United States Environmental Protection Agency

EPA's ToxCast Program for Predicting Hazard and Prioritizing the Toxicity Testing of Environmental Chemicals

December 12, 2007
Society for Risk Analysis 2007 Annual Meeting, Dec 9-12 2007, San Antonio, TX

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http://www.epa.gov/comptox/toxcast

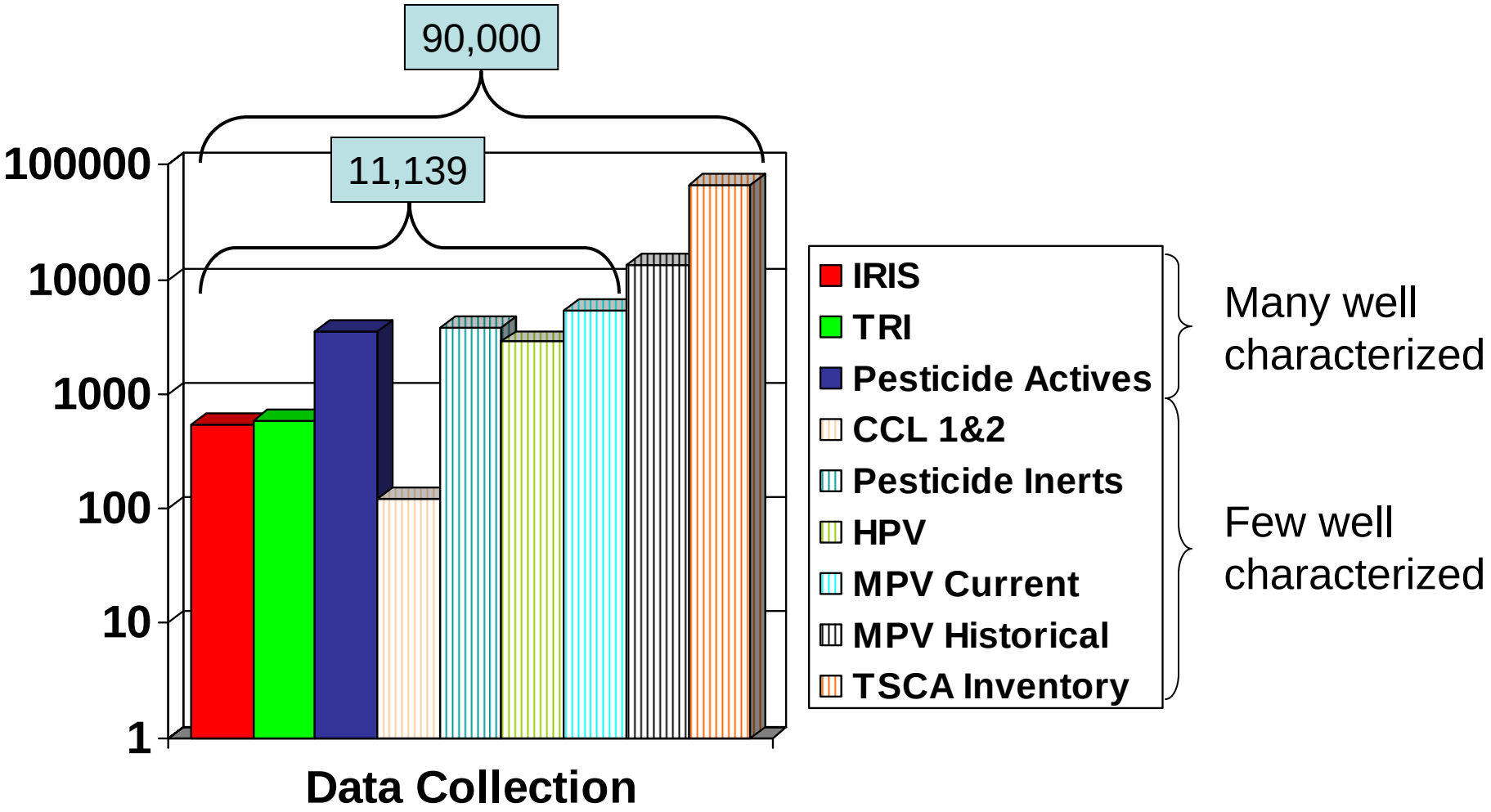
Office of Research and Development
National Center for Computational Toxicology

United States Environmental Protection Agency

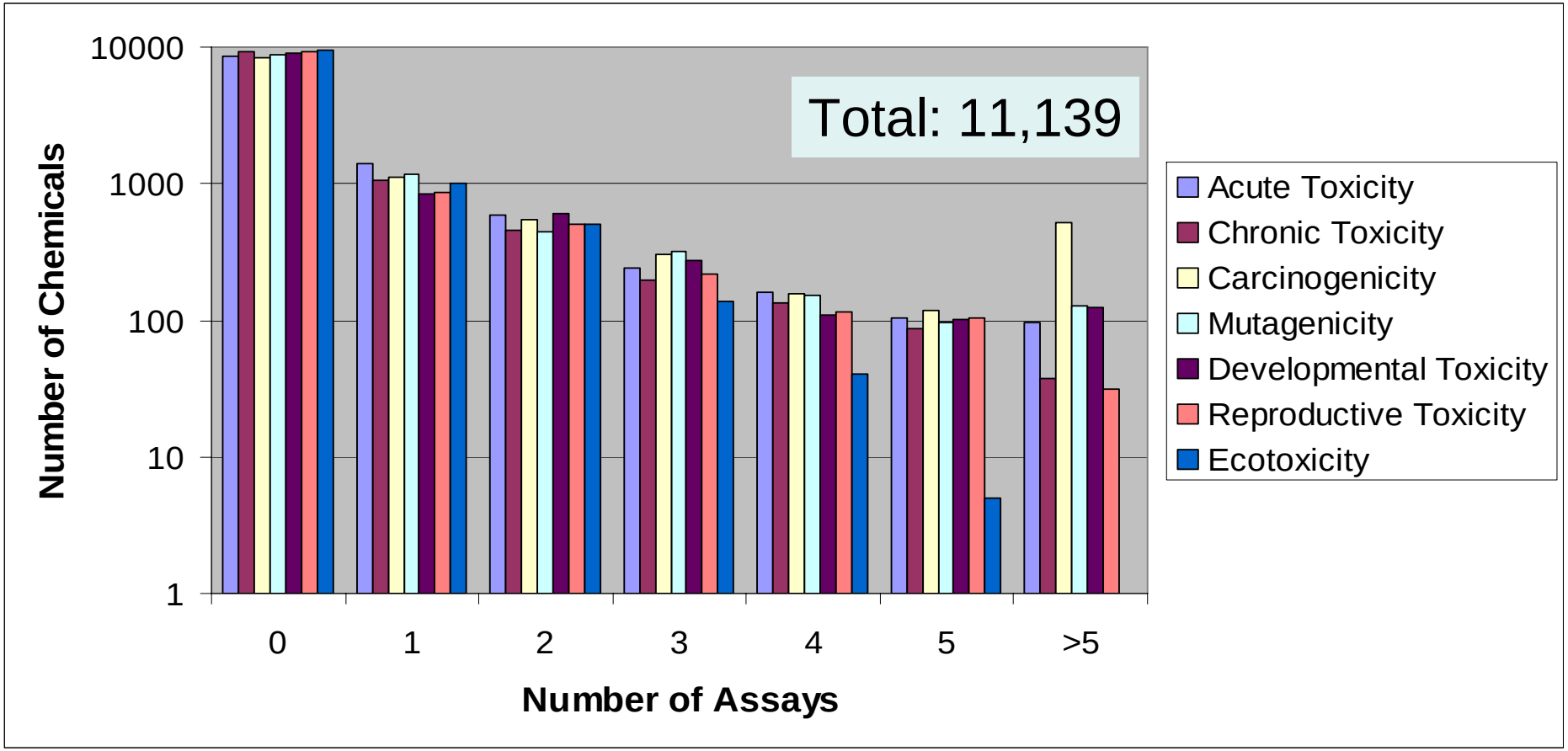
ToxCast Background

- Project of US EPA's National Center for Computational Toxicology
- Formulated to address chemical screening and prioritization needs
- Screening approach based on experience of the pharmaceutical industry
- Comprehensive use of a broad range of HTS technologies
- Phased approach to evaluate utility
- Committed to stakeholder involvement and publication of data

The Problems: Too Many Chemicals

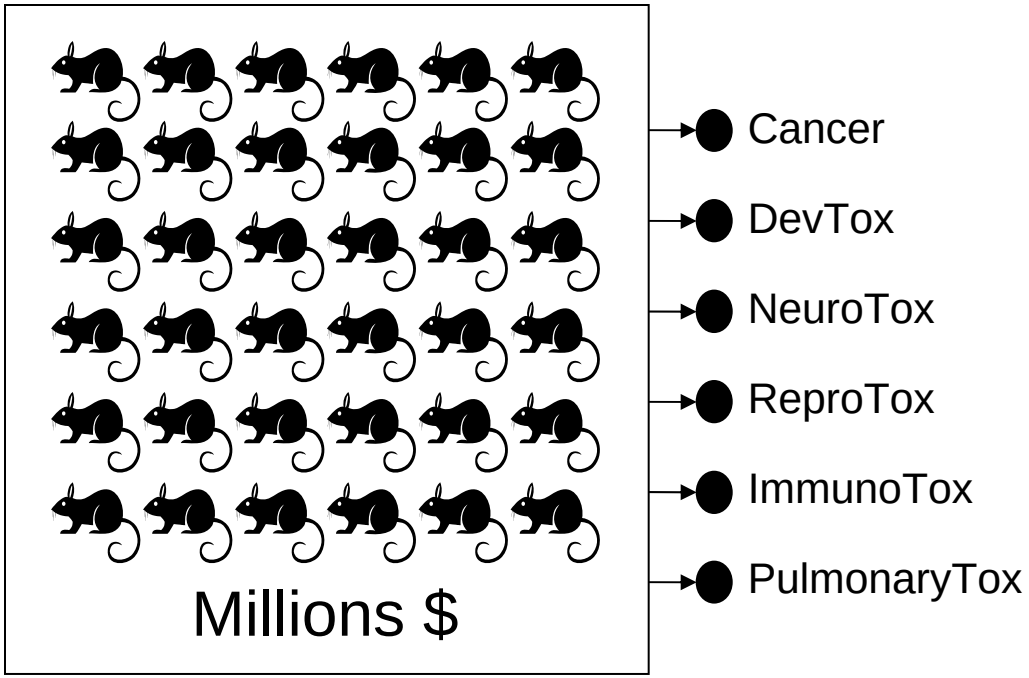


The Problems: Too Little Data



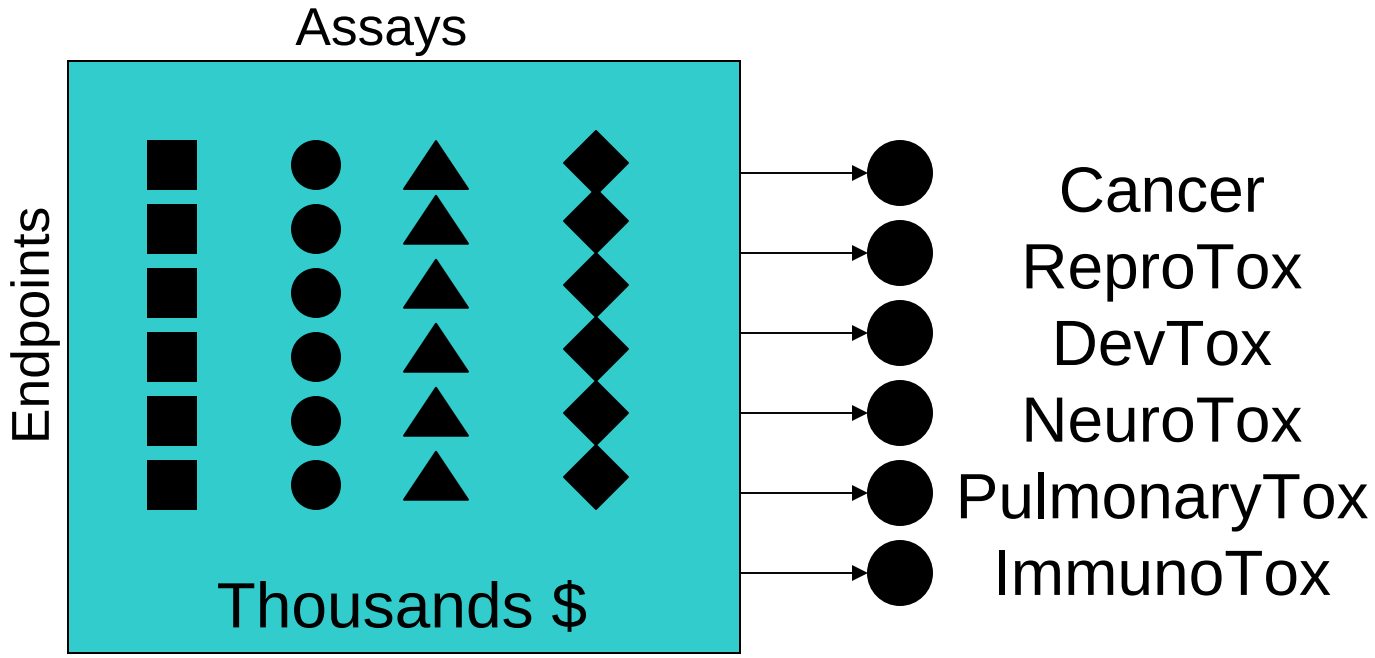
<10% have data for most tests

The Problems: Too High A Cost



The Solution: ToxCast™

Derive classifiers or signatures from hundreds of HTS, HCS and genomics assays to predict hazard…



… and use these toxicity predictions for prioritizing further testing of environmental chemicals.

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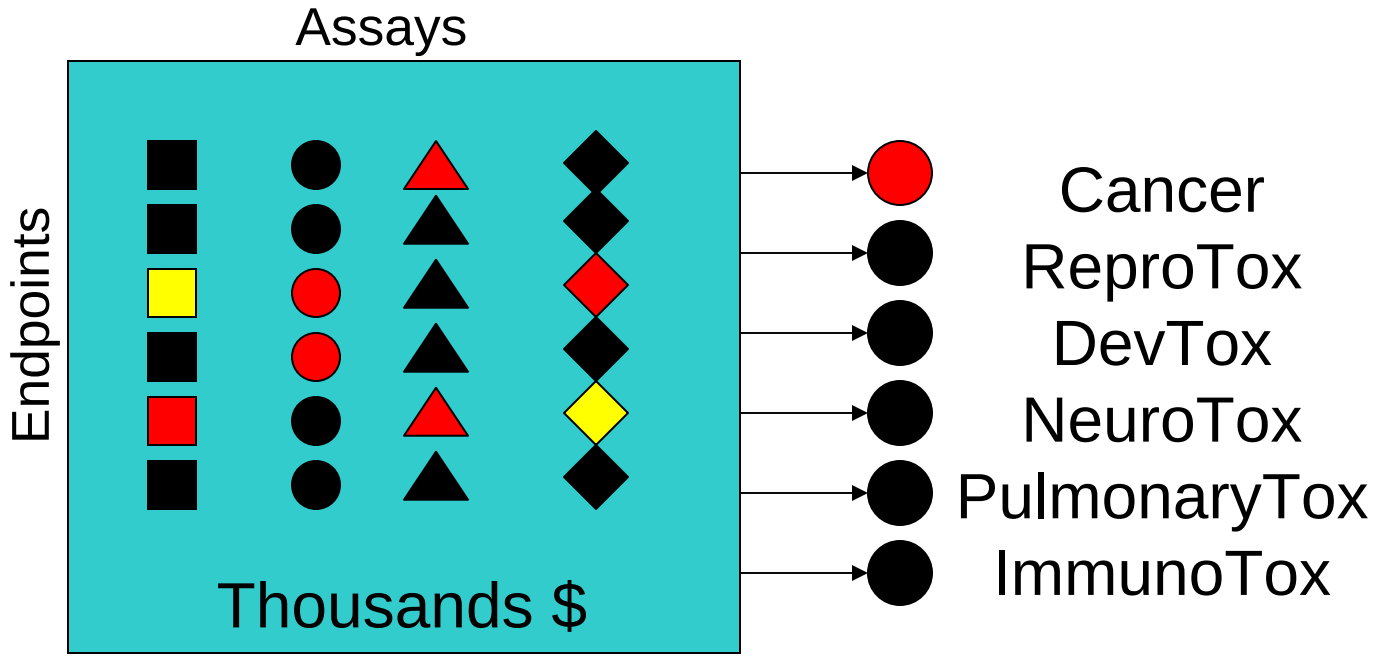

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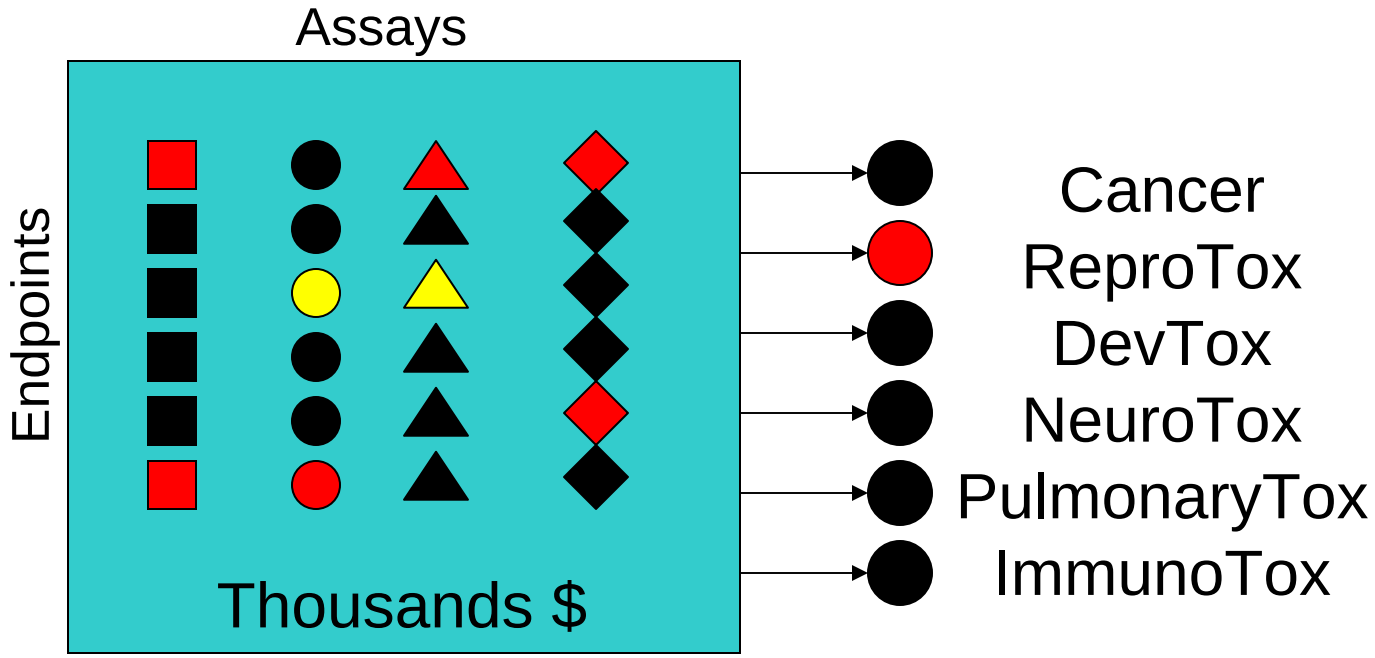

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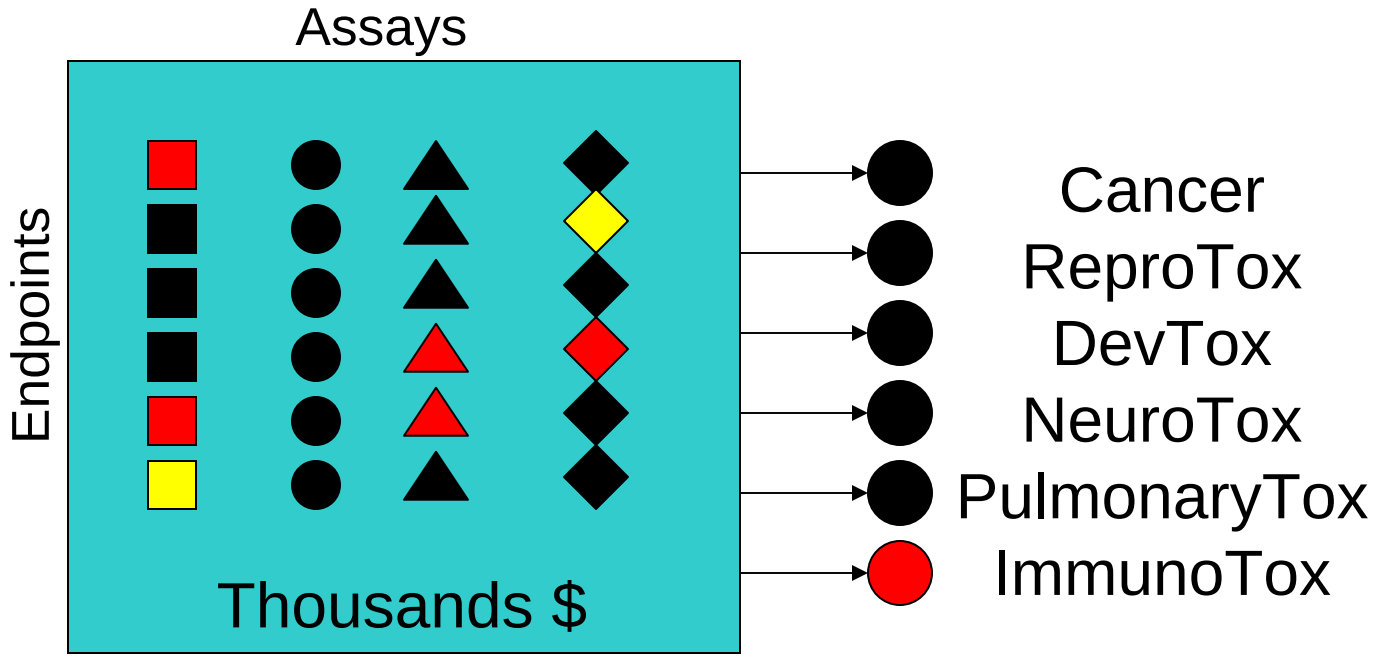

… and use these toxicity predictions for prioritizing further testing of environmental chemicals.

Phased Development of ToxCast Program

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
I	>300	Data Rich (pesticides)	Signature Development	>400	$20k	FY07-08
II	>1000	Expanded Structure and Use Diversity	Evaluation and Extension	>300	$15-20k	FY08-09
III	Thousands	Data poor	Prediction and Prioritization	>300	$10-15k	FY10-12

- Delivers an affordable, science-based system for categorizing chemicals
- Increasing confidence as database grows
- Identify potential mechanisms of action
- Refine and reduce use of animals in hazard identification and risk assessment

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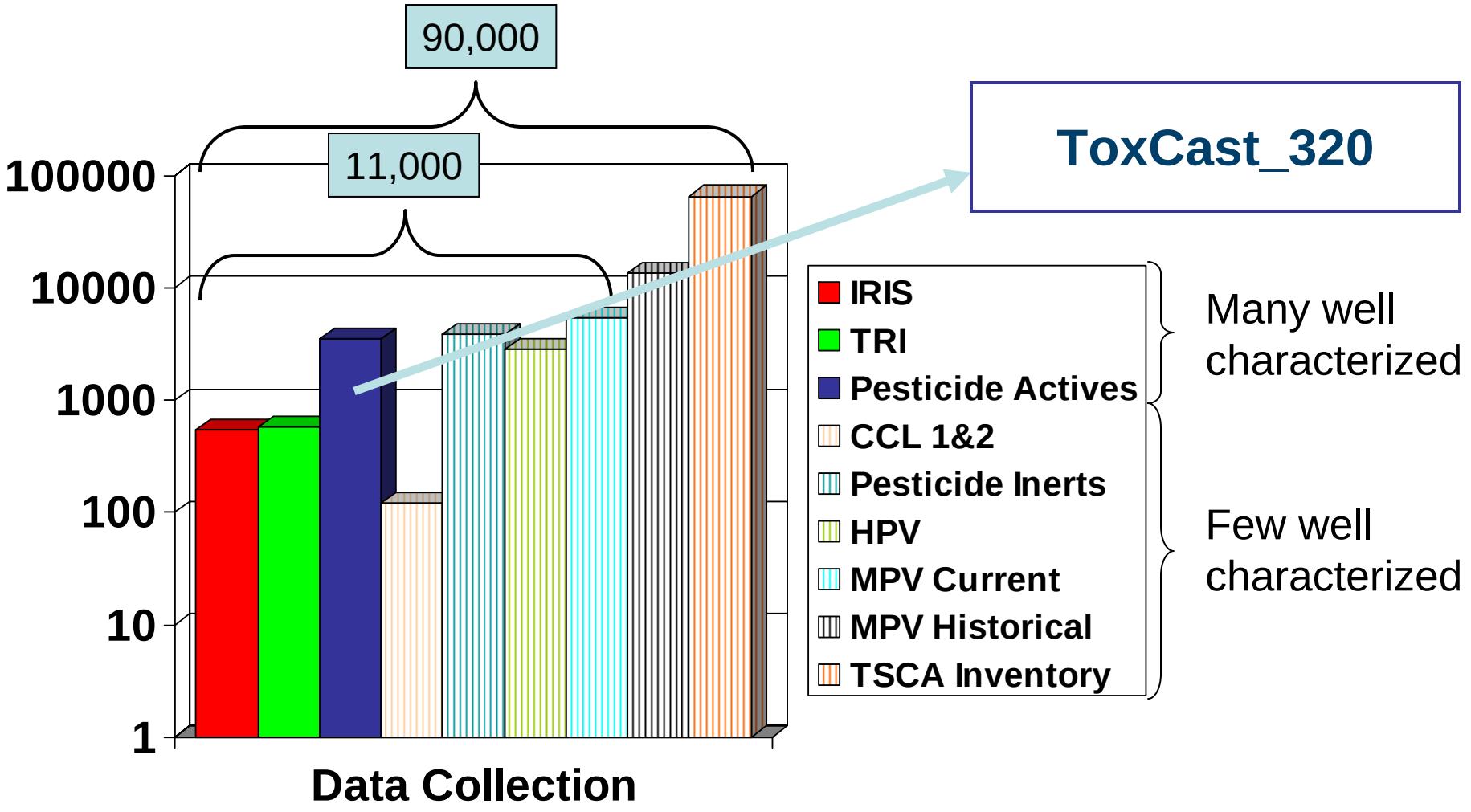
Components of ToxCast

1. **Chemicals = ToxCast_320 (Phase I); replicates for QC**
2. **Toxicity data = ToxRefDB**
3. **HTS data = 265 assays ; multiple endpoints and concentrations ; millions of datapoints; blinded chemical set**
4. **Predictive modeling = ACToR and ToxMiner**
5. **Toxicity predictions = specific to test, target and type**
6. **Chemical prioritizations = application of predictions with US EPA Program Offices and international partners**

ToxCast Phase I Chemicals



Chemical Classes Investigated in ToxCast Phase I

291 pesticide actives with complete toxicity datasets
30 Carbamates (plus one metabolite)
33 Organophosphates (plus several metabolites)
12 Pyrethroids
12 Triazines (plus one metabolite)
17 Azole Fungicides (plus one metabolite)
13 Organochlorines
7 Phthalates (and several metabolites)
14 HPVs, 11 HPV challenge
55 of 73 chemicals proposed for Tier 1 EDSP

ToxRefDB

Reference In Vivo Toxicology Database

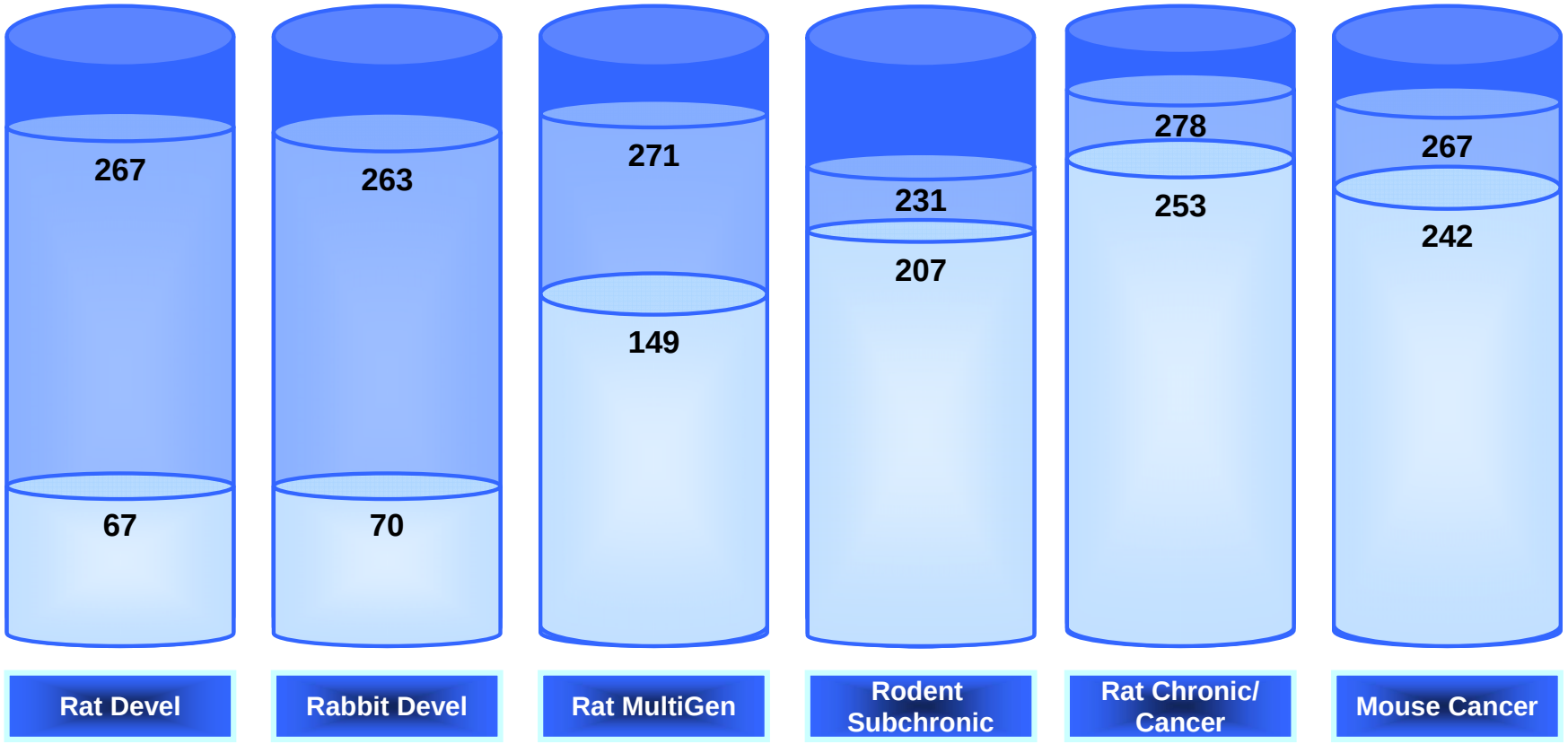
- Office of Pesticide Programs - Data Evaluation Records (DER)
  - 20 years of toxicology data from registration studies
  - Complete data package for >300 chemicals
    - SubChronic, Chronic, Cancer, Repro, DevTox
  - High quality, comparable data with significant QC
  - Incorporate into relational model with expert-developed controlled vocabularies
  - Integrate other primary toxicology sources (NTP, OPPT, European agencies)

First effort to capture, tabulate and mine this unique resource

ToxRefDB Data Entry Status

ToxCast Chemicals	320
Unique Chemicals	308
Pesticide Actives	291

Current as of November 1, 2007



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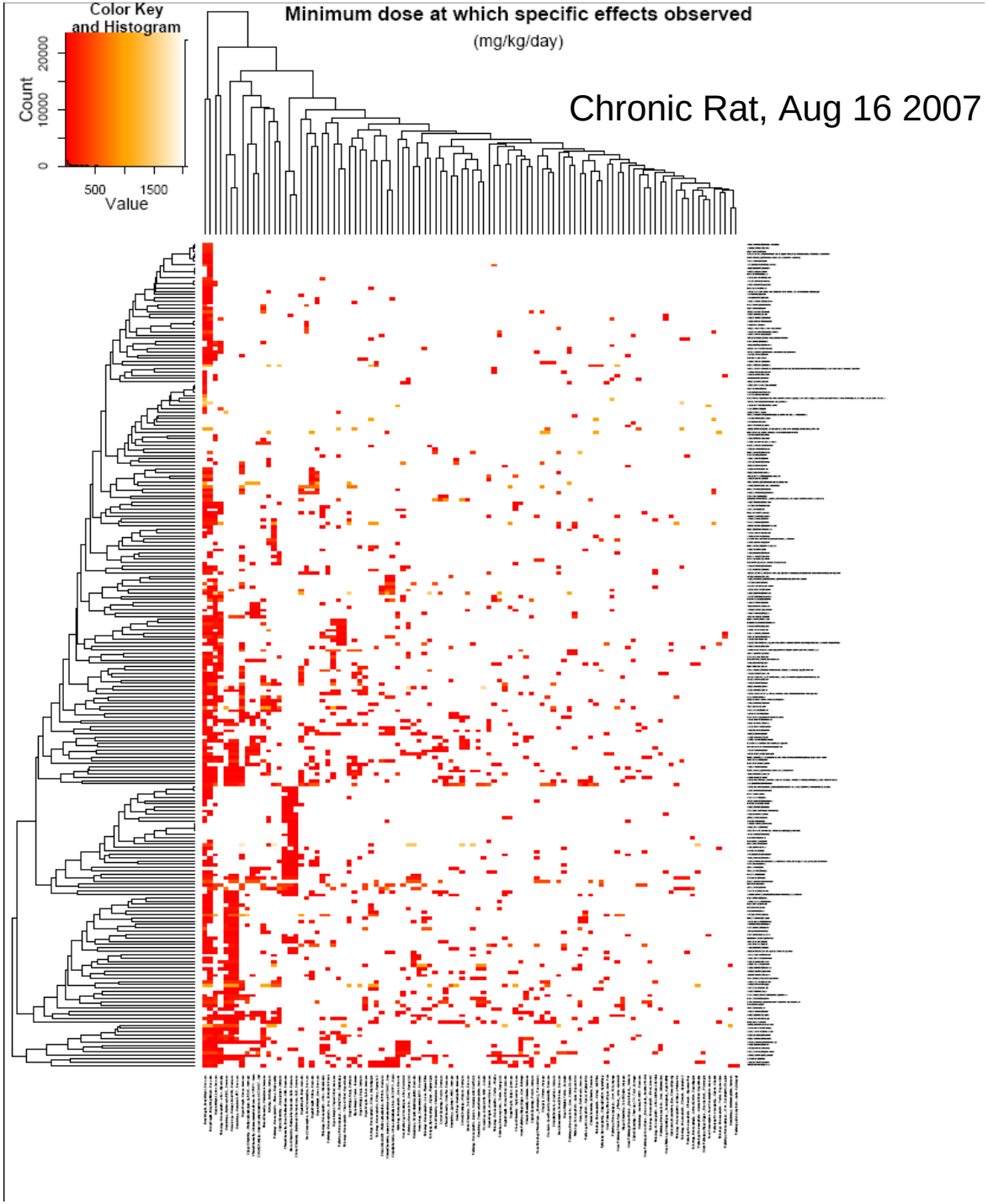
ToxRefDB

- Novel relationships between effects can be observed and calculated
- Formats toxicity data in manner conducive to linking HTS and genomic data
- Unlimited means for looking at toxicity data
  - By chemical(s)
  - By study type(s)
  - By effect(s)
  - By species
  - By dose



INITIAL 50 CHRONIC/SUB-CHRONIC ENDPOINTS FOR PREDICTION DERIVED FROM TOXREF

- selected for relevance, potency and power
- cancer and non-cancer effects and groups of effects
- target organs include liver, kidney, lung, mammary gland, testes, thyroid
- expansion to reproductive and developmental tests underway

ToxCast Phase I Systemic Endpoints	# of Chemicals (Total: 308)						*Missing Studies: 32(Chronic Rat) 52(Cancer Mouse) 84(90-day Rat)*
	Chronic Rat		Cancer Mouse		90-day Rat		
Effect Group/Endpoint	-	+	-	+	-	+	**Effect Group Description**
Anemia	194	74	221	35	140	84	Anemia diagnosis terms including hematocrit decrease
Anemia_NonSpecific	145	123	170	86	85	139	Anemia diagnosis terms and all associated hematological parameters
BodyWeightDecrease							
Brain_AllPathology							
Cholesterol_Increase							
Cholinesterase_Inhibition							
Kidney_AllProliferativeLesions							
Kidney_ChronicProgressiveNephr							
Kidney_Hyperlasia							
Kidney_InjuryBiomarkers							
Kidney_NeoplasticPathology							
Kidney_NonNeo_Pathology							
Kidney_PapillaryNecrosis							
Kidney_PreNeo_Pathology							
Kidney_TubuleNecrosisRegenerati							
Kidney_TubuleNeoplasms							
Kidney_WeightIncreaseEnlarged							
Liver_AllHepatocellularProliferative							
Liver_BiliaryNeoplasms							
Liver_Biliary_InjuryBiomarkers							
Liver_HepatocellularNeoplasms							
Liver_Hepatocyte_InjuryBiomarker							
Liver_Hypertrophy							
Liver_Injury_Biomarkers							
Liver_KupfferPathology							
Liver_NecrosisApoptosis							
Liver_NonProlBiliaryPathology	254	14	249	7	219	5	Non-neoplastic non-proliferative biliary pathology of the liver
Liver_NonProlHepatocellularPathology	159	109	155	101	122	102	Non-neoplastic non-proliferative hepatocyte pathology of the liver (default: hepatocyte)
Liver_ProlBiliaryPathology	245	23	237	19	215	9	All non-neoplastic biliary proliferative lesions
Liver_ProlHepatocellularPathology	228	40	215	41	212	12	All non-neoplastic hepatocellular proliferative lesions (default: hepatocyte)
Liver_WeightInc_Enlarged	145	123	118	138	77	147	Liver weight increase or grossly enlarged
Lung_Neoplasms	267	1	239	17	224	0	Malignant and benign neoplasms of the lung
Lung_NonProliferativeLesions	245	23	247	9	217	7	Non-neoplastic non-proliferative lesions of the lung
Lung_ProliferativeLesions	262	6	236	20	224	0	Non-neoplastic and neoplastic proliferative leasions of the lung
MammaryGland_AllPathology	245	23	253	3	221	3	Non-neoplastic and neoplastic pathology of the mammary gland
MammaryGland_Neoplasms	253	15	254	2	223	1	Malignant and benign neoplasms of the mammary gland
Stomach_AllPathology	241	27	234	22	212	12	Non-neoplastic and neoplastic lesions of the stomach
Stomach_Hyperplasia	260	8	248	8	216	8	Hyperplasia of the stomach at any site or cell type
Stomach_Inflammation	263	5	256	0	222	2	Inflammation of the stomach at any site or cell type
Stomach_Mineralization	261	7	255	1	224	0	Mineralization of the stomach at any site or cell type
Stomach_Neoplasms	266	2	255	1	224	0	Malignant and benign neoplasms of the stomach
Testes_AnyLesion	224	44	231	25	214	10	Non-neoplastic and neoplastic pathology of the testes
Testes_Atrophy	248	20	246	10	216	8	Testicular atrophy at any site or cell type
Testes_Hyperlasia	255	13	250	6	223	1	Testicular hyperplasia at any site or cell type
Testes_InterstitialNeoplasticLesions	246	22	256	0	224	0	Interstitial cell malignant and benign neoplasms in the testes (default: interstitial)
Testes_InterstitialProliferativeLesions	239	29	250	6	223	1	Interstitial cell non-neoplastic and neoplastic proliferative lesions in the testes (default: interstitial)
Testes_WeightDecreaseReducedSize	255	13	244	12	206	18	Testicular weight decrease or gross reduction in size
Thyroid_NeoplasticLesions	244	24	256	0	214	10	Malignant and benign neoplasms of the thyroid
Thyroid_NonProliferativePathology	240	28	245	11	210	14	Non-neoplastic non-proliferative lesions of the thyroid
Thyroid_PreNeoplasticLesions	244	24	256	0	214	10	All proliferative non-neoplastic lesions of the thyroid
Thyroid_ProliferativeLesions	229	39	255	1	214	10	All proliferative non-neoplastic and neoplastic lesions of the thyroid
Thyroid_WeightIncreaseEnlarged	235	33	249	7	193	31	Thyroid weight increase of grossly enlarged

ToxCast HTS Assays

United States Environmental Protection Agency

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

NOVASCREEN
a Caliper Life Sciences company

PHYLONIX
Zebrafish Assays for Drug Screening

attagene
The Home of TFomics™

BioSeek

Nine contracts and one IAG providing chemical procurement, biochemical assays, cellular reporter assays and genomics, complex human cell responses, and model organisms; capacity to screen up to 10,000 chemicals in over 400 assays by 2012

NCGC
NIH Chemical Genomics Center

CELLUMEN

ACEA Biosciences, Inc.

EXPRESSION ANALYSIS

Table 1. Assays and Endpoints Contained Within ToxCast Phase I Signature Development						
Assay Type	**# Assays**	**# Unique Endpoints**	**Assay Source**	**Comment**	**Source**	**Reference**
Biochemical	**240**	**240**	**Mostly human and rat**	**Enzyme inhibition, Ion channels, GPCRs, P450s, Nuclear receptors**	**NovaScreen**	www.novascreen.com
Transcription Factor Profiling	**2**	**67**	**HepG2 cells (human liver)**	**Nuclear receptor and transcription factor reporter gene assays**	**Attagene**	US Patent Application 20060160108 Populations of reporter sequences and methods of their use; www.attagene.com
Nuclear Receptor Modulation	**10+**	**10+**	**Human and rodent**	**Reporter gene assays**	**NIH Chemical Genomics Center**	Inglese et al 2006. Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. Proc Natl Acad Sci USA 103:11473-8; www.ncgc.nih.gov
Genomics	**1**	**22,000**	**Primary hepatocyte-Kupffer cell co-cultures**	**Illumina microrrays**	**In Vitro ADMET Laboratories (IVAL) and Expression Analysis**	Shi et al 2006. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. Nat Biotechnol. 2006 Sep;24(9):1151-61; www.expressionanalysis.com
Kinetic Cell Growth	**1**	**Kinetic**	**A549 cells (human lung)**	**Real time recording of electrical impedance**	**ACEA Biosciences**	Xing et al 2006. Microelectronic cell sensor assay for detection of cytotoxicity and prediction of acute toxicity. Toxicol In Vitro 20:995-1004; www.aceabio.com
Cytotoxicity and Biotransformation	**1**	**6**	**Primary human liver, lung and kidney cells**	**Shared metabolism across cell types**	**IVAL**	Li AP 2007. Human hepatocytes: isolation, cryopreservation and applications in drug development. Chem Biol Interact 168:16-29; www.invitroadmet.com
Complex cell culture	**8**	**87**	**Primary human cells**	**Many cell signaling pathways**	**Bioseek**	Berg et al 2006. Characterization of compound mechanisms and secondary activities by BioMAP analysis. J Pharmacol Toxicol Methods 53:67-74; www.bioseekinc.com
High content screening	**1**	**11**	**HepG2 cells (human liver)**	**Fluorescence imaging of cells**	**Cellumen**	Giuliano et atl 2006. Systems cell biology based on high-content screening. Methods Enzymol 414:601-19; www.cellumen.com
Fish development	**1**	**11**	**Zebrafish (Dana rerio)**	**Teratogenesis**	**Phylonix**	Parng et al 2007. Neurotoxicity assessment using zebrafish. J Pharmacol Toxicol Methods 55:103-112; www.phylonix.com
TOTAL	**265**	**22,433**				

ToxCast Biochemical High Throughput Screeening (HTS)

Number of Assays

30 Cytochrome P450s

81 GPCRs

22 Ion Channels

28 Kinases

24 Nuclear Receptors

19 Phosphatases

9 Transporters

27 Other Enzymes

240 total

- Binding or inhibition assays
- Human, rodent, other species
- Initially screened at 25 uM
- Concentration-response follow-up

EPA-NCGC: Screening Nuclear Receptors for Chemical Agonism and Antagonism

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NIH Chemical Genomics Center

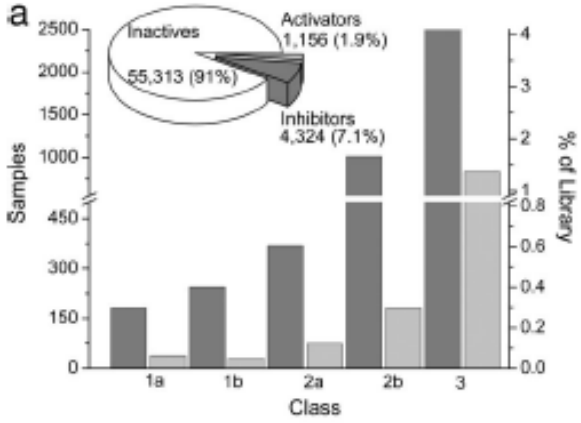
PNAS

Quantitative high-throughput screening: A titration-based approach that efficiently identifies biological activities in large chemical libraries

James Inglese*, Douglas S. Auld, Ajit Jadhav, Ronald L. Johnson, Anton Simeonov, Adam Yasgar, Wei Zheng, and Christopher P. Austin

NIH Chemical Genomics Center, National Human Genome Research Institute, Na

Communicated by Francis S. Collins, National Institutes of Health, Bethesda, MD



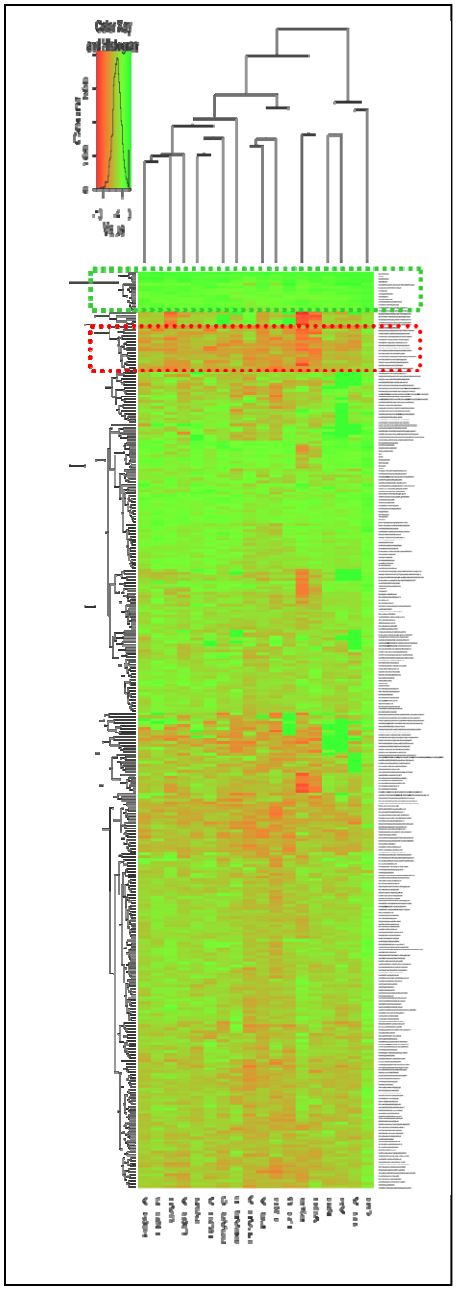
PNAS August 2006 vol 103 no 31 11473-11478

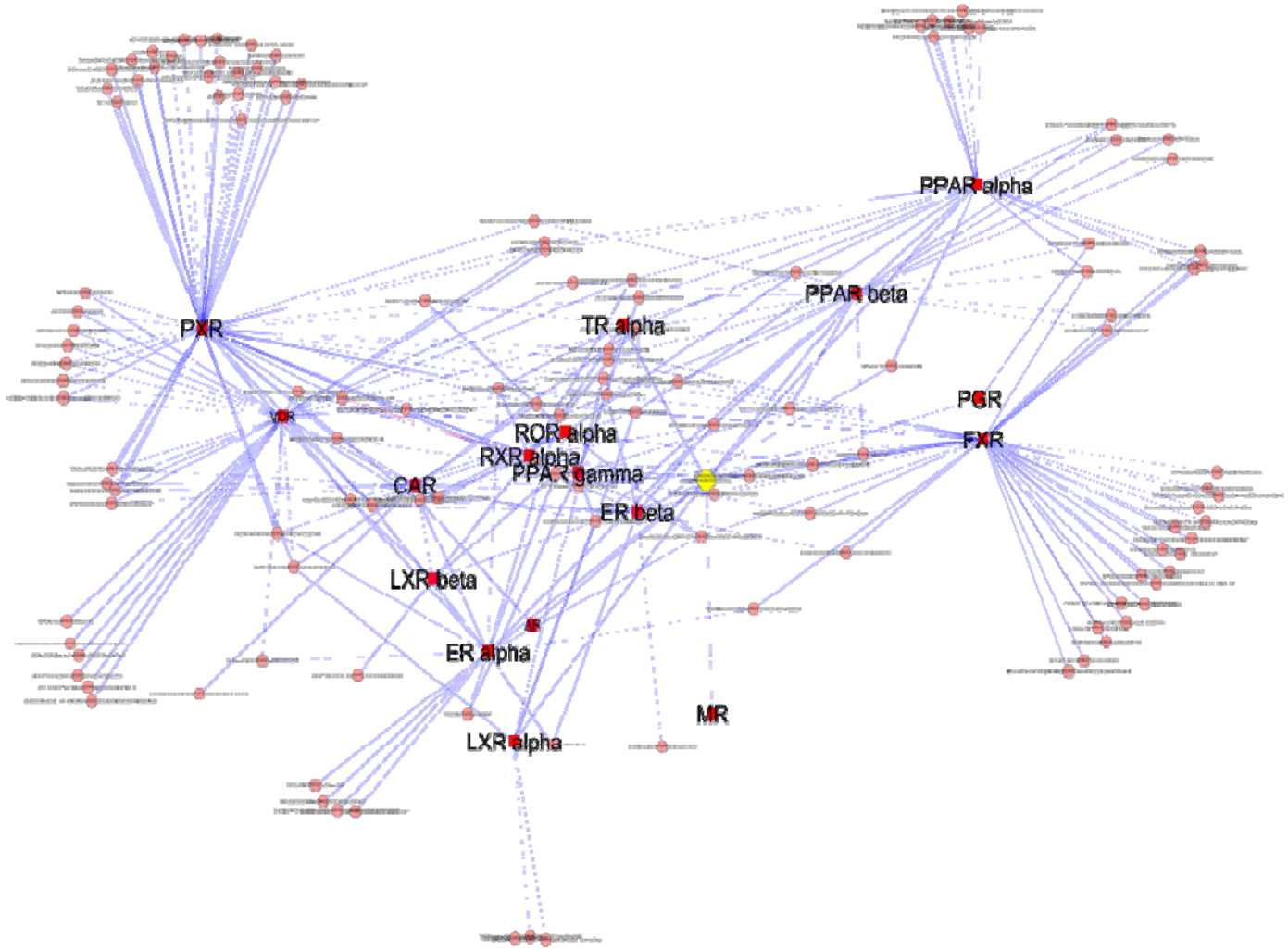
ToxCast-NCGC Reporter Gene Assays

- AR
- ERa
- GR
- FXR
- LXRb
- PPARd
- PPARg
- RXRa
- TRb
- VDR

- Invitrogen assays
- Human targets
- 1536well format
- 1408 chemicals
- 11 concentrations

ORD/NCCT: In Silico Affinity of ToxCast Chemicals to In Vitro Assay Targets





Transcription Factor Activity Profiling

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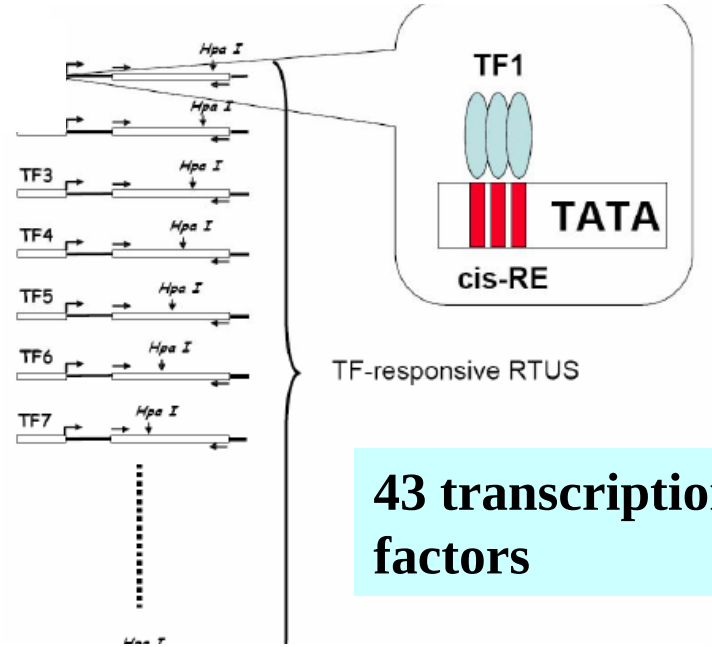
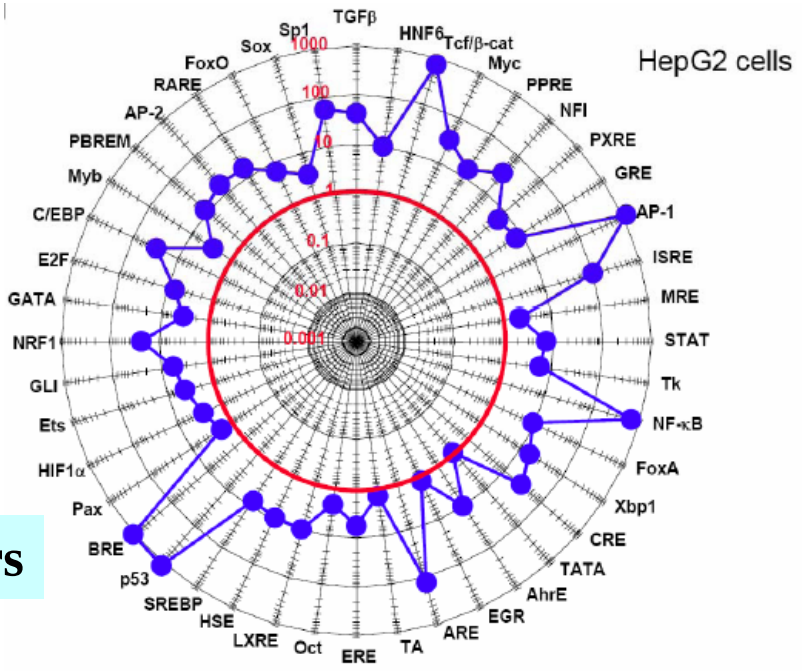
Cis-Factorial™ Biosensors

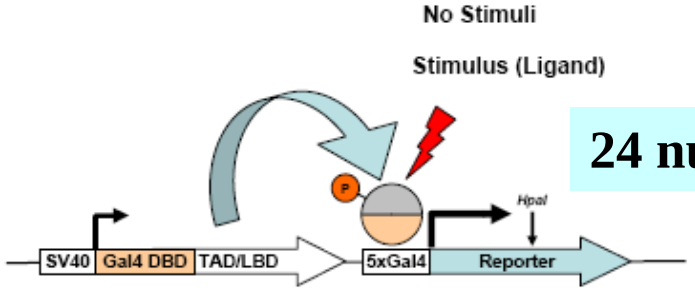


- HepG2 assays
- Initially screened at LC50/10
- Follow-up concentration response

43 transcription factors

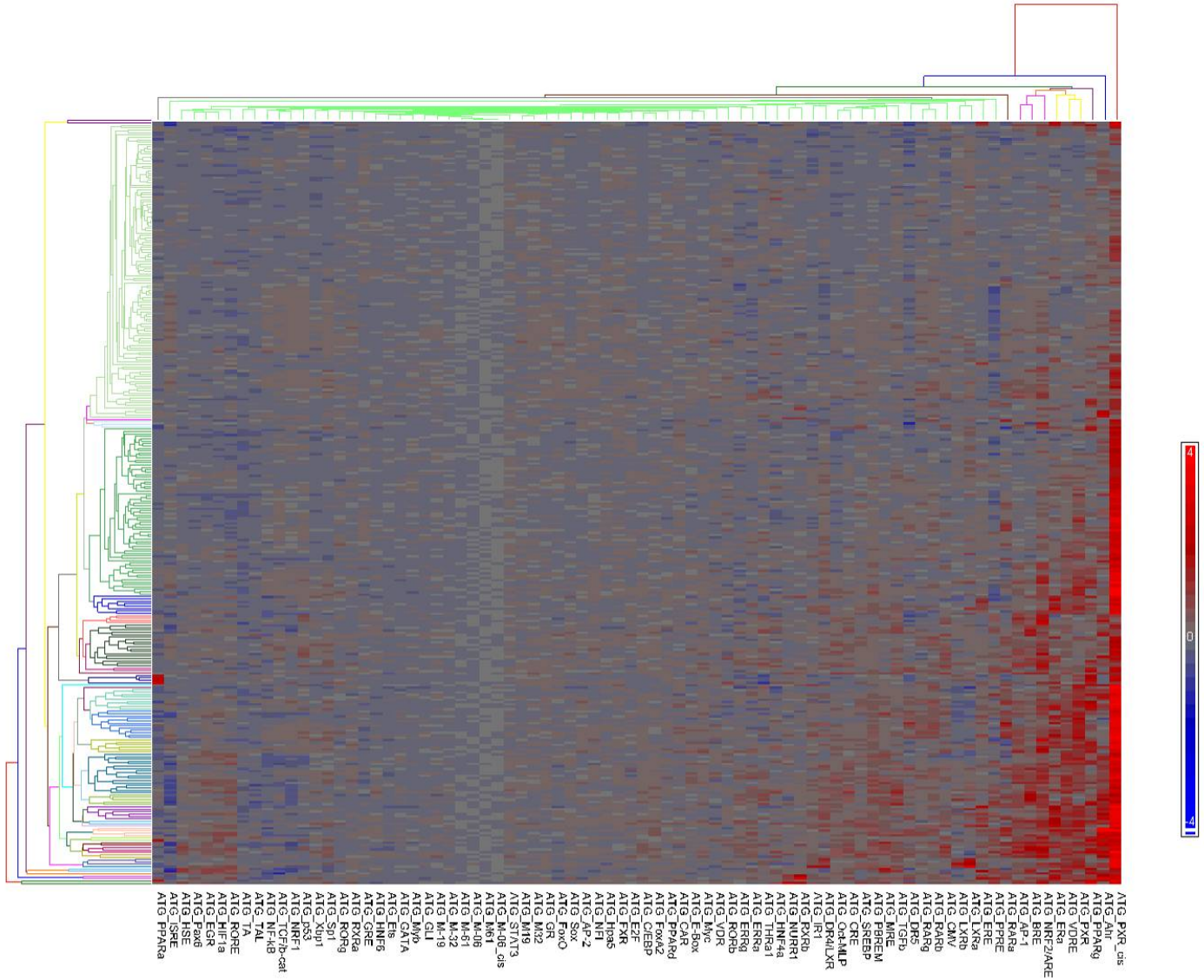


Trans-Factorial™ Biosensors



24 nuclear receptors

Initial ToxCast Screening Results from Attagene



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Toxicogenomic Profiling of Hepatocyte – Kupffer Cell Co-Cultures

- Testing human, mouse and rat
- Coordinating with NCCT Virtual Liver project
- Optimized ratio of Hepatocyte – Kupffer cells
- Collagen-Matrigel sandwich culture
- Concentration response

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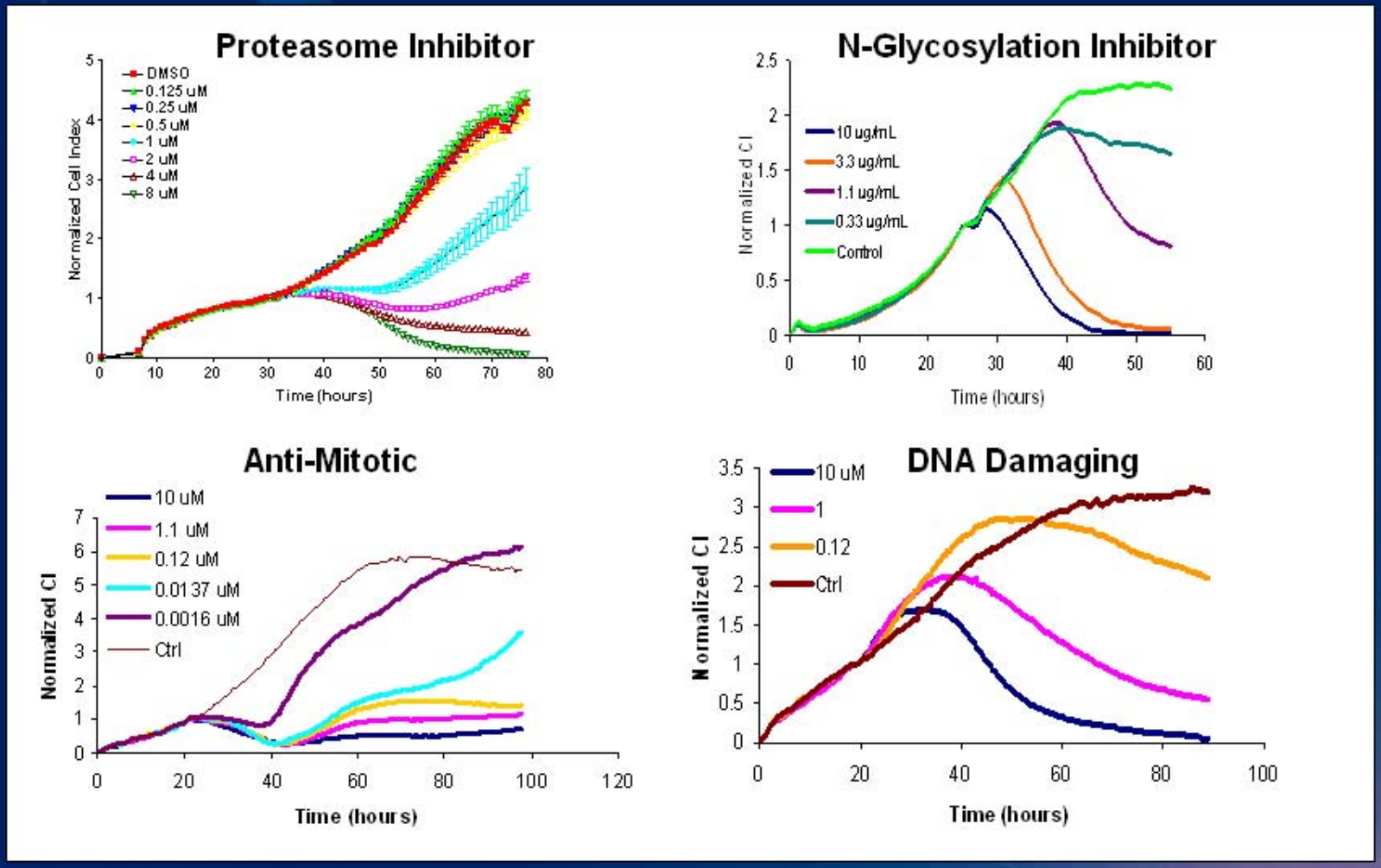
EXPRESSION ANALYSIS

Gene Expression Profiling

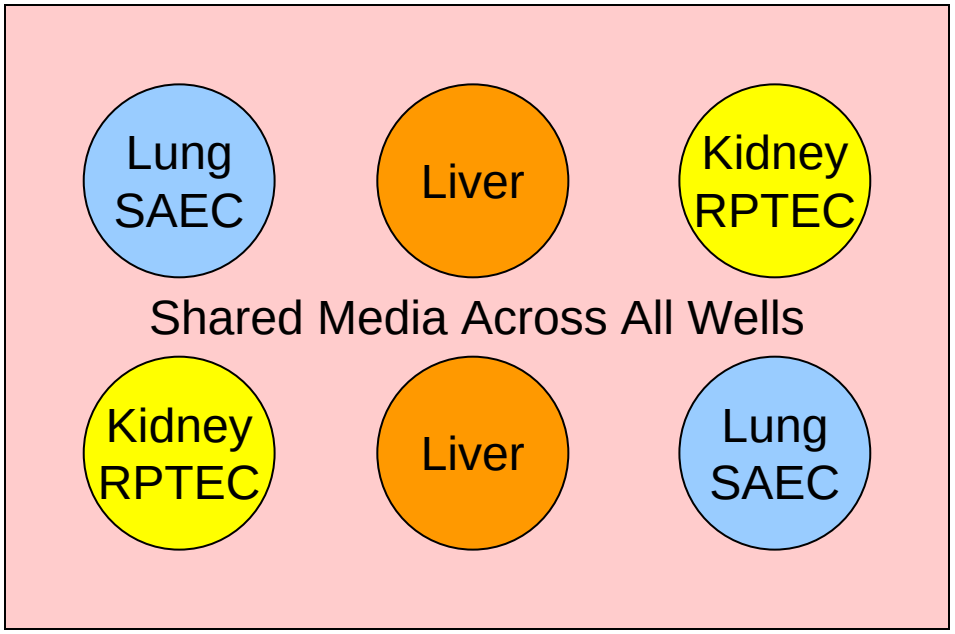
Rat arrays for 22,000 transcripts.
Customized chips- up to 1400 genes in 96well format.
Individual or multiplexed PCR (≤ 48 transcripts in parallel).

ACEA RT-CES™ Impedance-based Biomonitoring of Cellular Cytotoxicity

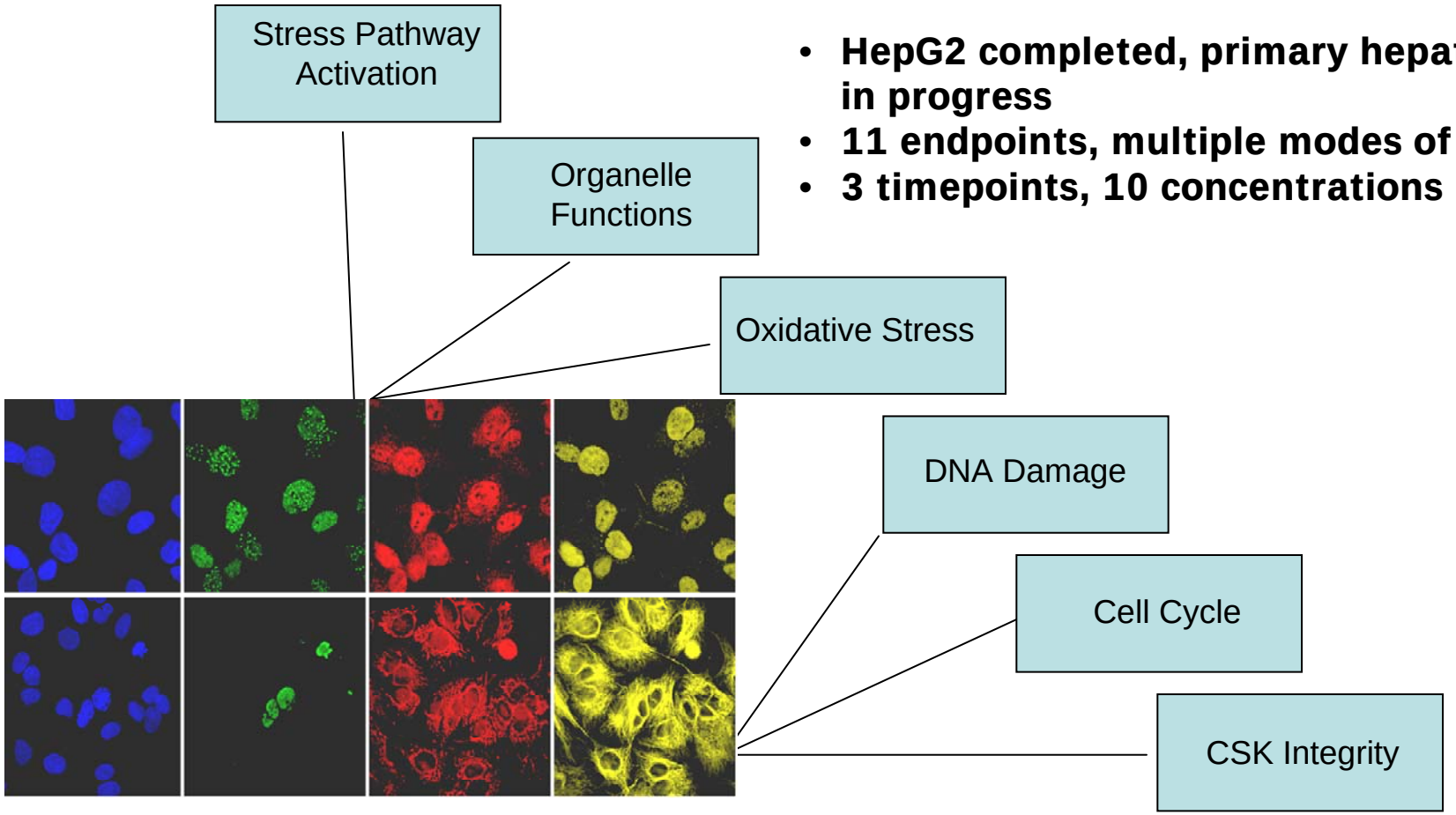


ACEA Biosciences, Inc.

ToxCast Human Integrated Multiple Organ Culture (IdMOC)



High Content Cytotoxicity Assessment



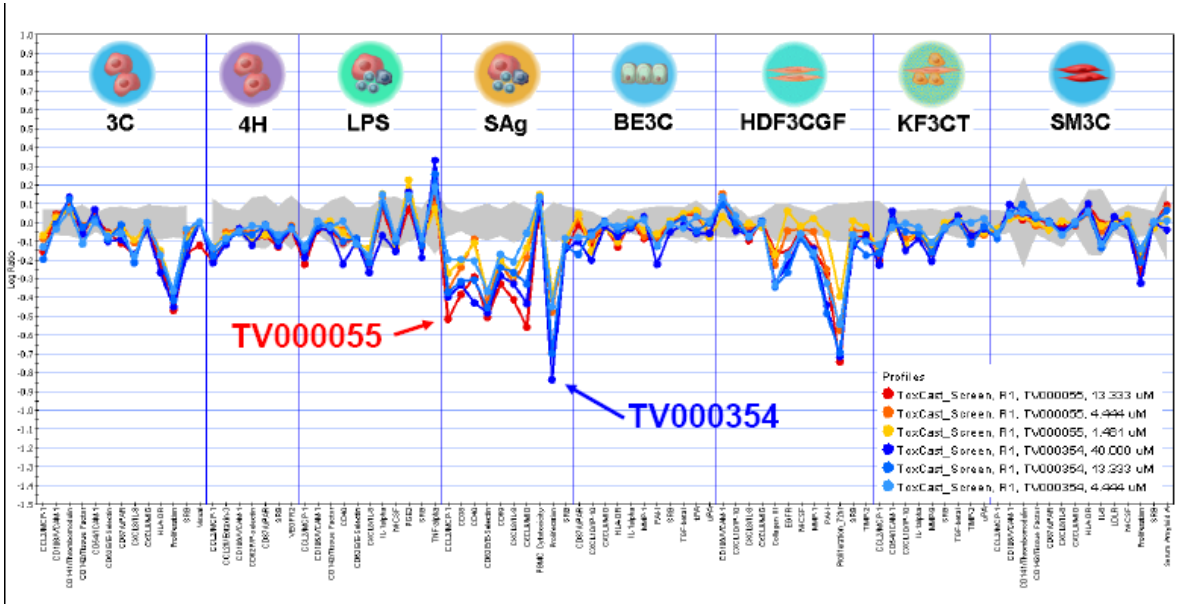
- **HepG2 completed, primary hepatocytes in progress**
- **11 endpoints, multiple modes of toxicity**
- **3 timepoints, 10 concentrations**

EPA United States Environmental Protection Agency

BioSeek

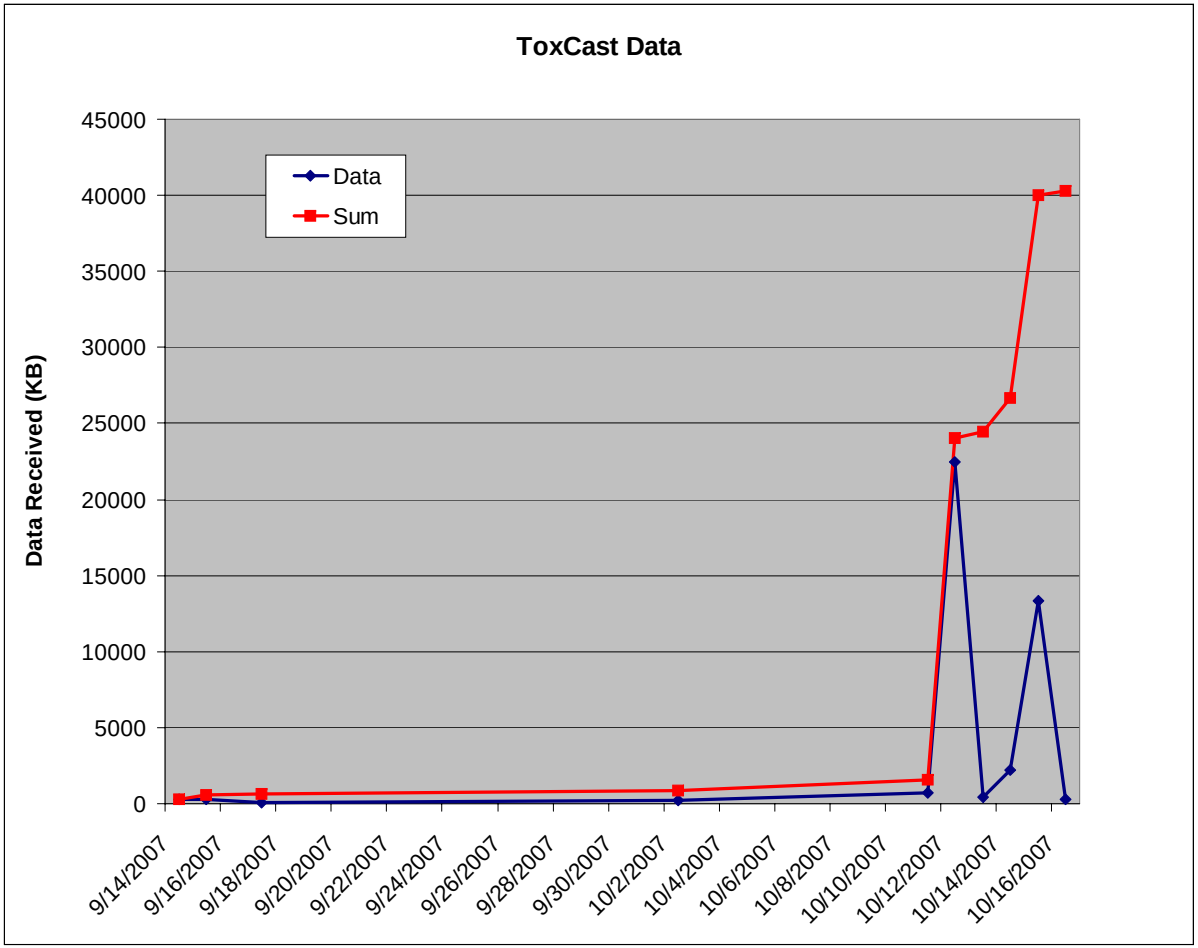
Biomarkers of Cell Function in Complex Human In Vitro Systems

System	Cell Types
3C	Endothelial cells
4H	Endothelial cells
LPS	Peripheral blood mononuclear cells + Endothelial cells
SAg	Peripheral blood mononuclear cells + Endothelial cells
BE3C	Bronchial epithelial cells
HDF3CGF	Fibroblasts
KF3CT	Keratinocytes + Fibroblasts
SM3C	Vascular smooth muscle cells

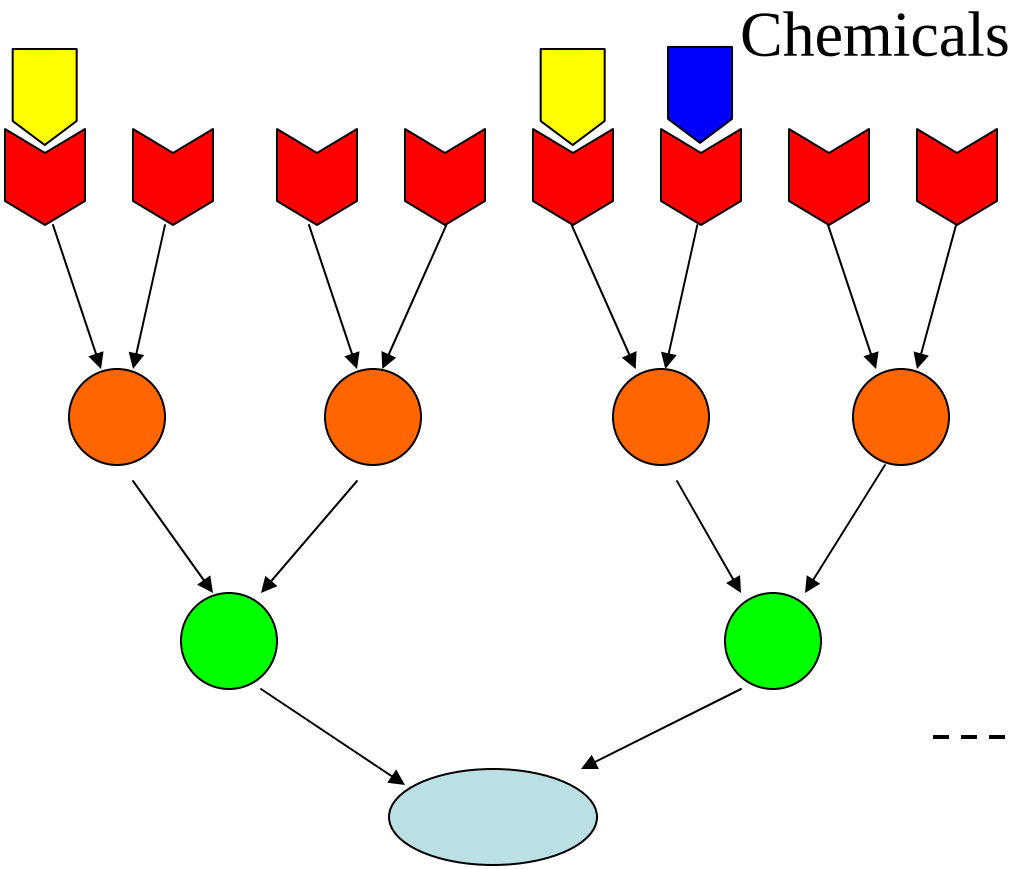


- 8 assays, 87 endpoints
- 4 concentrations
- 68% active

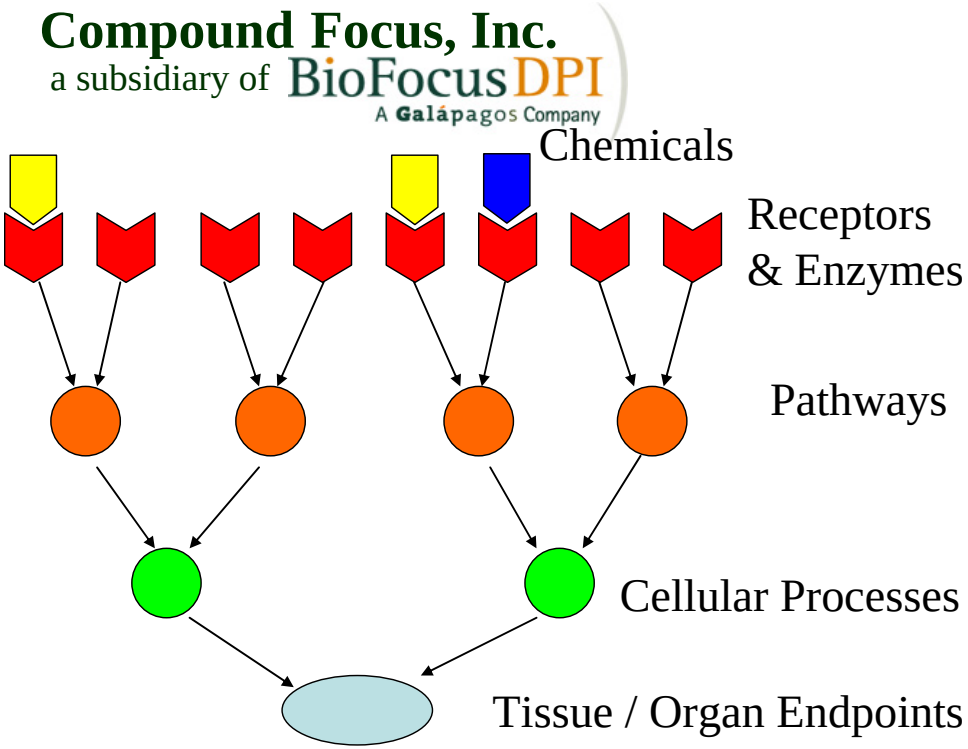
The Deluge of Data has Started……..



Biological Ontology of ToxCast



ToxCast Assay Data Organized by Ontology



NCGC NIH Chemical Genomics Center

NOVASCREEN a Caliper Life Sciences company

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EXPRESSION ANALYSIS

ACEA Biosciences, Inc.

iVAL

BioSeek

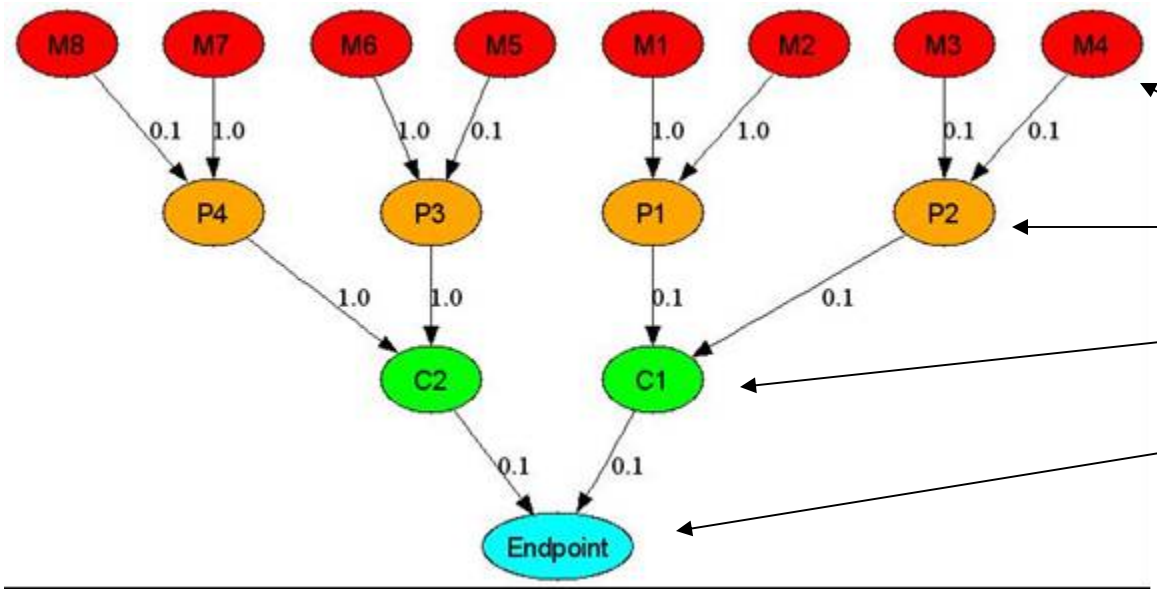
CELLUMEN

PHYLONIX Zebrafish Assays for Drug Screening

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY ToxRefDB COMPUTATIONAL TOXICOLOGY

United States Environmental Protection Agency

ToxCast Biological Ontology Used in Predictive Modeling



Direct Molecular Interactions

Pathway Processes

Cellular Processes

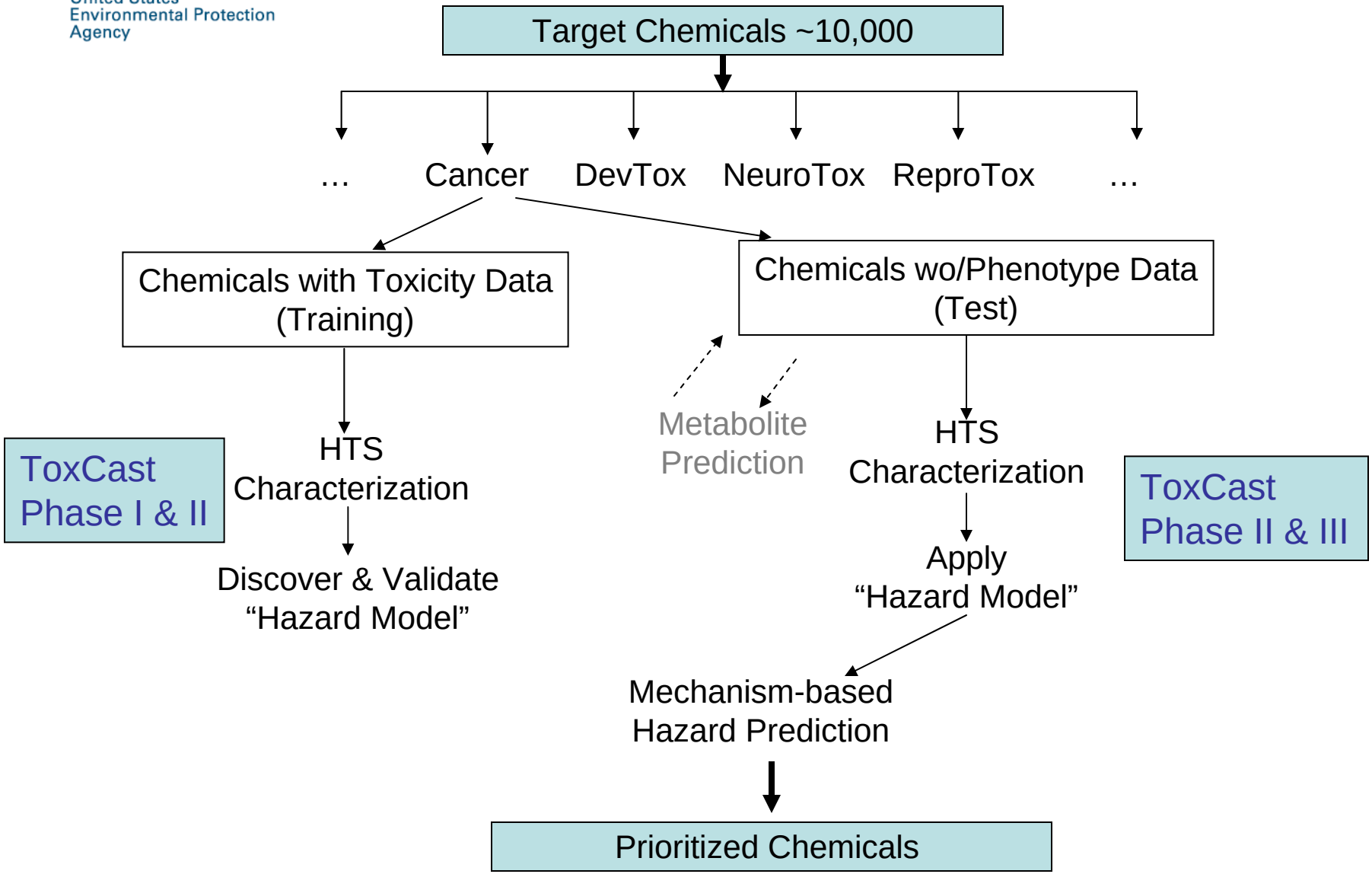
Tissue or Organ Endpoint

Each chemical will have a spectrum of activities for M-P-C-E nodes.
Predictive classifiers will include features from multiple data levels.

Predictive Classifiers for Toxicity

- Goal: Find “classifiers” that accurately predict endpoints
  - Evaluate multiple methods (NN, KNN, SVM, SLR, GA, CART)
- Use all available data
  - ToxRefDB
  - ToxCast HTS, HCS, genomics
  - Physicochemical properties, calculated properties
  - Data from ToxCast partners
- Properties of an ideal classifier
  - Accurate (low false positive & false negative rates)
  - Inexpensive and easy to measure for new chemicals
  - Easy to interpret and provides biological insight

ToxCast Screening and Prioritization



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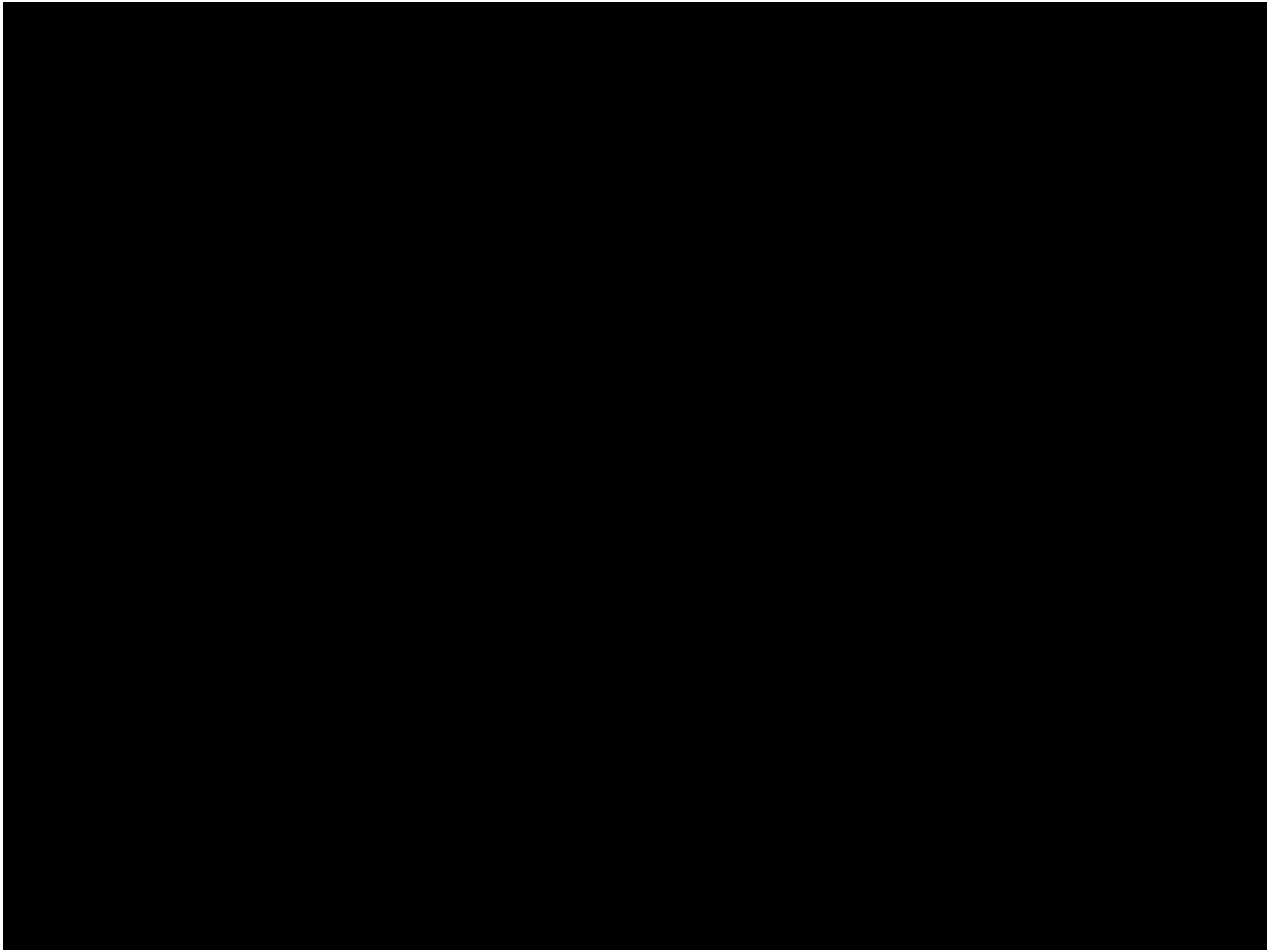
Next Steps for ToxCast

1. Complete ToxRefDB data population for ToxCast Phase I
2. Capture & QC all ToxCast Phase I assay data
3. Complete toxicity signature discovery for ToxCast Phase I
4. Prioritize chemicals for ToxCast Phase II
5. Capture toxicology data for ToxCast Phase II



ToxCast Internal EPA Partnerships

- Office of Pesticide Programs- ToxRefDB, antimicrobials, inerts
- Office of Pollution Prevention and Toxics- HPV, MPV, PFCs,
  - GLNPO
- Office of Water- chemical contaminant candidates
- Office of Research and Development- NHEERL, NERL, NCEA

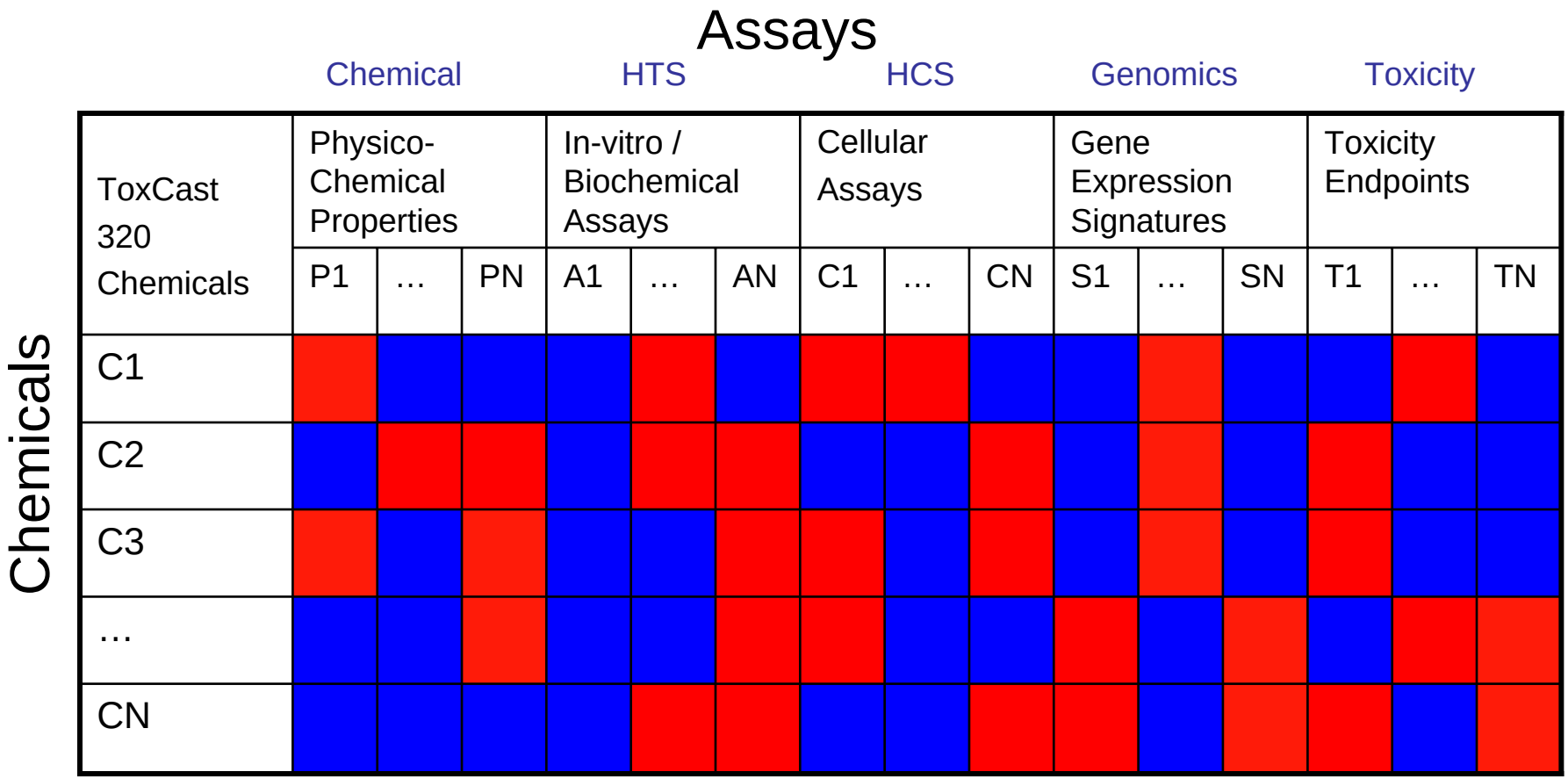
United States Environmental Protection Agency

ToxCast External Partnerships

- NIH Chemical Genomics Center
- NTP HTS Initiative
- Security and Prosperity Partnership: North American Coordination on Industrial Chemicals
- OECD Molecular Screening Project
- MOU with The Hamner Institutes of Life Sciences
- CRADA with L'Oreal
- mCRADA with Illumina

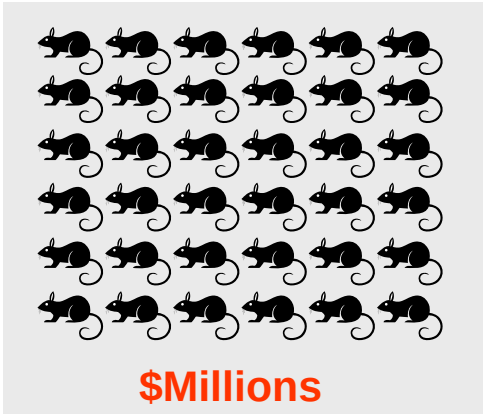
The ToxCast Data Matrix

[320 chemicals] x [265 assays] x [multiple endpoints] x [multiple concentrations] = millions of datapoints



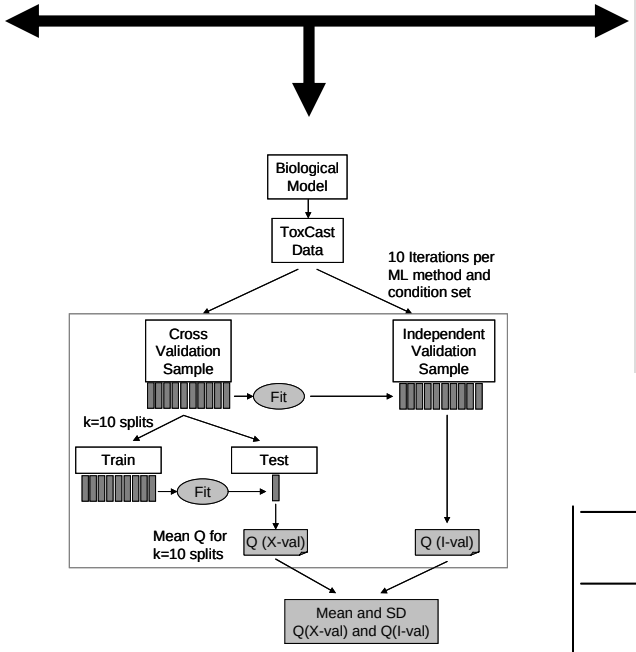
Bridging from Current Practice to the Future of Toxicity Testing

in vivo testing



$Millions

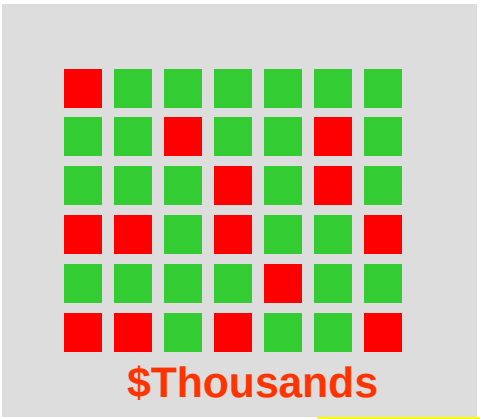
ToxRefDB



in silico analysis

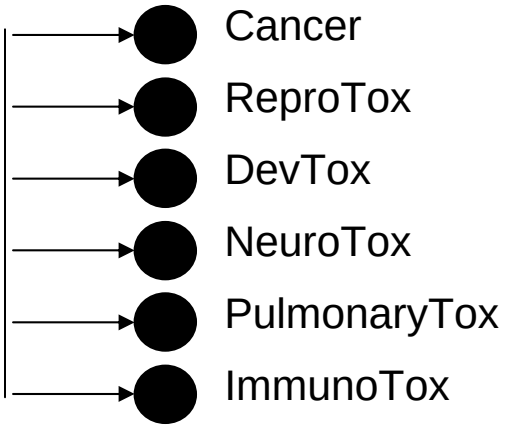
ToxMiner

in vitro testing



$Thousands

ToxCast



The ToxCast Team