United States Environmental Protection Agency

Alliance for Innovation & Sustainability Meeting
Dalton State College, GA, April 20, 2017

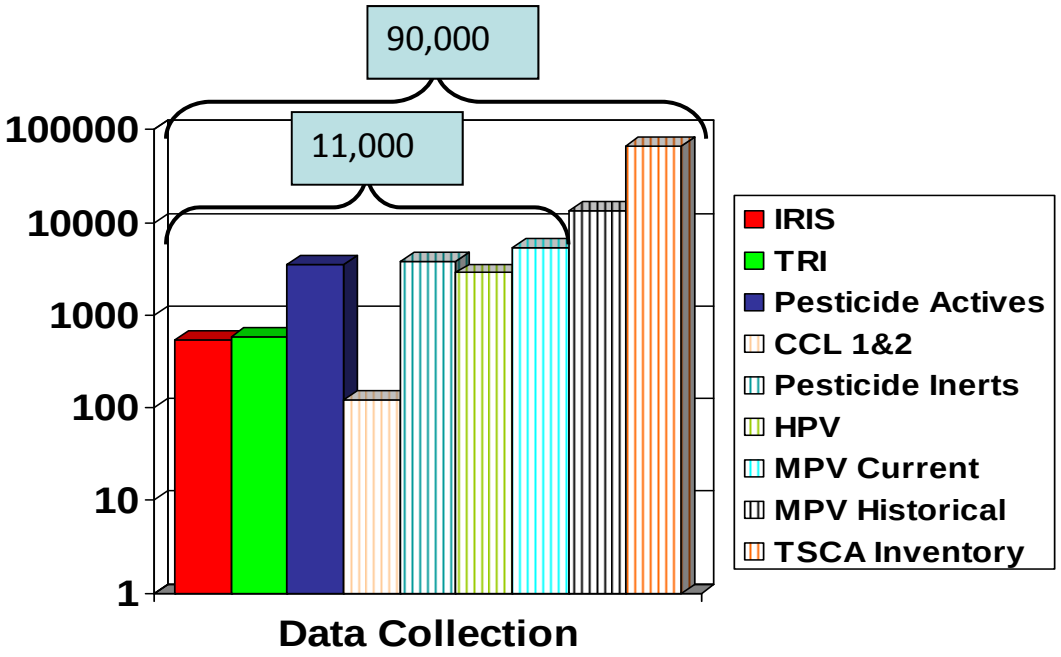
U.S. EPA's Computational Toxicology Program: *Innovation Powered by Chemistry*

Ann Richard
National Center for Computational Toxicology
Office of Research & Development
richard.ann@epa.gov

Office of Research and Development
National Center for Computational Toxicology

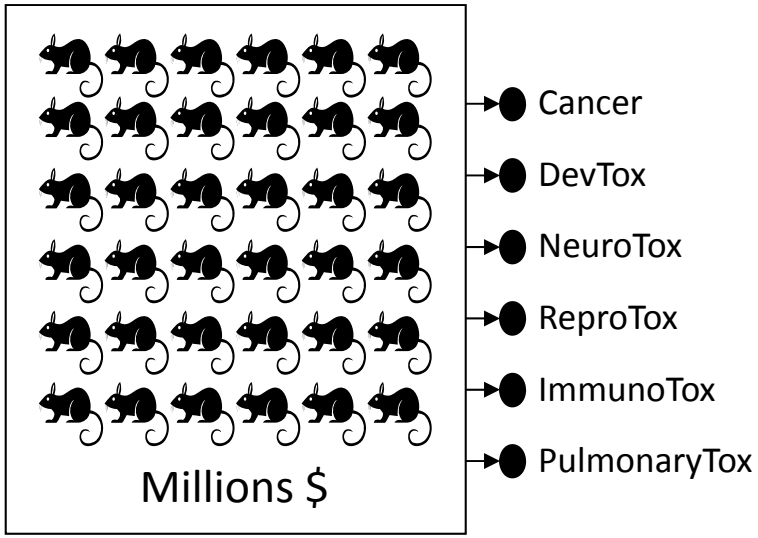
The Big Problem …..

Too Many Chemicals



…and not enough data.

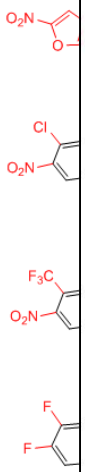
Too High a Cost



Too many endpoints
Too many mechanisms

Judson, et al EHP, 2008

Toxicity Testing in the 21st Century



THE NATIONAL

July 2007

REPORT IN BRIEF

Toxicity Testing in the 21st Century: A Vision and a Strategy

Advances in molecular biology, biotechnology, and other fields are paving the way for major improvements in how scientists evaluate the health risks posed by potentially toxic chemicals found at low levels in the environment. These advances would make toxicity testing quicker, less expensive, and more directly relevant to human exposures. They could also reduce the need for animal testing by substituting more laboratory tests based on human cells. This National Research Council report creates a far-reaching vision for the future of toxicity testing.

Toxicity tests on laboratory animals are conducted to evaluate chemicals—including medicines, food additives, and industrial, consumer, and agricultural chemicals—for their potential to cause cancer, birth defects, and other adverse health effects. Information from toxicity testing serves as an important part of the basis for public health and regulatory decisions concerning toxic chemicals. Current test methods were developed incrementally over the past 50 to 60 years and are conducted using laboratory animals, such as rats and mice. Using the results of animal tests to predict human health effects involves a number of assumptions and extrapolations that remain controversial. Test animals are often exposed to higher doses than would be expected for typical human exposures, requiring assumptions about effects at lower doses or exposures. Test animals are typically observed for overt signs of adverse health effects, which provide little information about biological changes leading to such health effects. Often controversial uncertainty factors must be applied to account for differences between test animals and humans. Finally, use of animals in testing is expensive and time consuming, and it sometimes raises ethical issues.

Today, toxicological evaluation of chemicals is poised to take advantage of the on-going revolution in biology and biotechnology. This revolution is making it increasingly possible to study the effects of chemicals using cells, cellular components, and tissues—preferably of human origin—rather than whole animals. These powerful new approaches should help to address a number of challenges facing the

National Academ

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.

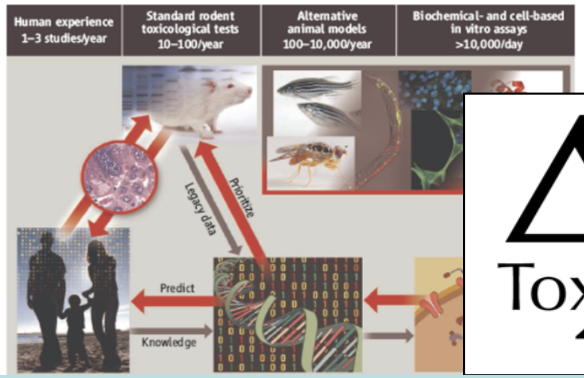
Francis S. Collins,[1*†] George M. Gray,[2*] John R. Bucher[3*]

In 2005, the U.S. Environmental Protection Agency (EPA), with support from the U.S. National Toxicology Program (NTP), funded a project at the National Research Council (NRC) to develop a long-range vision for toxicity testing and a strategic plan for implementing that vision. Both agencies wanted future toxicity testing and assessment paradigms to meet evolving regulatory needs. Challenges include the large numbers of substances that need to be tested and how to incorporate recent advances in molecular toxicology, computational sciences, and information technology; to rely increasingly on human as opposed to animal data; and to offer increased efficiency in design and costs (*1*–*5*). In response, the NRC Committee on Toxicity Testing and Assessment of Environmental Agents produced two reports that reviewed 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 (*6*, *7*). Although the NRC reports have laid out a solid theoretical rationale, comprehensive and rigorously gathered data (and comparisons with historical animal data) will determine whether the hypothesized improvements will be realized in practice. For this purpose, NTP, EPA, and the National Institutes of Health Chemical 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 vision and roadmap for the 21st century (*1*), which established initiatives to integrate high-throughput screening (HTS) and other automated screening assays into its testing program. In 2005, the EPA established the National Center for Computational Toxicology (NCCT). Through these initiatives, NTP and EPA, with the NCGC, are promoting the evolution of toxicology from a predominantly observational science at the level of disease-specific models in vivo to a predominantly predictive science focused on broad inclusion of target-specific, mechanism-based, biological observations in vitro (*1*, *4*) (see figure, below).

Toxicity pathways. In vitro and in vivo tools are being used to identify cellular responses after chemical exposure expected to result in adverse health effects (*7*). HTS methods are a primary means of discovery for drug development, and screening of >100,000 compounds per day is routine (*8*). However, drug-discovery HTS methods traditionally test compounds at one concentration, usually between 2 and 10 μM, and tolerate high false-negative rates. In contrast, in the EPA, NCGC, and NTP combined effort, all compounds are tested at as many as 15 concentrations, generally ranging from ~5 nM to ~100 μM, to generate a concentration-response curve (*9*). This approach is highly reproducible, produces significantly lower false-positive and false-negative rates than the traditional HTS methods (*9*), and facilitates multiassay comparisons. Finally, an informatics platform has been built to compare results among HTS screens; this is being expanded to allow comparisons with historical toxicologic NTP and EPA data (http://ncgc.nih.gov/pub/openhts). HTS data collected by EPA and NTP, as well as by the NCGC and other Molecular Libraries Initiative centers (http://mli.nih.gov/), are being made publicly available through Web-based databases [e.g., PubChem (http://pubchem.ncbi.nlm.nih.gov)]. In addition,



[1]Director, National Human Genome Research Institute (NHGRI), National Institutes of Health, Bethesda, MD 20892; [2]Assistant Administrator for the Office of Research and Development, U.S. Environmental Protection Agency, policies of their respective agencies.

†Author for correspondence. E-mail: francisc@mail.nih.gov

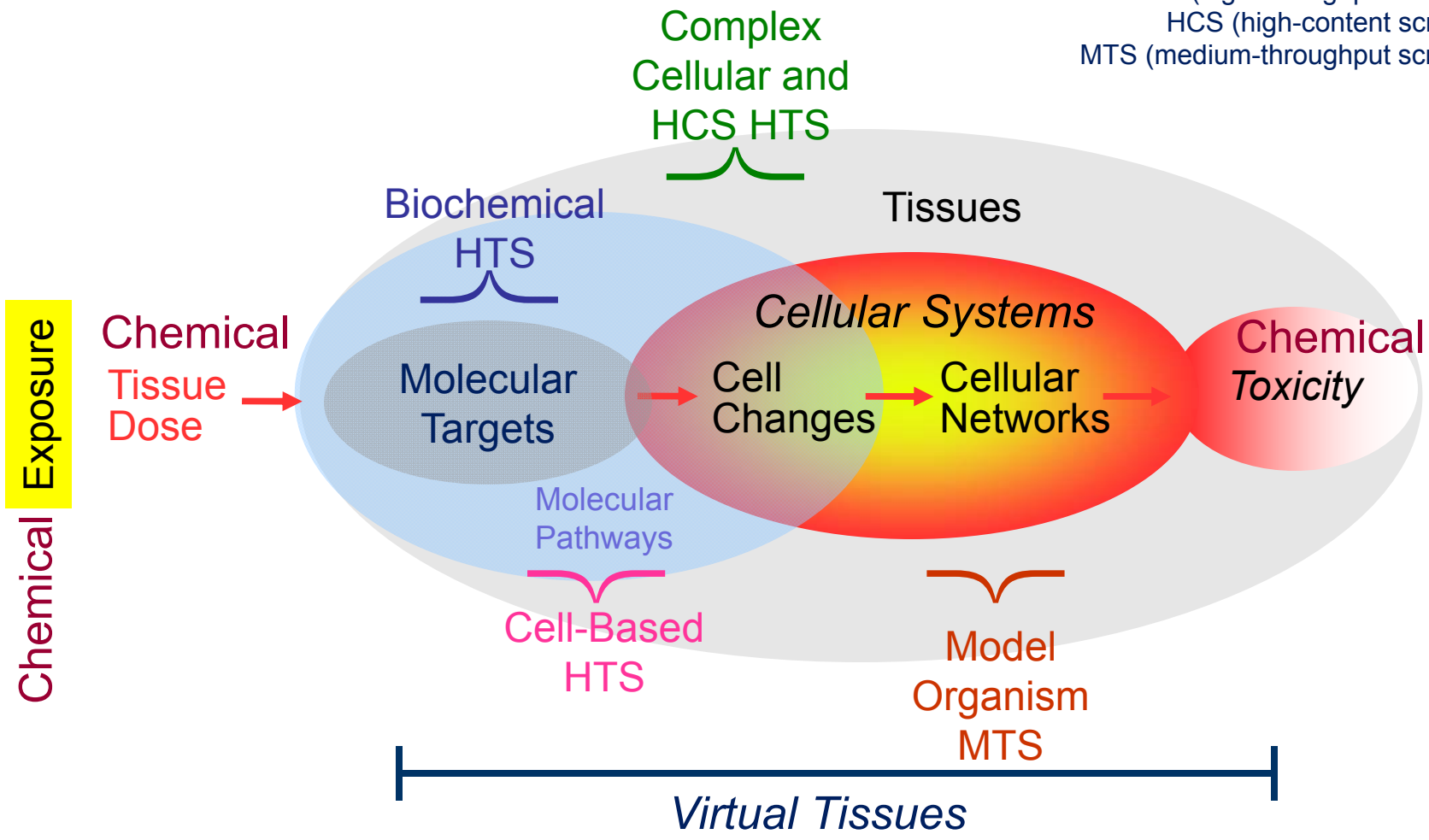
Transforming toxicology. The studies we propose will test whether high-throughput and computational toxicology approaches can yield data predictive of results from animal toxicity studies, will allow prioritization of chemicals for further testing, and can assist in prediction of risk to humans.

Tox21

EPAs Contribution: The ToxCast Research Program

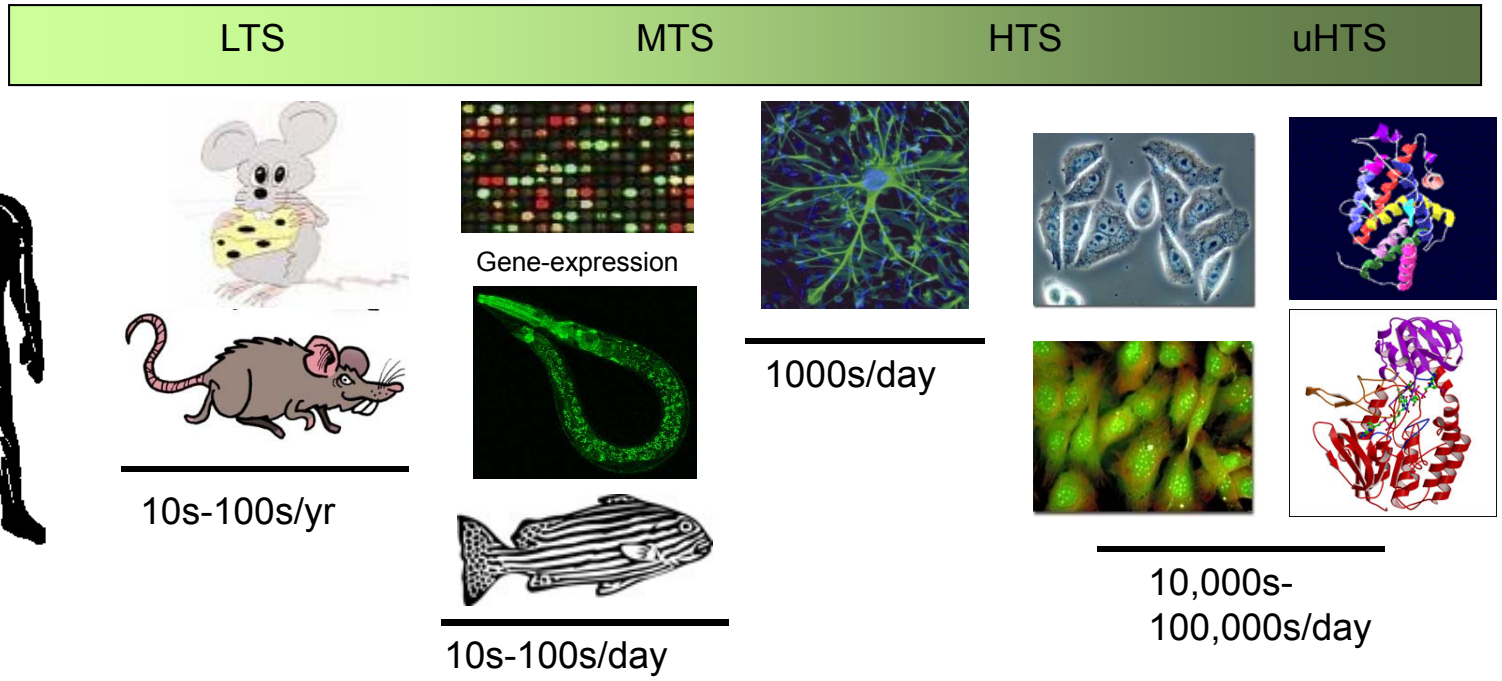
A Grand Challenge: Predicting Chemical Toxicity

HTS (high-throughput screening)
HCS (high-content screening)
MTS (medium-throughput screening)



High-Throughput Screening Assays

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

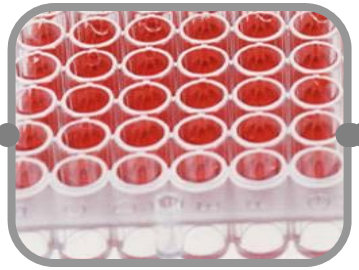


Human Relevance/
Cost/Complexity

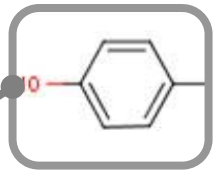
Throughput/
Simplicity

High Throughput Screening 101

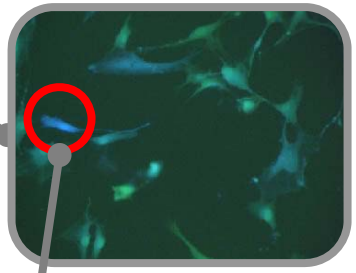
HTS Robotic Platform



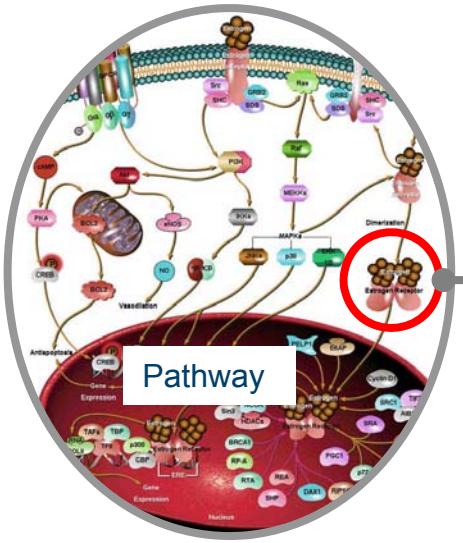
96-, 384-, 1536 Well Plates

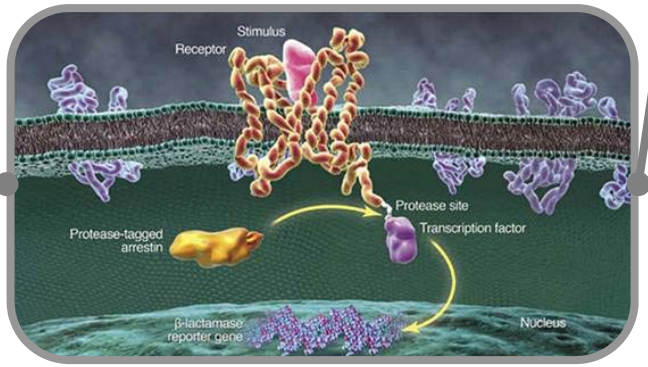


Chemical Exposure



Cell Population



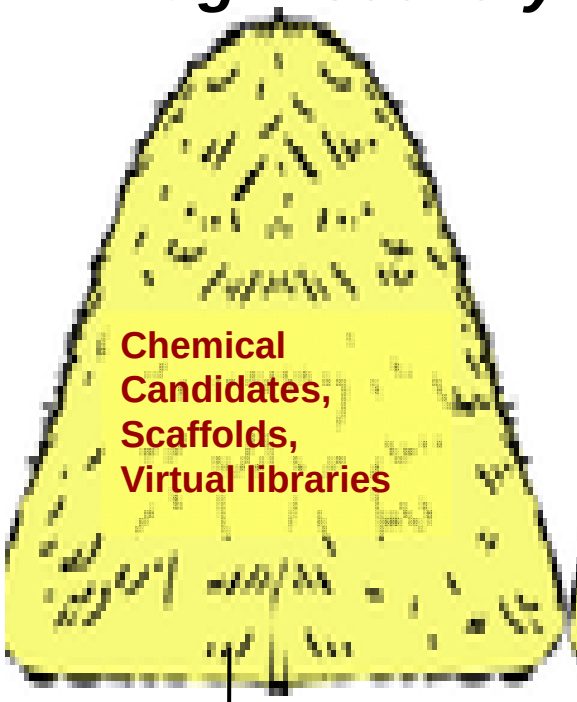


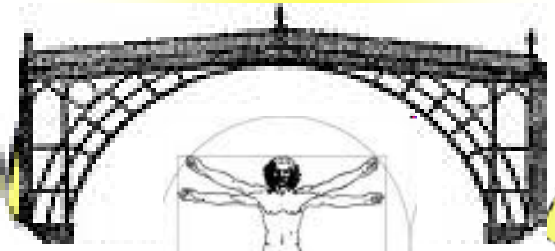
Assay Target Biology (e.g., Estrogen Receptor)

Drug Discovery

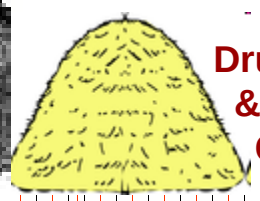
Chemical libraries
HTS screening
Bioprofiling
Chemical probes
Molecular profiling
Feature optimization
Cheminformatics
Chemogenomics
Systems biology
ADME

Chemical Candidates, Scaffolds, Virtual libraries

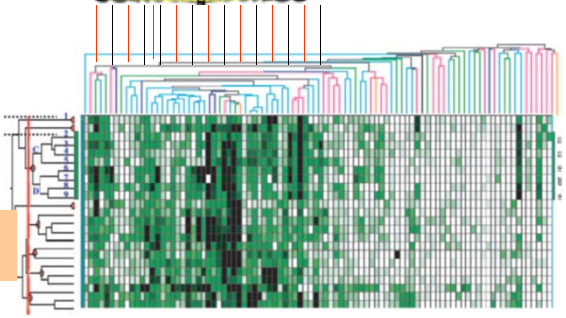




Toxicity Screening



Drugs, Industrial, & Environmental Chemicals



Therapeutic Endpoint

Polypharmacology | Polyfunctional

Low dose | High dose

Structure modification | Green chemistry

High specificity
High affinity

Chronic, Acute, Devel, Repro, Immuno, Neuro…tox

Low specificity
Low affinity

ToxCast Screening Assays

United States Environmental Protection Agency

Biochemical Assays

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

~800 Total Endpoints

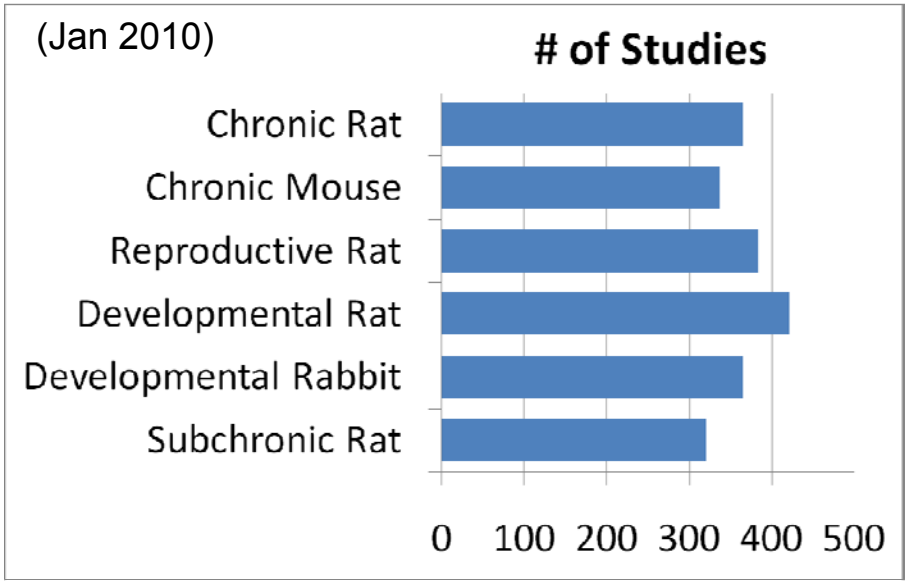
Cellular Assays

- 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
  - Rat hepatocytes
  - Mouse embryonic stem cells (Sid Hunter)
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

- Primarily Human/Rodent targets & cell lines
- Model organisms: Zebrafish embryo assay
- >50 external EPA collaborators (academics, govt, companies)

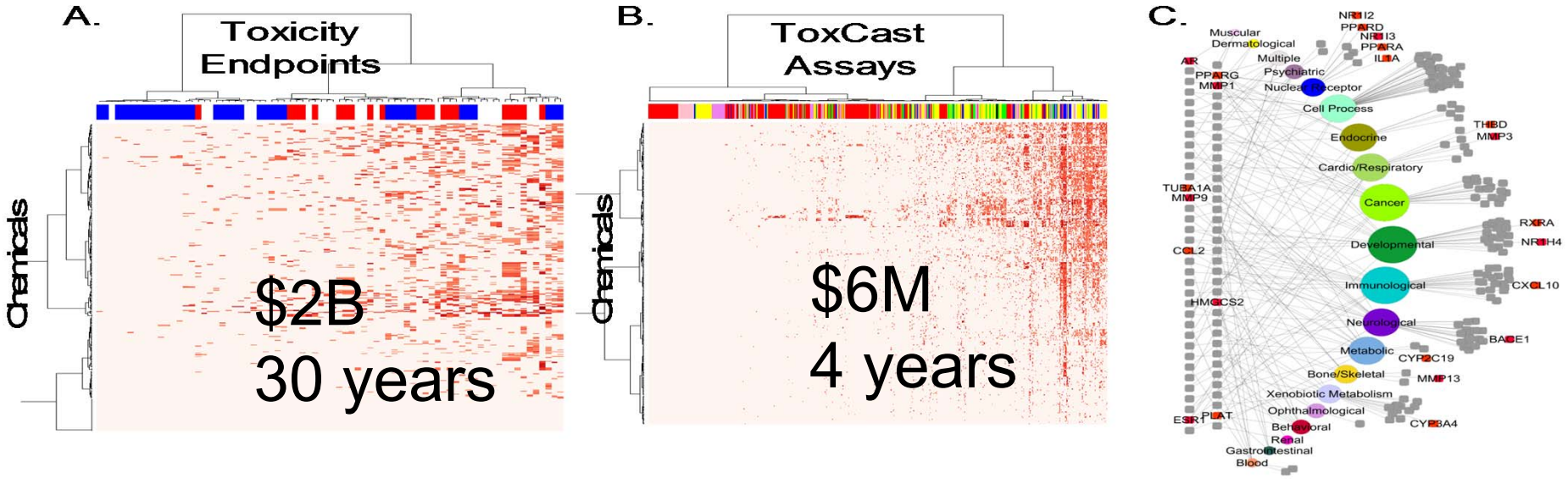
ToxRefDB (Toxicity Reference Database)

- Publically available toxicity reference database
- Captures 30 years & $2 billion of animal testing data
  - mostly pesticide registration data reports submitted to EPA
- Stores study design, dosing & observed treatment-related effects using standard vocabulary



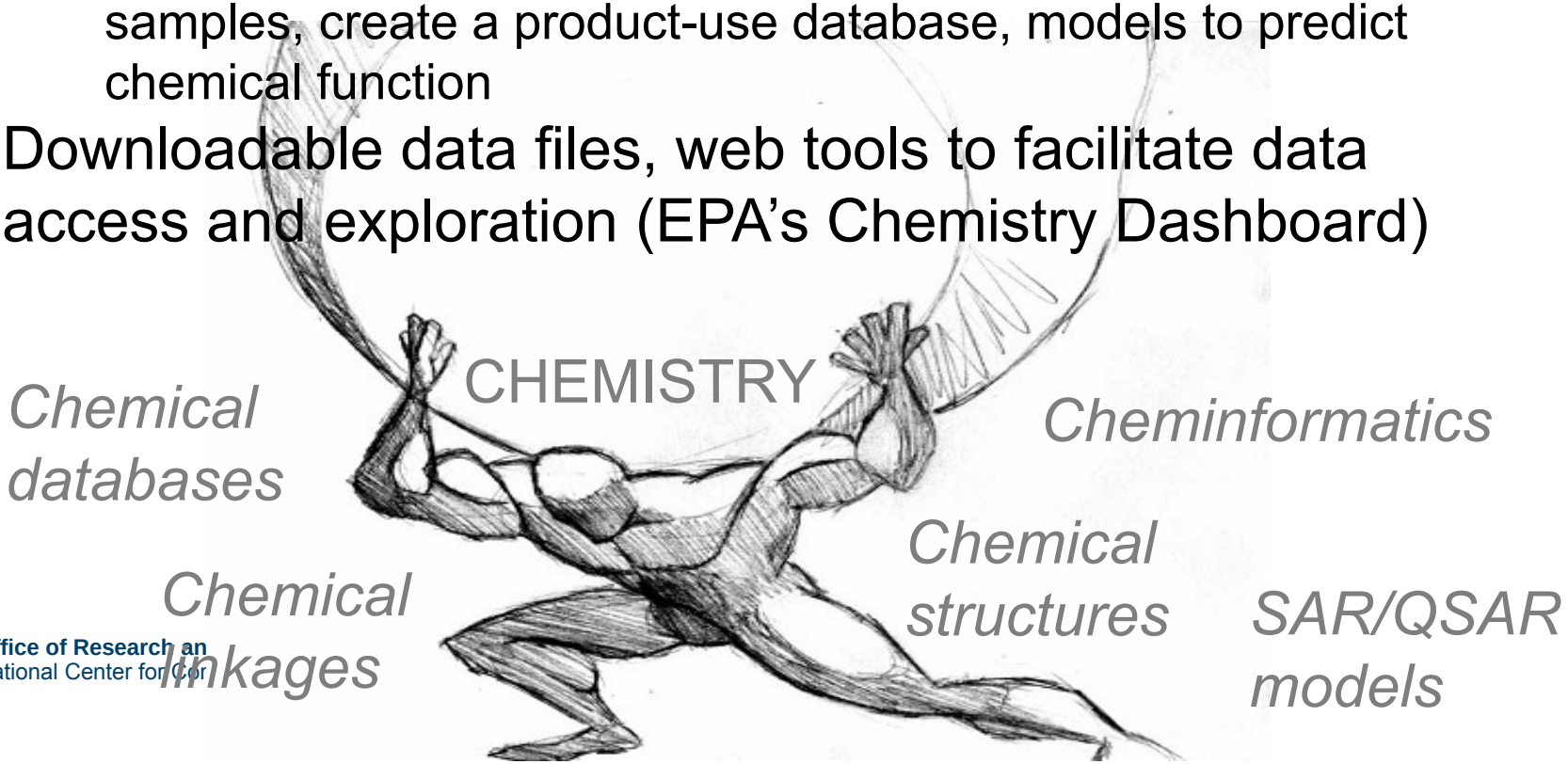
Using HTS data to predict Toxicity

ToxRefDB ← *ToxCast* → *Human Disease*



EPA's Computational Toxicology Research

- ToxCast (EPA) & Tox21 (Multi-Agency)
  - screening >4000 (ToxCast) to >10K (Tox21) environmentally relevant chemicals across 10's to 100's of HTS assays
- ExpoCast, CPCat, ToxRef DB
  - Exposure projects, non-targeted screening of environmental samples, create a product-use database, models to predict chemical function
- Downloadable data files, web tools to facilitate data access and exploration (EPA's Chemistry Dashboard)



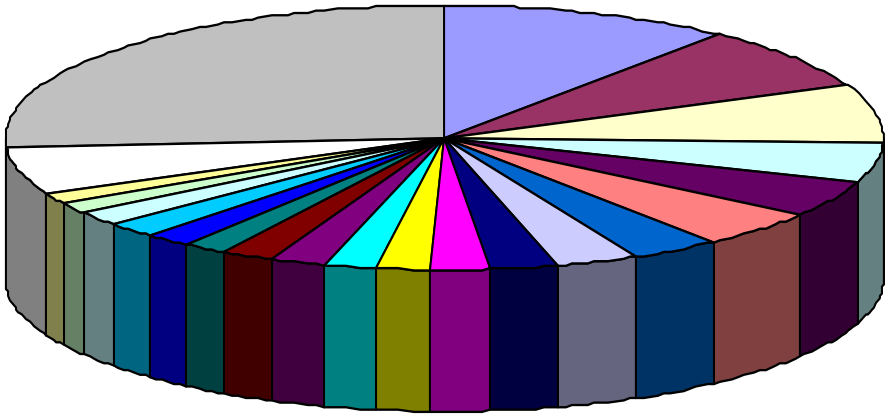
ToxCast Phase I

(309 Unique Chemicals)

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

- **276** Pesticides
- **16** Antimicrobials
- **9** Industrial Chemicals
- **8** Metabolites

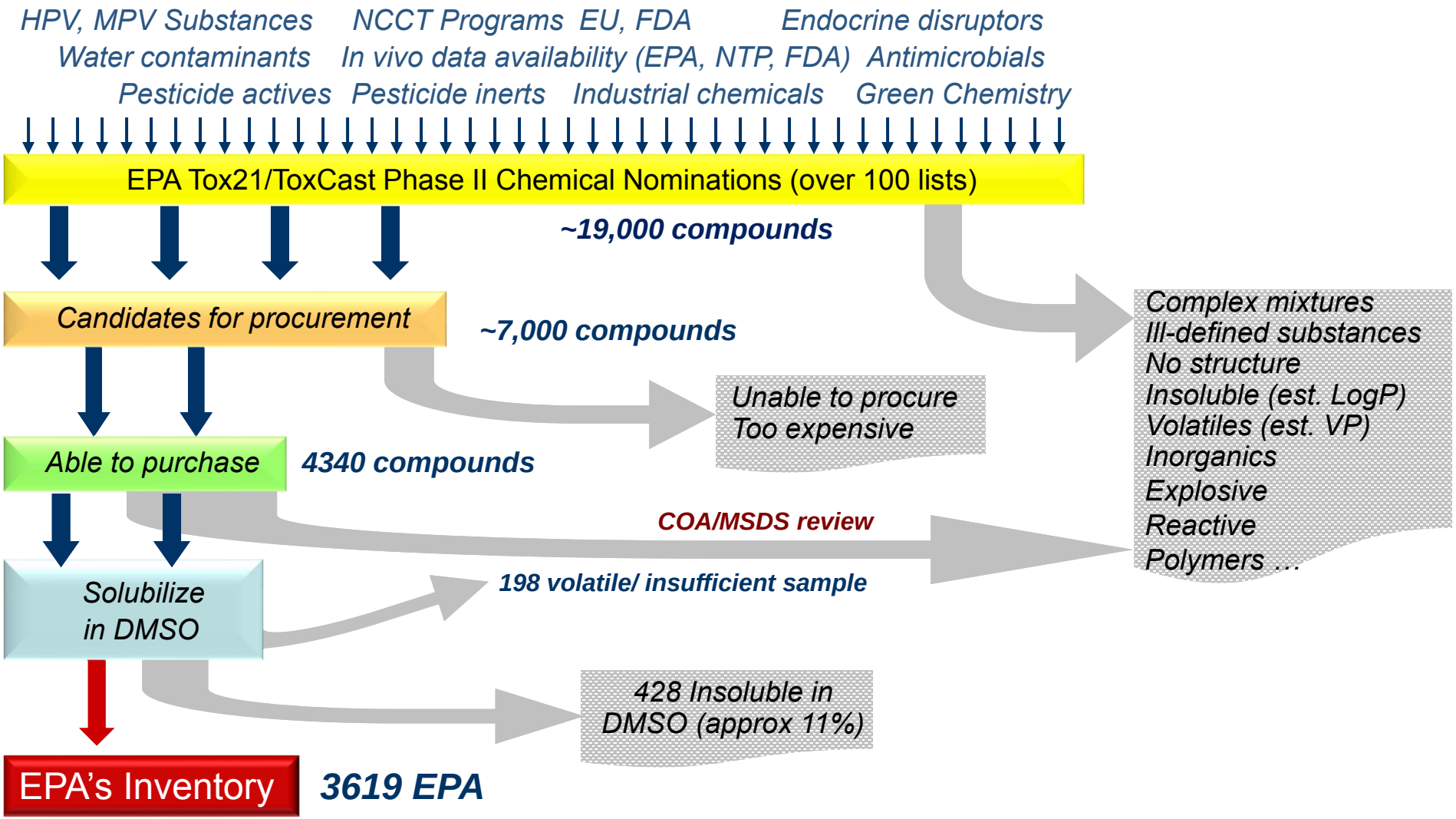
- **75** Chemical Classes



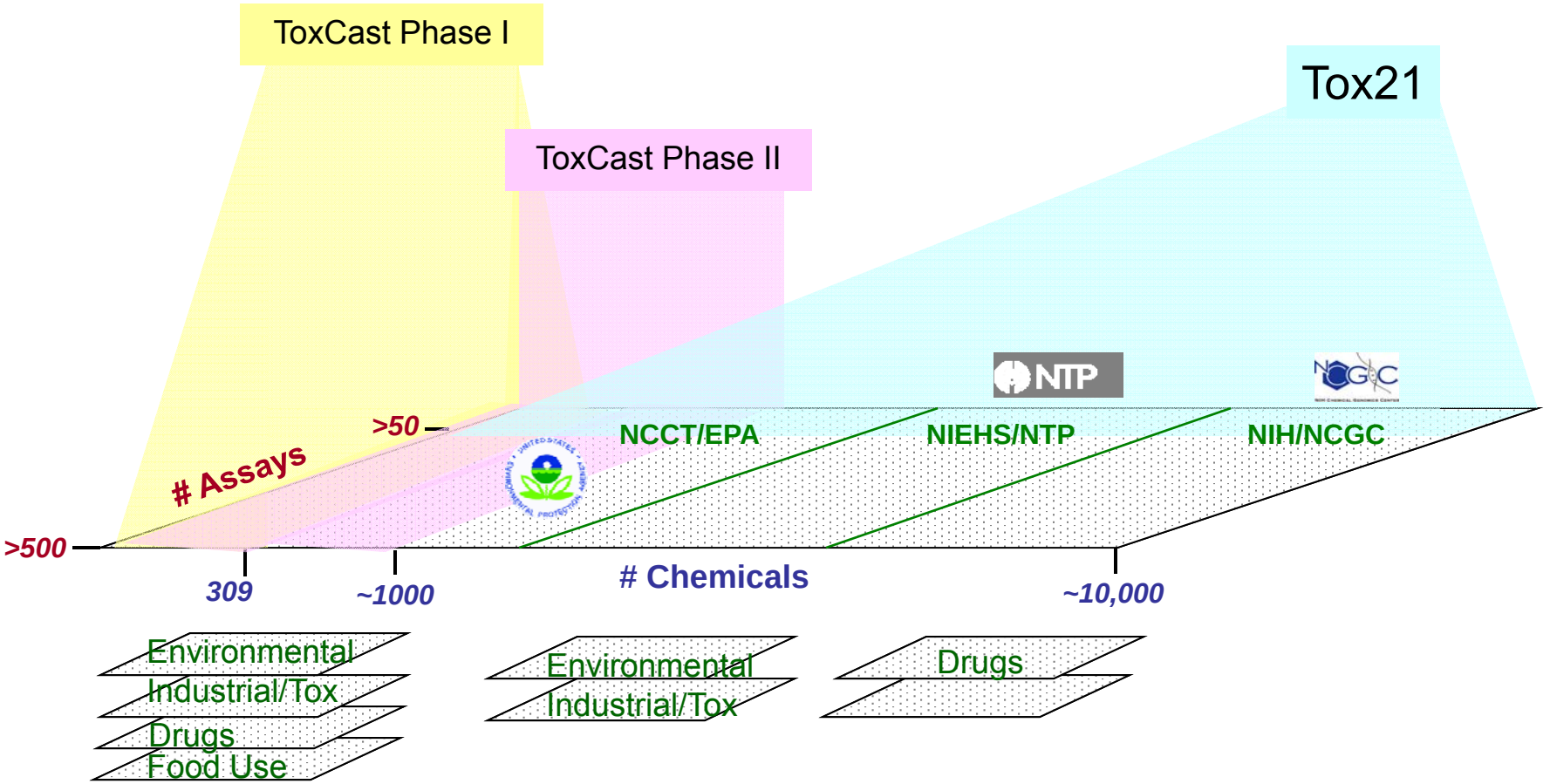
Chemical Class Distribution
(≥5/Class)

- 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)

EPA's ToxCast/Tox21 Library Construction



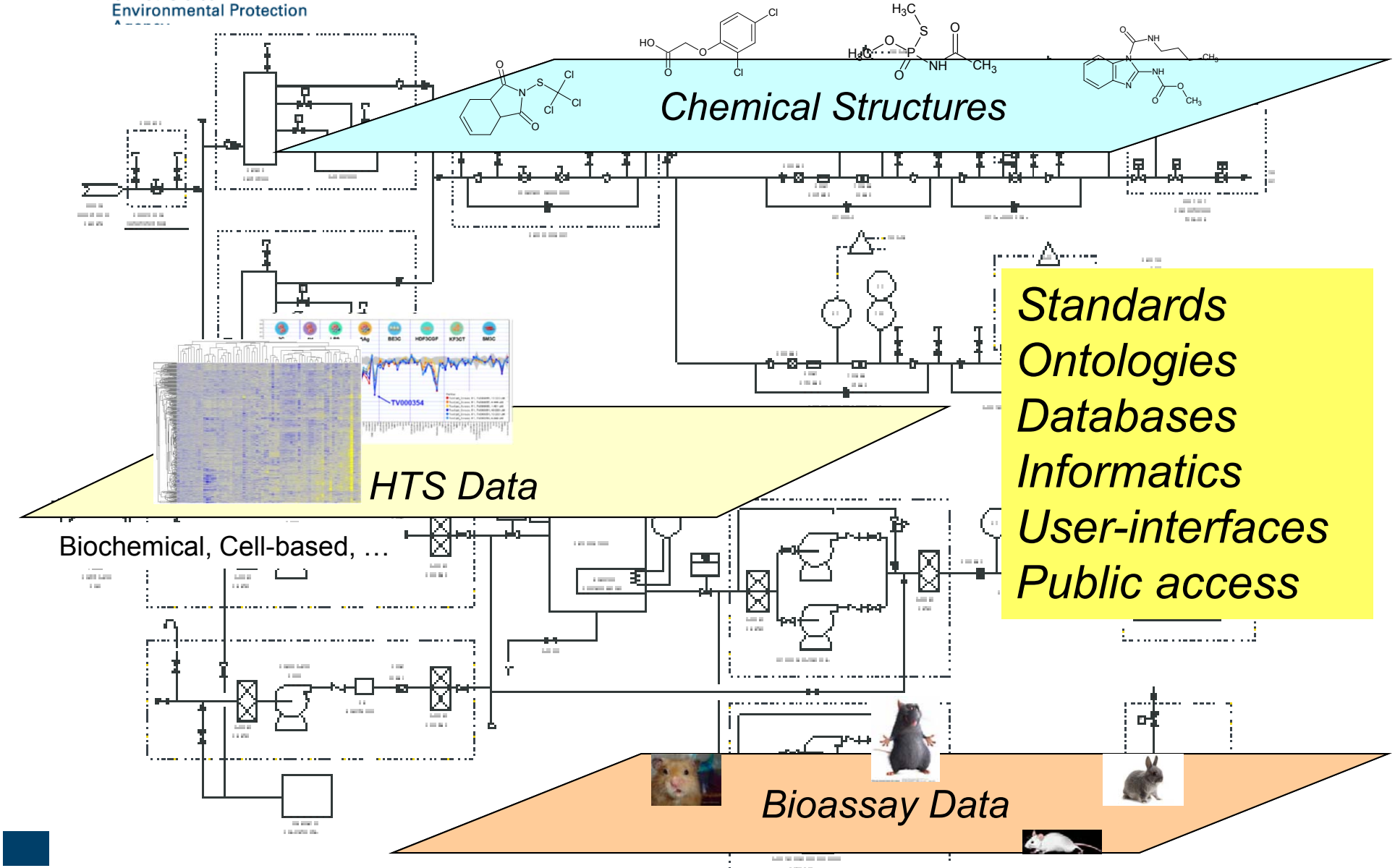
ToxCast/Tox21 Chemical Library



>8500 unique chemicals
Approx 100 HTS assays

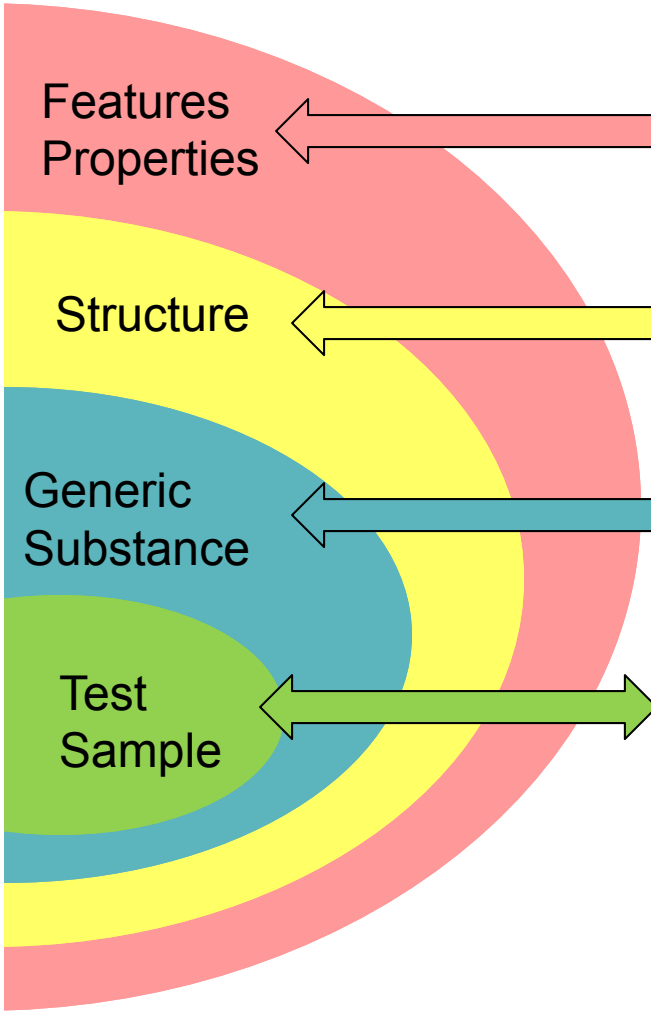
ToxCast: "Big Data" Informatics Challenges

United States Environmental Protection Agency



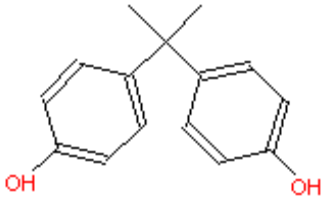
Standards
Ontologies
Databases
Informatics
User-interfaces
Public access

Chemical Elements to Data Integration: *Chemical representations → Uses*



Chemotypes, fingerprints, phys-chem properties, ...

Chemical analogs, Read-across, SAR modeling



SMILES
InChI

Structure searching & modeling

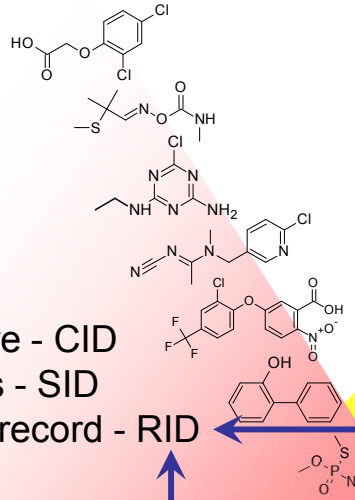
Chemical Name
CASRN

Public toxicity datasets

Supplier, Lot/Batch, physical description

Experimental Endpoint Data

ToxCast/Tox21 Chemical Registry



DSSTox

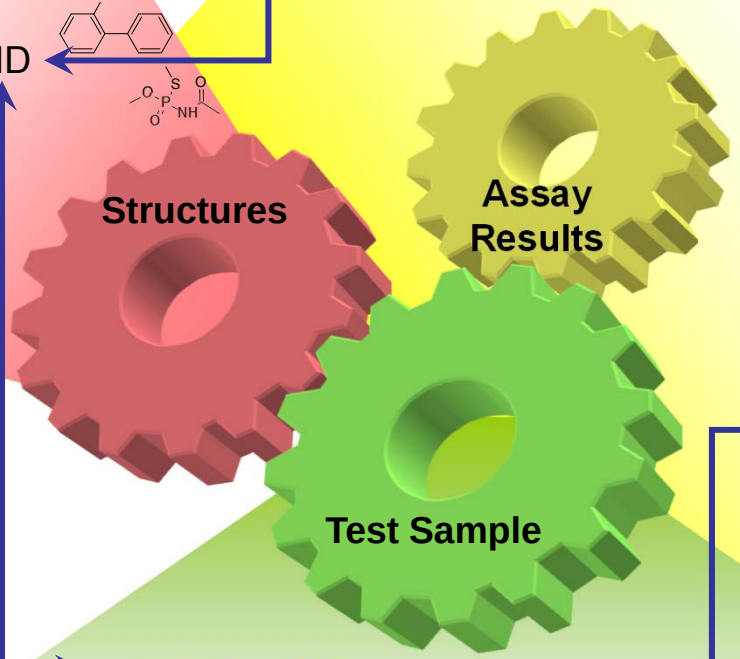
Chemical structure - CID
Substance details - SID
Project inventory record - RID

DSSTox RID

ACToR/InVitroDB

Assay name
Assay details
Assay outcome

Solution ID
Plate ID
Plate Address ID



DSSTox RID
Bottle ID (→ COA ID)
Solution ID (→ QC ID)

ChemTrack Database

ToxCast Chemical Landscape

United States Environmental Protection Agency

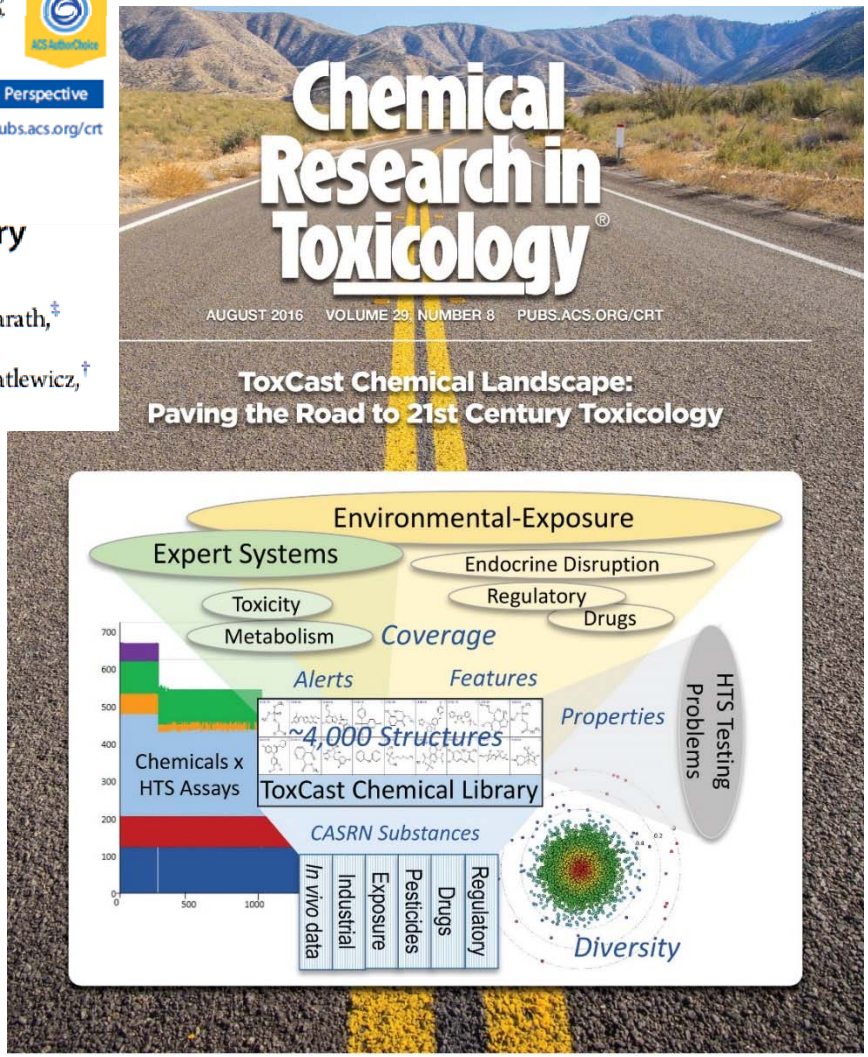
Chemical Research in Toxicology

Perspective

pubs.acs.org/crt

ToxCast Chemical Landscape: Paving the Road to 21st Century Toxicology

Ann M. Richard,[*,†] Richard S. Judson,[†] Keith A. Houck,[†] Christopher M. Grulke,[†] Patra Volarath,[‡] Inthirany Thillainadarajah,[§] Chihae Yang,[∥,⊥] James Rathman,[⊥,#] Matthew T. Martin,[†] John F. Wambaugh,[†] Thomas B. Knudsen,[†] Jayaram Kancherla,[▽] Kamel Mansouri,[▽] Grace Patlewicz,[†] Antony J. Williams,[‡] Stephen B. Little,[†] Kevin M. Crofton,[†] and Russell S. Thomas[†]



Open Access Perspectives article and Supporting Info files available for free download at:

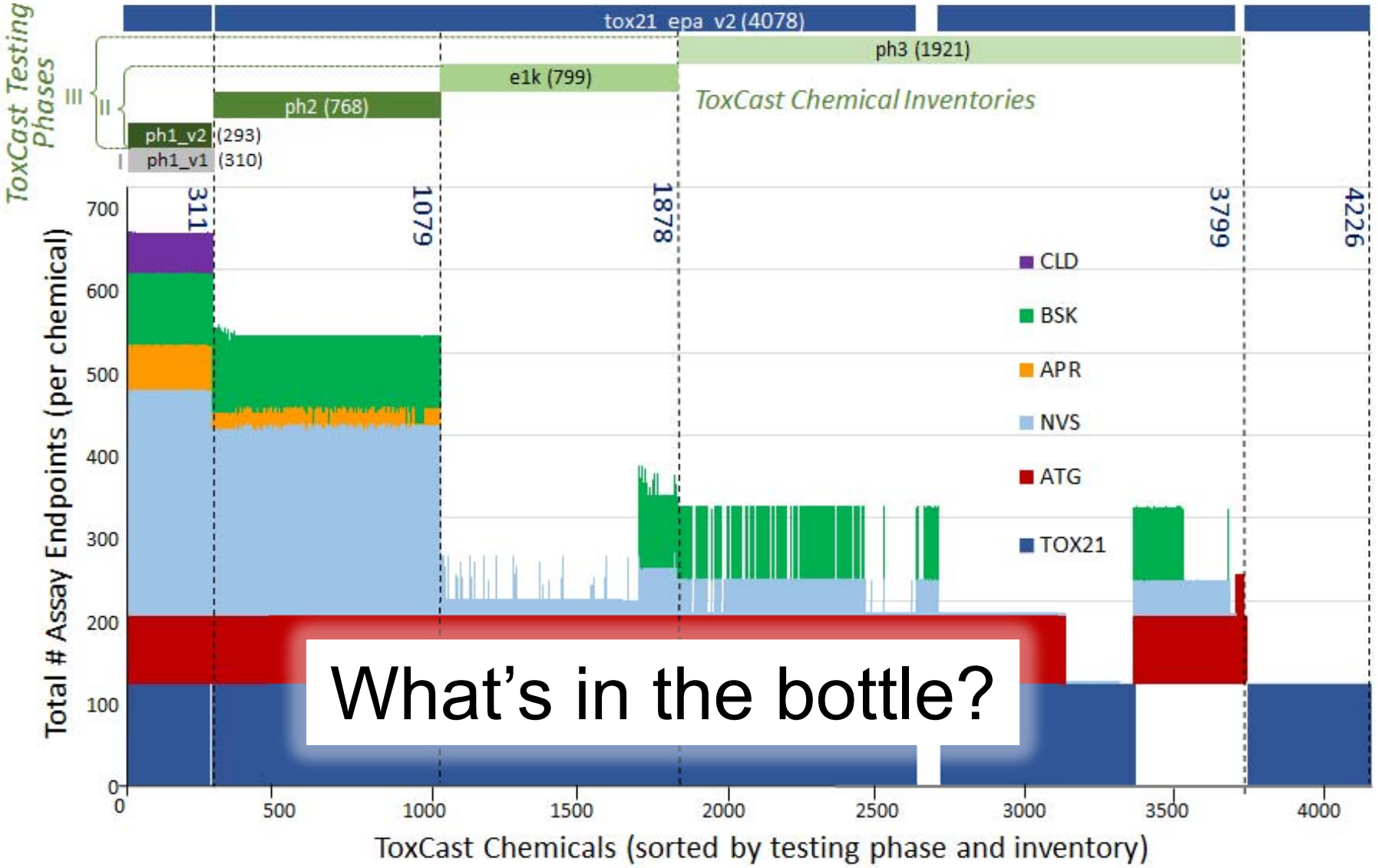
http://pubs.acs.org/doi/abs/10.1021/acs.chemrestox.6b00135

DOI: 10.1021/acs.chemrestox.6b00135

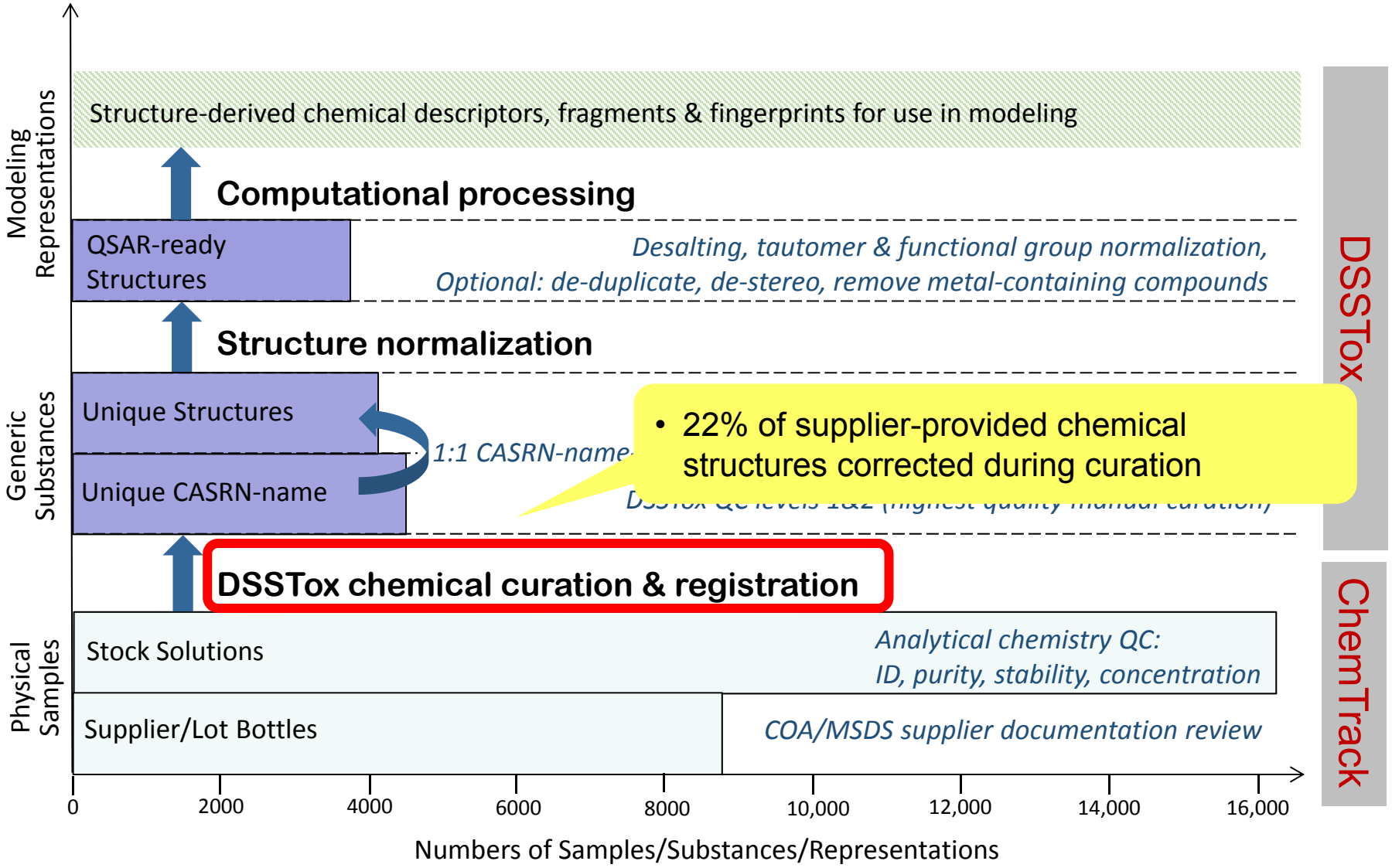
ChemResToxicol., 2016, 29, 1225−1251

ToxCast chemical x assay counts

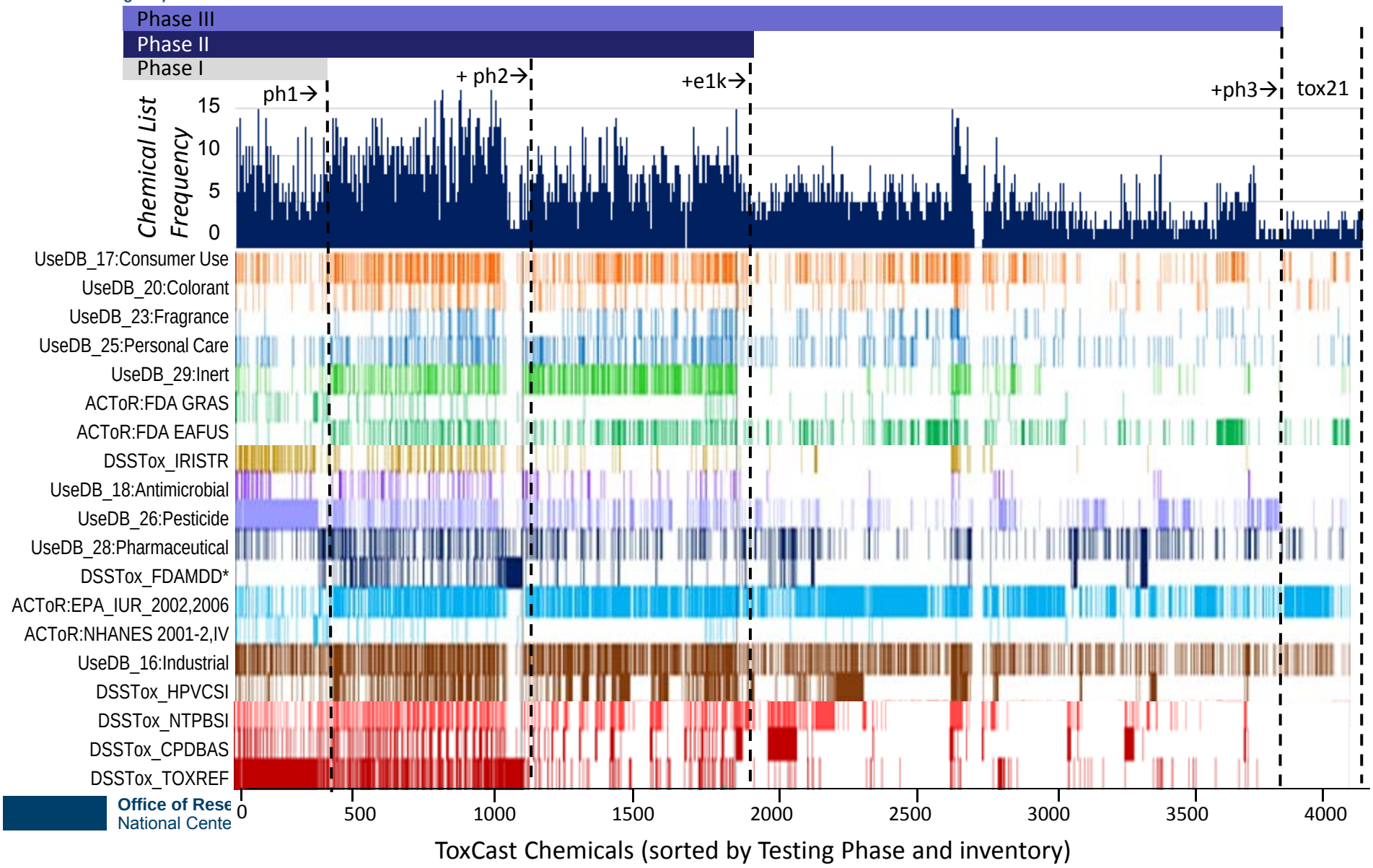
(Top 5 assay providers & Tox21, as of Jan 2016)



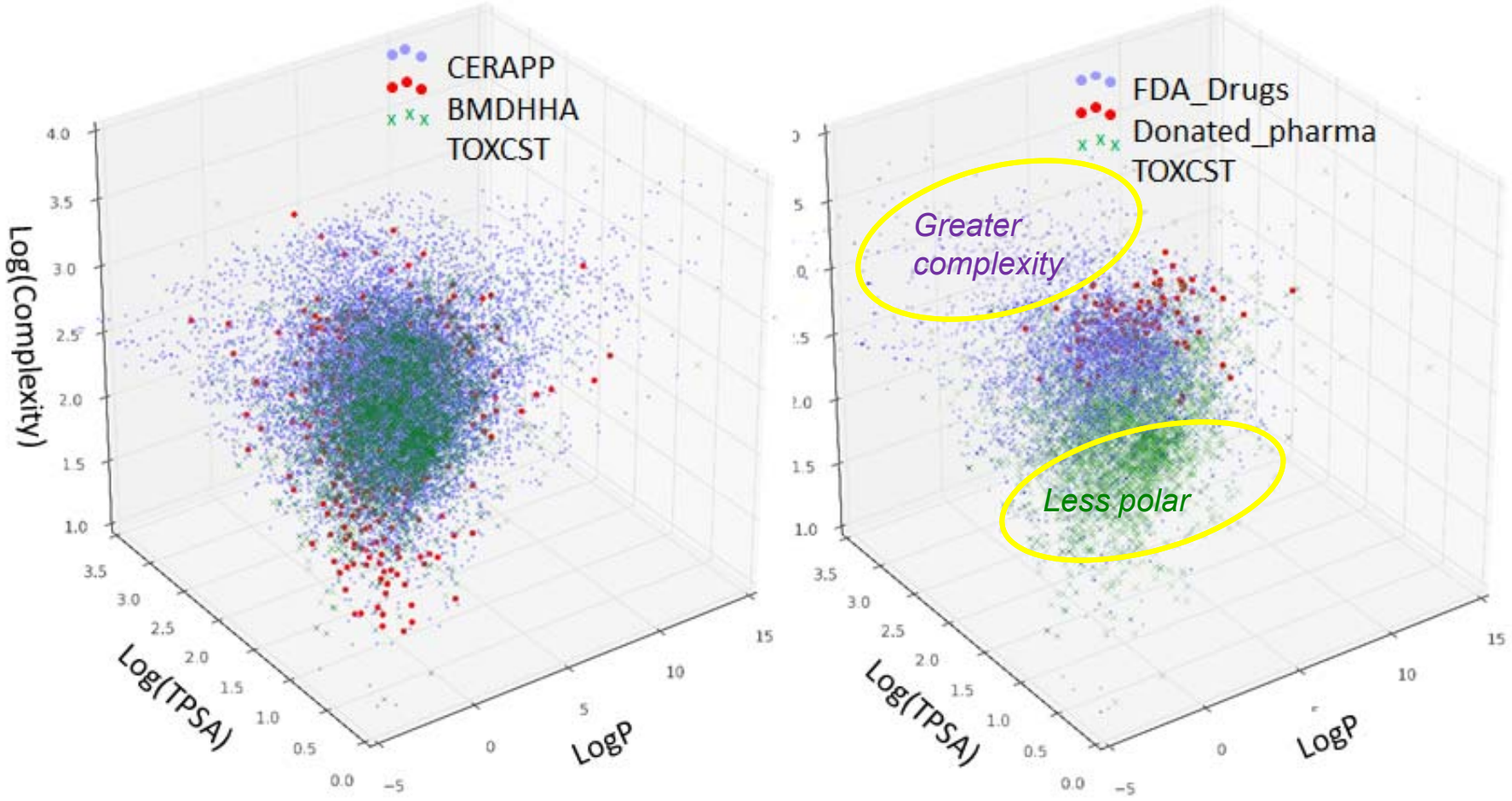
QC process for Chemical Registration & Characterization



What's in the library?



Comparison to potential target inventories based on computed properties



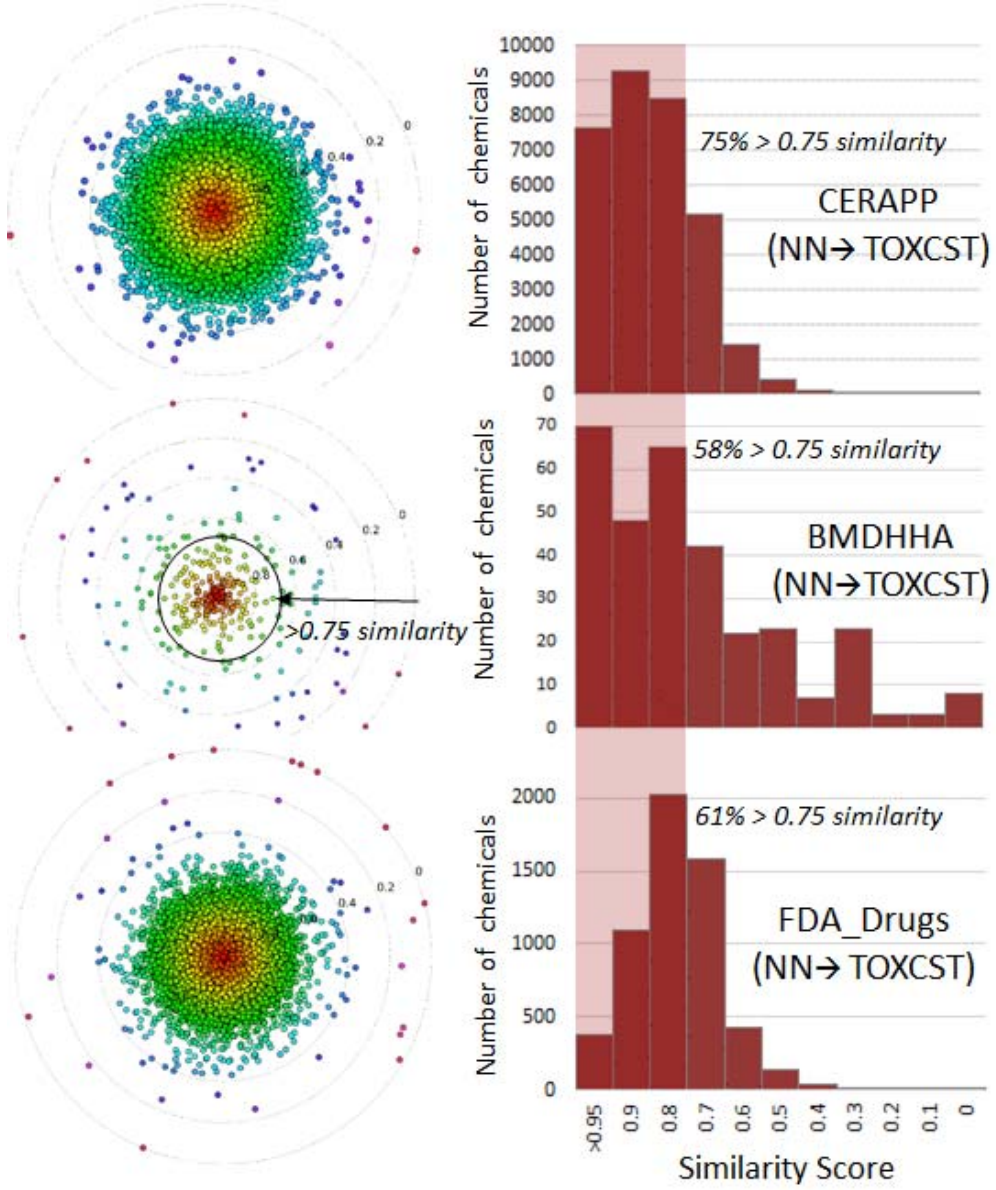
CERAPP – approx. 30,000 chemicals with predicted endocrine activity

BMDHHA – human health assessment benchmark doses available

FDA_Drugs – approx. 7000 marketed & discontinued drugs

Nearest neighbor similarity comparisons

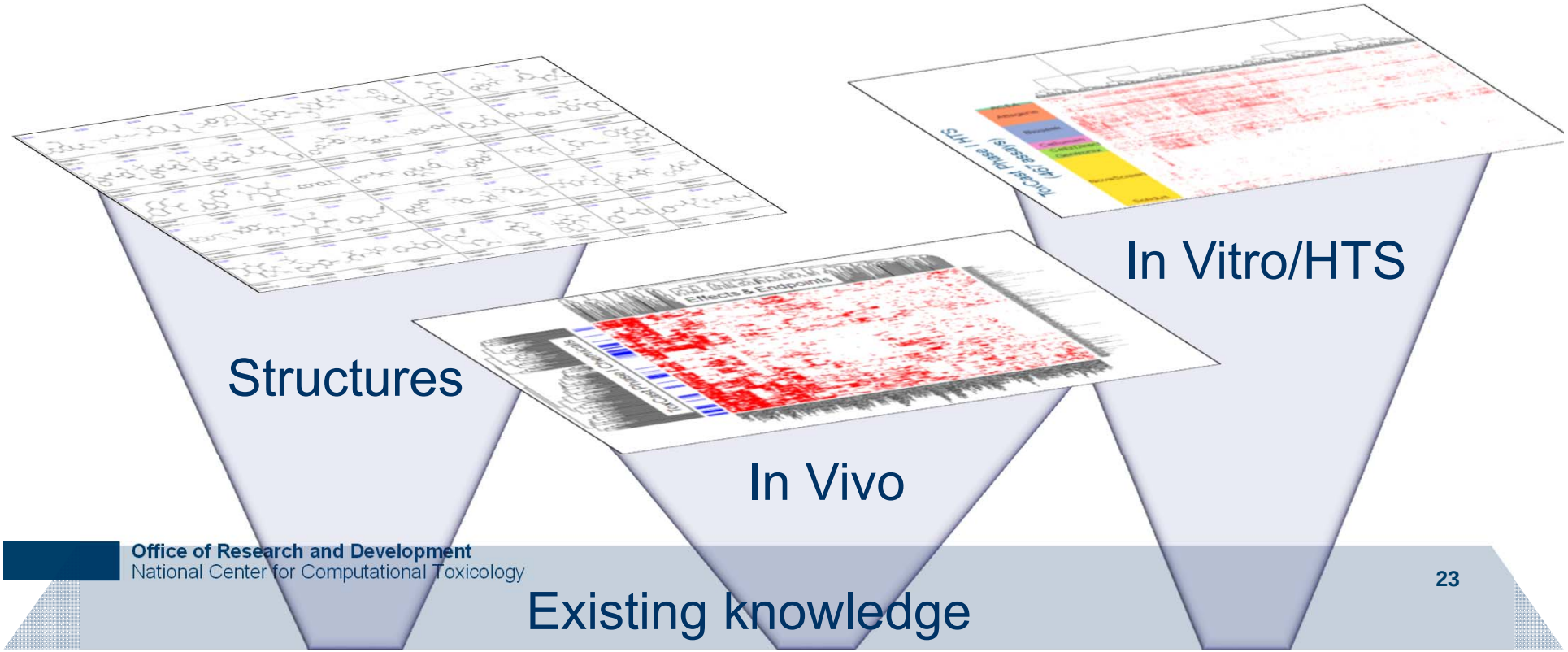
- 75% of CERAPP chemicals have a >75% similar TOXCST “analog”
- 58% of BMDHHA chemicals have a >75% similar TOXCST “analog
- 61% of FDA_Drugs chemicals have a >75% similar TOXCST “analog



ChemResToxicol., 2016, 29, 1225−1251

Toxicity Prediction Challenge

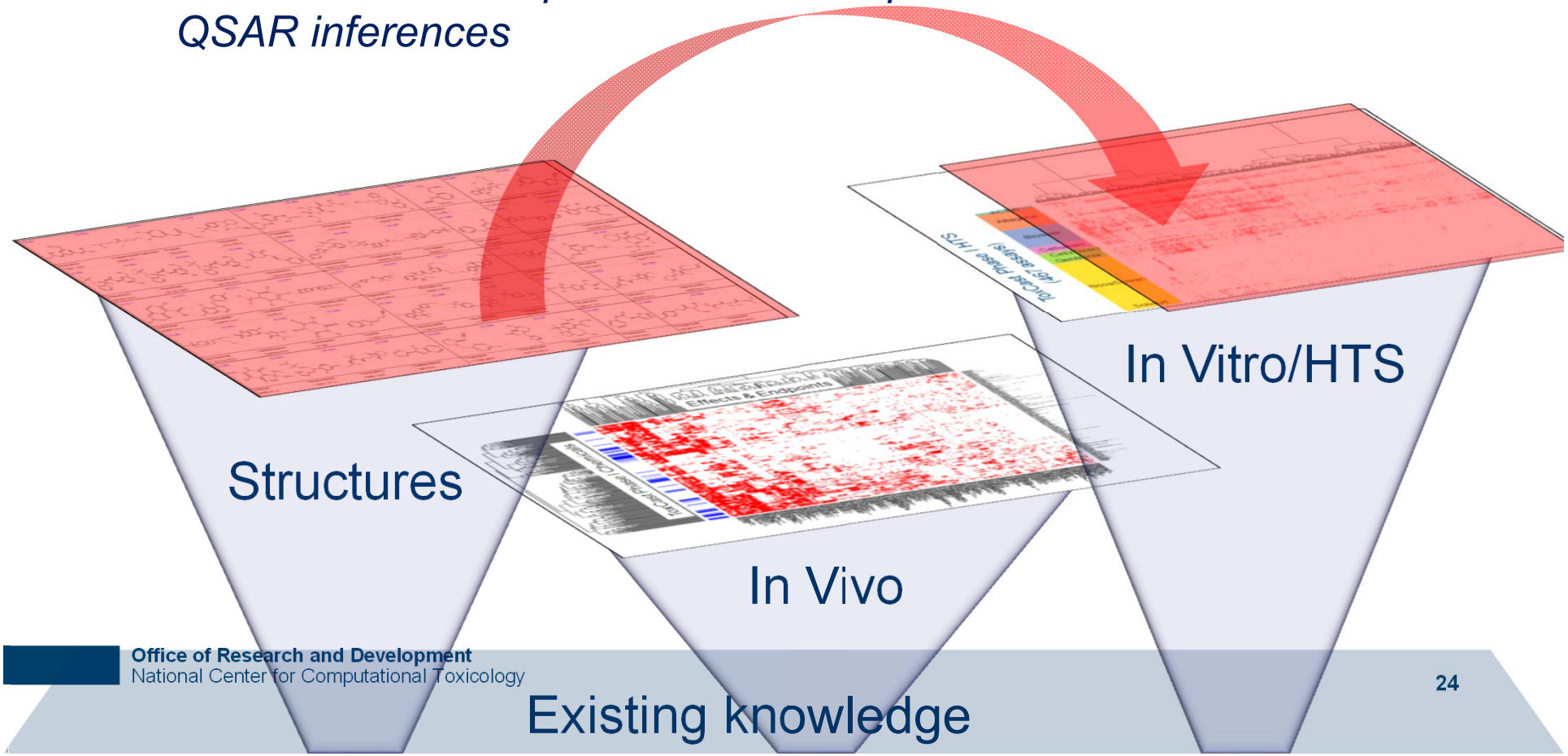
How do we make best use of all the data to improve predictive models?



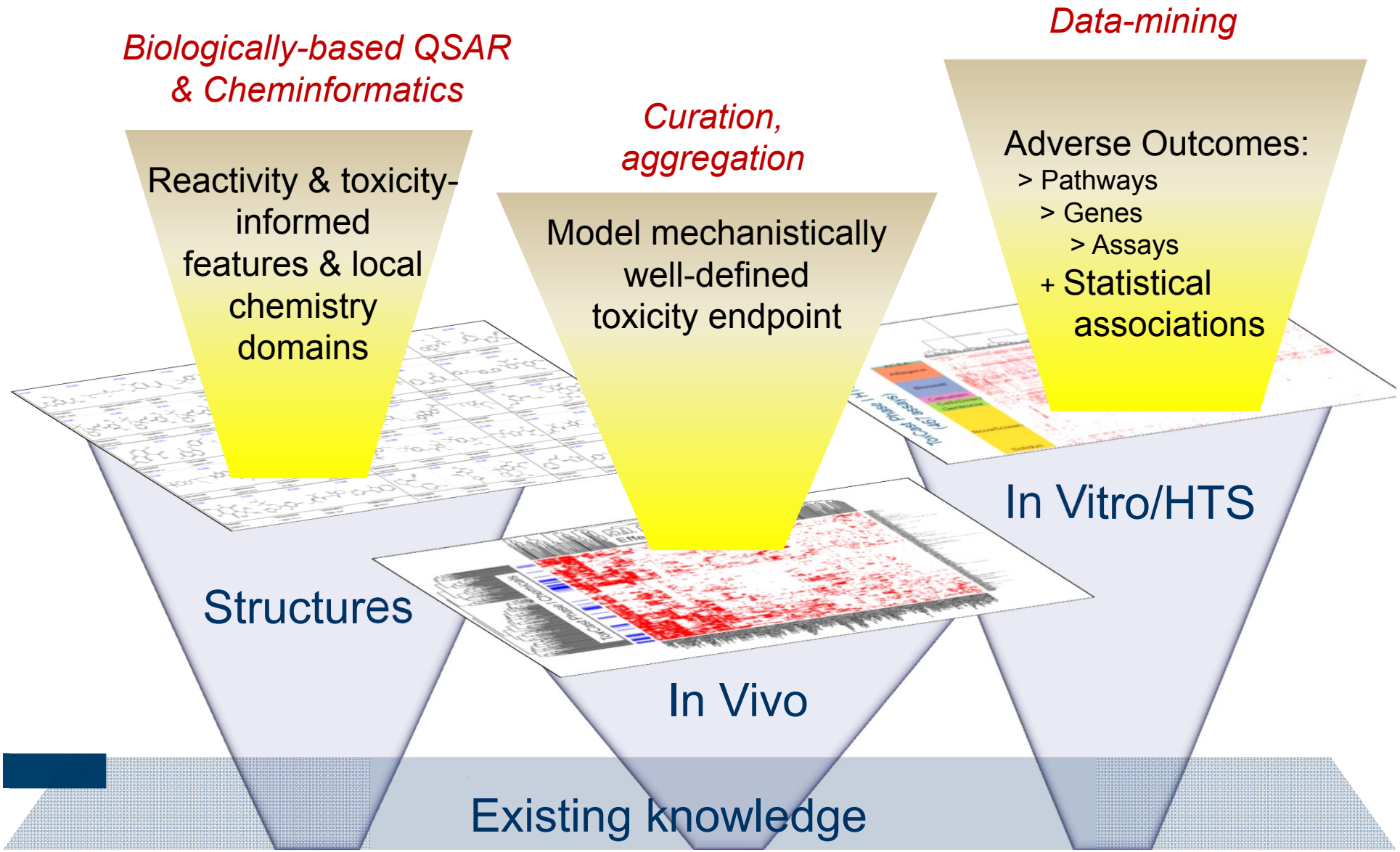
Toxicity Prediction Challenge

Chemical library serves to probe in vitro biology

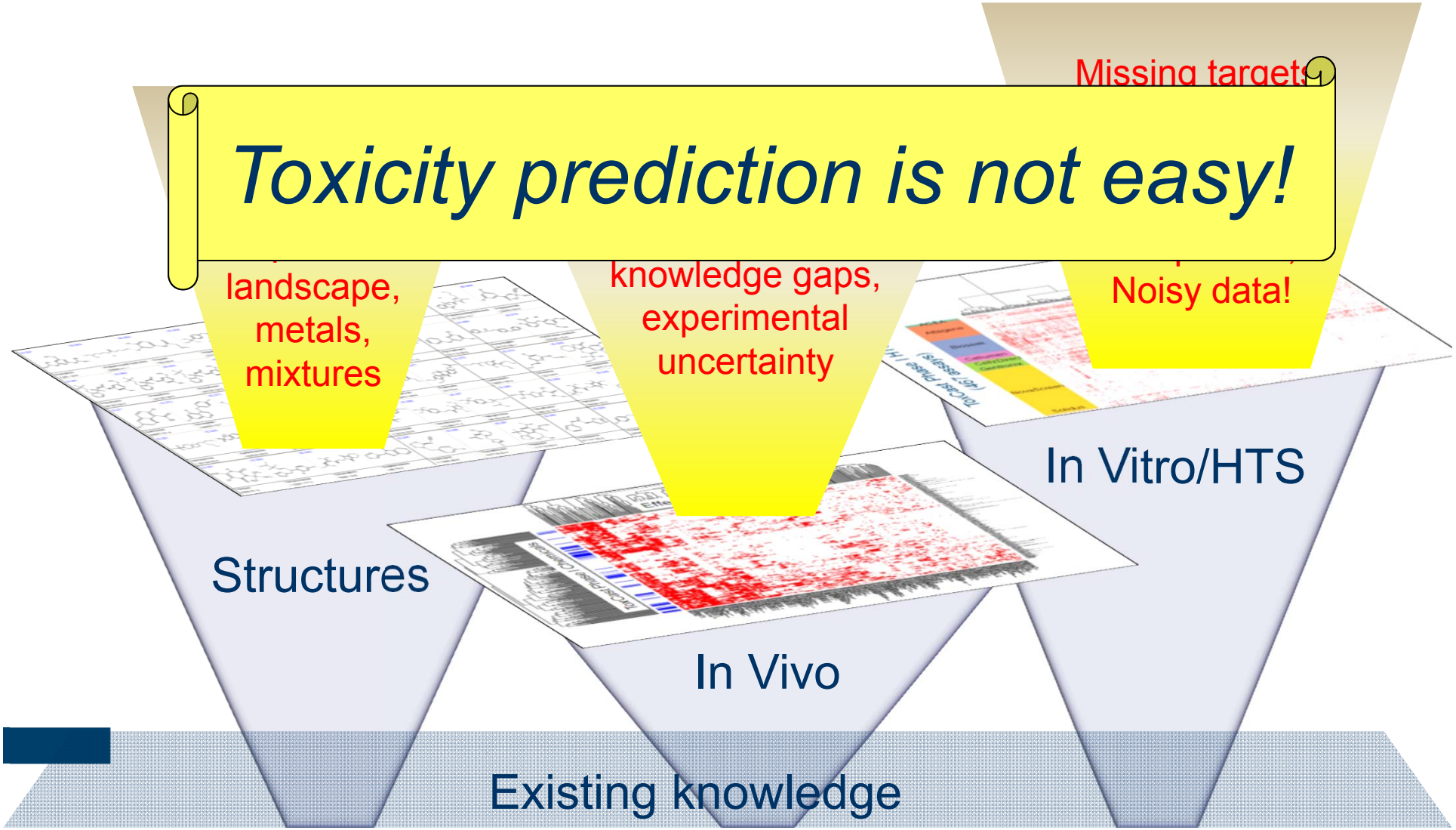
- *need sufficient diversity to sample wide range of target interactions, pathways, and MOAs*
- *need sufficient representation of important features to enable QSAR inferences*



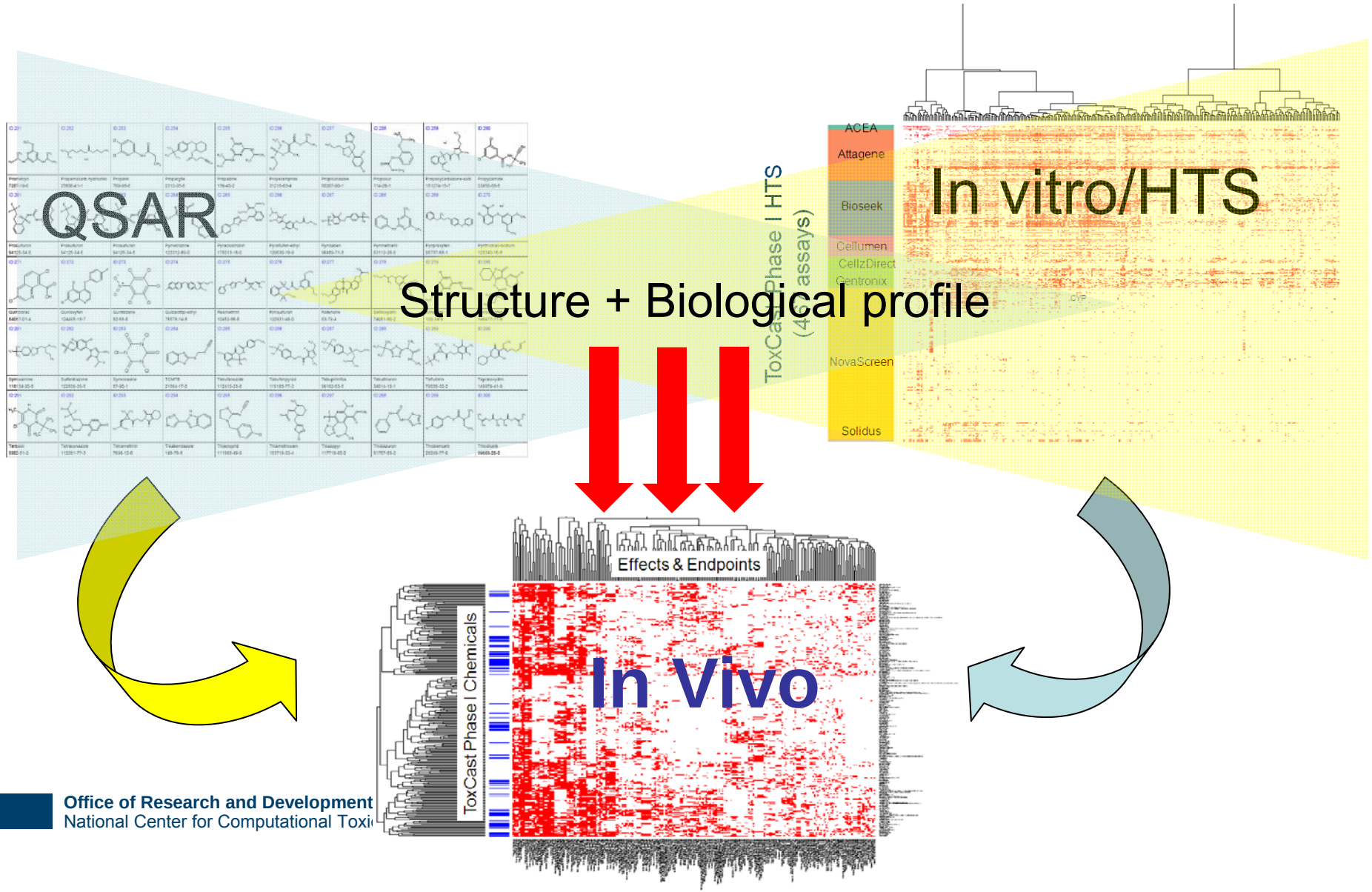
Toxicity Prediction Challenge



Toxicity Prediction Challenge



Combined Approaches



Structure vs. Bioactivity Similarity

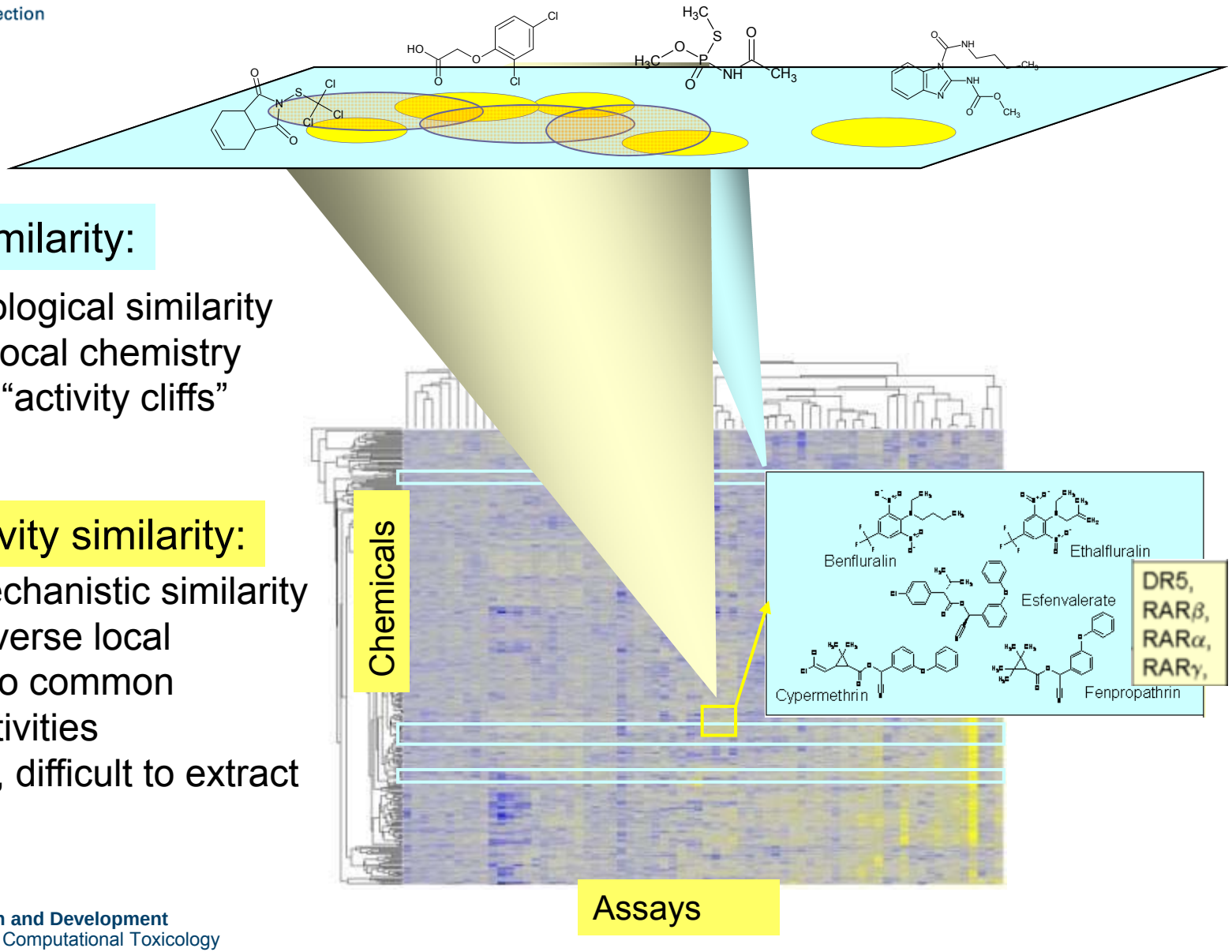
United States Environmental Protection Agency

Structure similarity:

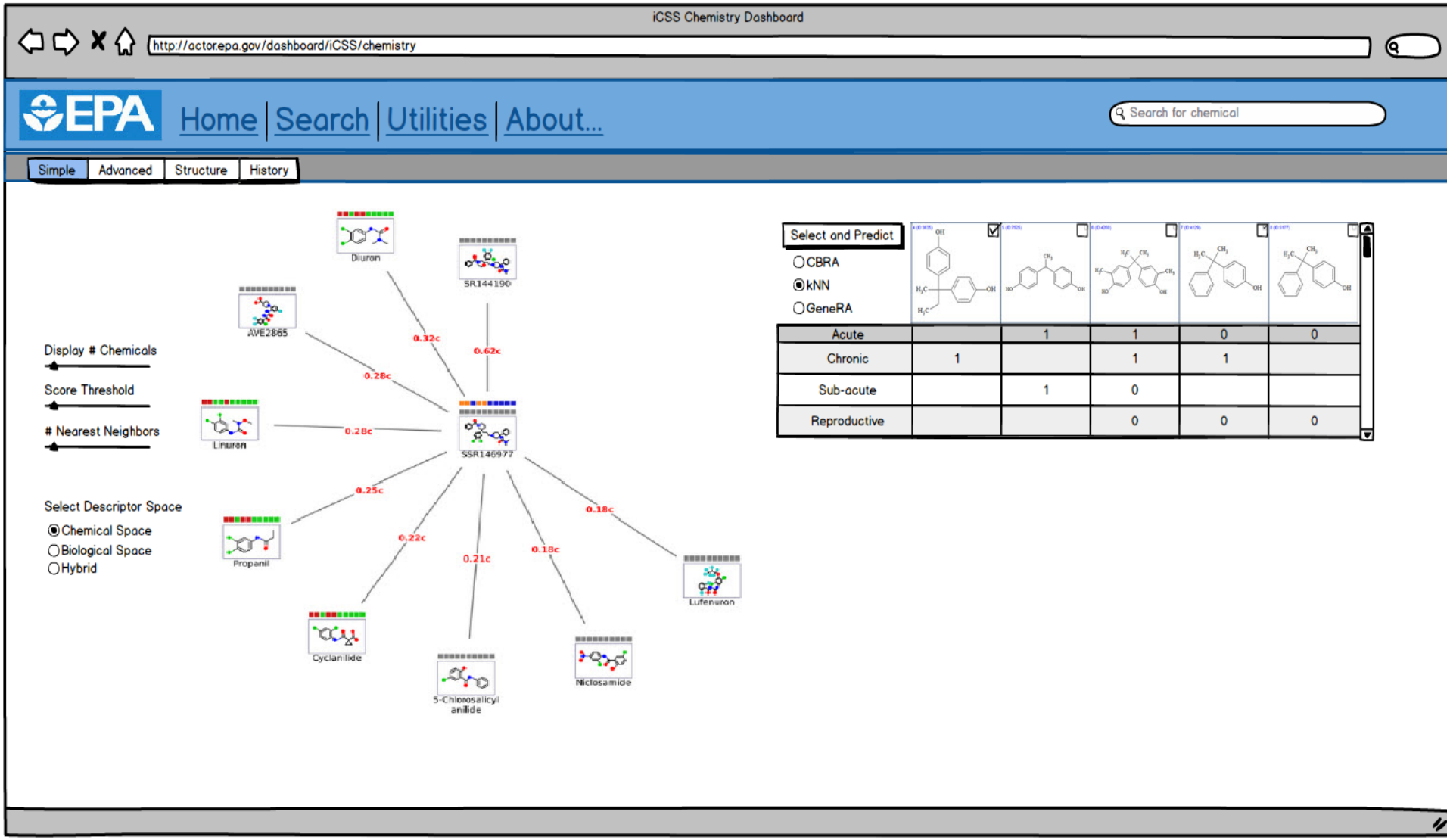
- implies biological similarity
- limited to local chemistry
- subject to “activity cliffs”

HTS bioactivity similarity:

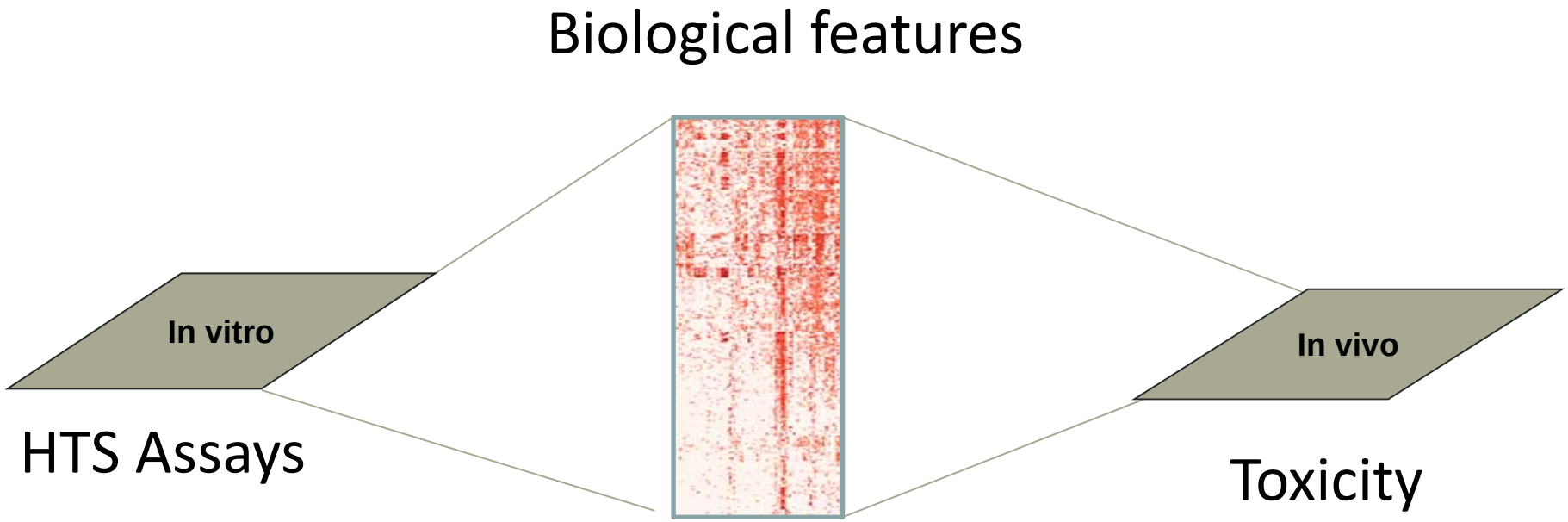
- implies mechanistic similarity
- can link diverse local chemistries to common biological activities
- noisy data, difficult to extract clear signal



Chemical “Read-Across” Explorer: Using bioactivity profiles & structure to find best analogs



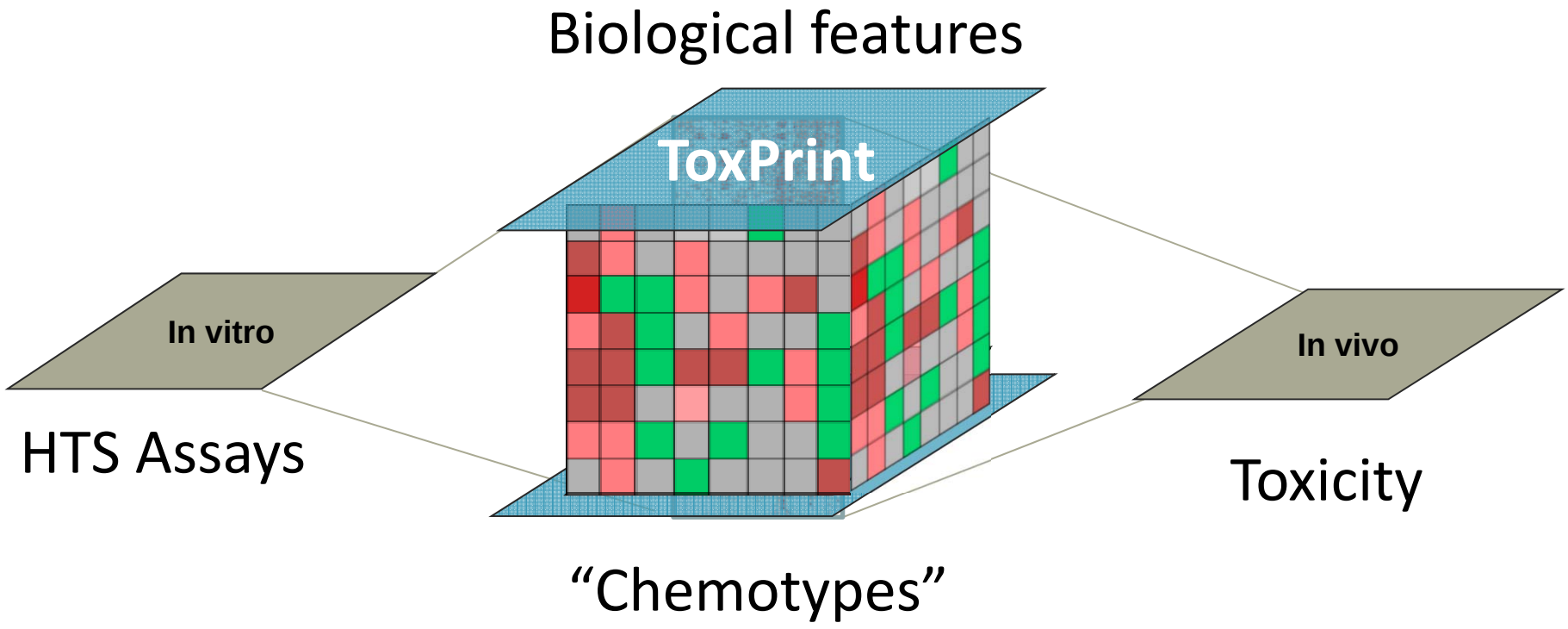
Structure-modeling using biologically informed chemical features



HTS results are used to inform feature selection, linking chemical features, or "Chemotypes" to toxicity

Slide courtesy of C. Yang

Structure-modeling using biologically informed chemical features



HTS results are used to inform feature selection, linking chemical features, or "Chemotypes" to toxicity

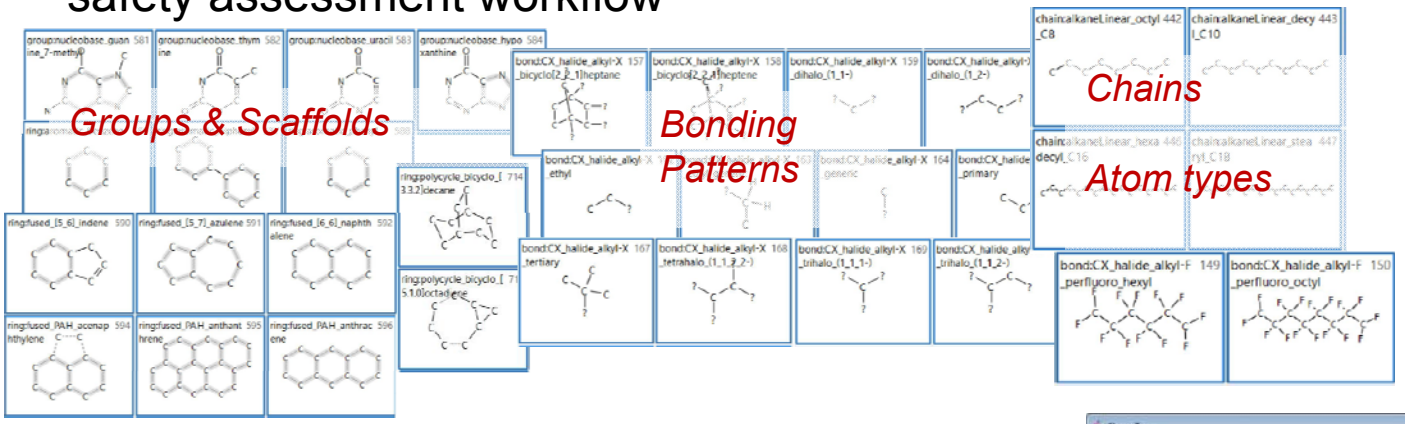
Slide courtesy of C. Yang

ToxPrints: A Public Set of Chemotypes

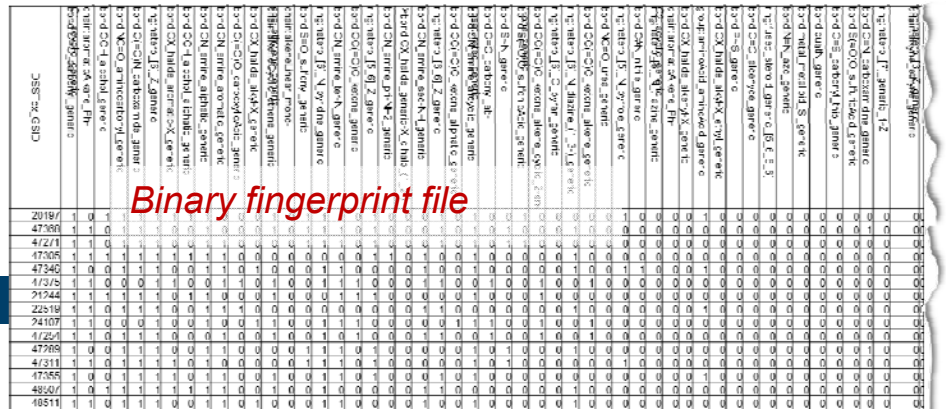
Yang, C. et al, J. *J. Chem. Inf. Model.* 55:510-28, **2015**.

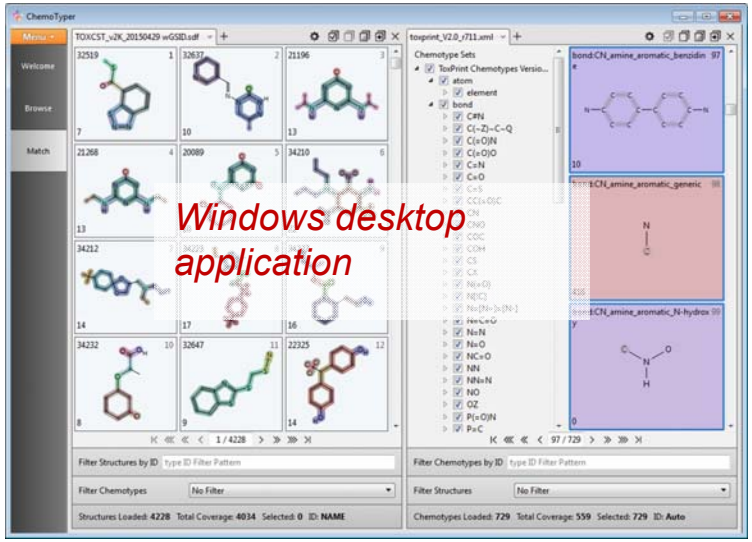
ToxPrints: http://www.toxprint.org

729 features important to EPA & FDA's "chemical exposure" landscape and safety assessment workflow

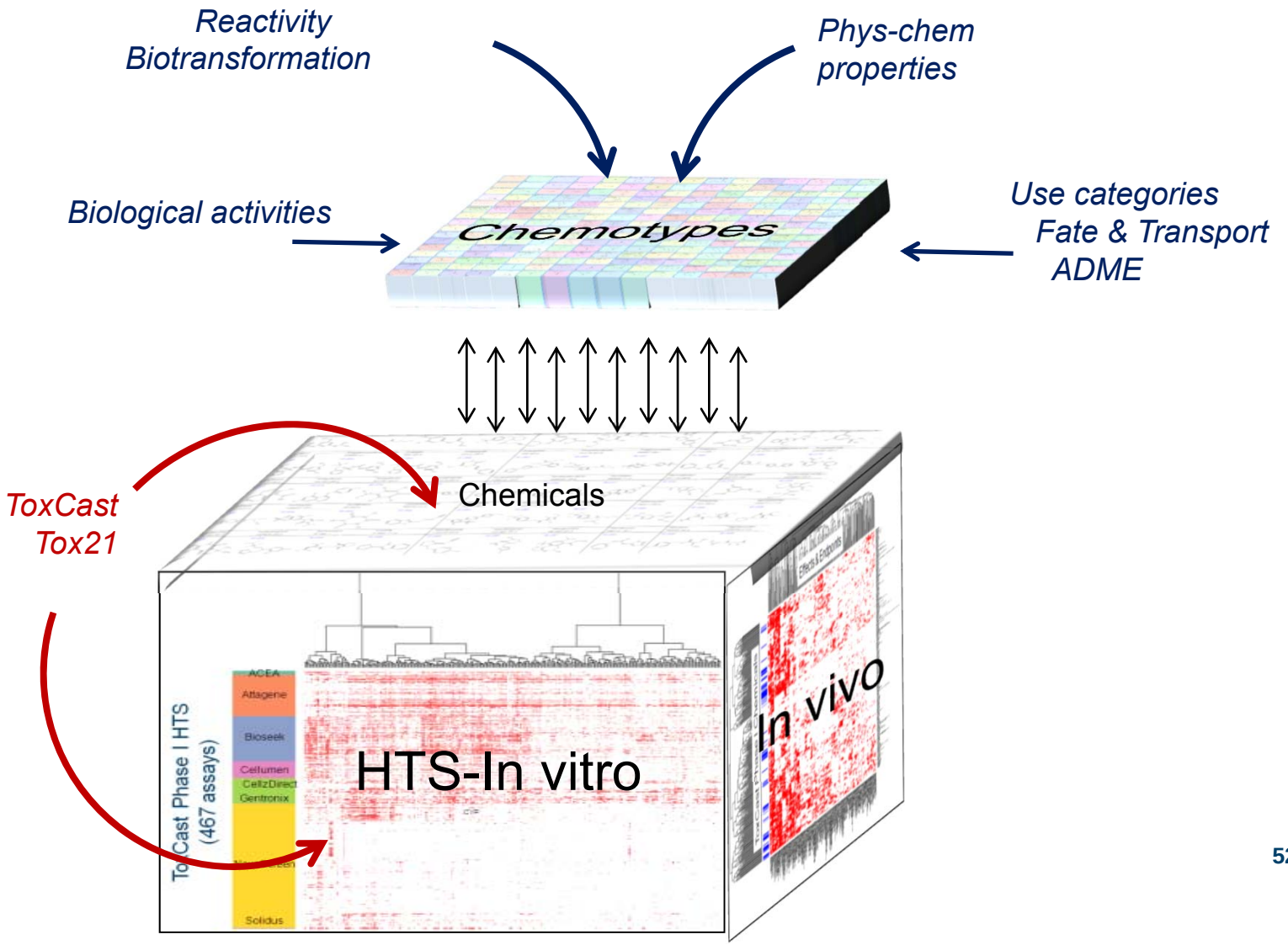


Chemotyper: http://www.chemotyper.org

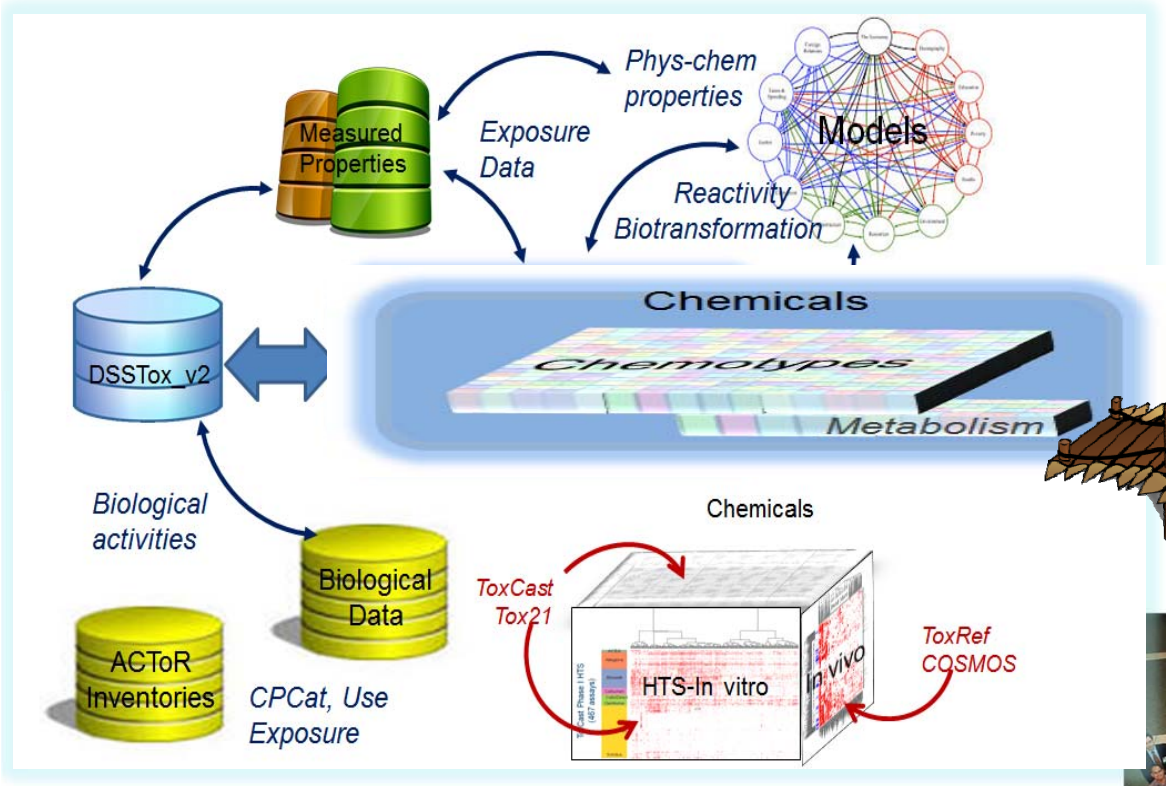




Building a public chemotype "knowledge- base"



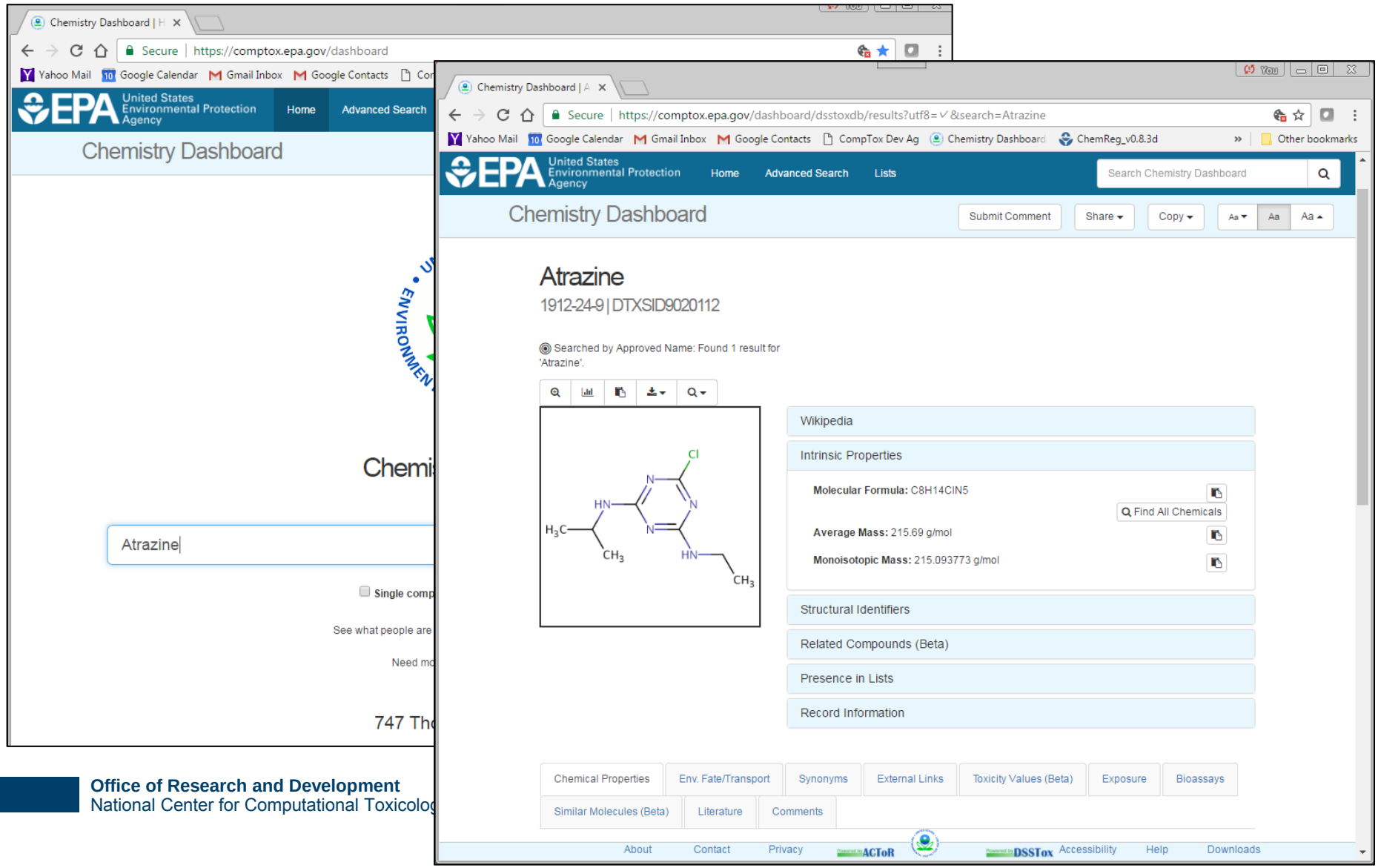
Chemotypes: Build bridges to domain experts & knowledge resources



- Toxicologists
- SAR modelers
- Chemists
- Bioinformaticists
- EPA Programs
- Risk assessors

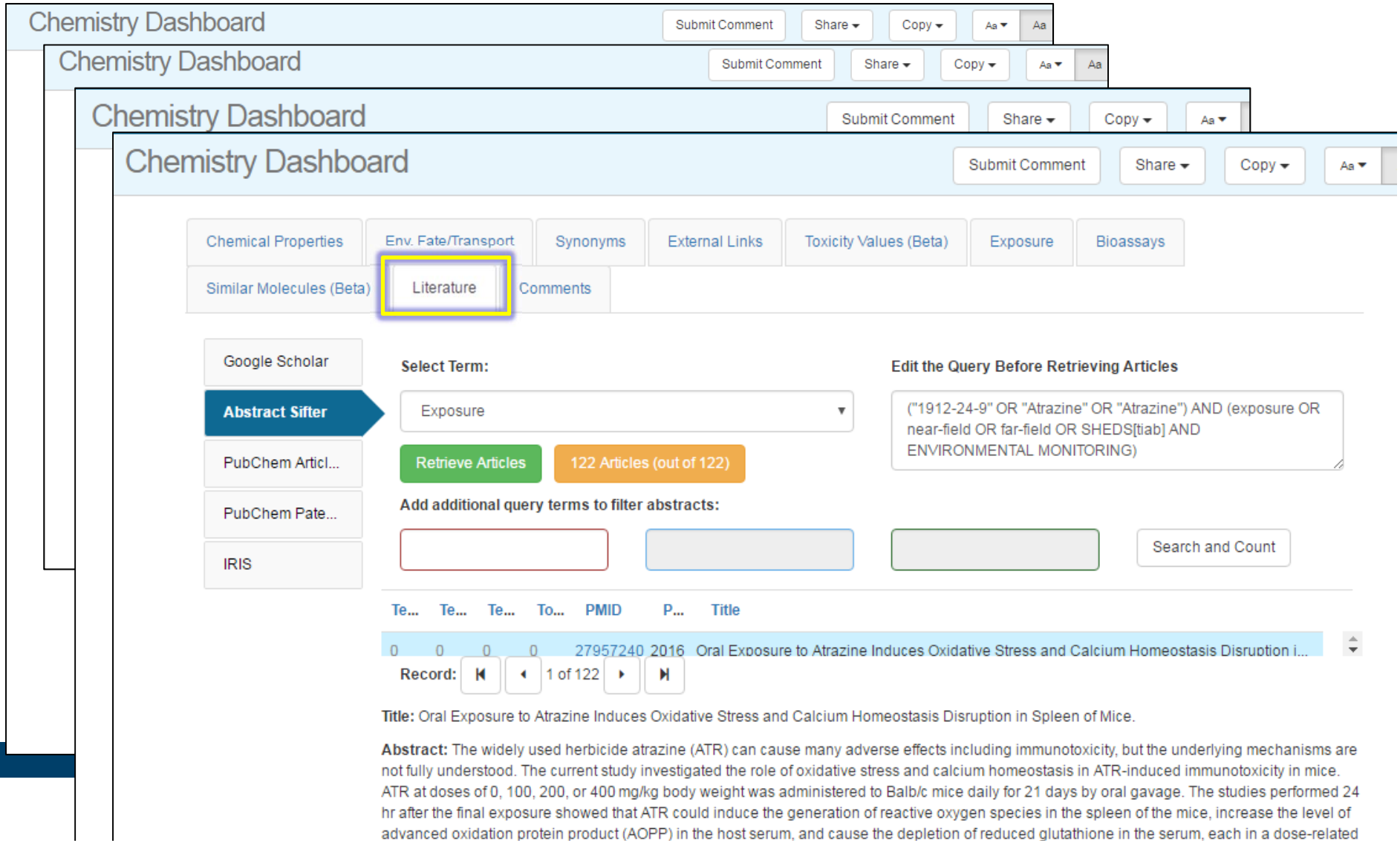
EPA's Chemistry Dashboard

https://comptox.epa.gov/dashboard



EPA's Chemistry Dashboard

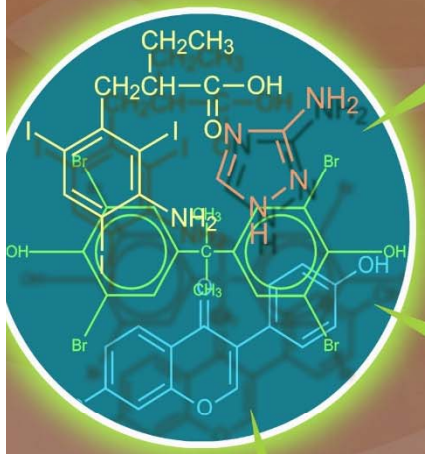
https://comptox.epa.gov/dashboard

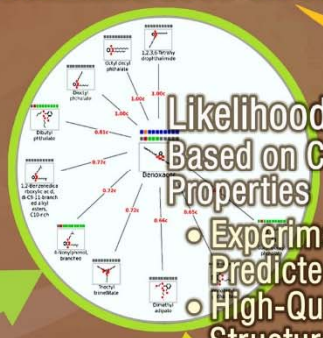


CompTox Online Dashboard

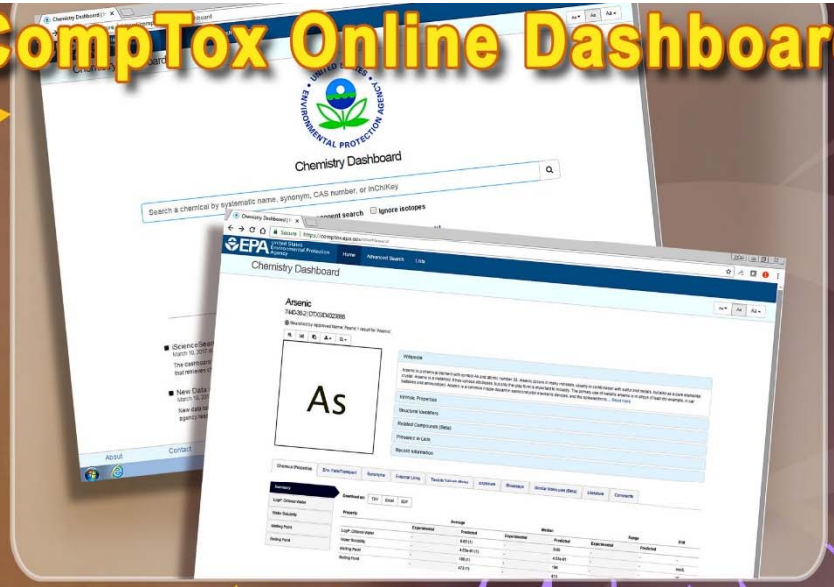
CHEMISTRY DATA

Thousands of CHEMICALS
Environment and Products





- Likelihood of Toxicity Based on Chemical Properties
  - Experimental and Predicted Values
  - High-Quality Chemical Structures



TOXICITY DATA

- High-Throughput Screening on Thousands of Chemicals and Interactions with Biological Processes
- Animal Toxicity Studies when Available

EXPOSURE DATA

- Overlay Toxicity and Exposure Data to Identify Chemicals Higher Likelihood of Potential Health Effects
  - Consumer Products
  - Predicted Total Human Exposure
  - Estimate Dose Values for Various Populations

SUPPORT TSCA ACTIVITIES

CORPORATE

CompTox Online Dashboard

Already IN USE

ENDOCRINE DISRUPTION

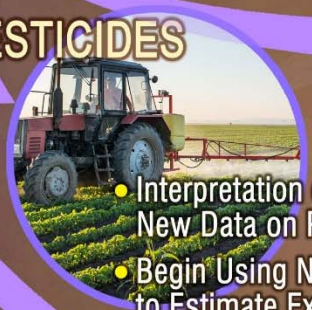
- OLD: 50-100 Chemicals per Year at Approximately $1M Each
- NEW: 1800 Chemicals in 3 Years at Approximately $30K Total

NEAR-TERM USE

CONTAMINATED SITE EVALUATION--RapidTox

- Data-Poor Chemicals Found on Site
- Integrate Related Chemical Properties Toxicity and Exposure

PESTICIDES



- Interpretation of New Data on Pesticides
- Begin Using New Models to Estimate Exposure and Dose

SUPPORT TSCA ACTIVITIES

CORPORATE

ThermoFisher SCIENTIFIC, Unilever, DOW

INTERNATIONAL

IMAP, ACCELERATED ASSESSMENT OF INDUSTRIAL CHEMICALS IN AUSTRALIA, Health Canada

STATES

- CALIFORNIA Prioritization for Biomonitoring Pesticides
- MINNESOTA Water Contaminants

Conclusions

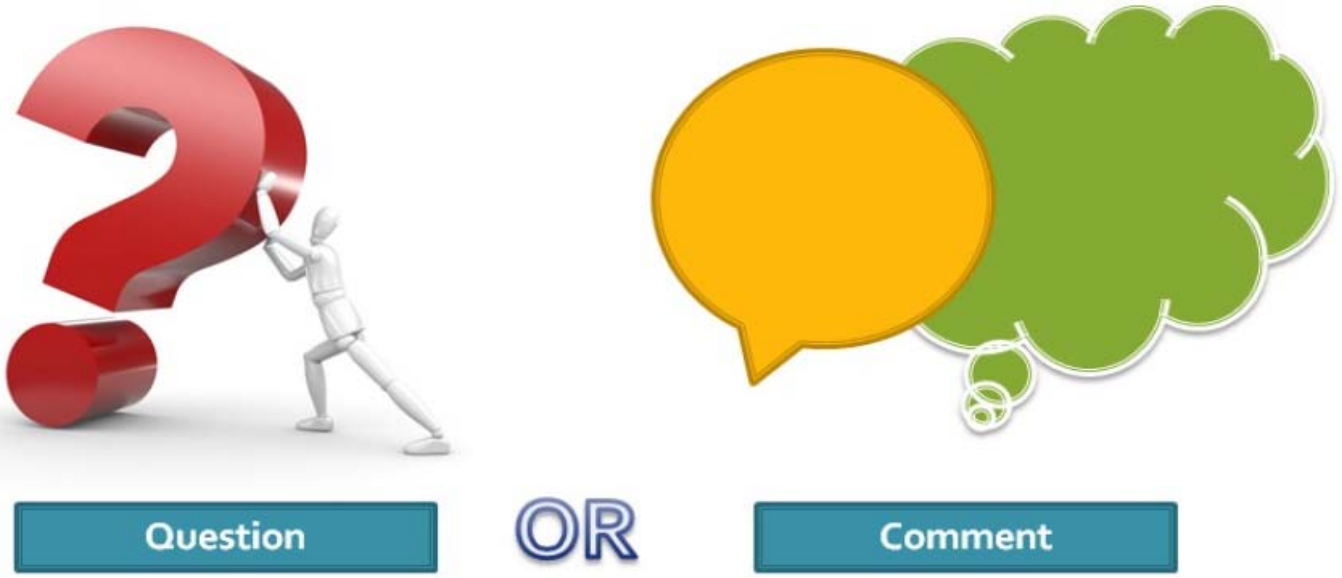
- EPA's ToxCast & Tox21 programs are helping to modernize & transform toxicity testing to be more efficient, sustainable and protective
- High priorities for program are community involvement, full public release of data, and web interfaces & tool development to support data analysis
- Much progress has been made, but plenty of opportunities remain to harness the power of chemistry towards improving predictive tox capabilities

Acknowledgements:

- EPA NCCT Chemistry Team
  - Chris Grulke (DSSTox, Chemotypes)
  - Indira Thillainadarajah (DSSTox)
  - Tony Williams (DSSTox, Chemistry Dashboard)
  - Grace Patlewicz (Read-across)
- Rest of the EPA ToxCast team >
  - Rusty Thomas (NCCT Director)
  - Kevin Crofton (NCCT Assoc. Director)
  - Keith Houck
  - Richard Judson
  - John Wambaugh
  - Tom Knudsen
  - Matt Martin

United States Environmental Protection Agency

Thank you for your attention



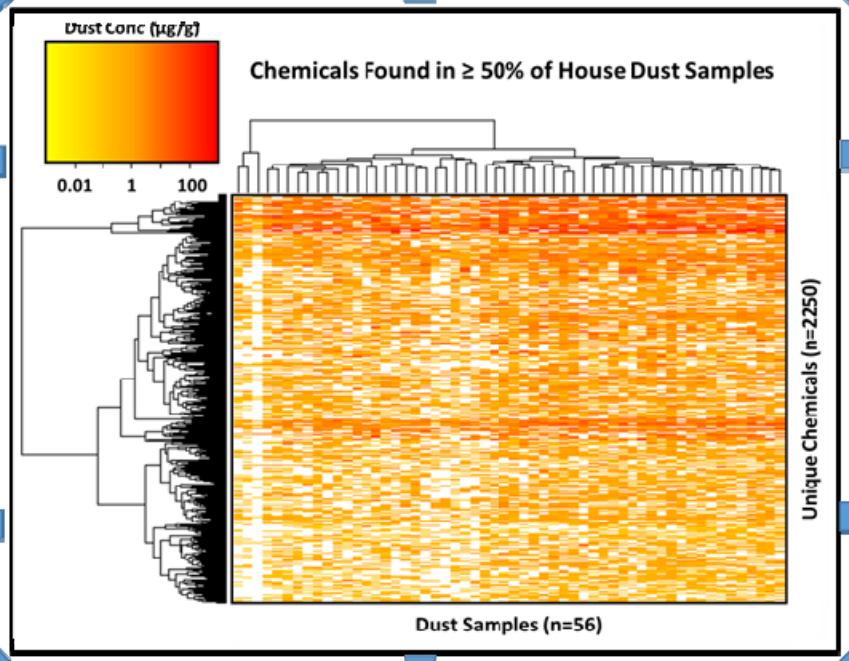
Suspect Screening Prelim Results

Piperine:
53/56 samples
Med conc=12 µg/g

Triclocarban:
54/56 samples
Med conc= 0.5 µg/g

DEET:
52/56 samples
Med conc= 2 µg/g

Propylparaben:
49/56 samples
Med conc= 2 µg/g



N-Dodecanoyl-N-methylglycine:
51/56 samples
Med conc= 40 µg/g

PFOA:
49/56 samples
Med conc= 0.5 µg/g

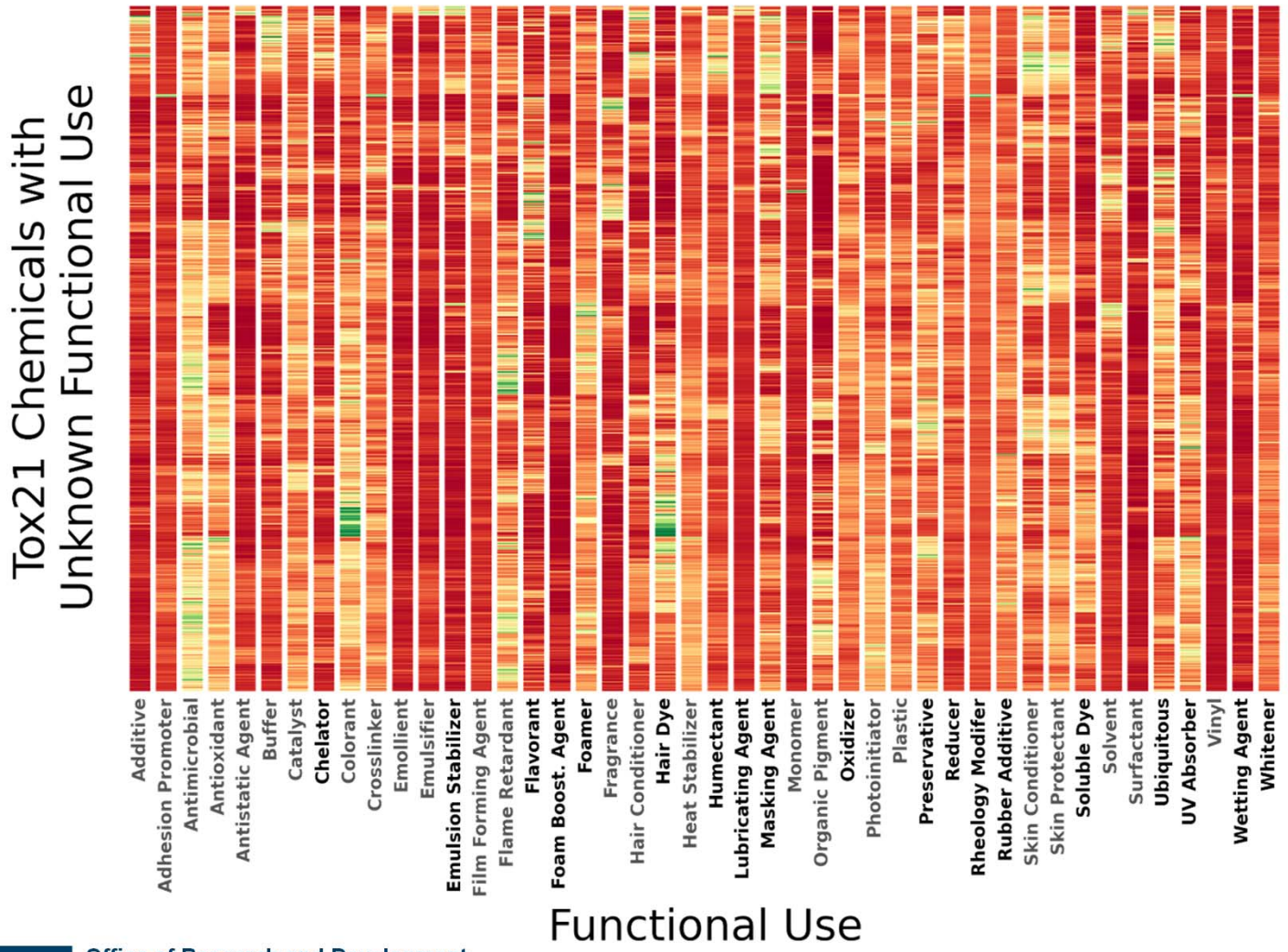
TDCPP:
40/56 samples
Med conc= 6 µg/g

Di(propylene glycol) dibenzoate:
35/56 samples
Med conc= 2 µg/g

C.I. Disperse Yellow 3:
33/56 samples
Med conc= 1 µg/g

Bisphenol S:
32/56 samples
Med conc= 0.5 µg/g

Predicting Functional Use of Chemicals



Material from Katherine Phillips

Literature Text Mining

United States Environmental Protection Agency

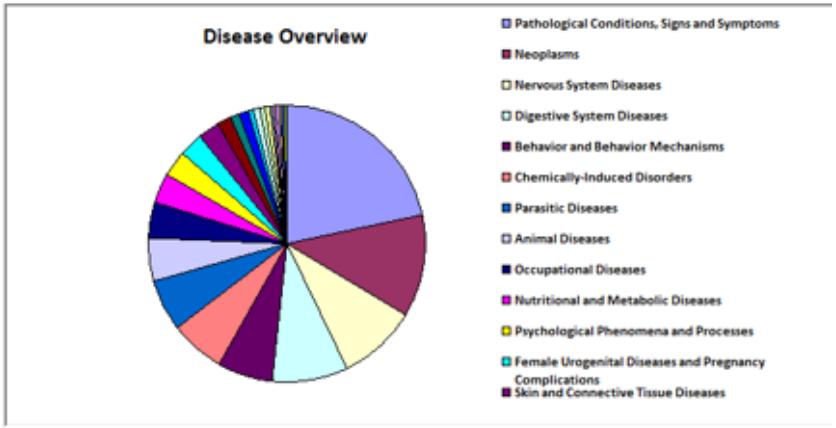
RapidTox Literature Dashboard

Pick a chemical: Dieldrin

(•) Diseases and Conditions ( ) Anatomy ( ) Processes ( ) Proteins, genes, hormones, etc. ( ) LitToxPi ( ) Risk and exposure abstract sifter

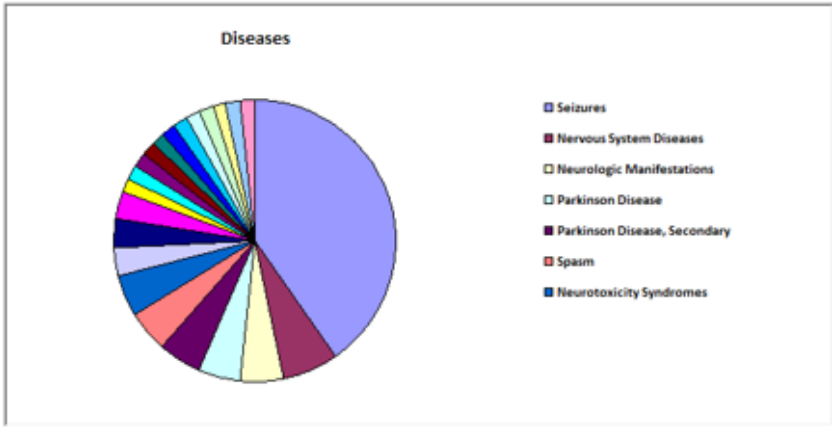
Top Level Diseases and Conditions

TopDescriptor	PMIDCt	Tox	Treat	Induced	Therapy
Pathological Conditions, Signs and Symptoms	115				
Neoplasms	64				
Nervous System Diseases	48				
Digestive System Diseases	48				
Behavior and Behavior Mechanisms	34				
Chemically-Induced Disorders	34				
Parasitic Diseases	33				
Animal Diseases	26				
Occupational Diseases	22				
Nutritional and Metabolic Diseases	19				
Psychological Phenomena and Processes	17				
Female Urogenital Diseases and Pregnancy Cc	15				
Skin and Connective Tissue Diseases	12				
Male Urogenital Diseases	9				
Congenital, Hereditary, and Neonatal Disease	6				



Diseases and Conditions

MeSH Term	PMIDCt	Tox	Treat	Induced	Therapy
Seizures	25				
Nervous System Diseases	4				
Neurologic Manifestations	3				
Parkinson Disease	3				
Parkinson Disease, Secondary	3				
Spasm	3				
Neurotoxicity Syndromes	3				
Tremor	2				
Neurodegenerative Diseases	2				
Parkinsonian Disorders	2				
Encephalomalacia	1				
Botulism	1				



Articles

Articles	Tox	Treat	Therap	Induc	Article	Articles
5351682					1969	Dieldrin: studies in a poisoned child.
14249895					1964	TOXICITY AND METABOLISM OF DIELDRIN IN RATS.
14340058					1965	EFFECTS OF DIELDRIN, PICROTOXIN AND TELODRIN ON THE METABOLISM OF AMMONIA IN BRAIN.
1797280					1991	Human aldrin poisoning.
20211759					2010	Reevaluation of the developmental toxicity of dieldrin by the use of fer[illegible]
2410825					1985	Effects of lindane-type insecticides in mammals: unsolved problems

Slide courtesy of Nancy Baker