

Identifying Toxicity Pathways with ToxCast High Throughput Screening and Applications to Predicting Developmental Toxicity

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ToxCast Phases I, II and III

FY07

FY08

FY09

FY10

FY11

FY12

Proof of Concept: ToxCast Phase I

Verification/Extension: Phase II

Reduce to Practice:
Phase III

ToxCast_320 Phase I Chemicals

309 unique structures

Replicates for QC

8 metabolites

291 total pesticide actives

273 registered pesticide actives

22 pesticide inerts

33 antimicrobials

56 Tier 1 EDSP

23 IUR

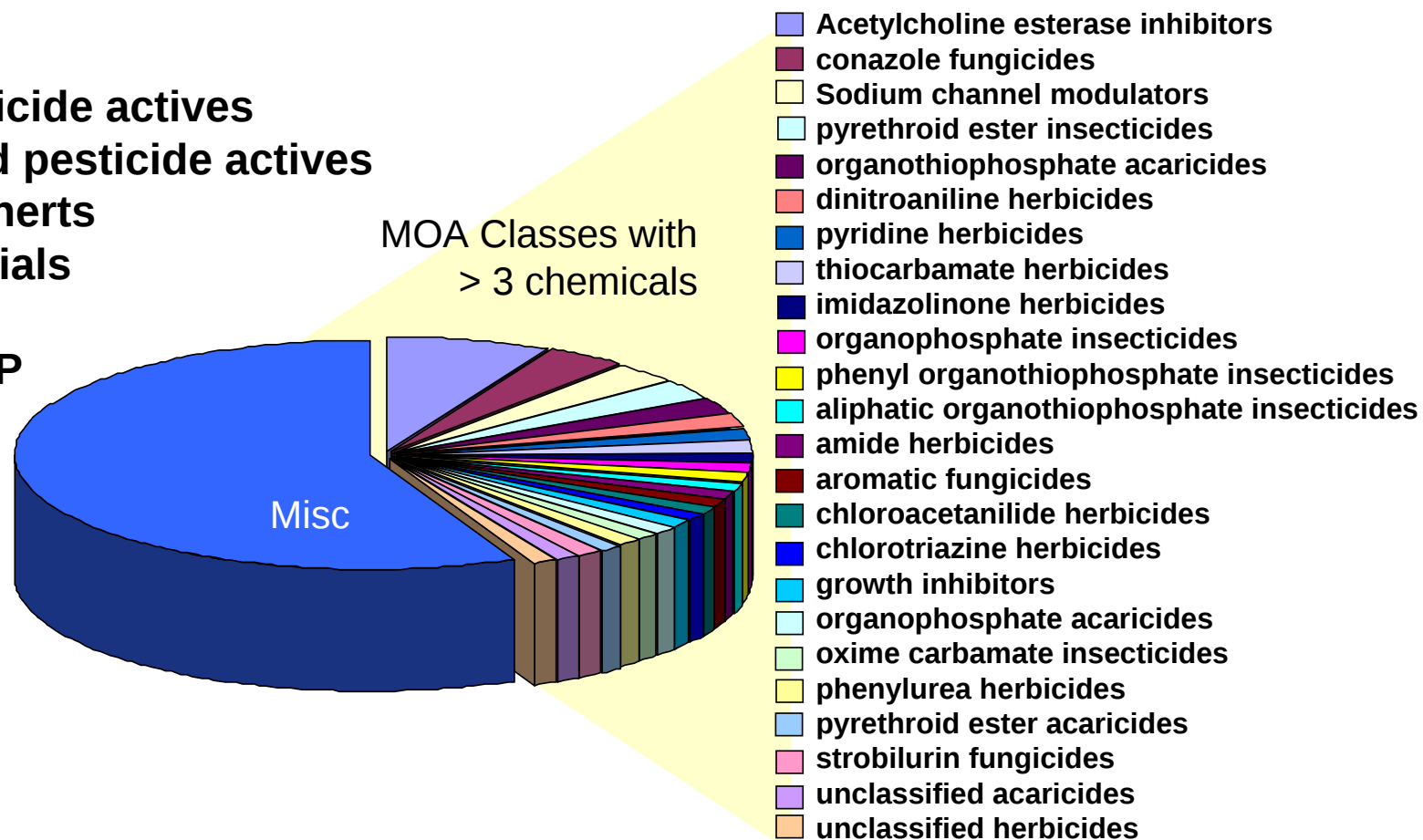
13 HPV

73 OW PCCL

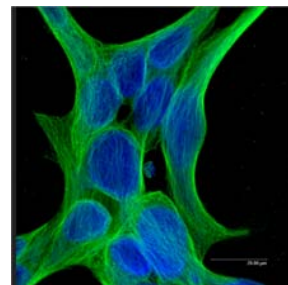
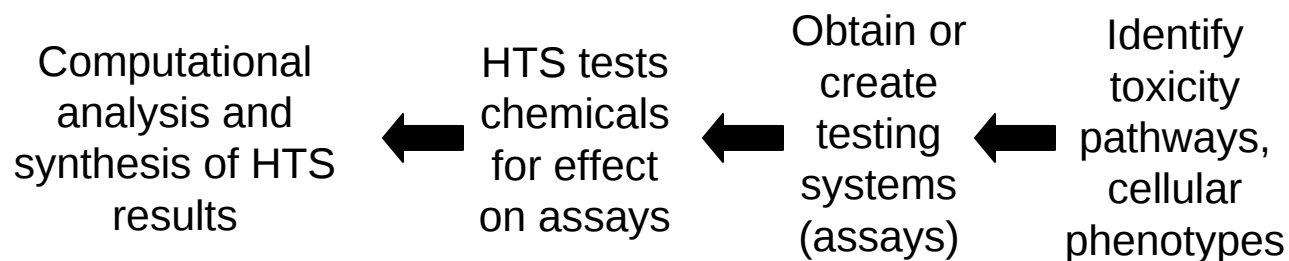
11 CCL1

10 CCL2

25 CCL3



Characterizing Effects of Environmental Chemicals on Molecular Targets and Biological Pathways: HT Toxicology





ToxCast Data Sources



attagene

The Home of TFomics™



BioSeek



6 contracts, 4 collaborations
467 assays, 534 endpoints



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

6 contracts, 4 collaborations
467 assays, 534 endpoints

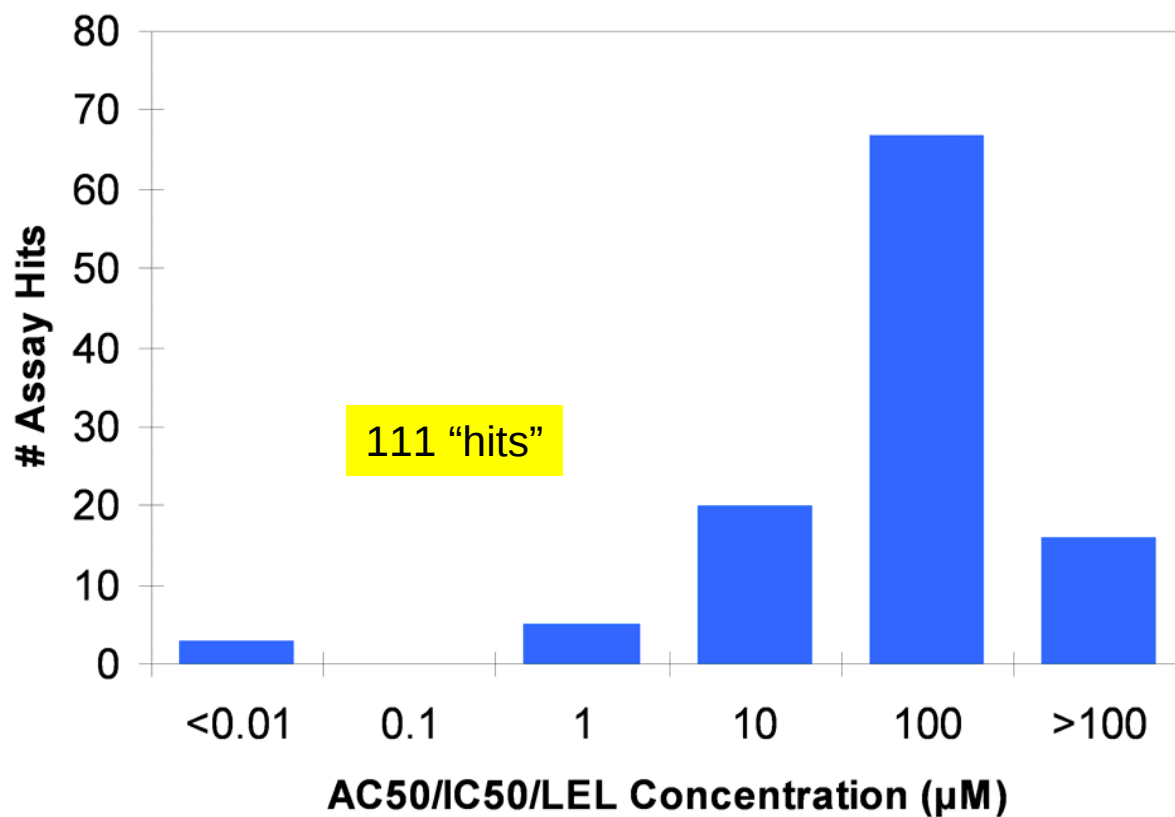
Cellular Assays

- 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 In vitro data (467 assays)

Bisphenol A

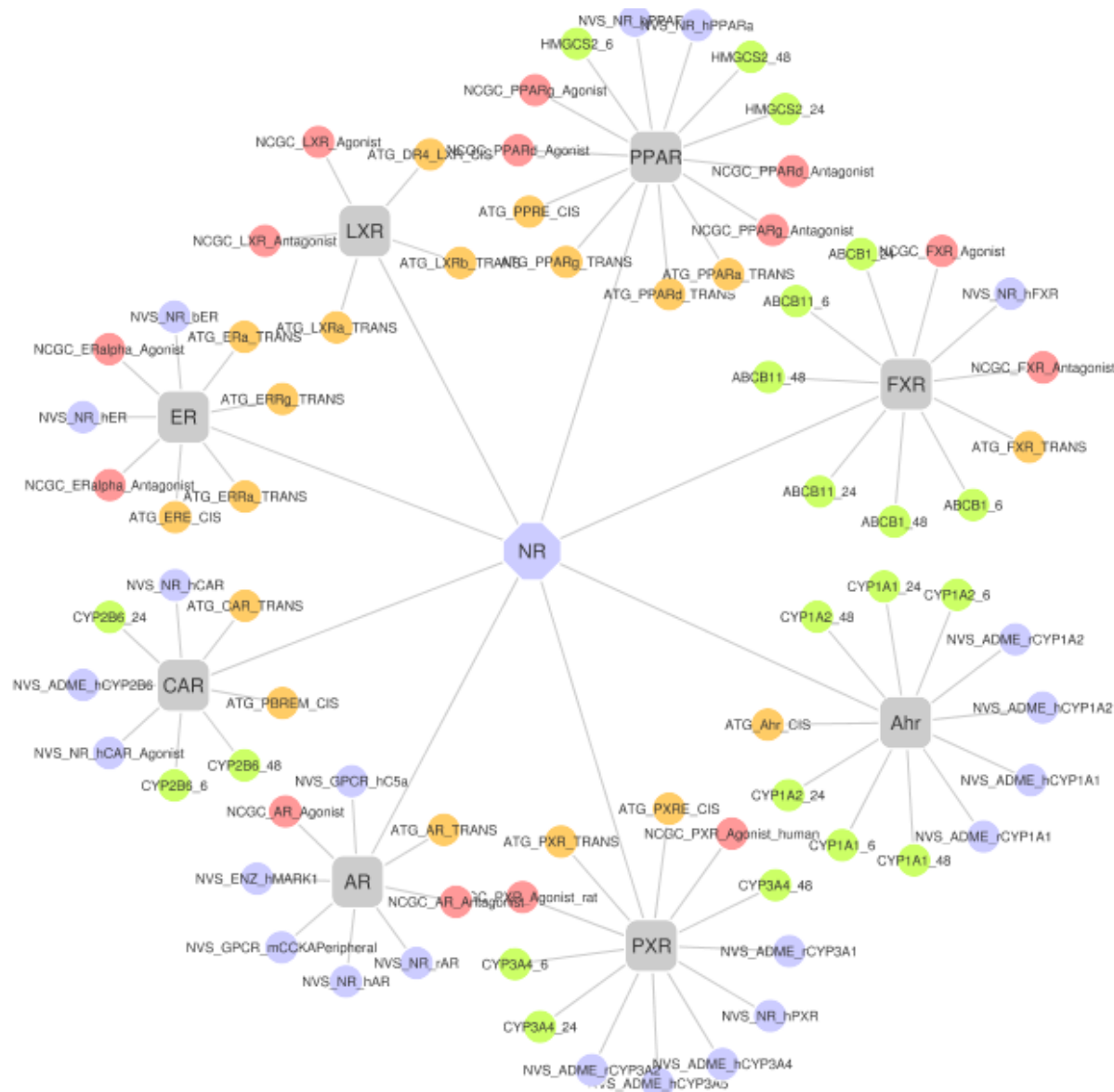
- Cell
- Multi
- Hum
- HCS
- qNPA
- XMEs
- Imper
- Geno



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

Multiple Assays per Endpoint



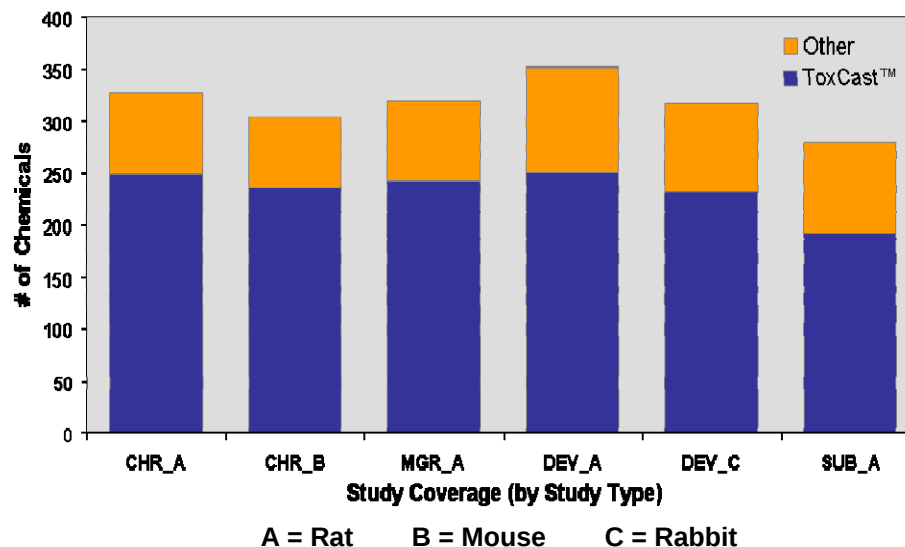


- [illegible]

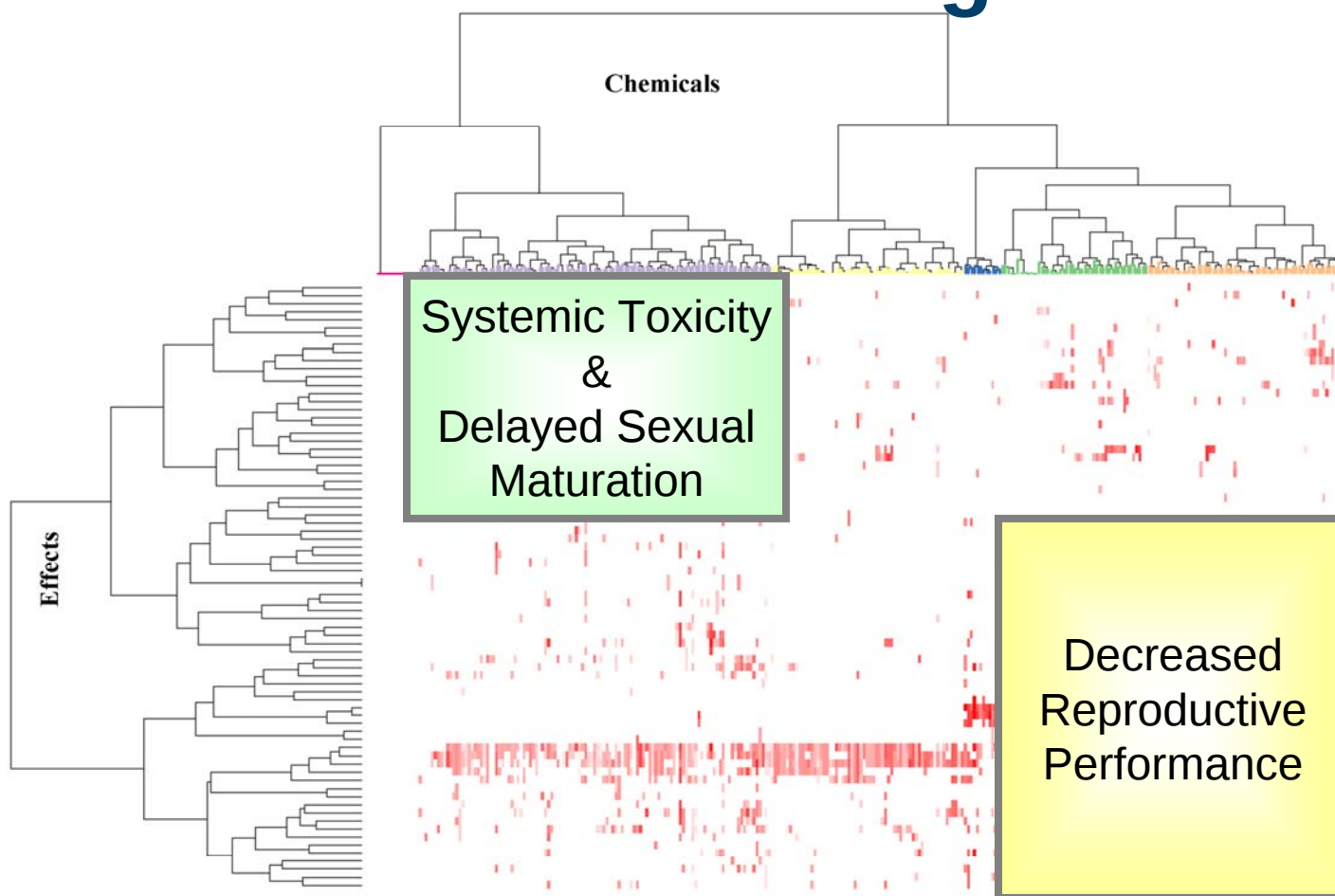
Office of Research and Development
National Center for Computational Toxicology



ToxRefDB

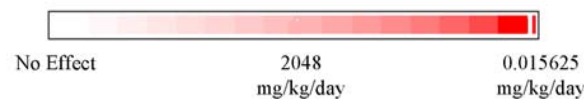
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Reproductive Toxicity Profiling



Chemical Clusters

- - 1: No Observed Toxicities
- - 2: General Systemic Toxicity & Delayed Sexual Maturation
- - 3: Limited Toxicity - Primarily Body Weight Changes
- - 4: Cholinesterase Inhibition
- - 5: Male Reproductive Toxicity & Decrease Reproductive Performance
- - 6: Offspring Viability & Decreased Reproductive Performance



Lowest Effect Level (LEL): $-\text{Log}_2(\text{LEL})$

<http://toxsci.oxfordjournals.org/cgi/reprint/kfp080>

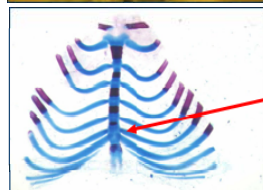
Associations of ToxCast Assays with ToxRefDB MGR Endpoints

ToxRefDB Endpoints	# Assays
MGR_Rat_Adrenal	7
MGR_Rat_Epididymis	10
MGR_Rat_Fertility	25
MGR_Rat_GestationalInterval	29
MGR_Rat_Implantations	32
MGR_Rat_Kidney	17
MGR_Rat_LactationPND21	10
MGR_Rat_LitterSize	29
MGR_Rat_LiveBirthPND1	35
MGR_Rat_Liver	25
MGR_Rat_Mating	3
MGR_Rat_Ovary	15
MGR_Rat_Prostate	7
MGR_Rat_Spleen	9
MGR_Rat_Testis	6
MGR_Rat_Thyroid	57
MGR_Rat_Uterus	7
MGR_Rat_ViabilityPND4	45

Profiling Developmental Toxicity



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

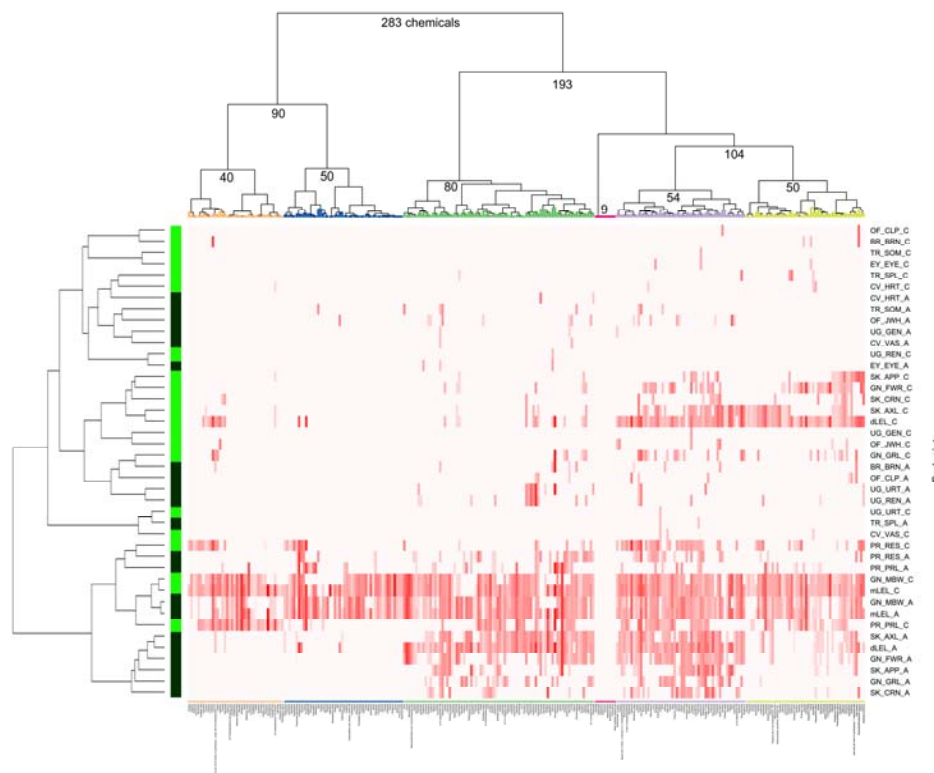


target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130



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

images from www.DevTox.org



ToxRefDB 387 chemicals, 751 prenatal studies,
988 total effects annotated (enhanced DevTox.org)

283 chemicals x 293 effects → **19 target**
systems from rat (■) and rabbit (■) studies

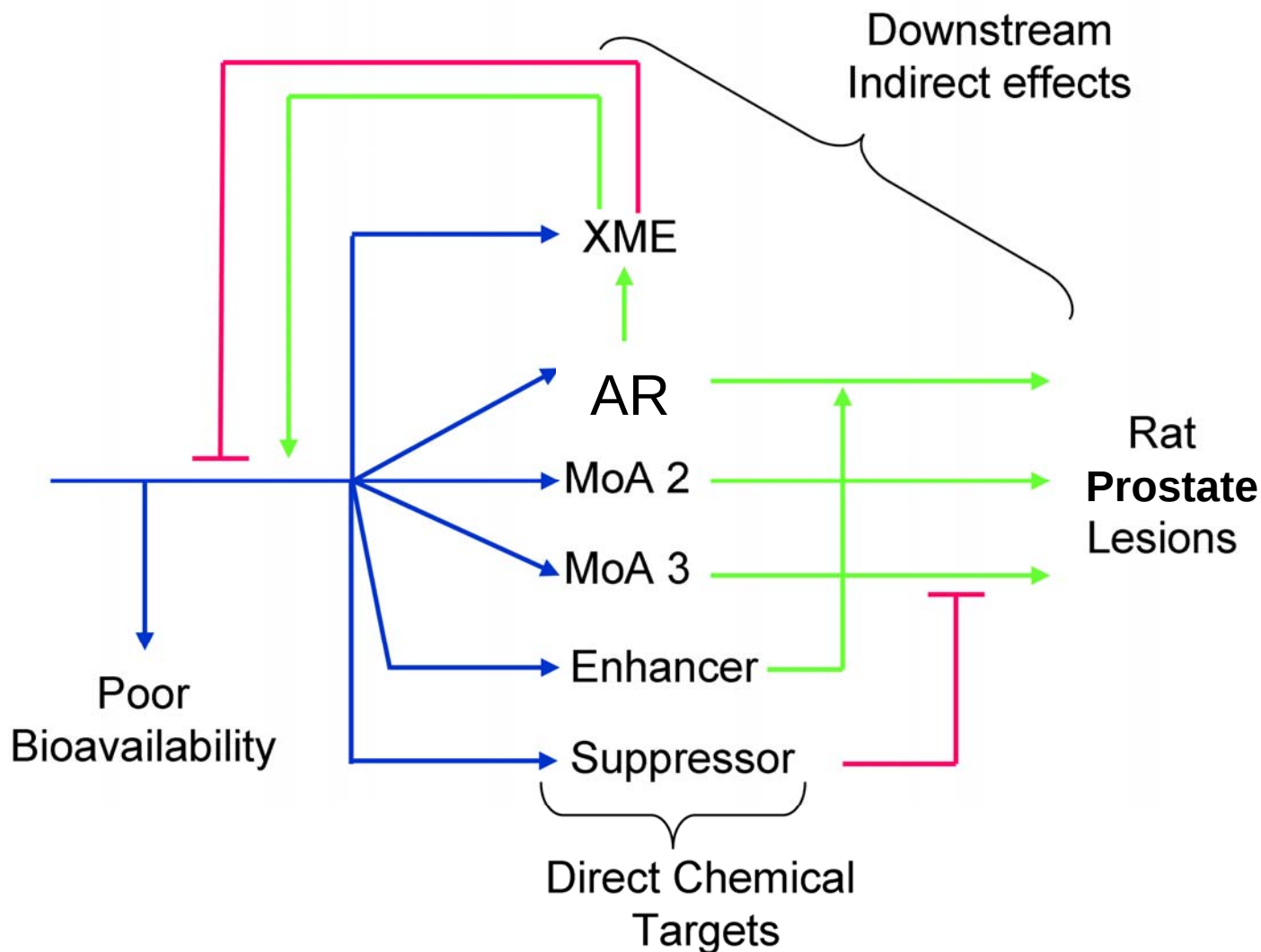
Associations of Individual ToxCast Assays with ToxRefDB DEV Endpoints

ToxRefDB Endpoints	# Assays
DEV_Rabbit_Cardiovascular_Heart	1
DEV_Rabbit_General_FetalWeightReduction	15
DEV_Rabbit_General_GeneralFetalPathology	13
DEV_Rabbit_Neurosensory_Brain	10
DEV_Rabbit_Neurosensory_Eye	4
DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss	11
DEV_Rabbit_PregnancyRelated_MaternalPregLoss	5
DEV_Rabbit_Skeletal_Appendicular	11
DEV_Rabbit_Skeletal_Axial	22
DEV_Rabbit_Skeletal_Cranial	6
DEV_Rabbit_Trunk_BodyWall	16
DEV_Rabbit_Trunk_SplanchnicViscera	2
DEV_Rat_Urogenital_Genital	55
DEV_Rat_Urogenital_Renal	55
DEV_Rat_Urogenital_Ureteric	28

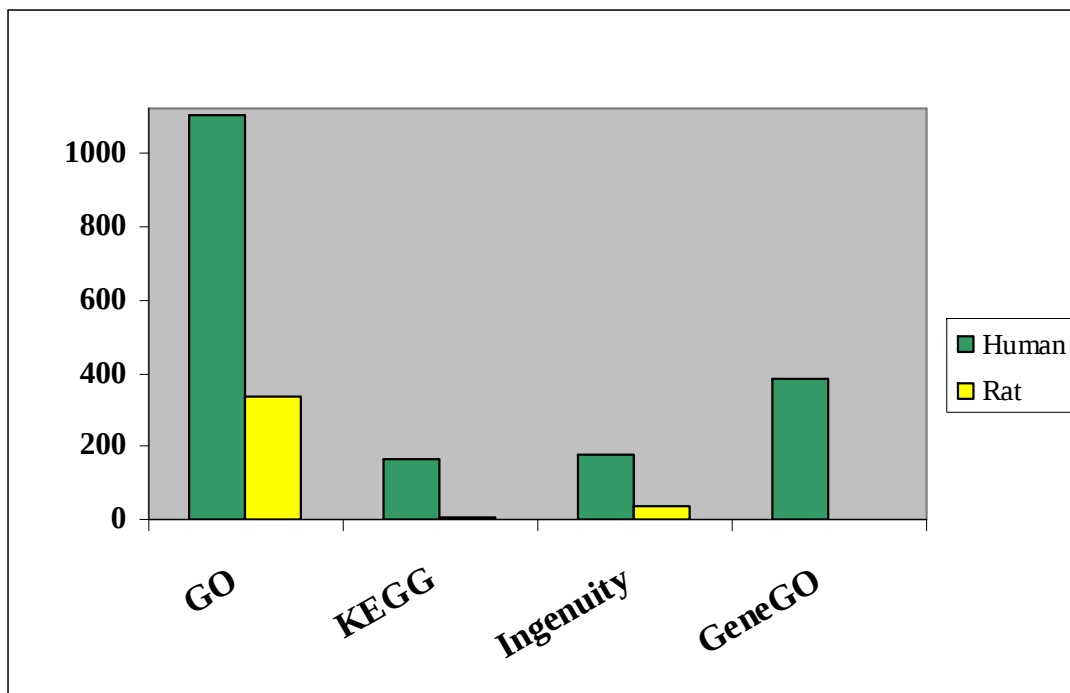
Examples of Some of the Strongest Associations- Multiple Sources, Targets, Pathways, Possible MOA

Assay	Endpoint	Relative Risk	RR.Per centile	Fisher.Test.
BSK_3C_MIG_down	MGR_Rat_Prostate	41.67	0.0288	0.0278884
NCGC_AR_Agonist	MGR_Rat_Prostate	41.67	0.0293	0.0278884
BSK_hDFCGF_MMP1_do	MGR_Rat_Prostate	12.70	0.0045	0.0042722
BSK_3C_Thrombomodulin_down	MGR_Rat_Prostate	11.80	0.0033	0.005274
BSK_BE3C_IL1a_down	MGR_Rat_Prostate	9.71	0.0103	0.0091086
ATG_NRF1_CIS	MGR_Rat_Prostate	9.64	0.0279	0.0270278
NVS_ADME_rCYP2C12	MGR_Rat_Prostate	7.32	0.0444	0.044971

Toxicity is Multi-Factorial; Toxicity Predictions Need to be Multi-Factorial



Pathways and Processes Identified by ToxCast Data



	<i>Human</i>	<i>Rat</i>
GO	1104	336
KEGG	166	9
Ingenuity	180	39
GeneGO	385	-
Total Unique Entrez GeneID	236	51

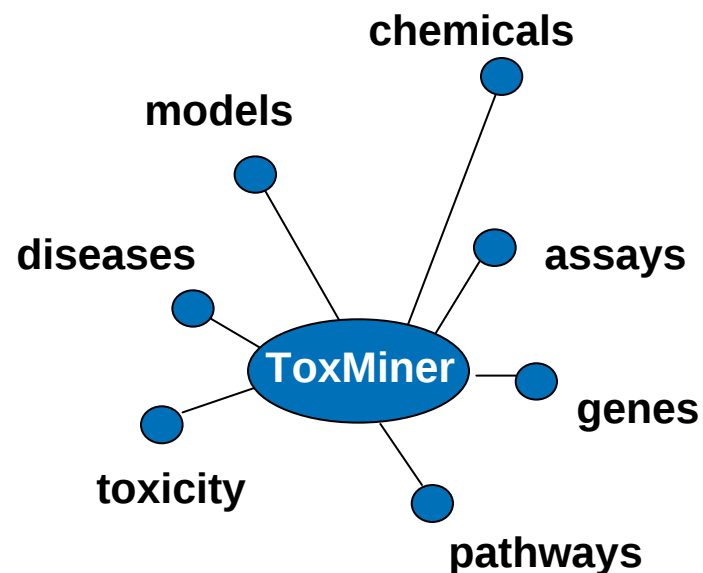
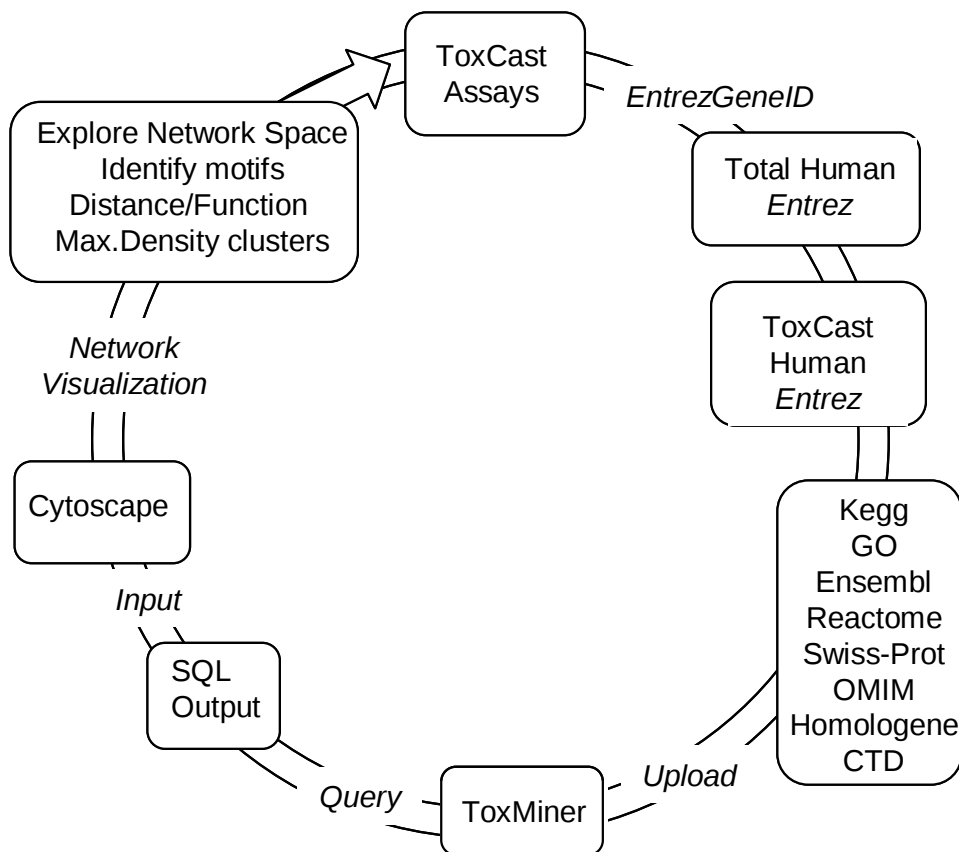
Identifying Pathways Associated with Reproductive and Developmental Toxicity (DEV)

Assay_1 ▼	Assay_2 ▼
PATH_Inguinity_Aryl_Hydrocarbon_Receptor_Signaling	DEV_Rat_Orofacial_CleftLipPalate
PATH_Inguinity_Glucocorticoid_Receptor_Signaling	DEV_Rat_Orofacial_CleftLipPalate
PATH_KEGG_hsa04080_Neuroactive_ligand_receptor_interaction	DEV_Rat_Orofacial_CleftLipPalate
PATH_Inguinity_Pi3K_AKT_Signaling	DEV_Rat_PregnancyRelated_EmbryoFetalLoss
PATH_Inguinity_Tight_Junction_Signaling	DEV_Rat_PregnancyRelated_EmbryoFetalLoss
PATH_KEGG_hsa04540_Gap_junction	DEV_Rat_PregnancyRelated_EmbryoFetalLoss
PATH_Inguinity_G_Protein_Coupled_Receptor_Signaling	DEV_Rat_Skeletal_Appendicular
PATH_KEGG_hsa04080_Neuroactive_ligand_receptor_interaction	DEV_Rat_Skeletal_Appendicular
PATH_KEGG_hsa04080_Neuroactive_ligand_receptor_interaction	DEV_Rat_Skeletal_Axial
PATH_Inguinity_FXR_RXR_Activation	DEV_Rat_Urogenital_Ureteric
PATH_Inguinity_PPARa_RXRa_Activation	DEV_Rat_Urogenital_Ureteric
PATH_KEGG_hsa00591_Linoleic_acid_metabolism	DEV_Rat_Urogenital_Ureteric

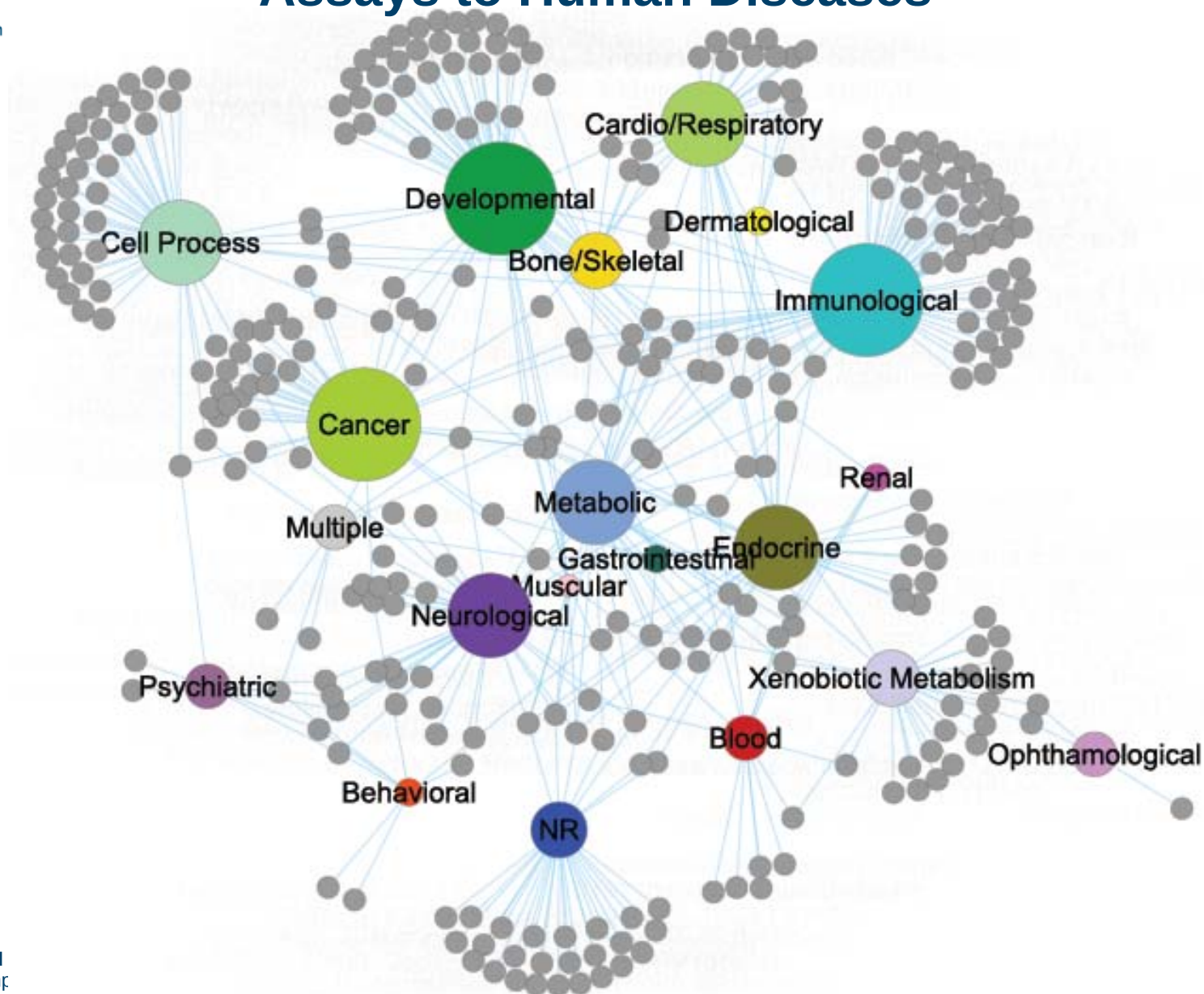
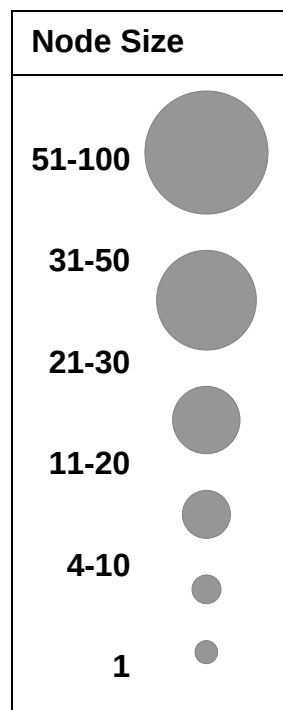
Identifying Pathways Associated with Reproductive and Developmental Toxicity (MGR)

Assay_1	Assay_2
PATH_KEGG_hsa03320_PPAR_signaling_pathway	MGR_Rat_Epididymis
PATH_KEGG_hsa00590_Arachidonic_acid_metabolism	MGR_Rat_GestationalInterval
PATH_KEGG_hsa00591_Linoleic_acid_metabolism	MGR_Rat_Implantations
PATH_KEGG_hsa00590_Arachidonic_acid_metabolism	MGR_Rat_LiveBirthPND1
PATH_KEGG_hsa03320_PPAR_signaling_pathway	MGR_Rat_Testis
PATH_Ingenuity_RAR_Activation	MGR_Rat_Thyroid
PATH_KEGG_hsa00590_Arachidonic_acid_metabolism	MGR_Rat_ViabilityPND4
PATH_KEGG_hsa00591_Linoleic_acid_metabolism	MGR_Rat_ViabilityPND4

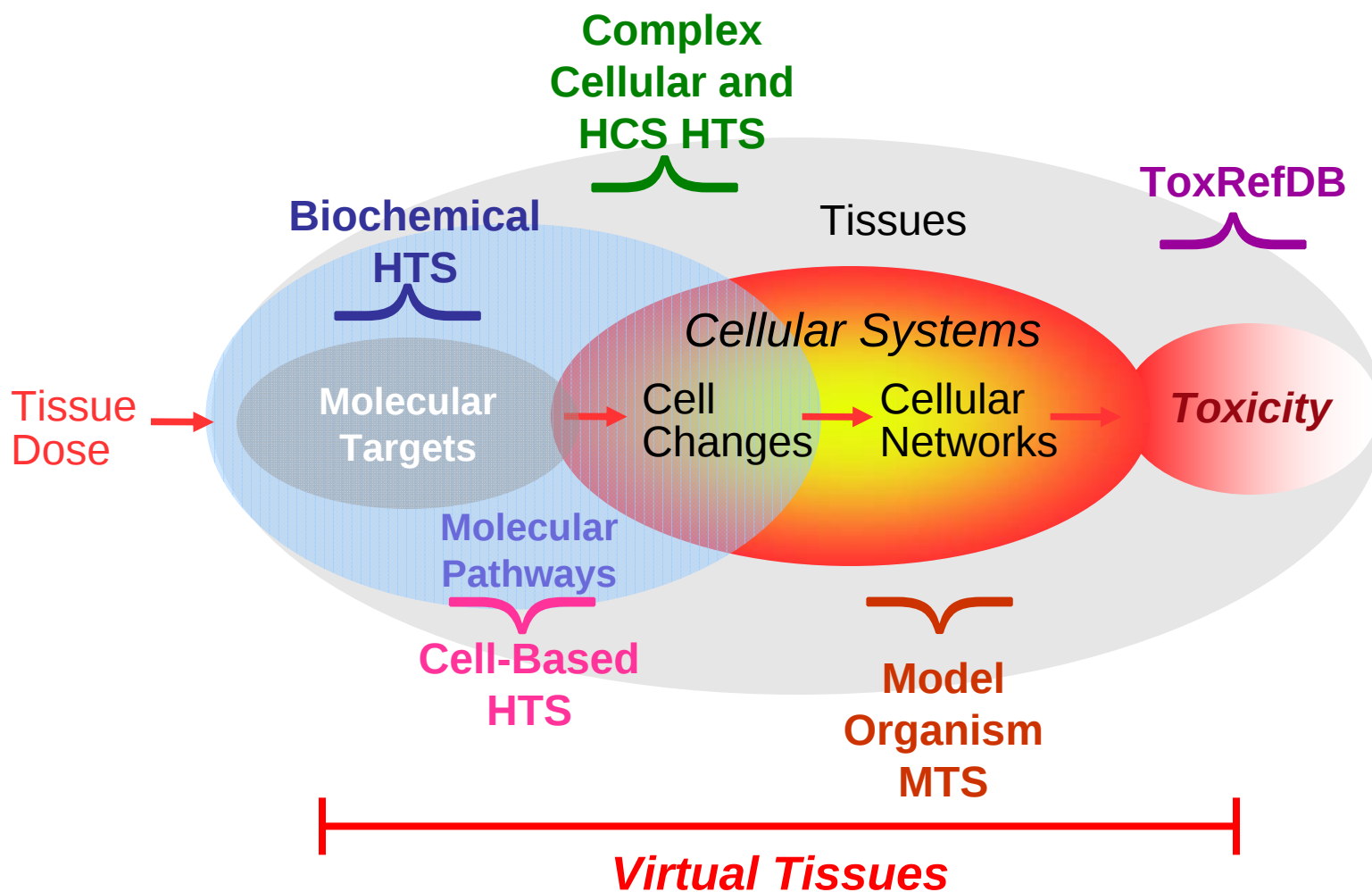
ToxMiner Pathway Database



ToxMiner Includes Mapping of ToxCast Assays to Human Diseases



Predicting Human Toxicity and Disease: The Grand Challenge in Toxicology



An Example Predictive Model for a Developmental Toxicity

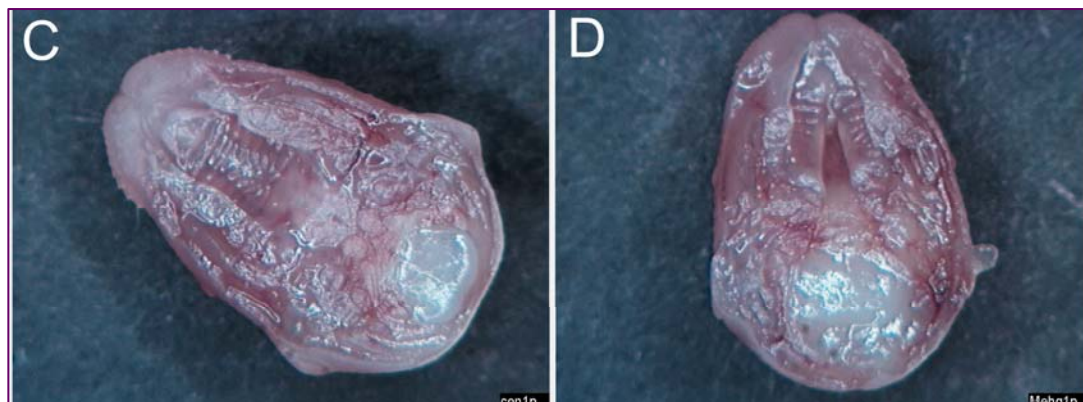
Cleft Palate

chemicals = 12 *

assays = 37

relative risk (avg) = 6.24

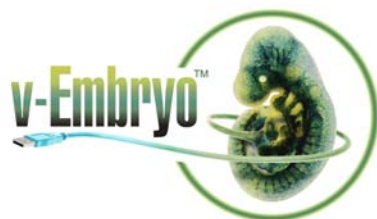
pathways (≥ 5 hits) = 13



PubMed co-occurrences (April 14, 2009)

PATHWAY	SIGNAL	cleft palate	palatal development
AhR	<i>hypoxia?</i>	23	16
GPCR	cAMP	25	32
GR	glucocorticoids	187	61
RAR/RXR	retinoids	189	83
Wnt	Wnt	18	22

* Cymoxanil, Cyproconazole, Dichlobenil, Tri-allate, Propiconazole, Spiroxamine, Triadimefon, Triclopyr, Fluazinam, Flusilazole, Mancozeb, Dibutyl phthalate



Moving Beyond Empirical Models- Multi-Scale Models of Developing Systems

- ❖ **Motivation:** computational models to navigate complex relationships in the embryo and predict key *in vivo* events from *in vitro* data
- ❖ **Research goal:** simulate embryonic tissues reacting to perturbation across chemical class, system, stage, genetic makeup, dose and time
- ❖ **Inputs:** detailed knowledge of biochemical targets, molecular pathways, cellular networks, and emergent phenotypes
- ❖ **Outputs:** modular reconstruction of specific systems (short-term) and the human embryo (long-term)

Some missing pieces:

Development of an In Vitro Technique to Use Mouse Embryonic Stem Cells (mESC) in Evaluating Effects of Xenobiotics

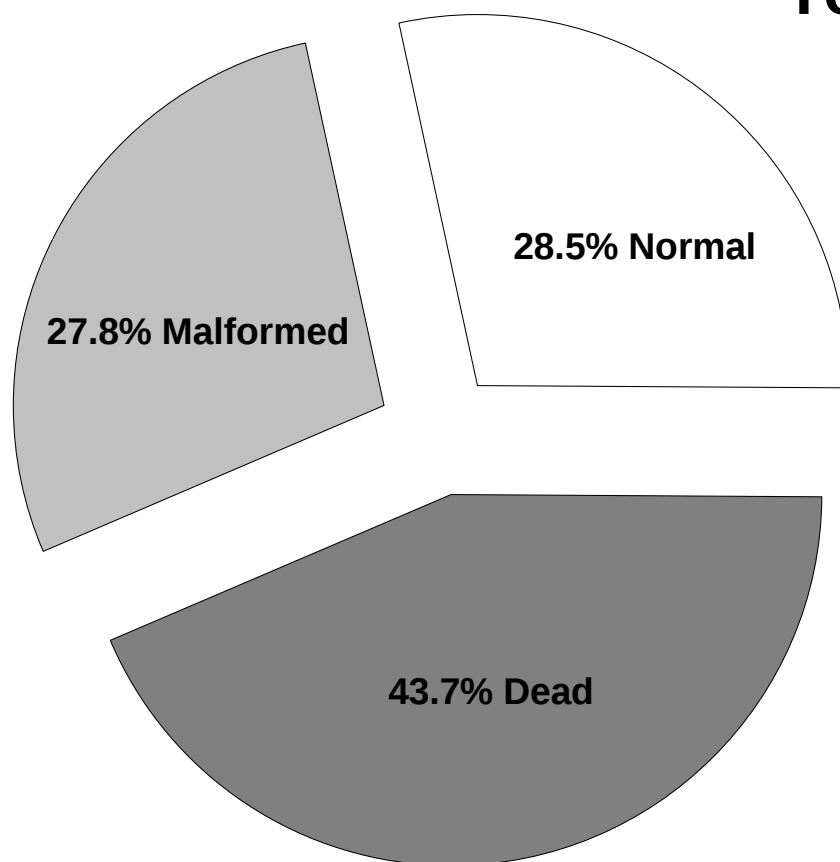
Barrier M, Jeffay S, Nichols HP, Slentz-Kesler K, Hunter ES

**US Environmental Protection Agency,
Research Triangle Park, NC, United States
Almac Diagnostics, Durham, NC, United States**

Abstract #32, Teratology 2009

Some missing pieces:

80 uM screening results-



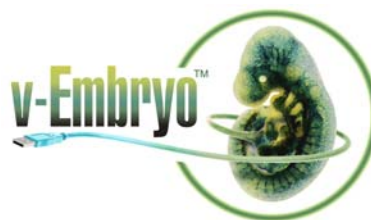
Developmental Effects of ToxCast_320 in Zebrafish

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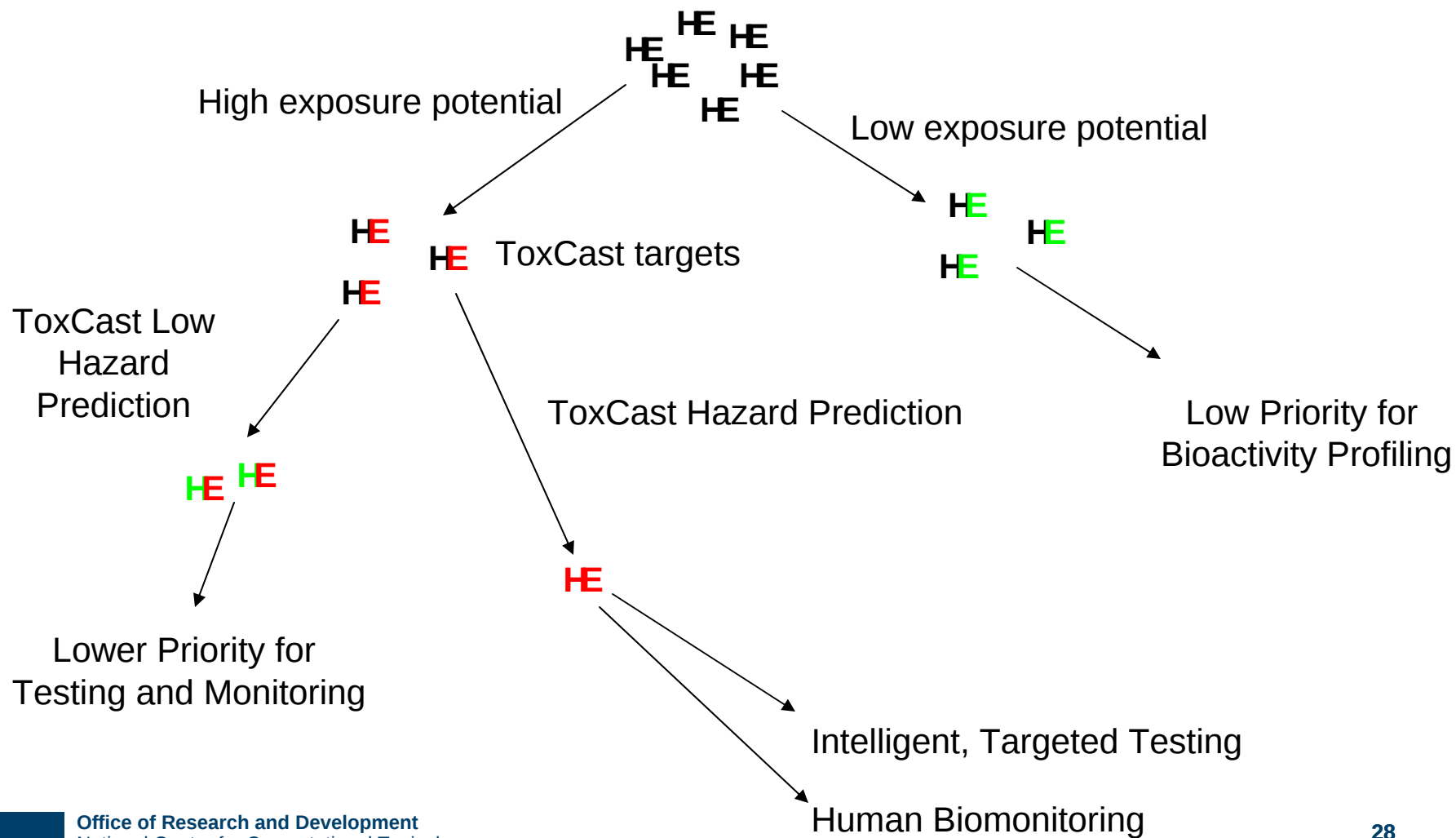


Marianne Barrier
Sid Hunter
Tom Knudsen
Michael Rountree
Amar Singh



Ray Tice +
Chris Austin +

The Future State: Using Hazard and Exposure Information for Prioritizing Testing and Monitoring



Conceptual Model for Incorporating Biotransformation in Predictive Modeling

