

#### **Species Considerations for Predictive and Mechanistic Modeling**

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

Office of Research and Development

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



Greetings from NCCT!



#### COMPUTATIONAL TOXICOLOGY TOXCast (NCCT)

Keith HouckVirBob KavlockTorDavid DixAmRichard JudsonMidMatt MartinRichardDavid ReifRoiAnn RichardNikJim RabinowitzNisShiobhan SneedKel(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

American Academy of Pediatrics

#### 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

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 COUNCIL ON ENVIRONMENTAL HEALTH KEY WORD environmental health ABBREVIATIONS TSCA—Toxic Substances Control Act EPA—Environmental Protection Agency

The American Academy of Pediatrics recommends that chemicalmanagement 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.

#### abstract

The American Academy of Pediatrics recommends that chemicalmanagement 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

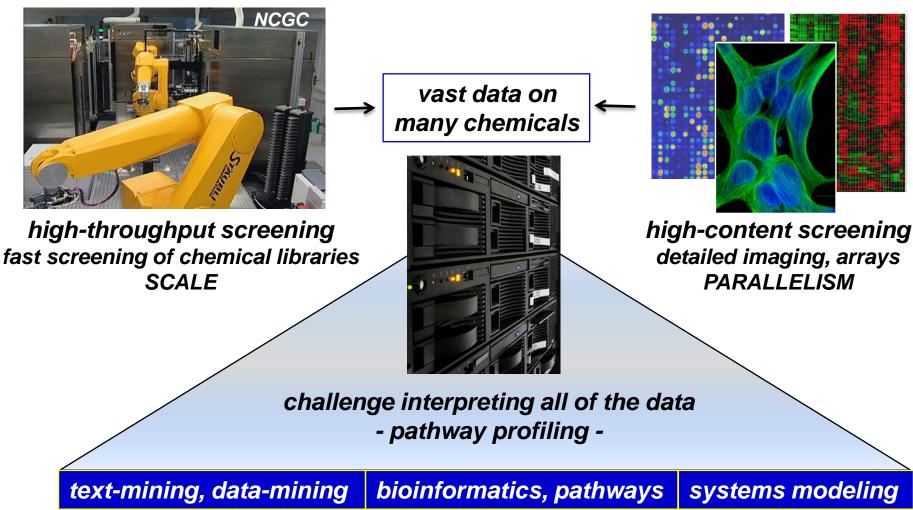


# **Computational Toxicology**

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

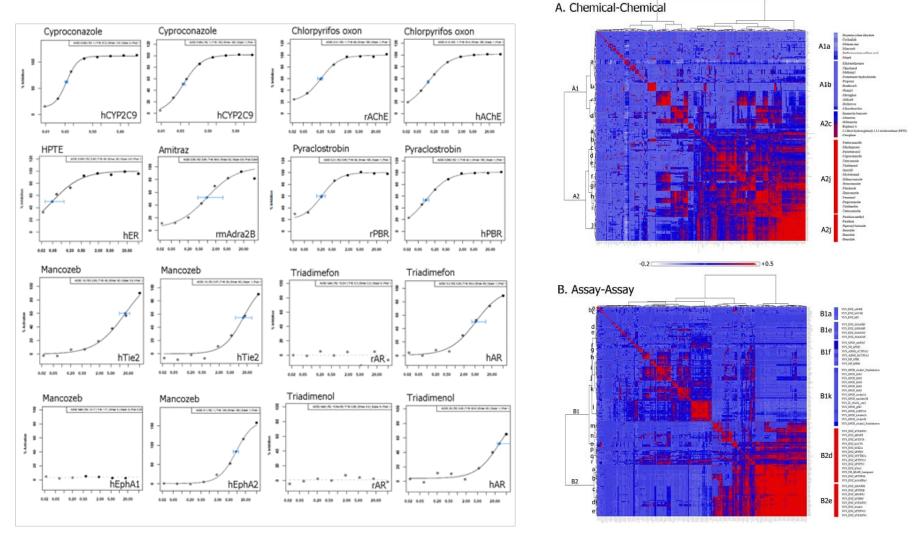


# **HTS paradigm**





#### **EXAMPLE:** biochemical HTS (292 assays)



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

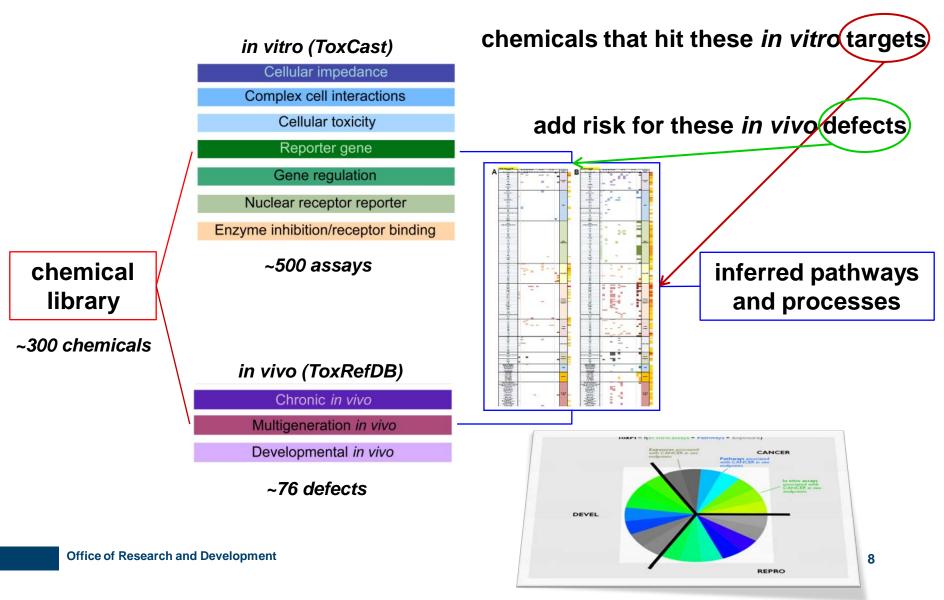


## **Predictive toxicology**

- *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









## ToxRefDB

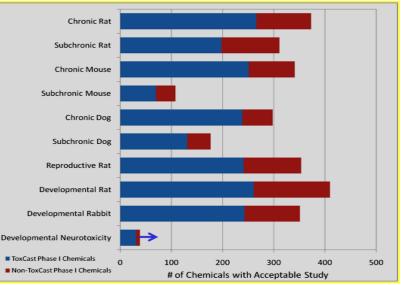
- computable database containing \$2B data from registrantsubmitted toxicology study results (DERs)
- source data from 3184 studies for 572 chemicals entered with controlled vocabulary and harmonized terminology
- database and web interface open to the public in 2010 <u>http://actor.epa.gov/toxrefdb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

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

MULTIGENERATIONAL REPRODUCTIVE Martin et al. (2009) Toxicol Sci

**PRENATAL DEVELOPMENTAL** Knudsen et al. (2009) Reprod Toxicol

**DEVELOPMENTAL NEUROTOXICITY** Sneed et al. (in preparation)



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## 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
- Iowest 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 Fields/Data Elements Utilizing Standardized

TOX CHEM NO. 4748

Vocabulary

Chemical

Target Region

Admin

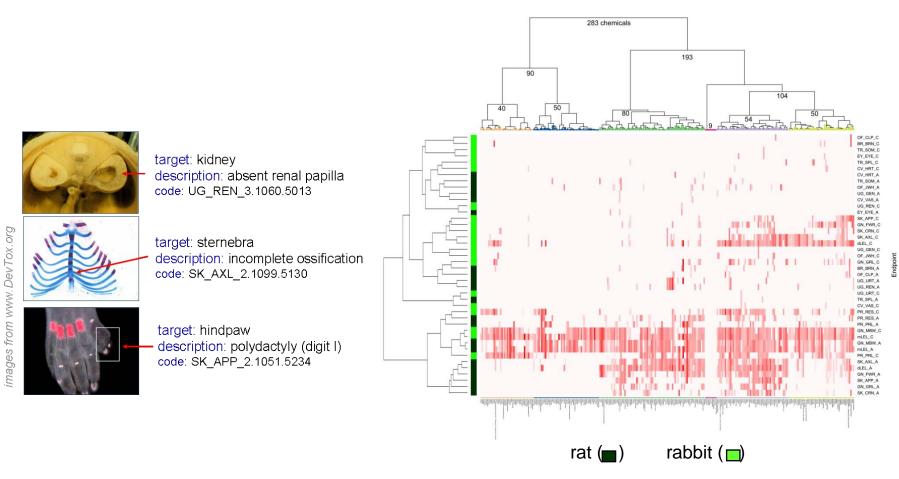
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# In vivo profiling





#### Query: cleft lip and cleft palate

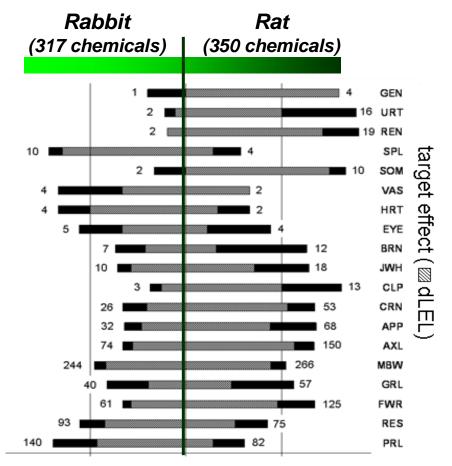
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	60207-90-1	Propiconazole			30.0	300.0	90.0
	<u>118134-30-8</u>	Spiroxamine			10.0	100.0	100.0
	<u>43121-43-3</u>	Triadimefon			10.0	100.0	100.0
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#### http://actor.epa.gov/toxrefdb



#### ToxRefDB: species dimorphism

- 53 of 283 chems specific (no mLEL) or sensitive (dLEL < mLEL) for DevTox</li>
- 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)





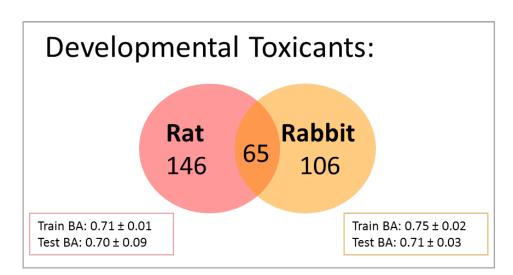
# **Species Considerations**

- 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**

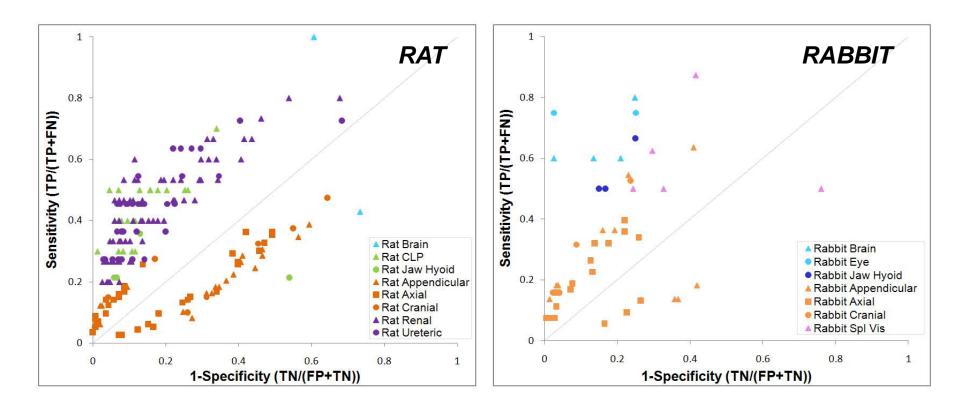
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507708 8038     505708 8038     505708 1058     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588     505708 10588	<ul> <li>SU400 SI + 5 tob 3 corp 1</li> <li>SU400 SI + 5 tob 3 corp 1</li> <li>SU13 - 54 H Regard (hr 1</li> <li>SU13 - 54 H Regard (hr 2</li> <li>Tob 54 H Regard (hr 2</li> </ul>				25 0.4 0.0 25 0.4 0.0 25 0.0 0.0 25 0.0	20 00 22 00 22 00 20 22 00 20 20 00 20 20 00 20 20 20 20 20 20 21 00 20 00 20 00 00 00 00 00 00 00 00 00 00 00 00 0	0         0	0         0         0         0         42           0         0         0         0         4         4           0         0         0         0         5         6           0         0         0         5         6         6           0         0         0         5         6         7         0           0         0         0         0         10         10         0         0           0         0         0         0         0         0         0         0         0           0         0         0         0         0         0         0         0         0           0				200 * 0 * 0	LAND OF ANY	The state	
6 5 5 5 5 5 5 5 5 5 5 5 5 5	State 21 - 5 toto 3 over 1     State 20 - 5 toto 3 over 1     State 20 - 5 toto 3 over 1     State 20 - 5 over 20     State 20 -					42 00 41 44 00 410000000000000000000000000000000		0         0         0         0         42           0         0         0         1         1         1           0         0         0         0         1         1         1           0         0         0         0         1         1         1         1           0         0         0         0         1         1         1         1         1           0         0         0         0         0         1				200 * 0 * 0	LAND OF ANY	The state	
500704, 5003     500704, 5003     500704, 5500     500704, 12540     500704, 12540     500704, 12540     500704, 12541     500704, 1254     500704     5000     500704	School 20 + 5 teds 2 cares 1     School 20 + 5 teds 2     School 20 + 5 teds     School 20 + 5 teds     Schoo				25 04 08 14 04 08 15 04 08 15 05 08 15 04 08 15 06 08 16 00 00 16 00 00 16 00 000000000000000	20 00 110 00 100 100 00 100 10 00 100 10 00 100 1	0         0	0         0         0         0         42           0         0         0         1         1         1           0         0         0         0         1         1         1           0         0         0         0         1         1         1         1           0         0         0         0         1         1         1         1         1           0         0         0         0         0         1				200 * 0 * 0	LAND OF ANY	The state	
500705-8008     500705-8008     505705-8008     505705-15500     505705-15500     505705-15500     505705-15500     505705-155     505705-1550     505705     505705-1550     505705-1550     505705-1550     505705	Sodo 22 - 3 tado 2 dara 1     Sodo 22 - 3 tado 2 dara 1     deba 2 dara 2     deba 2 dara 2     deba 2 deba 2 dara 2     deba 2 deba 2 deba 2     deba 2 deba 2     deba 2 deba 2     deba 2 deba 2     deba 2			1 000 000 000 000 000 000 000 000 000 0	25 04 08 05 06 08 15 06 08 15 07 08 08 07 08 10 0000000000	30         0.00         35           40         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35           50         0.00         35		0         0         0         0         42           0         0         0         1         1         1           0         0         0         0         1         1         1           0         0         0         0         1         1         1         1           0         0         0         0         1         1         1         1         1           0         0         0         0         0         1				200 * 0 * 0	LAND OF ANY	The state	
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500702-3038     500702-3038     500702-3039     500702-3039     500702-3039     500702-3039     500702-3039     500702-3039     500702-3039     500702-3039     500702-3039     500702-3039     500702-3049	Sodo 22 - 3 tado 2 dara 1     Sodo 22 - 3 tado 2 dara 1     deba 2 dara 2     deba 2 dara 2     deba 2 deba 2 dara 2     deba 2 deba 2 deba 2     deba 2 deba 2     deba 2 deba 2     deba 2 deba 2     deba 2			1 000 000 000 000 000 000 000 000 000 0	0.5 0.4 0.5 0.4 0.5 0.4 0.5 0.4 0.5 0.4 0.5 0.4 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5	52 00 41 51 00 41 52 00 41 52 00 41 52 00 41 52 00 55 53 00 55 54 00 55 55 00 55 55 00 55 55 00 55 55 00 55 55 00 55 55 000						200 * 0 * 0	LAND OF ANY	India of the	
200700, 8038     200700, 8038     200700, 2038     200700, 20380	1048/01/2 (0.00 pm)     1048/01/2 (0.00 pm)     1041/2 (0.00 pm)				25 0.4 0.5 0.4 0.5 0.4 0.5 0.4 0.5 0.4 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5	2 0 0 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	India of the	
SCTCH, 8038     SCTCH, 8038     SCTCH, 8034     SCTCH, 1550     SCTCH, 15	1000 01 0 100 0 2000     10 100     10 100 0 2000     10 100     100     10 100     100     10 100     100     100     100				25 04 00 55 04 00 15 00 00 15 00 00 15 00 00 15 00 00 15 00 00 15 04 00 15 00 00 10 00 00000000	42         0.0         41           11         11         0           12         0.0         0           11         0.0         0           12         0.0         0           12         0.0         0           13         16         0           14         0.0         1           14         0.0         1           15         0.0         0           14         0.0         1           15         0.0         0           14         0.0         1           15         0.0         0           16         0.0         0           17         0.0         0           18         0.0         0           19         0.0         0           10         0.0         0           10         0.0         0           10         0.0         0           10         0.0         0           10         0.0         0           10         0.0         0						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	India of the	
60704,803     60704,803     60704,803     60704,804     60704     607     60704,804     60704,804     60704     607     60704,804     60704,804     60704     607     60704     607	5000 00 10 1000 2000     10 1000 2000     10 1000 2000     10 1000 2000     10 1000 2000     10 1000 2000     10 1000 2000     10 1000     10 1000     10 1000     10 100     10     10 100     10 100     10 100     10 100     10 100     10     10 100     10 100     10     10 100     10     10 100     100     10     10     10     10     10     10     10     10     10				05 04 00 17 04 00 17 04 00 17 04 00 18 01 00 10 01 00 11 00 00 15 00	10 00 22 0 00 22 10 00 20 10 00 2						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
SCPCA 803	10400 201 9 table 2 cern 1     10400 201 9 table 2 cern     1     2013.044 Anony      1     2013.044 Anony      1     2013.044 Anony      1     2013.044 Anony      1     2014.044 Anony      2014.044 Anoony      2014.044 Anony      2014.0					55         0.0         42           55         0.0         62           54         0.0         62           12         0.0         62           12         0.0         62           13         0.0         62           14         0.0         62           13         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           14         0.0         62           15         0.0         62           16         0.0         62           16         0.0         62           16         0.0         62           16         0.0         62           16         0.0         62           16         0.0         62           16         0.0						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	India of the	
SCTCA 803	BOOD 21-0 tota 2 cm     B				25 0.4 0.5 14 0.4 0.5 14 0.4 0.5 14 0.4 0.5 15 0.5 0.5 15 0.5 0.5 15 0.4 0.5 15	40 00 25 0 00 0 25 0 00 0 0 0 0 0 0 0 0 0 0 0 0						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
SCICL ACIA     SCICL     S	A         5540 53 + 2 Maio 2 years         1           A         5540 53 + 2 Maio 2 years         1           A         9540 53 + 2 Maio 2 years         1           A         9740 53 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54 + 10 Maio 2 years         1           A         9740 54				25 84 88 14 70 4 15	54 00 41 55 00 41 56 00 41 51 00 5 51 00 50 5 50 50 50 50 50 50 50 50 50 50 50 50 50 5						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
SCPC1, 803     SCPC1, 1039	8         2048/201         7 Auto Am         1           9         1048/201         7 Auto Am         1           9         1049/201         7 Am         1           9         1049/201         7 Am         1           9         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1           1         1049/201         1         1         1           1         1049/201         1         1         1           1         1049/201         1         1         1         1           1         1049/201         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1         1				25 04 00 15 07 04 15 07 00 15 07 00 15 04 00 15 05 00 15 00 15 00 15 00 15 00 15 00 15 000 15 000 15	40         50         52           40         60         52           40         60         60           41         60         60           42         60         60           42         60         60           43         60         60           44         60         60           44         60         60           45         60         60           46         60         60           47         60         60           48         60         60           49         60         60           40         60         60           40         60         60           40         60         60           40         60         60           40         60         60           40         60         70           40         60         70           40         60         70           40         60         70           40         70         70           40         70         70           40         70         70						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
SOTON , 803     SOTON , 804     SOTON , 8	<ul> <li>Stodd G1 / Stodd G2 / Stodd G2</li></ul>				25 04 08 15 04 08 15 04 08 17 04 08 17 04 08 17 04 08 18 08 08 18 08	54 00 41 55 00 41 14 00 56 14 00						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
SCTON , ROB     SCTON , R	A         15405 03 / 9 Maio 2 years         1           A         15405 03 / 9 Maio 2 years         1           A         15405 03 / 9 Maio 2 years         1           A         15405 03 / 9 Maio 2 years         1           A         15405 03 / 9 Maio 2 years         1           A         15405 03 / 9 Maio 2 years         1           A         15405 03 / 9 Maio 2 years         1           A         15405 04 / 9 Maio 2 years         1           A         15405 04 / 9 Maio 2 years         1           A         15405 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         15406 04 / 9 Maio 2 years         1           A         1540				25         0.4         0.6           1.5         0.6         0.6           1.5         0.6         0.6           1.5         0.6         0.6           1.5         0.6         0.6           1.5         0.6         0.6           1.5         0.7         0.6           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7           1.5         0.7         0.7     <	52         0.0         42           54         0.0         42           54         0.0         42           54         0.0         42           54         0.0         42           54         0.0         10           52         0.7         10           52         0.7         10           54         0.0         10           52         0.7         10           54         0.0         10           54         0.0         11           54         0.0         11           50         0.0         11           50         0.0         11           50         0.0         11           50         0.0         11           50         0.0         11           50         0.0         11           50         0.0         12           51         0.0         12           52         0.0         12           50         0.0         12						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	No.	
2         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2013           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014           3         200701, 2014	Stold 01 - 0 tello 2 term 1     Stold 02 - 0 tello 2 term 1     Stold 03 - 0 tello 2 term 1     Stold 04 - 0 term 1     S					52         00         51           52         00         51           57         00         51           57         00         51           52         00         52           52         00         52           13         60         52           44         00         51           44         00         52           44         00         52           44         00         52           44         00         52           44         00         52           45         00         52           46         00         52           47         00         52           48         00         52           48         00         52           48         00         52           52         00         52           52         00         52           54         50         52           55         56         56           56         50         57           57         56         56           58         50         57						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
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2         SCPC0, 3034           2         SCPC0, 3034           3         SCPC0, 1346           4         SCPC0, 1346           5         SCPC0, 1446           5         SCPC0, 1447           5         SCPC0, 1448           5         SCPC0, 1448           5         SCPC0, 1449           6	Sold 21 - 2 Hold Stern 1     Sold 23 - 2 Hold Stern 1     Sold 24 - 2 Hold Stern 1     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24 - 4 Hold 24 - 4 Hold 24     Hold 24     Hold 24 - 4 Hold					52         0.0         54           54         0.0         54           54         0.0         54           54         0.0         54           1         0.0         54           1         0.0         54           1         0.0         54           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52           1         0.0         52 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td>200 * * * * * * * * * * * * * * * * * *</td> <td>LAND OF ANY ANY ANY ANY ANY ANY ANY ANY ANY ANY</td> <td>The state</td>						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
2         557°C0 . 8038           2         557°C0 . 8038           3         80700 . 41°C           3         80700 . 11°C	8         25005 02 9 1/min 2 0/min         2           4         25005 02 9 1/min 2 0/min         2           5         25005 02 9 1/min 2 0/min         2           6         25005 02 9 1/min 2 0/min         2           6         25005 02 9 1/min 2 0/min         2           7         25005 02 9 1/min 2 0/min         2           8         25005 02 9 1/min 2 0/min         2           9         25005 02 9 1/min 2 0/min         2           9         25005 02 9 1/min         2           9         25005 02 00/min         2           9         25000 02 00/min         2<					452         0.0         45           55         67         68           67         68         68           67         68         68           67         68         68           67         68         68           68         68         68           62         69         68           62         60         68           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         67           64         68         68           64         68         68           64         68         68           64         68         68           64         68         68           64         68         68 <td></td> <td></td> <td></td> <td></td> <td></td> <td>200 * * * * * * * * * * * * * * * * * *</td> <td>LAND OF ANY ANY ANY ANY ANY ANY ANY ANY ANY ANY</td> <td>No.</td>						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	No.	
2           2         SSCPC0_AC34           3         SSCPC0_AC34           3         SSCPC0_AC34 </td <td>8         20480 - 20 - 20 - 40 - 40 - 40 - 40 - 40 - 4</td> <td></td> <td></td> <td></td> <td></td> <td>442         0.0         41           51         0.0         42           52         0.0         3           41         0.0         3           42         0.0         3           42         0.0         3           43         0.0         3           44         0.0         3           45         0.0         3           45         0.0         3           44         0.0         3           45         0.0         3           44         0.0         3           44         0.0         3           45         0.0         3           44         0.0         3           45         0.0         3           46         0.0         3           47         0.0         3           48         0.0         3           48         0.0         3           49         0.0         3           49         0.0         3           49         0.0         3           40         0.0         3           41         0.0         3<td></td><td></td><td></td><td></td><td></td><td>200 * * * * * * * * * * * * * * * * * *</td><td>LAND OF ANY ANY ANY ANY ANY ANY ANY ANY ANY ANY</td><td>No.</td></td>	8         20480 - 20 - 20 - 40 - 40 - 40 - 40 - 40 - 4					442         0.0         41           51         0.0         42           52         0.0         3           41         0.0         3           42         0.0         3           42         0.0         3           43         0.0         3           44         0.0         3           45         0.0         3           45         0.0         3           44         0.0         3           45         0.0         3           44         0.0         3           44         0.0         3           45         0.0         3           44         0.0         3           45         0.0         3           46         0.0         3           47         0.0         3           48         0.0         3           48         0.0         3           49         0.0         3           49         0.0         3           49         0.0         3           40         0.0         3           41         0.0         3 <td></td> <td></td> <td></td> <td></td> <td></td> <td>200 * * * * * * * * * * * * * * * * * *</td> <td>LAND OF ANY ANY ANY ANY ANY ANY ANY ANY ANY ANY</td> <td>No.</td>						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	No.	
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2         50000, 3003           2         50000, 3003           3         500000, 4003           3	8         20400 - 20 - 30 - 40 - 40 - 40 - 40 - 40 - 40 - 4					442         0.0         44           55         0.0         44           55         0.0         45           61         0.0         45           61         0.0         45           61         0.0         45           62         0.0         60           62         0.0         60           63         0.0         60           64         0.0         60           64         0.0         60           64         0.0         60           65         0.0         60           64         0.0         60           64         0.0         60           64         0.0         60           64         0.0         60           64         0.0         60           64         0.0         60           65         0.0         70           64         0.0         60           65         0.0         70           64         0.0         70           65         0.0         70           64         0.0         70           70         10						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	
2         55°C0, AG38         55°C0, AG38           2         55°C0, AG38         55°C0, AG38           2         55°C0, AG38         55°C0, AG38           3         55°C0, AG38         55°C0, AG38           4         55°C0, AG48         55°C0, AG48           5         55°C0, AG48         55°C0, AG48           6	8         2000 20 - 9 table 20 mm 1.           4         2000 20 - 9 table 20 mm 1.           4         4000 23 - 10 table 20 mm 1.           4         4000 23 - 10 table 20 mm 1.           5         700 20 table 20 mm 1.           6         700 20 table 20 mm 1.           6         700 20 table 20 mm 1.           700 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 table 20 mm 1.         700 20 table 20 mm 1.           700 20 table 20 table 20 mm 1.         700 20 mm 1.           700 20 table 20 mm 1.         700 20 mm 1.           700 20 table 20 mm 1.         700 20 mm 1.           700 20 table 20 mm 1.         700 20 mm 1.					52         0.0         52           52         0.0         52           52         0.0         52           52         0.0         52           52         0.0         52           52         0.0         52           52         0.0         52           54         0.0         52           54         0.0         52           54         0.0         52           54         0.0         52           54         0.0         52           55         0.0         52           55         0.0         52           55         0.0         52           55         0.0         52           56         0.0         52           57         1.0         52           58         0.0         52           51         0.0         52           52         0.0         52           52         0.0         52           52         0.0         52           52         0.0         52           54         0.0         52           54         0.0						200 * * * * * * * * * * * * * * * * * *	LAND OF ANY	The state	



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

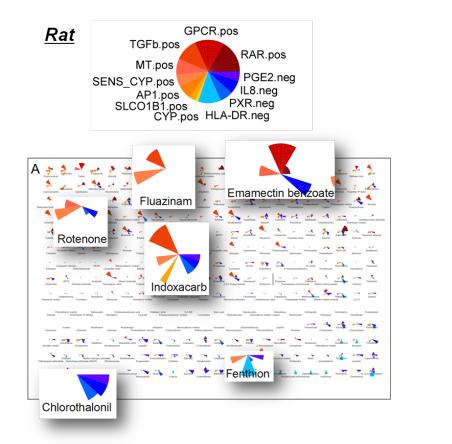


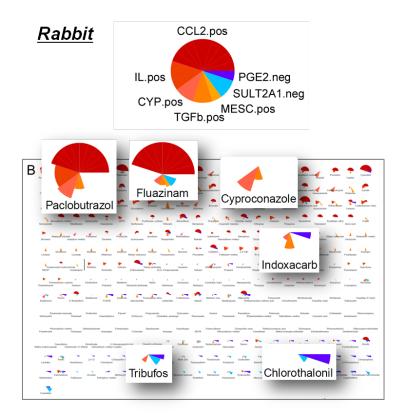
#### **Sensitivity analysis**





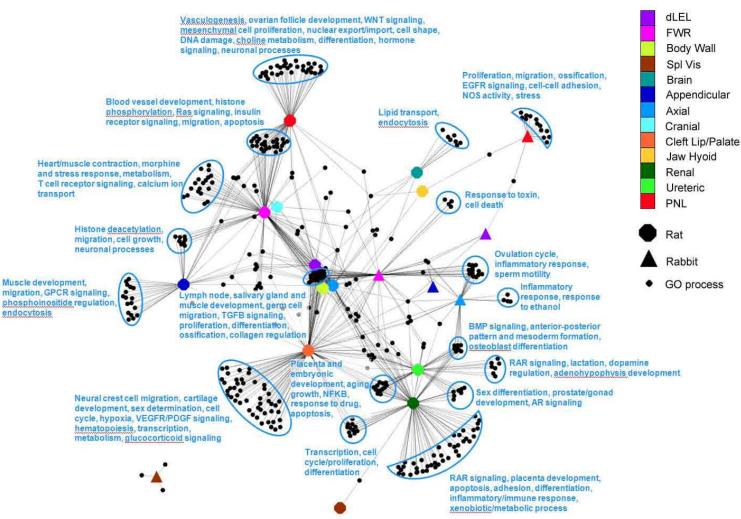
#### **ToxPi visualization**





### **GO Biological Process**





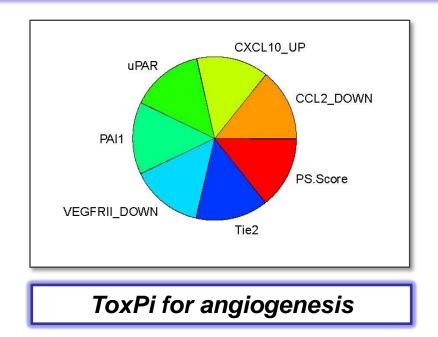
**Office of Research and Development** 

#### SOURCE: Sipes et al. 2011 (submitted)



## **Blood vessel development**

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



SOURCE: Kleinstreuer et al. (submitted)

	Ķ	<b>bV</b>	DC	s			Scol	res for		ToxCast by Overa		-l chemi	icals							
Pyridaben	nylene bisithecya	inate) Fyraclostrobin	Flumetralin	Propargite	Triffoxystrobin	Zoxamide	Abamedin	Maneb (2	Z,E)-Fenpyrotim	s-Bioallethrin	Etoxazolo	Chlorothalonil	Fenoxycarb	Thiram	Imazali	Diniconazole	Thiodicarb	Cyazofamid	Butafenacil	Lactofen
Azamethiphos	indoxacarb	Metiramainc	Butralin	Spirodiclofen	enzoquat metilsu	Dimethomorph Ifate	S-lodo-2		Icarbamate	Rimsulfuron	Tetramethrin	Quinoxyten	Naled	Niclosamide	Alachlor	Spiraxamine	Triclosan	Prochloraz	Triflumizole	Fluoxastrobin
Diazoxon	Bisphenol A	Tebu fenpyrad	Dazomét	Hexythiazox	Pacicbutrazol	Methoxychlor	Resmethrin Oxyte		Propetamphos ydrate	Chlorpyrifos axe	Teoucirimfos	Myclobutanii	Cyanazine	MGK		Metribuzin	Tetraconazole Perflu		Phosalone nic acid	Diquat dibromide
Pendimethalin	Bromoxynil	Prodiamine	Fenitrothion	Benomyl	Folpet	Cournaphos F	iumiclorac-penty	Prallethrin	Fluezinam	Tribulos	Thiazopyr	Bensulide	Dichlerves	Bentazone	Captafol	Fludioxonil	Difenoconazole	Acetochicr	Forchlorfenuro	Dithiopyr
Butachlor	Oxyfluorfen Per	fluoreoctancic a	Profenofos	Mancozeb d	-cis,trans-Alleth	rin Famcxadone	Aldicarb	Flufenpyr-ethy	Fenpropathrin	Flurcxypyr-mep	Dicofol Nyl	Esfenvalerate	Simazine	Marka International Internatio	Marka Trifuralin	No. 1990	Pyrimethanil	Flusilazole	Atrazine	Diclosulam
Methyl isothiocyar	rata 6	Parathion-methy	-	Cvfluthrin	►	Fenarimel	~	Captan	<b>14</b>	Eenthion	₩	Dicrotophos	Math	yl hydrogen phti		Dichloran	►	10dinafop-propa	red .	Metolachlor
		9	2%	ρV	DCs	wit	th ai	nim	al d	lata	had	d de	vel	on nac	ieni	tal e	effec	cts		
Disulfaton		9	hyl	Ametryn	(ske		loride	nal	fori		tion		r fe	Fluferacet	los	S)		Cts Thiobencarb		Guintozene
Disulfaton	Anilazine	iophanate-meth	hyl TCMTB	Ametryn	(ski Prope Chlorpyrifos=med		al n	Propyzamide	<b>fori</b> Ethoprop	mai	tion	S O	r fe	Flufenacet		S)	Triadimencl		Fenamiphos	
Disulfeton			hyl	Ametryn	(sk) Prope Chlorpyrifos-met		horide Dibutyl phthalate Oryzalin	nal Propyzamide	Ethoprop	mai	Boscalid	Clopyrelid-olamin	Pyriproxylen	Flufenacet	los	Diazinon		Thiobencarb	Fenamiphos	٩,
~	Anilazine	iophanate-meth	nyl TCMTB	Ametryn	(sk) Prope Chlorpyrifos-met		horide Dibutyl phthalate Oryzalin	Propyzamide	Ethoprop	Fenoxaprop-ett	Boscalid	Clopyralid-olamin	Pyriproxylen	Flufenacet	IOS:	Diazinon	Triadmencl	Thiobencarb	4	4
4 Amitraz	Anilazine V Icaridin	iiophanate-meth Image: Carbaryl	nyl TCMTB	Ametryn C Ett Propanil	(Ski Propr Chlorpyrifos-med Anametsulfuron me H	elet mocarb hydroch hyd btyd alosul furon-metl Methidathion	horide Dibutyl phthalate Oryzalin	Propyzamide	Ethoprop	Fenoxaprop-ett	Boscalid Diphenylamini Prop	Ctopyrelid-otermin	Pyripraxylen Pyripraxylen Eutylate odium	Flufenacet Flufenacet Penoxsularm	Fluthiacet-meth	biazinon yı thyl Malaoxon	Triadmencl & Novaluron	Thiobencarb Daminozide Cicrophene	Nethyl cellusolv	ve Mevinphos
Amitraz	Anilazine Rearidin	iophanate-meth Carbaryl Ç Isocallutole	yt TCAITB	Ametryn C Propanil Cloprop	(Ski Prope Charpyrilos-med charpersulturon me H Morturezon Napropamide	elet mocarb hydroch hyd btyd alosul furon-metl Methidathion	Horide Dibutyi phthalate Diryzalin hyl Metr	Propyzamide	Ethoprop Azinphos-method Cyanamide Lindane	Fenoxaprop-eth yi Flutolanii	vi Boscalid Diphenylamini- Prop Ethotumesate	Ctopyrelid-otermin	r fe Pyriproxylan Dutylate odum	Flufenacet	Fluthiacet-meth	S) Diszinon yl thyl Melaoxon Cohexadione-ca yl	Triadmenci Veraluron	Thiobencarb Daminozide Clorophene yl	Nethyl cellusolv	/e Mevinphos
Amitraz P Bromacil	Anilazine Rearidin Anilazine Icaridin Imazethapyr	iiophanate-meth Carbaryl V Isocallutole	yt TCAITB	Ametryn Cepropanil Cloprop	(Ski Prope Charpyrilos-med charpersulturon me H Morturezon Napropamide	elettion mocarb hydroch hyd hyd Nethidethion Form	Aloride Dibutyl pithalate Oryzalin hyl Metr etanate hydrochi	Propyzamide	Ethoprop Azinphos-method Cyanamide Lindane	Fenoxeprop-et yl Flutolanii Triclopyr	vi Boscalid Diphenylamini- Prop Ethotumesate	Ctopyrelid-olemii	r fe Pyriproxylan Dutylate odum	Flufenacet	Futhiacet-meth itsulfuron-me infoenuron-meth c	yl Diacinon yl Malaoxon Vyl ohexadione-ca yyl zine	Triadmencl Novaluron Icium Quizalofop-eth	Thiobencarb Daminozide Clorophene S	Methyl cellusol A Isazofos	re Mevinphos Dichlobenil
Amitraz Promacil Bendiocarb	Anilazine Raridin Anilazine Icaridin Anilazine Fenbuconazole	icphenate-meth Carbaryl Exactalutole	ryf TCAITB Pirimicarb Propoxur Propoxur Propoxur Pri	Ametryn Et <sup>e</sup> Propanil Cloprop	(sk( Proper chlorpyrilos-med h	eletti Imocarb hydroch yd Debyl Jobulfuron-metl Jobulfuron-metl Form Furmetsulam	toride Dibutyl phthalate Dibutyl phthalate Oryzalin Oryzalin Metr etanate hydrochl	Propyzamide * am-sodium hy Picloram ortide Linuron	Ethoprop Azinphos-meth cryanamide Lindane	Penoxeprop-eet y Plutolani Triclopyr 4 2-Phenytohenri Metsulfuron-met	bionaria Diphenylamini Diphenylamini Prop Ethotumesate	Ctopyrelid-olemii Ctopyrelid-olemii oporycerbezone-s Pyraflufen-ethy Chlorethoxyfos	r fe Pyripraxysin Butylate odium	Flufenacet Protocolum Penoxsular Methoxyfenacid Ge- Fenhexarrid	Futhiacet-meth	S) Discinon yl Walaoxon V thyl Malaoxon V v v v v v v C Cyromazine	Triadmend Novaturon Cuizatofop-eth Thiabendazole	Thiobencarb Daminacide Clorophene Sulfentrazone	Methyl cellusolu A Isazofos Trichlorfon	* Mevinphos Dichiobenii Malathion
Amirraz Fromacil Bendiocarb A	Anilazine Anilazine Icaridin Anilazine Imazethapyr Fenbuconazole Symclosene Anilazine	icphanate-meth Carbaryl V Clothianidin Clothianidin Triadimebri	nyi TCMTB Pirimicarb Proposur Diethyltduamidd Pri Diethyltduamidd	Ametryn Et <sup>e</sup> Propanil Cloprop e misulfuron-me thiciazuron	(Ski Propu chlorpyfilos-med armétulituron mm H e Norfurazon Napropamide tryf	Pletion Impeato hydroch yd Nethidathion Form Furmetsulam	Abride Dicuyl prithalate Dicuyl prithalate Corpaalin Meter V V V V V V V V V V V V V V V V V V V	Propyzamide * am-sodium hy Picloram ortide Linuron č	Etioprop Etioprop Azinphos-meth drate Cyanamide Lindane	Fenovaprop-ett Felulani Fululani Calenti Calen	Boscalid Diphenylaminin Prop Ethotumesate Clopyrašid	Chlorethacyfos	r fe Pyriparylan Butylate odurn Li Cinmethylin Bifenazate	Flufenacet Prufenacet Penoxsulari Methoxyfenacidd Ge Fenhexarrid	Futhiacet-meth	S) Discinon yl Walaoxon V thyl Malaoxon V v v v v v v C Cyromazine	Thiadmend Vovaluron Courselosop-eth Chizalosop-eth Thiabendazele 2,4-D Clomazone	Thiobencarb Daminacide O aminacide Sulfentrazone	Methyl cellusol Isazofos Trichlorfon Cixasulfuron Nitrapyrin	re Mevinphos Dichiobenii Malathion

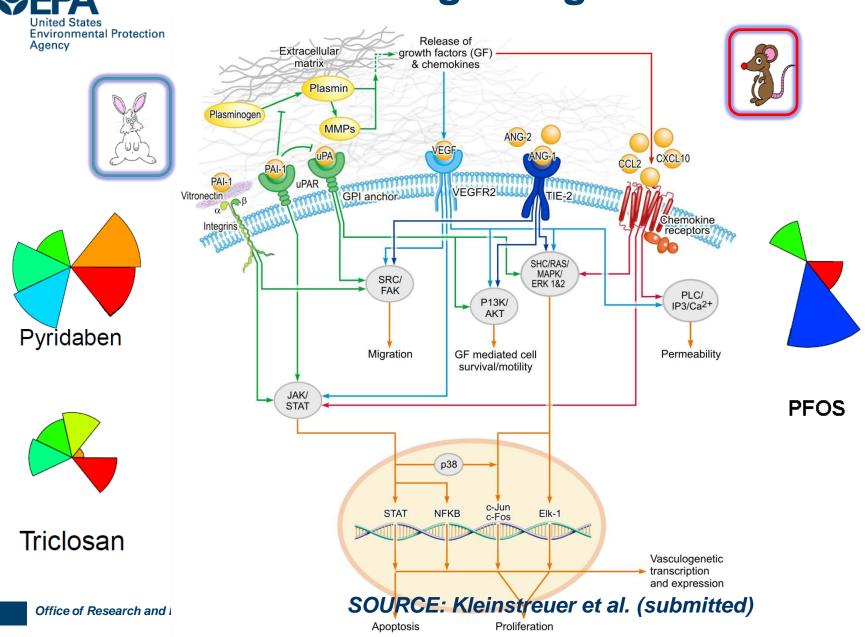
Acibenzolar-S-Methyl Sethonydim Etridiazole Pymetrozine Pymetrozine Piweitryl phthalate SOURCE: Kleinstreuer et, al. (submitted cc12\_DOVN

PS.Score

Tie2

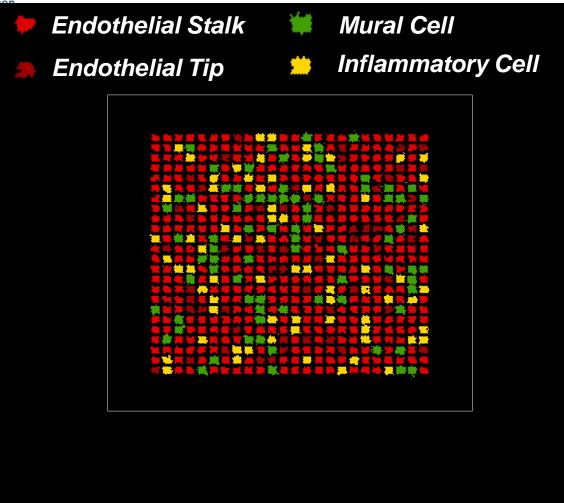
VEGFRII\_DOWN

## **Vascular Signaling Network**



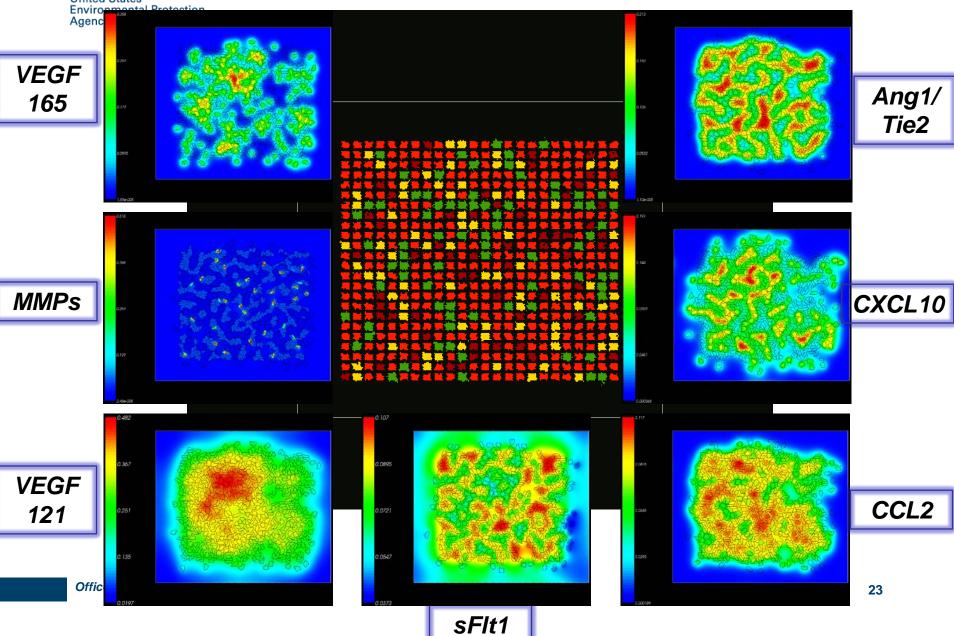


#### **Vascular Plexus**



#### **SEPA** United States Environmental Pro

#### **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

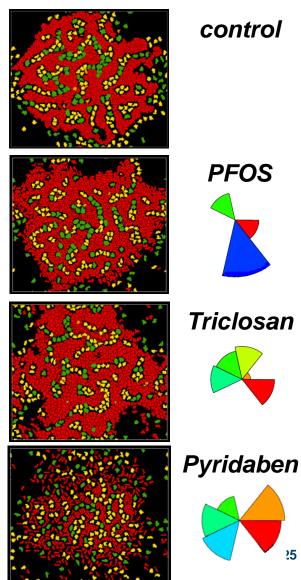
endothelial connectivity (plexus), in-degree (branching), vessel uniformity (width), sprouting

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







# **ToxRefDB Predictive modeling**

#### **ATTRIBUTES**

- searchable public database (<u>www.epa.gov/ncct/toxrefdb/</u>)
- 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