

Developing Predictive Bioactivity Signatures of Carcinogenesis Using ToxCast HTS Data

COMPUTATIONAL

TOXICOLOGY

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY







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



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/







- 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

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ToxRefDB



CHRONIC/CANCER (CHR)

Martin et al. (2008) Environ HIth 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



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SOURCE: Matt Martin, NCCT, 2009



Rat Chronic Bioassay Results



Martin, et al EHP, 2008



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

Martin, et al, 2009



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 (<u>www.epa.gov/ncct/toxrefdb/</u>)

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 ¹⁰ 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 (2): Multiple Assays and Technologies per Target





Confidence Builders (3): Pathway Based Analysis





Data Analysis: What is a hit?

Attagene ERa_TRANS





Biochemical HTS from Novascreen





TV000626:"2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane







TV000517:3-lodo-2-propynylbutylcarbamate TV000719:3-lodo-2-propynylbutylcarbamate

TV000541:Cyproconazole



TV000783:Mancozeb

qHTS from the NCGC on NRs Environmental Protection



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Attagene: cis and trans Assays





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TV000003



TV000391

17

CellzDirect: Data Examples

CYP1A1-AhR CYP2B6-CAR HMGCS2-PPARa CYP1A1 (6hr) - EC50: 0.2002 --30 HMGCS2 (6hr) - EC50: 14.22 CYP2B6 (6hr) - EC50: 0.08564 -CYP1A2 (6hr) - EC50: 0.1746 HMGCS2 (24hr) - EC50: 4.115 CYP2B6 (24hr) - EC50: 0.05418 -2500 -CYP1A1 (24hr) - EC50: 0.8615 HMGCS2 (48hr) - EC50: 8.509 CYP2B6 (48hr) - EC50: 0.04235 ŝ -CYP1A2 (24hr) - EC50: 0.3658 -CYP1A1 (48hr) - EC50: 0.6212 • 25 CYP1A2 (48hr) – EC50: 0.1125 2000 Fold Induction / Control Fold Induction / Control Fold Induction / Control 20 1500 9 15 Ŧ Ŧ 1000 2 ŝ 500 ŝ 0 0.020 0.005 0.050 0.200 0.500 2.000 0.002 0.005 0.020 0.050 0.200 0.500 0.5 1.0 2.0 5.0 10.0 20.0 50.0 200.0 Log Concentration (µM) Log Concentration (µM) Log Concentration (µM) Donor 776 Donor 778 Donor 776 1 ۸ Donor 776 Donor 778 Donor 778 Properamphos EC50 = 2.511 uM || % Efficacy 98.65% Prodiamine EC50 = 16.631 uM || % Efficacy 75.07% Coumaphos EC50 = 1.548 uM || % Efficacy 214.6% Lactofen EC50 = 2.965 uM || % Efficacy 131.2% -Propetamphos EC50 = 3.167 uM || % Efficacy 59.81% Bensulfuron-methyl EC50 = 0.074 uM || % Efficacy 68.58% Pyrithiobac-sodium EC50 = 1.75 uM || % Efficacy 62.30% Properation of the second state of the second 300 20 Phosalone EC50 = 15.78 uM || % Efficacy 199.0% Chloroneb EC50 = 0.372 uM || % Efficacy 95.33% Lindane EC50 = 0.769 uM || % Efficacy 123.4% Indoxacarb EC50 = 0.016 uM || % Efficacy 58.18% Fenofibric Acid EC60 = 8.509 uM || % Efficacy 100.0%
Fenofibric Acid EC50 = 3.885 uM || % Efficacy 100.0% 2 Phenobarbital ECS0 = 404.314 uM || % Efficacy 100.0%
Phenobarbital ECS0 = 617.241 uM || % Efficacy 100.0% Tebupirimfos EC50 = 0.449 uM || % Efficacy 38.41% Rifampicin EC50 = 6.138 uM || % Efficacy 100.0%
Rifampicin EC50 = 9.5 uM || % Efficacy 100.0% 250 00 48hr Control Cont 200 8 48hr 2 Induction / 150 60 Ā plo" plo 8 \$ 10 8 20 ------5e-03 5e-02 5e-01 5e+00 5e+01 1e-02 1e-01 1e+00 1e+01 1e+02 1e-02 1e+00 1e+02 Log Concentration (µM) Log Concentration (µM) Log Concentration (µM)



Metabolic Activity from Solidus





HCS

qNPAs

Impedance

Genotoxicity

XMEs

Cell Free HTS

Multiplexed TF



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

Judson et al, EHP, submitted

Minimum Human Pathways in ToxCast Phase I



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Environmental Protection

Judson et al, EHP, submitted¹



"Hits" per Chemical As a Function of AC50/LEC Cutoff



9 Chemicals have at least 20 hits at an AC50 of <30µM

Judson et al, EHP, submitted₂



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

Minimal Pathways, 30 uM



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Judson et al, EHP, submitted



Activity of Conazoles Against Minimal Pathway Set



I:LPS/IL-1 Mediated Inhibition of RXR Function K:Metabolism of xenobiotics by cytochrome P450 I:Aryl-Hydrocarbon Receptor Signaling K:Linoleic acid metabolism I:Glucocorticoid Receptor Signaling I:Hepatic Fibrosis/Hepatic Stellate Cell Activation K:Cytokine cytokine receptor interaction K:Cell adhesion molecules/CAMs K:Hematopoietic cell lineage K:Complement and coagulation cascades I:Acute Phase Response Signaling K:Tryptophan metabolism K:Neuroactive ligand receptor interaction I:Wnt beta catenin Signaling K:MAPK signaling pathway



Rat Liver Histopathology from Chronic Bioassays



N = 248 Chemicals

Source: Judson et al, submitted



Rat Liver Tumor Correlations lmaz 2,5-l dipro Pym Flud Vinc Sim Тер Cart Diet Oxa Dic Teb Dich Cyc Dim Ace Ame Mev Mala Lac sox

	AF	PF	PP	нм	сс
athion					
inphos					
stryn					
azine					
ochlor					
ethenamid					
lozolin					
anilide					
lobenil					
ioxonil					
zalil					
^D yridinedicarboxylic acid, ppyl ester					
etrozine					
Jenpyrad					
aflutole					
ofen					
ofop-methyl					
diazon					
hylhexyl phthalate					
baryl					
aloxydim					

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Fisher's Exact test, p<0.01

Judson, et al, submitted

Gene Networks Associated with Progression of Rat Liver Tumor Endpoints Environmental Protection

United States

Agency



Judson et al, EHP, submitted



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
la	320	Data Rich (pesticides)	Signature Development	552	\$20k	FY07-09
lb	15	Nanomaterials	Pilot	166	\$10K	FY09
lla	>300	Data Rich Chemicals	Validation	>400	~\$20 -25k	FY09-11
llb	>100	Known Human Toxicants	Extrapolation	>400	~\$20 -25k	FY09-11
lic	>300	Expanded Structure and Use Diversity	Extension	>400	~\$20 -25k	FY09-11
lld	>12	Nanomaterials	PMN	>200	~\$15-20K	FY10-11
111	Thousands	Data poor	Prediction and Prioritization	>300	~\$15-20k	FY11 -12
FY07	FYO	8 FY09	FY1	.0	FY11	FY12

Proof of Concept: ToxCast

Tox21

Verification/Extension

Reduce to Practice



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