EPA
United States
Environmental Protection
Agency

Species Considerations for Predictive and Mechanistic Modeling

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National Center for Computational Toxicology

This work was reviewed by EPA and approved for presentation but does not necessarily reflect official Agency policy.

7th Workshop on Terminology in Developmental Toxicology
Berlin, Germany
May 4-6, 2011

Office of Research and Development

Greetings from NCCT!

ToxCast (NCCT)

Keith Houck
Bob Kavlock
David Dix
Richard Judson
Matt Martin
David Reif
Ann Richard
Jim Rabinowitz
Shiobhan Sneed
(and all those pictured)

Virtual Embryo (NCCT)

Tom Knudsen
Amar Singh (LHM)
Michael Rountree (SSC)
Richard Spencer (LHM)
Rob DeWoskin (NCEA)
Nikal Kleinstreuer
Nisha Sipes
Kelly Chandler (NHEERL)

Virtual Liver (NCCT)

Imran Shah
John Wambaugh
Woody Setzer
John Jack

Scope of the Problem

PEDIATRICS

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

Policy Statement Chemical-Management Policy: Prioritizing Children's Health
COUNCIL ON ENVIRONMENTAL HEALTH
Pediatrics published online Apr 25, 2011;
DOI: 10.1542/peds.2011-0523

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

FROM THE AMERICAN ACADEMY OF PEDIATRICS

Organizational Principles to Guide and Define the Child Health Care System and/or Improve the Health of all Children

Policy Statement—Chemical-Management Policy: Prioritizing Children's Health

COUNCIL ON ENVIRONMENTAL HEALTH

KEY WORD
environmental health

ABBREVIATIONS
TSCA—Toxic Substances Control Act
EPA—Environmental Protection Agency

abstract FREE

The American Academy of Pediatrics recommends that chemical-management policy in the United States be revised to protect children and pregnant women and to better protect other populations. The Toxic Substance Control Act (TSCA) was passed in 1976. It is widely recognized to have been ineffective in protecting children, pregnant women, and the general population from hazardous chemicals in the marketplace. It does not take into account the special vulnerabilities of children in attempting to protect the population from chemical hazards. Its processes are so cumbersome that in its more than 30 years of existence, the TSCA has been used to regulate only 5 chemicals or chemical classes of the tens of thousands of chemicals that are in commerce. Under the TSCA, chemical companies have no responsibility to perform premarket testing or postmarket follow-up of the products that they produce; in fact, the TSCA contains disincentives for the companies to produce such data. Voluntary programs have been inadequate in resolving problems. Therefore, chemical-management policy needs to be rewritten in the United States. Manufacturers must be responsible for developing information about chemicals before marketing. The US Environmental Protection Agency must have the authority to demand additional safety data about a chemical and to limit or stop the marketing of a chemical when there is a high degree of suspicion that the chemical might be harmful to children, pregnant women, or other populations. *Pediatrics* 2011;127:983–990

INTRODUCTION
Over the past several decades, tens of thousands of chemicals have

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SOURCE: Pediatrics, April 25, 2011

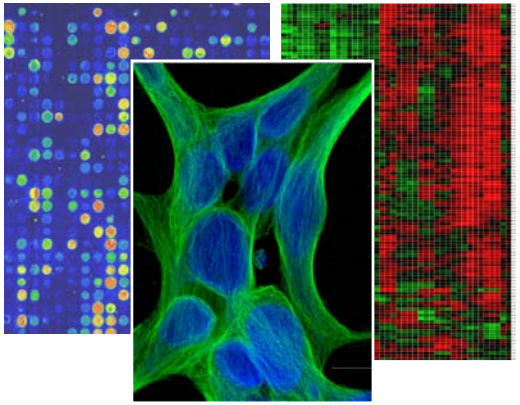
Computational Toxicology

- critical data lacking on the potential toxicity of tens of thousands of chemicals in commerce and the environment
- **20th Century** paradigm: based mainly on animal testing is too slow, expensive, and uses a lot of critters
- **21st Century** paradigm: prioritize chemicals based on chemical structure, exposure metrics, *in vitro* profiling
- ToxCast™: EPA's part of US federal Tox21 consortium (EPA, NCGC, NTP, FDA) for high-throughput screening (HTS)

HTS paradigm

high-throughput screening
fast screening of chemical libraries
SCALE

vast data on many chemicals

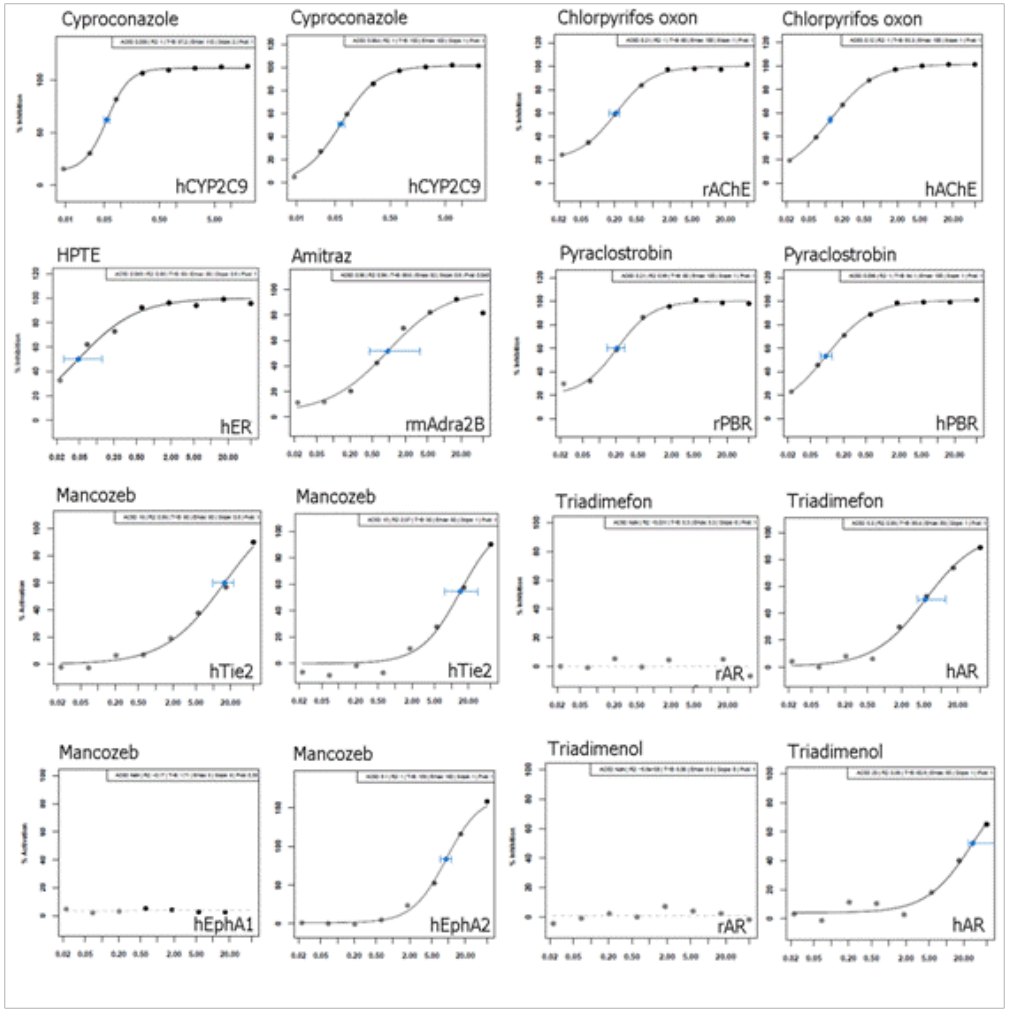


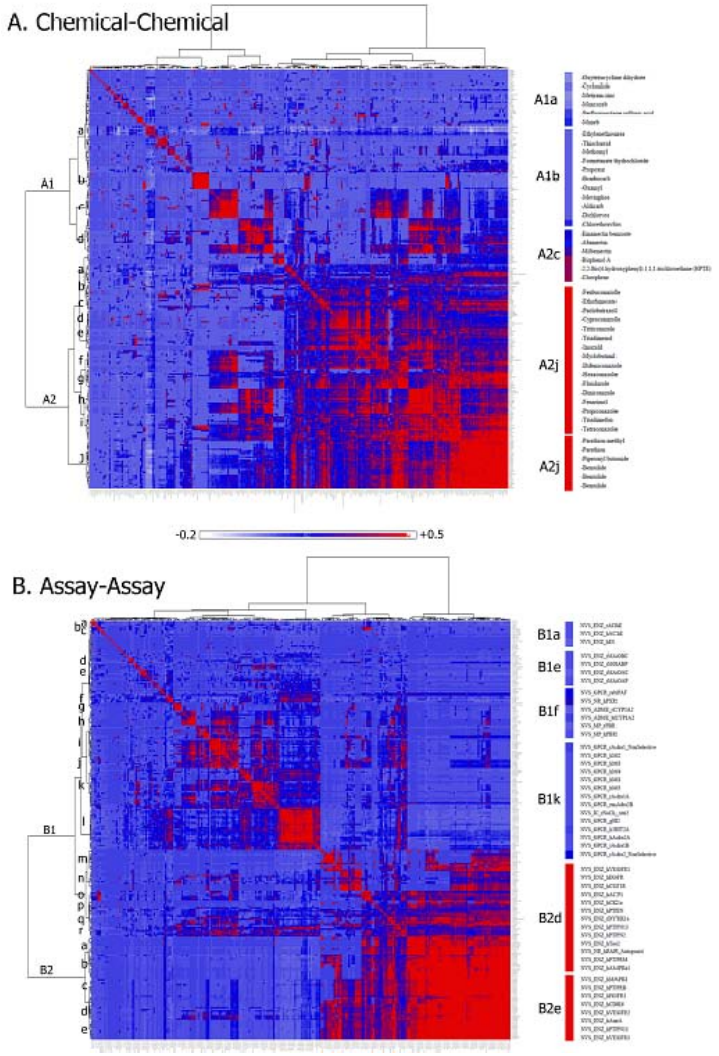
high-content screening
detailed imaging, arrays
PARALLELISM

challenge interpreting all of the data
- pathway profiling -

text-mining, data-mining	***bioinformatics, pathways***	***systems modeling***

EXAMPLE: biochemical HTS (292 assays)





SOURCE: Knudsen et al. (2011) Toxicology (in press)

Predictive toxicology

- *in vitro* technologies for HTS are providing vast amounts of data for a ‘human toxicology project’ akin to the HGP
- need to structure *in vivo* data, where available, in a computable format to derive predictive signatures
- models would link hierarchical relationships from chemical classes to adverse outcomes …
- … and balance sensitivity/specificity to predict developmental toxicity solely from *in vitro* profiling data

ToxCastDB

United States Environmental Protection Agency

chemical library

~300 chemicals

in vitro (ToxCast)

- Cellular impedance
- Complex cell interactions
- Cellular toxicity
- Reporter gene
- Gene regulation
- Nuclear receptor reporter
- Enzyme inhibition/receptor binding

~500 assays

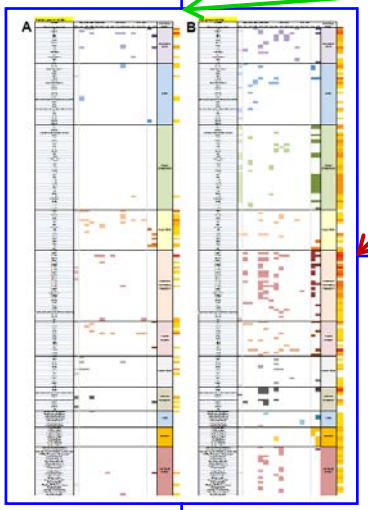
in vivo (ToxRefDB)

- Chronic *in vivo*
- Multigeneration *in vivo*
- Developmental *in vivo*

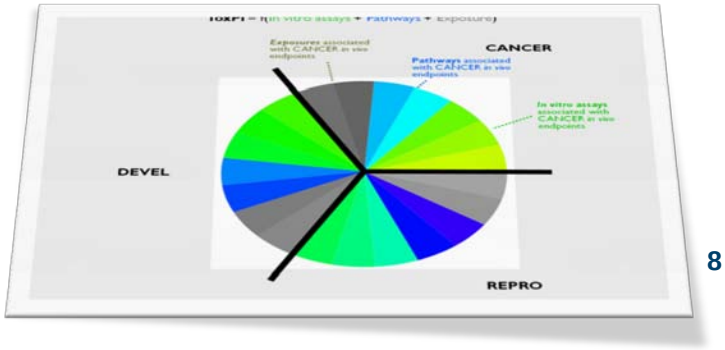
~76 defects

chemicals that hit these *in vitro* targets

add risk for these *in vivo* defects



inferred pathways and processes



ToxRefDB

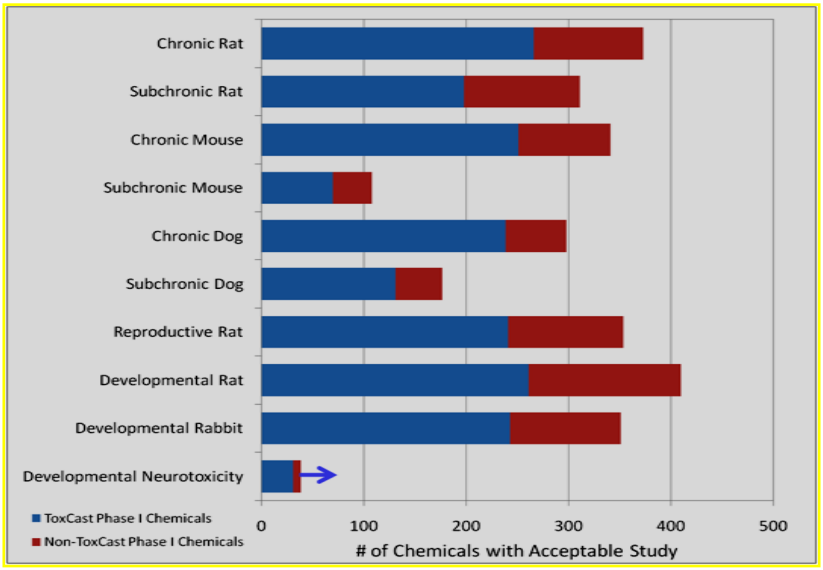
- computable database containing $2B data from registrant-submitted toxicology study results (DERs)
- source data from 3184 studies for 572 chemicals entered with controlled vocabulary and harmonized terminology
- database and web interface open to the public in 2010 **http://actor.epa.gov/toxrefdb**

CHRONIC/CANCER
Martin et al. (2009) Environ Hlth Persp

MULTIGENERATIONAL REPRODUCTIVE
Martin et al. (2009) Toxicol Sci

PRENATAL DEVELOPMENTAL
Knudsen et al. (2009) Reprod Toxicol

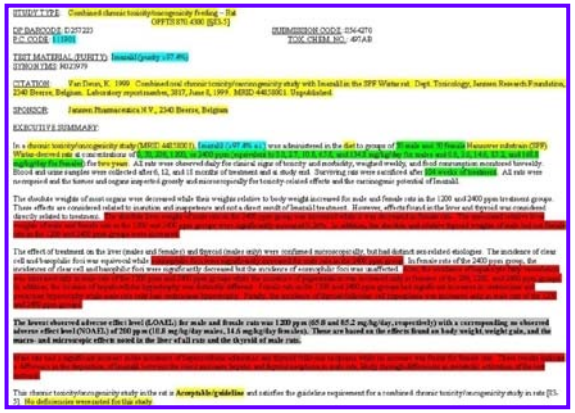
DEVELOPMENTAL NEUROTOXICITY
Sneed et al. (in preparation)



ToxRefDB_prenatal

- **prenatal studies:** 751 studies (870.3700) mostly rat and rabbit, testing 387 chemicals (283 in both species)
- **annotation:** 988 terms for maternal and fetal effects based on an enhanced *DevTox.org* thesaurus
- **lowest effect levels:** mg/kg/day administered dose for maternal (mLEL) and developmental (dLEL) endpoints
- **developmental effects:** further categorized by LEL (cLEL) for 18 systems in a computable format

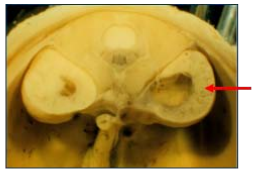
Data Evaluation Record (DER)



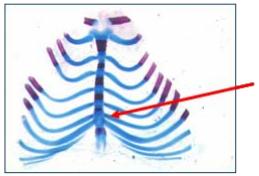
Fields/Data Elements Utilizing Standardized Vocabulary

- Chemical
- Study Quality
- Study Type
- Method & Route of Admin
- Species
- Strain
- Generation
- Dosing Period
- Gender
- Dosing Duration
- Effect Type
- Effect Target
- Effect Description
- Target Cell Type
- Target Region

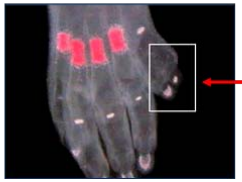
In vivo profiling



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

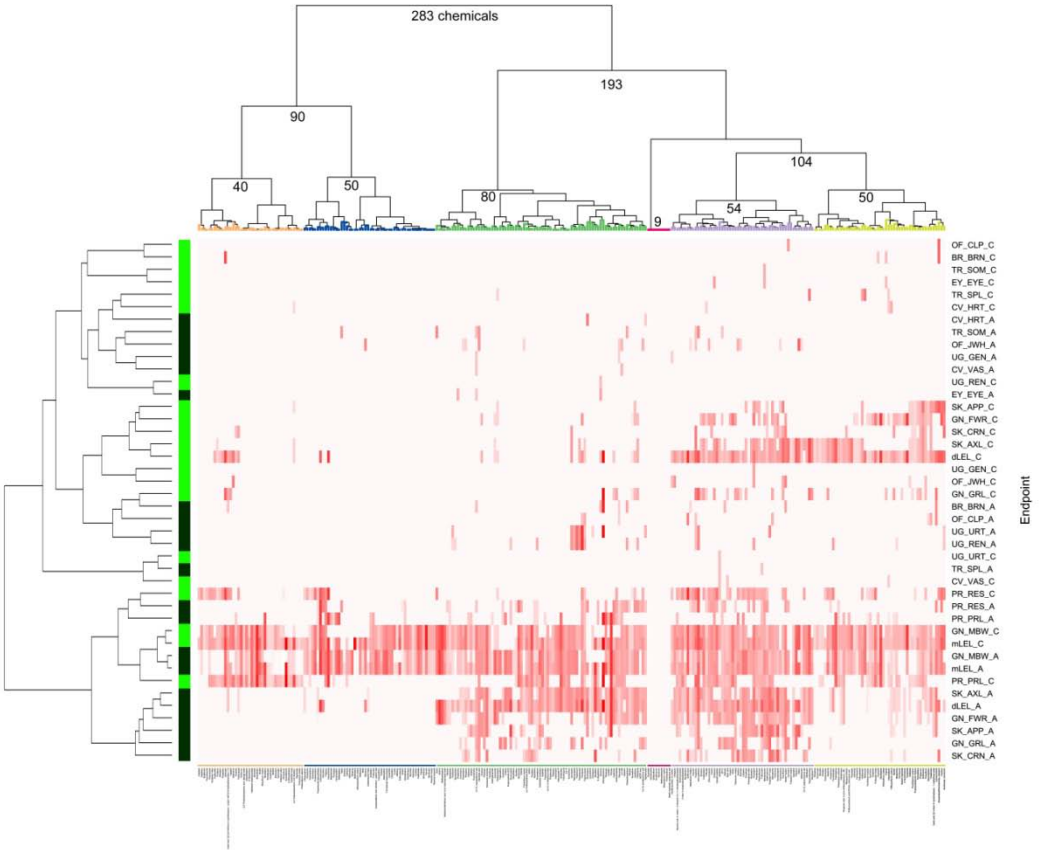


target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130



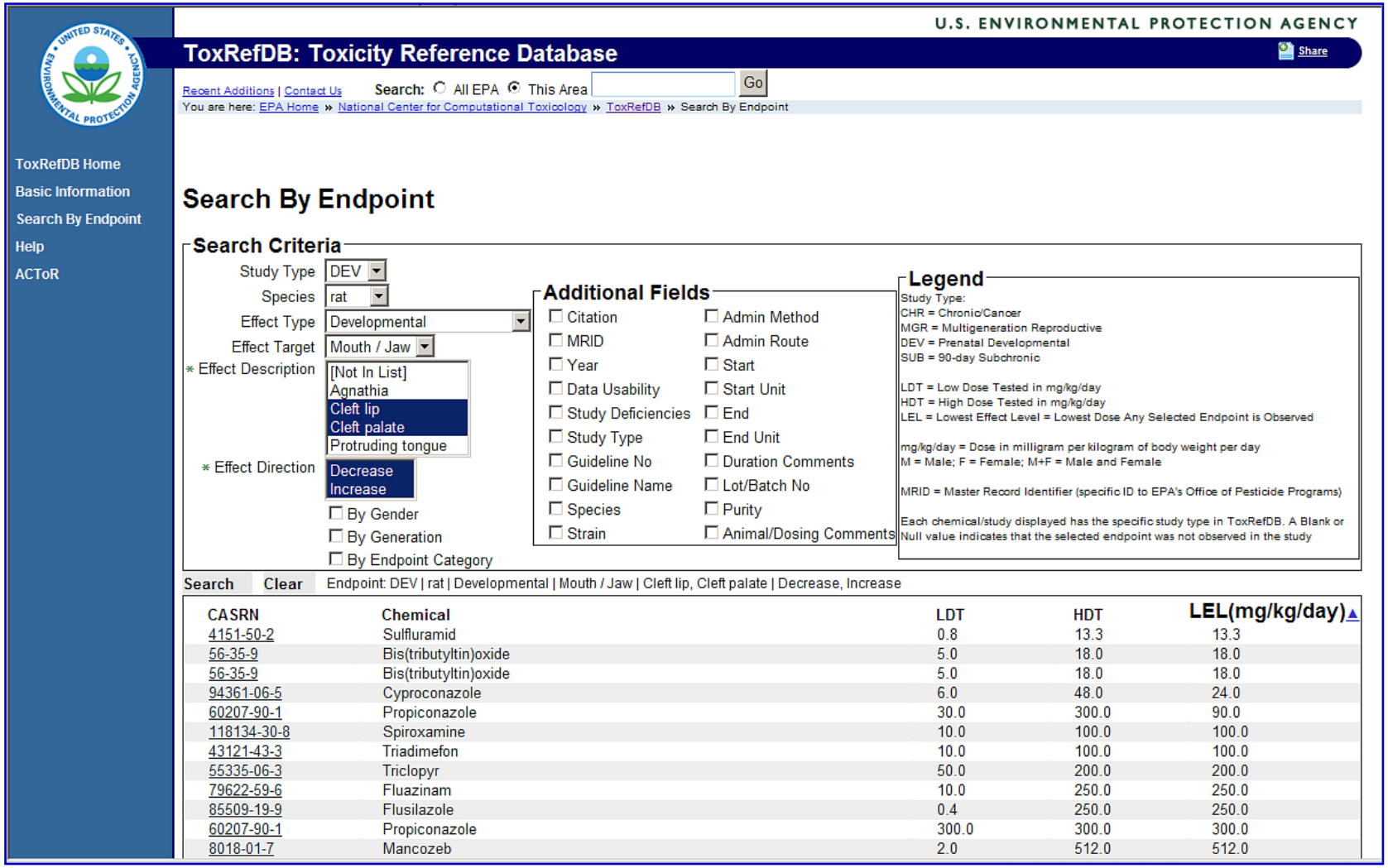
target: hindpaw
description: polydactyly (digit I)
code: SK_APP_2.1051.5234

images from www.DevTox.org



SOURCE: Knudsen et al. (2009) Reprod Toxicol 28: 209-219

Query: cleft lip and cleft palate



U.S. ENVIRONMENTAL PROTECTION AGENCY

ToxRefDB: Toxicity Reference Database

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Basic Information
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Help
ACToR

Search By Endpoint

Search Criteria

Study Type: DEV
Species: rat
Effect Type: Developmental
Effect Target: Mouth / Jaw
* Effect Description: [Not In List], Agnathia, Cleft lip, Cleft palate, Protruding tongue
* Effect Direction: Decrease, Increase

☐ By Gender
☐ By Generation
☐ By Endpoint Category

Additional Fields

☐ Citation ☐ Admin Method
☐ MRID ☐ Admin Route
☐ Year ☐ Start
☐ Data Usability ☐ Start Unit
☐ Study Deficiencies ☐ End
☐ Study Type ☐ End Unit
☐ Guideline No ☐ Duration Comments
☐ Guideline Name ☐ Lot/Batch No
☐ Species ☐ Purity
☐ Strain ☐ Animal/Dosing Comments

Legend

Study Type:
CHR = Chronic/Cancer
MGR = Multigeneration Reproductive
DEV = Prenatal Developmental
SUB = 90-day Subchronic

LDT = Low Dose Tested in mg/kg/day
HDT = High Dose Tested in mg/kg/day
LEL = Lowest Effect Level = Lowest Dose Any Selected Endpoint is Observed

mg/kg/day = Dose in milligram per kilogram of body weight per day
M = Male; F = Female; M+F = Male and Female

MRID = Master Record Identifier (specific ID to EPA's Office of Pesticide Programs)

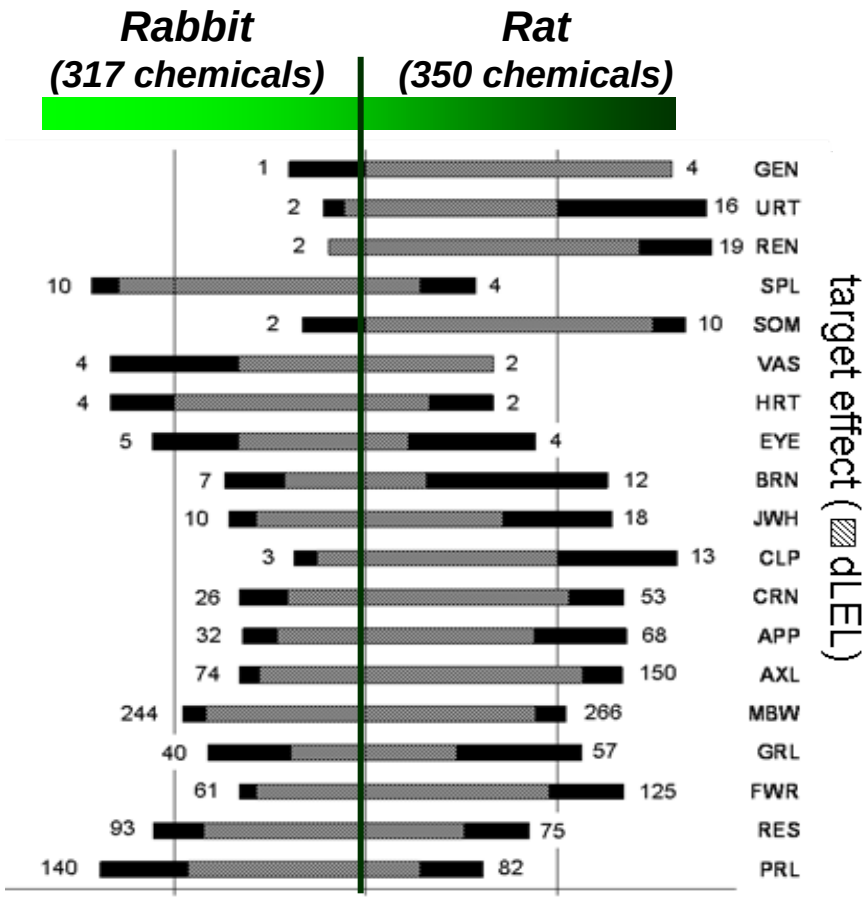
Each chemical/study displayed has the specific study type in ToxRefDB. A Blank or Null value indicates that the selected endpoint was not observed in the study

Search Clear Endpoint: DEV | rat | Developmental | Mouth / Jaw | Cleft lip, Cleft palate | Decrease, Increase

CASRN	Chemical	LDT	HDT	LEL(mg/kg/day)
4151-50-2	Sulfluramid	0.8	13.3	13.3
56-35-9	Bis(tributyltin)oxide	5.0	18.0	18.0
56-35-9	Bis(tributyltin)oxide	5.0	18.0	18.0
94361-06-5	Cyproconazole	6.0	48.0	24.0
60207-90-1	Propiconazole	30.0	300.0	90.0
118134-30-8	Spiroxamine	10.0	100.0	100.0
43121-43-3	Triadimefon	10.0	100.0	100.0
55335-06-3	Triclopyr	50.0	200.0	200.0
79622-59-6	Fluazinam	10.0	250.0	250.0
85509-19-9	Flusilazole	0.4	250.0	250.0
60207-90-1	Propiconazole	300.0	300.0	300.0
8018-01-7	Mancozeb	2.0	512.0	512.0

ToxRefDB: species dimorphism

- 53 of 283 chems specific (no mLEL) or sensitive (dLEL < mLEL) for DevTox
- rat was more sensitive based on mg/kg/day administered dose
- primary expressions of DevTox:
  - FWR & skeletal defects (**rat** > rabbit)
  - pregnancy/fetal loss (**rabbit** > rat)
  - urinary tract defects (**rat** > rabbit)
  - CNS defects (**rabbit** > rat)



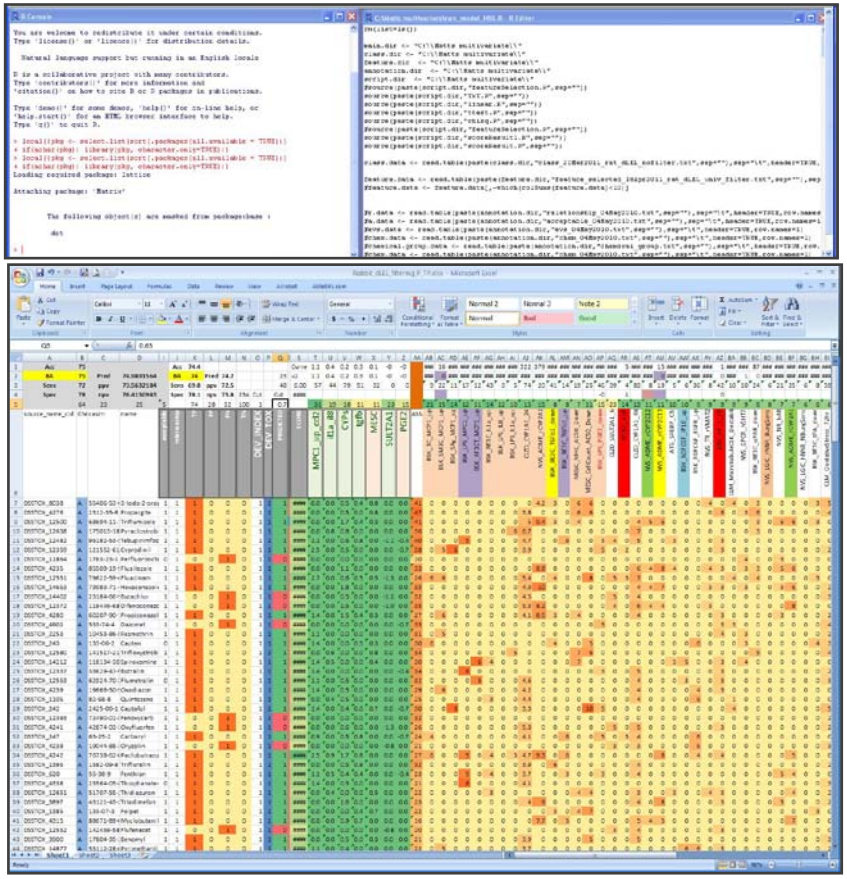
SOURCE: Knudsen et al. (2009) Reprod Toxicol 28: 209-219

Species Considerations

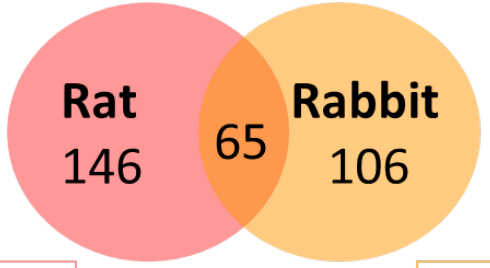
- relative sensitivity and specificity of maternal and fetal parameters in comparing responses between species

- species differences could arise from multiple factors:
  - genetic background (species, strain, individual)
  - variation in embryology (timing, pathways)
  - mode of placentation
  - differences in maternal behavior or ADME
  - dosing and evaluation schema

Predictive DevTox model



Developmental Toxicants:



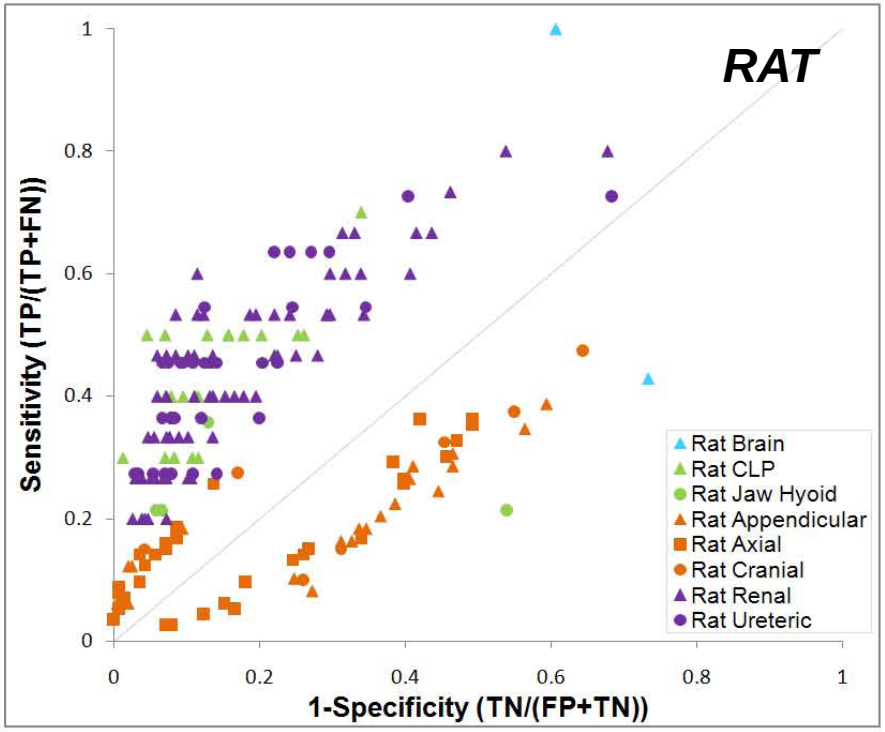
Train BA: 0.71 ± 0.01
Test BA: 0.70 ± 0.09

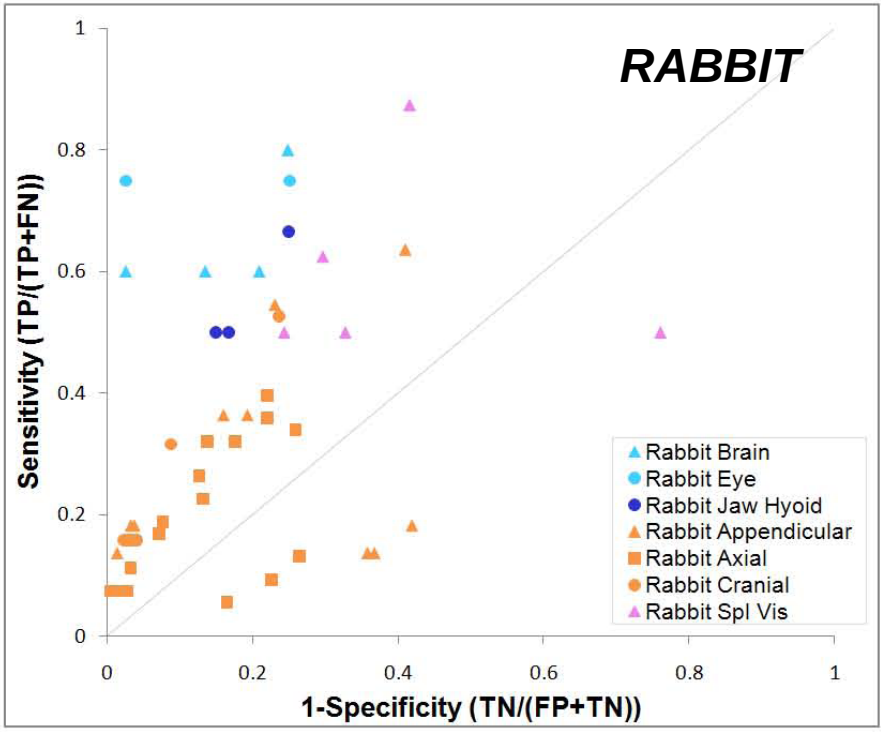
Train BA: 0.75 ± 0.02
Test BA: 0.71 ± 0.03

659 assay feature space
271 chemical space
→ 187 dLELs, 60 negatives

SOURCE: Sipes et al. 2011 (submitted)

Sensitivity analysis

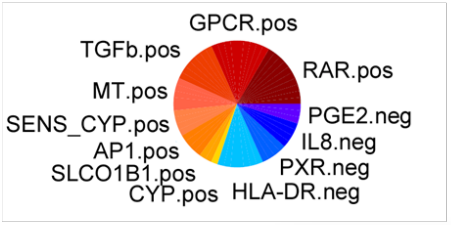


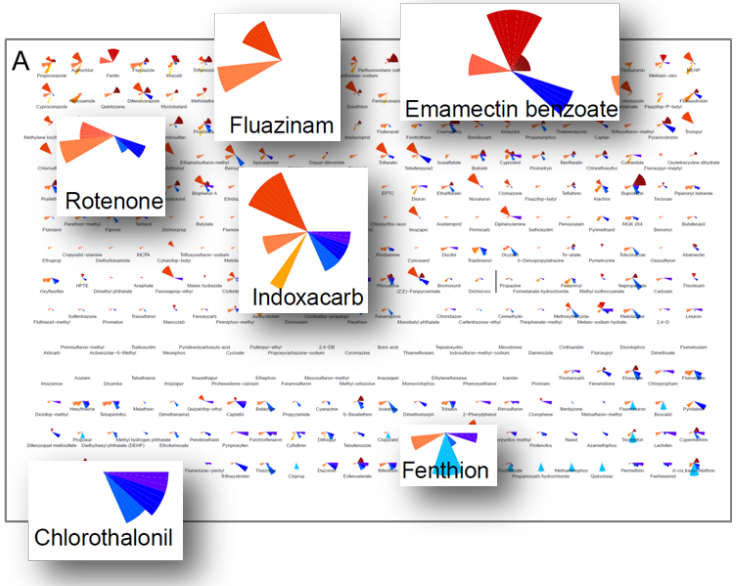


SOURCE: Sipes et al. 2011 (submitted)

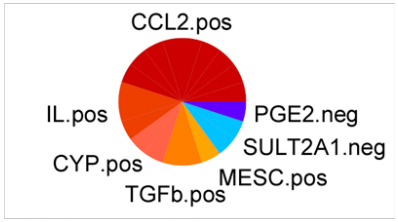
ToxPi visualization

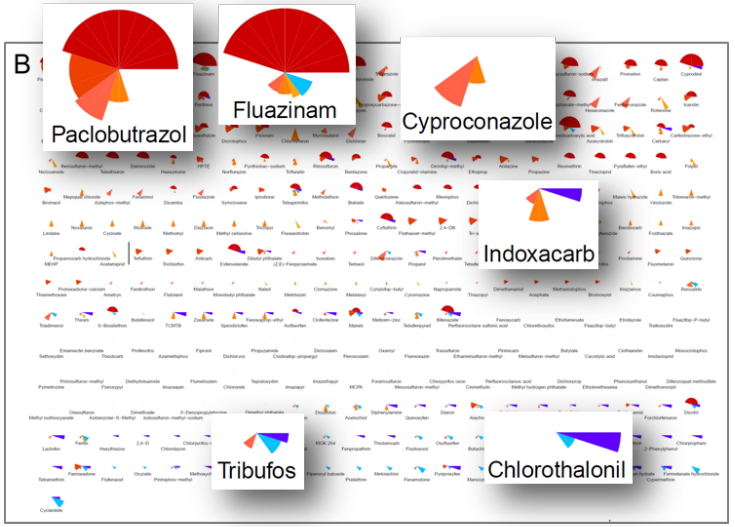
Rat





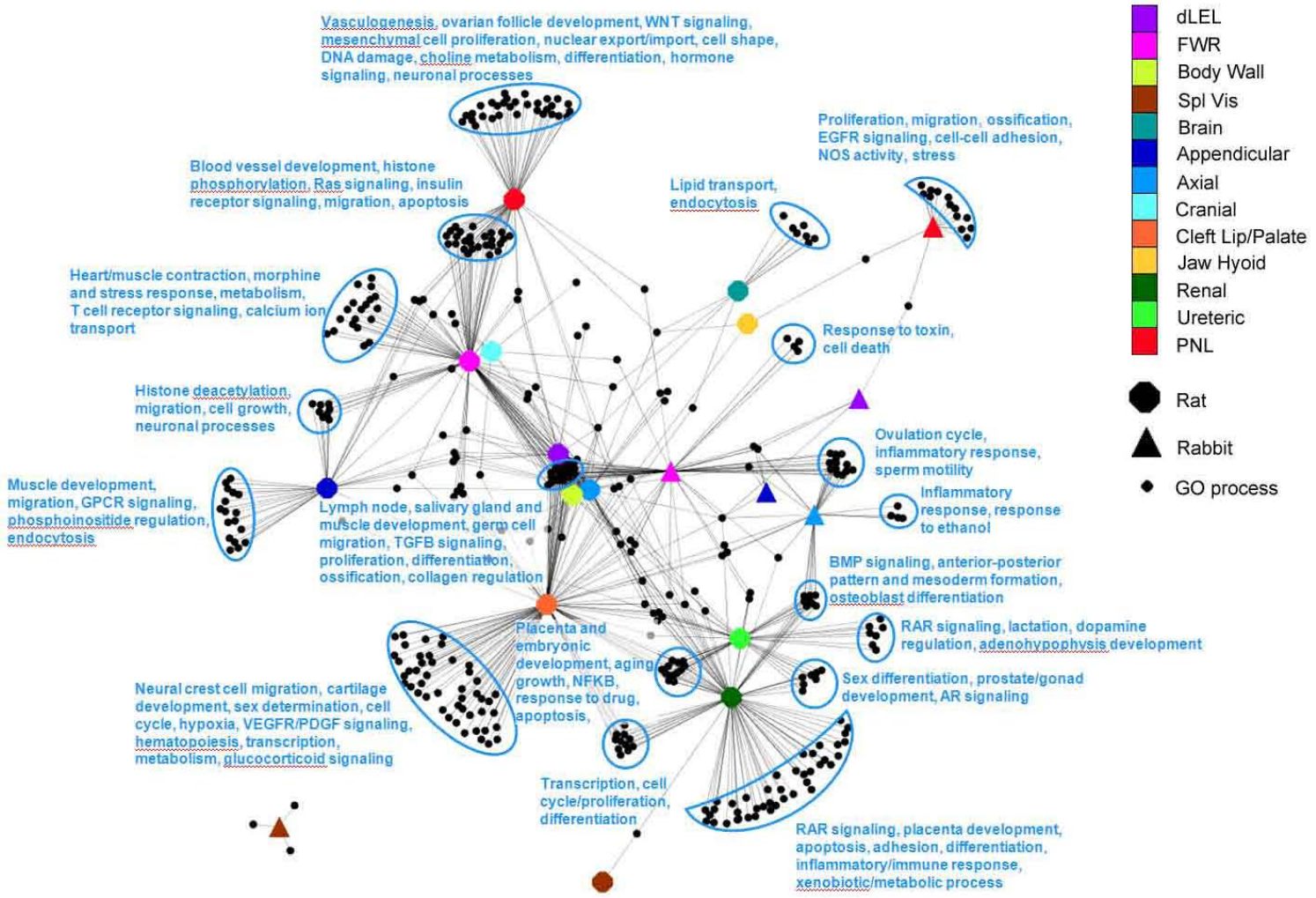
Rabbit





SOURCE: Sipes et al. 2011 (submitted)

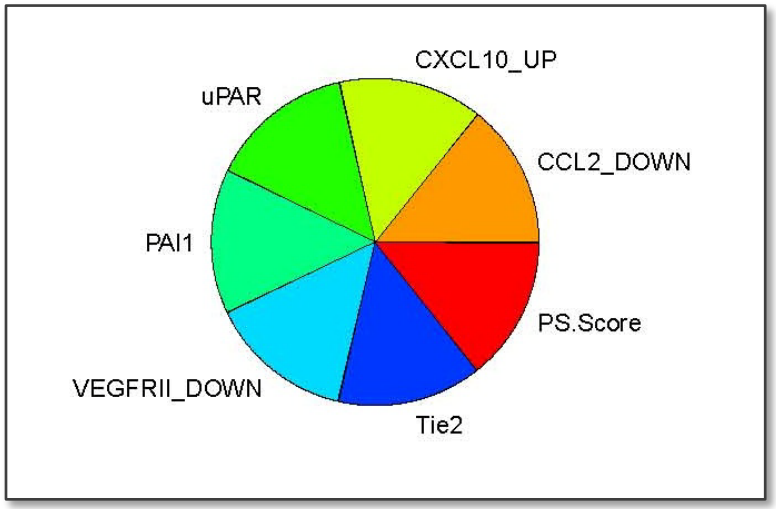
GO Biological Process



SOURCE: Sipes et al. 2011 (submitted)

Blood vessel development

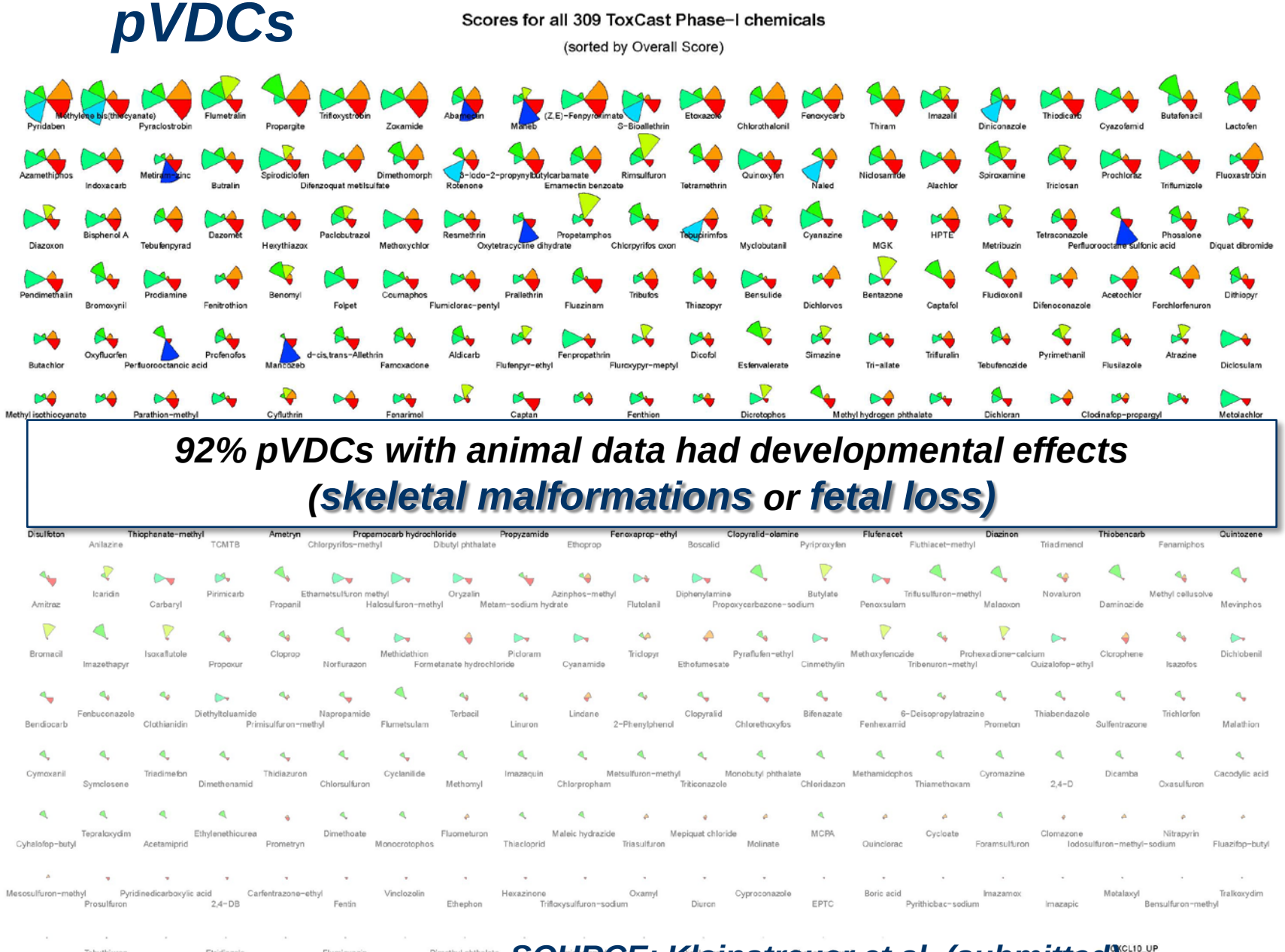
VT-KB: ***~80 distinct ToxCast assay targets map to 3 key systems in vascular development***



ToxPi for angiogenesis

SOURCE: Kleinstreuer et al. (submitted)

pVDCs

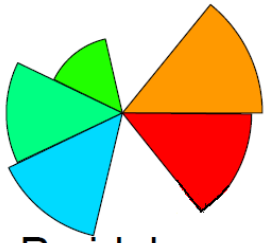


92% pVDCs with animal data had developmental effects (skeletal malformations or fetal loss)

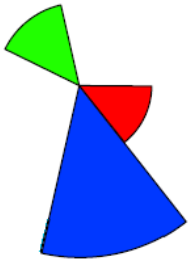
SOURCE: Kleinstreuer et al. (submitted)

Vascular Signaling Network

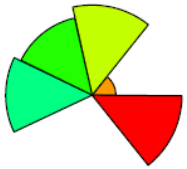
United States Environmental Protection Agency



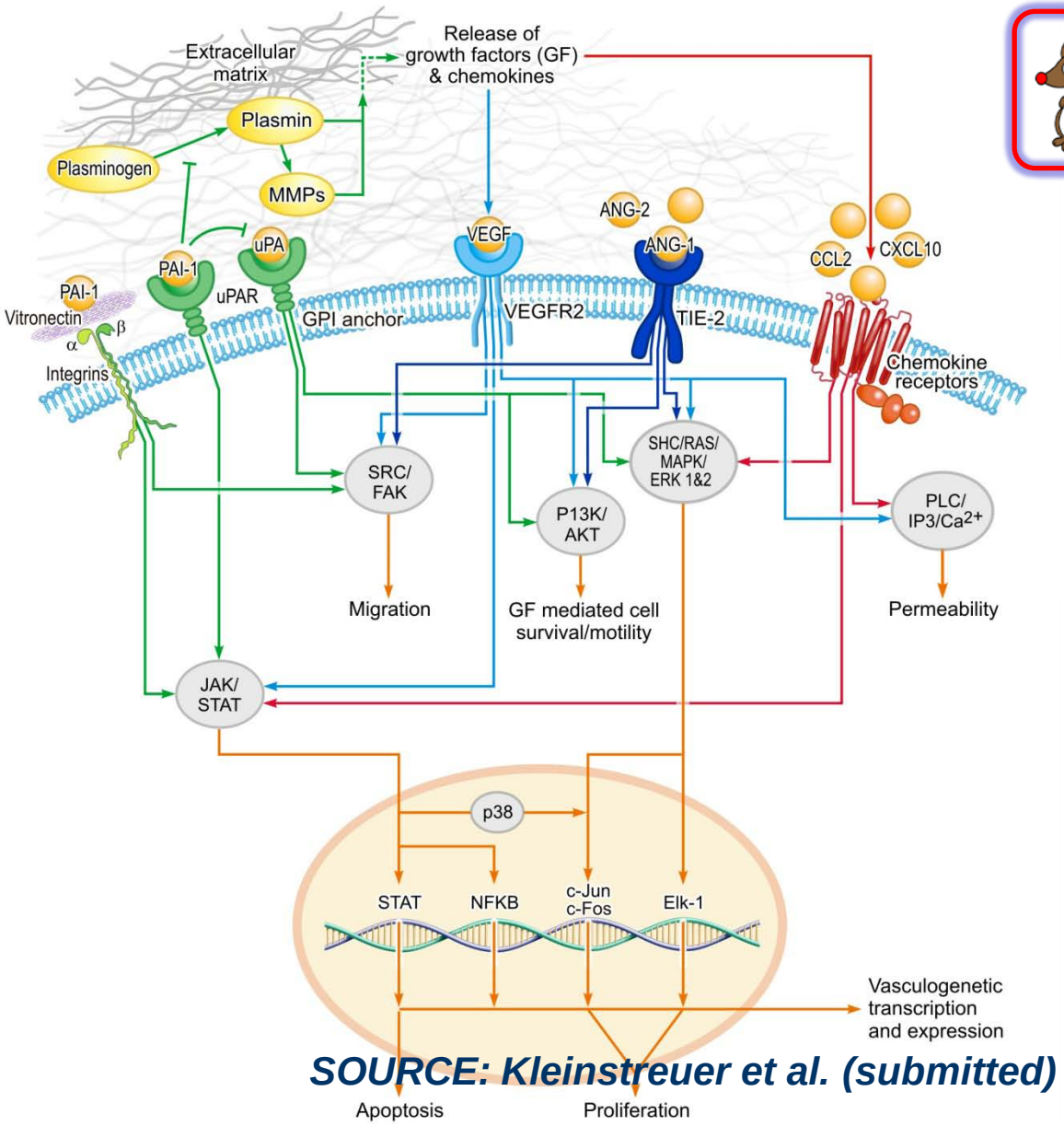
Pyridaben



PFOS

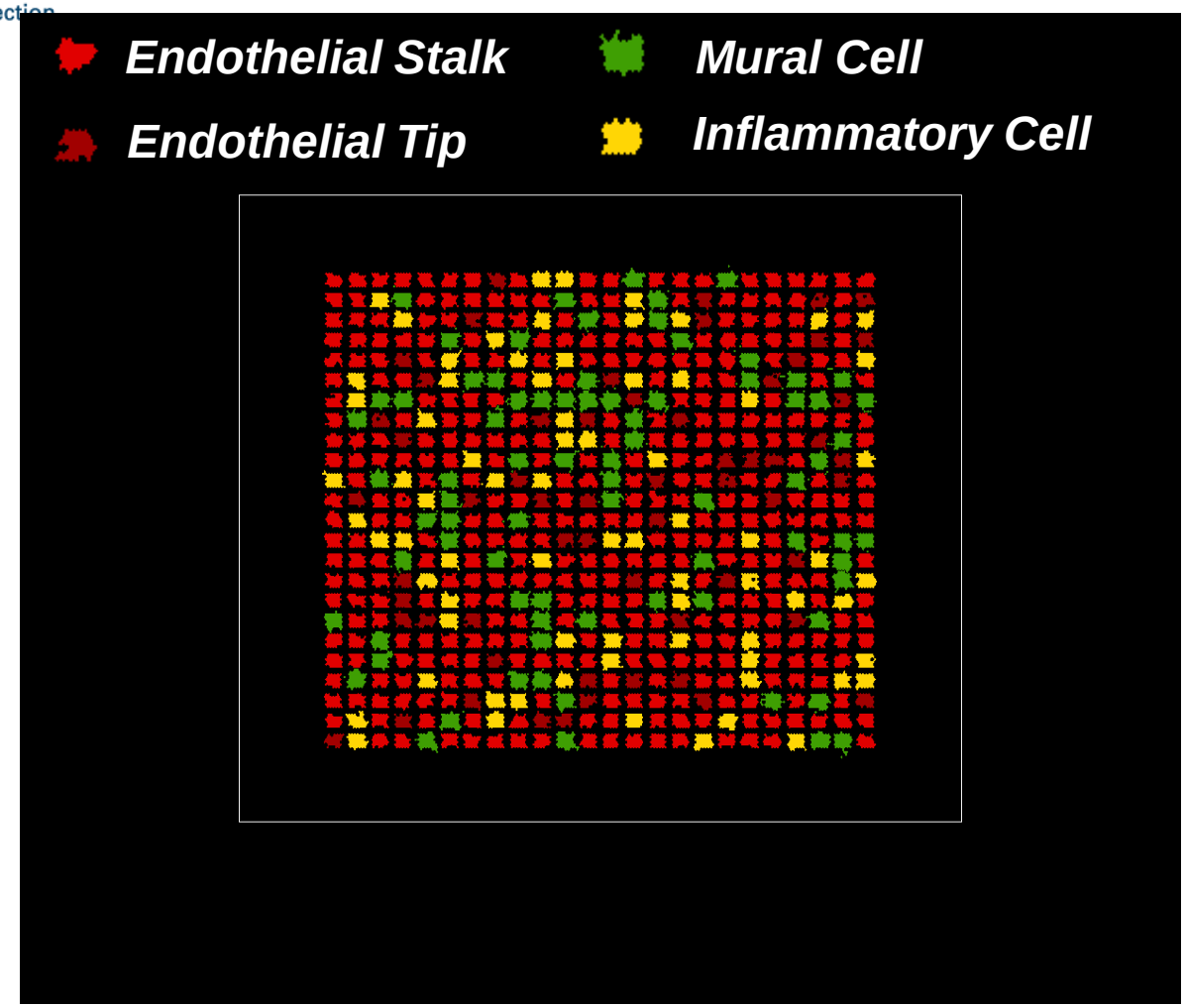


Triclosan



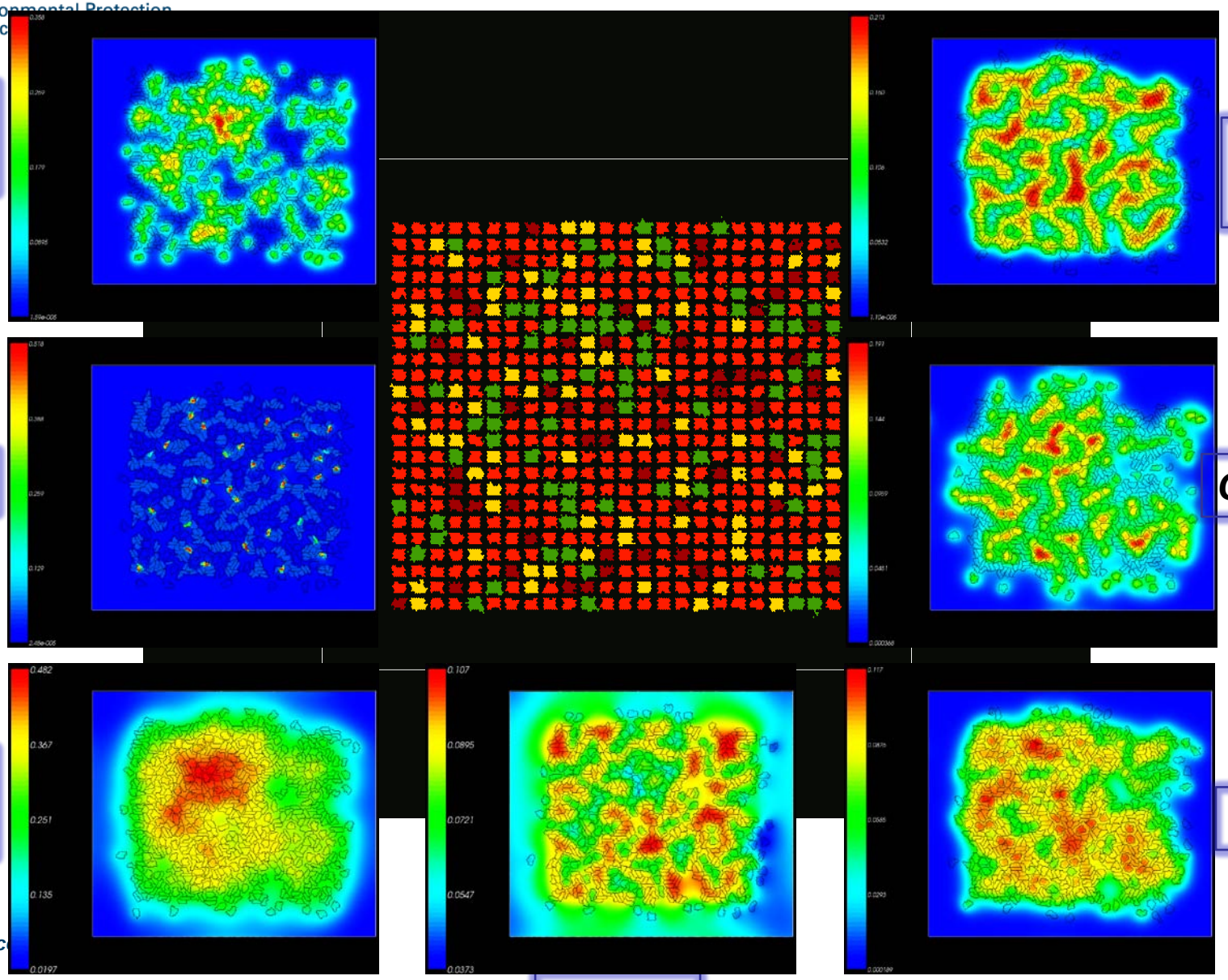
SOURCE: Kleinstreuer et al. (submitted)

Vascular Plexus



SOURCE: Kleinstreuer et al. (in preparation)

Vascular Plexus



Reconstructing toxicity

ToxCast Target	Cell behavior	Model Parameters
Tie2	Ang1 receptor expressed by ECs, controls MC adhesion and proliferation	Tie2.secretion (ECs), chemotactic strength (MC)
CXCL10	competitive binding with VEGF on heparan glycan site of ECs,t	CXCL10.secretion (IC, MC)
uPAR	EC receptor controlling interaction and movement along extra-cellular matrix	Motility (ECs,t)
Endothelial Proliferation	Increase in EC volume and mitosis in response to growth factors	ECs.targetVolume, mitotic threshold
VCAM1	Cellular adhesion molecule influencing MC, Ecs adherence	JMC,ECs
Flt1 (VEGFR1)	Decoy receptor expressed by ECs, increased expression adjacent to ECt, sequesters VEGF	Flt1.secretion (ECs), VEGF.fieldcoupling
PBMC Cytotox	Increase in apoptosis rate of IC	IC.apoptosisrate
CCL2	Growth factor and chemoattractant for ECs,t, MC	CCL2.secretion, chemotaxis (IC, MC, ECs,t)
Mural Proliferation	Increase in MC volume and mitosis in response to growth factors	MC.targetVolume, mitotic threshold
KDR (VEGFR2)	Major signalling receptor for VEGF: controls cell growth, chemotaxis, secretion, tip cell expression	ECs.targetVolume, VEGFsol.secretion (ECs), chemotactic strength (ECt), ECt.celltype, JECt,ECs

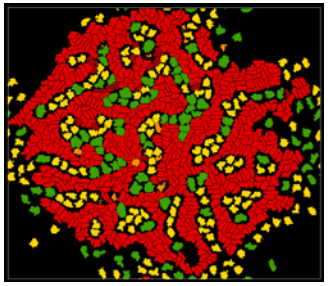
In silico predictions

endothelial connectivity (plexus), in-degree (branching), vessel uniformity (width), sprouting

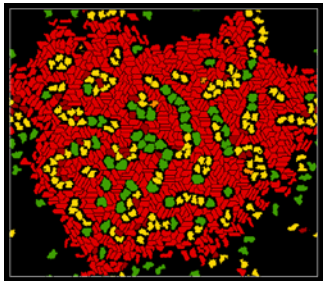
weakened mural adhesion to nascent vessels; alters endothelial growth and stimulates spreading behavior

endothelial hyperplasia with decreased cell migration and polarization; leads to thicker vascular cords

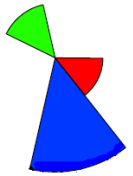
little to no vessel formation

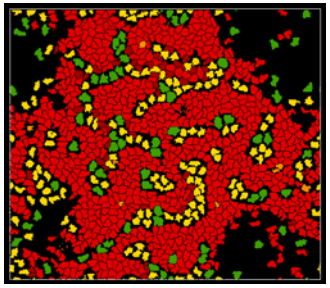


control

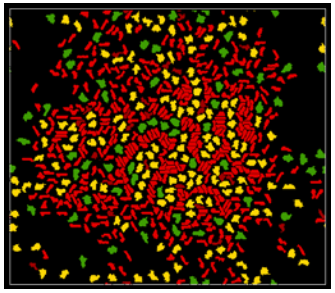


PFOS





Triclosan



Pyridaben

ToxRefDB Predictive modeling

ATTRIBUTES

- searchable public database (www.epa.gov/ncct/toxrefdb/)
- DERs are reasonably well understood studies
- puts >$2B worth of legacy data into a computable form
- *in vivo* database anchoring HTS *in vitro* assays
- enables comparison of endpoints between species

LIMITATIONS

- endpoints aggregated as independent features
- data largely qualitative (mLELs, dLELs, cLELs)
- not all ToxCast chemicals represented in ToxRefDB
- not all ToxRefDB chemicals represented in ToxCast
- study design differences for species consideration