

# Computational embryology as an integrative platform for predictive DART

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*Symposium: Update on Integrated Approaches to Testing and Assessment (IATA) for DART*  
45th Conference of the European Teratology Society, September 7, 2017, Budapest

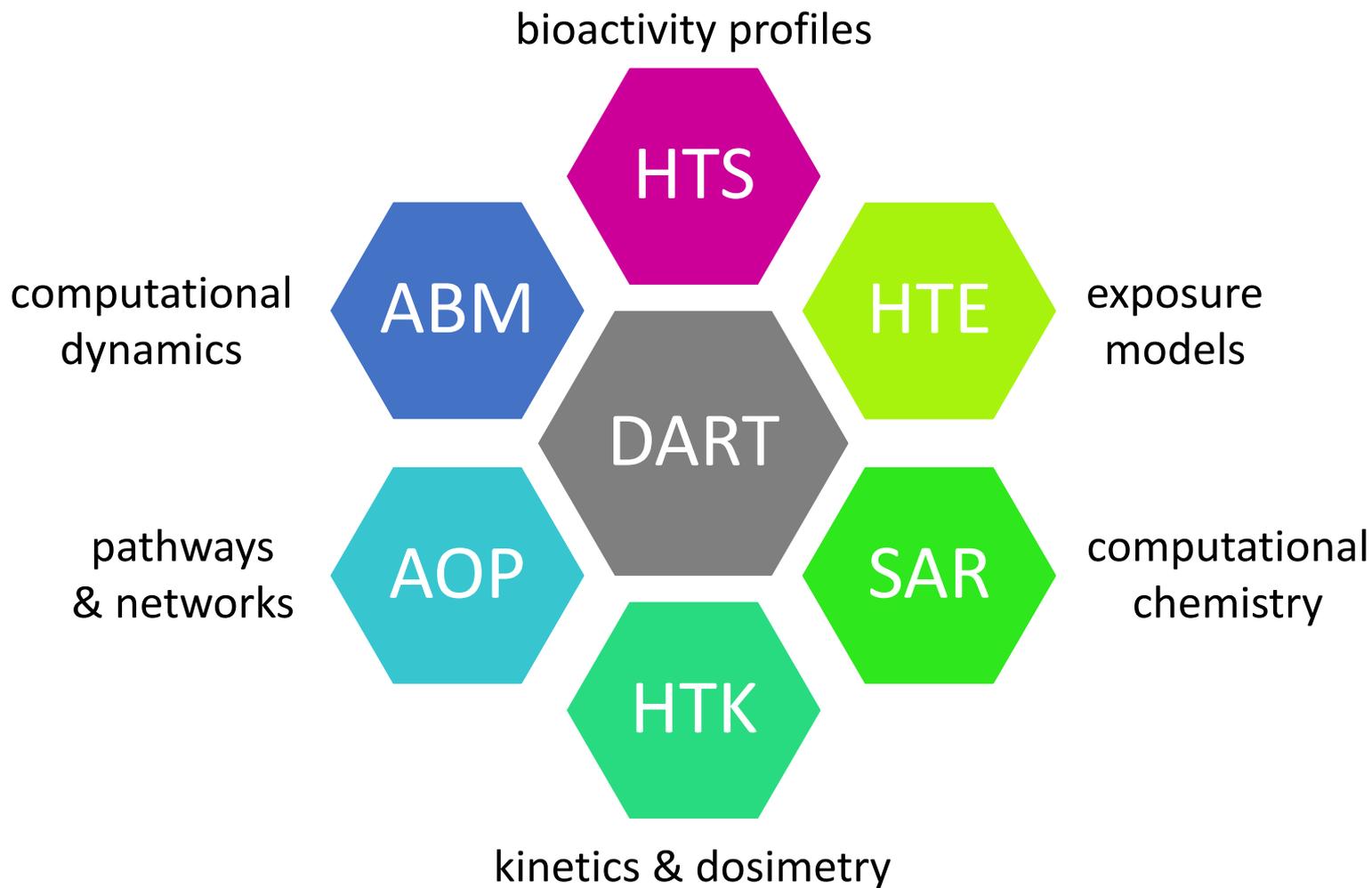
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# Mechanistically-informed IATAs for DART:

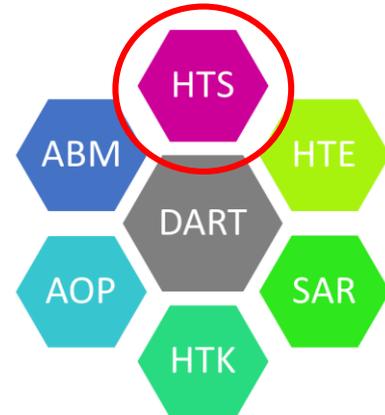
*opportunities and challenges*

- Advances in biomedical, engineering, and computational sciences enable rapid and cost-effective profiling of large chemical libraries.
- Considerable mechanistic knowledge exists about embryogenesis but must be collected, synopsized, and assimilated into AOPs.
- AOP-based IATAs will have a well-defined endpoint, purpose, rationale, information stream, organization, and uncertainty.
- Computational models to support regulatory application: weight-of-evidence and guidance to hypothesis-based data generation.

# IATA synthesis and integration



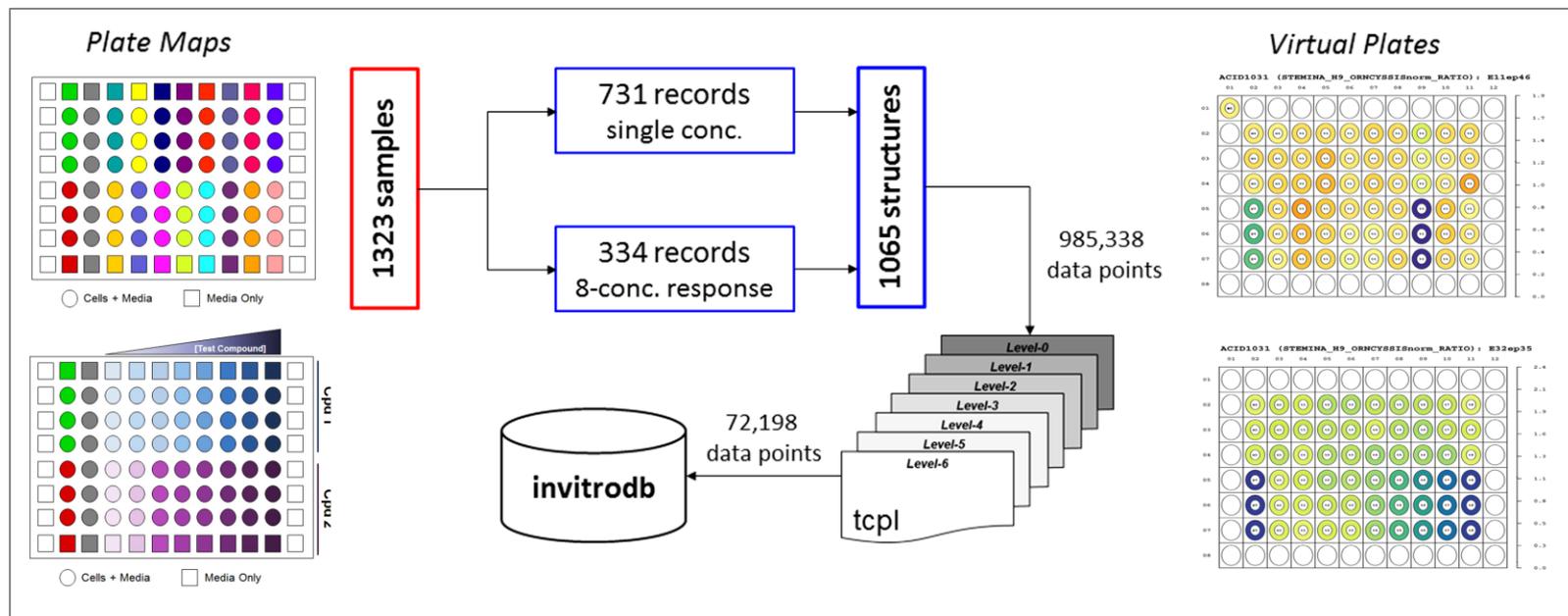
# High-throughput screening



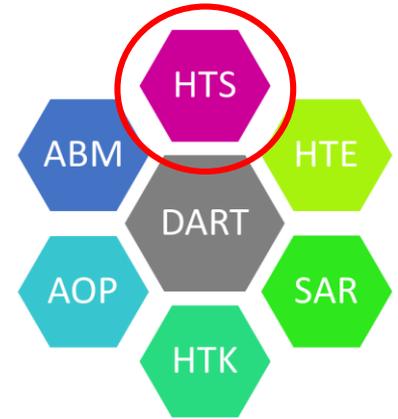
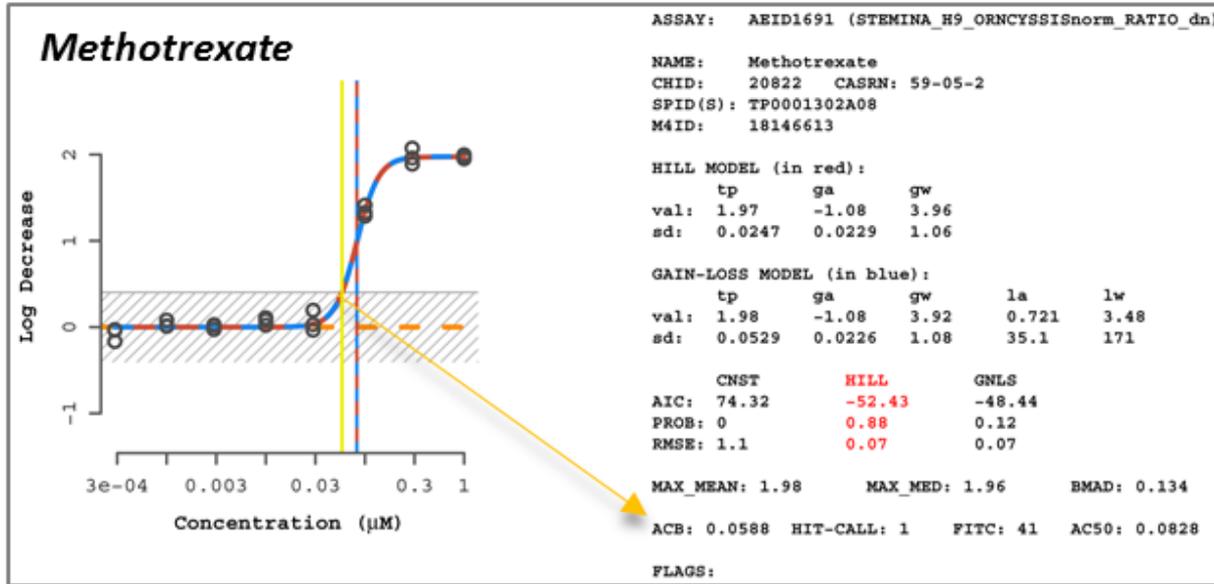
- Large data streams from high-throughput profiling of chemicals for *in vitro* bioactivity (ToxCast/Tox21).

<https://actor.epa.gov/dashboard/#Chemicals>

- New platform: ToxCast library (1065 chemicals) tested in a pluripotent H9 human embryonic stem cell assay.



# ToxCast STM platform



**INPUT:** ratio of *ornithine* secreted to *cystine* utilized, by LC-MS-MS.

**OUTPUT:** exposure-based 'Teratogen Index' (TI) = 0.06 μM for MTX.

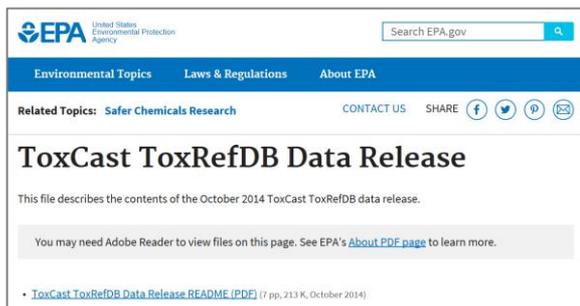
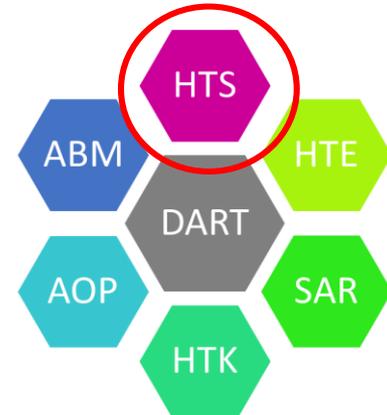
- 181 of 1065 ToxCast chemicals (17% tested) gave a positive 'hit'.
- 83% accurate classifying conventional DevTox anchors (n = 30).

Anchor	TI (μM)	Class
all-trans-Retinoic acid	0.003	TP
Cytarabine hydrochloride	0.054	TP
Methotrexate	0.059	TP
Diphenhydramine hydrochloride	0.588	TP
Thalidomide	1.267	TP
5-Fluorouracil	2.021	TP
Carbamazepine	2.294	TP
Busulfan	2.313	TP
Amiodarone hydrochloride	5.101	TP
Dexamethasone sodium phosphate	37.680	TP
Hydroxyurea	74.935	TP
Valproic acid	154.955	TP
MEHP	166.595	TP
Salicylic acid	513.436	TP
Rifampicin	2.464	FP
5,5-Diphenylhydantoin	1000000	FN
Boric acid	1000000	FN
Cyclophosphamide monohydrate	1000000	FN
Ethylene glycol	1000000	FN
1,2-Propylene glycol	246664	TN
Acrylamide	1000000	TN
Aspirin	1000000	TN
Butylparaben	1000000	TN
Caffeine	1000000	TN
D-Camphor	1000000	TN
Dimethyl phthalate	1000000	TN
Isoniazid	1000000	TN
Retinol	1000000	TN
Saccharin	1000000	TN
Sodium L-ascorbate	1000000	TN

## Common DevTox Anchors

TP	14
FP	1
FN	4
TN	11
n	30
Sensitivity	0.778
Specificity	0.917
Accuracy	83.3%
Mathew's cc	0.680
F1 score	0.47

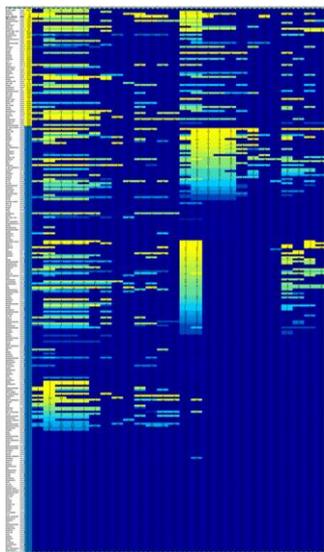
# Performance of STM vs ToxRefDB fetal endpoints



<https://www.epa.gov/chemical-research/toxcast-toxrefdb-data-release>

**INPUT:** concordance anchor for 272 chemicals tested in pregnant rats & rabbits

- positives = dLEL  $\leq$  125 mg/kg/d in both species
  - negatives = no dLEL  $\geq$  1000 mg/day in both species
- }  $n = 146$  compounds



ToxRefDB\_fetal Anchor

TP	9
FP	19
FN	15
<u>TN</u>	<u>103</u>
n	146
Sensitivity	0.375
Specificity	0.844
<b>Accuracy</b>	<b>76.7%</b>
Mathew's cc	0.206
F1 score	0.005

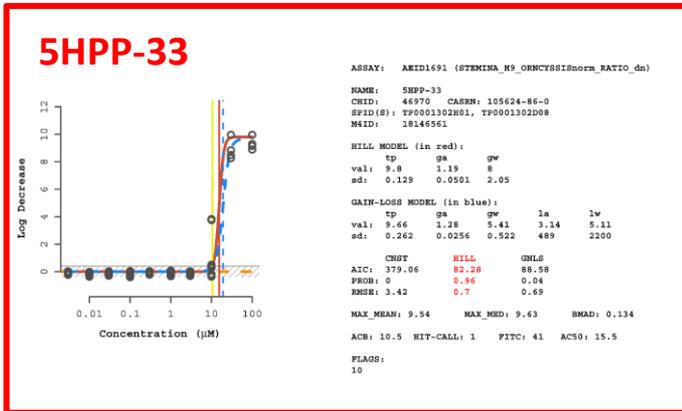
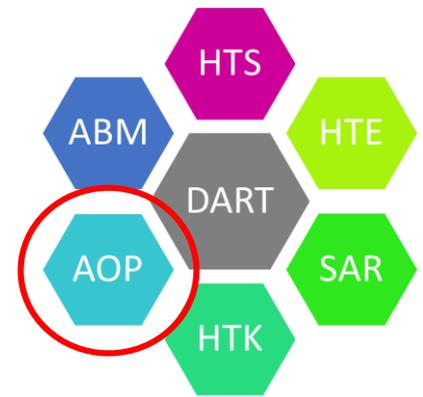
**OUTPUT:**

- 77% accurate classifying 146 compounds concordant in rat-rabbit studies.
- Strong NPV (high specificity) but weak PPV (low sensitivity).
- Machine learning with 865 ToxCast assays to find missing sensitivity?

# Vascular Disruption (pVDCs)

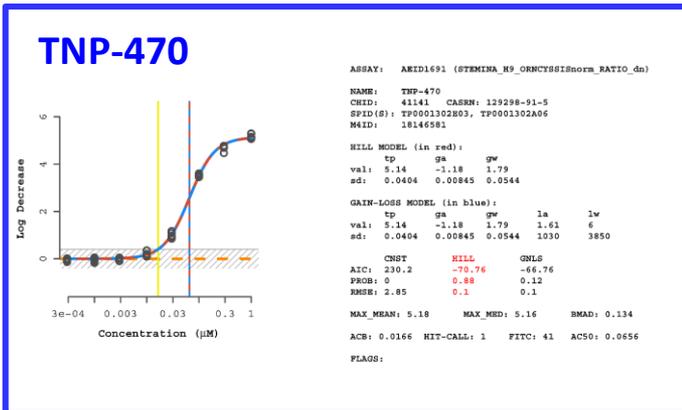
**INPUT:** exposure-based TI predicted by the STM assay.

**OUTPUT:** margin between hazard prediction and rat WEC effect.



TI = 10.48  $\mu\text{M}$

AC50 (embryo lethality) = 21.2  $\mu\text{M}$

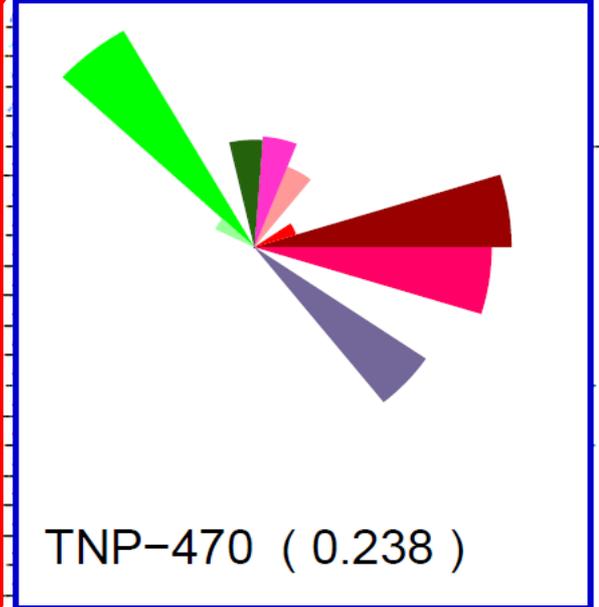
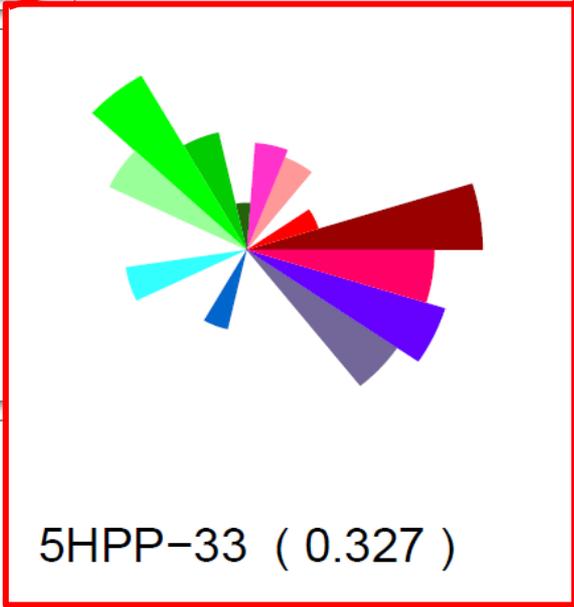
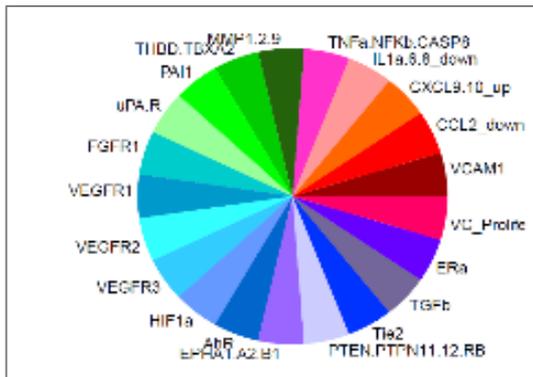
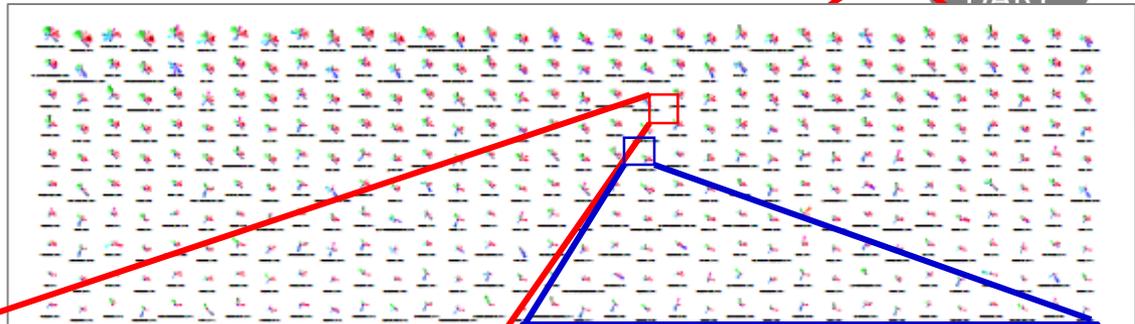
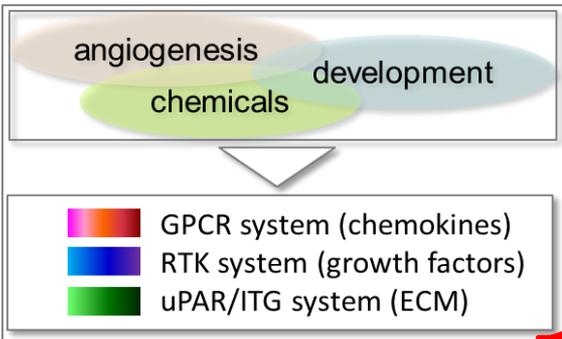
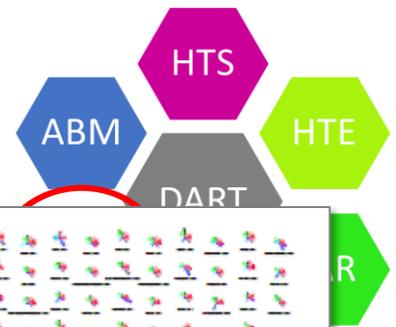


TI = 0.017  $\mu\text{M}$



AC50 (dysmorphogenesis) = 0.038  $\mu\text{M}$

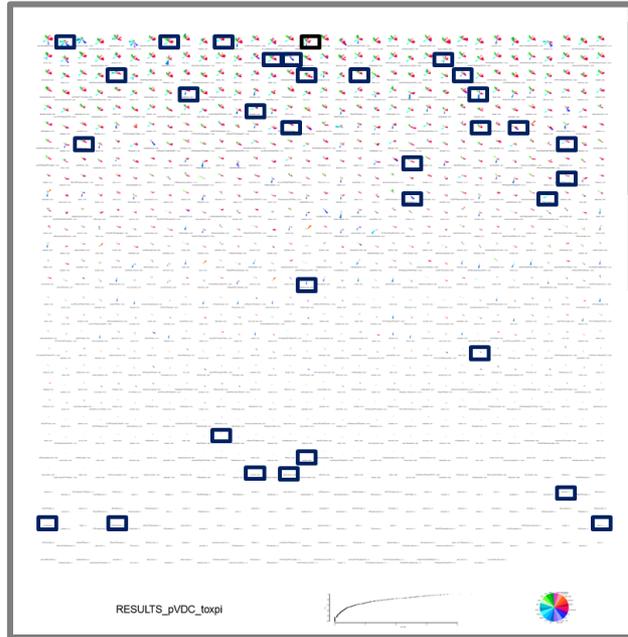
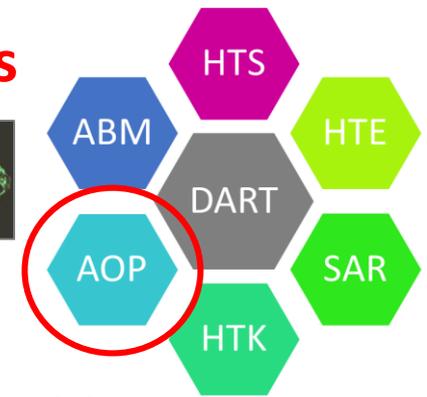
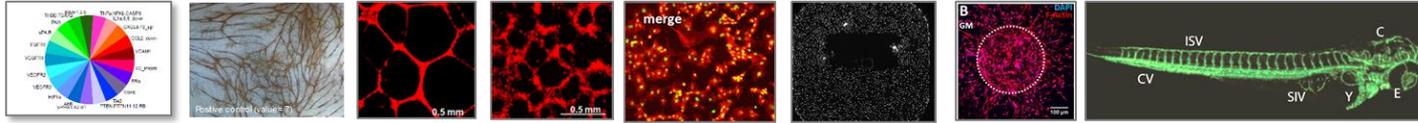
# AOP-based chemical prioritization



<http://epa.gov/ncct/ToxPi>  
<http://comptox.unc.edu/toxpi.php>



# Qualification against 8 diverse angiogenesis platforms



	A	B	C	D	E	F	G	H	I	J
Decane	inactive									
1,2,3-Trichloropropane	inactive									
Pymetrozine	inactive									
Methimazole	inactive									
Imazamox	inactive									
D-Mannitol	inactive									
Methylparaben	inactive									
Valproic acid	inactive									
Tris(2-ethylhexyl) phosphate	inactive									
PFOS	inactive									
1,2,4-Trichlorobenzene	inactive									
Methanolamine	inactive									
INP-470	inactive									
Sodium dodecylbenzenesulfonate	inactive									
4-Nonylphenol, branched	inactive									
Tris(2-chloroethyl) phosphate	inactive									
2,4-Diaminotoluene	inactive									
Tris(1,3-dichloro-2-propyl)phosphate	inactive									
Oxytetracycline dihydrate	inactive									
Celecoxib	inactive									
Quercetin	inactive									
C.I. Solvent Yellow 14	inactive									
Triclosan	inactive									
Bisphenol AF	inactive									
Docosate sodium	inactive									
tert-Butylhydroquinone	inactive									
Haloperidol	inactive									
Cladribine	inactive									
Triclocarban	inactive									
Pyridaben	inactive									
1-Hydroxypyrene	inactive									
Disulfiram	inactive									
Fluzinam	inactive									
Bisphenol A	inactive									
Phenolphthalein	inactive									
UCYI galact...	inactive									
SHPP-33	inactive									

- A ToxPi [1]
- B FICAM tubulogenesis [2]
- C Synthetic tubulogenesis [3]
- D Matrigel tubulogenesis [3]
- E nuCTNB [4]
- F EC migration [4]
- G angiogenic sprouting [5]
- H TG-zebrafish [1]
- I Vala tubulogenesis [2]
- J aggregate (B to I)

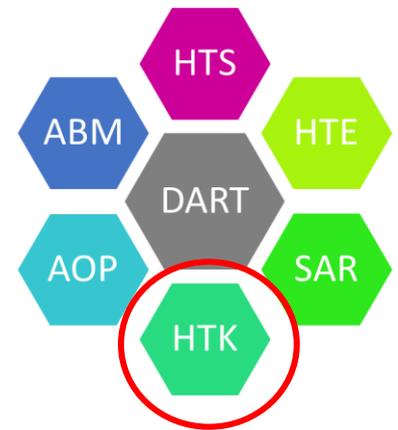
**Predicted (ToxCast)**

inactive  
active

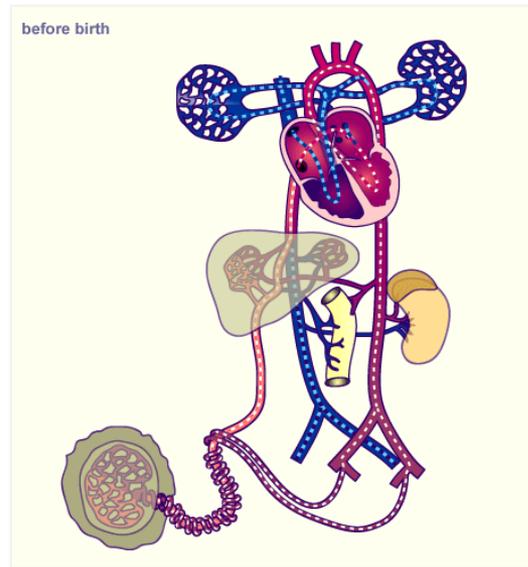
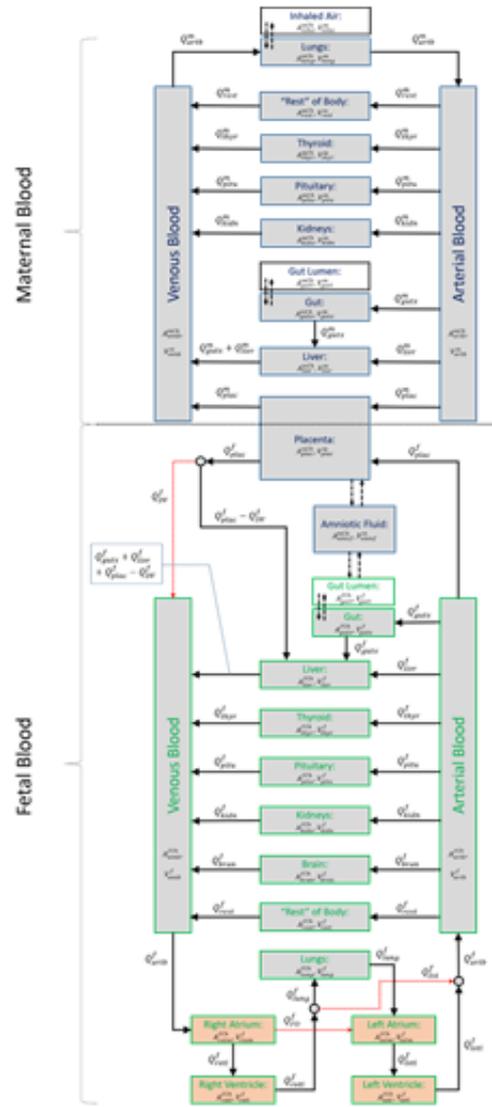
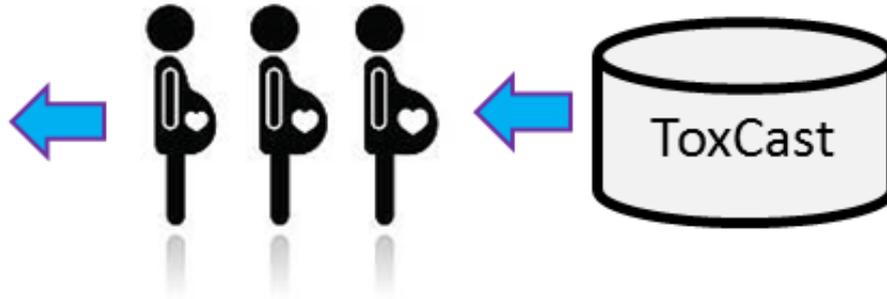
**Empirical (angiogenesis)**

[1] Tal et al. *Reprod Toxicol* (2017); [2] Knudsen et al., *in prep*; [3] Nguyen et al. *Nature Bioengineering* (2017); [4] Belair et al. (2016) *Acta Biomaterialia*.

# High-throughput kinetics

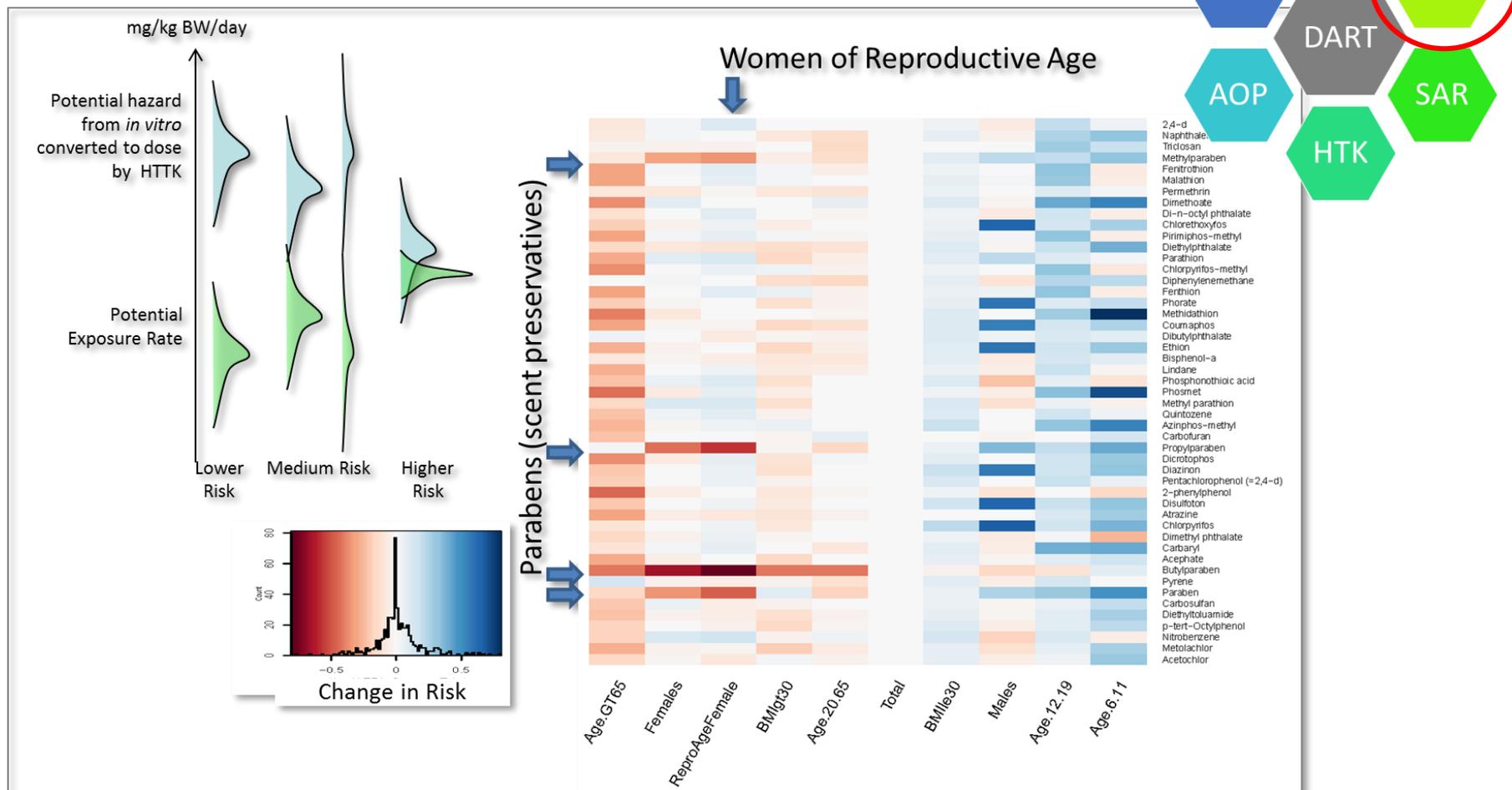


## Comprehensive HTTK Pregnancy Model



- **INPUT:** metrics from human pregnancy (> 1<sup>st</sup> trimester), SBML solver in Tellurium.
- **OUTPUT:** parameterized to run 585 ToxCast chemicals for fetal dosimetry.

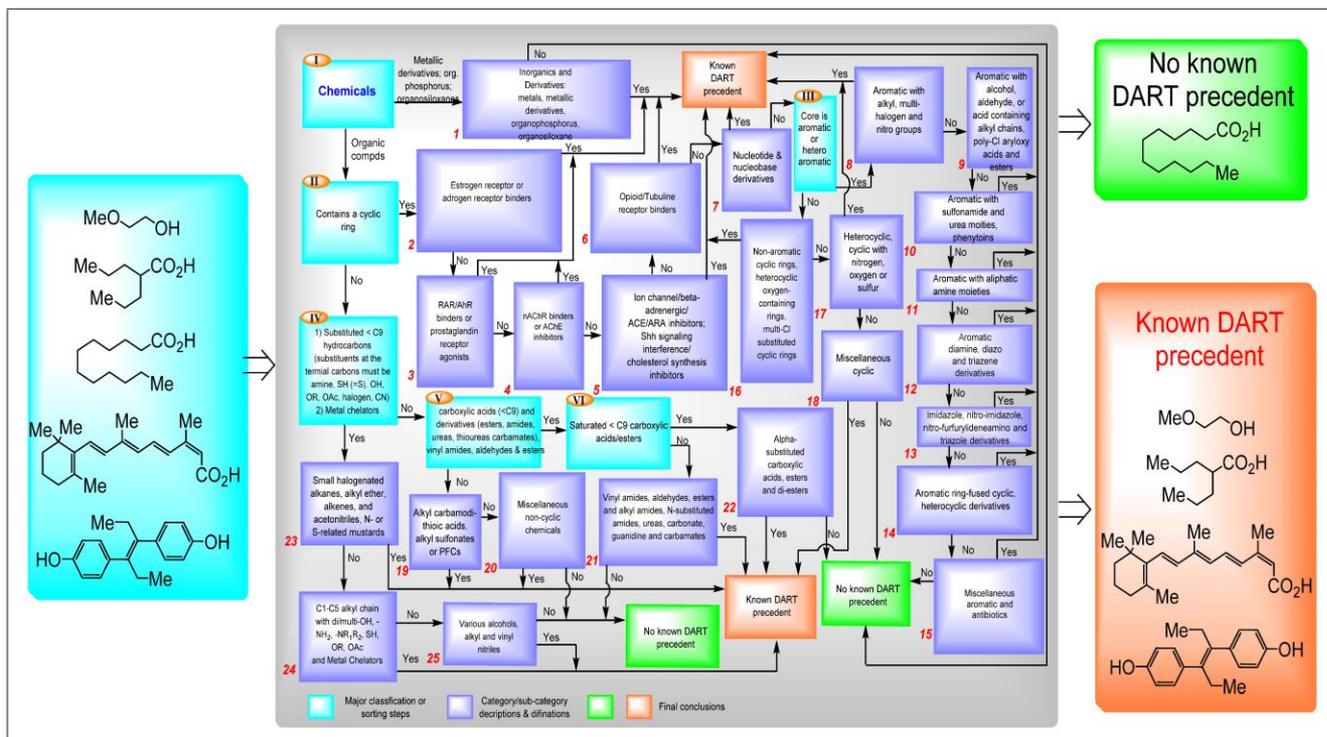
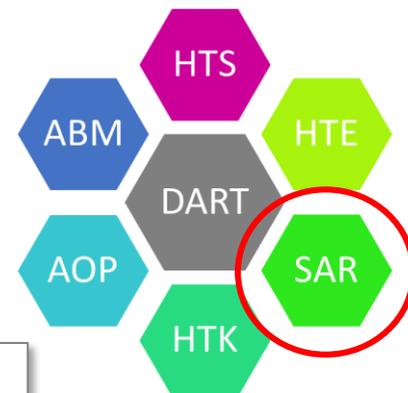
# High-throughput exposure ratio



**INPUT:** HTS data on bioactivity profiles (eg, AC50) and exposure for specific subpopulations.  
**OUTPUT:** margin between bioactivity and exposure for WOCBP.

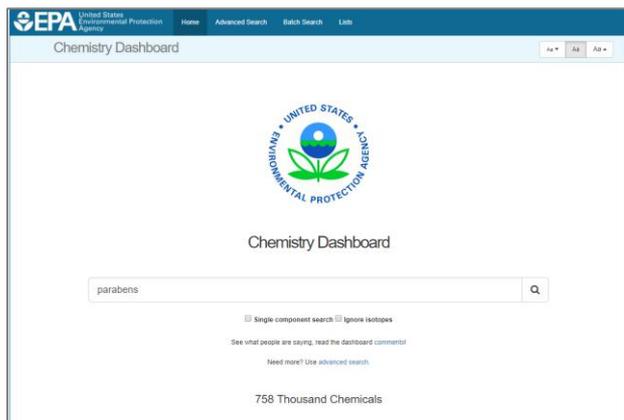
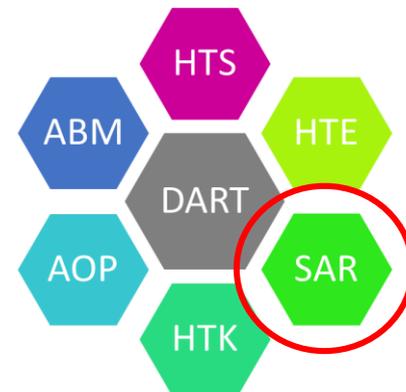
# Structure-activity relationships

**INPUT:** 716 chemicals with DART endpoints grouped into receptor binding and chemical domains (example – 5 parabens).

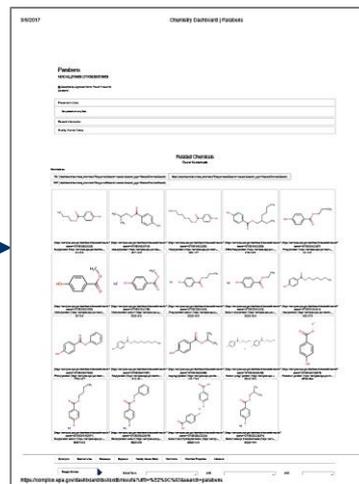


**OUTPUT:** classification tree based on whether or not a chemical has receptor-binding properties and structural features consistent with known DART.

# Mining the literature for SAR information



INPUT: 'parabens'



<https://comptox.epa.gov/dashboard>

OUTPUT: all literature on 19 'parabens' and 'embryotoxicity'

Select Term: Embryo and embryonic development

Retrieve Articles 0 Articles

Add additional query terms to filter abstracts:

toxic placenta rat

Edit the Query Before Retrieving Articles

("NOCAS\_879989" OR "Parabens") AND (embryo OR Embryonic Structures OR fetus OR Embryonic and Fetal Development)

Search and Count

toxic	placenta	rat	Total	PMID	PubYr	Title
5	0	0	5	27775672	2016	Screening the Toxicity of Selected Personal Care Products Using Embryo Bioassays: 4-MBC, Propylparaben and Triclocarban.
3	0	0	3	24095706	2013	Embryonic exposure of medaka ( <i>Oryzias latipes</i> ) to propylparaben: effects on early development and post-hatching growth.
2	0	3	5	28324817	2017	Developmental toxicity and induction of vitellogenin in embryo-larval stages of zebrafish ( <i>Danio rerio</i> ) exposed to methyl Paraben.
2	0	8	10	22372636	2012	Mixtures of endocrine disrupting contaminants modelled on human high end exposures: an exploratory study in rats.
2	0	3	5	15334527	2004	Developmental toxicity evaluation of butylparaben in Sprague-Dawley rats.

Record: 1 of 44

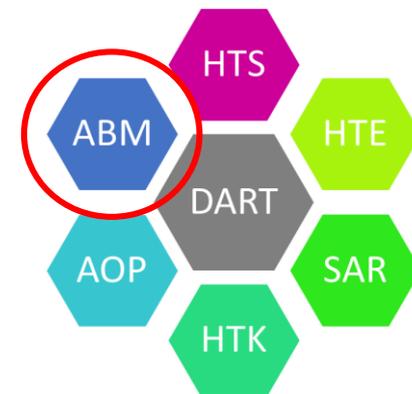
Title: Screening the Toxicity of Selected Personal Care Products Using Embryo Bioassays: 4-MBC, Propylparaben and Triclocarban.

Abstract: Recently, several emerging pollutants, including Personal Care Products (PCPs), have been detected in aquatic ecosystems, in the ng/L or µg/L range. Available toxicological data is limited, and, for certain PCPs, evidence indicates a potential risk for the environment. Hence, there is an urgent need to gather ecotoxicological data on PCPs as a proxy to improve risk assessment. Here, the toxicity of three different PCPs (4-Methylbenzylidene Camphor (4-MBC), propylparaben and triclocarban) was tested using embryo bioassays with *Danio rerio* (zebrafish) and *Paracentrotus lividus* (sea urchin). The No Observed Effect Concentration (NOEC) for triclocarban was 0.256 µg/L for sea urchin and 100 µg/L for zebrafish, whereas NOEC for 4-MBC was 0.32 µg/L for sea urchin and 50 µg/L for zebrafish. Both PCPs impacted embryo development at environmentally relevant concentrations. In comparison with triclocarban and 4-MBC, propylparaben was less toxic for both sea urchin (NOEC = 160 µg/L) and zebrafish (NOEC = 1000 µg/L). Overall, this study further demonstrates the sensitivity of embryo bioassays as a high-throughput approach for testing the toxicity of emerging pollutants.

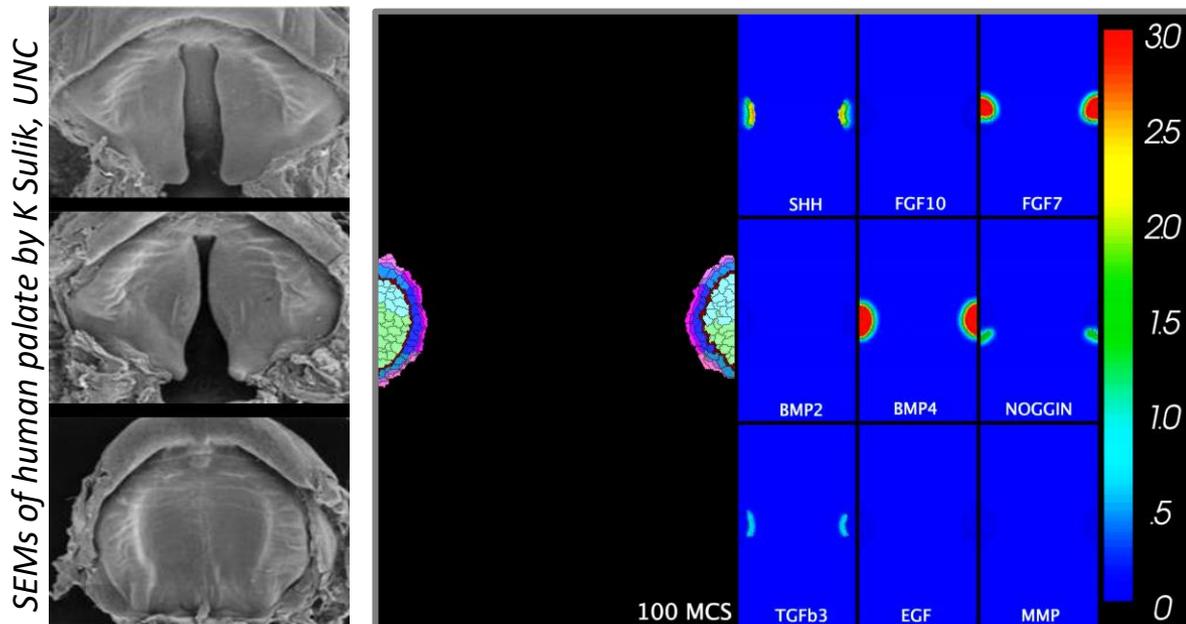
# Agent-Based Models

Executed with [CompuCell3D.org](http://CompuCell3D.org) modeling environment

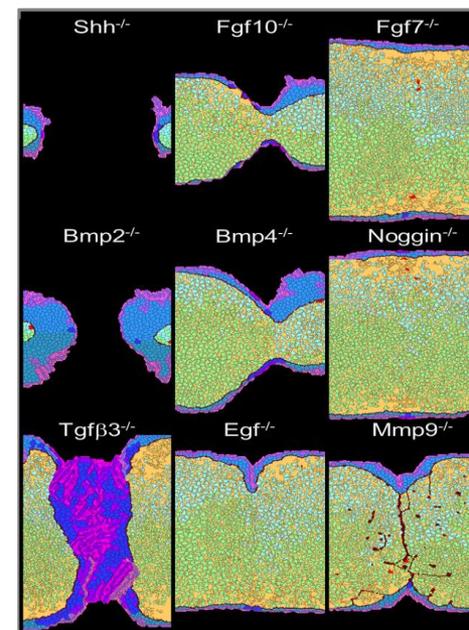
- *reconstruct development cell-by-cell, interaction-by-interaction*
- *pathogenesis following electronic knockdown (cybermorphs)*
- *impute ToxCast data into a computer simulation (example – palate)*
- *return quantitative predictions of where, when and how the defect arises.*



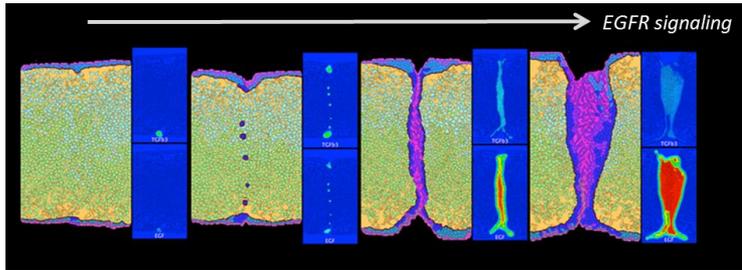
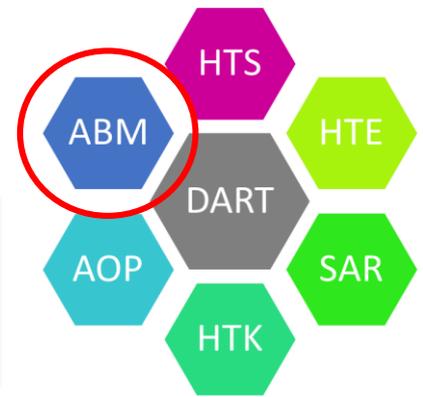
Palatal fusion in a virtual system



Cybermorphs

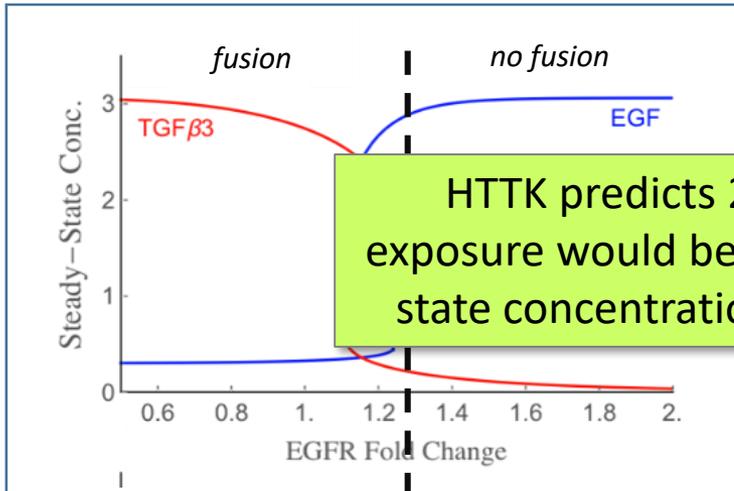


# Imputing ToxCast data

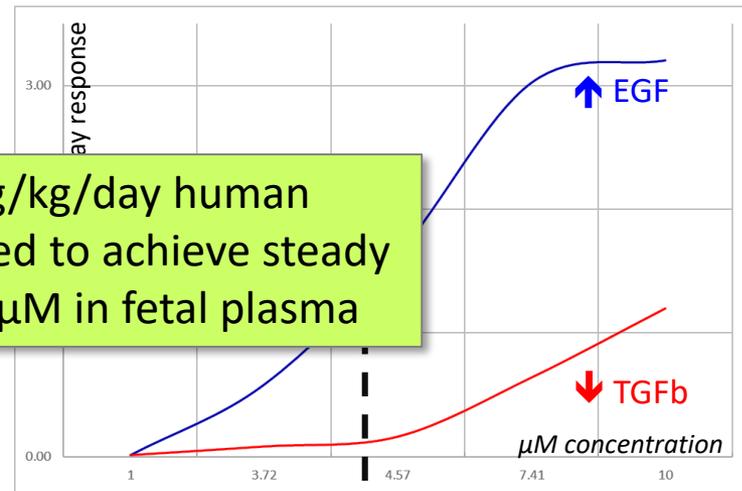


**Captan → cleft palate**  
 ToxRefDB NOAEL = 10 mg/kg/day (rabbit)  
 LOAEL = 30 mg/kg/day

**INPUT:** ToxCast HTS data



HTTK predicts 2.39 mg/kg/day human exposure would be required to achieve steady state concentration of 4 μM in fetal plasma

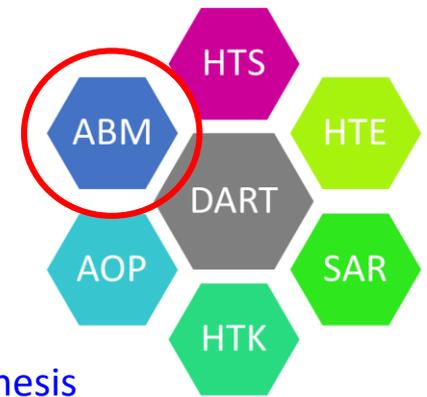


**OUTPUT:** tipping point predicted by computational dynamics (hysteresis switch)

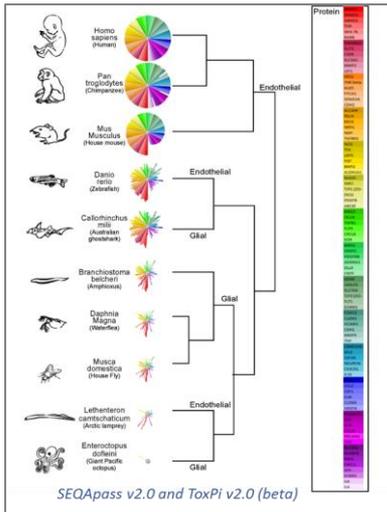
**OUTPUT:** tipping point mapped to ToxCast concentration response (4 μM for Captan)

# In silico framework for hypothesis-based testing

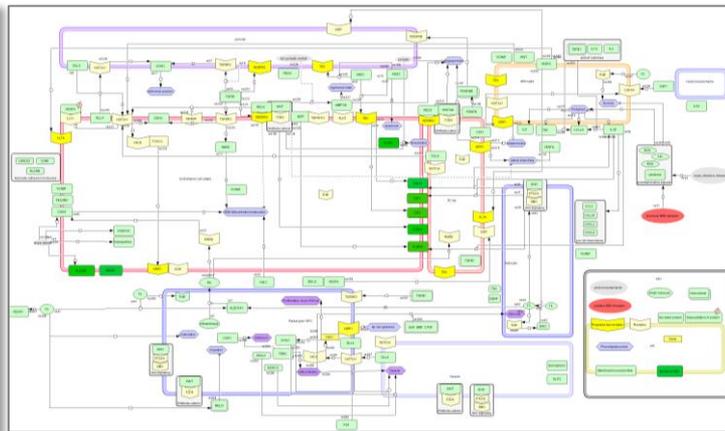
- Blood-brain-barrier (BBB) development
- driven by >90 genes and > 5 cell types



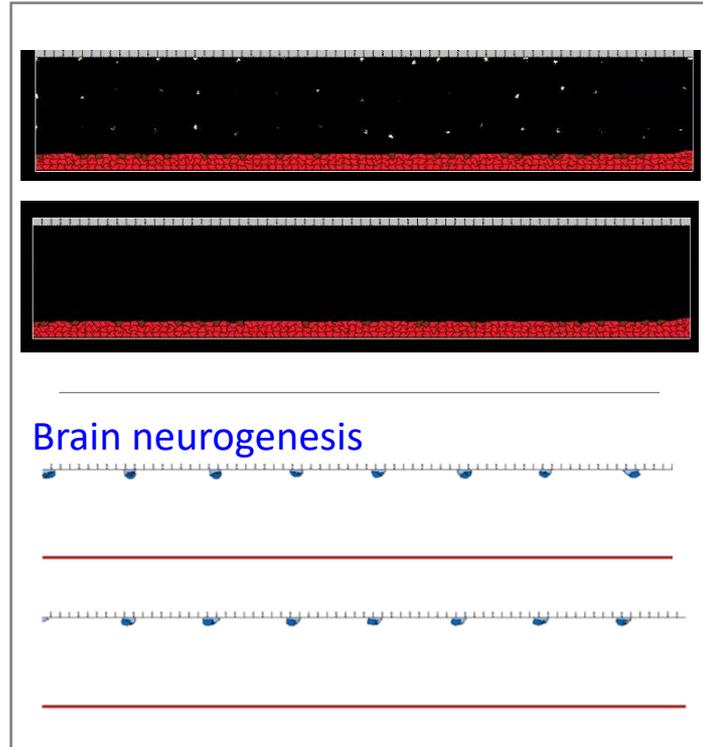
## BBB Phylogeny



## BBB Ontogeny



## Brain angiogenesis

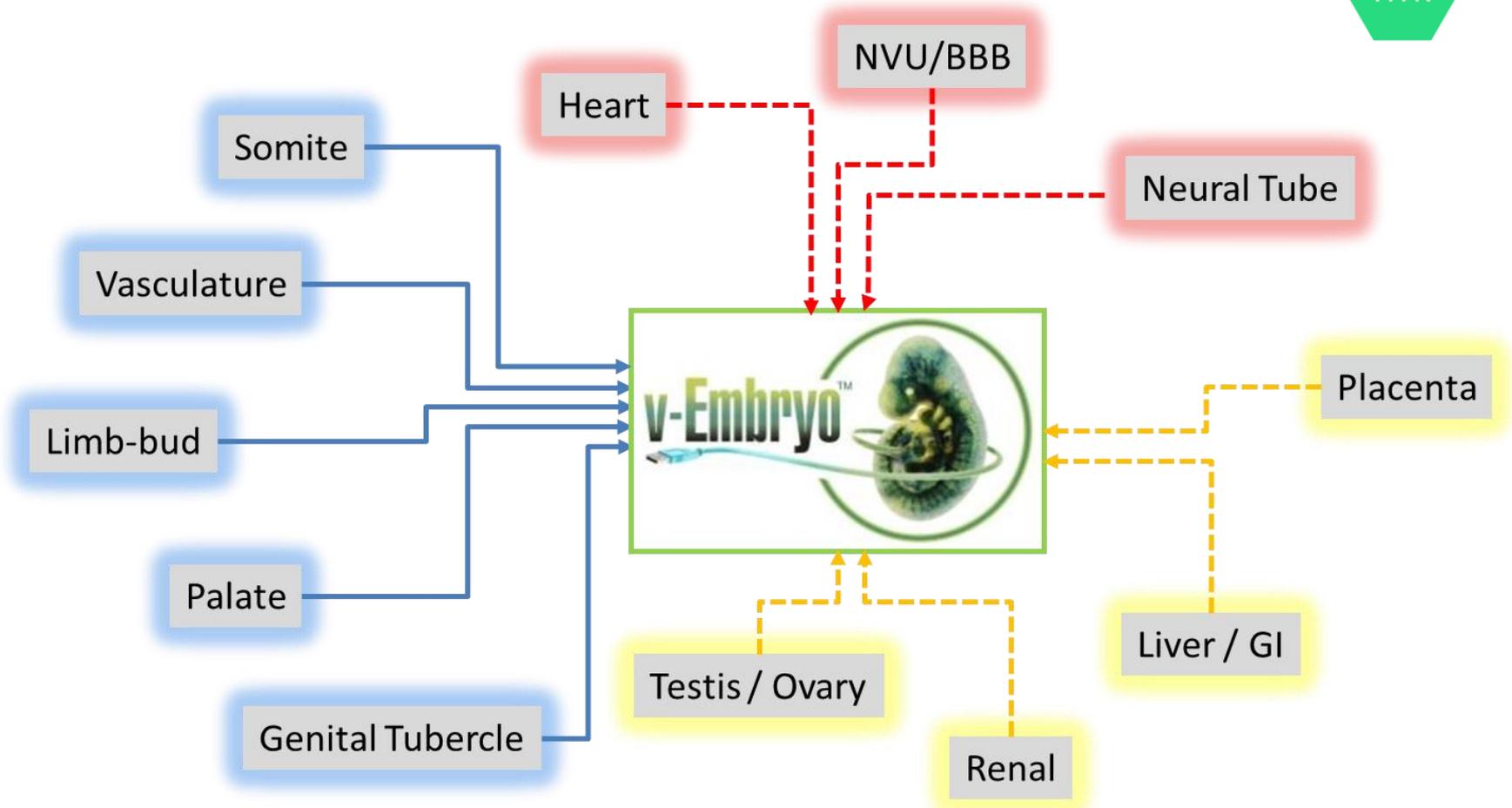


## Brain neurogenesis

We are building and testing computer models formulated around novel hypotheses such as *'chemical disruption of microglia perturb neurovascular development'*.

# Grand Challenge: a predictive virtual embryo

*how far must 'computational embryology' advance to predict developmental toxicity in lieu of animal testing?*





# National Center for Computational Toxicology

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