

Modeling the disruption of vascular development in a Virtual Embryo using ToxCast HTS Bioactivity Profiles

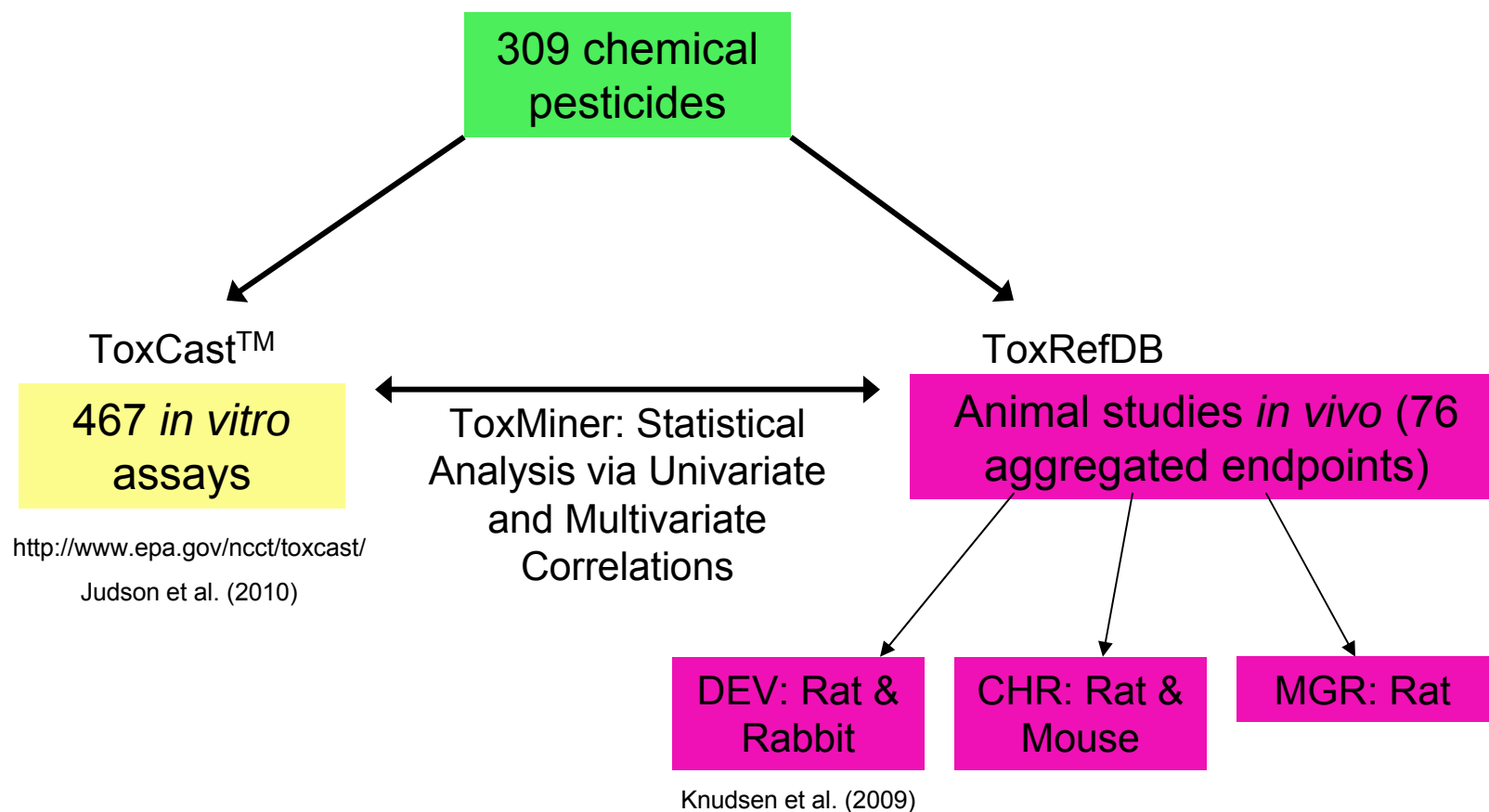
Nicole Kleinstreuer

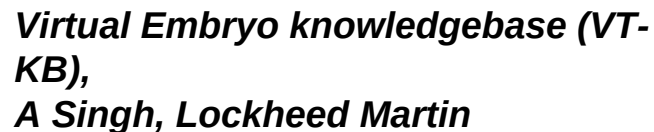
USEPA/ORD/NCCT (<http://www.epa.gov/ncct/>)

Virtual Embryo (<http://www.epa.gov/ncct/v-Embryo/>)

Objective:

derive a 'toxicity signature' for vascular disruption in developmental toxicity



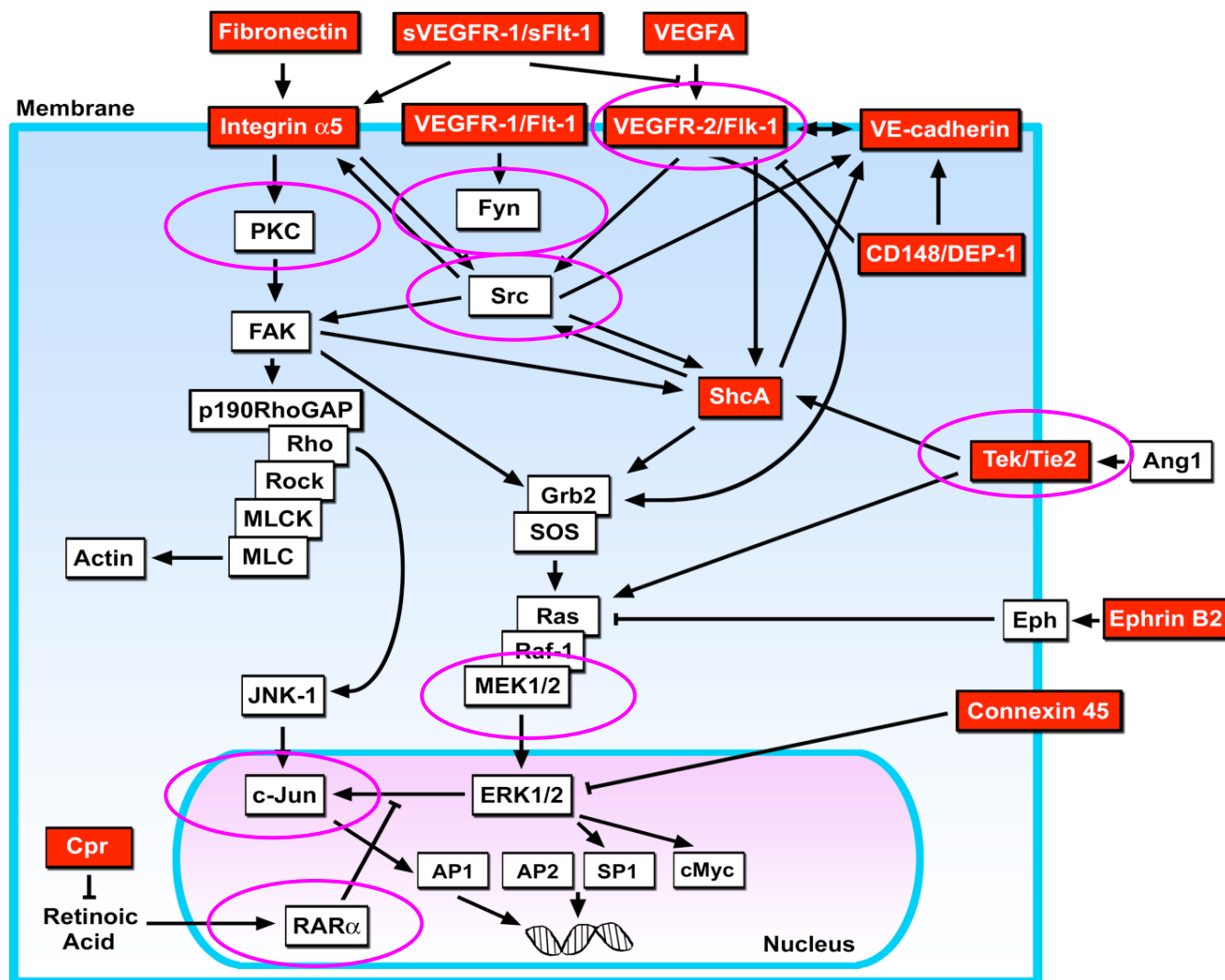


Key formative processes: vasculogenesis / angiogenesis



- endothelial proliferation, cell migration & chemotaxis
 - RTK signaling: VEGF, VEGF-r, TIE2
 - chemokine signaling: CCL2, CXCL10
- neovascular stabilization:
 - endothelial cell (EC) adhesion
 - vascular smooth muscle cell (VSMC) recruitment
- extracellular matrix (ECM) degradation
 - plasminogen activating system (PAS)
 - matrix metalloproteinases (MMPs)

1. VEGF signaling



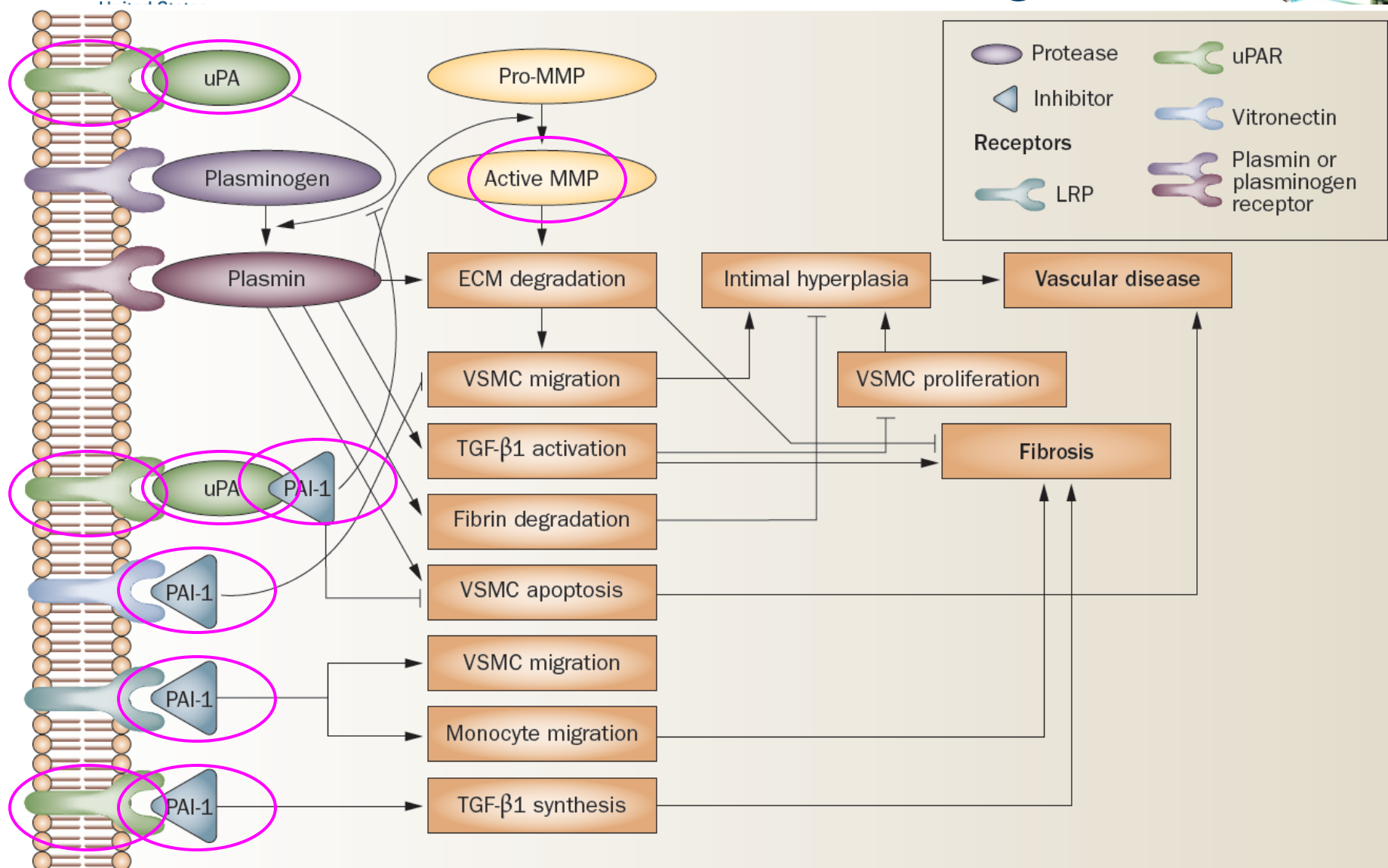
2. Chemokine signaling



Angiogenic	Receptor	Angiostatic	Receptor
CXCL1 (Gro-a)	CXCR2	CXCL4 (PF-4)	CXCR3B
CXCL2 (Gro-b)	CXCR2	CXCL9 (Mig)	CXCR3B
CXCL3 (Gro-g)	CXCR2	CXCL10 (IP-10)	CXCR3B
CXCL5 (ENA-78)	CXCR2	CXCL11 (I-TAC)	CXCR3B
CXCL6 (GCP-2)	CXCR2		
CXCL7 (NAP-2)	CXCR2		
CXCL8 (IL-8)	CXCR2		
CCL2 (MCP-1)	CCR2		
CCL11 (Eotaxin)	CCR3		
CCL16 (HCC-4)	CCR1		

Adapted from Keeley et al. (2008) Chemokines as Mediators of Neovascularization. *Arterioscler Thromb Vasc Biol* 28:1928-1936

3. ECM remodeling



Assay inclusion criteria

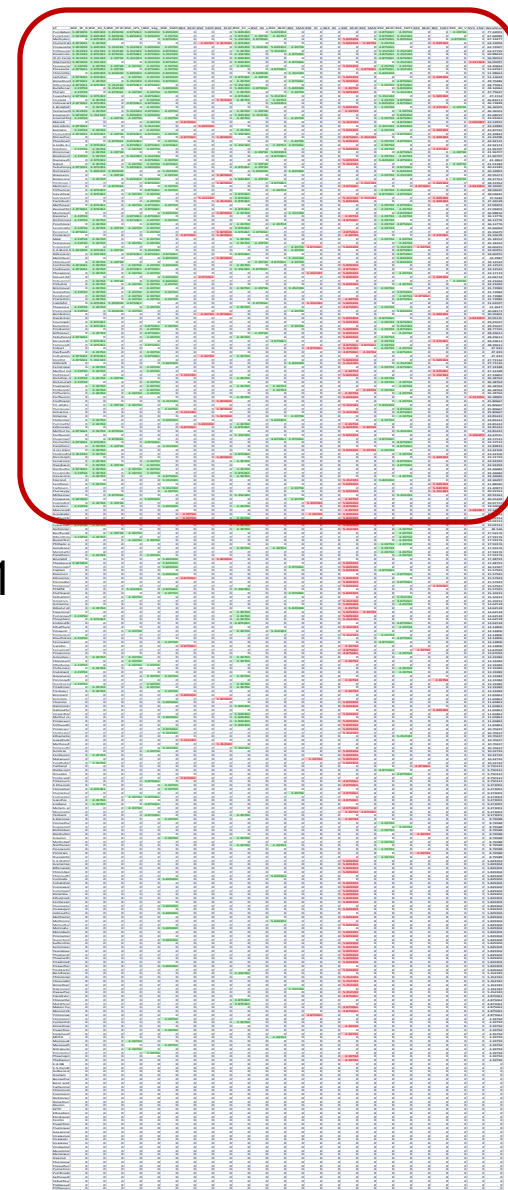


I. Select ToxCast™ assay targets by literature co-occurrence with ‘vasculogenesis’ & ‘angiogenesis’:

- **downregulation** of VEGFR2 receptor (KDR/FLK1)
- biochemical Tie2 assay
- **upregulation** of CXCL10 (pro-inflammatory, anti-angiogenic)
- **downregulation** of CCL2 (pro-angiogenic chemokine)
- **up-** or **down-**regulation of PAS via uPAR or PAI1/SERPINE1

II. Log transform AC50s, multiply by weighted coefficients (k_i), prioritize ToxCast™ chemicals:

- assay Score = $[-\log_{10}(\text{AC}_{50}(\mu\text{M})/100000)] = \text{AS}_i$
- signature Score = $\sum k_i * \text{AS}_i$





ToxRefDB developmental defects for putative 'Vascular Disruptor Chemicals':

- 15% of VDCs had no prenatal study in ToxRefDB
- 81% VDCs with data had developmental endpoints of **skeletal malformation** or **fetal loss** (69% overall)
- 7.5% VDCs showed **no prenatal effect** in rat or rabbit; ~ 4% had no effect in one species, **different developmental effect** in other

Pyraclostrobin	DEV_Rat_Skeletal_Axial;DEV_Rat_Ur;DEV_Rabbit_PregnancyRelated
Spirooxime	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Perfluorobutylamine	DEV_Rat_Skeletal_Axial;DEV_Rat_Ur;DEV_Rabbit_Skeletal_Axial
Butyltin	DEV_Rat_PregnancyRelated_Matern;DEV_Rabbit_General_FetalWei
Metribuzin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_Skeletal_Axial
Diquat dibromide	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_GeneralF
Trifluralin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Reformin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Trifluralin	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_Cardiovascular_H
Sinazoline	DEV_Rat_General_GeneralFetalPath;DEV_Rabbit_General_FetalWei
Oxadiazon	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Hexaconazole	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Cyprodinil	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_Skeletal_Axial
Flumetralin	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Propargite	No recorded effect;DEV_Rabbit_Skeletal_Axial
Trifluorostrobin	No recorded effect;DEV_Rabbit_Skeletal_Axial
Fenoxycarb	No recorded effect;DEV_Rabbit_Skeletal_Axial
Etoxaolol	No recorded effect;DEV_Rabbit_Skeletal_Axial
Indoxacarb	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Dazomet	No recorded effect;DEV_Rabbit_General_GeneralF
Pyrimethanil	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Fenitrothion	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_General_FetalWei
Folpet	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_Macrosomery_Br
Pyridazin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Leisofen	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Thiamet	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
(Z,E)-Fenprophimate	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Emamectin benzoate	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Maneb	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Fluxastrobin	DEV_Rat_Skeletal_Appendicular;DEV_Rabbit_PregnancyRelated
Dicofenylpyrid	DEV_Rat_General_GeneralFetalPath;DEV_Rabbit_PregnancyRelated
Tebuconazole	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
S-Bioallethrin	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Myclobutanil	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Cyazofamid	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Benomyl	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Tetraconazole	DEV_Rat_General_GeneralFetalPath;DEV_Rabbit_PregnancyRelated
Difenoconazole	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Thiodicarb	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
3-Iodo-2-propenylbutylcarbamate	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Acetochlor	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Hexythiazox	DEV_Rat_Skeletal_Appendicular;DEV_Rabbit_PregnancyRelated
Cyfluthrin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Alcibac	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Methyl Isothiocyanate	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Fenitrothion	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Fluraldox	DEV_Rat_General_GeneralFetalPath;DEV_Rabbit_General_FetalWei
Alfentra	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Permethrin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Permethrin-methyl	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Clodinafop-propargyl	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Fenamidone	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Thiazopyr	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Propazine	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Mancozeb	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Acaphate	DEV_Rat_Skeletal_Appendicular;DEV_Rabbit_PregnancyRelated
Dichloran	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Buprofezin	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Methylene bithiocyanate	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Abamectin	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Zoxamide	No recorded effect;DEV_Rabbit_PregnancyRelated
Dinethomorph	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Butafenacil	No recorded effect;DEV_Rabbit_PregnancyRelated
Tetramethrin	No recorded effect;DEV_Rabbit_PregnancyRelated
Rimsulfuron	No recorded effect;DEV_Rabbit_PregnancyRelated
Naled	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Chlorothalonil	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Nitrofen	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Difenoquat metilsulfate	No recorded effect;DEV_Rabbit_PregnancyRelated
Rotenone	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Trifloxan	No recorded effect;DEV_Rabbit_PregnancyRelated
Metiram-zinc	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Invalit	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Azamectin	No recorded effect;DEV_Rabbit_PregnancyRelated
Propanil	No recorded effect;DEV_Rabbit_PregnancyRelated
Cyanazine	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
2,2-Bis(4-hydroxyphenyl)-3,3,1	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Chlorpyrifos oxon	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Fenchone	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Fluoxifen	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Spirodiclofen	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Phosalone	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Alachlor	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Bisphenol A	No recorded effect;DEV_Rabbit_PregnancyRelated
Tebuconazole	DEV_Rat_PregnancyRelated_Matern;DEV_Rabbit_PregnancyRelated
Tribufos	No recorded effect;DEV_Rabbit_PregnancyRelated
Pendimethalin	No recorded effect;DEV_Rabbit_PregnancyRelated
Pralithrin	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Captafol	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Diazoxon	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Flumetralin-pentyl	No recorded effect;DEV_Rabbit_PregnancyRelated
Bentazone	No Developmental Rat Study in ToxRefDB;DEV_Rabbit_PregnancyRelated
Quinoxifen	No recorded effect;DEV_Rabbit_PregnancyRelated
Butachlor	No recorded effect;DEV_Rabbit_General_FetalWei

Thiram	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
(Z,E)-Fenprophimate	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Emamectin benzoate	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Maneb	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated
Fluxastrobin	DEV_Rat_Skeletal_Appendicular;DEV_Rabbit_PregnancyRelated
Dicofenylpyrid	DEV_Rat_General_GeneralFetalPath;DEV_Rabbit_PregnancyRelated
S-Bioallethrin	DEV_Rat_PregnancyRelated_Embryo;DEV_Rabbit_PregnancyRelated
Myclobutanil	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Cyazofamid	DEV_Rat_Skeletal_Axial;DEV_Rabbit_PregnancyRelated
Benomyl	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_Urogenital_Renal
Tetraconazole	DEV_Rat_General_GeneralFetalPath;DEV_Rabbit_PregnancyRelated
Difenoconazole	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_General_FetalWei
Thiodicarb	DEV_Rat_General_FetalWeightRedu;DEV_Rabbit_PregnancyRelated

Hypothesis



Multiple components in the VEGF, PAS and chemokine pathways are targets for ToxCast™ chemicals

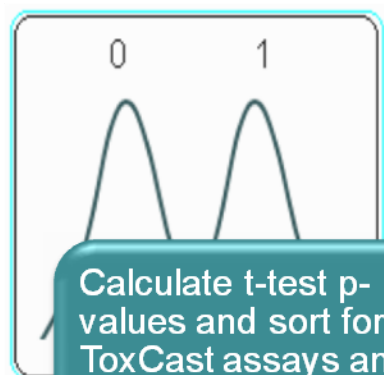
corollary 1:

in vitro bioactivity profiles can be used to mine a ‘toxicity signature’ for *in vivo* vascular development

corollary 2:

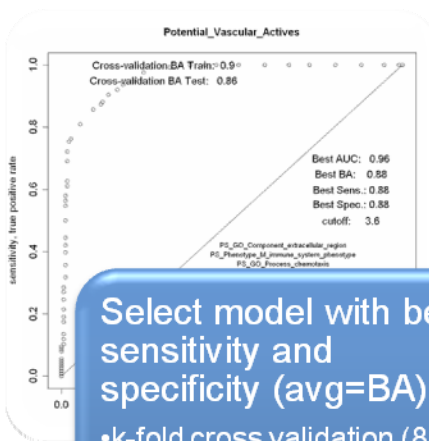
in vitro signature can inform mechanistic models during chemical disruption of embryonic vascular development

Multivariate Machine Learning Analysis



Calculate t-test p-values and sort for all ToxCast assays and Pathway Scores

- PS: Kegg, Ingenuity, GO, Mouse Phenotypes, Pathway Commons, etc.



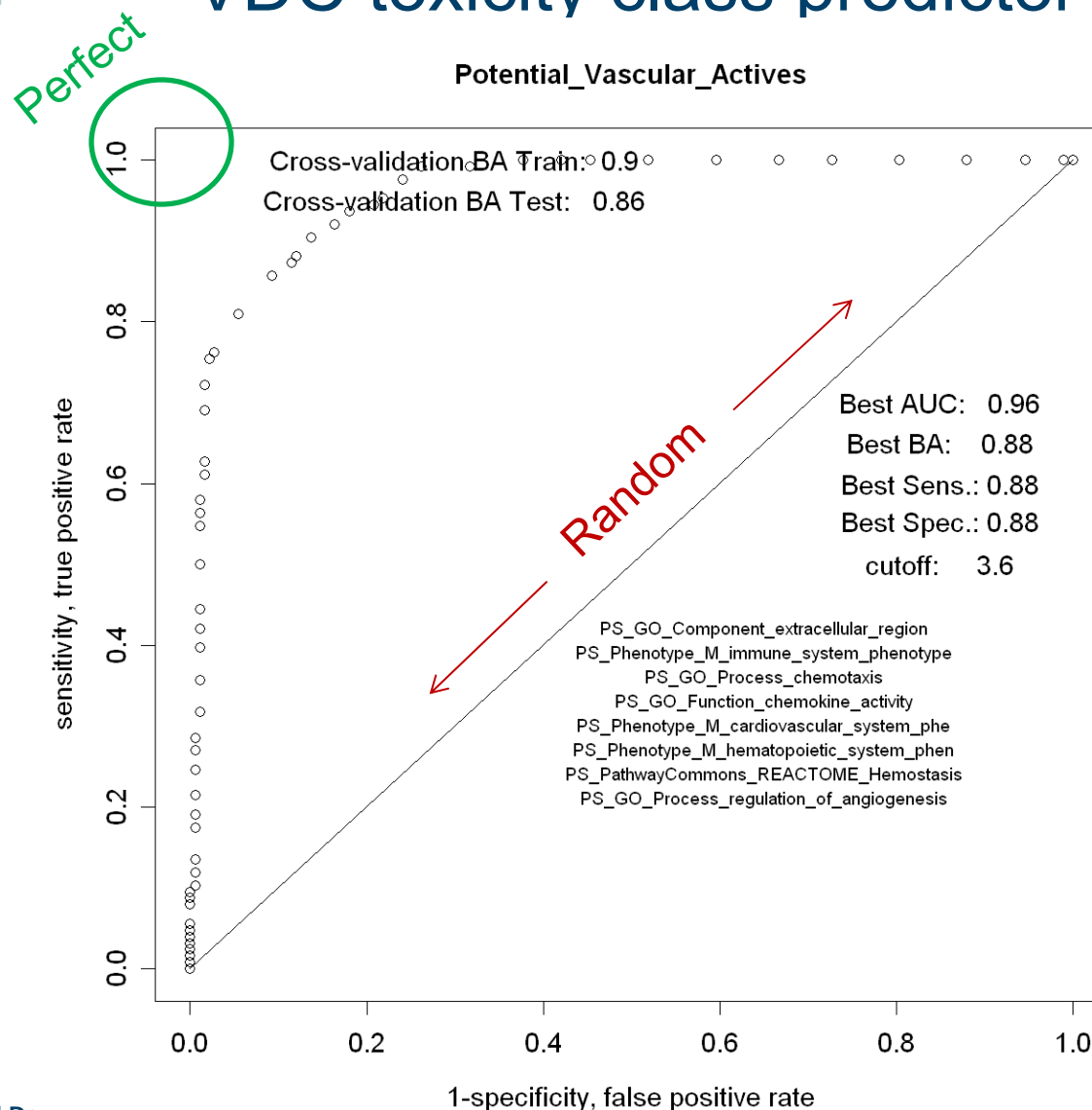
Select model with best sensitivity and specificity (avg=BA)

- k-fold cross validation (80% training, 20% test for each iteration)
- Calculate ROC curve as function of number of features included and cutoff value (hit vs. no-hit)



Multivariate Signature allows for prediction of toxicity endpoints based on *in vitro* assay results and Pathway Scores

Pathway-level signature: VDC toxicity class predictor





True Positives: defined as chemicals with weighted assay score > mean for 6 targets (*VEGFR2*, *Tie2*, *CCL2*, *CXCL10*, *uPAR*, *PAI-1*)

Predicted Positives: chemicals belonging to the toxicity class defined by pathway disruption.

- GO: *processes* = regulation of angiogenesis, chemotaxis; *component* = extracellular region; *molecular function* = chemokine activity
- Mouse Phenotype: cardiovascular, hematopoietic, immune system
- PathwayCommons: Hemostasis



“False Negatives”



Chemical Name	Predicted Positives (MLR Pathway Signature)	Training Set (Weighted Assay Score)	RAT DEV Phentoype	RABBIT DEV Phentoype
Acephate	0	1	;DEV_Rat_Skeletal_Appendicular;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Acifluorfen	0	1	No Developmental Rat Study in ToxRefDB	No Developmental Rabbit Study in ToxRefDB
Atrazine	0	1	;DEV_Rat_Orofacial_JawHyoid;DEV_Rat_PregnancyRelated_MaternalPregLoss;DEV_Rat_Skeletal_Cranial	;DEV_Rabbit_General_GeneralFetalPathology;DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss
Bentazone	0	1	No Developmental Rat Study in ToxRefDB	No Developmental Rabbit Study in ToxRefDB
Carboxin	0	1	No recorded effect	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Clodinafop-propargyl	0	1	;DEV_Rat_General_FetalWeightReduction;DEV_Rat_General_GeneralFetalPathology;DEV_Rat_Skeletal_Appendicular;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Cyanazine	0	1	No recorded effect	No Developmental Rabbit Study in ToxRefDB
Cypermethrin	0	1	No recorded effect	No recorded effect
Diclosulam	0	1	No recorded effect	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Dicrotophos	0	1	No recorded effect	;DEV_Rabbit_General_FetalWeightReduction;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Flufenpyr-ethyl	0	1	No recorded effect	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Fluroxypyr	0	1	;DEV_Rat_PregnancyRelated_MaternalPregLoss	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Fosthiazate	0	1	No recorded effect	No recorded effect
Isoxaben	0	1	;DEV_Rat_General_GeneralFetalPathology;DEV_Rat_PregnancyRelated_EmbryoFetalLoss	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Myclobutanil	0	1	;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Perfluorooctanoic acid	0	1	No Developmental Rat Study in ToxRefDB	No Developmental Rabbit Study in ToxRefDB
Propazine	0	1	;DEV_Rat_General_FetalWeightReduction;DEV_Rat_General_GeneralFetalPathology;DEV_Rat_Orofacial_JawHyoid;DEV_Rat_Skeletal_Axial;DEV_Rat_Skeletal_Cranial	No recorded effect
Simazine	0	1	;DEV_Rat_General_GeneralFetalPathology;DEV_Rat_Skeletal_Axial;DEV_Rat_Skeletal_Cranial	;DEV_Rabbit_General_FetalWeightReduction;DEV_Rabbit_General_GeneralFetalPathology;DEV_Rabbit_Skeletal_Axial
Tebufenozide	0	1	No recorded effect	No recorded effect

"False Positives"



Chemical Name	Predicted Positives (MLR Pathway Signature)	Training Set (Weighted Assay Score)	RAT_DEV_Phentoype	RABBIT_DEV_Phentoype
3-Iodo-2-propynylbutylcarbamate	1	0	;DEV_Rat_General_FetalWeightReduction;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Amitraz	1	0	;DEV_Rat_PregnancyRelated_EmbryoFetalLoss	No Developmental Rabbit Study in ToxRefDB
Bendiocarb	1	0	;DEV_Rat_PregnancyRelated_EmbryoFetalLoss	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Captan	1	0	No Developmental Rat Study in ToxRefDB	;DEV_Rabbit_General_FetalWeightReduction;DEV_Rabbit_General_GeneralFetalPathology;DEV_Rabbit_Neurosensory_Brain;DEV_Rabbit_Neurosensory_Eye;DEV_Rabbit_Orofacial_JawHyoid;DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss;DEV_Rabbit_Skeletal_Appendicular;DEV_Rabbit_Skeletal_Axial;DEV_Rabbit_Skeletal_Cranial;DEV_Rabbit_Trunk_BodyWall;DEV_Rabbit_Trunk_SplanchnicViscera
Chlorpyrifos-methyl	1	0	No recorded effect	No Developmental Rabbit Study in ToxRefDB
Clofentezine	1	0	No recorded effect	No Developmental Rabbit Study in ToxRefDB
Clorophene	1	0	No recorded effect	;DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss
d-cis,trans-Allethrin	1	0	;DEV_Rat_PregnancyRelated_MaternalPregLoss	No Developmental Rabbit Study in ToxRefDB
Dibutyl phthalate	1	0	;DEV_Rat_General_FetalWeightReduction;DEV_Rat_Orofacial_CleftLipPalate;DEV_Rat_PregnancyRelated_EmbryoFetalLoss;DEV_Rat_PregnancyRelated_MaternalPregLoss	No Developmental Rabbit Study in ToxRefDB
Fipronil	1	0	No recorded effect	No recorded effect
Fluazinam	1	0	;DEV_Rat_General_FetalWeightReduction;DEV_Rat_General_GeneralFetalPathology;DEV_Rat_Orofacial_CleftLipPalate;DEV_Rat_Orofacial_JawHyoid;DEV_Rat_PregnancyRelated_EmbryoFetalLoss;DEV_Rat_Skeletal_Appendicular;DEV_Rat_Skeletal_Axial;DEV_Rat_Skeletal_Cranial;DEV_Rat_Trunk_BodyWall;DEV_Rat_Urogenital_Renal;DEV_Rat_Urogenital_Ureteric	;DEV_Rabbit_General_GeneralFetalPathology;DEV_Rabbit_PregnancyRelated_EmbryoFetalLoss;DEV_Rabbit_PregnancyRelated_MaternalPregLoss;DEV_Rabbit_Skeletal_Axial;DEV_Rabbit_Skeletal_Cranial
Formetanate hydrochloride	1	0	No recorded effect	No recorded effect
Metam-sodium hydrate	1	0	No Developmental Rat Study in ToxRefDB	No Developmental Rabbit Study in ToxRefDB
Milbemectin (mix of >70 percent Milbemycin)	1	0	No Developmental Rat Study in ToxRefDB	No Developmental Rabbit Study in ToxRefDB
Pirimiphos-methyl	1	0	No Developmental Rat Study in ToxRefDB	No recorded effect
Quintozene	1	0	;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_General_FetalWeightReduction;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
TCMTB	1	0	;DEV_Rat_PregnancyRelated_EmbryoFetalLoss;DEV_Rat_Skeletal_Appendicular;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss
Tefluthrin	1	0	;DEV_Rat_General_GeneralFetalPathology;DEV_Rat_Skeletal_Appendicular	;DEV_Rabbit_Skeletal_Axial
Thiophanate-methyl	1	0	No Developmental Rat Study in ToxRefDB	;DEV_Rabbit_PregnancyRelated_MaternalPregLoss;DEV_Rabbit_Skeletal_Appendicular;DEV_Rabbit_Skeletal_Axial
Triadimenol	1	0	;DEV_Rat_Skeletal_Axial;DEV_Rat_Urogenital_Renal;DEV_Rat_Urogenital_Ureteric	No recorded effect
Tri-allate	1	0	;DEV_Rat_General_FetalWeightReduction;DEV_Rat_Orofacial_CleftLipPalate;DEV_Rat_Skeletal_Axial;DEV_Rat_Skeletal_Cranial	No Developmental Rabbit Study in ToxRefDB

No Developmental Effect Recorded in ToxRefDB



Chemical Name	Predicted Positives (MLR Pathway Signature)	True Positives (Weighted Assay Score)	RAT_DEV_Phentoype	RABBIT_DEV_Phentoype
Benfluralin	1	1	No recorded effect	No recorded effect
Dithiopyr	1	1	No recorded effect	No recorded effect
Esfenvalerate	1	1	No recorded effect	No recorded effect
MGK	1	1	No recorded effect	No recorded effect
Tribufos	1	1	No recorded effect	No recorded effect
Triclosan	1	1	No recorded effect	No recorded effect
Zoxamide	1	1	No recorded effect	No recorded effect
Cypermethrin	0	1	No recorded effect	No recorded effect
Fosthiazate	0	1	No recorded effect	No recorded effect
Tebufenozide	0	1	No recorded effect	No recorded effect
Fipronil	1	0	No recorded effect	No recorded effect
Formetanate hydroch	1	0	No recorded effect	No recorded effect

Potential VDCs Lacking Developmental Data



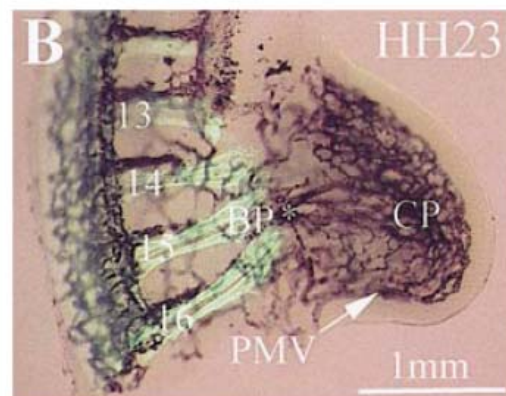
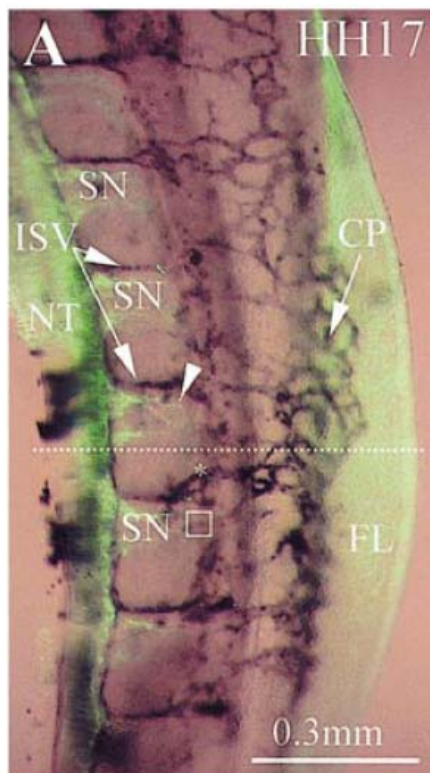
Chemical Name	Predicted Positives (MLR Pathway Signature)	True Positives (Weighted Assay Score)	RAT_DEV_Phentoype	RABBIT_DEV_Phentoype
2,2-Bis(4-hydroxyphenyl)-1,1,1-trichloroethane	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Abamectin	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Bromoxynil	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Captafol	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Chlorpyrifos oxon	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Coumaphos	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Diazoxon	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Fluroxypyr-meptyl	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Forchlorfenuron	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Imazalil	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Methoxychlor	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Methyl hydrogen phthalate	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Niclosamide	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Oxytetracycline dihydrate	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Parathion	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Prochloraz	1	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Acifluorfen	0	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Bentazone	0	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Perfluorooctanoic acid	0	1	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Metam-sodium hydrate	1	0	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB
Milbemectin (mix of >70 percent Milbemycin A)	1	0	No Dev Rat Study in ToxRefDB	No Dev Rabbit Study in ToxRefDB

Summary

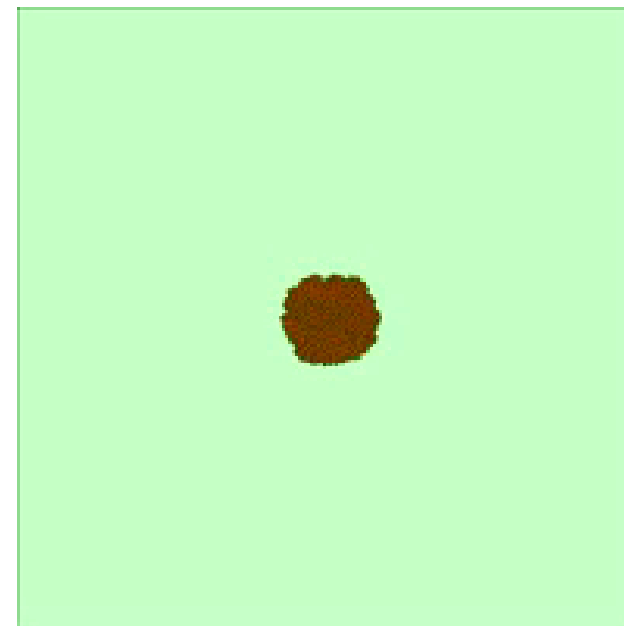


- HTS data is used to develop an *in vitro* toxicity signature for “Vascular Disruptor Chemicals” (VDCs)
- Broad pre-natal developmental activity correlated in ToxRefDB with putative VDCs
- *in vitro* signature will inform mechanistic models (computational and experimental) during chemical disruption of embryonic vascular development

Computational (*in silico*): vasculogenesis model



Bates, Taylor, and Newgreen (2002) The Pattern of Neurovascular Development in the Forelimb of the Quail Embryo. *Dev Bio* 249:300-320

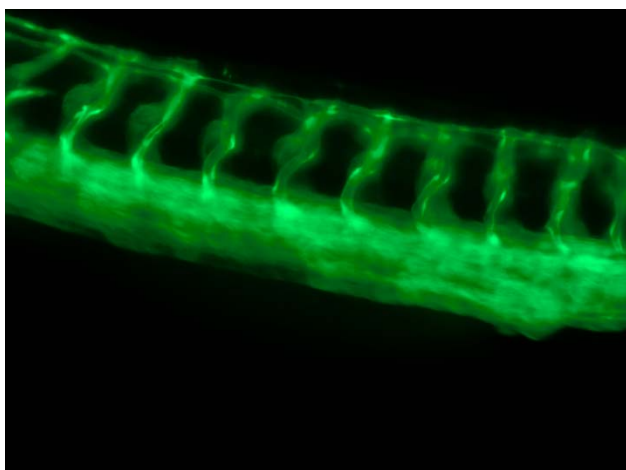


Merks, Perryn, Shirinifard, and Glazier (2008) Contact-Inhibited Chemotaxis in De Novo and Sprouting Blood-Vessel Growth. *PLoS Comput Biol* 4(9): e1000163

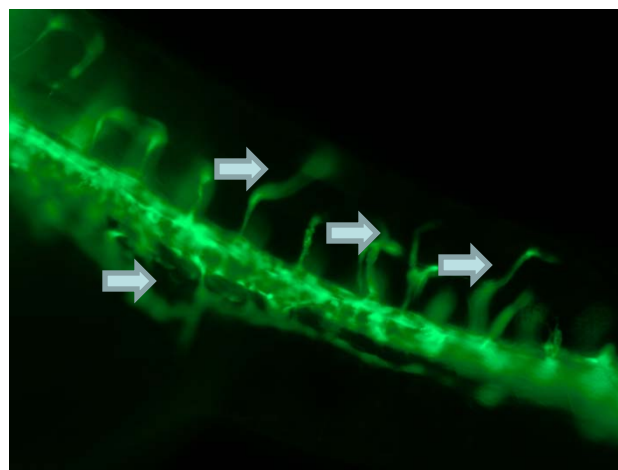
Zebrafish Vascular System (Fli1 marker)



72 h wild type (20x)



72 h treated 1 mg/ml (20x)



*Unpublished Images Courtesy of Maria
Bondesson, TIVS & Catherine
McCollum, UH*

Intersomitic vessel disruption is apparent in treated group

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