United States Environmental Protection Agency

EPA's ToxCast Program:
From Research to Application

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OECD Molecular Screening
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Paris, France

Office of Research and Development
National Center for Computational Toxicology

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

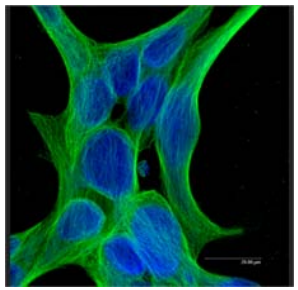
ToxCast™ Background

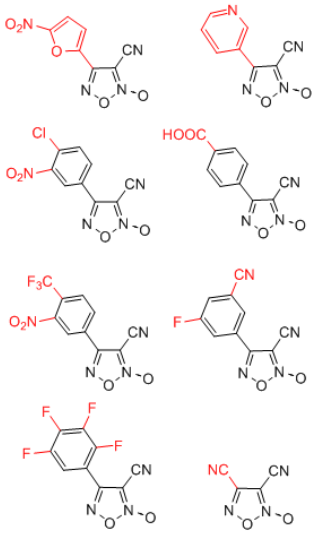
- Research program of EPA's National Center for Computational Toxicology
- Addresses chemical screening and prioritization needs for pesticidal inerts, antimicrobials, CCLs, HPVs and MPVs
- Comprehensive use of HTS technologies
- Coordinated with NTP and NHGRI/NCGC via Tox21

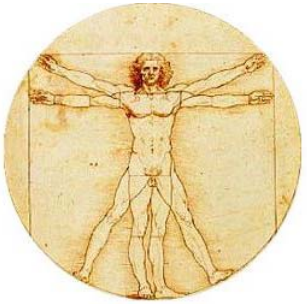
- Committed to stakeholder involvement and public release of data
  - Chemical Prioritization Community of Practice
  - NCCT website- http://www.epa.gov/ncct/

HTS in Drug Development

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







HTS in Toxicology

Test prioritized chemicals in animals

Categorize as inactive subject to further testing

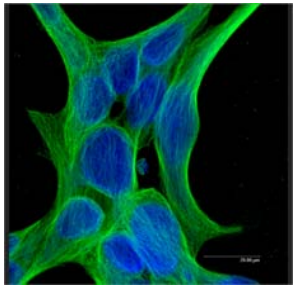
Computational analysis & Synthesis of HTS results

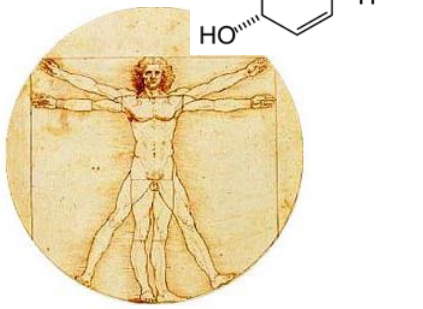
HTS tests chemicals for effect on assays

Obtain or create testing systems ("assays")

Identify toxicity pathways, cellular phenotypes

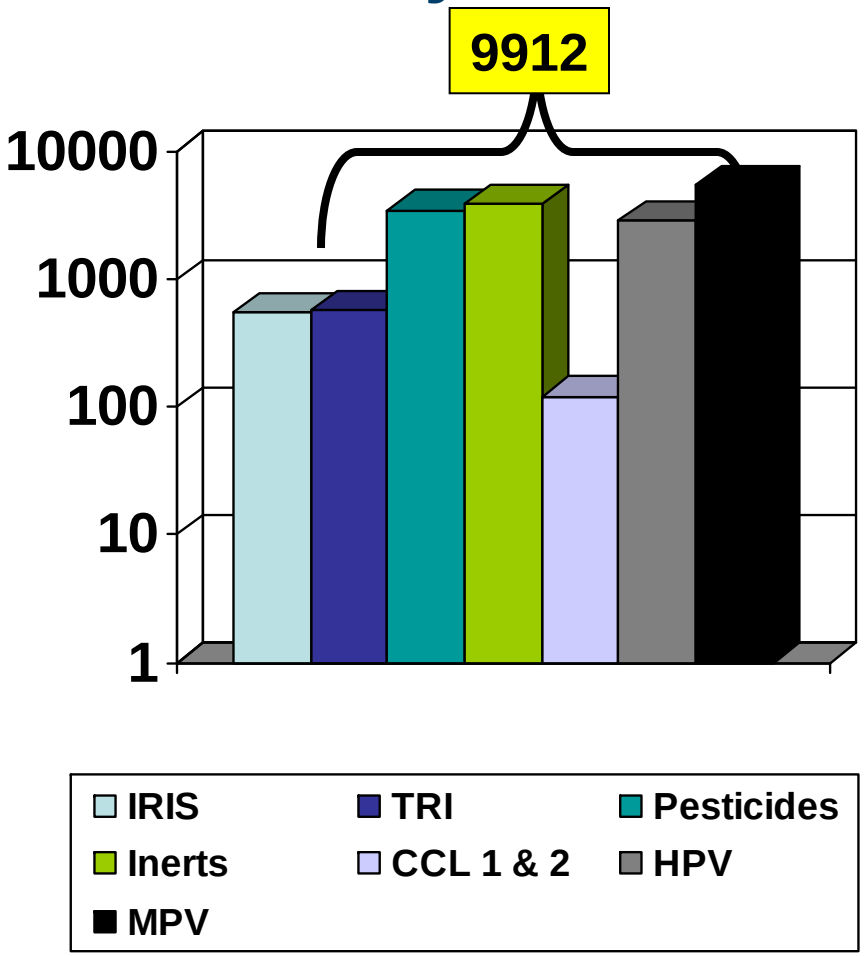
Chemicals with known or suspected toxicity/ activity



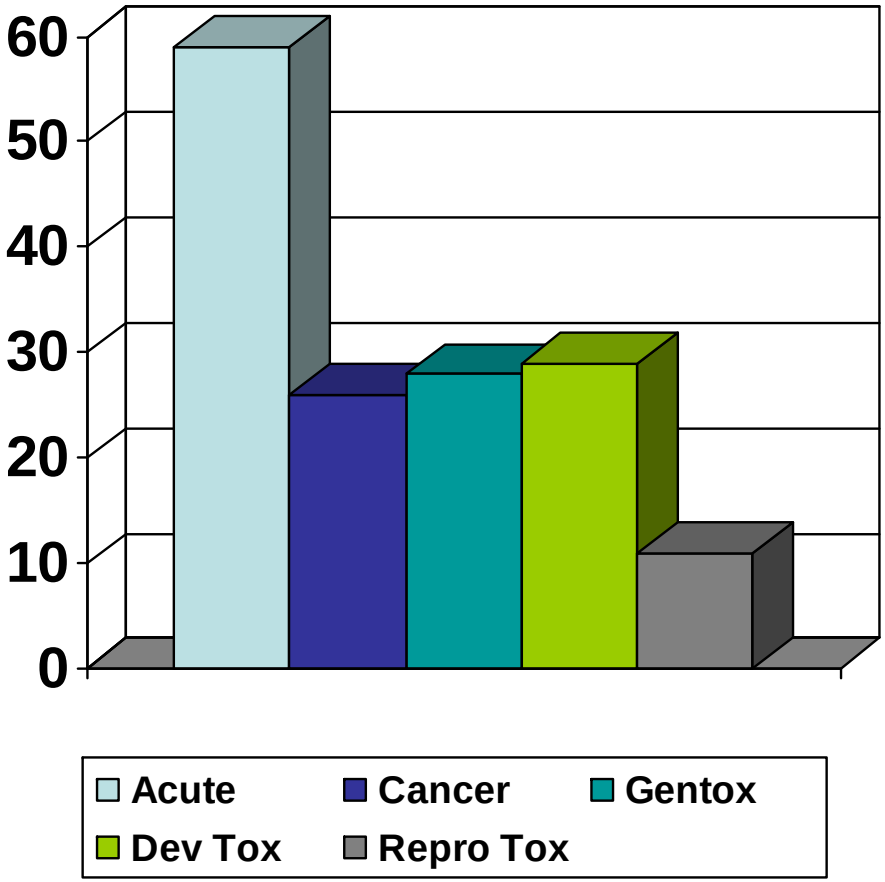


EPA's Need for Prioritization

Too Many Chemicals



Too Little Data (%)



Chemical Prioritization
Pesticides: Current Status

- Antimicrobials (300 Total)
  - ~100 have undergone (re-)registration since 1996 (FQPA)
  - Limited to no toxicity information
  - Limited regulatory capacity for requesting toxicity data
  - Current practice:
    - *Food-use to non-food-use chemicals*
    - *Chemical groupings by structure similarity*
  - Potential need:
    - *Biologically-based support for toxicity data requests*
    - *Re-registration prioritization*
    - *Biologically driven chemical groupings*

- Inerts ('Other' Ingredients (>4500 Total))
  - Legislative mandate to (re)assess all 'other' ingredients
  - ~700 Currently re-assessed (~2500 previously assessed)
  - Limited to no toxicity information
  - Limited to no regulatory capacity for requesting toxicity data
  - Current practice:
    - *Limited use of QSAR models*
    - *Use limited available information in categorical assessment*
    - *Tackle recognizably safe chemicals 1st (GRAS, etc.)*
  - Potential need:
    - *Prioritization & Classification of Ingredients*
    - *Biologically driven chemical groupings*
    - *Targeted testing of chemicals/groups*

Chemical Prioritization
Industrial: Current Status

- HPV (~3500 Total)
  - >1Million lbs production/importation
  - 2200 Part of HPV Challenge
  - Wide range of toxicological data availability
  - Limited to no regulatory capacity for requesting toxicity data
  - Current practice:
    - *HPV Categories (Chemical groupings by structural similarity)*
    - *Use of QSAR models*
  - Potential need:
    - *Biologically driven chemical groupings*
    - *Rapid evaluation of chemicals with no toxicity information*

- MPV (~2800)
  - >25,000 lbs production/importation
  - Wide range of toxicological data availability (primarily SIDS)
  - ChAMP expanded to MPVs
  - Current practice:
    - *Hazard-based (screening-level documents)*
    - *Consider QSAR estimates*
    - *Consider Canada's categorization results*
  - Potential need:
    - *Enhance use of models with screening data*
    - *Rapid evaluation of chemicals with no toxicity information*

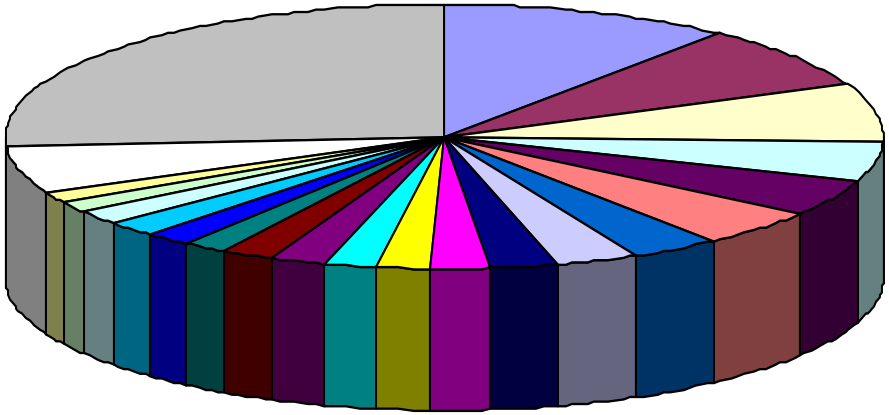
ToxCast_320

309 Unique Chemicals

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

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

- **75** Chemical Classes



Chemical Class Distribution
(≥5/Class)

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

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ToxCast 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

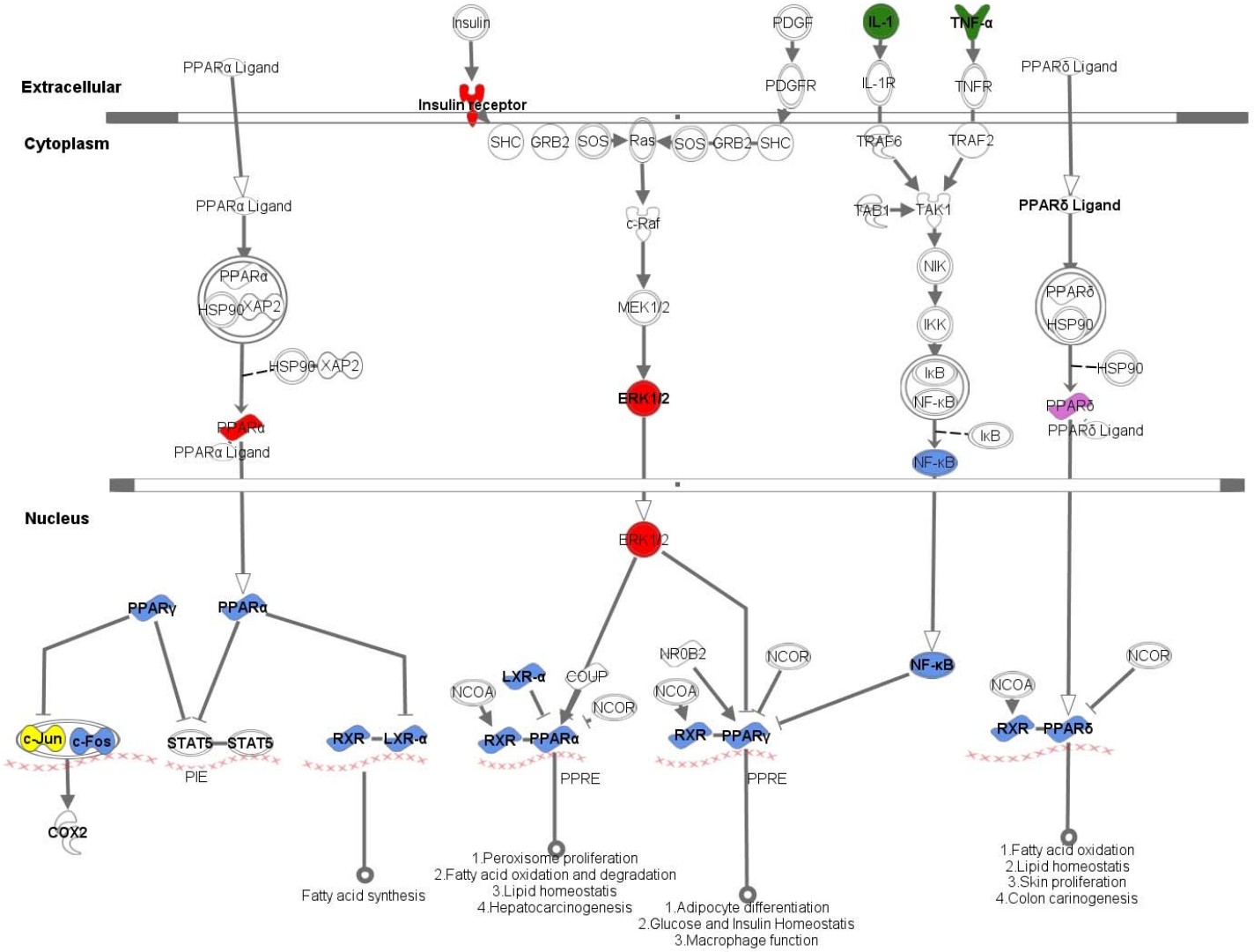
467 Total Endpoints

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
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

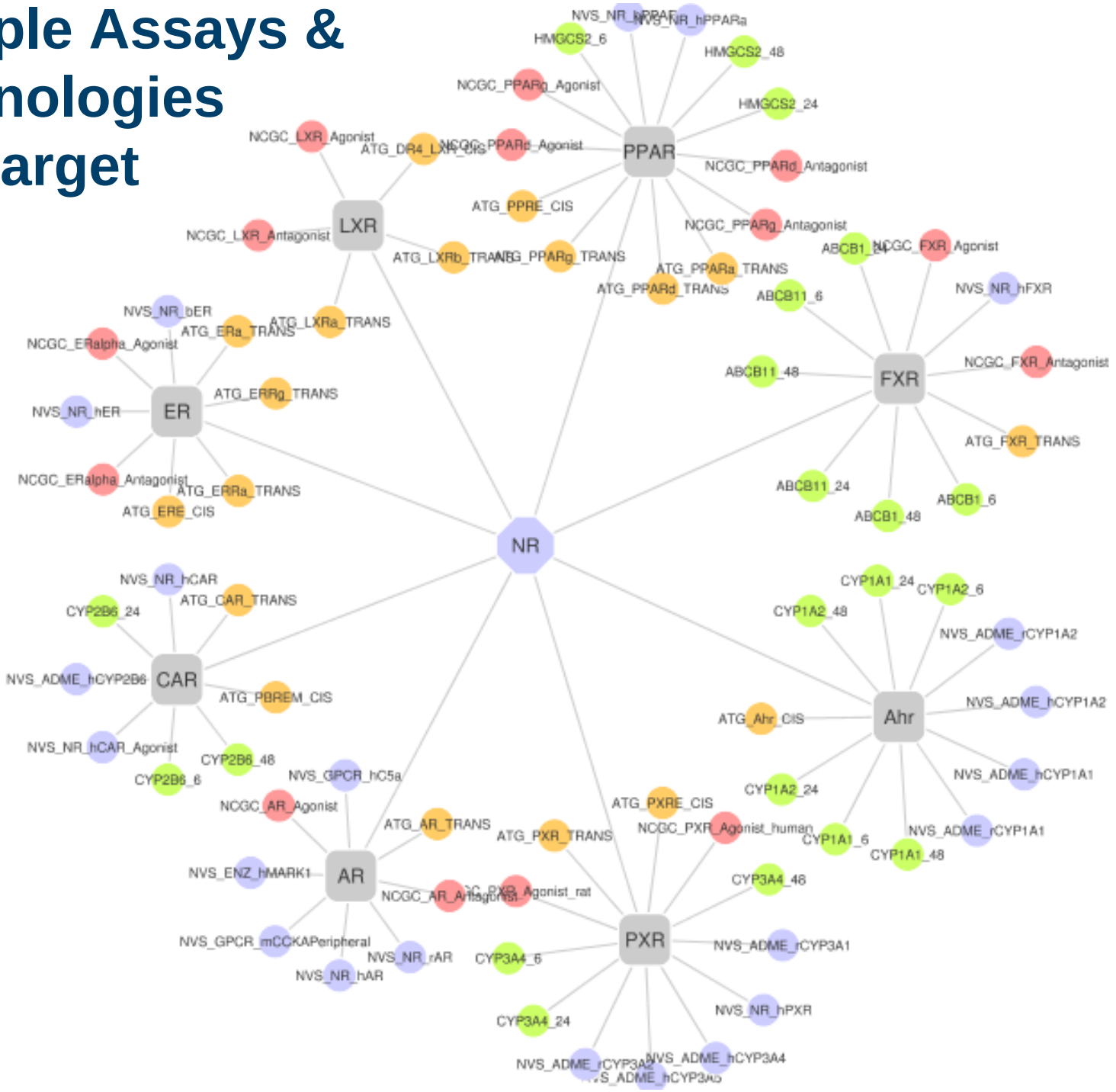
ToxCast: Pathway Coverage

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



PPAR Signaling Pathway

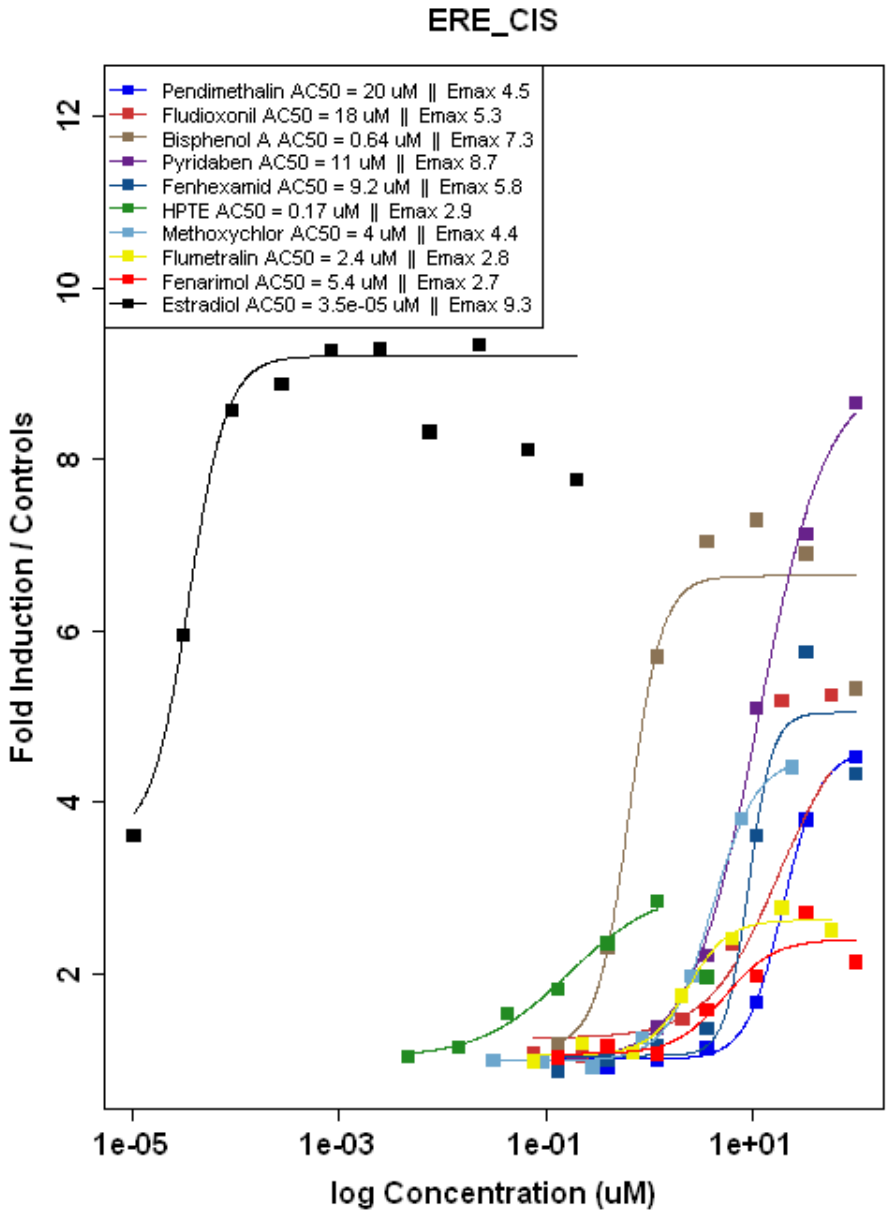
Multiple Assays & Technologies Per Target

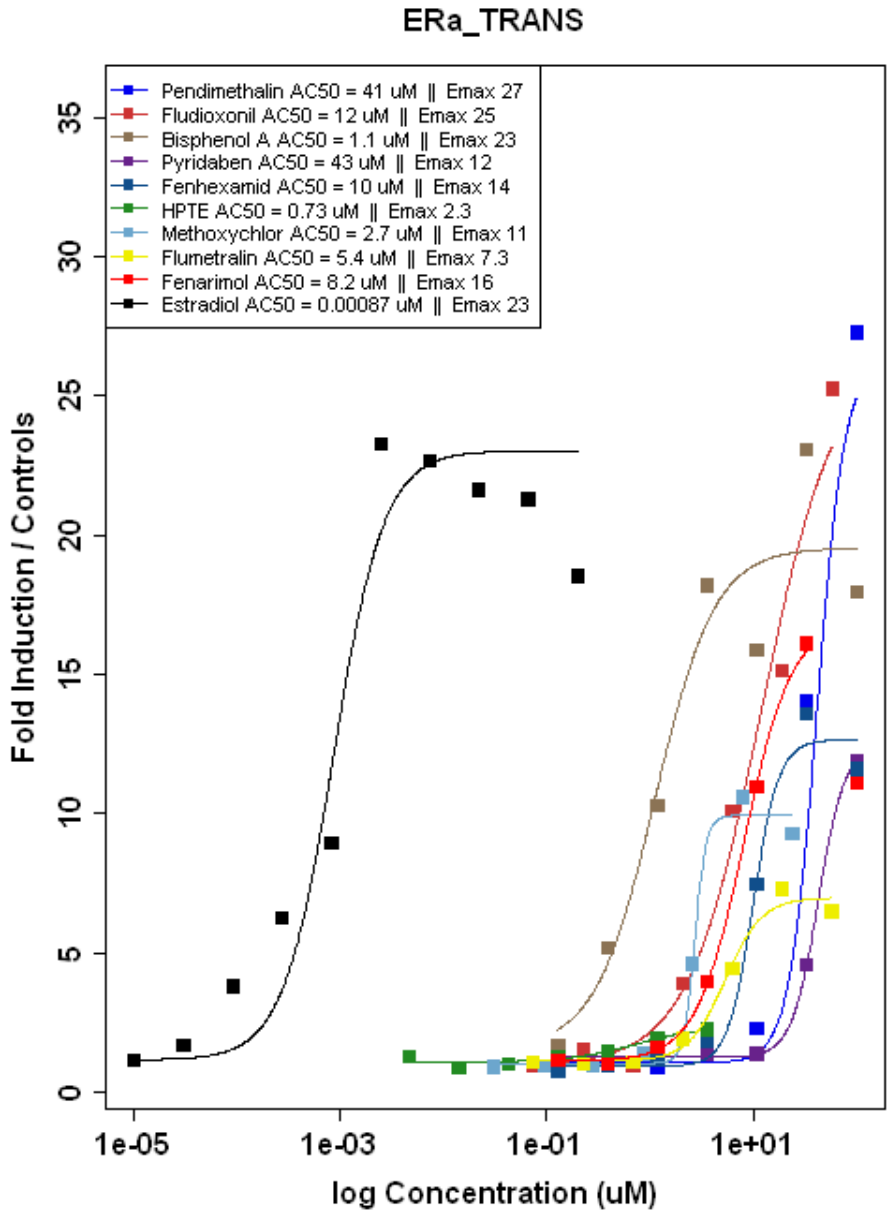


Some Expected Results…

- Estrogen receptor (ER)
  - Bisphenol A, Methoxychlor, HPTE
- Androgen Receptor (AR)
  - Vinclozolin, Linuron, Prochloraz
- PPAR
  - PFOA, PFOS, Diethylhexyl Phthalate, Lactofen
- Mitochondrial Poisons
  - Azoxystrobin, Fluoxastrobin, Pyraclostrobin
- Acetylcholinesterase Inhibition
  - Multiple organophosphorus pesticides

What is a hit?


ERE_CIS
Pendimethalin AC50 = 20 uM || Emax 4.5
Fludioxonil AC50 = 18 uM || Emax 5.3
Bisphenol A AC50 = 0.64 uM || Emax 7.3
Pyridaben AC50 = 11 uM || Emax 8.7
Fenhexamid AC50 = 9.2 uM || Emax 5.8
HPTE AC50 = 0.17 uM || Emax 2.9
Methoxychlor AC50 = 4 uM || Emax 4.4
Flumetralin AC50 = 2.4 uM || Emax 2.8
Fenarimol AC50 = 5.4 uM || Emax 2.7
Estradiol AC50 = 3.5e-05 uM || Emax 9.3
Fold Induction / Controls
2
4
6
8
10
12
1e-05
1e-03
1e-01
1e+01
log Concentration (uM)

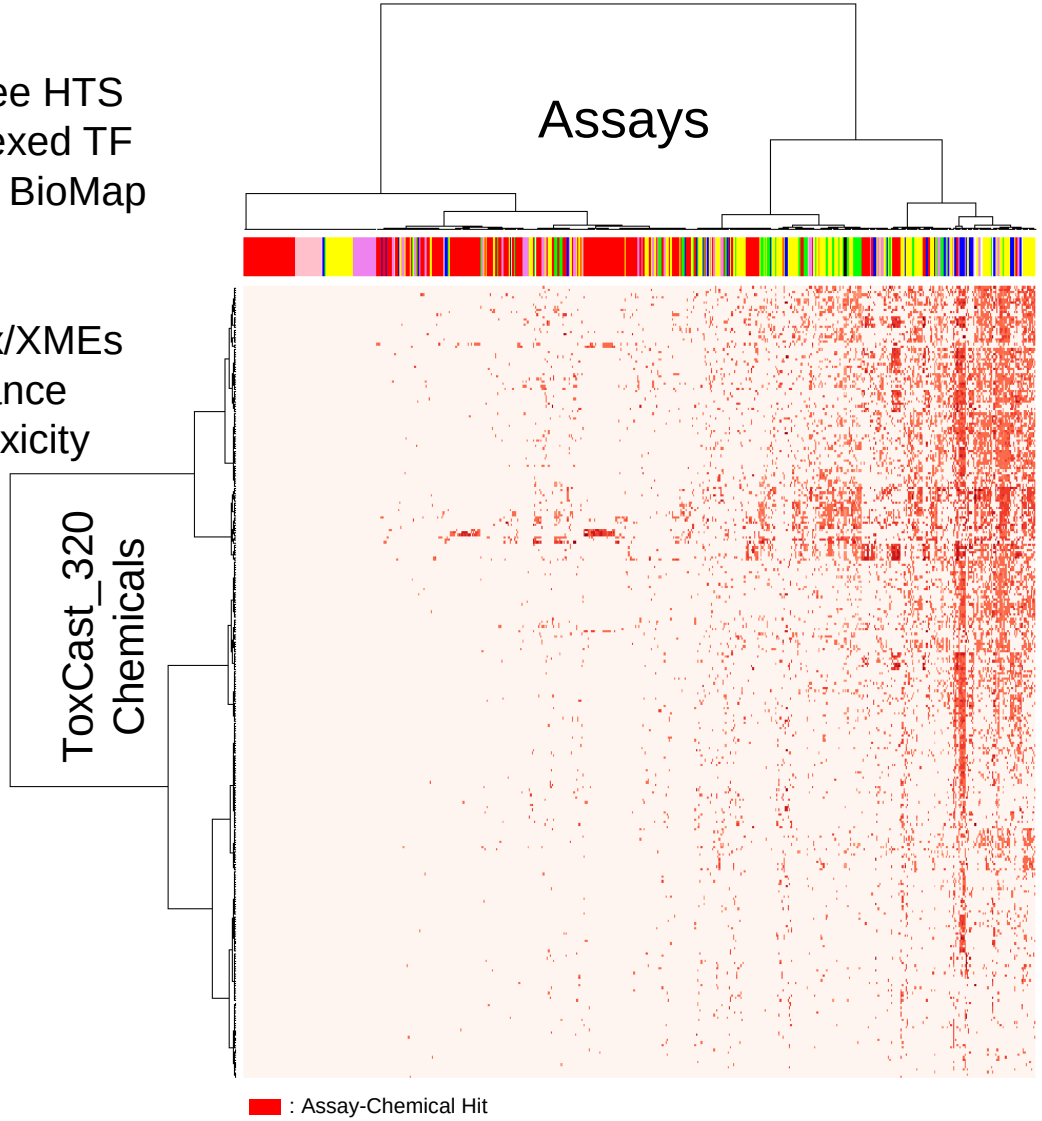

ERa_TRANS
Pendimethalin AC50 = 41 uM || Emax 27
Fludioxonil AC50 = 12 uM || Emax 25
Bisphenol A AC50 = 1.1 uM || Emax 23
Pyridaben AC50 = 43 uM || Emax 12
Fenhexamid AC50 = 10 uM || Emax 14
HPTE AC50 = 0.73 uM || Emax 2.3
Methoxychlor AC50 = 2.7 uM || Emax 11
Flumetralin AC50 = 5.4 uM || Emax 7.3
Fenarimol AC50 = 8.2 uM || Emax 16
Estradiol AC50 = 0.00087 uM || Emax 23
Fold Induction / Controls
0
5
10
15
20
25
30
35
1e-05
1e-03
1e-01
1e+01
log Concentration (uM)

ToxCast Phase I Assay Hits
(n=624 measurements)

United States Environmental Protection Agency

Novascreen (Knudsen et al, NCB, submitted)	Cell Free HTS
Attagene (Martin et al, CRT, submitted)	Multiplexed TF
Bioseek (Houck et al, JBS, published)	Human BioMap
Cellumen (Houck et al, In prep)	HCS
CellzDirect (Rotroff et al, TAP, submitted)	qNPAs
Solidus (Ryan et al, In prep)	Cytotox/XMEs
ACEA (Judson et al, In prep)	Impedance
Gentronix (Knight et al, RTP, published)	Genotoxicity

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

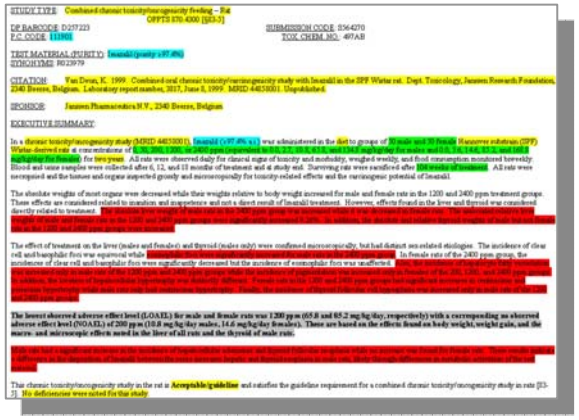


ToxRefDB

- Relational phenotypic/toxicity database
  - Stores Guideline In Vivo Laboratory Animal Toxicology Data
  - All Treatment-Related Effects at All Dose Levels Captured
- Provides in vivo anchor for ToxCast predictions
- Focus: 3 study types
  - Chronic/Cancer Rat and Mouse (Martin, et al, EHP 2008)
  - Rat Multigeneration Reproductive Toxicity (Martin, et al, ToxSci 2009)
  - Rat & Rabbit Developmental Toxicity (Knudsen, et al, ReproTox 2009)
- Two types of synthesis
  - Supervised (common individual phenotypes)
  - Unsupervised (machine based clustering of phenotype patterns)

ToxRefDB Endpoint Coverage

data evaluation records

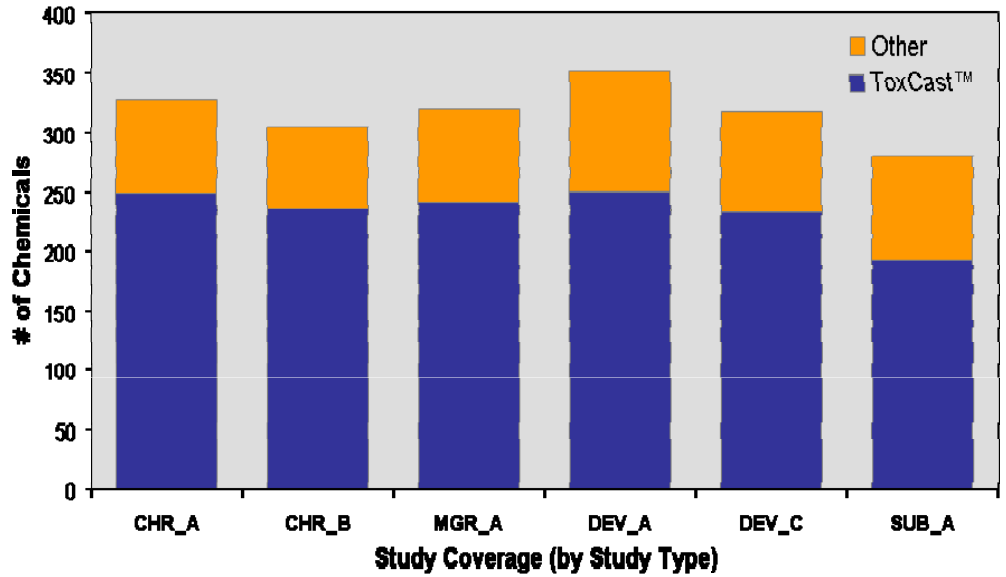


ToxRefDB

CHRONIC/CANCER (CHR)
Martin et al. (2008) Environ Hlth Persp
doi:10.1289/ehp.0800074

MULTIGENERATION REPRODUCTIVE (MGR)
Martin et al. (2009) Toxicol Sci
doi: 10.1093/toxsci/kfp080

PRENATAL DEVELOPMENTAL (DEV)
Knudsen et al. (2009) Reprod Toxicol
doi: 10.1016/j.reprotox.2009.03.016



A = Rat B = Mouse C = Rabbit

ToxRefDB in Predictive Modeling

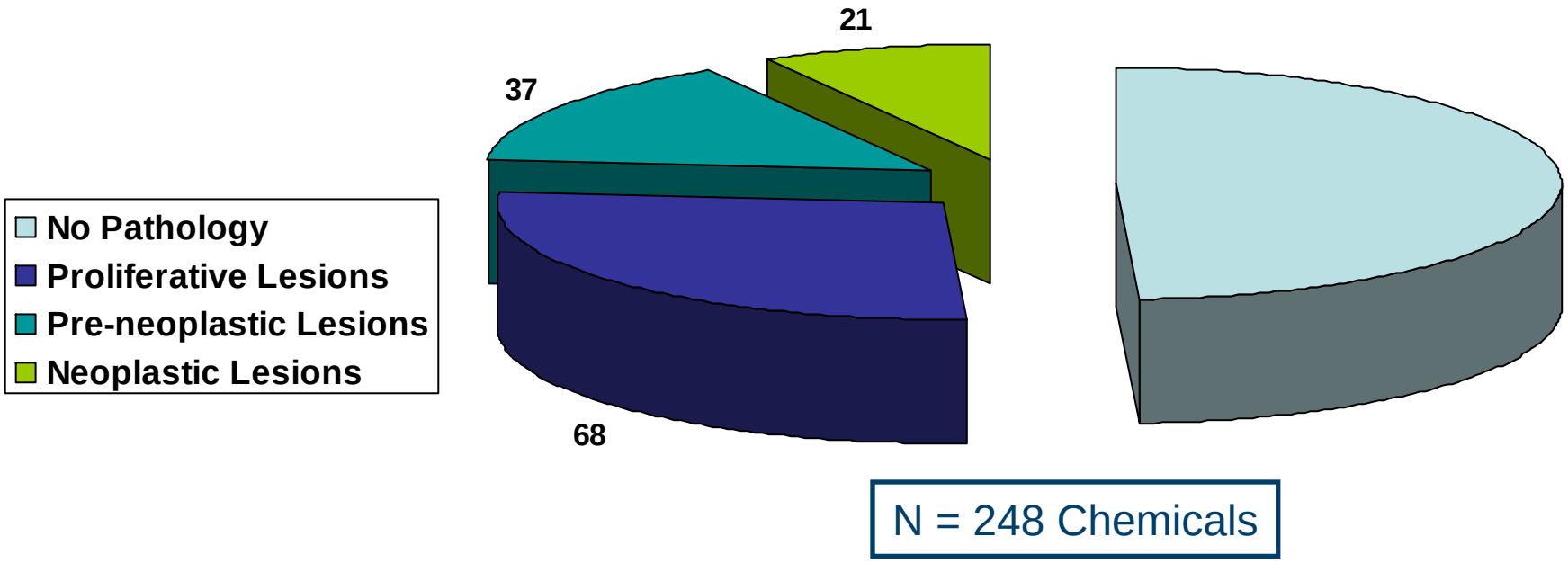
STRENGTHS

- Source data from >2,000 guideline studies
- Puts >$2B worth of legacy data into a computable form
- *in vivo* database anchoring HTS *in vitro* assays
- Enables comparison of endpoint incidence between species
- Searchable database will be public (www.epa.gov/ncct/toxrefdb/)

LIMITATIONS

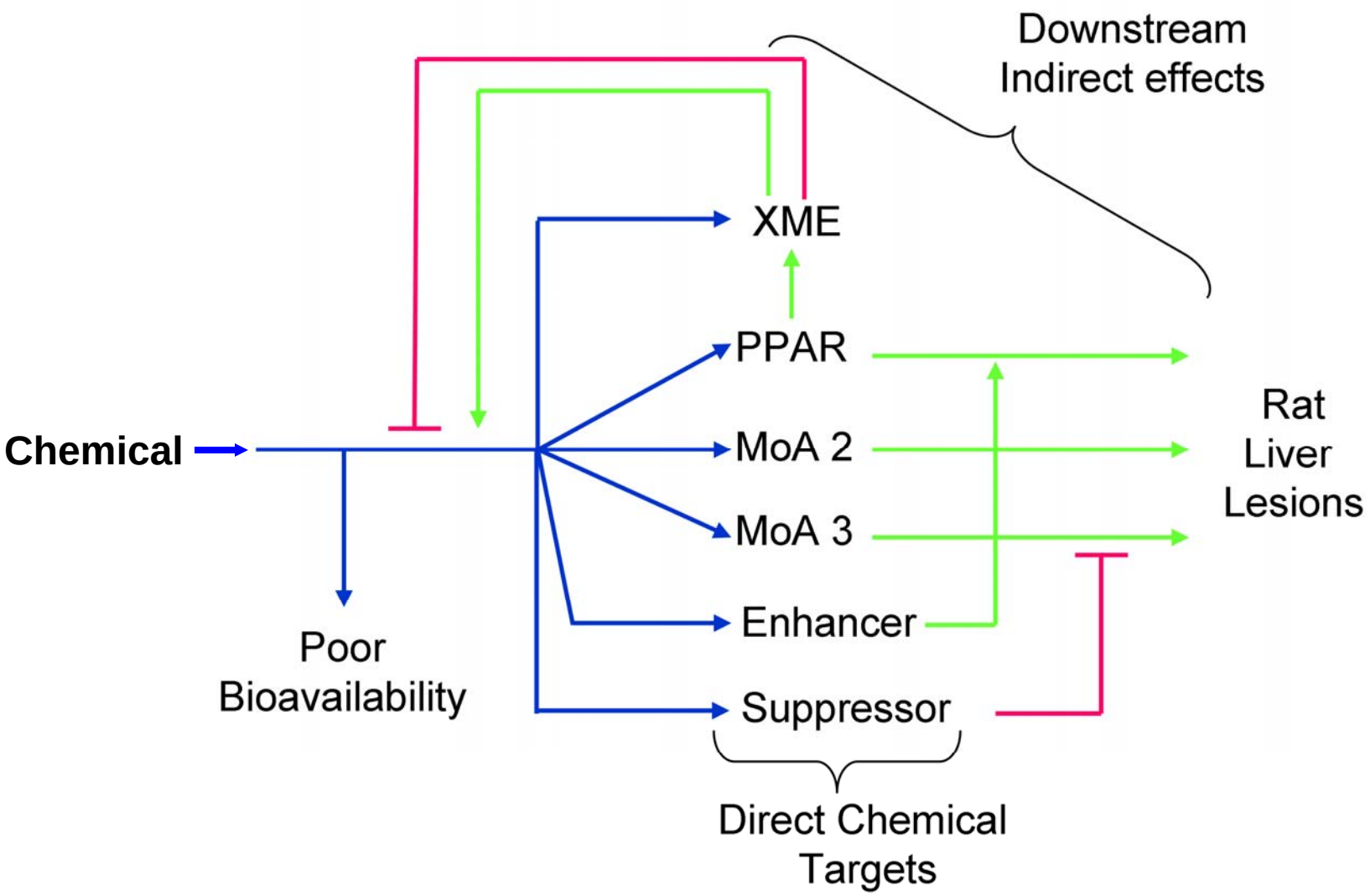
- Endpoints aggregated as independent features
- Data largely qualitative (LELs, LOAELS)
- Not all ToxCast™ chemicals represented in ToxRefDB
- Not all ToxRefDB chemicals represented in ToxCast™
- Species dimorphism may link to biology or study design
- Limited mode of action information available in source DERs
- Not all endpoints routinely measured/captured

Rat Liver Histopathology from Chronic Bioassays

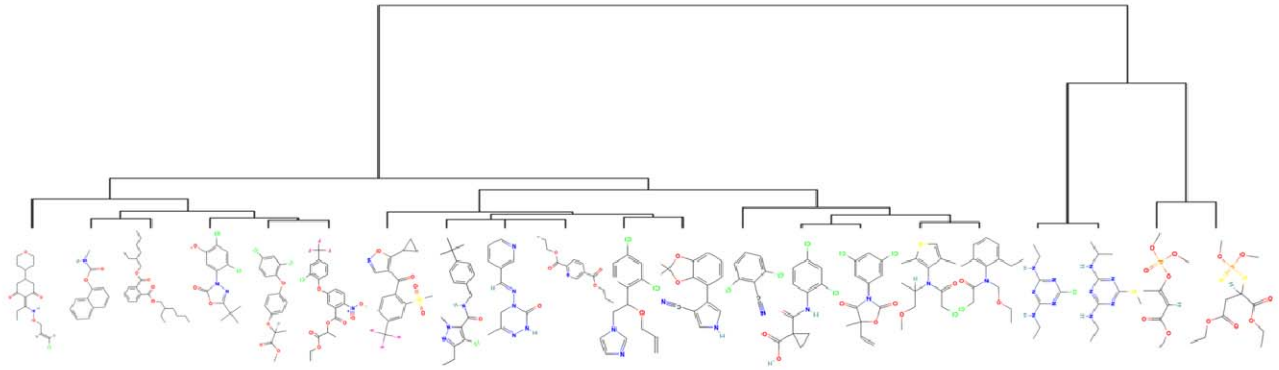


N = 248 Chemicals

Predicting Toxicity Is Not Easy



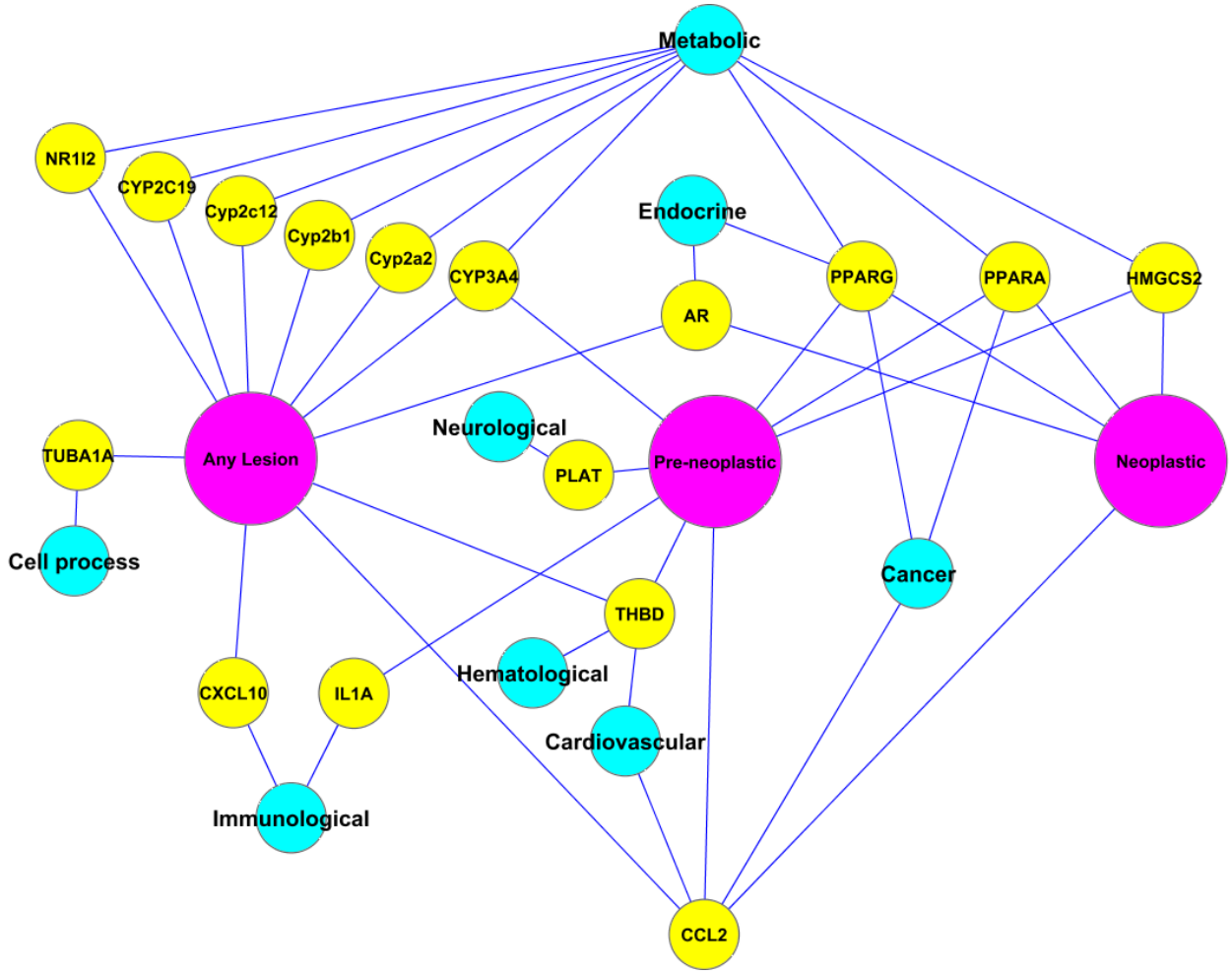
Rat Liver Tumor Correlations



Tepraloxydim	Carbaryl	Diethylhexyl phthalate	Oxadiazon	Diclofop-methyl	Lactofen	Isoxaflutole	Tebufenpyrad	Pymetrozine	2,5-Pyridinedicarboxylic acid, dipropyl ester	Imazalil	Fludioxonil	Dichlobenil	Cyclanilide	Vinclozolin	Dimethenamid	Acetochlor	Simazine	Ametryn	Mevinphos	Malathion	
			■	■	■									■	■	■				■	AR
		■		■	■					■											PPARA
	■	■	■	■	■	■	■		■	■	■		■	■	■	■	■	■	■	■	PPARG
					■		■						■			■		■			HMGCS2
			■	■		■			■								■		■		CCL2

Fisher's Exact test, $p<0.01$

Gene Networks Associated with Progression of Rat Liver Tumor Endpoints



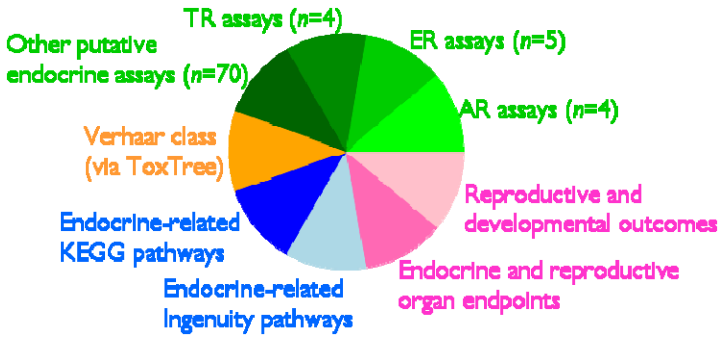
Some Challenges Faced or to be Faced

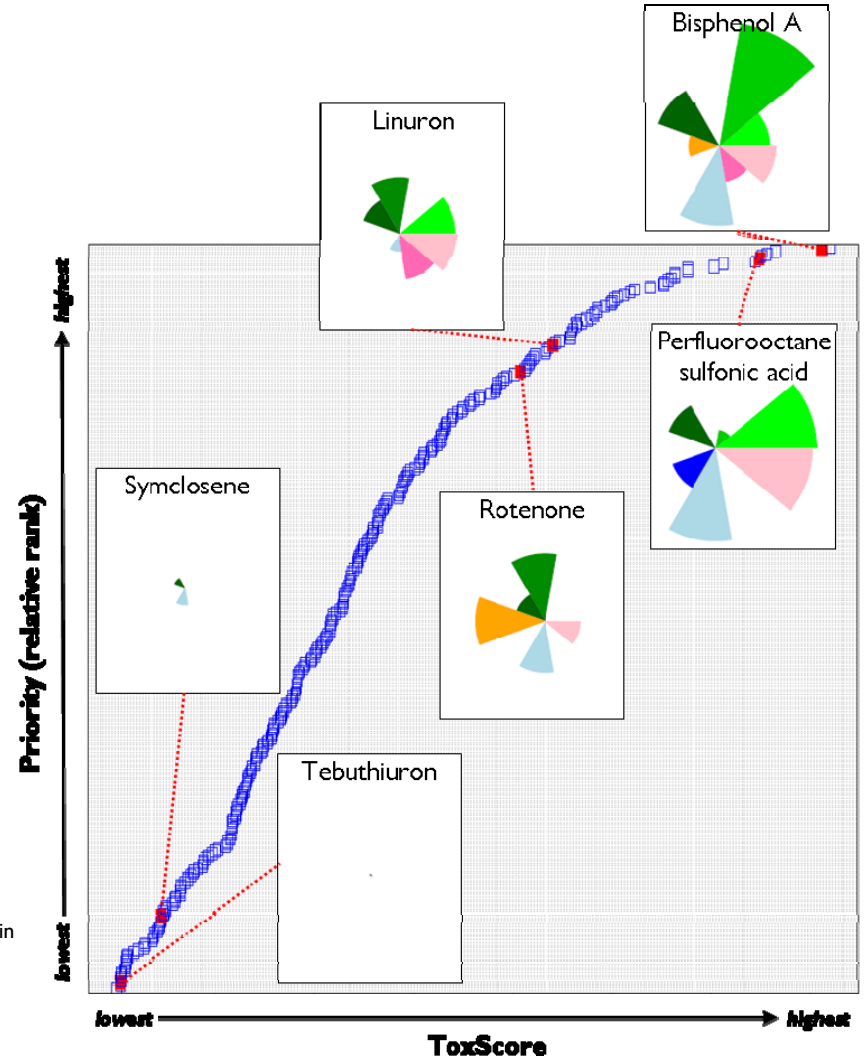
- Quality control of the chemical library
  - Acceptable purity, stability, and organization
- Defining/normalizing conc. response ranges
- Definition/Calculation of a hit
  - Minimum fold change; minimum r-squared; limit on Hill function
- Interpretation of hits and causality
  - Statistical vs. biological relevance
  - Association vs. causation
- Assay performance
  - Replicates, artifacts
- Sufficient coverage of biological pathways
  - Including those that represent tissue level processes
- Incorporation of metabolic competency
- Establishment of target prediction
  - Pathway perturbation
  - Rodent bioassay data
  - Rodent mechanistic studies
  - Human effects
- Sufficient representation of positives to predict against

Potential Application to Chemical Programs:
Endocrine Profiling & Prioritization

EDSP (Currently 67)

- 53 in ToxCast_320
- Tiered testing program
- Regulatory capacity to request data under FFDCA
- Mandate to test all chemicals
- Current practice:
  - *Exposure based chemical selection*
  - *Not selected based on potential endocrine disruption*
  - *Two-tier system*
- Potential need:
  - *Pre-screen for ER, AR, or TR activity*
  - *Priority setting/targeted testing once expanded to evaluating all chemicals*



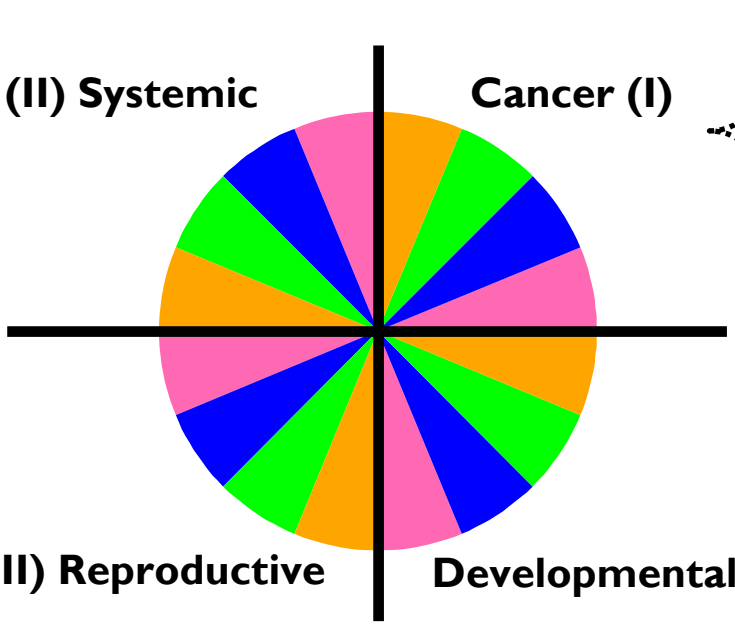


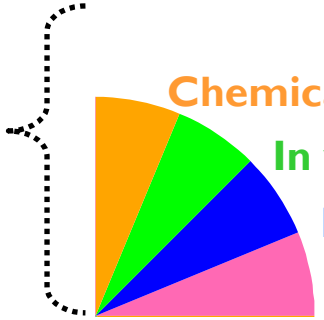
Each chemical signature/ gives a priority score (ToxScore) that can be ranked along any domain

ToxScore = f(In vitro assays + Chemical properties + Pathways + In vivo endpoints)

ToxCast Hazard-Based Prioritization

ToxScore = f(Chemical properties + In vitro assays + Pathways + Dosimetry)





Chemical properties (e.g. benzene fragment)

In vitro assays (e.g. p53 activation)

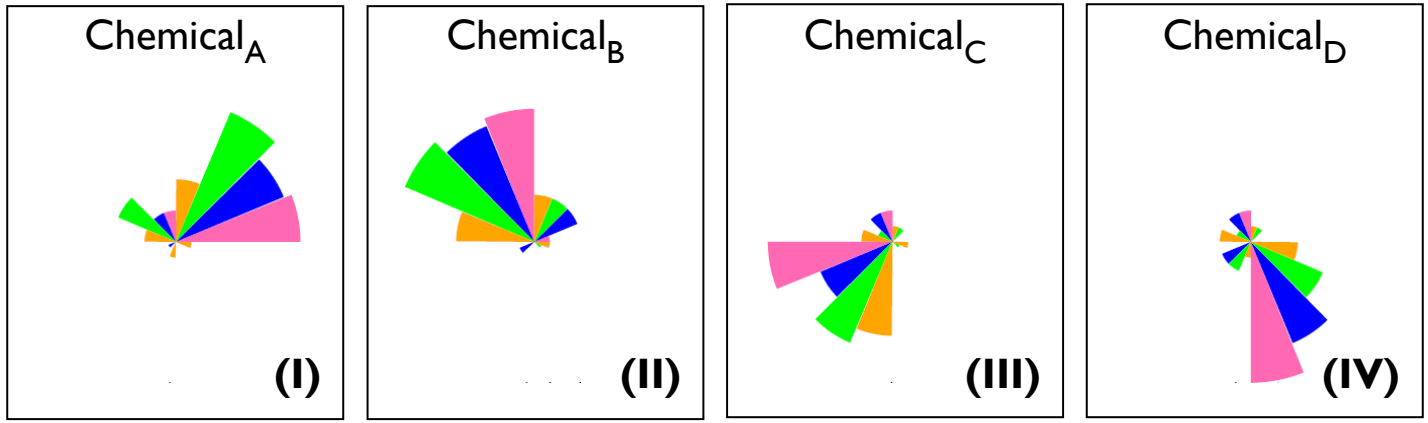
Pathways (e.g. DNA damage repair)

Dosimetry (e.g. biotransformation)

Features for each toxicity sector are selected for specific prioritizations; different chemical properties, assays, pathways and dosimetry for specific types of toxicity testing.

$$\text{ToxScore} = \sum_{1}^{C} w_c * \text{chemProp}_c + \sum_{1}^{I} w_i * \text{assay}_i + \sum_{1}^{P} w_p * \text{pathway}_p + \sum_{1}^{E} w_e * \text{dosimetry}_e$$

Example ToxScores prioritizing chemicals for Cancer, Systemic, Reproductive or Developmental testing, respectively.



Reif and Dix, Unpublished

Prioritization Product Timeline

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Cost per Chemical	Target Date
Ia	320	Data Rich (pesticides)	Signature Development	552	$20k	FY07-09
Ib	15	Nanomaterials	Pilot	166	$10K	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	~$20 -25k	FY09-11
IIb	>100	Known Human Toxicants	Extrapolation	>400	~$20 -25k	FY09-11
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	~$20 -25k	FY09-11
IId	>12	Nanomaterials	PMN	>200	~$15 -20K	FY10-11
III	Thousands	Data poor	Prediction and Prioritization	>300	~$15 -20k	FY11 -12

FY07 FY08 FY09 FY10 FY11 FY12

Proof of Concept: ToxCast

Verification/Extension

Reduce to Practice

Tox21

Phase II Plans

- Done in conjunction with Tox21 10k Library
  - Subset of 700 will seed Phase II
- Chemical Diversity
  - More food use active pesticides (~100-200)
  - Pesticidal antimicrobials & inerts (~100-200)
  - Failed pharmaceuticals (preclinical and clinical, ~100-150)
  - “Green” chemicals
  - HPV Categories
  - Liver toxicants
  - OECD Molecular Screening Group nominations
- Evaluation of Phase I Assays
- Additional assays via competitive procurements, collaborative partners…