United States Environmental Protection Agency

Species-Specific Predictive Signatures of Developmental Toxicity using the ToxCast Chemical Library

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2nd Species Workgroup
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Office of Research and Development

Disclosure

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Disclaimer: views are those of the presenter and do not necessarily reflect Agency policy nor imply endorsement of software used here.

High-throughput screening (HTS)

- Toxicity Testing in the Twenty-first Century: A Vision and a Strategy - *National Academy of Sciences (2007)*
  http://iccvam.niehs.nih.gov/docs/about_docs/NAS-Tox21.pdf

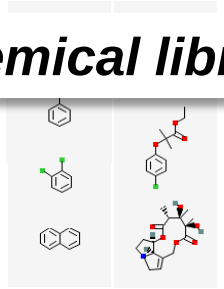
- Move away from animal testing to HTS
  - Understand how chemicals perturb cellular functions
  - Broader coverage of chemicals and biological activities
  - Reduce cost and time for testing
  - Use fewer animals

- Establish relationships between *in vitro* perturbation (toxicity pathways) and *in vivo* outcomes (adverse outcome pathways)

  - **ToxCast™ Program: Chemical prioritization and predictive model development**

Predictive Model Development

Chemical library

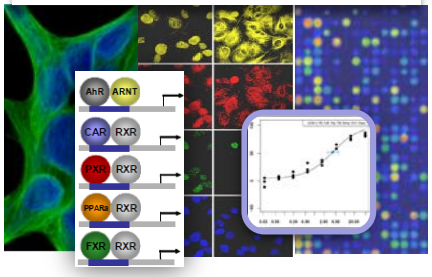


310

1060 ToxCast Phase I, II

- food use pesticides, food additives, failed pharmaceuticals, and alternative plasticizers

in vitro testing

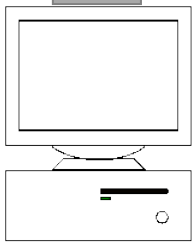


www.epa.gov/ncct/toxcast/

Predictive Model

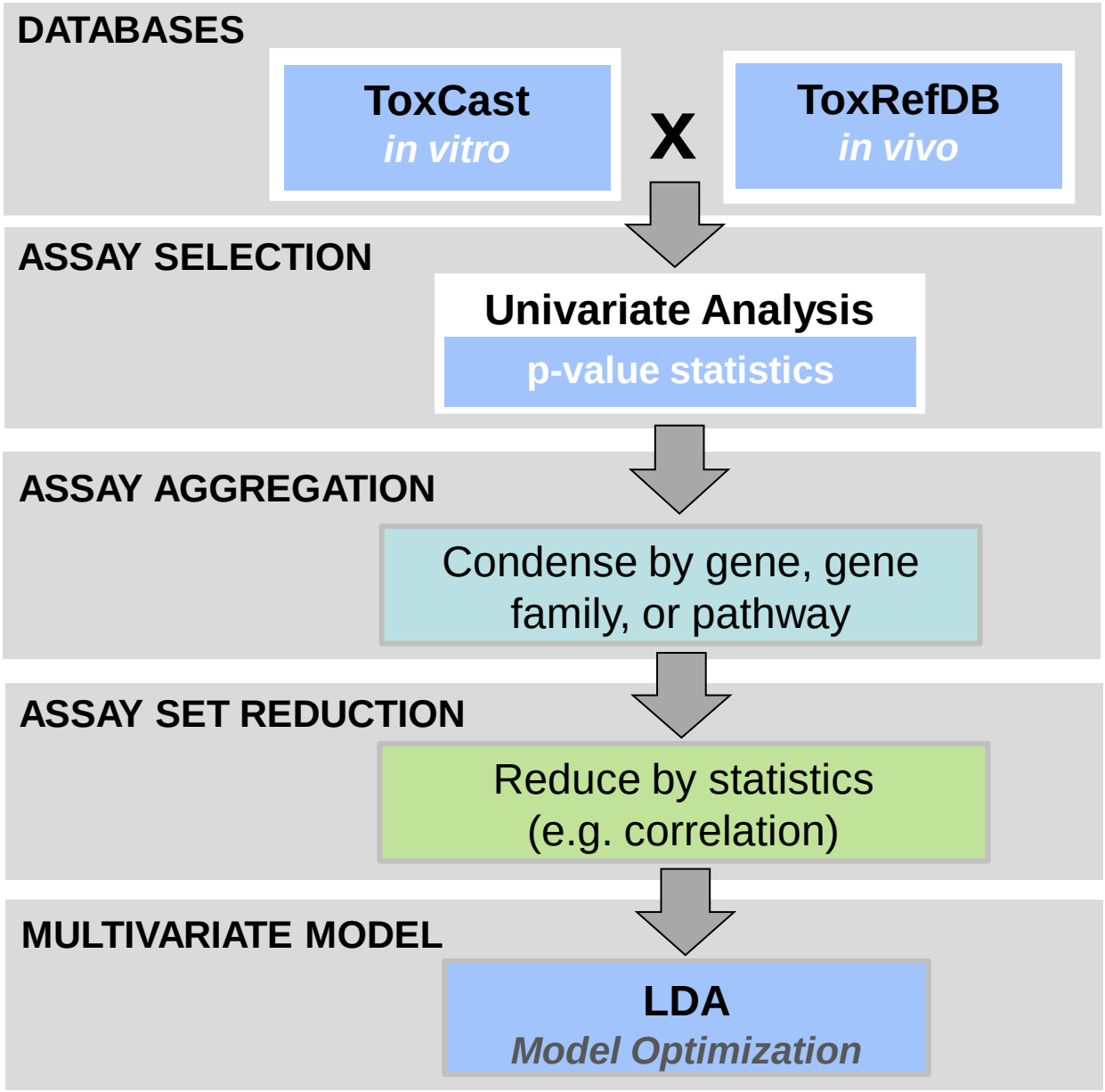
in vivo data

www.epa.gov/ncct/toxrefdb/



Bioinformatics

Predictive Model Development Workflow



Databases

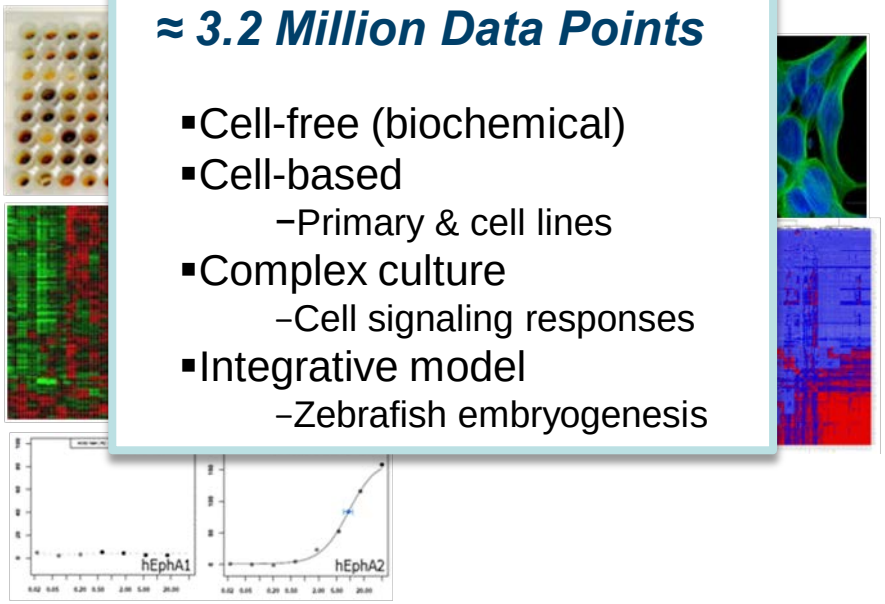
DATABASES

ToxCast *in vitro* **X** **ToxRefDB** *in vivo*

HTS Data

≈ 3.2 Million Data Points

- Cell-free (biochemical)
- Cell-based
  - Primary & cell lines
- Complex culture
  - Cell signaling responses
- Integrative model
  - Zebrafish embryogenesis



Phase I chemicals tested 271 (87%)

- 251 Prenatal Rat
- 234 Prenatal Rabbit
- 214 overlap (79%)

Developmental Effects (dLEL)

- Fetal weight reduction
- Malformations
  - *(e.g. cleft lip, eye & skeletal defects)*
- Prenatal loss

Sipes et al 2011

Spectrum of Developmental Endpoints

Endpoint Class	*Number of Chemicals*		
	Rat	*Rabbit*	*Overlap*
Developmental (dLEL)	**146**	**106**	**65**
Fetal Weight Reduction (FWR)	**87**	**45**	**20**
Malformation (MAL)*	**127**	**75**	**39**
Skeletal (axial)	113	53	25
Skeletal (appendicular)	49	22	7
Skeletal (cranial)	40	19	3
Urogenital (renal)	15	2	0
Urogenital (ureteric)	11	2	0
Jaw/Hyoid	14	6	0
Cleft Lip/Palate	10	2	0
Neurosensory (eye)	2	4	0
Neurosensory (brain)	7	5	0
Body Wall (somatic)	5	1	0
Viscera (splanchnic)	4	8	0
Cardiovascular (heart)	2	3	0
Cardiovascular (major vessels)	1	3	0
Prenatal Loss (PNL)	**86**	**136**	**47**

Sipes et al 2011

Predictive Model Assay Selection

What assays are statistically associated with the in vivo endpoints?

ASSAY SELECTION

Univariate Analysis

p-value statistics

Endpoints (*in vivo*)

ToxRefDB

Endpoint Class	Number of Chemicals		
	Rat	Rabbit	Overlap
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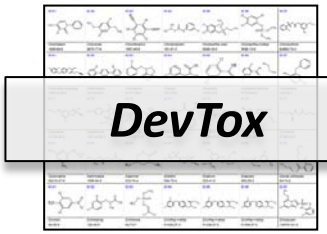
dLEL

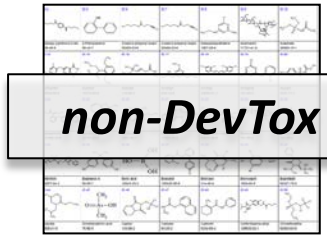
no dLEL

Chemicals (*ToxCast*)

310 compounds



DevTox

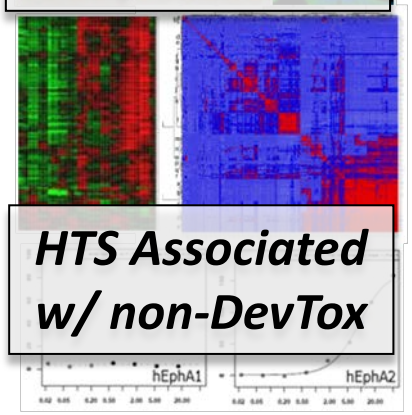


non-DevTox

Effects (*HTS assays*)

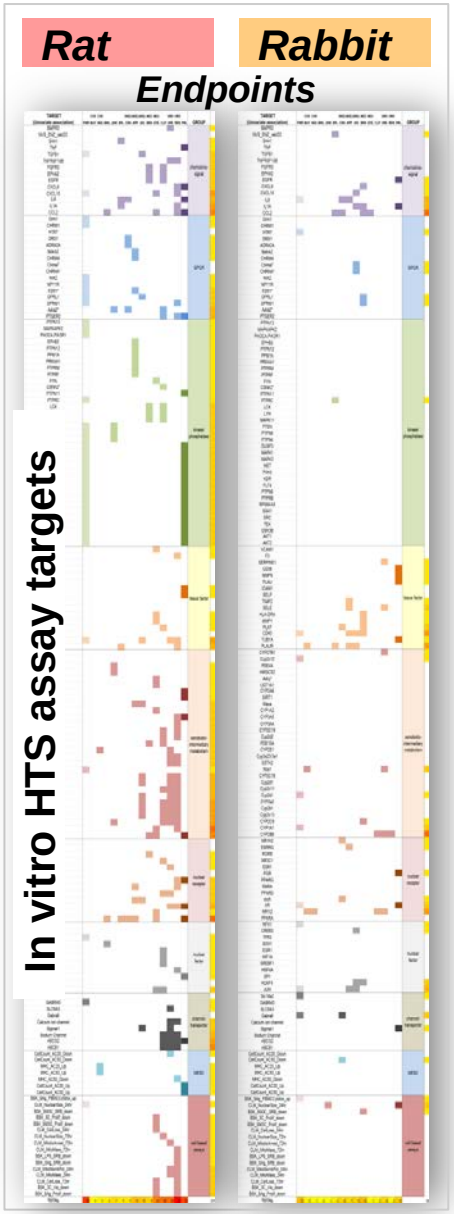
ToxCastDB

HTS Associated w/ DevTox



HTS Associated w/ non-DevTox

Assay Set Aggregation & Reduction



ASSAY AGGREGATION

Condense by gene, gene family, or pathway

ASSAY SET REDUCTION

Reduce by statistics (e.g. correlation)

Assay Set 1 (e.g. IL)
Assay 2 – BSK_BE3C_IL1a_up
Assay 5 – BSK_LPS_IL1a_up
Assay 39 – BSK_LPS_IL8_up

Assay Set 2 (e.g. GPCRs)
Assay 6 – NVS_Opiate_mu
Assay 155 – NVS_hORL1
Assay 276 – NVS_hM1

…

Assay Set X

MULTIVARIATE MODEL

LDA
Model Optimization

code from Martin et al 2011

Species-Specific DevTox Predictive Model Features

MULTIVARIATE MODEL

LDA
Model Optimization

Rat_{PM}

Features	Description	Weight
RAR	Retinoic Acid receptor	0.58
GPCR	G-Protein-Coupled Receptors	0.55
TGFβ	Transforming Growth Factor β	0.38
MT	Microtubule organization	0.30
SENS_CYP	Cytochrome P450 (sensitive)	0.26
AP1	Activator protein 1	0.24
SLCO1B1	Organic anion transporter 1B1	0.11
CYP	CYPs (other)	0.06
HLA-DR	MHC complex	-0.38
PXR	Pregnane X receptor	-0.24
IL8	Interleukin 8	-0.23
PGE2	Prostaglandin E2 response	-0.18

5 fold cross validation balanced accuracies: 71% Rat_{PM}, 74% $Rabbit_{PM}$

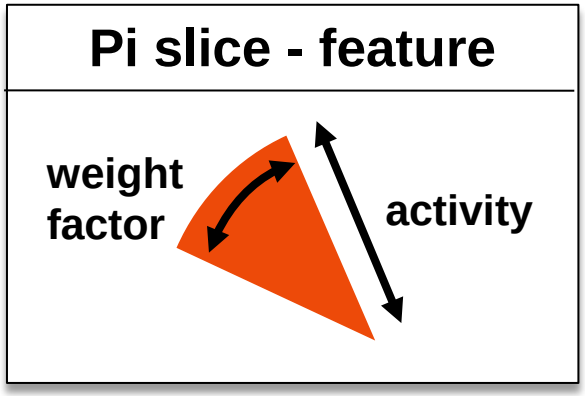
Chemical Rank Order Visualization

Sipes et al 2011

ToxPi Visualization

Toxicity Prioritization Index (Reif et al 2010)

- Graphical view of multiple parameters
- Intended for quick comparisons



Rat_{PM}

Features	Weight
RAR	0.58
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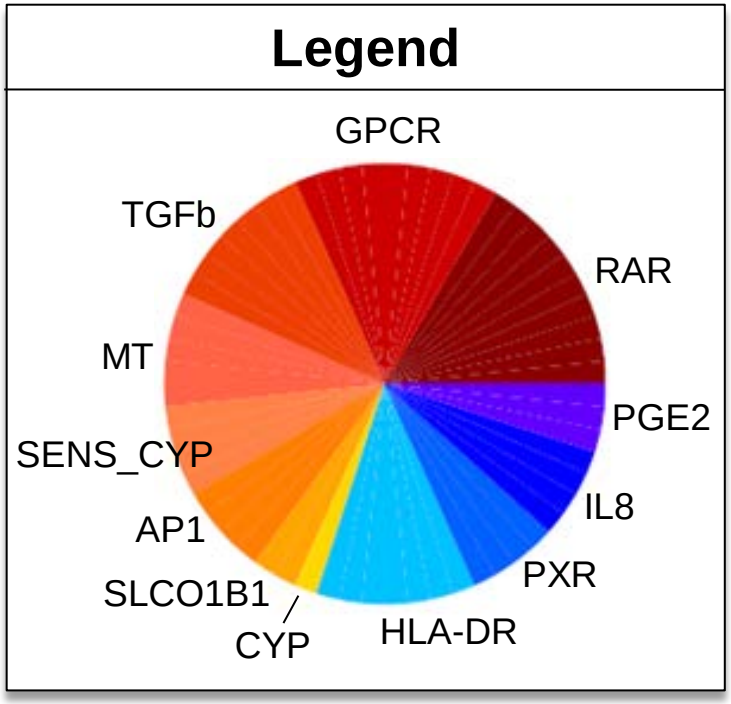
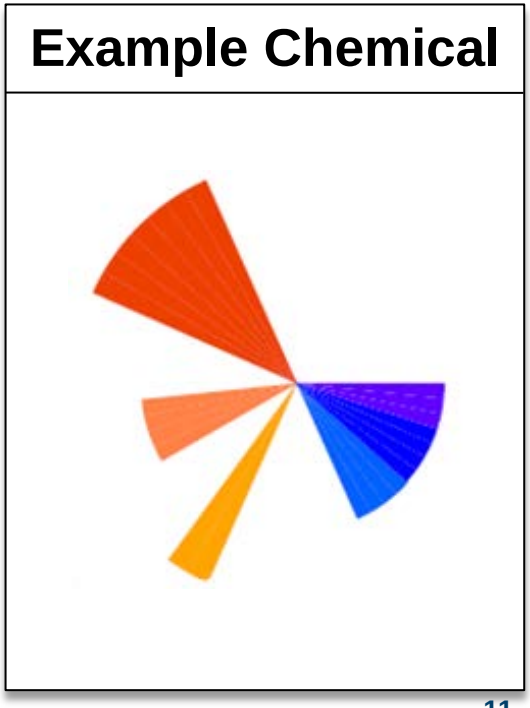
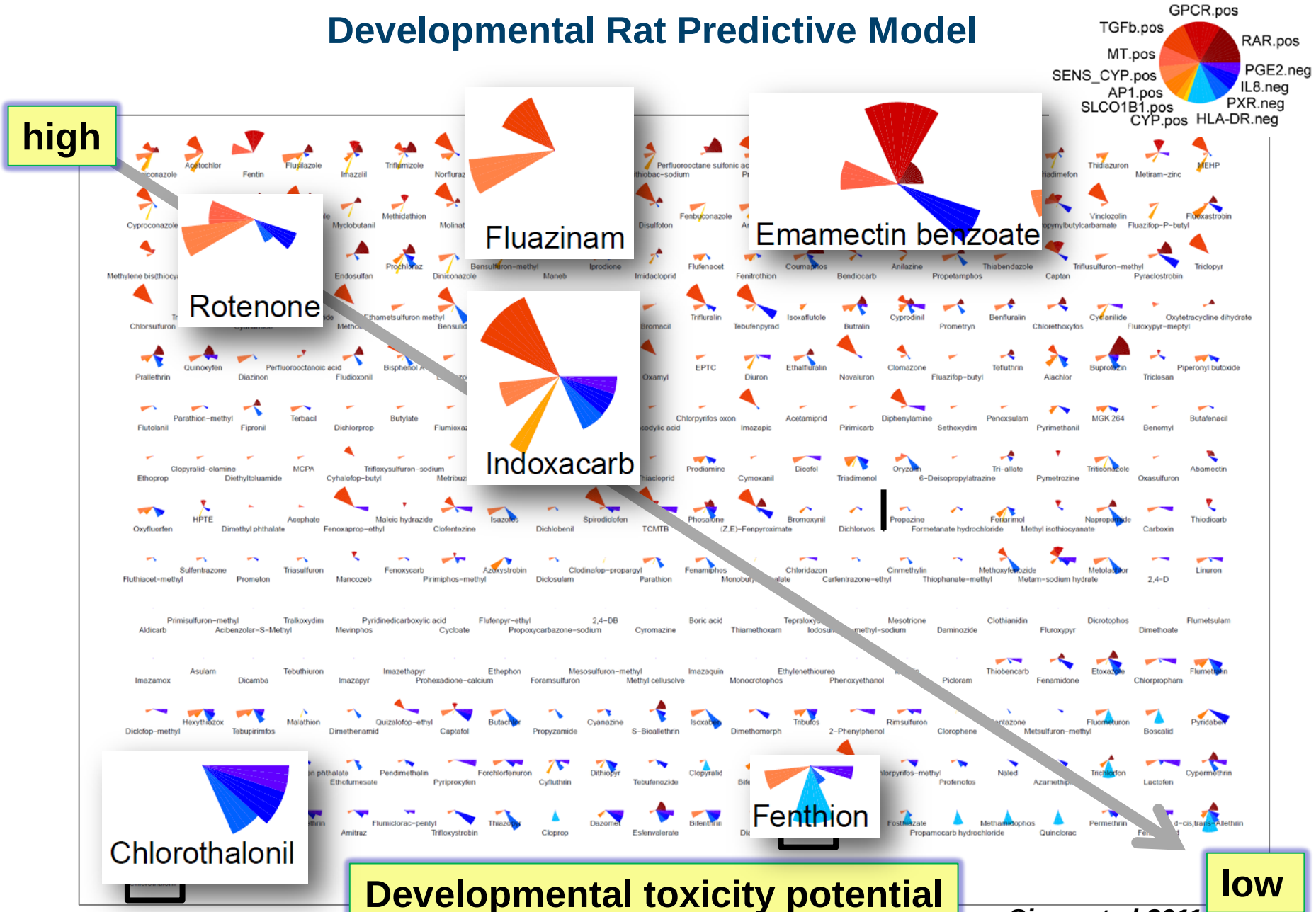




Ex. ToxCast Phase I Chemical Rank Order

Developmental Rat Predictive Model



Sipes et al 2011

Does each 1st generation species model contribute unique information?

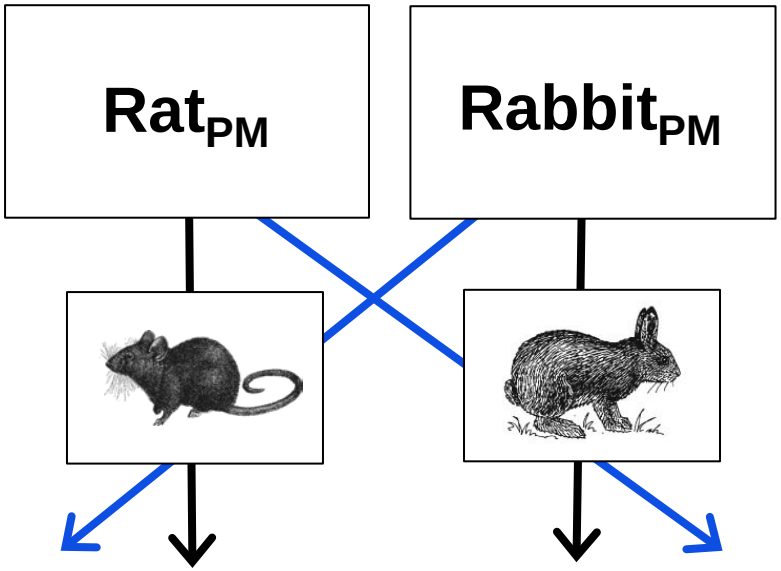
If yes:

- Predictive model assay targets may also be species-specific contributing to differential toxicity

If no:

- Predictive model assay targets may be redundant and could contribute to the same pathways involved in developmental toxicity

Are the Rat and Rabbit Models Unique?



Ability to correctly predict positives

Ability to correctly predict negatives

Conclusions

1) These models are species-specific

2) They are giving different information

Sipes et al 2011

Can we use the models to prioritize testing for one species?

Feasibility for using predictive models for animal model replacements to:

- Reduce animal use
- Decrease cost, increase throughput of chemical testing

Evaluation of Current Predictive Models

- **Chemicals tested in both species (214)**
- **Developmental toxicants = Developmental toxicant in rat OR rabbit (154)**
  - Rat only (61), Rabbit only (28), Both (65)

Rat	**in vivo**	**in vivo**	**PM**	**PM**
Rabbit	**in vivo**	**PM**	**in vivo**	**PM**
BA (%)	100	85	74	64
FP (#)	0	15	21	30
FN (#)	0	9	26	33

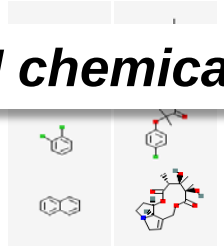
Conclusion: The predictive models may allow us to focus testing on one species

1) Run rat in vivo prenatal guideline study
2) Run rabbit predictive model when needed as a follow-up

Alternative Workflows

Forward Validation (Preliminary)

Phase II chemical library



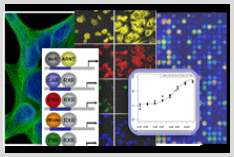
~700

in vivo data

Expanded ToxRefDB (anticipated fall 2012)

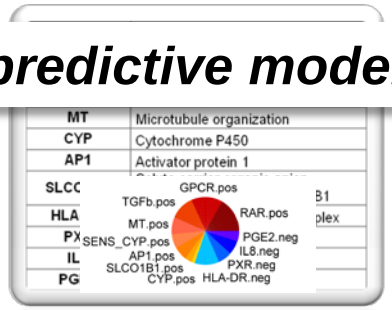
+

in vitro data



Expanded ToxCastDB (anticipated fall 2012)

1st generation predictive models (rat & rabbit)



Top 15 Phase II Chemicals

Preliminary analysis based on data received to date

- **data from 70% of 1st generation predictive model (PM) assays/features**

no study available
developmental toxicity observed
no developmental toxicity observed

Ranked by Rat_{PM}	ToxRefDB_rat	ToxRefDB_rabbit	Lit Dev Tox
Colchicine			
Crystal violet			
Dieldrin			
Dimethyl malonate			
Dodecyltrimethylammonium chloride			
Mercuric chloride			
Nitrobenzene			
Octanoic acid			
$PharmaX_1$			
Phenylmercuric acetate			
Sodium dodecylbenzenesulfonate			
Sodium tetradecyl sulfate			
trans-Retinoic acid			
Tributyltin chloride			
Tributyltin methacrylate			

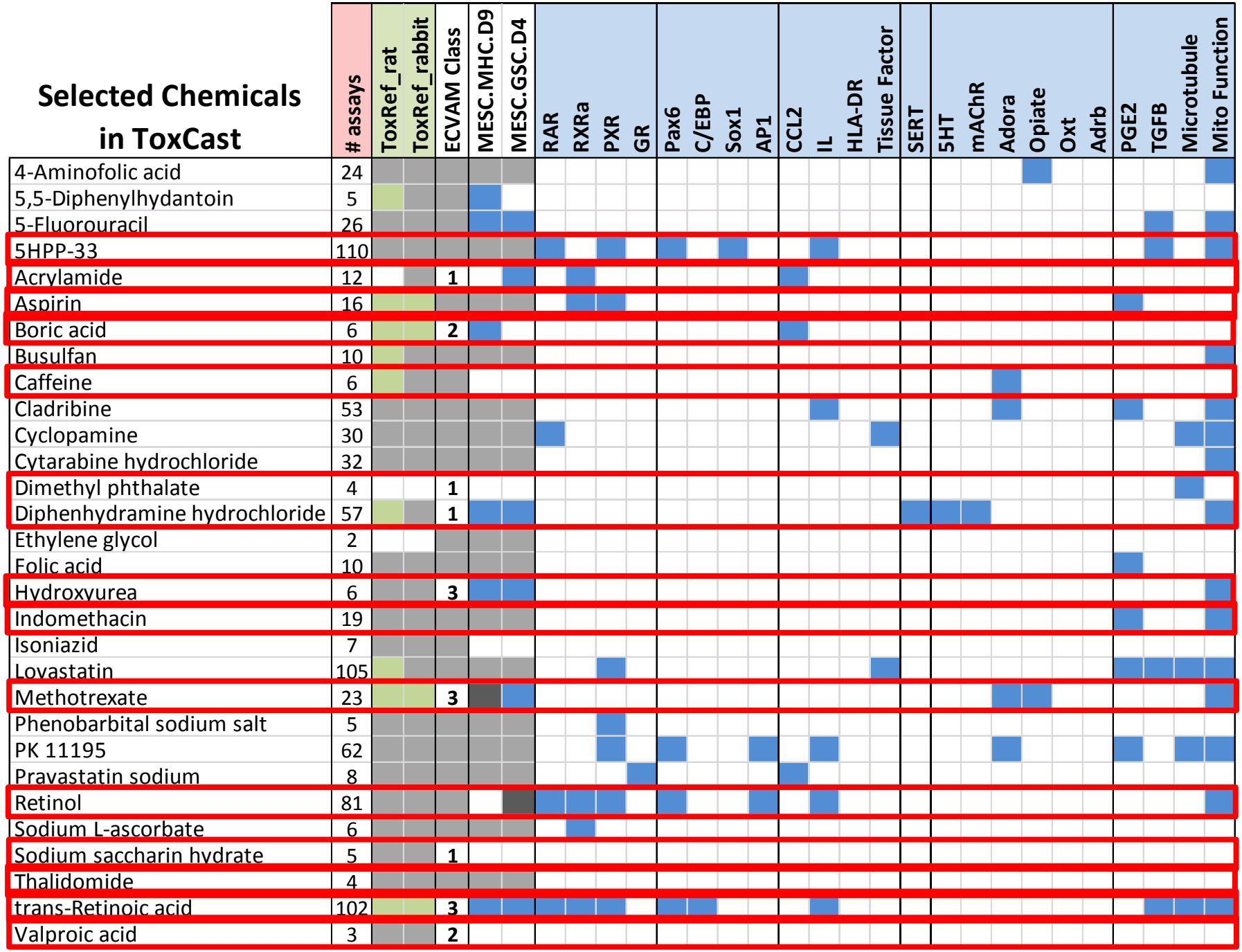
Ranked by $Rabbit_{PM}$	ToxRefDB_rat	ToxRefDB_rabbit	Lit Dev Tox
1,4-Dichlorobenzene			
2-(Perfluorohexyl)ethyl methacrylate			
2,4,6-Tris(tert-butyl)phenol			
Acrylamide			
Biphenyl			
Diethanolamine			
Diethylene glycol monomethyl ether			
Isophthalic acid			
Kepone			
Oryzalin			
$PharmaX_2$			
$PharmaX_3$			
$PharmaX_4$			
$PharmaX_5$			
$PharmaX_6$			

Potential Assays for Addition in 2nd Gen PM

Preliminary analysis based on 162 Phase II chemicals

Top Assays Associated with Developmental Toxicity

Species	Most associated assays
Rabbit	Nuclear Receptors (RAR, RXRa) Transcription Factors (Sox1, Pax6, C/EBP) Serotonin Transporter (SERT) GPCR (Adora) Mitochondrial Function
Rat	Glucocorticoid Receptor (GR) Serotonin Transporter (SERT) GPCRs (Adrb, Adora, 5HT, mAChR, Oxt) Platelet Tissue Factor


Selected Chemicals in ToxCast
assays
ToxRef_rat
ToxRef_rabbit
ECVAM Class
MESC.MHC.D9
MESC.GSC.D4
RAR
RXRa
PXR
GR
Pax6
C/EBP
Sox1
AP1
CCL2
IL
HLA-DR
Tissue Factor
SERT
5HT
mAChR
Adora
Opiate
Oxt
Adrb
PGE2
TGFB
Microtubule
Mito Function
4-Aminofolic acid 24
5,5-Diphenylhydantoin 5
5-Fluorouracil 26
5HPP-33 110
Acrylamide 12 1
Aspirin 16
Boric acid 6 2
Busulfan 10
Caffeine 6
Cladribine 53
Cyclopamine 30
Cytarabine hydrochloride 32
Dimethyl phthalate 4 1
Diphenhydramine hydrochloride 57 1
Ethylene glycol 2
Folic acid 10
Hydroxyurea 6 3
Indomethacin 19
Isoniazid 7
Lovastatin 105
Methotrexate 23 3
Phenobarbital sodium salt 5
PK 11195 62
Pravastatin sodium 8
Retinol 81
Sodium L-ascorbate 6
Sodium saccharin hydrate 5 1
Thalidomide 4
trans-Retinoic acid 102 3
Valproic acid 3 2

Conclusions

- Species predictive models of developmental toxicity give >70% BA
  - Species-specific assay targets may be contributing to differential pathways of developmental toxicity
- It is feasible to use predictive models in conjunction with animal data
  - Prioritize chemicals for testing in one species
  - Rat in vivo followed by rabbit predictive model
- Early findings indicate we can bring additional assays into predictive models for a broader range of developmental toxicants in Phase II

Thank you!

http://www.epa.gov/ncct/