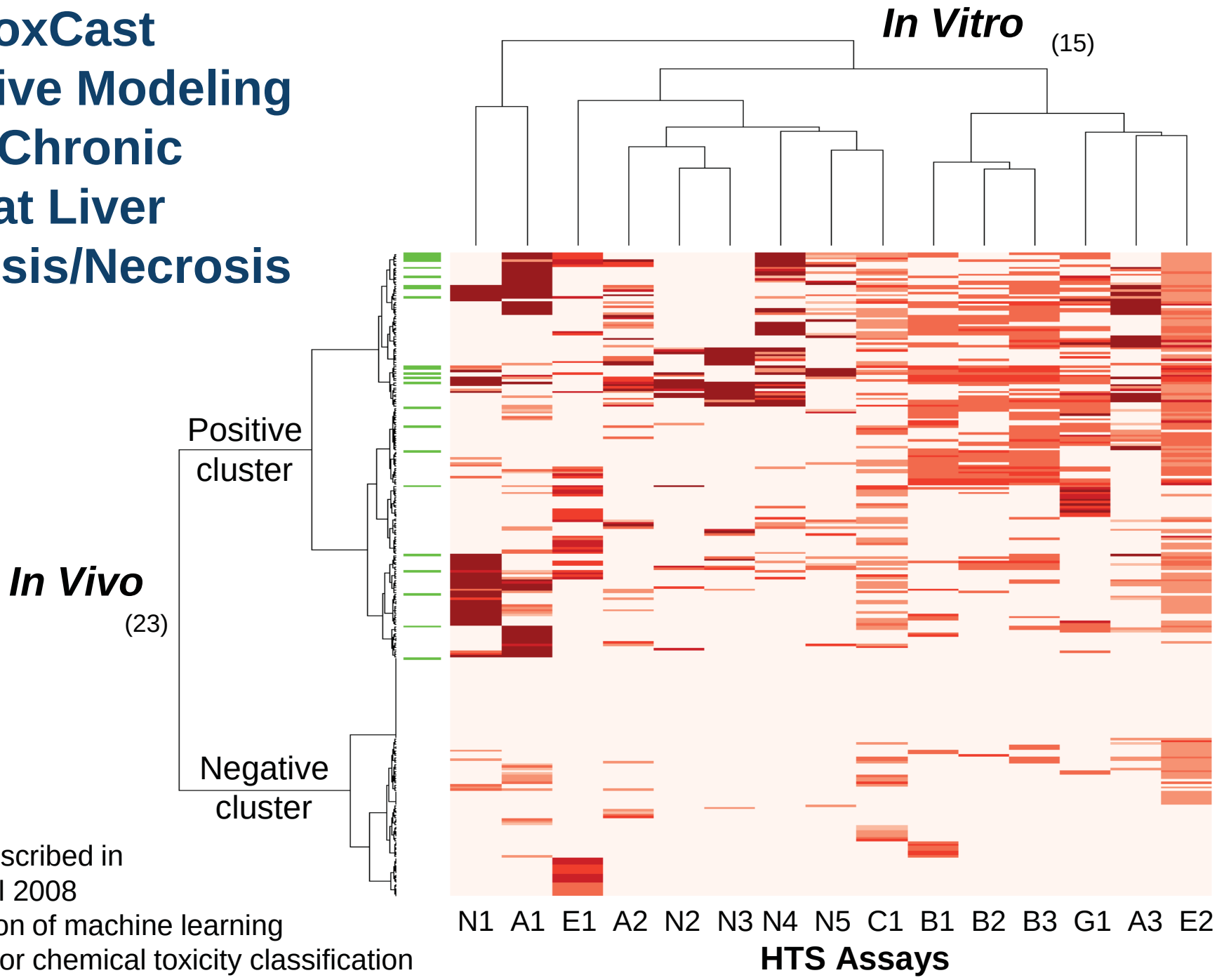
Multiple Assays per Endpoint



ToxCast: Data Publication & Exploration



ToxCast Predictive Modeling of Chronic Rat Liver Apoptosis/Necrosis



Methods described in
Judson et al 2008
A comparison of machine learning algorithms for chemical toxicity classification using a simulated multi-scale data model.
BMC Bioinformatics 9:241

ToxCast In Vitro/In Vivo Correlation Examples

Calculate Univariate Associations with Rat Liver Proliferative Lesions

- Significance Tests:
  - T-test (treat *in vitro* as continuous)
  - Chi-squared (treat *in vitro* as dichotomous, using 100μM as the cutoff)
- Significant associations are:
  - PPARA
  - PPARG
  - HMGCS2 (regulated by PPAR)
  - RXRA (dimerizes with PPAR)
  - CCL2
  - CCL26

PPAR signaling and Rodent Liver Tumors

- PPAR is involved with lipid and fatty acid metabolism
- Xenobiotics can activate PPAR
  - Leads to peroxisome proliferation and hepatocyte hypertrophy

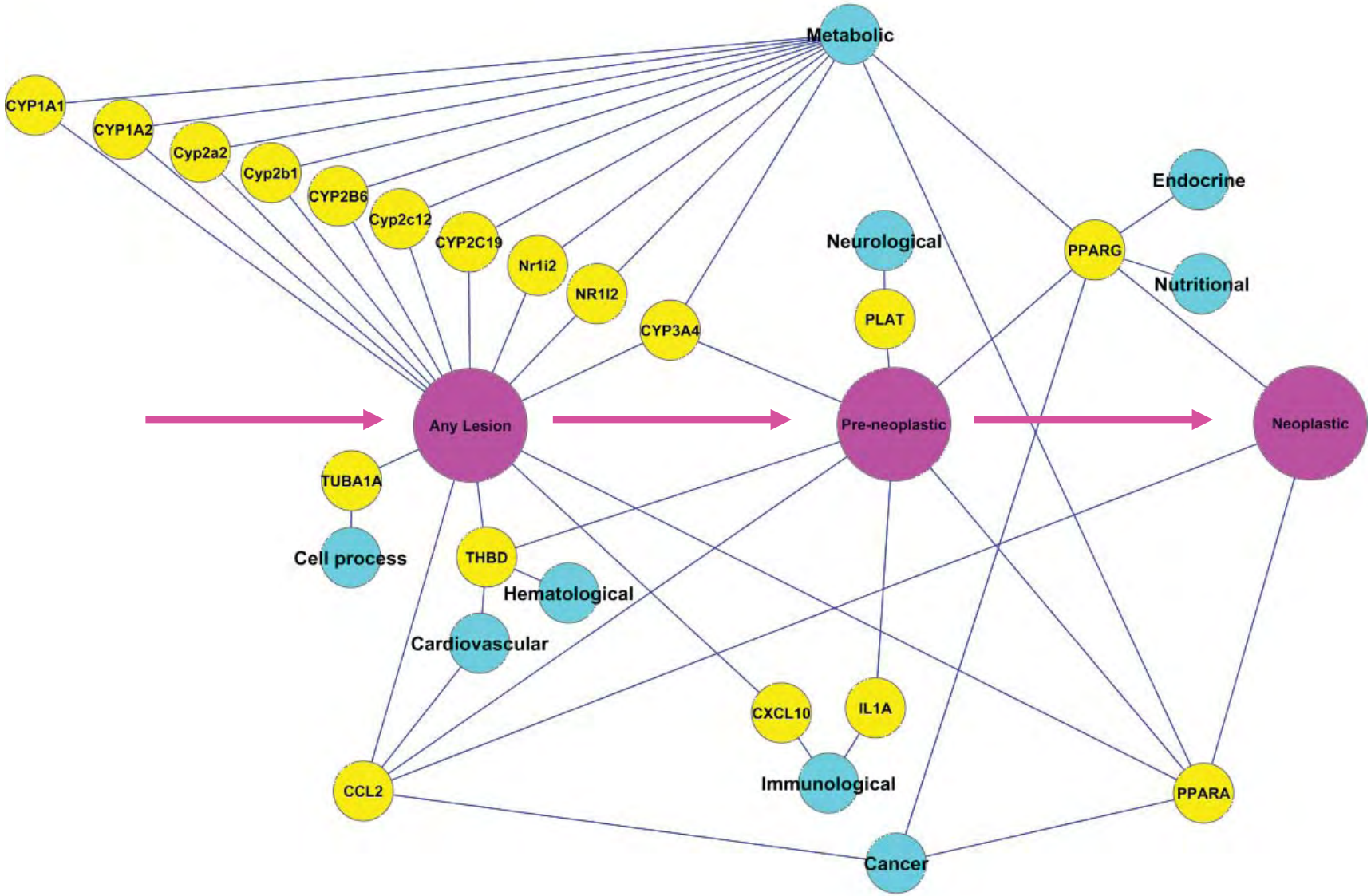
- PPAR-driven liver tumorigenesis does not seem to act in humans
  - But PPAR-driven hepatotoxicity is of concern (FDA)
  - PPAR is a target for human drugs to treat metabolic syndrome / diabetes

- 3 isoforms
  - PPARA / PPARα
  - PPARG / PPARγ
  - PPARD / PPARδ

CCL2 Associations with Environmental Chemicals and Liver Toxicity are Novel

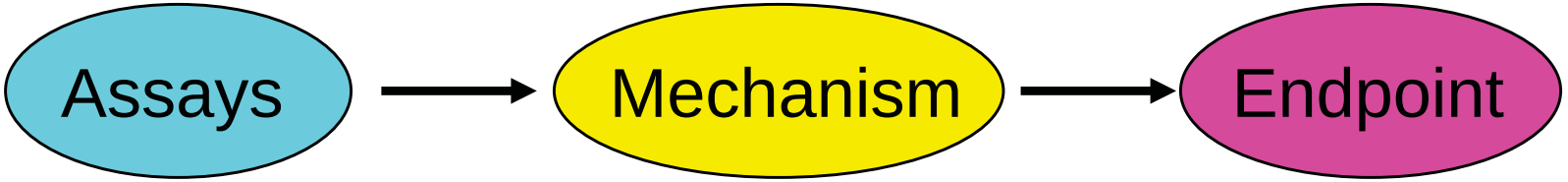
- Chemokine (C-C motif) ligand 2
- Drives angiogenesis and tumor cell invasion
- Seen in both humans and rodents
- Increased CCl2 levels associated with
  - Human Prostate cancer severity and progression
  - Human Gastric carcinomas
  - Human Oral carcinomas
  - Human Breast cancer
  - Human Thyroid cancer
  - Rat cholestatic liver injury
- May be related to PPAR signaling

Rat Liver Disease Progression Links



Links Drawn for Univariate Associations with p<0.01

Toxicity Signature Definition

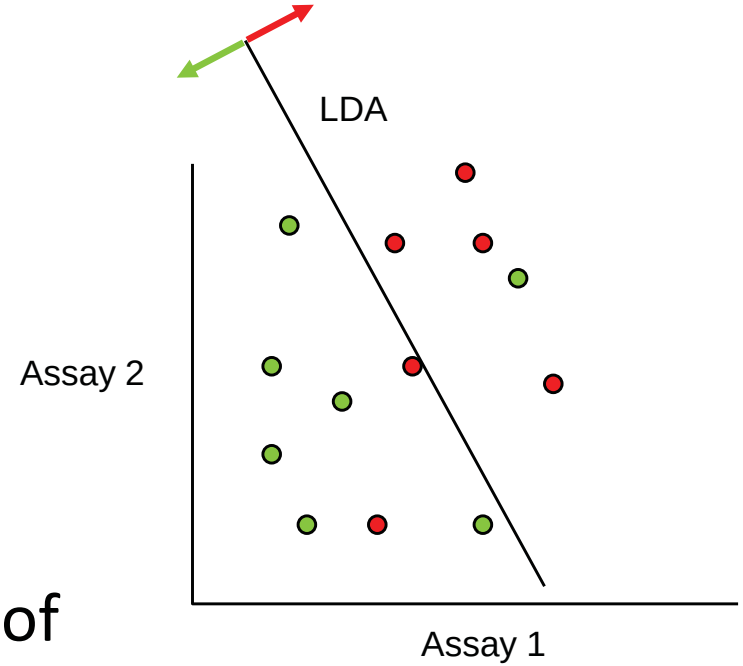


- An algorithm that takes as its input
  - A chemical
  - One or more *in vitro* assay measurement or *in silico* parameters
- And returns
  - A classification for that chemical for a toxicity endpoint

- Other terms
  - Model
  - Classifier

Association Analysis /Signatures

- Use Machine Learning methods
  - SLR: Stepwise Logistic Regression
  - LDA: Linear Discriminant Analysis
  - SVM: Support Vector Machines
  - Many others
- For each binary endpoint, build models of form
  - *Predictor* = *F*(assay values)
  - If
    - *Predictor* for a chemical meets criteria
  - Then
    - Predict endpoint to be positive for the chemical



	+ Truth	-
+ Test	TP	FP
-	FN	TN

Machine Learning Process

- ML Methods used
  - SVM – Support Vector Machines
  - NNET – Neural Networks
  - LDA – Linear Discriminant Analysis
  - SLR – Stepwise Logistic Regression

} Seemed to consistently overfit
Consistent with unbalanced data set

- Use AC50/LEC Data and log transform
- T-test Feature Selection
  - $p<0.1$ for cutoff
  - Accept maximum of n(chemical)/10 feature
- Use 5-fold cross validation
- Evaluate performance using balanced accuracy (BA)
  - BA=average of sensitivity and specificity

SLR Signature:
Rat Liver Proliferative Lesions

Assay	Coefficient	Gene	Gene Name
Intercept	-2.86		
ATG_PPARg_TRANS	0.298	PPARG	peroxisome proliferator-activated receptor gamma
NVS_ADME_hCYP3A4	0.614	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4
CLM_OxidativeStress_24hr	0.403	H2AFX	H2A histone family, member X (oxidative stress)
BSK_SM3C_MCP1_up	0.331	CCL2	chemokine (C-C motif) ligand 2
BSK_BE3C_IL1a_down	0.389	IL1A	interleukin 1, alpha
ATG_RORg_TRANS	0.51	RORC	RAR-related orphan receptor C
BSK_BE3C_tPA_up	0.386	PLAT	plasminogen activator, tissue
CLM_Hepat_Steatosis_24hr	0.181		
ATG_PPARa_TRANS	0.254	PPARA	peroxisome proliferator-activated receptor alpha
CLM_MitoticArrest_24hr	-0.322		
CLM_p53Act_72hr	0.28	TP53	tumor protein p53
ATG_Sp1_CIS	0.195	SP1	Sp1 transcription factor
ATG_NRF2_ARE_CIS	-0.171	NFE2L2	nuclear factor (erythroid-derived 2)-like 2 (oxidative stress)

Start with 624 Assay measurements, 3 p-chem, 103 chemical structure class variables
Genes associated with tumors or liver disease in red

Signature Performance – Proliferative Lesions

In vivo data

Signature	+	-
+	31	11
-	30	176

Sensitivity=51%
Specificity=94%

- 248/309 chemicals had rat data in ToxRefDB (used for model building)
- 8 other chemicals were predicted to be positive
  - PFOA: Causes rat liver adenomas
  - PFOS: Causes rat liver adenomas
  - Diniconazole: rat liver hypertrophy
  - Chlorothalonil: rat liver enlargement, kidney tumors
  - TCMTB: testicular and thyroid adenomas
  - No data for Niclosamide, Methylene bis(thiocyanate), Phenoxyethanol

Examine False Positives

- Look for data outside of ToxRefDB for highest scoring false positives

- Fenpyroximate
  - Liver hypertrophy in a rat 90-day subchronic study
- Bromoxynil
  - Non-proliferative lesions (2 year rat study)
  - Liver adenomas (2 year mouse study)
- Cyproconazole
  - Hepatocellular adenomas and carcinomas in mice
- Tribufos
  - Liver hemangiosarcomas in male mice

United States Environmental Protection Agency

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

- Phase 1 ToxCast data made available to analysis partners prior to full public release
  - >500 HTS assays – categorical (1/0)
  - 76 “bioassay” endpoints from ToxRefDB for modeling
  - Chemical structure SD file (DSSTox), chemical information files (descriptors)
- Over 200 registered attendees, 60 presenters
- Wide variety of prediction schemes
  - *In vitro* → *In vivo*
  - Chemical descriptors → *In vivo* (SAR)
  - Chemical descriptors + *In vitro* → *In vivo*
- Wide variety of approaches
  - Statistics, clustering, machine learning, particle swarm, etc.

United States Environmental Protection Agency

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

Impressions, Conclusions, Lessons…

- ToxCast Phase I data set poses highly challenging problems for prediction methods
- Global associations (*in vitro* to *in vivo*) trends not readily apparent → must go local to see meaningful associations
- Statistical means for dealing with highly dimensional, sparse, unbalanced data needed → new methods proposed
- Use of chemical descriptors and features improve model performance when combined with HTS (Is this accounting for ADME??)
- Public data availability and transparency successful in engaging wide range of researchers and capabilities in early analysis
- PASS, LAZAR, ToxTree indicate limited applicability of prior SAR carcinogenicity prediction models (based on public data) to ToxCast Phase I chemical space → reinforces need to enrich public data space, improve models

United States Environmental Protection Agency

ToxCast Development

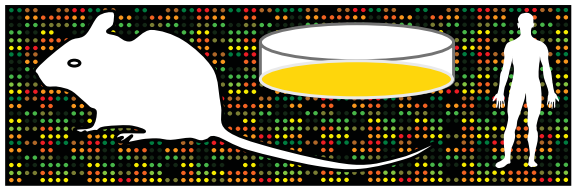
Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
Ia	320	Data Rich (pesticides)	Signature Development	>500	$20k	FY07-08
Ib	15	Nanomaterials	Pilot	166	$10K	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	~$20-25k	FY09
IIb	>100	Known Human Toxicants	Extrapolation	>400	~$20-25k	FY09
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	~$20-25k	FY10
IId	>12	Nanomaterials	PMN	>200	~$15-20K	FY09-10
III	Thousands	Data poor	Prediction and Prioritization	>300	~$15-20k	FY11-12

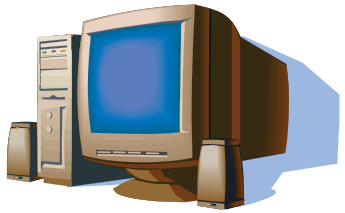
January 2009

EPA United States Environmental Protection Agency

Tox21 Collaboration

NCGC Tox21 EPA





National Health and Environmental Effects Research Laboratory

National Center for Computational Toxicology

- Combined HTS plates (6x1408) high interest chemicals
- Joint assay development
- Use of NCGC HTS testing capabilities
- EPA informatics (ACToR/DSSTox)

National Toxicology Program
Department of Health and Human Services

Biomolecular Screening Branch

NCGC
NIH Chemical Genomics Center

Toxicology Project Team

IJC Work Group addressing Chemicals of Emerging Concern

Alan Waffle P. Eng

June 23, 2009

Great Lakes Binational Toxics Strategy

Introduction

- October 2007 - IJC establishes priorities within context of the 2007-2009 Nearshore Framework Priority, including a Priority on Chemicals of Emerging Concern

- 2 tasks are concurrently undertaken to address the Priority on Chemicals of Emerging concern:

  - A multi Board Work Group is brought together, and,
  - A list is defined to identify chemicals being addressed

Workgroup Defined

Charge:

Assess current scientific and policy information to identify gaps and new approaches that could be applied to existing binational and domestic policy frameworks

Workplan:

- Review current scientific **literature** with focus on water quality
- Review international and national programs/**policies** for mgmt
- **Assessment and analysis** to address gaps with relevance to Great Lakes

Co-chairs:

a) Ted Smith, US EPA

b) Gary Klecka, DOW Chemical

List of Chemicals of Emerging Concern

- Synthetic Musks
- Fluorinated Surfactants
- Brominated Diphenyl Ethers
- Other Flame Retardants
- Alkylphenol Ethoxylates
- Chlorinated Paraffins
- Pharmaceuticals, Veterinary Drugs and Personal Care Products
- Current Use Pesticides

Definition of Chemicals of Emerging Concern

- The emphasis on research and monitoring has shifted from the analysis of “legacy pollutants” to a wide array of new chemicals being discovered in the environment

- While it has been known that many substances used by society enter the environment, improvements in instrumentation and analytical methodology have brought increased awareness to the presence and potential risk that these chemicals may pose

- The term "chemicals of emerging concern" has come to define the emerging awareness of the presence in the environment of many chemicals used by society, along with concern over the risk that these chemicals may pose to the health of humans and ecosystems

Literature Analysis

- Literature search conducted to identify recent research regarding ecological exposures to a wide variety of potential contaminants in relevant environmental media with emphasis placed on wastewater treatment plants, as well as rural and urban pollution
- Reported concentrations were assembled into a Database, statistically analyzed and compared with currently available regulatory standards, guidelines or criteria
- Findings attached

Analytical Findings - Literature

8 Findings:

- Shift in focus from industrial point sources to dispersed, non-point releases of chemicals & substances
- Many CEC's have been detected in environmental media, although many are present at trace levels
- Detection abilities surpass abilities to understand implications of such findings
- Limited surveillance for many CEC's
- Availability of data varies among different classes of compounds
- Regulatory criteria are not available for many CEC's
- Significant scientific gaps in our ability to interpret monitoring data
- Wastewater treatment plants have been identified as an important source of contaminants to surface waters

Recommendations - Literature

4 Recommendations:

- Enhance binational communication, coordination, and cooperation on the design and implementation of monitoring programs for CEC's to set common objectives
- Developed appropriate tools in order to adequately assess exposures and impacts of CEC's
- Enhance ongoing research programs to resolve a number of significant gaps in current state-of-the-science
- Thorough analysis of the performance of wastewater treatment plants required and recommended for the next biennial cycle

Policy Analysis

- Evaluate “gaps” in national, state/provincial, and regional policies and programs that address identification, assessment, prevention, and control of the range of emerging CEC’s with a focus on prevention-oriented programs and policies

- Report prepared for consideration by those attending expert consultation. Final findings (once expert input considered) are attached.

Analytical Findings - Policy

8 Findings:

- Industrial chemicals (US & Canada) subject to pre-manufacturing notification, review, and approval by the federal governments
- International treaties exist for identification, assessment, and management of persistent organic pollutants. *
- Voluntary stewardship initiatives on both sides address some of the CEC's in the report.
- Gaps exist in assessment and management for certain classes of chemicals
- There are concerns regarding adequacy of some waste management practices
- Wastewater treatment is essential component to controlling a wide diversity of chemicals
- Chemical-by-chemical analyses in biota does not by itself constitute a sufficient basis to assess toxicant stress.
- GLWQA serves an important purpose in bringing together the US and Canada to exercise co-custodial responsibilities

Recommendations - Policy

11 Recommendations:

- A renewed GLWQA should include underlying principles/processes by which the Parties would establish priorities, rather than a specific list of substances
- Emphasis should be placed on moving upstream & adopting sustainable solutions to design, production and consumption of CEC's
- Prenotification programs should be continuously improved & made more robust
- Premanufacturing notification level of review should be conducted
- Adoption of enhanced wastewater treatment technologies should be implemented

Recommendations – Policy Cont.

- Strict regulations & enforcement should be put into place for waste & nutrient management practices
- New policies need to be developed to manage CEC's with new and innovative scientifically sound approaches
- Risk communication regarding CEC's should be carefully designed
- Consumer education and incentives should be provided to encourage conservation and environmentally sound consumer choices
- A Canadian Great Lakes National Program Directorate or Office should be established within Environment Canada mirroring U.S. Environmental Protection Agency's Great Lakes National Program Office
- Further emphasis should be placed on gaining knowledge and understanding of human health effects

Great Lakes Restoration Initiative

GLBTS Status Update

June 23, 2009

Background

- The U.S. Environmental Protection Agency, together with its federal agency partners, is developing a new Great Lakes Restoration Initiative
- The Initiative begins in 2010 by identifying $475 million for programs and projects strategically chosen to target the most significant environmental problems in the Great Lakes ecosystem.
- Funds will be used to strategically implement both federal projects and prioritized/competitive grants.

Five Focus Areas

- Toxic Substances and Areas of Concern
- Invasive Species
- Nearshore Health and Nonpoint Source Pollution
- Habitat and Wildlife Protection and Restoration
- Accountability, Monitoring, Evaluation, Communication, and Partnerships

Toxic Substances/Areas of Concern

- Restore Areas of Concern/Remediate Contaminated Sediments
- Strategic Pollution Prevention and Reduction Projects
- Protect Human Health through Safer Fish Consumption
- Measuring Progress and Assessing New Toxic Threats

Restore AOCs/Remediate Contaminated Sediments

Accelerate the rate of sediment clean-up in AOCs and other locations throughout the Great Lakes basin through programs such as the Great Lakes Legacy Act, Water Resources Development Act, and Natural Resource Damage Assessment. Restore and delist AOCs through strategic actions identified in Remedial Action Plans to restore individual beneficial uses.

Strategic Pollution Prevention and Reduction Projects

- Implement projects/actions delivering toxic reductions/pollution prevention for substances targeted by the Great Lakes Binational Toxics Strategy
- Sustainable Collection Programs
- Reduce the use of mercury in products and better manage mercury product wastes
- Promote and implement sustainable green practices at the household and community level
- Develop/foster adoption of green chemistry/green engineering practices
- Foster adoption of innovative products that would reduce the use and release of toxic substances

Protect Human Health through Safer Fish Consumption

Continue to protect Great Lakes fish consumers with sound and sensible advice provided through robust State and tribal fish advisory programs. Work closely with the Great Lakes medical and health communities to educate the general public regarding the benefits and risks of Great Lakes fish consumption.

Measuring Progress and Assessing New Toxic Threats

Measure progress in cleaning up toxics in the Great Lakes environment through comprehensive monitoring. Identify significant sources of new toxics through robust surveillance, lab and field studies, and modeling in order to devise and implement effective control strategies.

Measures of Progress

- AOC beneficial use impairments removed.
- Cubic yards (in millions) of contaminated sediment remediated in the Great Lakes.
- Pollution (in pounds, potentially entering the Great Lakes) reduced through prevention and waste minimization projects.
- Annual percentage decline for the long term trend in average concentrations of Legacy pollutants in Great Lakes wildlife and of atmospheric deposition.

2011 and Beyond

- Public Meetings in July
- Plan due to OMB Sept 1