EPA
United States
Environmental Protection
Agency

Predictive Signatures of Developmental Toxicity Modeled with HTS data from ToxCast™ Bioactivity Profiles

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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

Office of Research and Development
National Center for Computational Toxicology

SOT, abstract 1382, 03/09/2010

EPA United States Environmental Protection Agency

ToxCast™

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

- project to profile the bioactivity of hundreds to thousands of environmental chemicals using *in vitro* HTS assays,
- mine for *in vivo* correlations by training *in vitro* bioactivity profiles against compounds with evident toxicity,
- build and test computational (*in silico*) models for 'toxicity signatures' that predict subtending biological pathways,
- and prioritize chemicals that inform mechanistic models during chemical disruption (e.g., embryonic development)

ToxCast™ bioactivity profiling

United States Environmental Protection Agency

Biochemical HTS assays

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

309 chemicals
471 endpoints

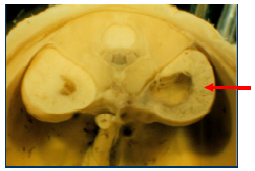
Cell-based assays

- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
  - **J1 mouse ES cells (ACDC)**
- 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

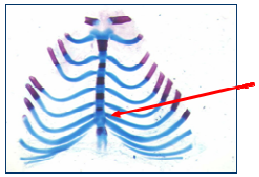
Profiling developmental toxicity

ToxRefDB: >30 yrs of toxicity data worth >$2B

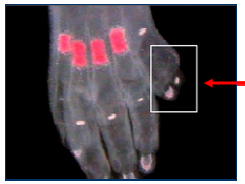
in vivo bioassays
(target, description)



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

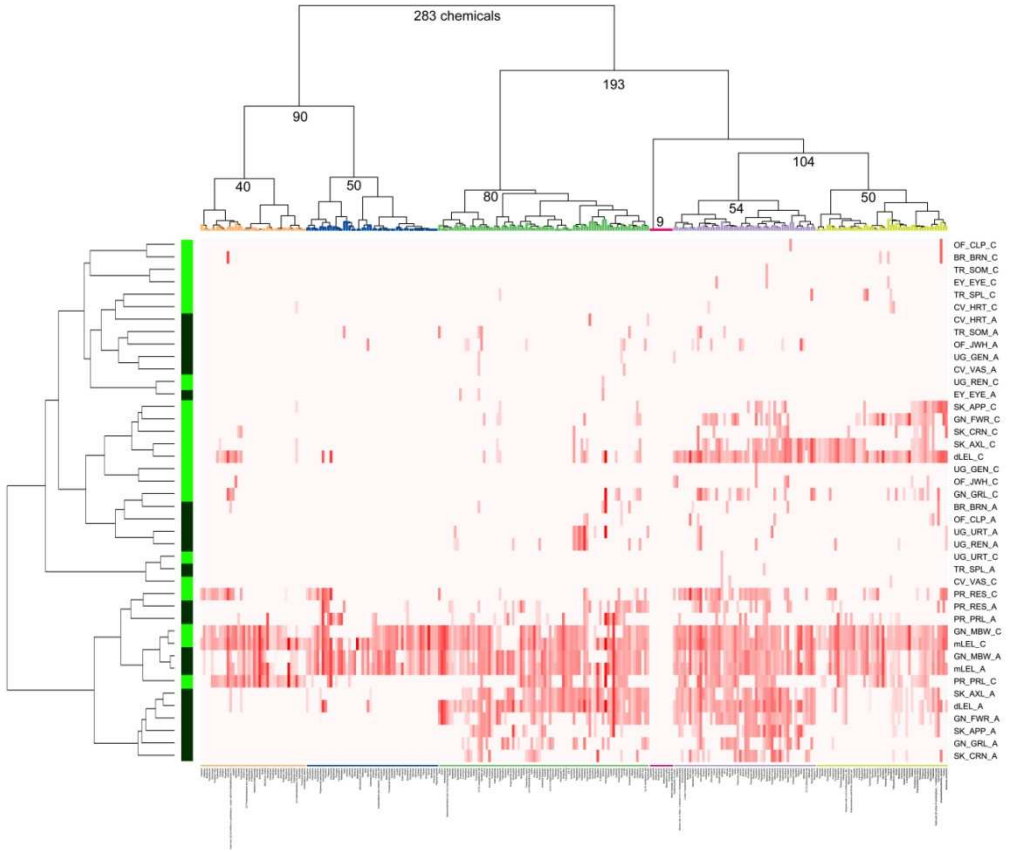


target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130



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

images from www.DevTox.org



ToxRefDB 387 chemicals, 751 prenatal studies, 988 effects annotated

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

SOURCE: Knudsen et al. (2009) Repro Tox 28: 209-19

Developmental effects (ToxRefDB):
cLEL based on mg/kg/day administered dose

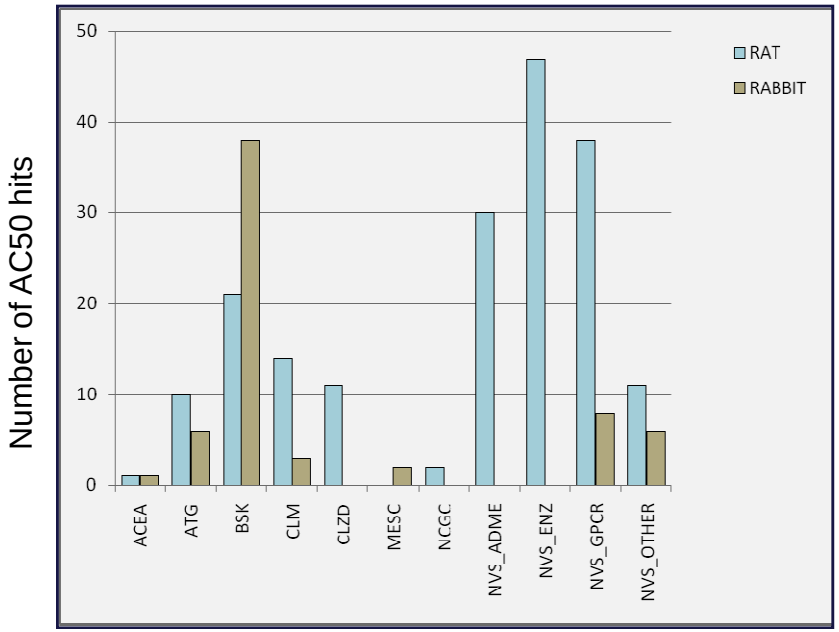
CRITICAL ENDPOINT	NUMBER of CHEMICALS		
	rabbit	***rat***	***overlap***
Developmental (global)	111	153	70
Skeletal_Axial	55	118	18
FetalWeightReduction	49	92	6
Skeletal_Appendicular	24	50	7
Skeletal_Cranial	21	41	1
Embryo-Fetal losses	33	35	5
Urogenital (renal, ureteric)	3	19	0
JawHyoid	8	14	0
CleftLipPalate	2	11	0
Neurosensory (brain and eye)	6	8	0
BodyWall (somatic)	1	6	0
Viscera (splanchnic)	9	4	0
Cardiovascular (heart, major vessels)	6	3	0

SOURCE: http://www.epa.gov/NCCT/toxrefdb/

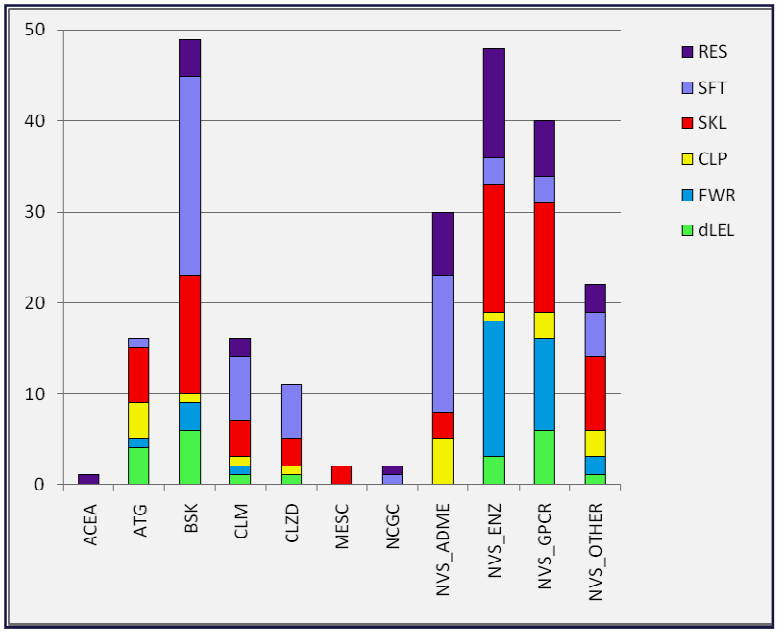
Workflow slide - placeholder

Assay-DevTox associations:
distribution by HTS assay platform

Aggregated by species



Stratified by system



894 total univariate DevTox associations from ToxMiner v16

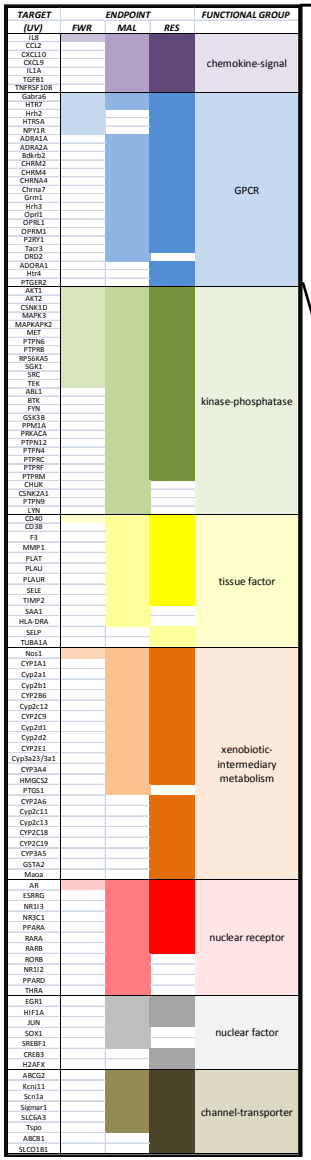
DevTox targets in ToxCast™

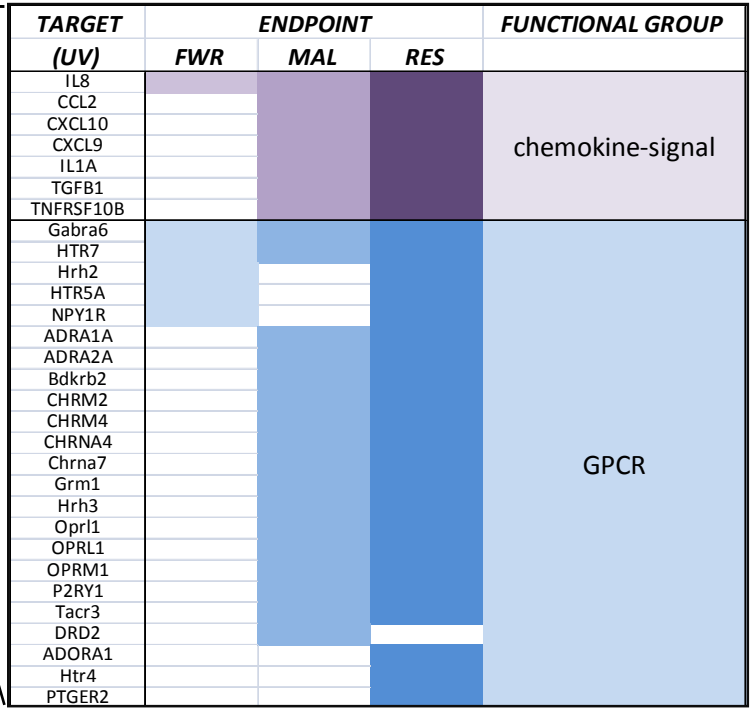
nonredundant ***assays*** (154 annotated by target gene function tested) selected by significant AC50 - cLEL correlation and mapped across the prenatal 'penetrance spectrum':

FWR fetal weight reduction
MAL abnormalities and variations
RES resorptions-fetal death

TARGETS	FWR	MAL	RES
assays	21	98	104
pathways	75	70	11

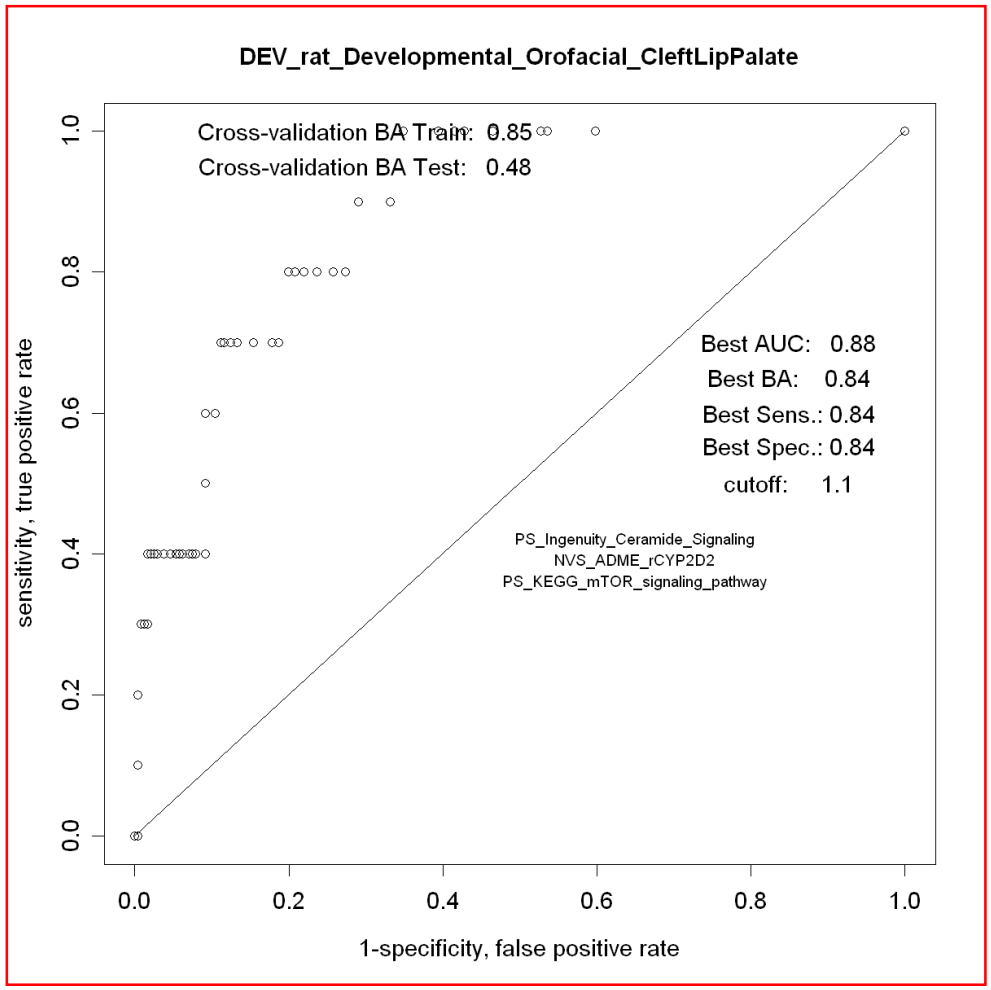
pathways inferred from 'perturbation score' (PS)

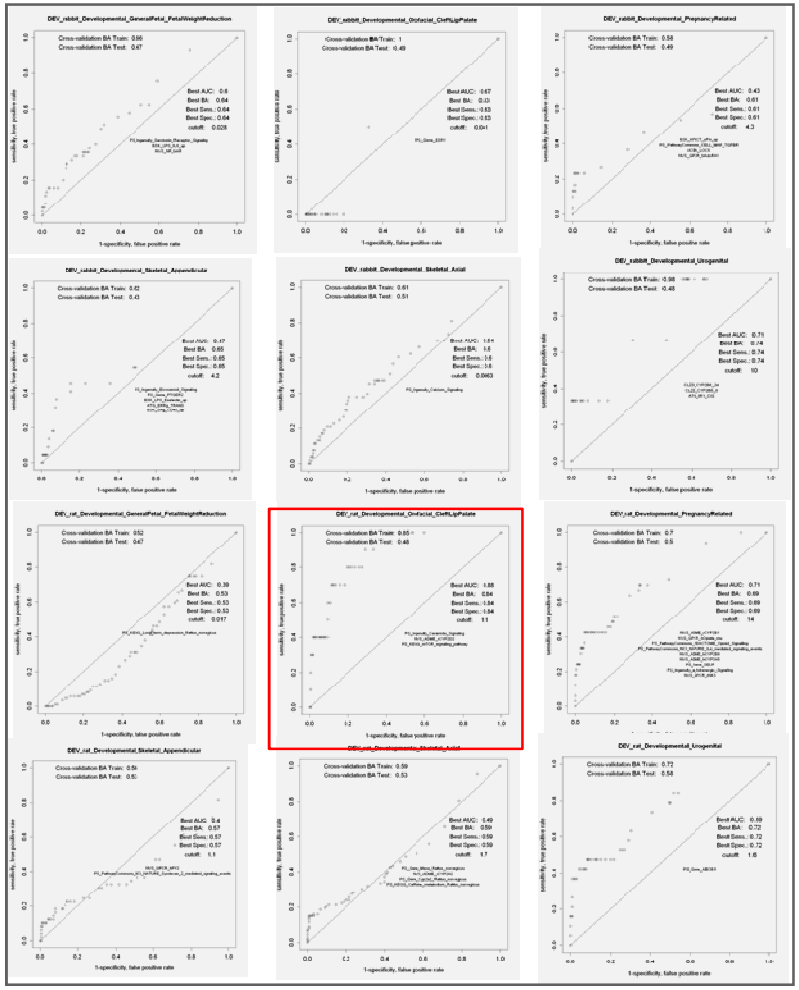




SOURCE: ToxMiner v16, NCCT

Signature detection





SOURCE: NCCT – N Kleinstreuer, built with 'linmod from R Judson [presented 03/08/2010, abstract 96]

Multivariate - placeholder

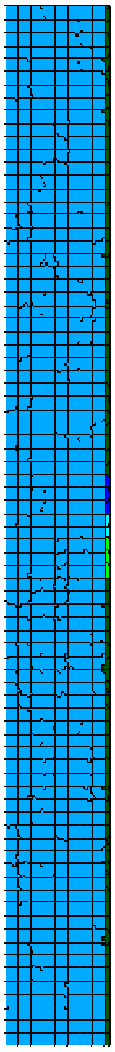
Pathways - placeholder

Predictive signatures: *challenges*

- predictive modeling of an effect is complicated by the inherent nonlinearity of biological systems
- even homogeneous cell populations *in vitro* can display complex responses to environmental chemicals
- toxicity in an intact organism results from numerous complex and inter-related events at a multi-cellular scale
- Holy Grail: *in silico* reconstruction of tissues to evaluate biological plausibility of predictive signatures

Cell networks - placeholder

Modeling Morphogenesis



cell growth & death

induced by FGFs from the AER

Polarized limb outgrowth

*Modeled in www.**CompuCell3d**.org environment*
N Poplawski (chick limb) → M Rountree (mouse limb)

Summary

- toxicity in the embryo is an expression of complex and interwoven events that follow from cellular perturbation
- ToxCast™ is a resource to compile *in vitro* signatures into computational models that are diagnostic of *in vivo* toxicity
- systems-level models that recapitulate *in vivo* biology can be used to assess the plausibility of diagnostic signatures
- multicellular 'virtual tissues' can help bridge the gap between *in vitro* profiling and *in vivo* response

Research Network

Virtual Embryo (NCCT)
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Jim Glazier (Indiana U)

EU interactions
Virtual Physiome
ChemScreen (2010)

Virtual Liver (NCCT)
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Woody Setzer
John Jack

ToxCast™ (NCCT)
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Keith Houck
Matt Martin
David Reif
Ann Richard
Jim Rabinowitz
Holly Mortensen

http://www.epa.gov/ncct/v-Embryo/