

Developing Predictive Bioactivity Signatures of Carcinogenesis Using ToxCast HTS Data

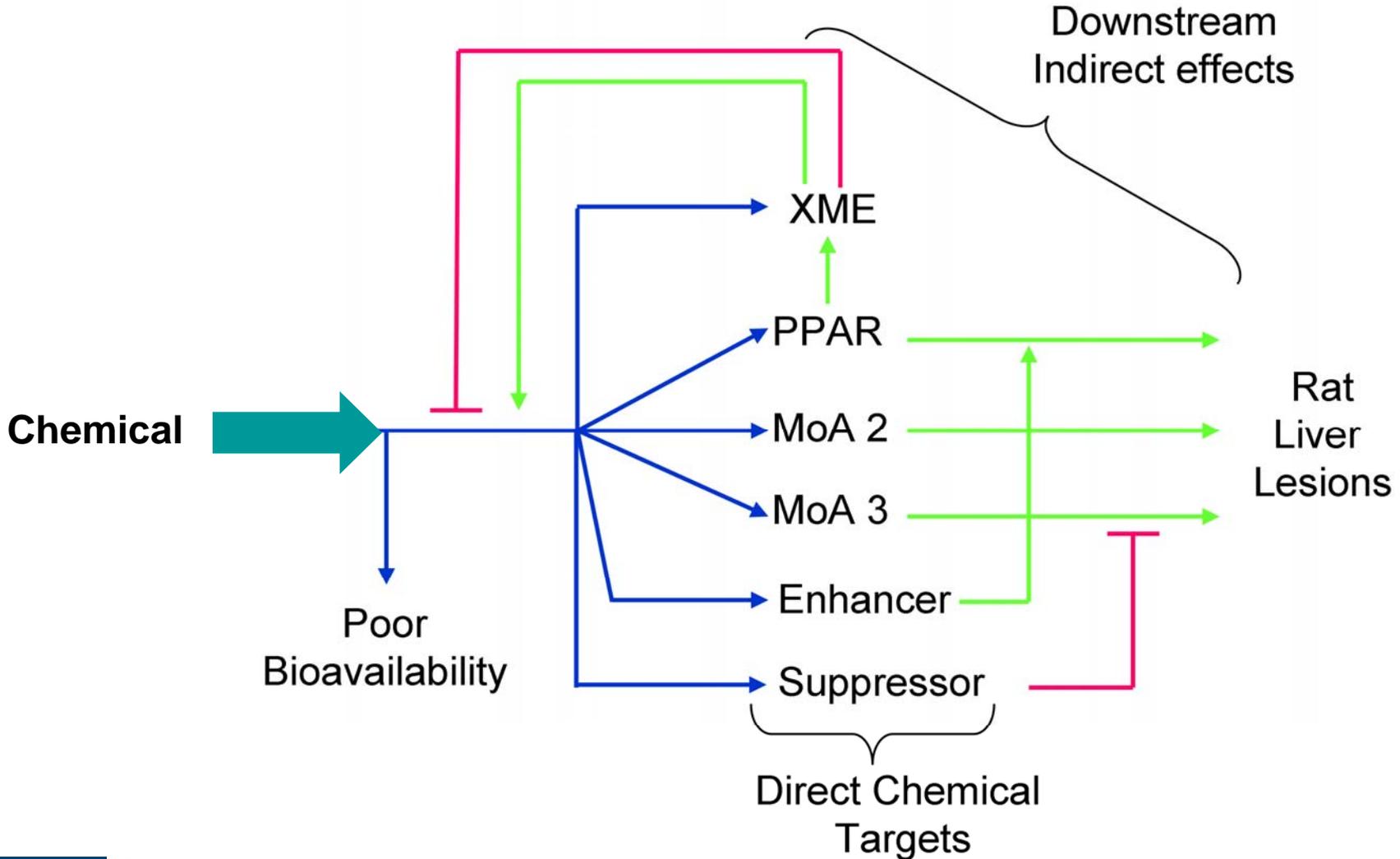
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY



**COMPUTATIONAL
TOXICOLOGY**

Predicting Toxicity Will Not Be Easy

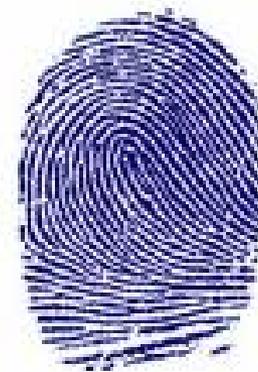


Key Challenges Of Pathway Profiling

- Find the Toxicity Pathways
 - Hepato vs developmental neurotoxicity
- Obtain HTS Assays for Them
 - Including metabolic capability
- Screen Chemical Libraries
 - Coverage of p-chem properties
- Link Results to in vivo Effects
 - Gold standard and dosimetry

ToxCast™ Background

- Research program of EPA's National Center for Computational Toxicology
- Addresses chemical screening and prioritization needs for pesticidal inerts, anti-microbials, 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/>



The ToxCast_320

309 Unique Structures

Replicates for QC

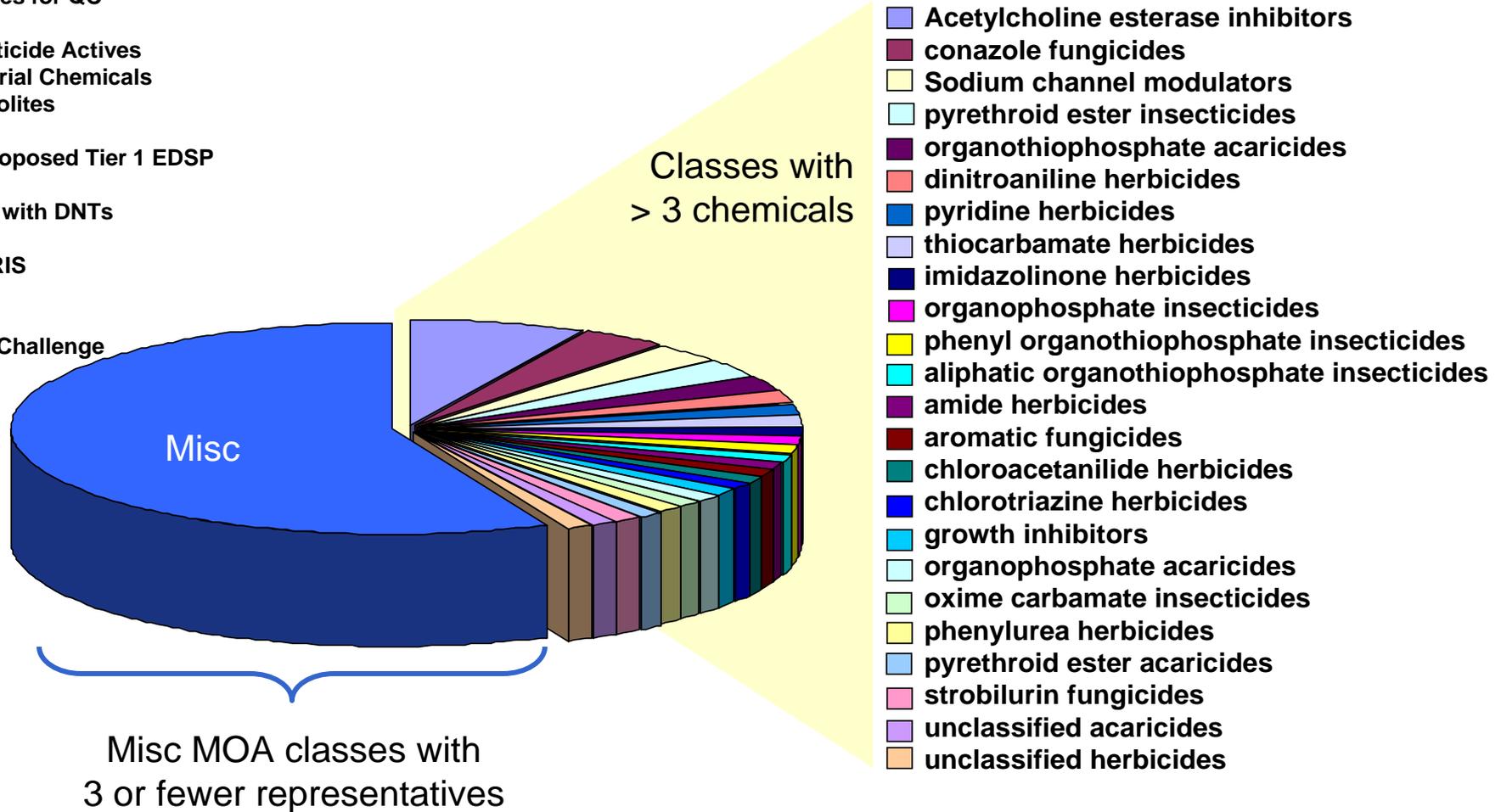
291 Pesticide Actives
9 Industrial Chemicals
8 Metabolites

56/73 Proposed Tier 1 EDSP

53 of 80 with DNTs

122 in IRIS

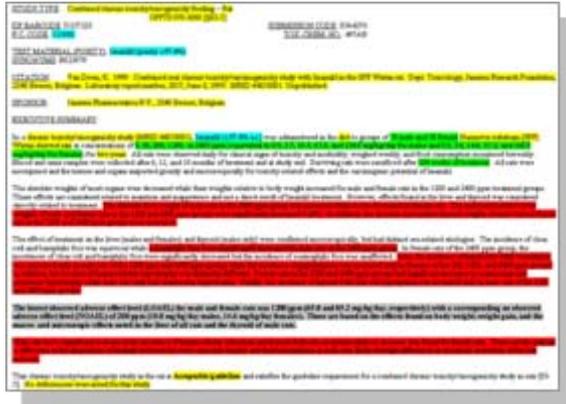
14 HPV
11 HPV Challenge



- Relational phenotypic/toxicity database
- Provides in vivo anchor for ToxCast predictions
- Three study types
 - Chronic/Cancer Rat and Mouse (Martin, et al, EHP 2008)
 - Rat multigenerational Reproduction (Martin, et al, 2009)
 - Rat & Rabbit Developmental Toxicity (Knudsen, et al, 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)

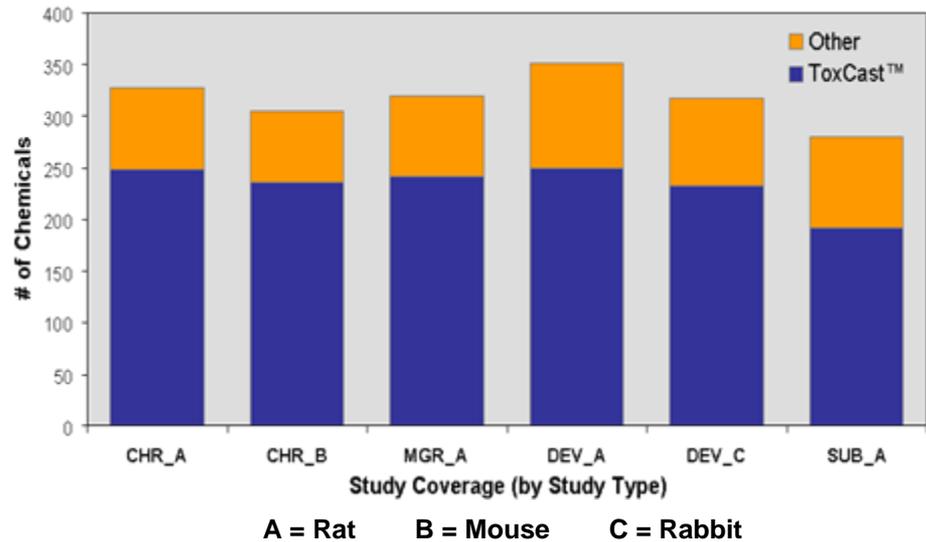
Martin et al. (2008) Environ Hlth Persp
doi:10.1289/ehp.0800074

MULTIGENERATION REPRODUCTIVE (MGR)

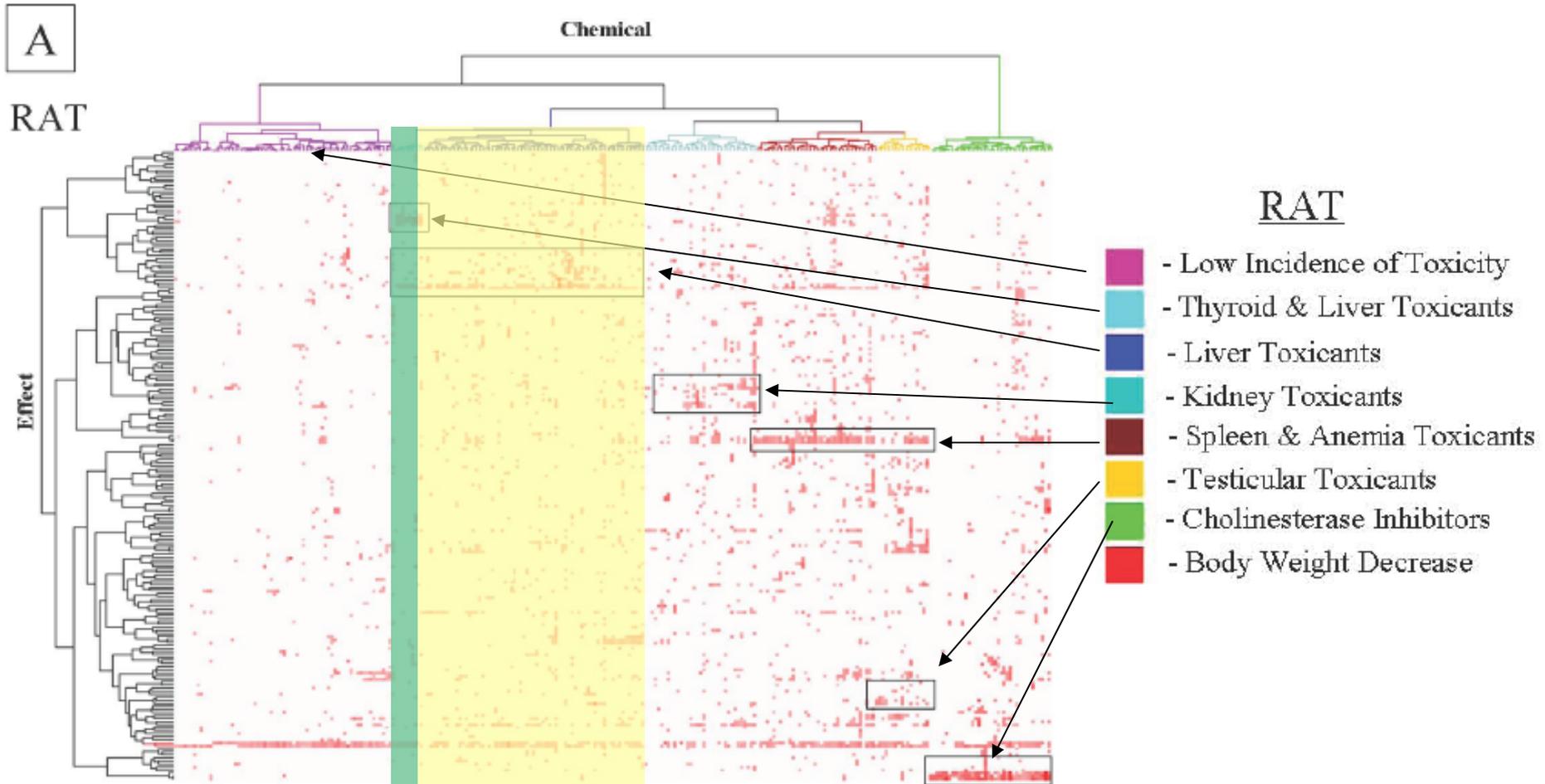
Martin et al. (2009) Toxicol Sci
doi: 10.1093/toxsci/kfp080

PRENATAL DEVELOPMENTAL (DEV)

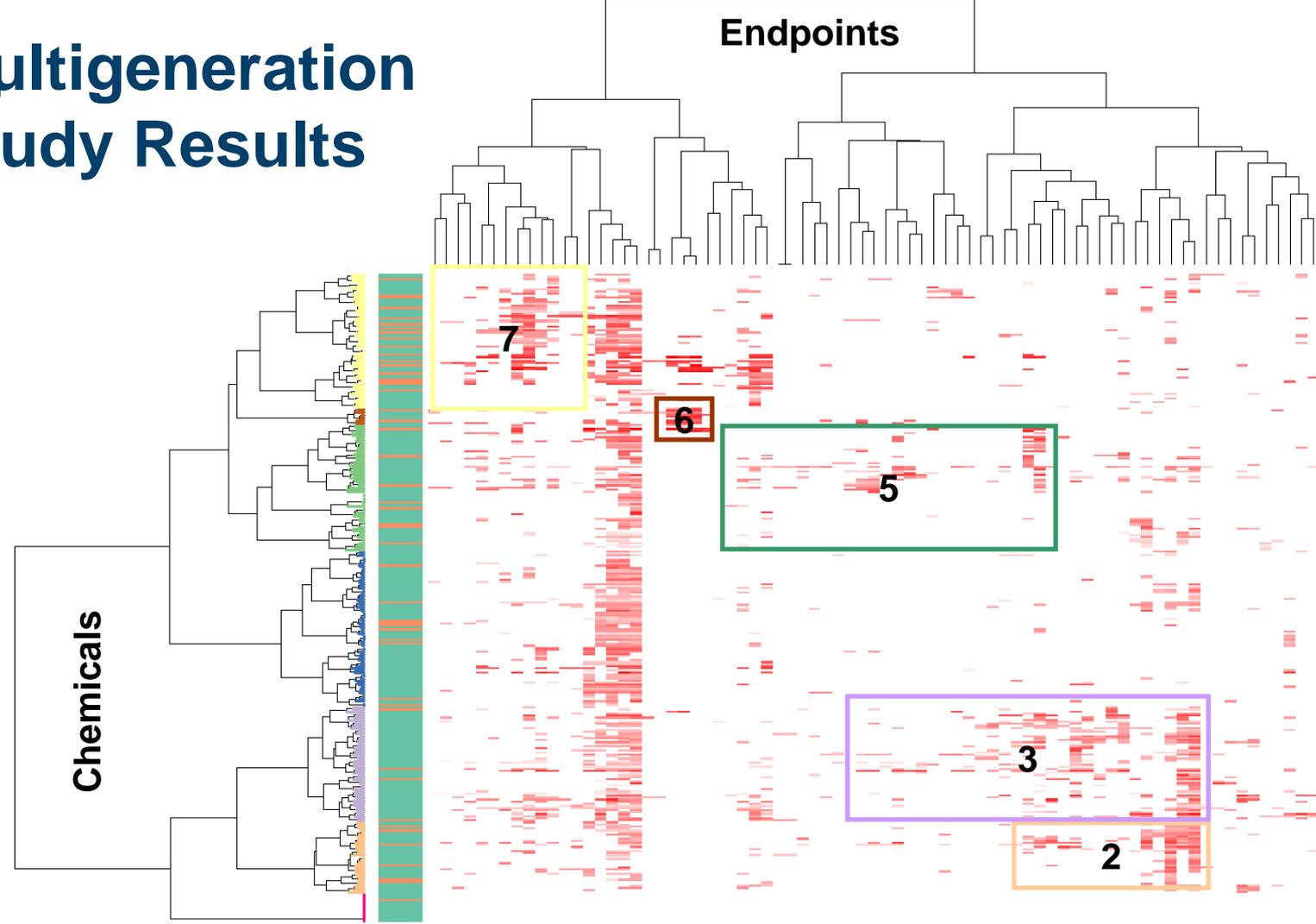
Knudsen et al. (2009) Reprod Toxicol
doi: 10.1016/j.reprotox.2009.03.016



Rat Chronic Bioassay Results



Multigeneration Study Results



| CHEMICAL CLUSTER # (Top 10 Weighted Endpoints) | | | | | | |
|--|---|--|---|---|--|--|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | Adrenal Pathology AGD Kidney Pathology Liver Pathology Liver Pathology (g) Liver Weight Pituitary Weight Thyroid Pathology Thyroid Weight Uterine Weight | ALP Heart Weight Lung Weight Pituitary Weight Sperm Morphology Spleen Pathology Spleen Pathology (g) Spleen Weight Thymus Weight VO | | AGD Bladder Pathology Epididymal Pathology Epididymal Weight HCT HGB Prostate Weight Testis Pathology Testis Pathology (g) Testis Weight | Birth Index ChE (Brain) ChE (Brain-regional) ChE (Plasma) ChE (RBC) Epididymal Pathology Lung Pathology Lung Pathology (g) Prostate Pathology Water Consumption | Clinical Signs Eye Developmental Fetal Mortality Lactation Index Litter Weight Live Birth Index Lung Pathology Lung Pathology (g) Stomach Pathology (g) Viability Index |

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

ToxCast Assays

Biochemical Assays

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

467 Endpoints

Cellular Assays

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

Confidence Builders: 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

Confidence Builders (3): Pathway Based Analysis

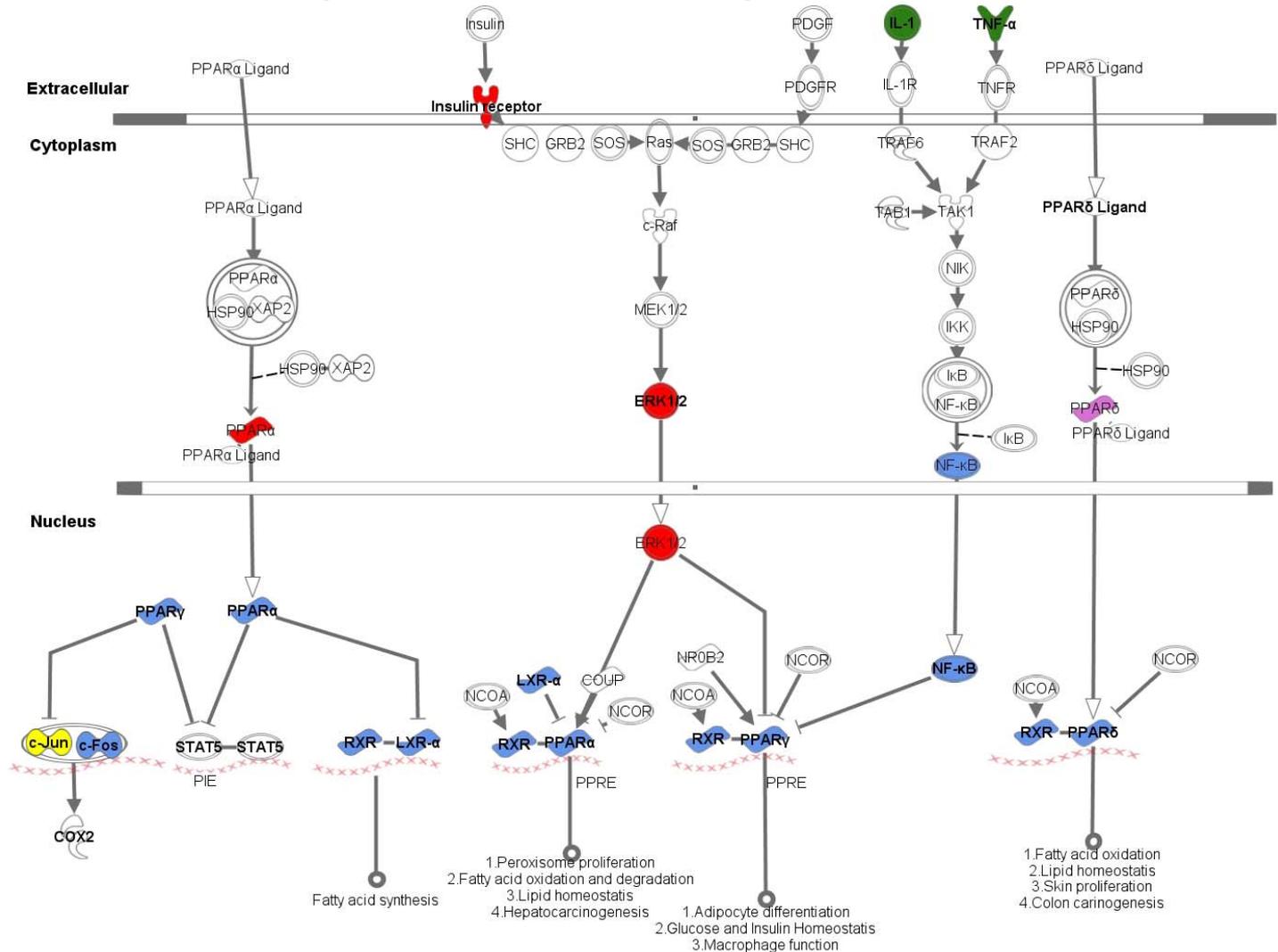
● Biologically Multiplexed Activity Profiling (BioMAP)

● Multiplex Transcription Reporter Assay

● Cell-based HTS Assays

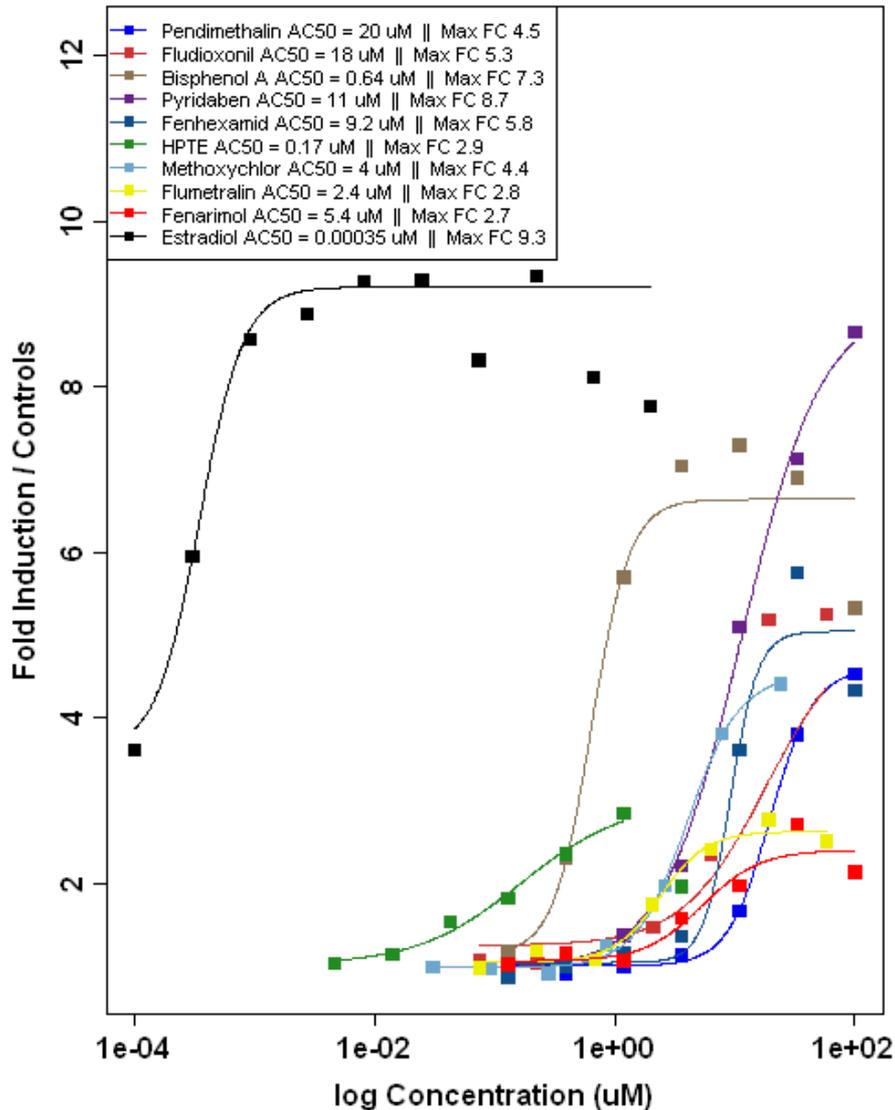
● Cell-free HTS Assays

● High Content Cell Imaging Assays

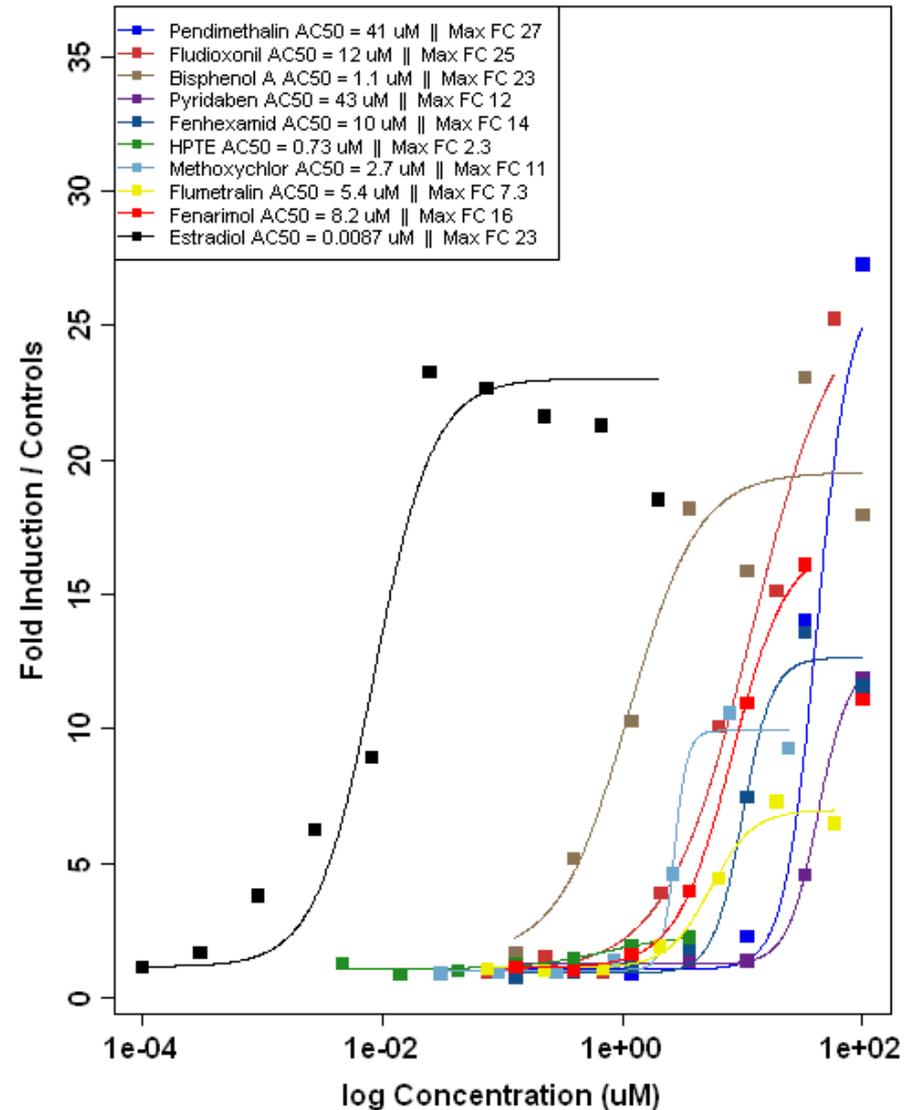


Data Analysis: What is a hit?

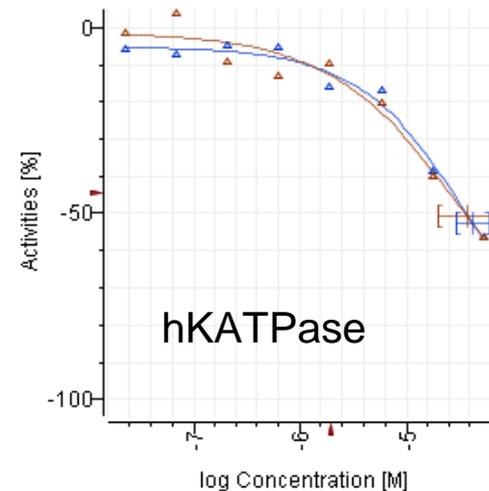
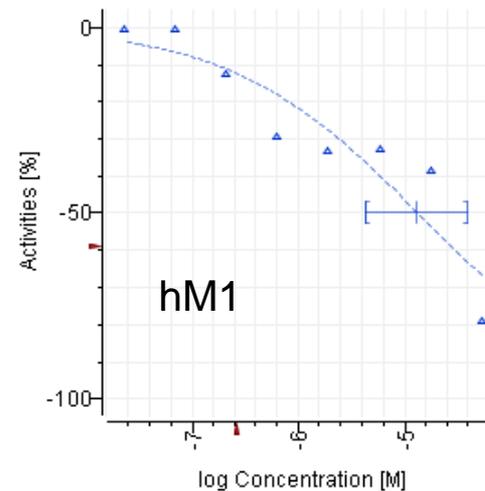
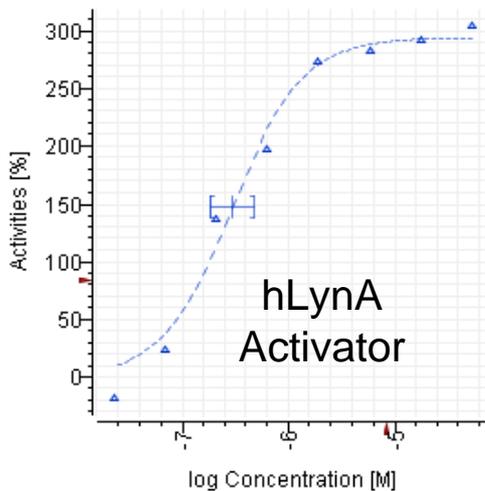
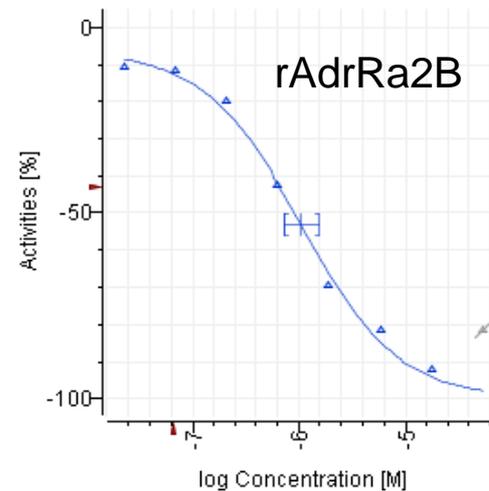
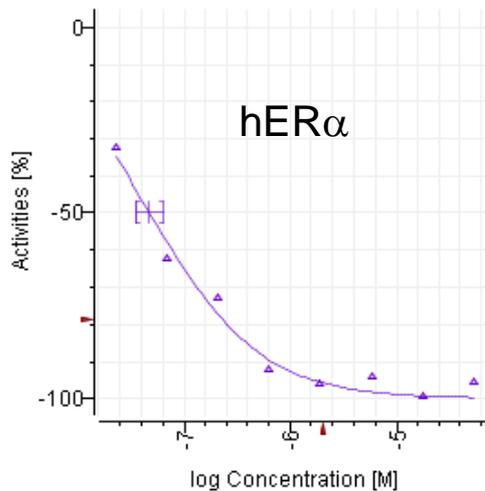
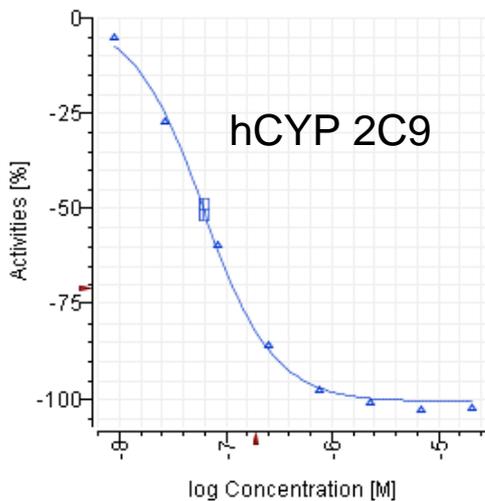
Attagene ERE_CIS



Attagene ERa_TRANS

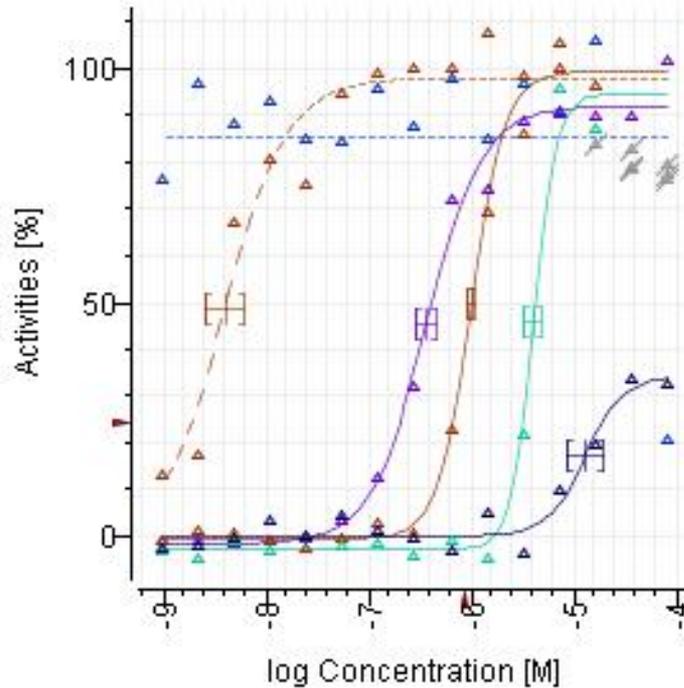


Biochemical HTS from Novascreen

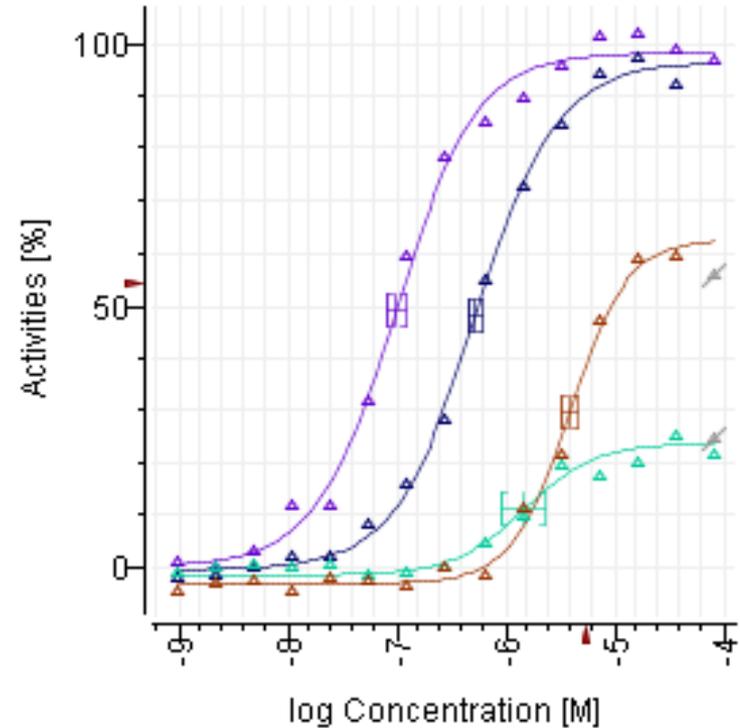


qHTS from the NCGC on NRs

ER α



PPAR γ

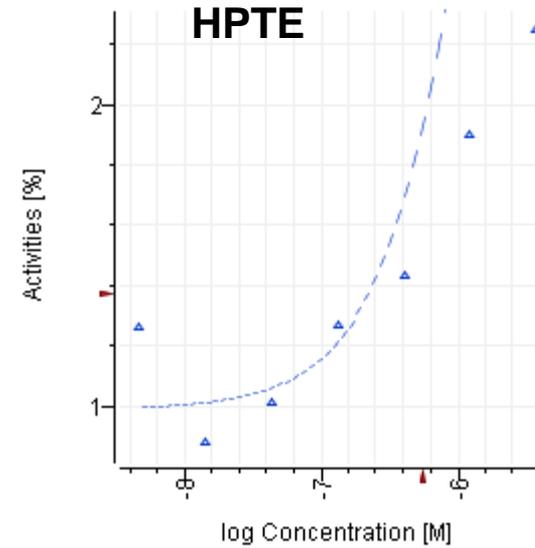
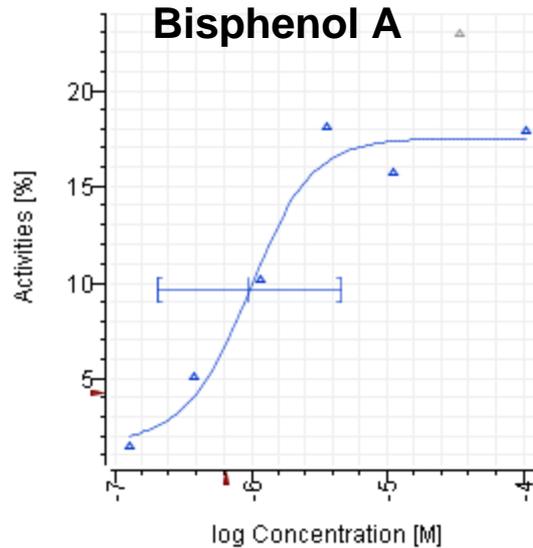


- NCGC00090749-04
- NCGC00161666-02
- NCGC00023462-04
- NCGC00025156-10
- NCGC00090965-03
- NCGC00164033-01

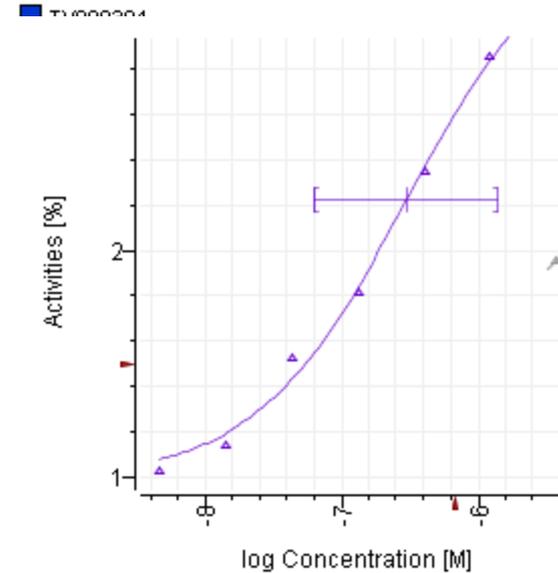
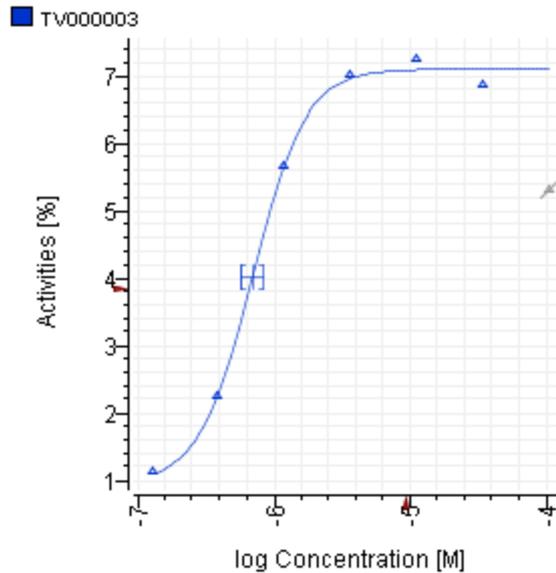
- NCGC00164420-01
- NCGC00093991-03
- NCGC00164230-01
- NCGC00022570-07

Attagene: *cis* and *trans* Assays

trans: ERa

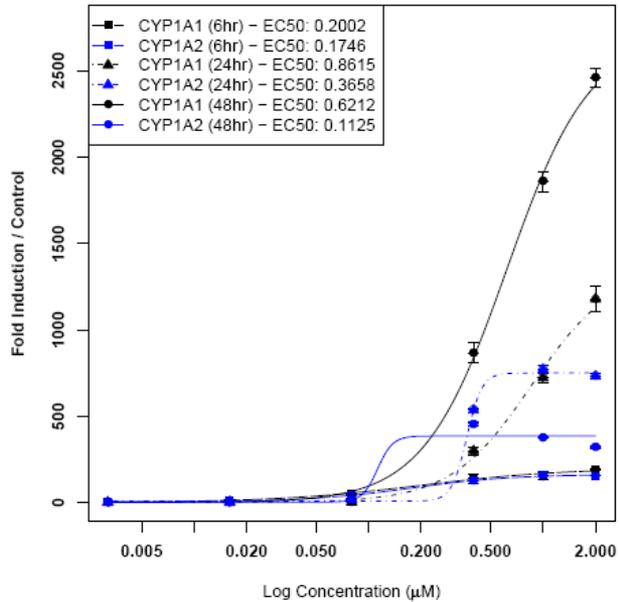


cis: ERE

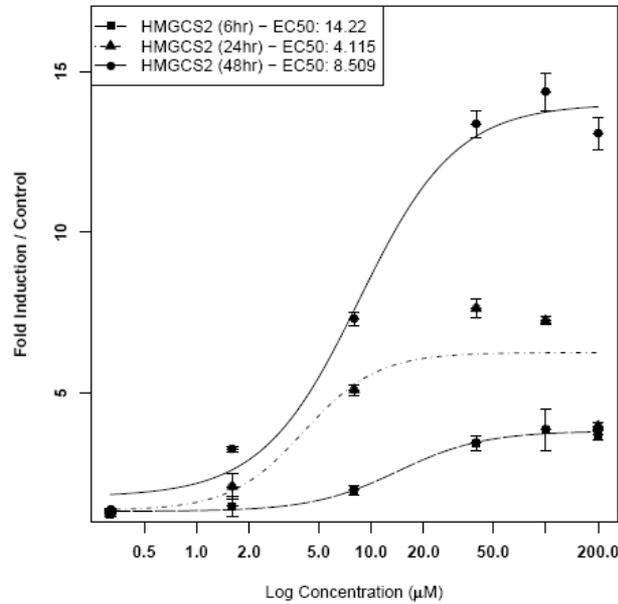


CellzDirect: Data Examples

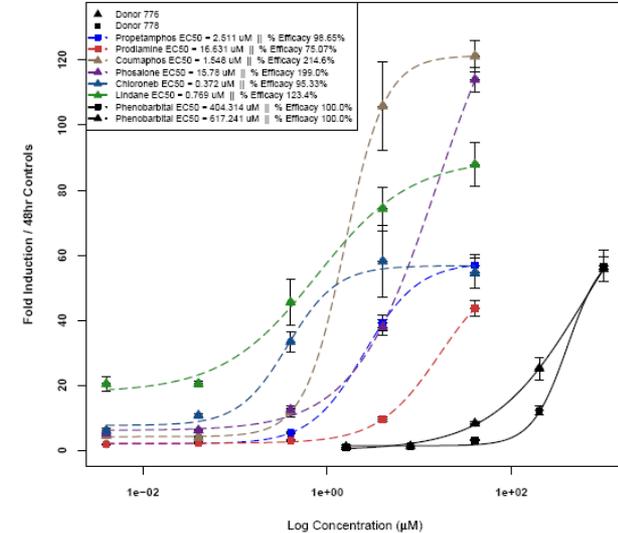
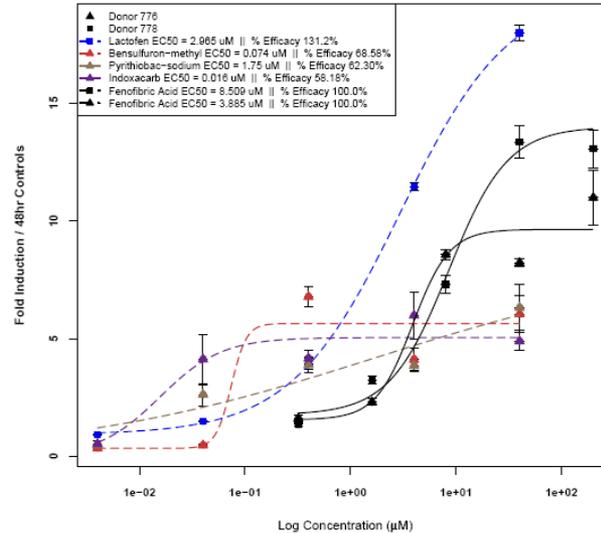
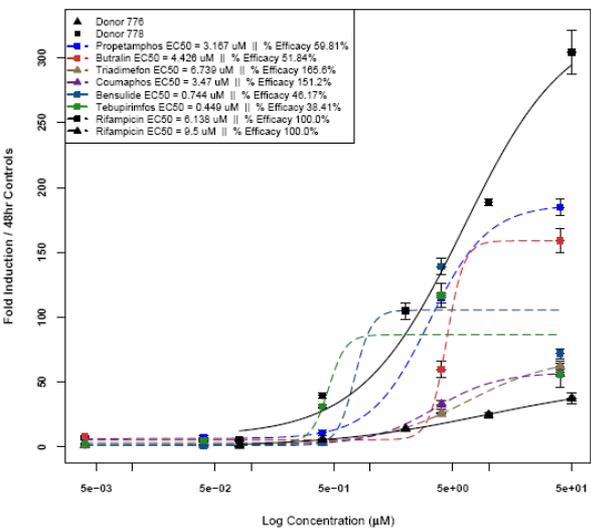
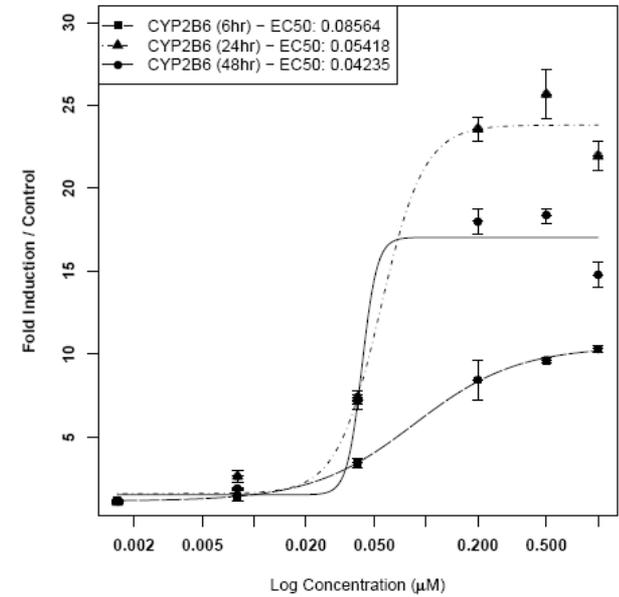
CYP1A1-AhR



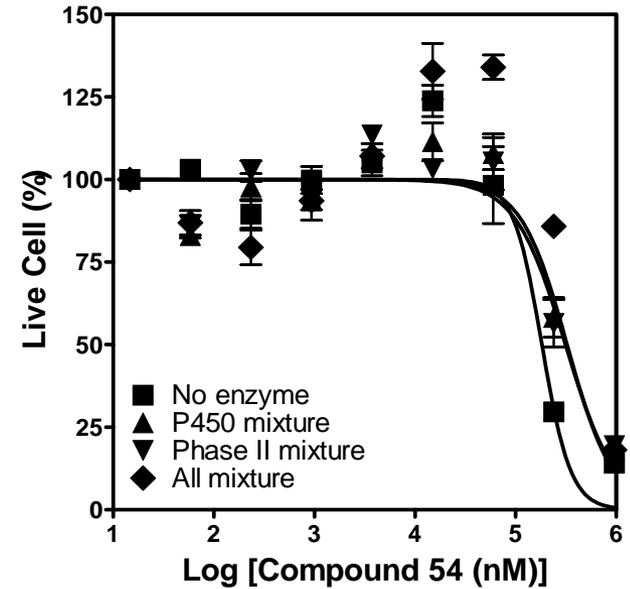
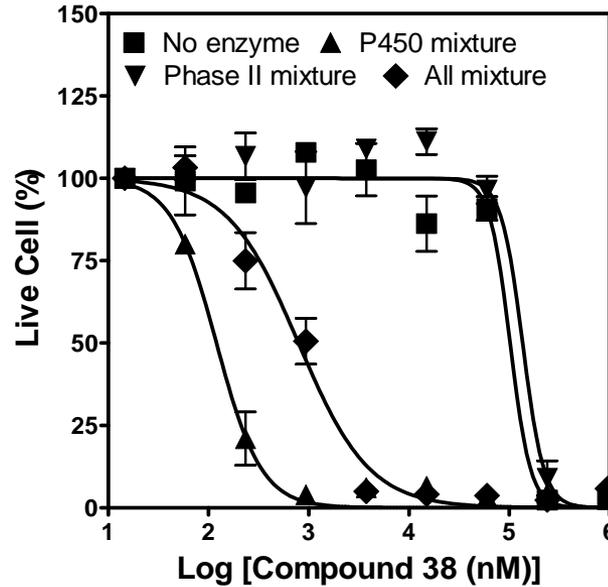
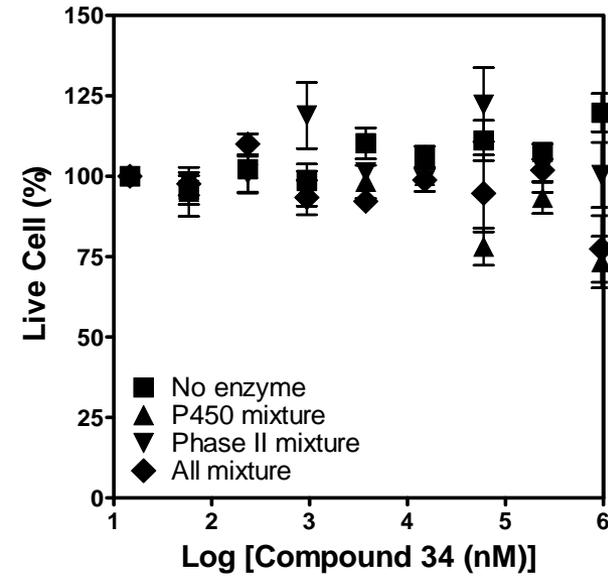
HMGCS2-PPAR α



CYP2B6-CAR

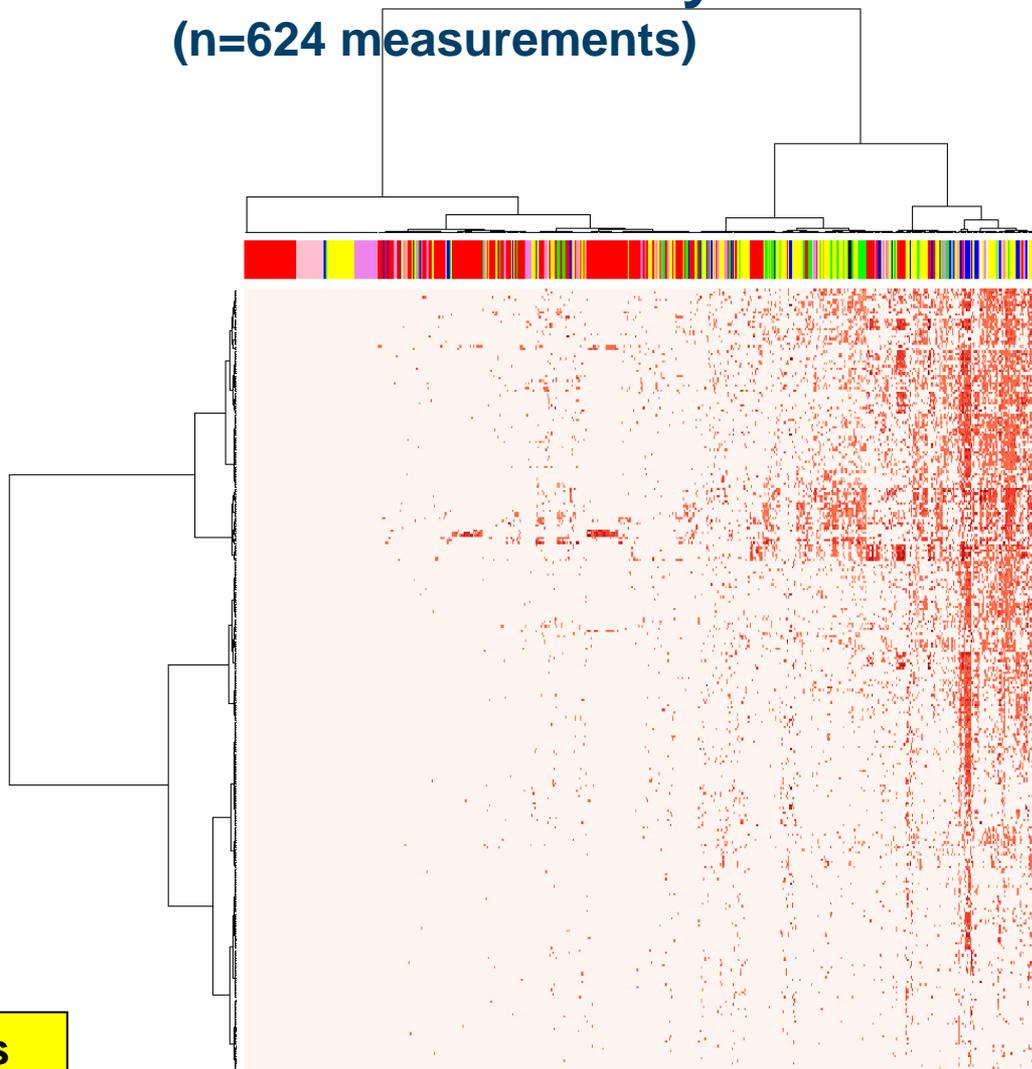


Metabolic Activity from Solidus



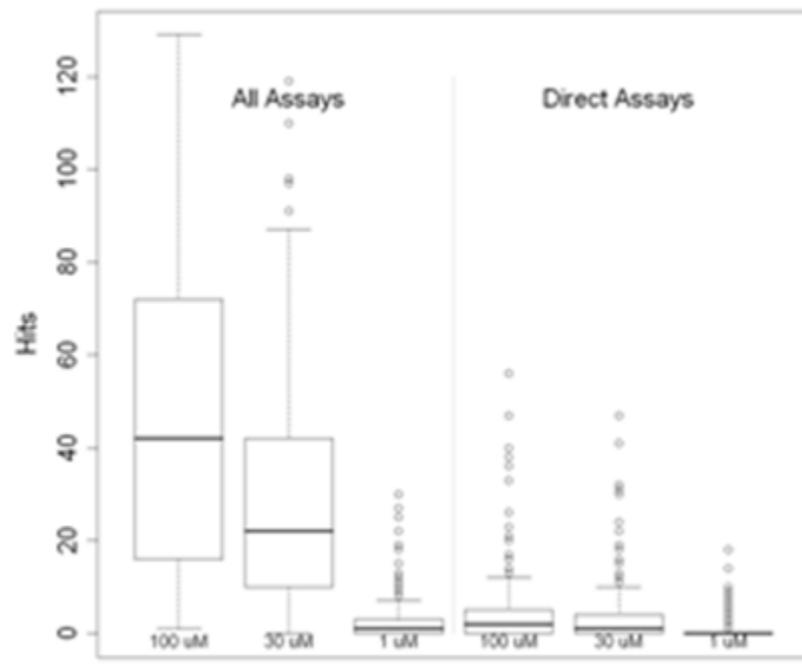
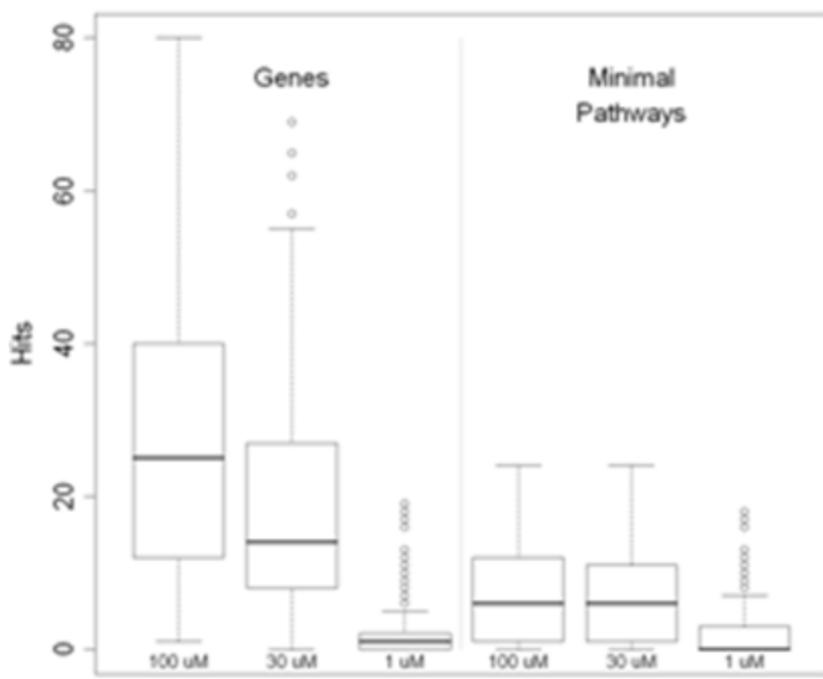
ToxCast Phase I Assay Hits (n=624 measurements)

-  Cell Free HTS
-  Multiplexed TF
-  Human BioMap
-  HCS
-  qNPAs
-  XMEs
-  Impedance
-  Genotoxicity



**828 Assay-Chemical Pairs
had AC50s of less than 1 μ M**

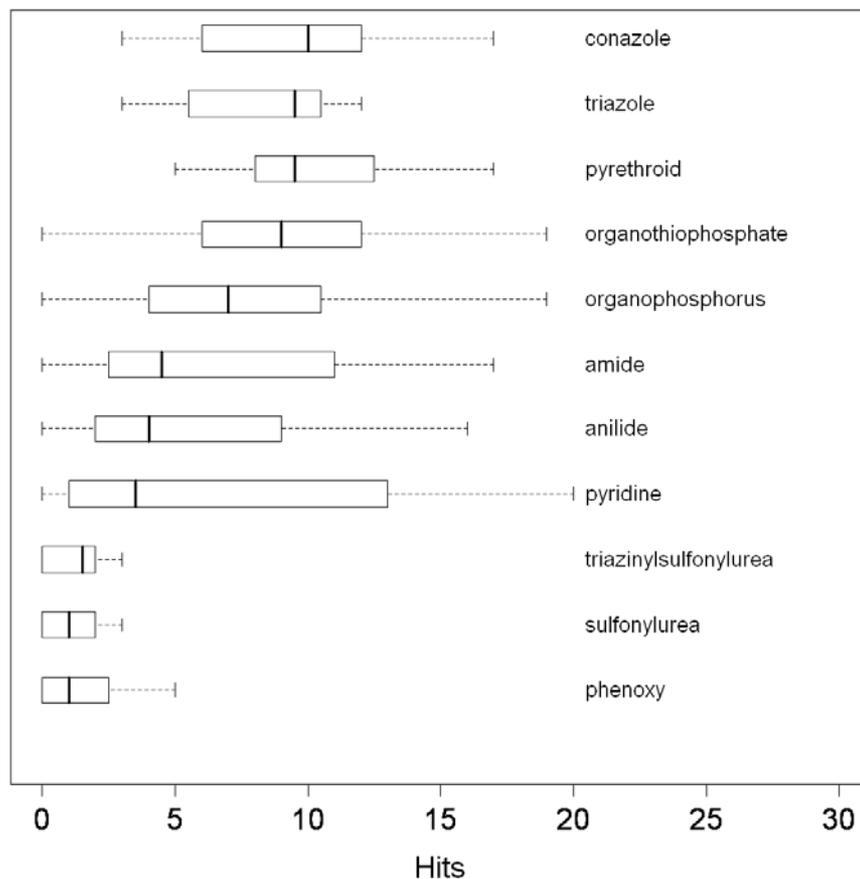
“Hits” per Chemical As a Function of AC50/LEC Cutoff



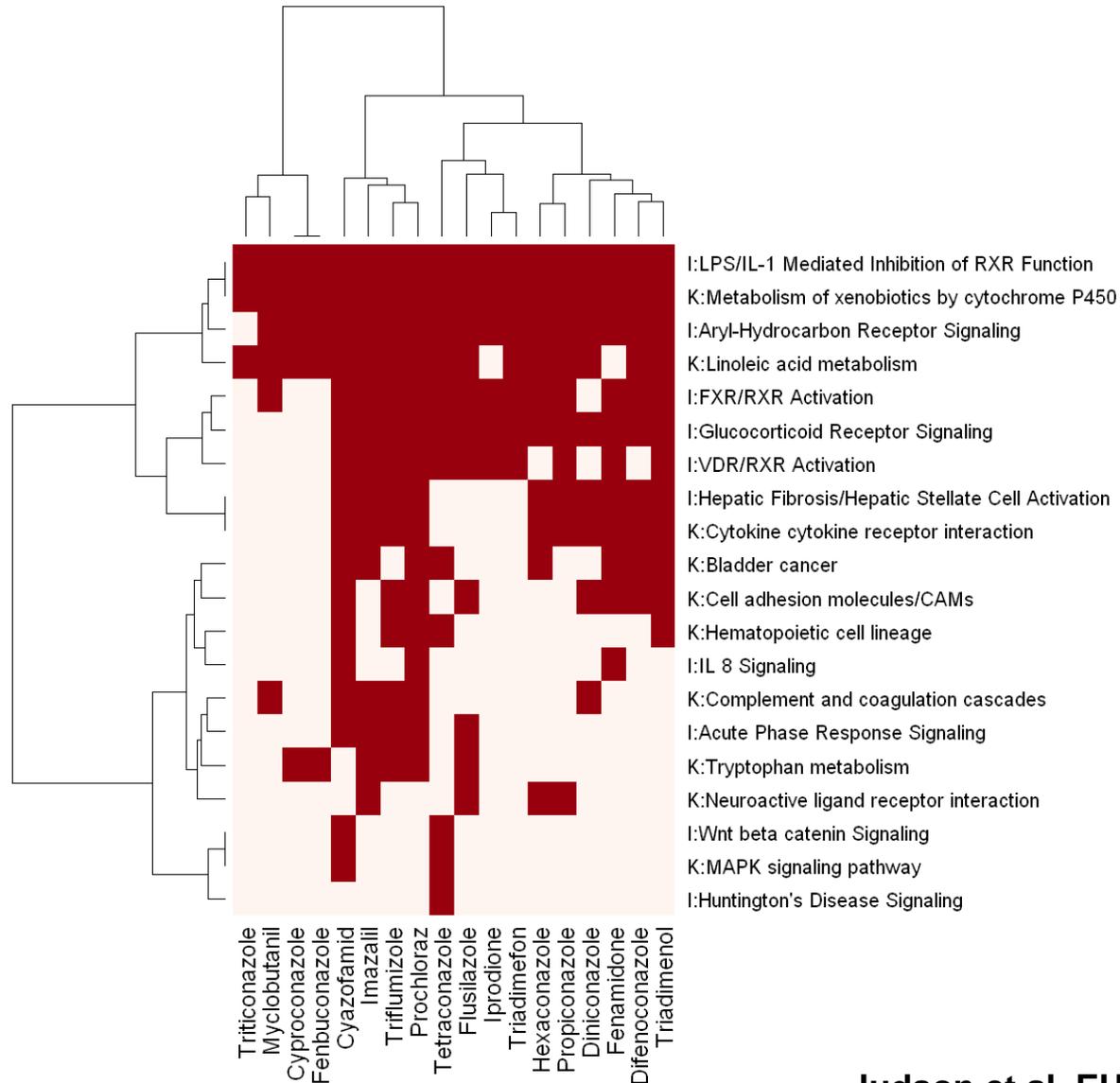
9 Chemicals have at least 20 hits at an AC50 of <math><30\mu\text{M}</math>

“Hit” Distribution for Chemical Classes Against 33 Minimal Pathways (at least 10 chemicals per class)

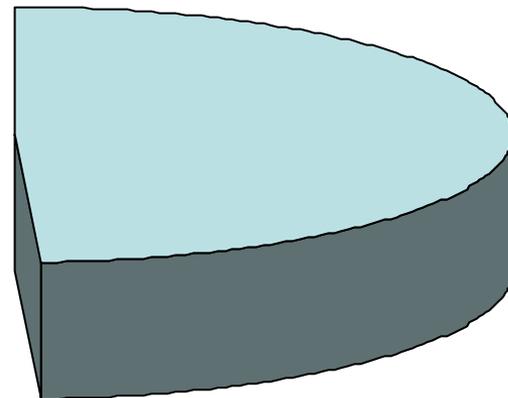
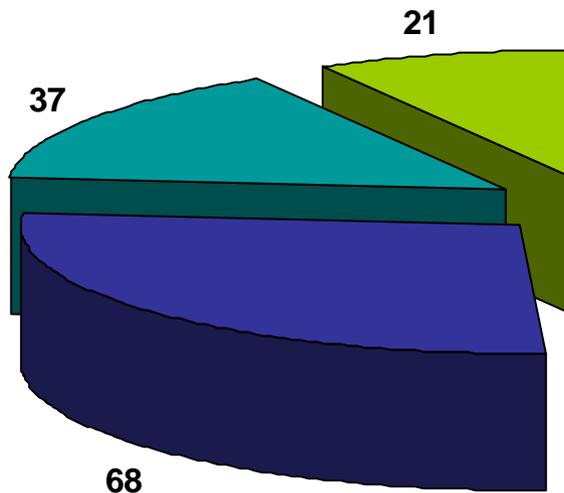
Minimal Pathways, 30 uM



Activity of Conazoles Against Minimal Pathway Set

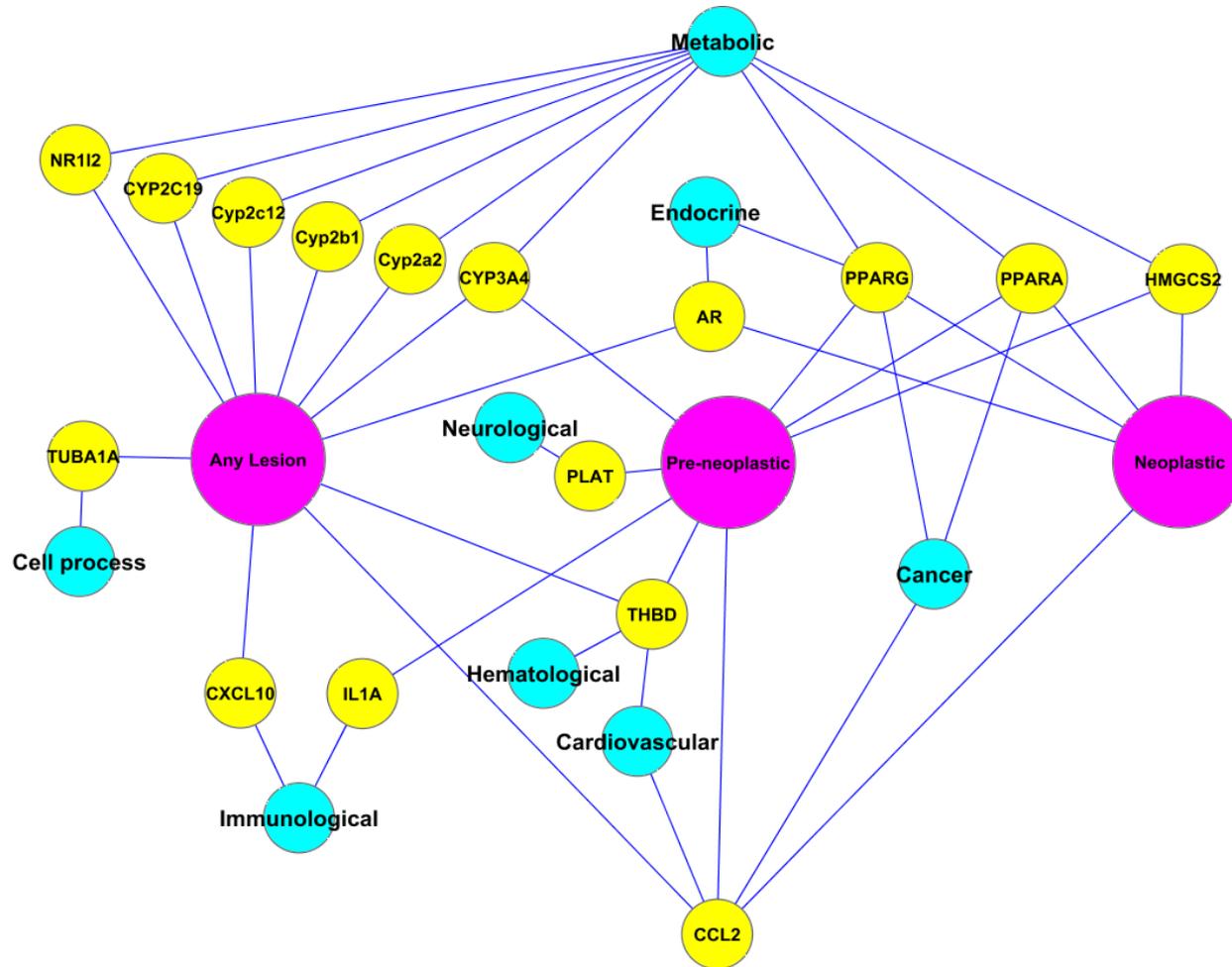


Rat Liver Histopathology from Chronic Bioassays



N = 248 Chemicals

Gene Networks Associated with Progression of Rat Liver Tumor Endpoints



Some Challenges Faced or to be Faced

- **Organizing the chemical library**
- **Quality control of the chemical library**
 - **Acceptable purity, stability**
- **Defining concentration response ranges to the assayed**
- **Definition/Calculation of a hit**
 - **Minimum fold change; minimum r-squared; limit on Hill function**
- **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**

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 pesticides (~100-200)
 - Failed pharmaceuticals (preclinical and clinical, ~100-150)
 - “Green” chemicals
 - HPV Categories
 - Liver toxicants (~150)
 - OECD Molecular Screening Group nominations
- Evaluation of Phase I Assays
- Addition of new assays via competitive procurements
- Timing
 - Chemical procurement completed 4thQ FY09
 - Launch of Assays, 1st Q FY10
 - Results Available early FY11