ToxCast: Using HTS To Identify Profiles of Biological Activity

Robert Kavlock
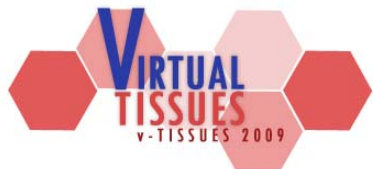*Director, EPAs National Center for Computational Toxicology*

Office of Research and Development
National Center for Computational Toxicology

WC7
Rome, Italy
September 2 2009

Acknowledgements

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

COMPUTATIONAL TOXICOLOGY

VIRTUAL TISSUES v-TISSUES 2009

Virtual Embryo
- Tom Knudsen
- Michael Rountree
- Nisha Snipes
- Richard Spenser (LHM)
- **NHEERL**
  - Sid Hunter
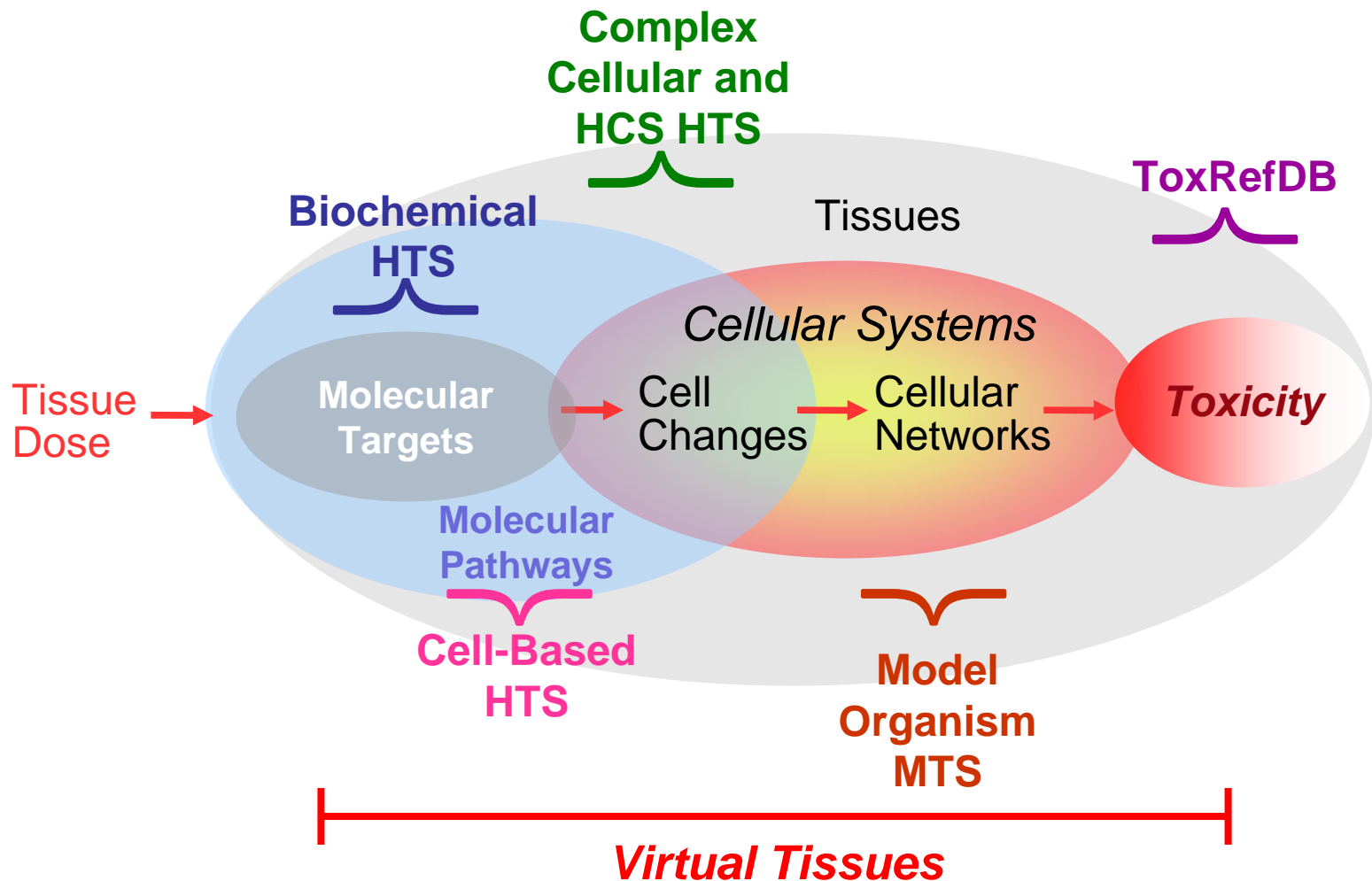 - Kelly Chandler
  - Stephanie Padilla
  - Chris Lau
  - John Rogers

Virtual Liver
- Imran Shah
- John Wambaugh
- John Jack
- **NHEERL**
  - Chris Corton

ToxCast
- David Dix
- Keith Houck
- Matt Martin
- Richard Judson
- Holly Mortenson
- David Reif
- Woody Setzer
- **NHEERL +**
- **NTP**
  - Ray Tice+
- **NCGC**
  - Chris Austin +

v-Embryo™

Predicting Human Toxicity:
The Grand Challenge in Toxicology

EPA's Need for Prioritization

Too Many Chemicals

Too Little Data (%)

9912

Legend (Too Many Chemicals):
- IRIS
- TRI
- Pesticides
- Inerts
- CCL 1 & 2
- HPV
- MPV

Legend (Too Little Data):
- Acute
- Cancer
- Gentox
- Dev Tox
- Repro Tox

Judson, et al *EHP* (2009)

EPA Strategic Plan (2009)

The U.S. Environmental Protection Agency's Strategic Plan for Evaluating the Toxicity of Chemicals
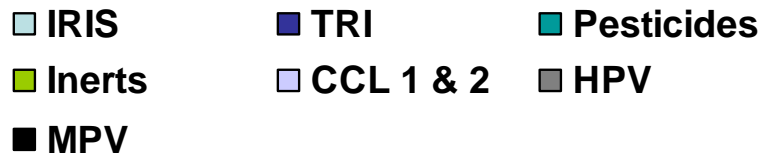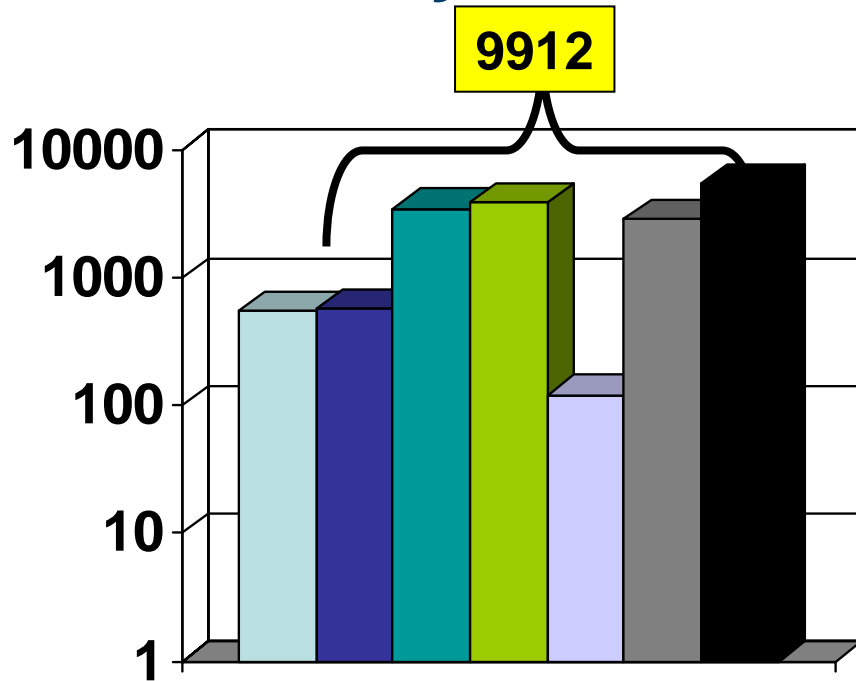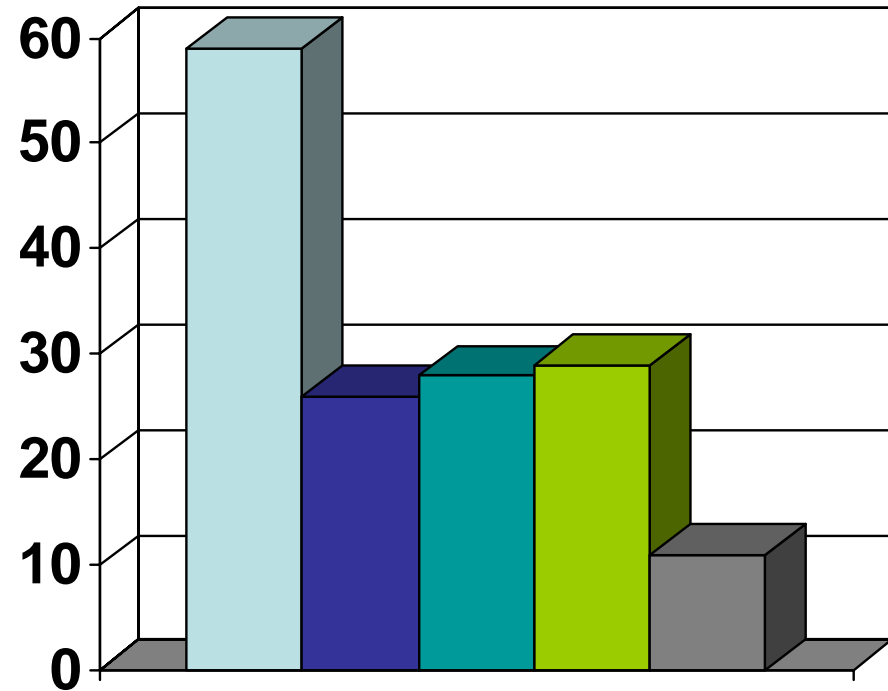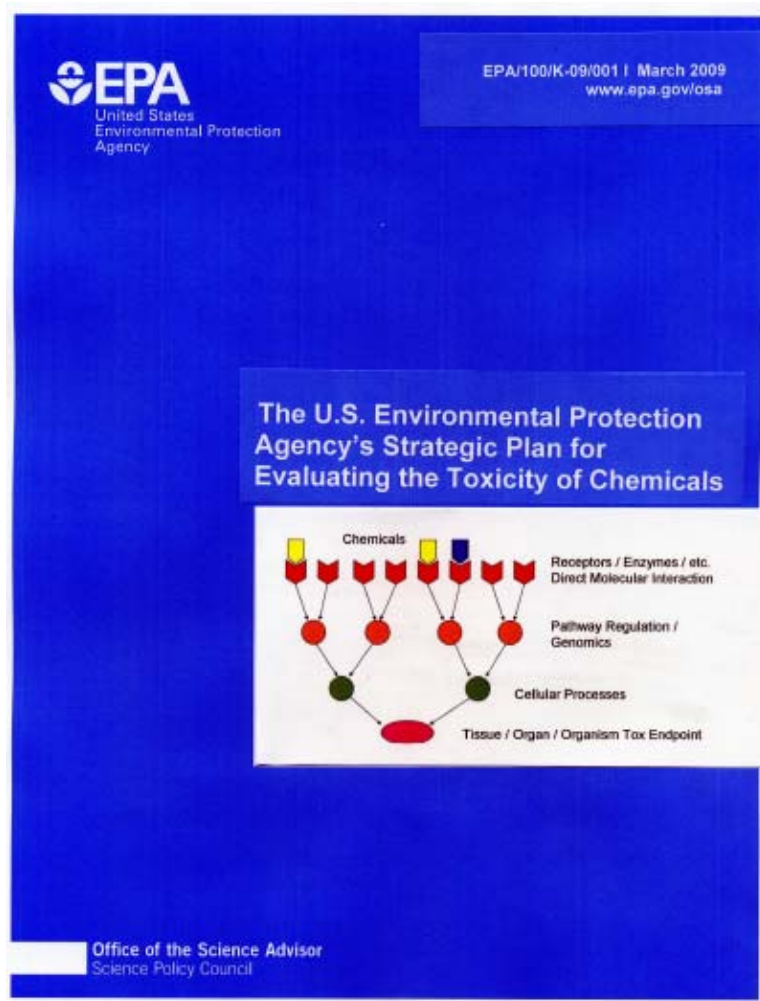
Strategic Goals
- **Toxicity Pathway ID and Screening**
- **Toxicity Based Risk Assessment**
- **Institutional Transition**

Predicting Toxicity Will Not Be Easy
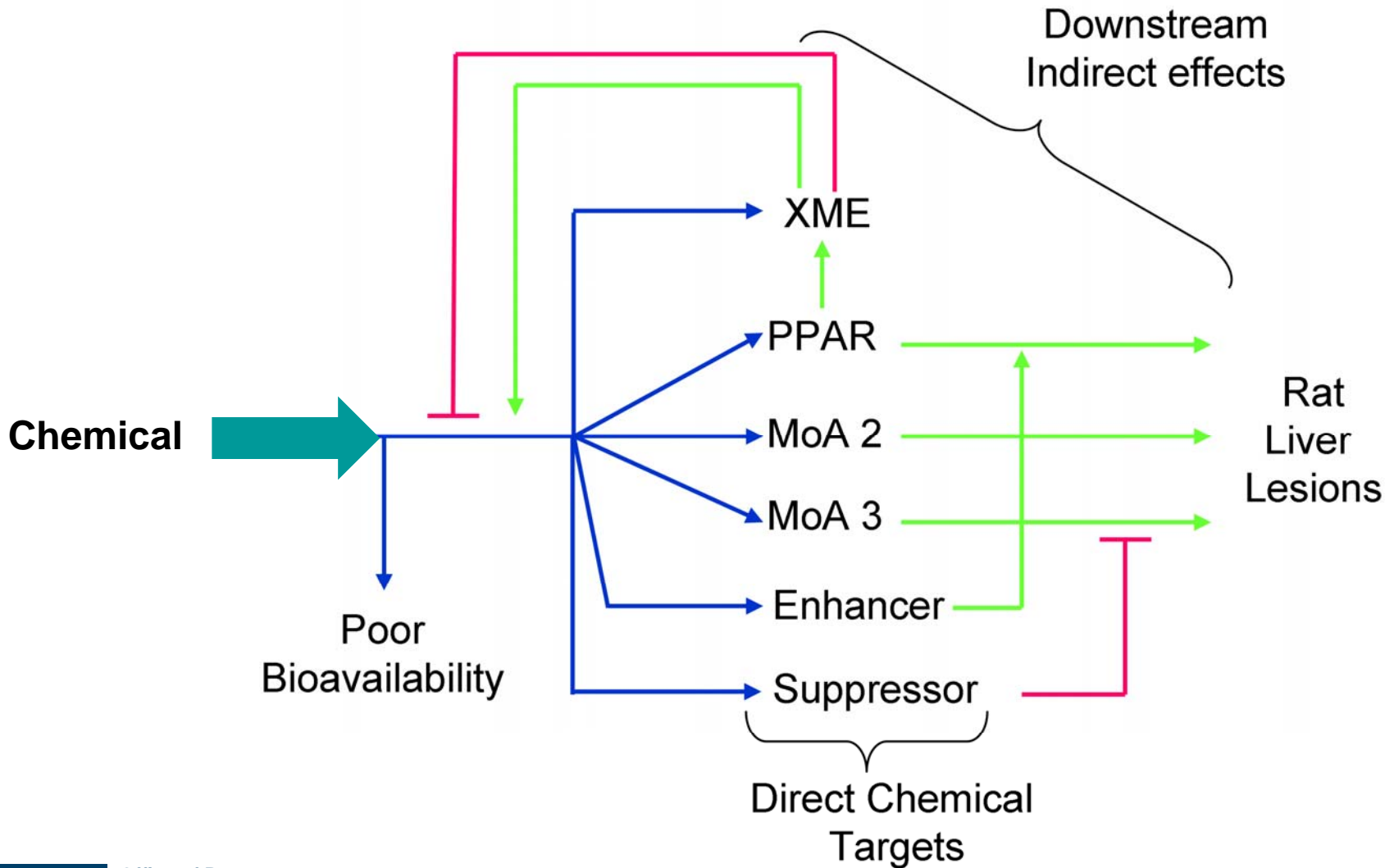
Key Challenges Of Pathway Profiling

- Find the Toxicity Pathways
  - Hepato vs developmental nuerotoxicity

- 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

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

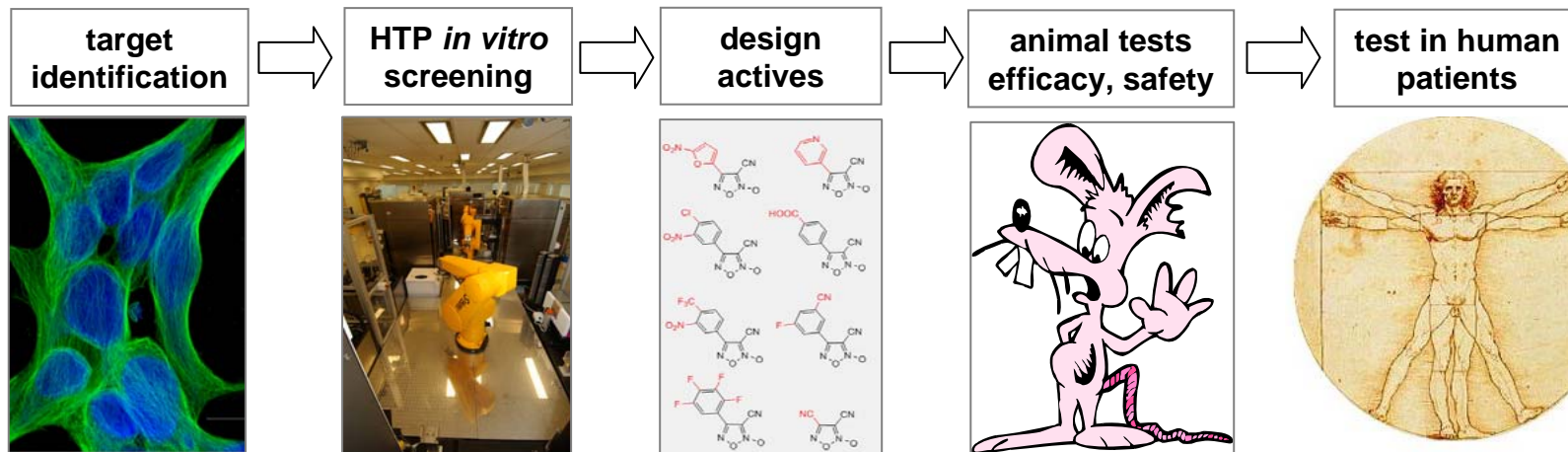FY07   FY08   FY09   FY10   FY11   FY12

Proof of Concept: ToxCast
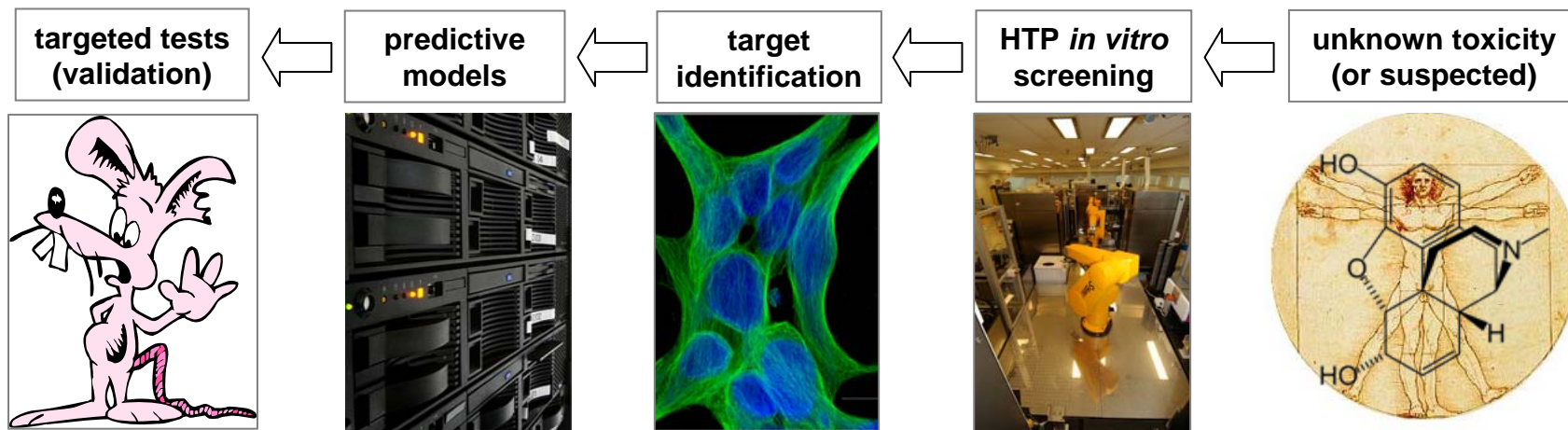
Verification/Extension

Reduce to Practice

Tox21

High-throughput screening (HTS):
building from the drug-discovery paradigm

drug development

| target identification | → | HTP *in vitro* screening | → | design actives | → | animal tests efficacy, safety | → | test in human patients |

environmental chemicals

| targeted tests (validation) | ← | predictive models | ← | target identification | ← | HTP *in vitro* screening | ← | unknown toxicity (or suspected) |

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
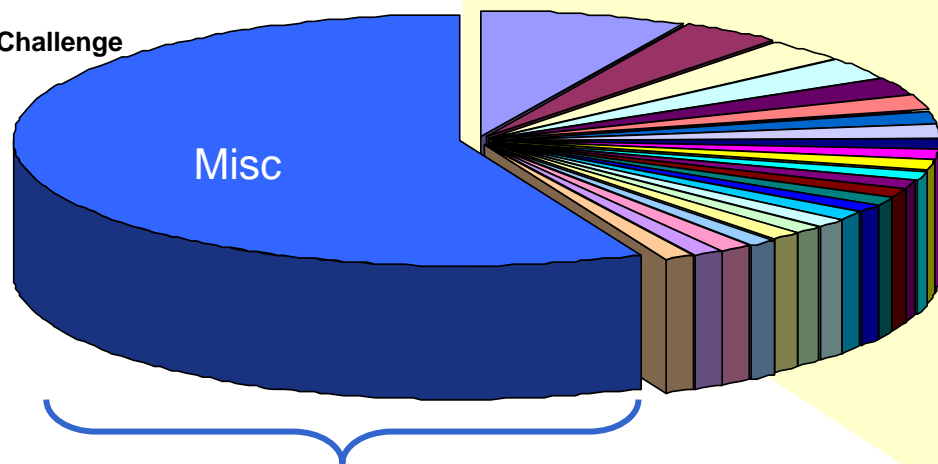
Classes with
> 3 chemicals

Misc

Misc MOA classes with
3 or fewer representatives

- Acetylcholine esterase inhibitors
- conazole fungicides
- Sodium channel modulators
- pyrethroid ester insecticides
- organothiophosphate acaricides
- dinitroaniline herbicides
- pyridine herbicides
- thiocarbamate herbicides
- imidazolinone herbicides
- organophosphate insecticides
- phenyl organothiophosphate insecticides
- aliphatic organothiophosphate insecticides
- amide herbicides
- aromatic fungicides
- chloroacetanilide herbicides
- chlorotriazine herbicides
- growth inhibitors
- organophosphate acaricides
- oxime carbamate insecticides
- phenylurea herbicides
- pyrethroid ester acaricides
- strobilurin fungicides
- unclassified acaricides
- unclassified herbicides

Classification based on OPPIN

Physical-Chemical Properties

QikProp properties for 289 chemicals
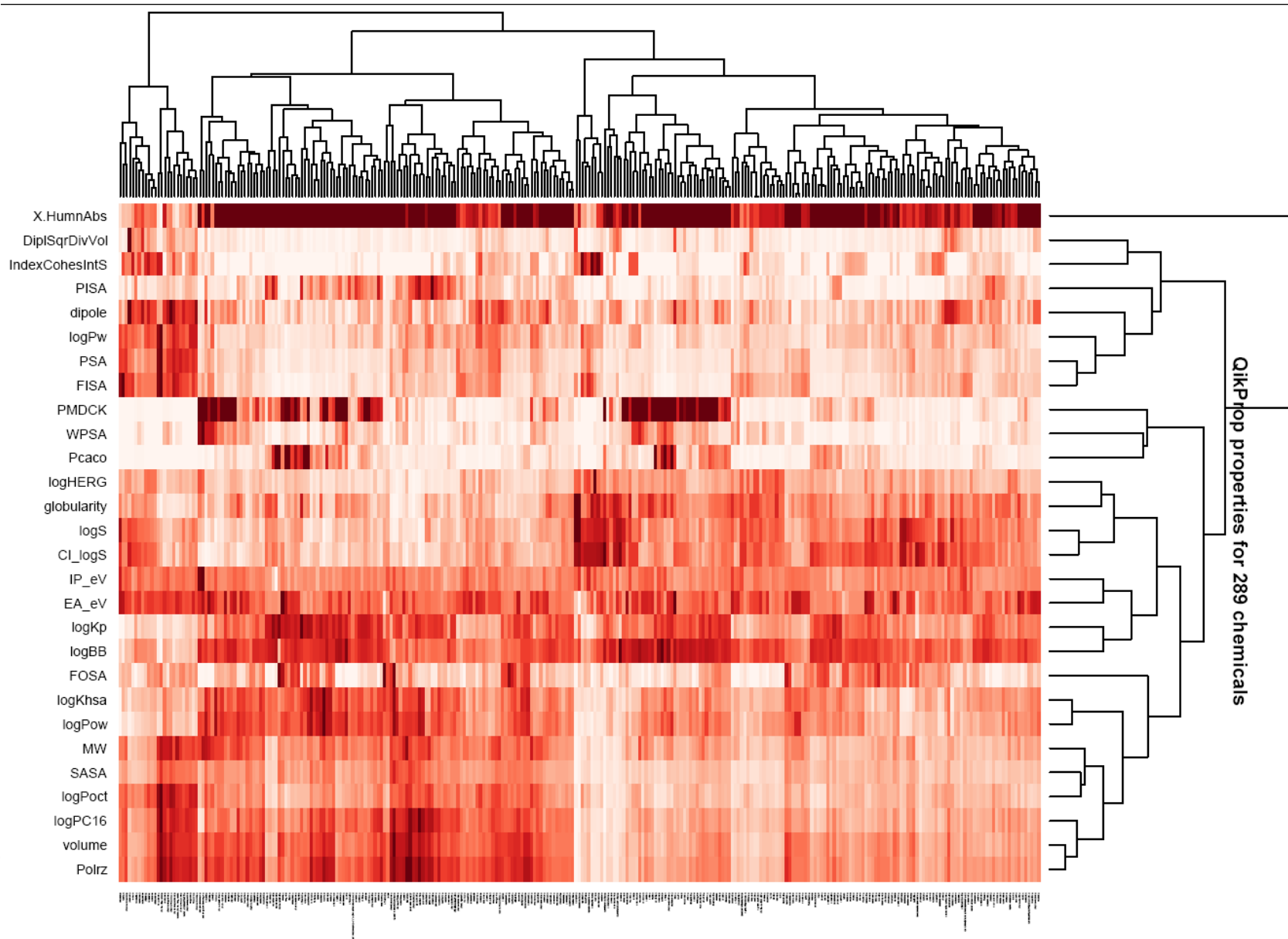
ToxRefDB Endpoint Coverage

data evaluation records

ToxRefDB
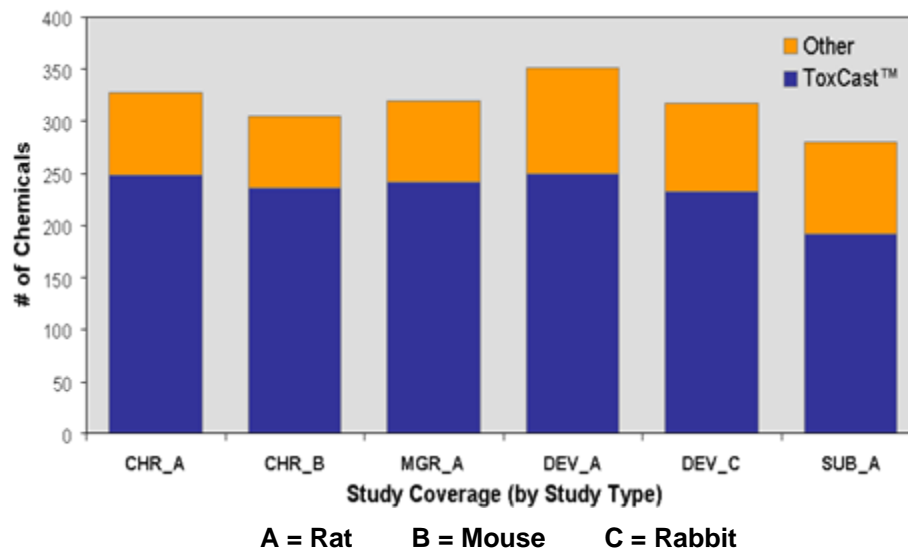

CHRONIC/CANCER (CHR)
Martin et al. (2008) Environ Hlth Persp
doi:10.1289/ehp.0800074

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

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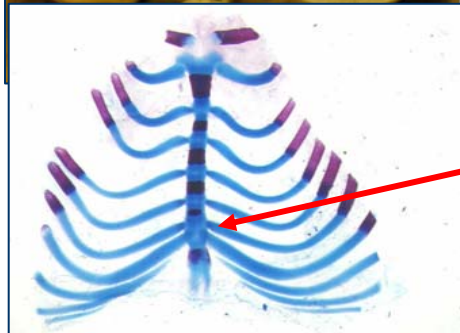Knudsen et al. (2009) Reprod Toxicol
doi: 10.1016/j.reprotox.2009.03.016

A = Rat   B = Mouse   C = Rabbit

SOURCE: Matt Martin, NCCT, 2009

Simple Annotations

images from www.DevTox.org

target: kidney
description: absent renal papilla
code: UG_REN_3.1060.5013

target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130

target: hindpaw
description: polydactyly (digit I)
code: SK_APP_2.1051.5234

ToxRefDB Multigeneration Studies

114 effects induced by least 5 chemicals

Liver Pathologies

Liver and Kidney Wts

Pup body wt

Lowest Effect Level (LEL): -Log$_2$(LEL)

No Effect — 2048 mg/kg/day — 0.015625 mg/kg/day

Martin et al, *ToxSci*, submitted

ToxRefDB Multigeneration Studies

Thyroid effects

Cholinesterase effects

Neurological effects

Lowest Effect Level (LEL): -Log₂(LEL)

No Effect   2048   0.015625
             mg/kg/day   mg/kg/day

Martin et al, *ToxSci*, 2009

ToxRefDB Multigeneration Studies

F1 Actives

Topramezone
Fipronil
Fenpropathrin
Bendiocarb
Fluvalinate
Thiacloprid
Ethoprop
Fenbuconazole
Mancozeb
Maneb
Spinosad
Thiophanate-methyl
Triforine
Ethofenprox
Butyl benzyl phthalate
Asulam

Thyroid effects

Lowest Effect Level (LEL): -Log$_2$(LEL)

No Effect   2048 mg/kg/day   0.015625 mg/kg/day

Martin et al, *ToxSci*, 2009

Digitizing Legacy *in Vivo* Data in ToxRefDB
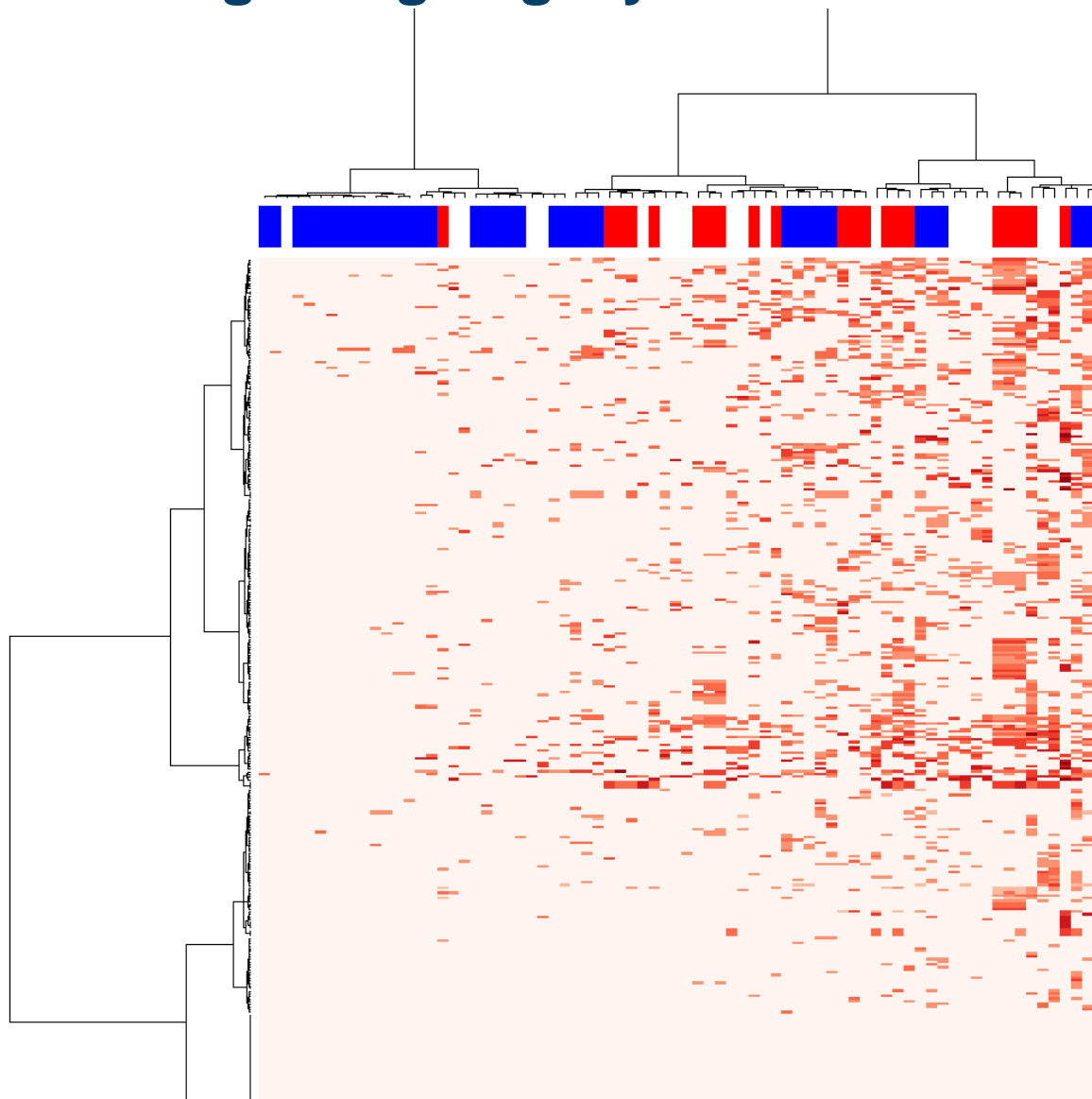
Chronic/Cancer
Multigenation
Developmental

Chemicals

Martin et al 2009a,b
Knudsen et al 2009

30 years and more than $2B worth of data

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

Biochemical HTS from Novascreen
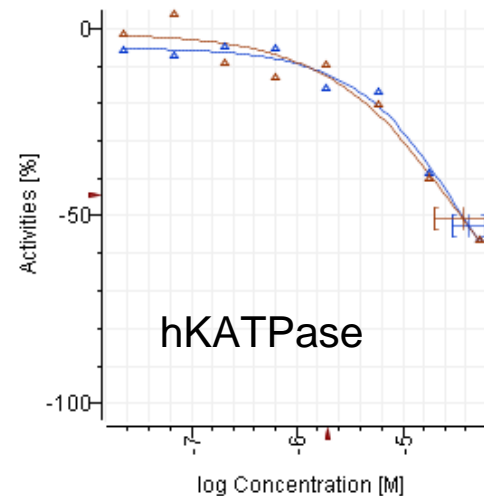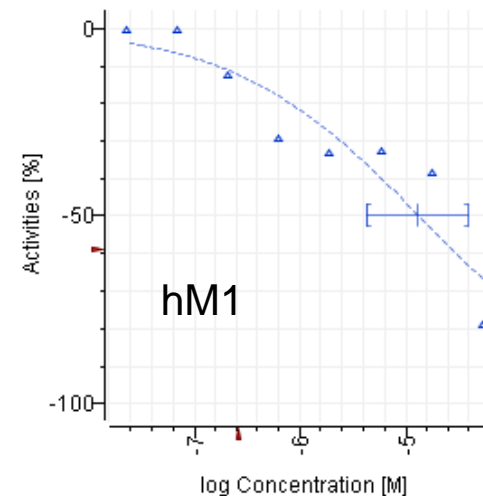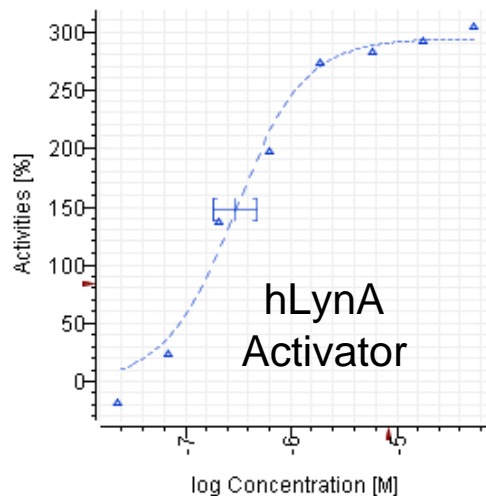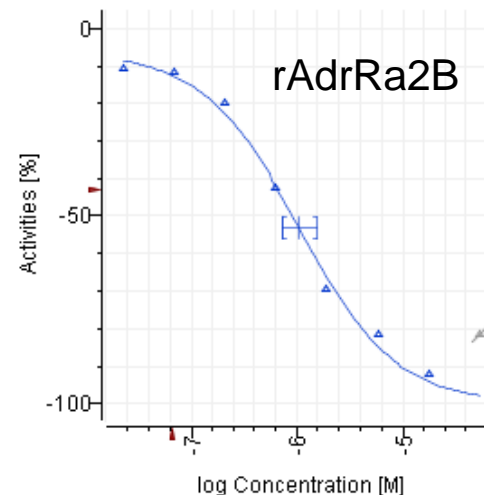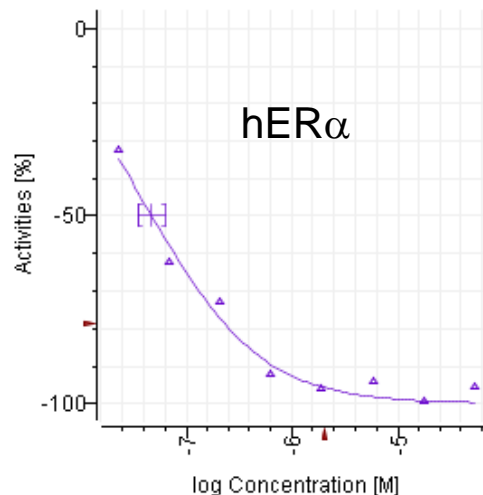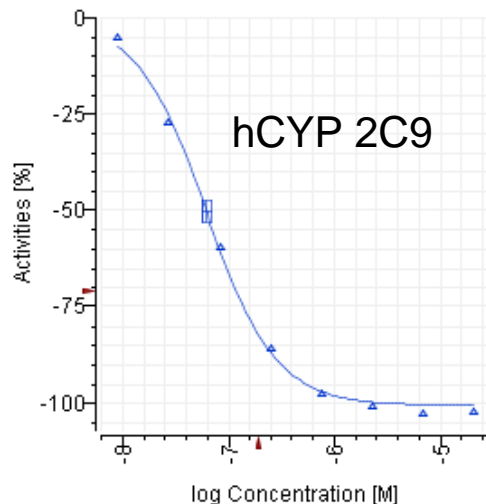
qHTS from the NCGC on NRs

ERα

PPARγ
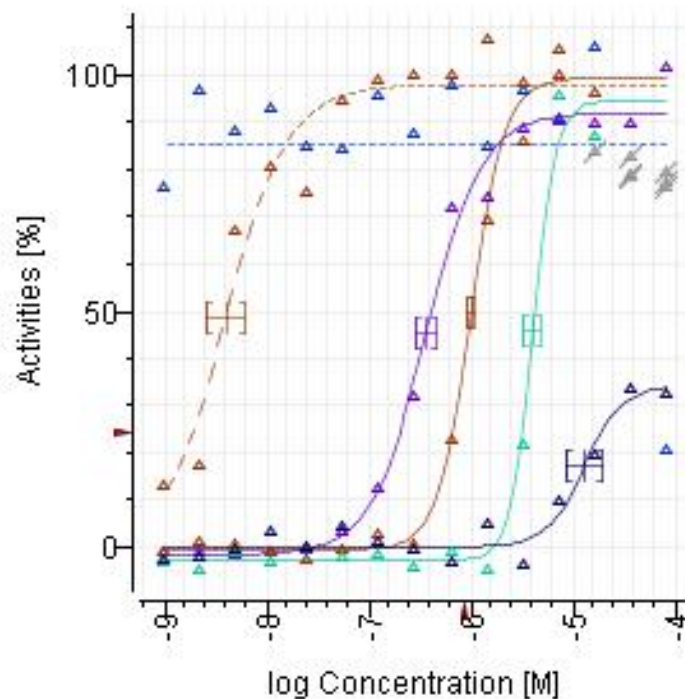
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NCGC00161666-02
NCGC00023462-04
NCGC00025156-10
NCGC00090965-03
NCGC00164033-01
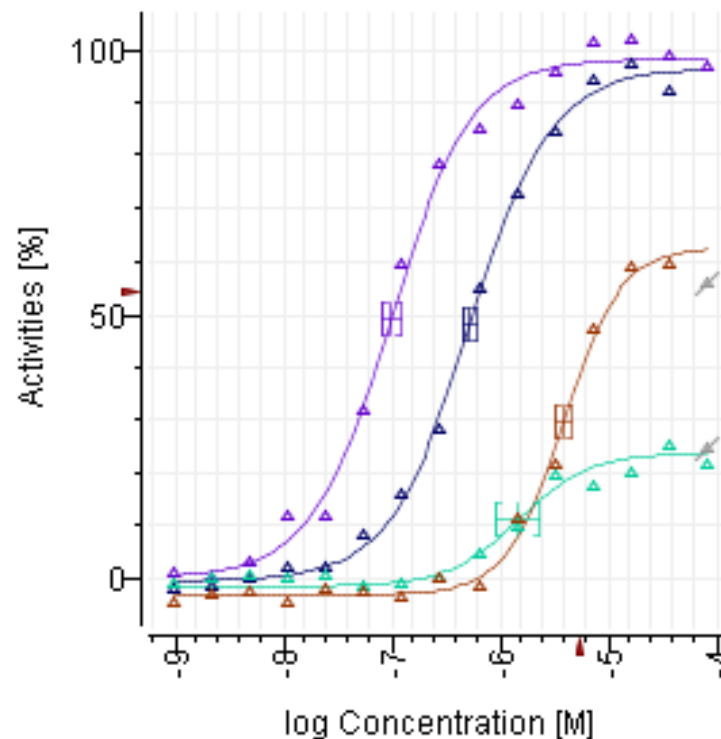
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NCGC00022570-07

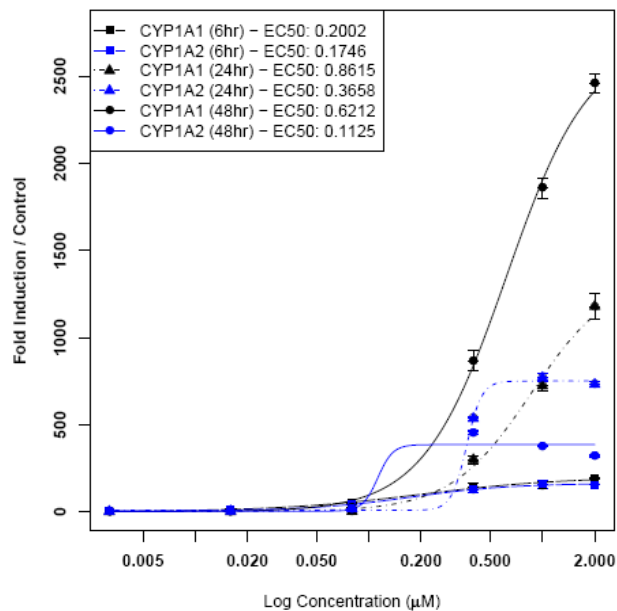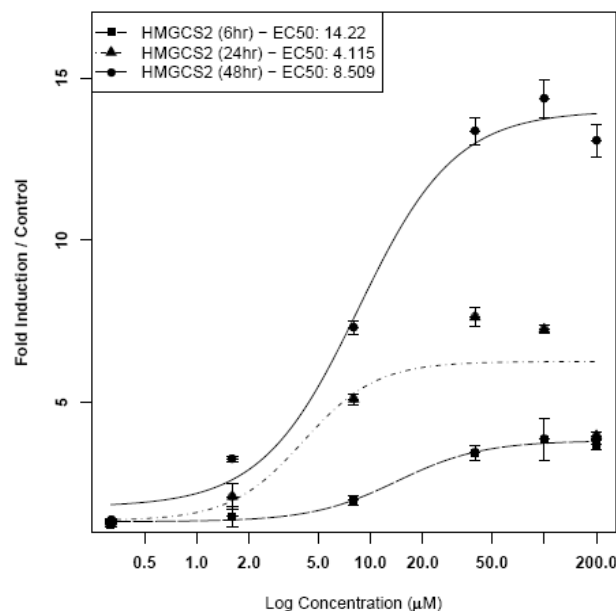# Attagene: *cis* and *trans* Assays
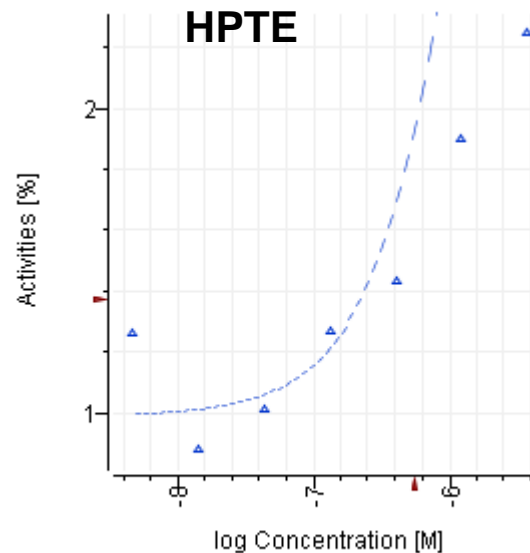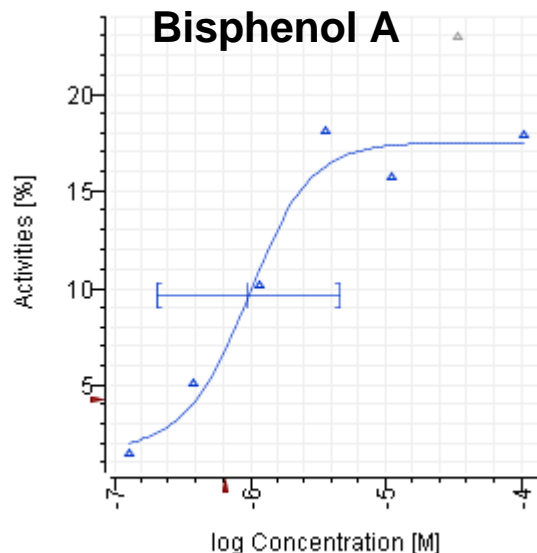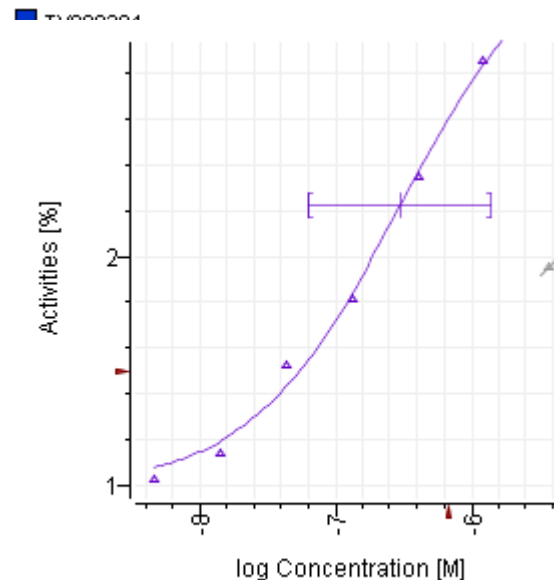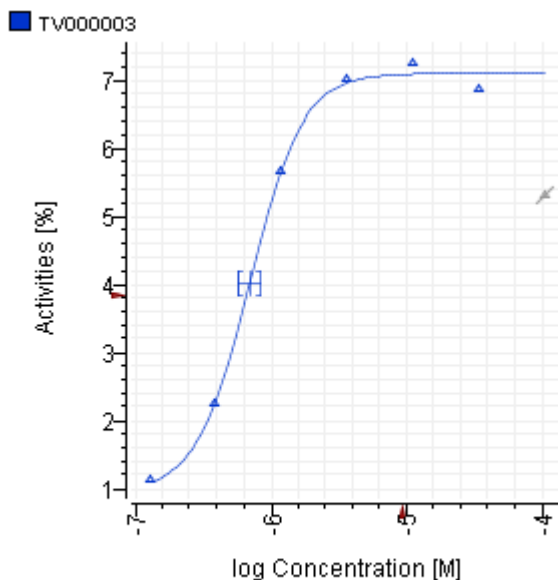
Transcription Factor Profiling from Attagene
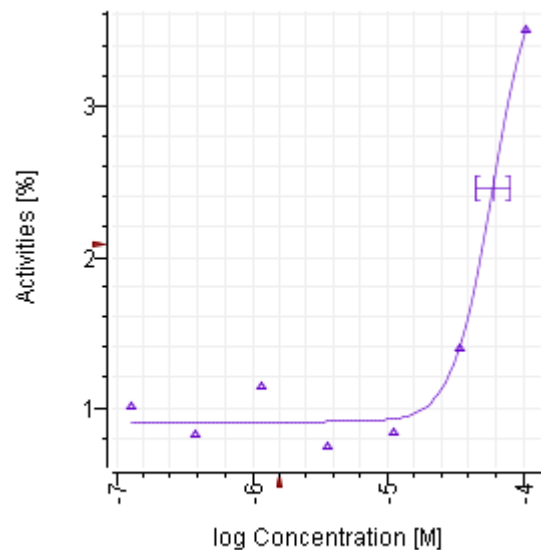
cis: HIF1a
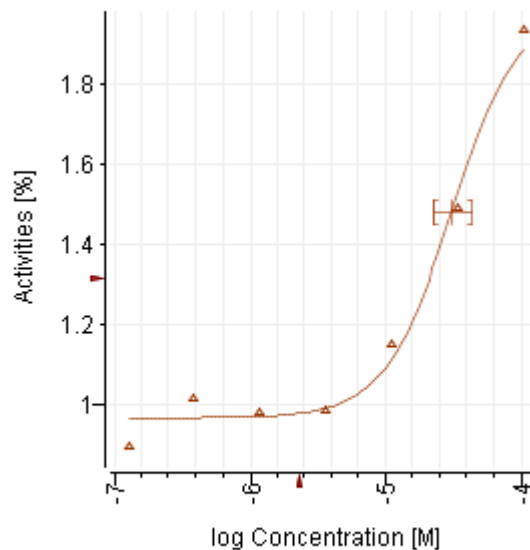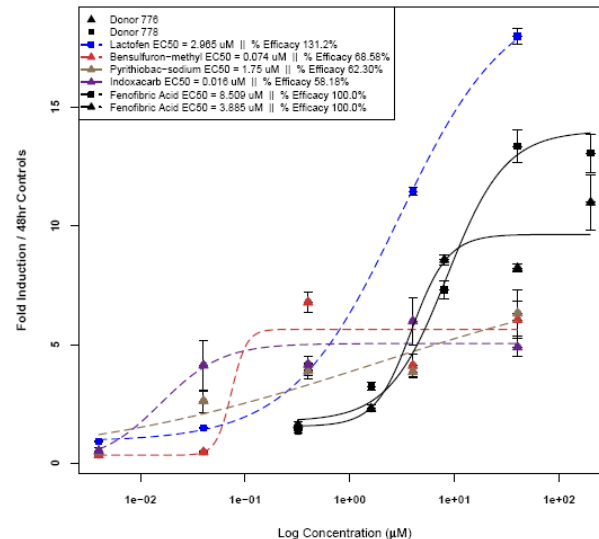
cis: myc
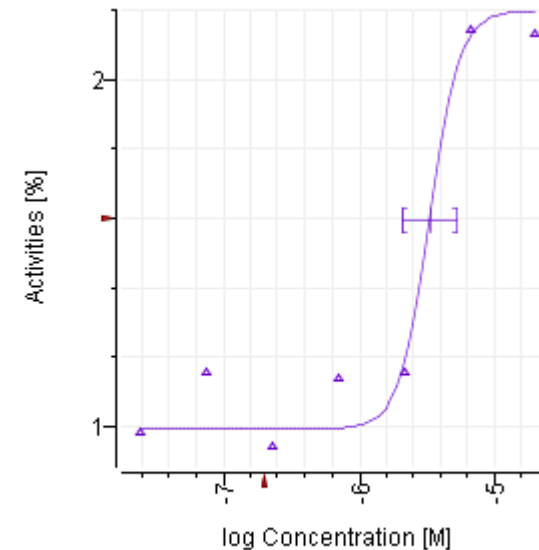
cis: TGFβ

CellzDirect: Data Examples
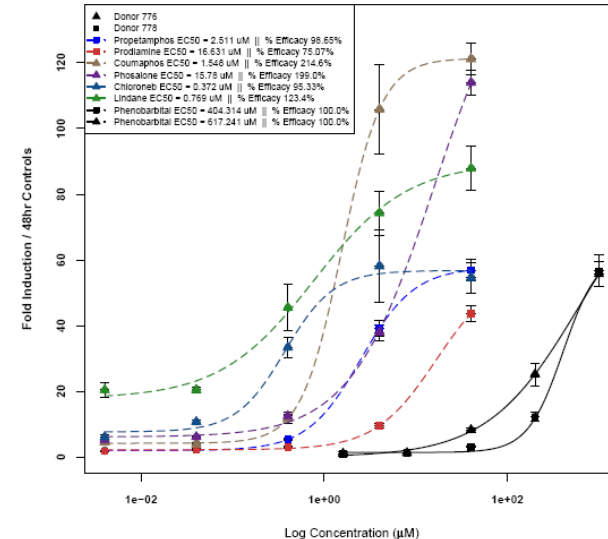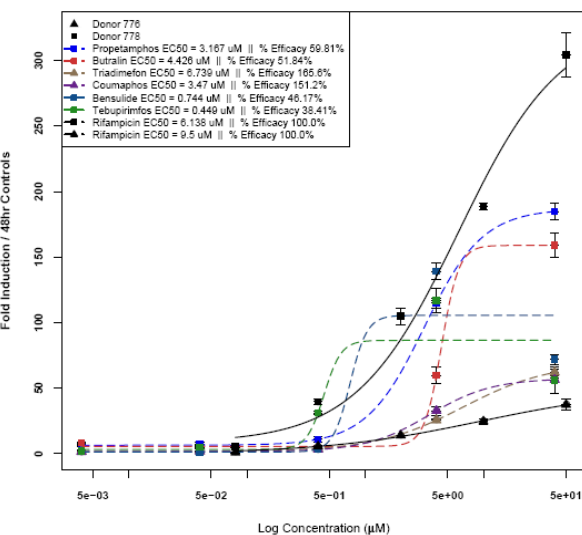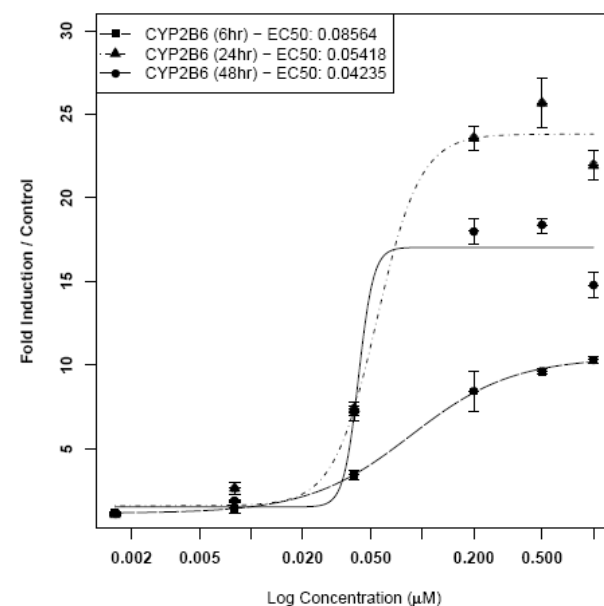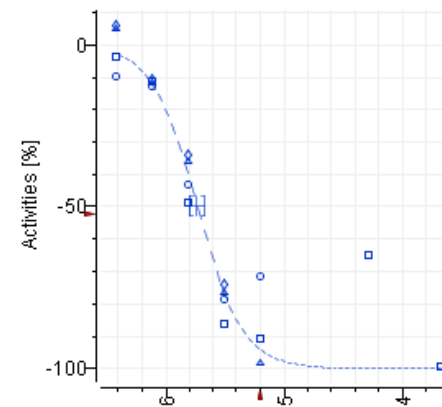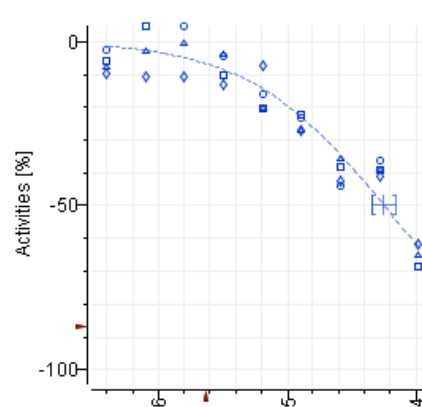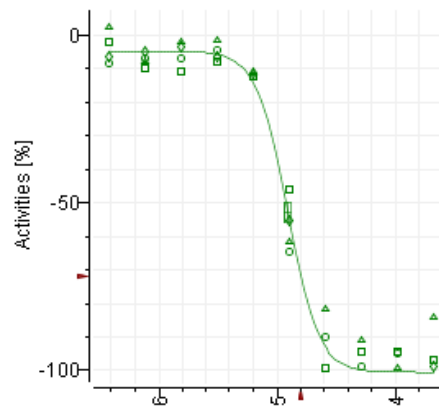
HCS from Cellumen

Attagene ERE_CIS

- Pendimethalin AC50 = 20 uM ‖ Max FC 4.5
- Fludioxonil AC50 = 18 uM ‖ Max FC 5.3
- Bisphenol A AC50 = 0.64 uM ‖ Max FC 7.3
- Pyridaben AC50 = 11 uM ‖ Max FC 8.7
- Fenhexamid AC50 = 9.2 uM ‖ Max FC 5.8
- HPTE AC50 = 0.17 uM ‖ Max FC 2.9
- Methoxychlor AC50 = 4 uM ‖ Max FC 4.4
- Flumetralin AC50 = 2.4 uM ‖ Max FC 2.8
- Fenarimol AC50 = 5.4 uM ‖ Max FC 2.7
- Estradiol AC50 = 0.00035 uM ‖ Max FC 9.3

Attagene ERa_TRANS

- Pendimethalin AC50 = 41 uM ‖ Max FC 27
- Fludioxonil AC50 = 12 uM ‖ Max FC 25
- Bisphenol A AC50 = 1.1 uM ‖ Max FC 23
- Pyridaben AC50 = 43 uM ‖ Max FC 12
- Fenhexamid AC50 = 10 uM ‖ Max FC 14
- HPTE AC50 = 0.73 uM ‖ Max FC 2.3
- Methoxychlor AC50 = 2.7 uM ‖ Max FC 11
- Flumetralin AC50 = 5.4 uM ‖ Max FC 7.3
- Fenarimol AC50 = 8.2 uM ‖ Max FC 16
- Estradiol AC50 = 0.0087 uM ‖ Max FC 23
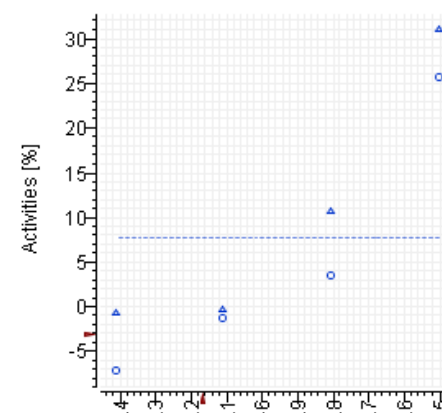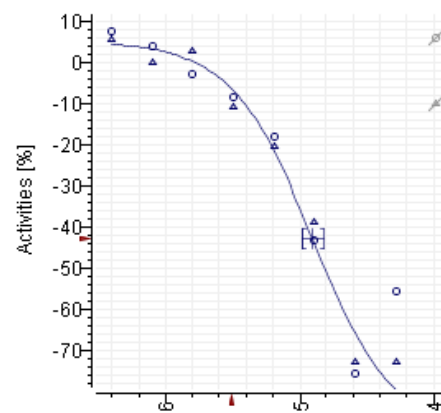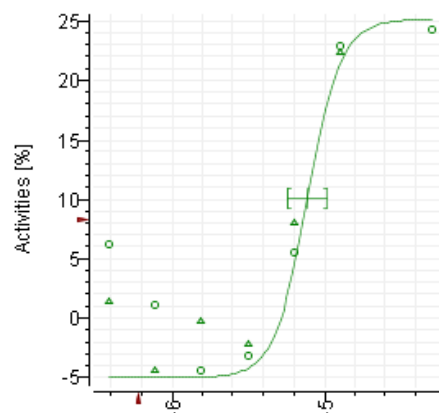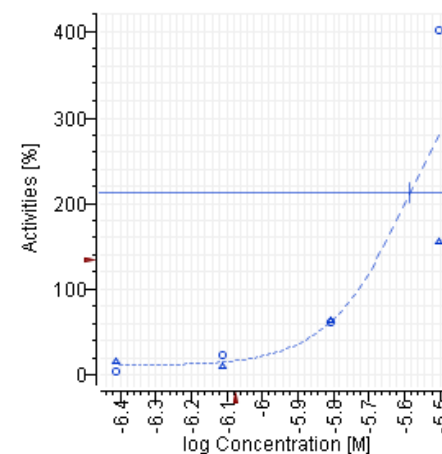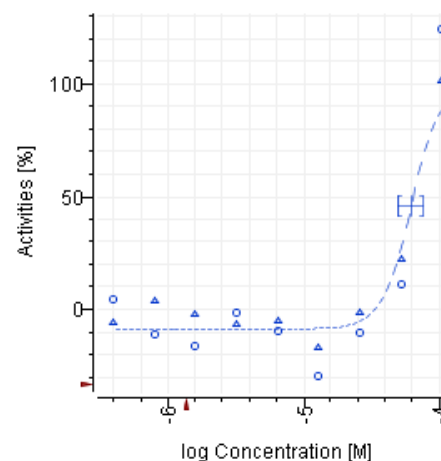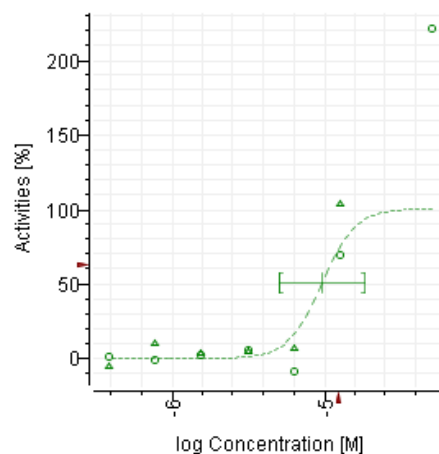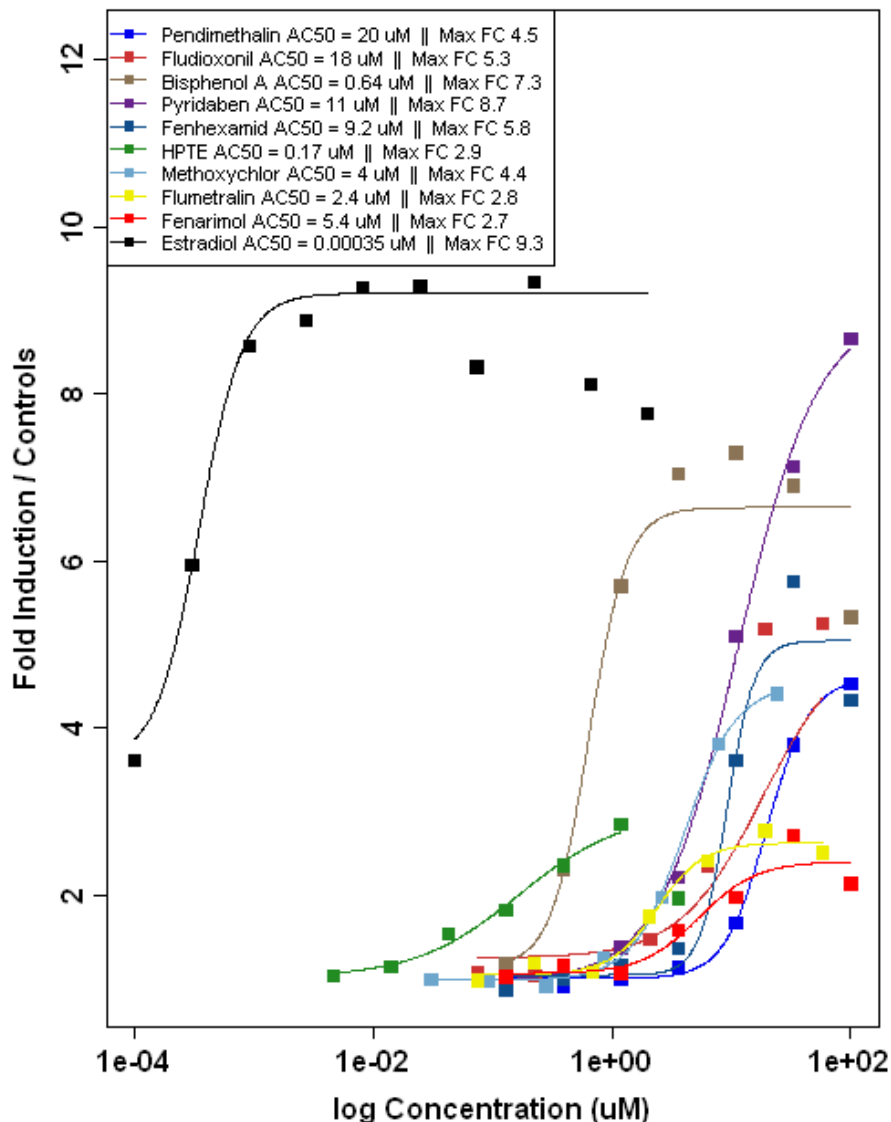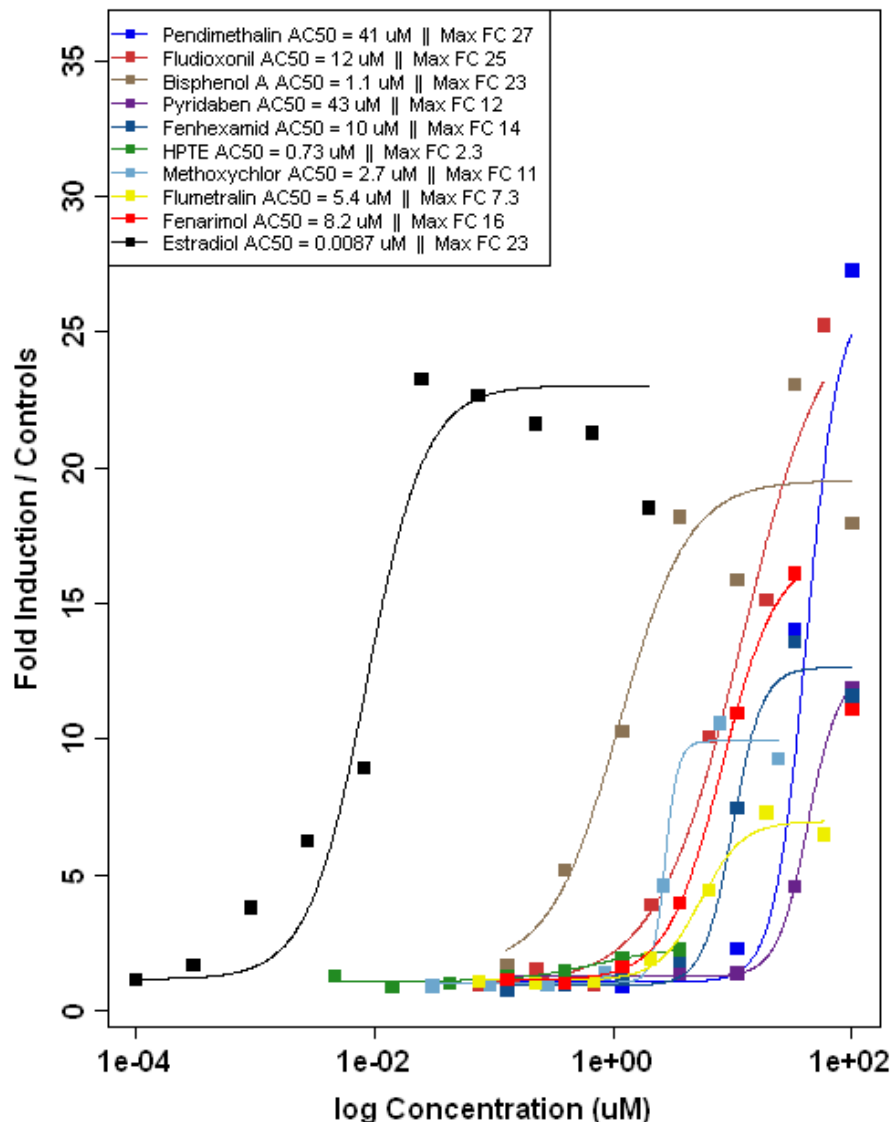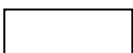
ToxCast Phase I Assay Hits
(n=624 measurements)

Legend:
- Cell Free HTS
- Multiplexed TF
- Human BioMap
- HCS
- qNPAs
- XMEs
- Impedance
- Genotoxicity
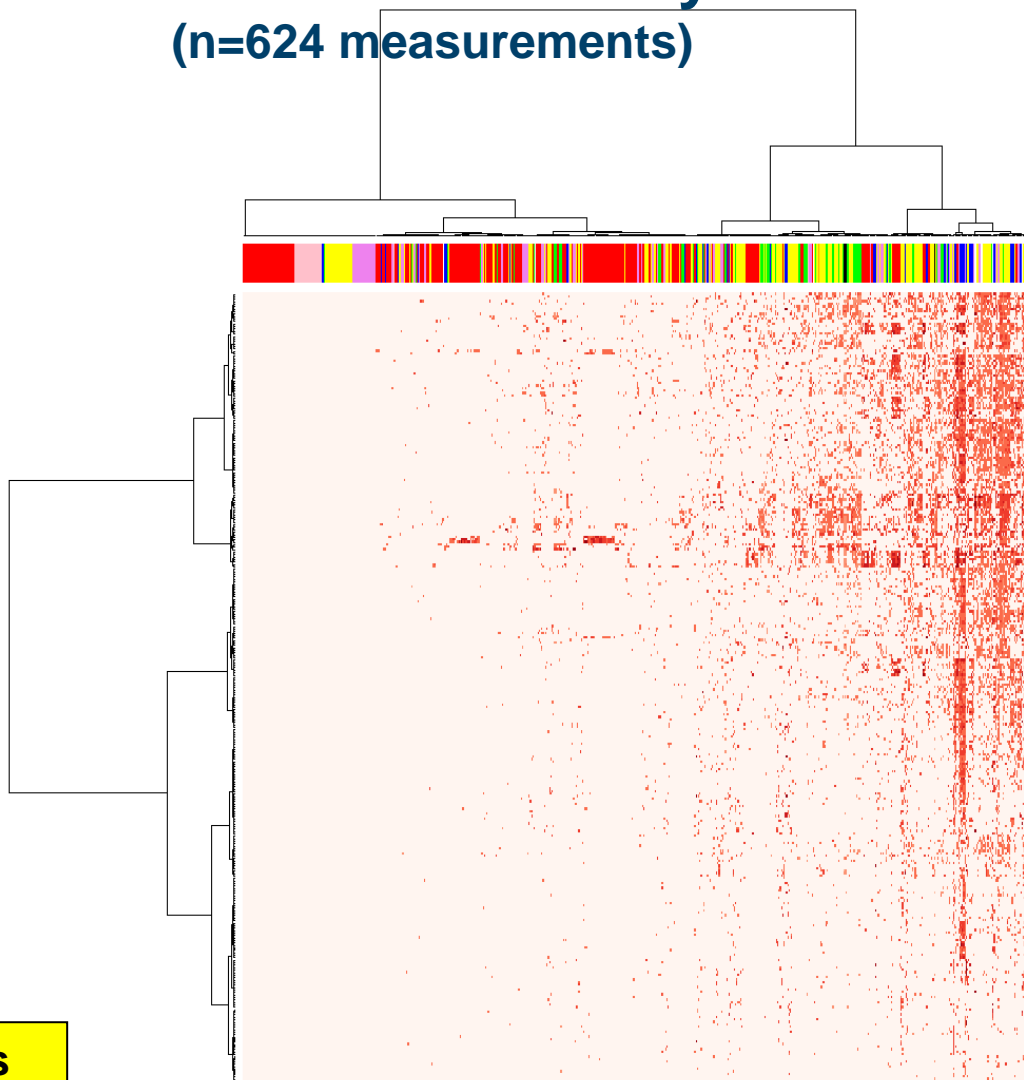
828 Assay-Chemical Pairs had AC50s of less than 1μM

Judson et al, EHP, submitted

ToxCast Phase I & TDAS Lessons Learned

- High quality HTS data is obtainable

- A number of expected observations were found, as were a number of unexpected ones

- Multiple assays per biological pathway are important to include

- Many chemicals in the library interact with a number of targets

- The in vitro and in vivo data sets are complicated and will require extensive data analysis to determine optimal approaches

TDAS Lessons Learned (Cont'd)

- Endpoint aggregation and/or progression should be examined

- Interpret results in context (chemistry$\leftarrow\rightarrow$toxicology$\leftarrow\rightarrow$biology$\leftarrow\rightarrow$ . . . .)

- Incorporation of external knowledge

- Prioritization scores based on hazard potential are feasible

- Metabolism remains a challenge to incorporate in many assays

- Greater numbers of chemicals and assays are needed

- **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**

Challenges (Cont'd)

- **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**

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

EPA

MATERIALS TRANSFER AGREEMENT

EPA:

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Pfizer:
Pfizer Inc, having a principal place of business at 235 East 42nd Street, New York, ("Pfizer") New York, 10017 and its Affiliates

WHEREAS the EPA wishes to obtain Pfizer Compounds to use in certain test assay panels, and whereas Pfizer wishes to have Pfizer Compounds evaluated on such test panels, the parties agree as follows:

_____

"Affiliate" means any corporation, firm partnership or other entity which directly or indirectly controls, is controlled by, or is under common control with either of the parties.

1. EPA agrees to receive Pfizer's compounds, listed in Exhibit B, in any form or any of its intermediates and derivatives ("Pfizer Compound"), in order to perform the research activities, further described in Exhibit A, and known as the "ToxCast™ Program."

2. The Pfizer Compounds:
   a. are the property of Pfizer and all existing rights including, without limitation, patent rights in or to the Pfizer Compounds will remain the property of the Pfizer.
   b. will be used with caution and for research purposes only, and shall not be used for research involving human subjects.
   c. will be used only by the EPA in the ToxCast™ Program described below, under suitable containment conditions.
   d. will not be used for screening, production or sale, for which a commercialization license may be required.

Both Pfizer and EPA agree to comply with all applicable laws, rules, guidelines and regulations applicable to the use, storage, shipping and the handling of the Pfizer Compounds and ToxCast™ Program.