Computational embryology as an integrative platform for predictive DART

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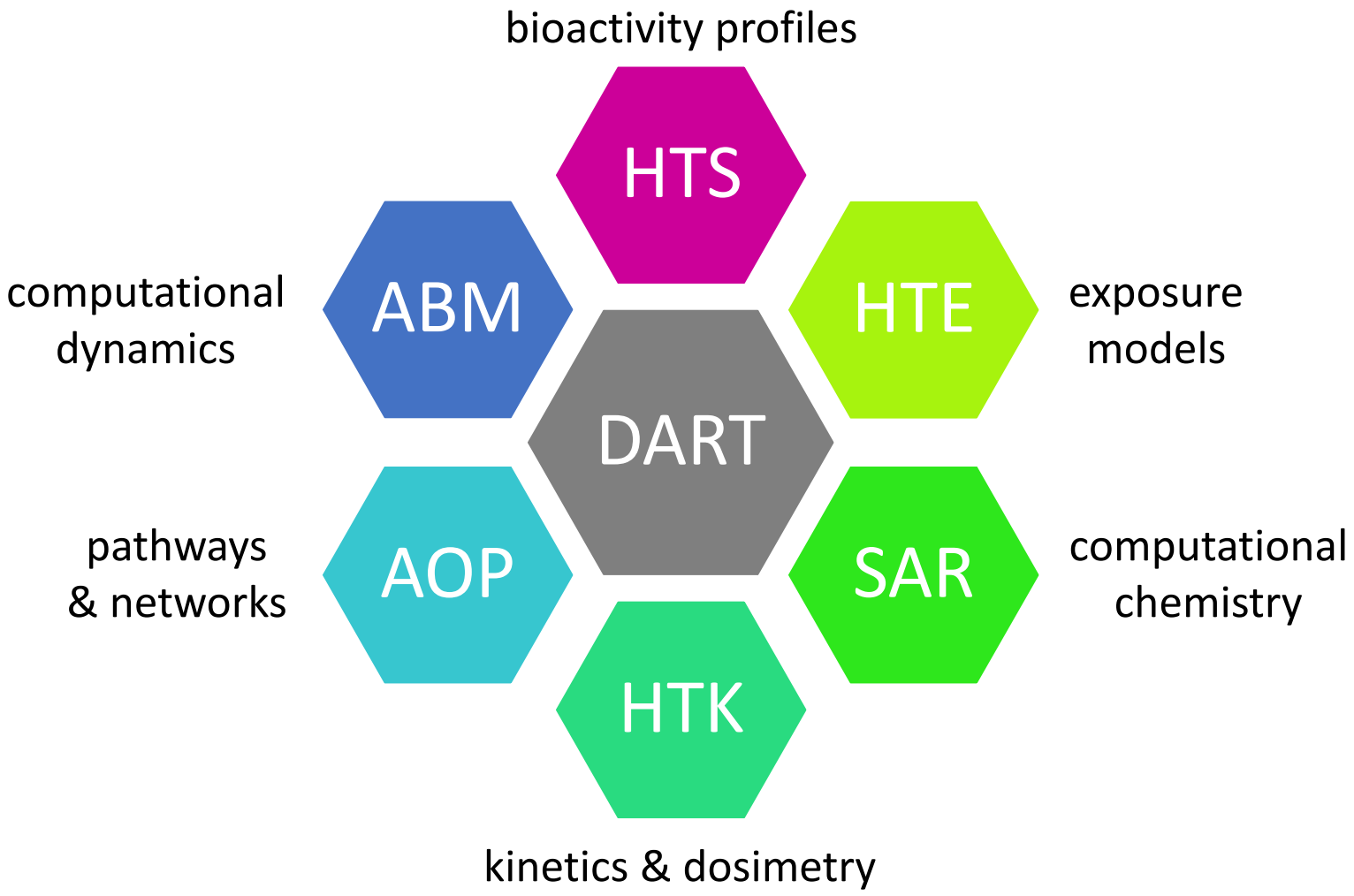
Symposium: Update on Integrated Approaches to Testing and Assessment (IATA) for DART
45th Conference of the European Teratology Society, September 7, 2017, Budapest

Mechanistically-informed IATAs for DART:

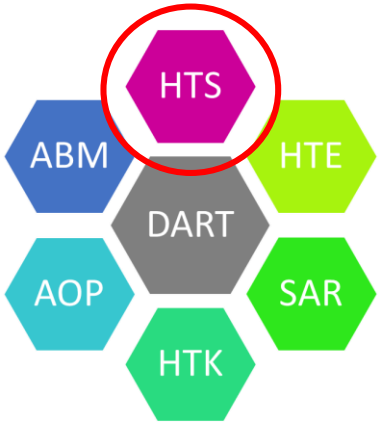
opportunities and challenges

- 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



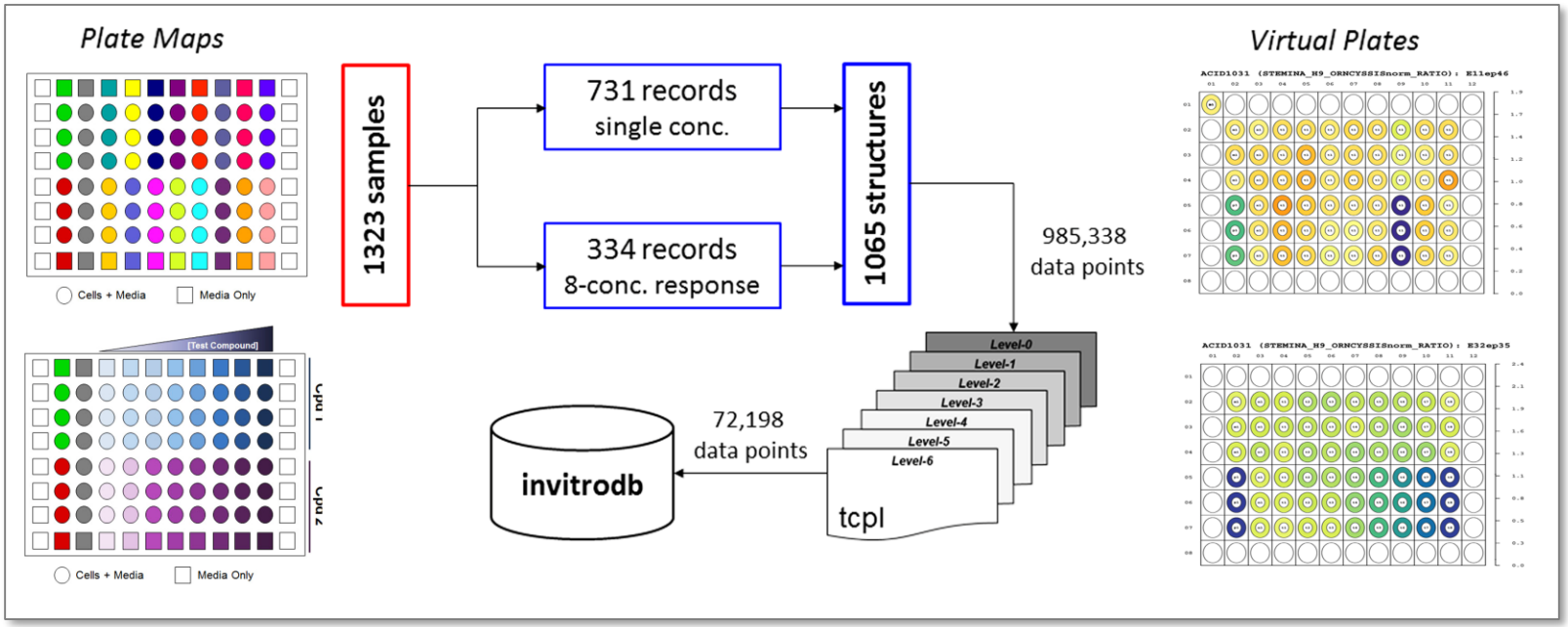
High-throughput screening



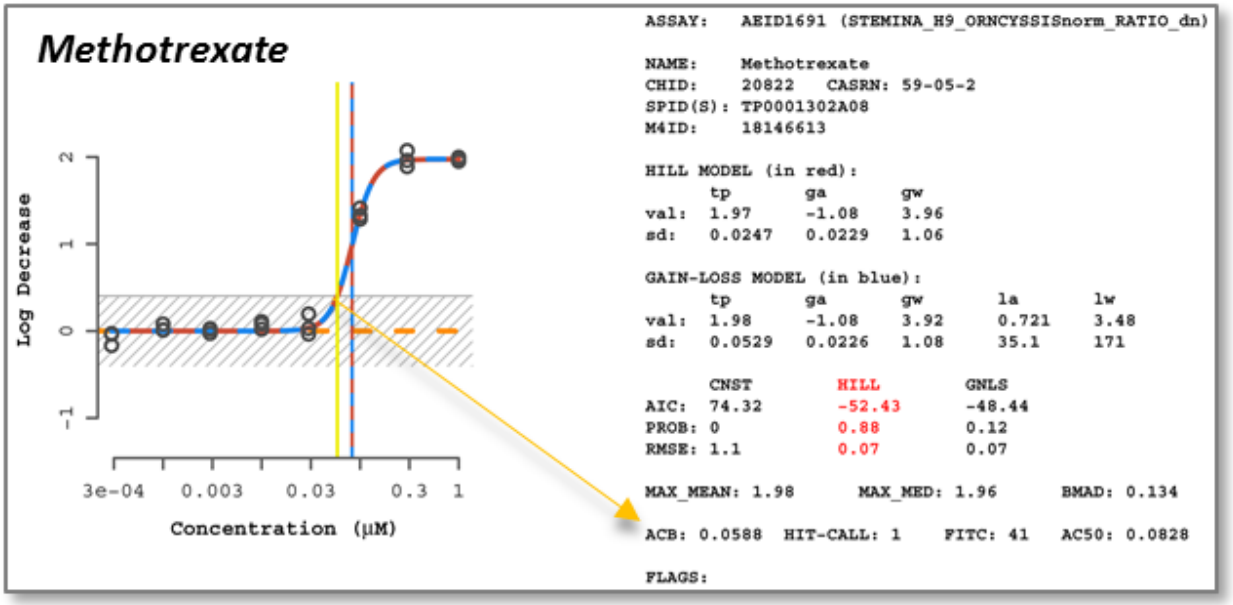
- Large data streams from high-throughput profiling of chemicals for *in vitro* bioactivity (ToxCast/Tox21).
  https://actor.epa.gov/dashboard/#Chemicals

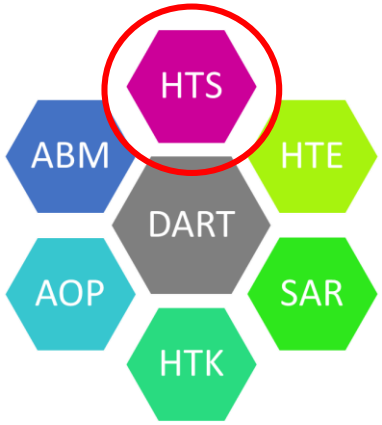
- New platform: ToxCast library (1065 chemicals) tested in a pluripotent H9 human embryonic stem cell assay.

Stemina BIOMARKER DISCOVERY



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).

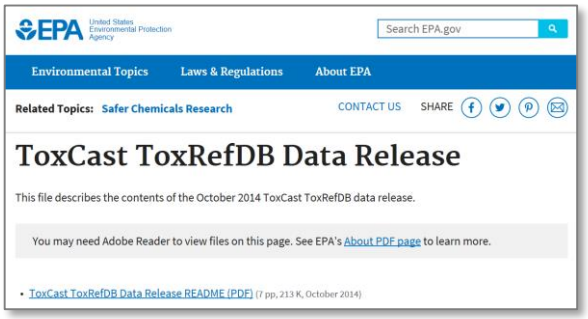
Anchor	TI (µM)	Class
all-trans-Retinoic acid	0.003	TP
Cytarabine hydrochloride	0.054	TP
Methotrexate	0.059	TP
Diphenhydramine hydrochloride	0.588	TP
Thalidomide	1.267	TP
5-Fluorouracil	2.021	TP
Carbamazepine	2.294	TP
Busulfan	2.313	TP
Amiodarone hydrochloride	5.101	TP
Dexamethasone sodium phosphate	37.680	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

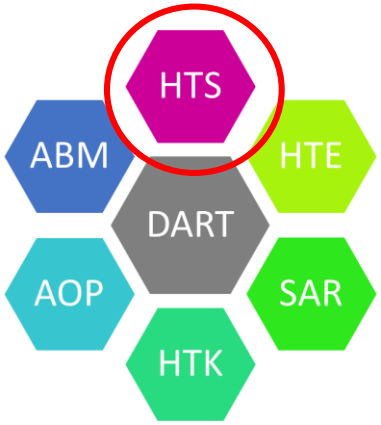
TP	14
FP	1
FN	4
TN	11
n	30
Sensitivity	0.778
Specificity	0.917
Accuracy	83.3%
Mathew's cc	0.680
F1 score	0.47

Knudsen et al. (2017) manuscript in preparation

Performance of STM vs ToxRefDB fetal endpoints



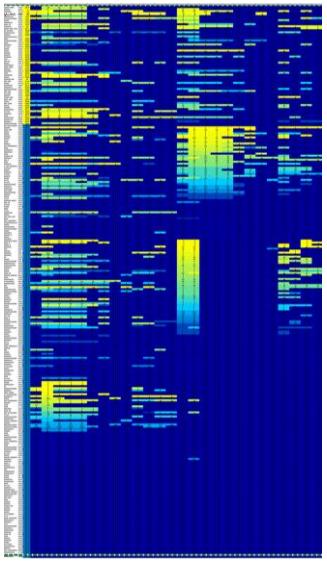
https://www.epa.gov/chemical-research/toxcast-toxrefdb-data-release



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

- positives = dLEL ≤ 125 mg/kg/d in both species
- negatives = no dLEL ≥ 1000 mg/day in both species

} *n = 146 compounds*



ToxRefDB_fetal Anchor

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

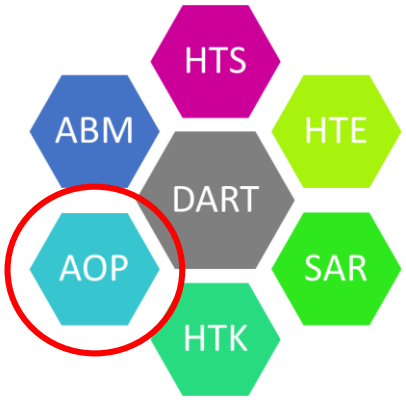
OUTPUT:

- 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?

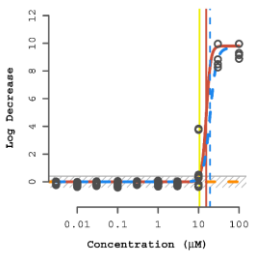
Knudsen et al. (2017) manuscript in preparation

Vascular Disruption (pVDCs)

INPUT: exposure-based TI predicted by the STM assay.
OUTPUT: margin between hazard prediction and rat WEC effect.



5HPP-33



ASSAY: AEID1691 (STEMINA_H9_ORNCYSSISnorm_RATIO_dn)

NAME: 5HPP-33
CHID: 46970 CASRN: 105624-86-0
SPID(S): TP0001302H01, TP0001302D08
M4ID: 18146561

HILL MODEL (in red):

	tp	ga	gw
val:	9.8	1.19	8
sd:	0.129	0.0501	2.05

GAIN-LOSS MODEL (in blue):

	tp	ga	gw	la	lw
val:	9.66	1.28	5.41	3.14	5.11
sd:	0.262	0.0256	0.522	489	2200

	CNST	HILL	GNLS
AIC:	379.06	82.28	88.58
PROB:	0	0.96	0.04
RMSE:	3.42	0.7	0.69

MAX_MEAN: 9.54 MAX_MED: 9.63 BMAD: 0.134

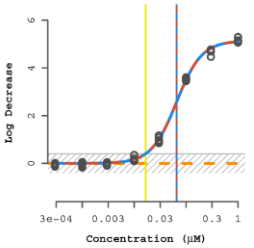
ACB: 10.5 HIT-CALL: 1 FITC: 41 AC50: 15.5

FLAGS:
10

TI = 10.48 µM

AC50 (embryolethality) = 21.2 µM

TNP-470



ASSAY: AEID1691 (STEMINA_H9_ORNCYSSISnorm_RATIO_dn)

NAME: TNP-470
CHID: 41141 CASRN: 129298-91-5
SPID(S): TP0001302E03, TP0001302A06
M4ID: 18146581

HILL MODEL (in red):

	tp	ga	gw
val:	5.14	-1.18	1.79
sd:	0.0404	0.00845	0.0544

GAIN-LOSS MODEL (in blue):

	tp	ga	gw	la	lw
val:	5.14	-1.18	1.79	1.61	6
sd:	0.0404	0.00845	0.0544	1030	3850

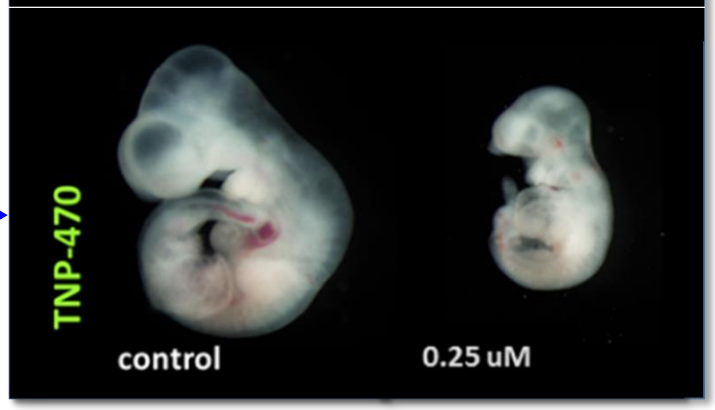
	CNST	HILL	GNLS
AIC:	230.2	-70.76	-66.76
PROB:	0	0.88	0.12
RMSE:	2.85	0.1	0.1

MAX_MEAN: 5.18 MAX_MED: 5.16 BMAD: 0.134

ACB: 0.0166 HIT-CALL: 1 FITC: 41 AC50: 0.0656

FLAGS:

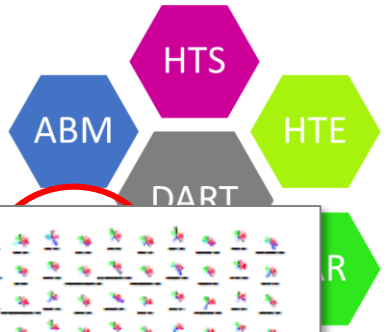
TI = 0.017 µM

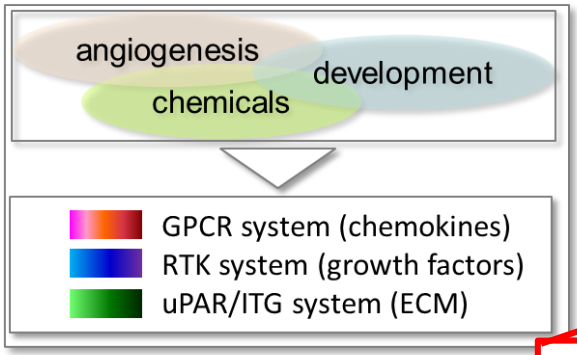


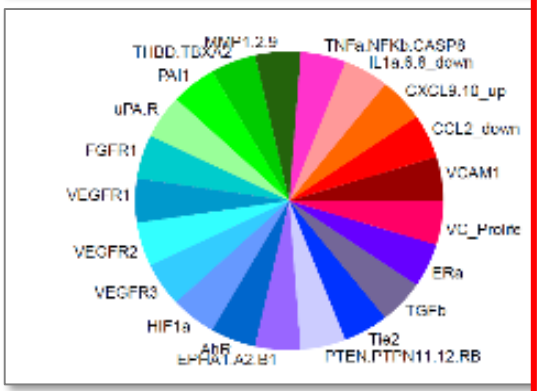
AC50 (dysmorphogenesis) = 0.038 µM

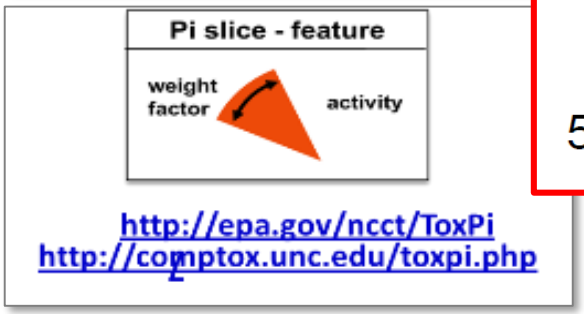
Ellis-Hutchings (2017) Reprod Toxicol.

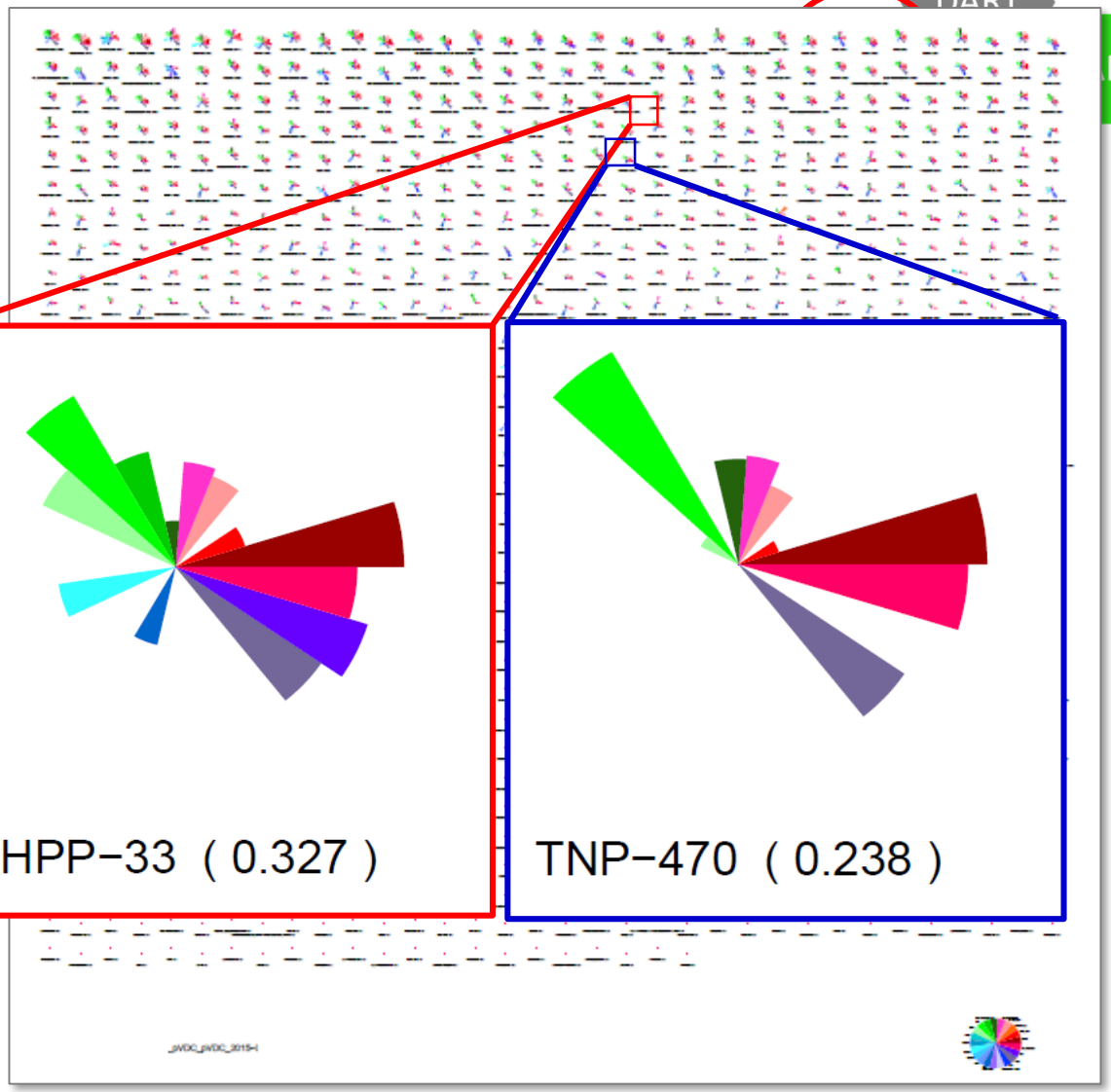
AOP-based chemical prioritization





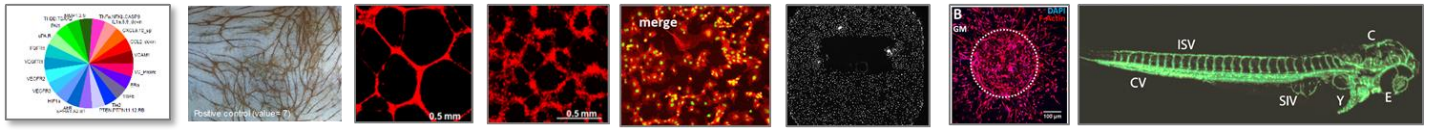


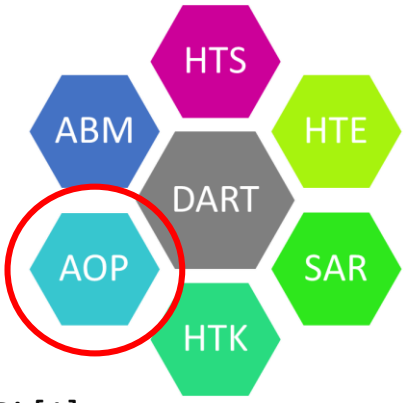


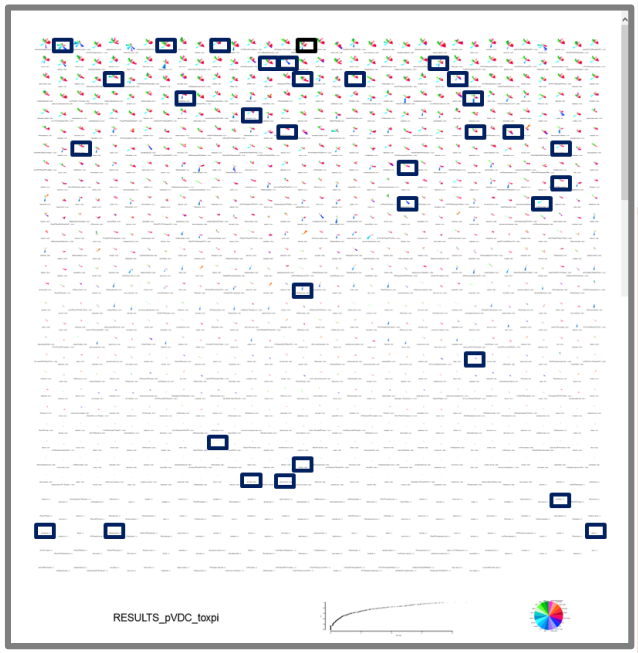


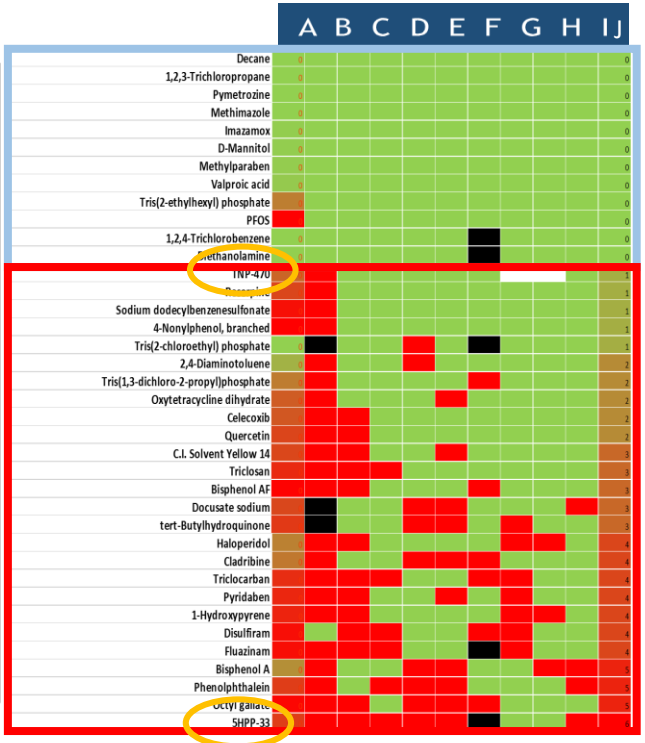
Knudsen and Kleinstreuer (2011) Birth Defects Res C

Qualification against 8 diverse angiogenesis platforms









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)

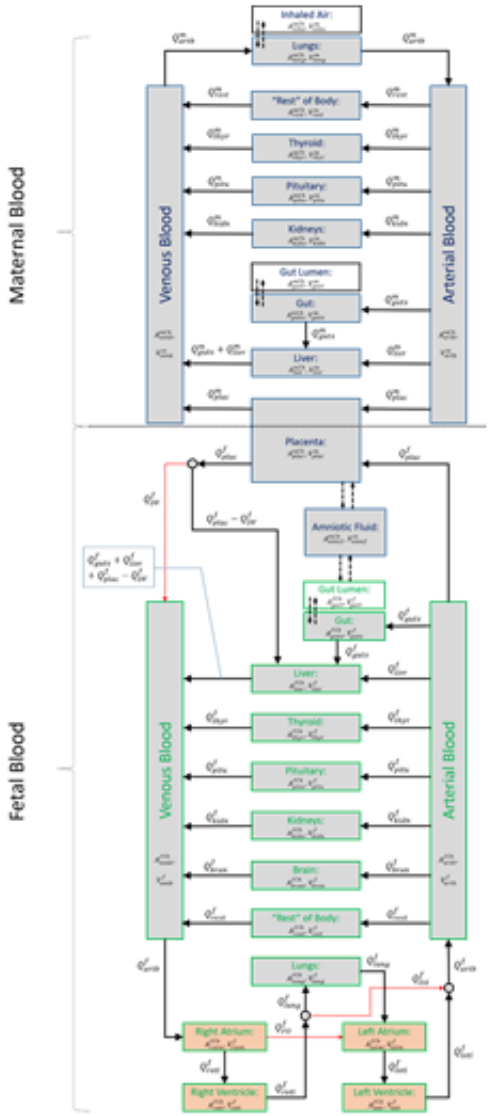
Predicted (ToxCast)

inactive
active

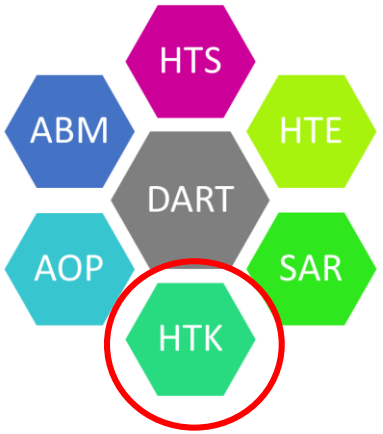
Empirical (angiogenesis)

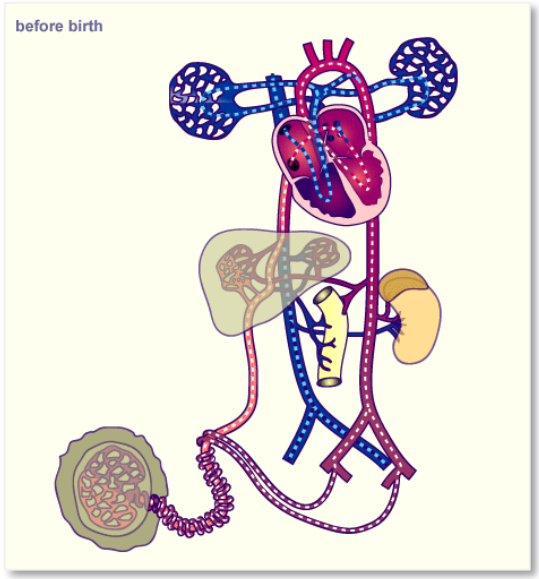
[1] Tal et al. Reprod Toxicol (2017); [2] Knudsen et al., in prep; [3] Nguyen et al. Nature Bioengineering (2017); [4] Belair et al. (2016) Acta Biomaterialia.

High-throughput kinetics



Comprehensive HTTK Pregnancy Model

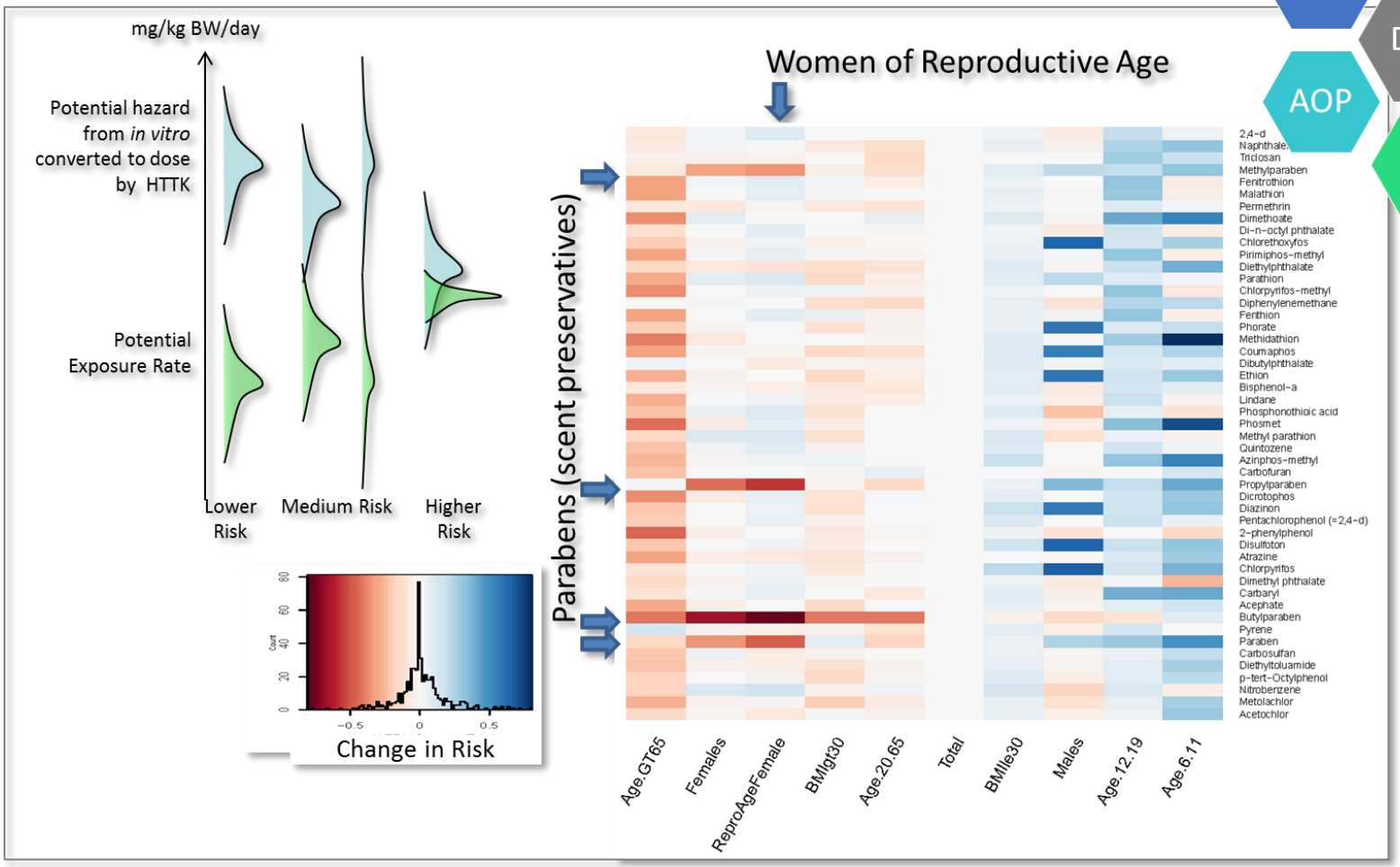


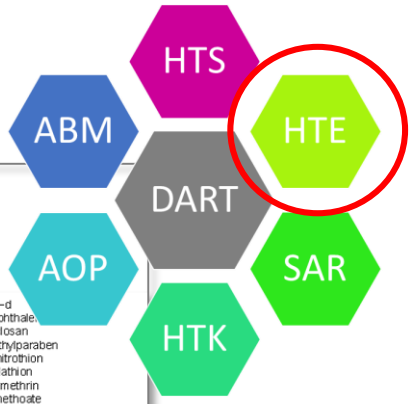


- **INPUT:** metrics from human pregnancy (> 1st trimester), SBML solver in Tellurium.
- **OUTPUT:** parameterized to run 585 ToxCast chemicals for fetal dosimetry.

Kapraun et al. (2017) (manuscript in preparation).

High-throughput exposure ratio

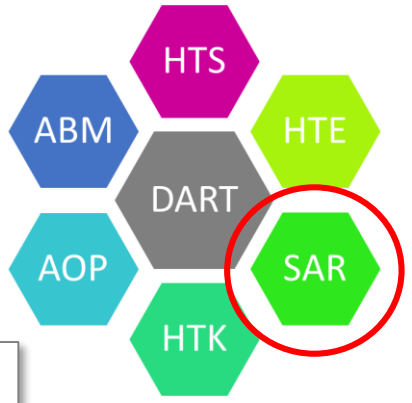




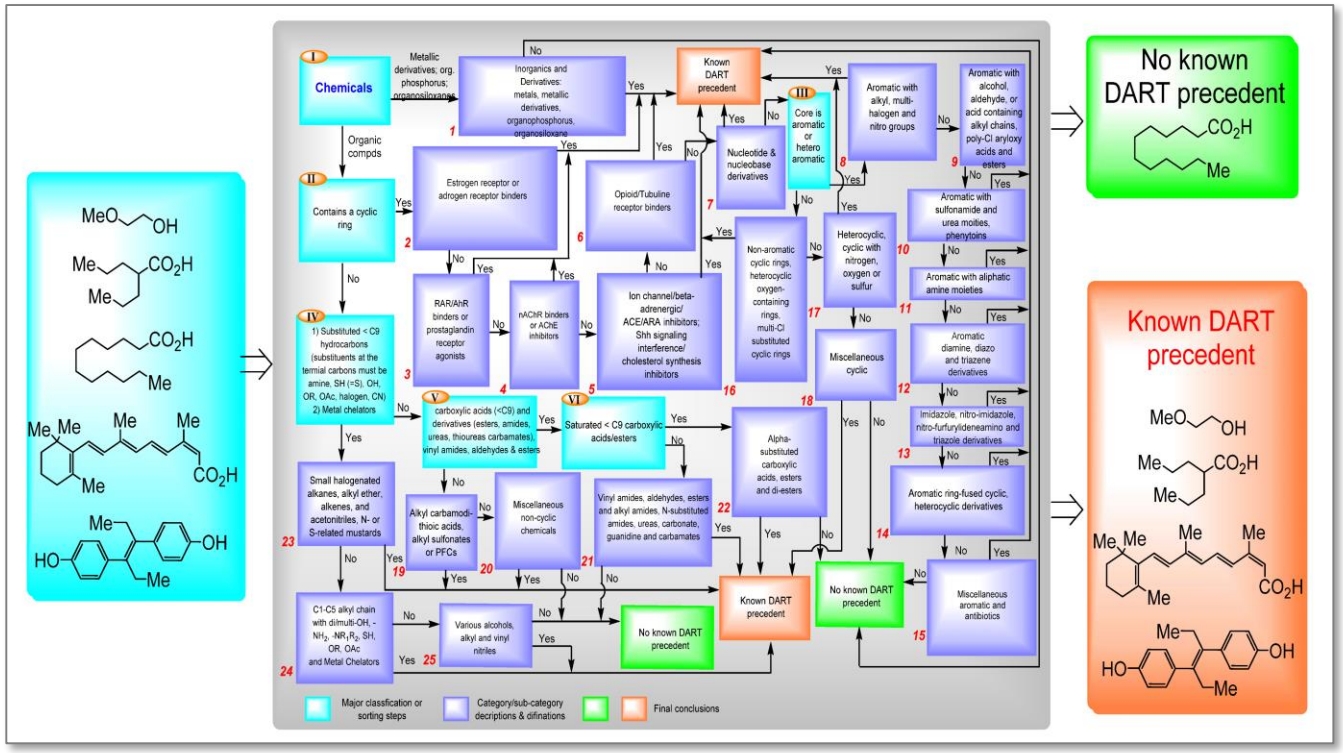
INPUT: HTS data on bioactivity profiles (eg, AC50) and exposure for specific subpopulations.
OUTPUT: margin between bioactivity and exposure for WOCBP.

Ring et al. (2017) Environment International

Structure-activity relationships



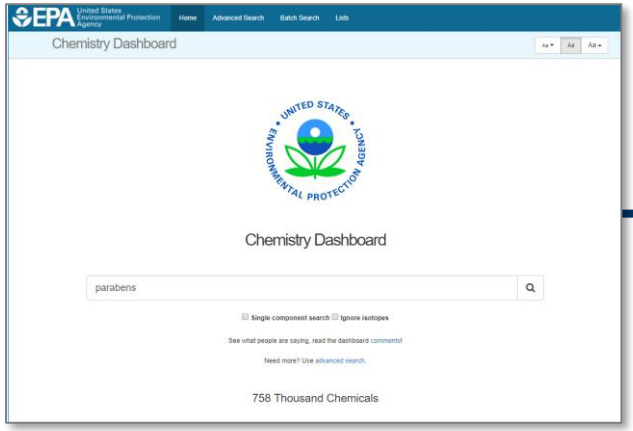
INPUT: 716 chemicals with DART endpoints grouped into receptor binding and chemical domains (example – 5 parabens).



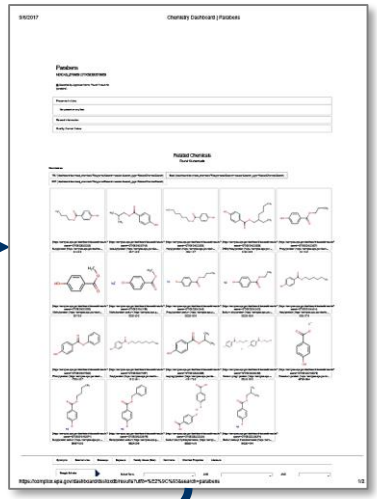
OUTPUT: classification tree based on whether or not a chemical has receptor-binding properties and structural features consistent with known DART.

Wu et al (2013) Chem Res Toxicol

Mining the literature for SAR information

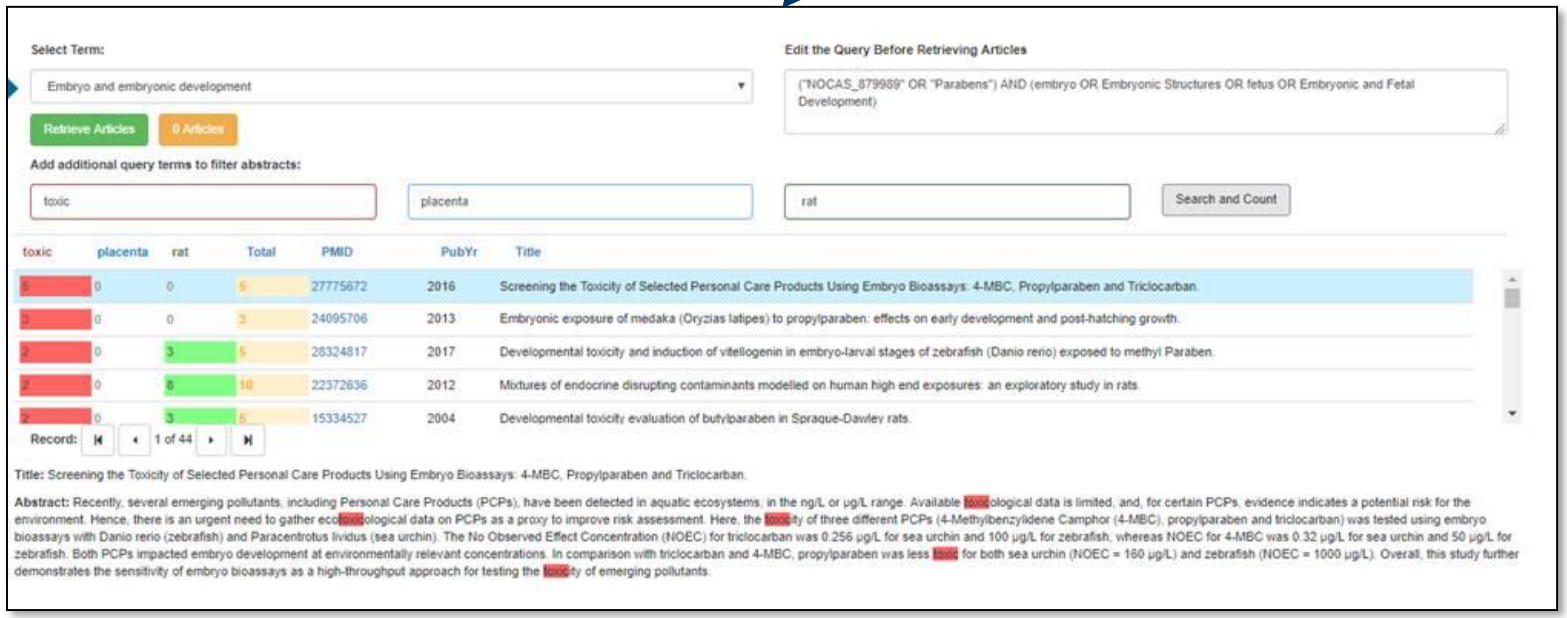


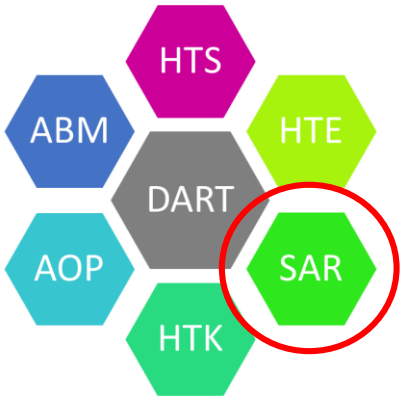
INPUT: 'parabens'



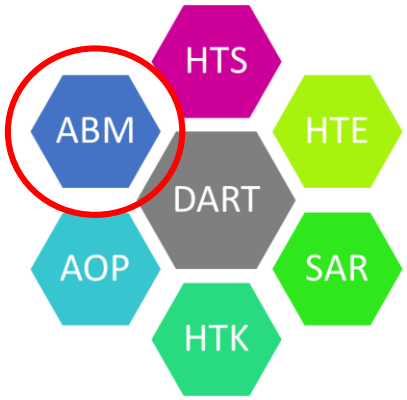
https://comptox.epa.gov/dashboard

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





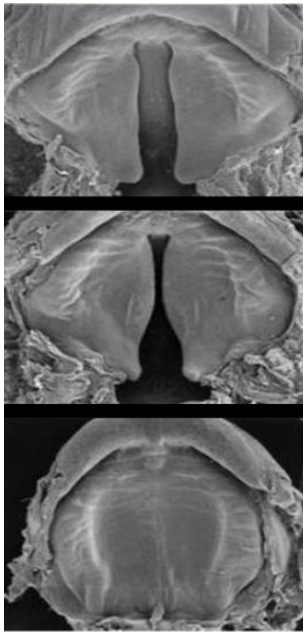
Agent-Based Models



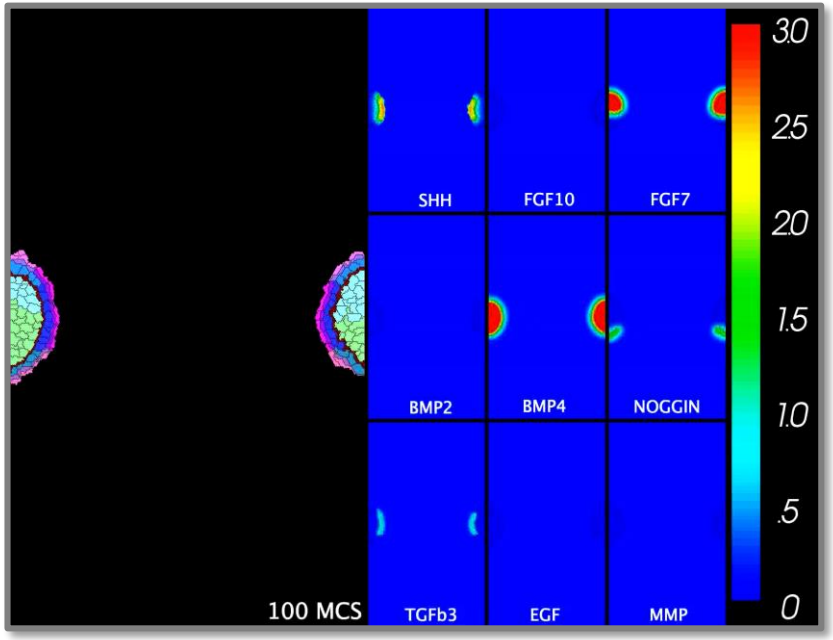
Executed with **CompuCell3D.org** modeling environment

- *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.*

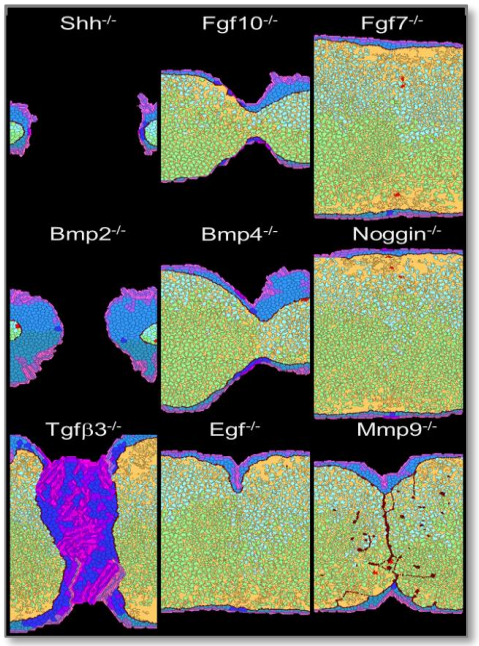
SEMs of human palate by K Sulik, UNC



Palatal fusion in a virtual system

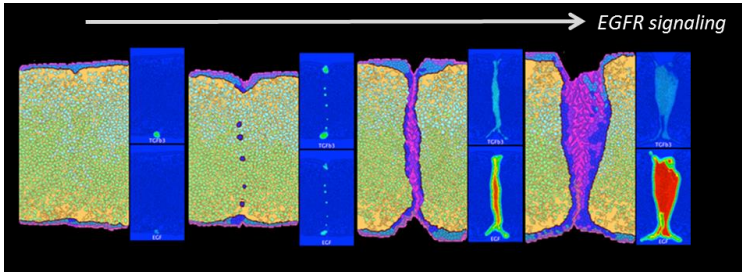


Cybermorphs



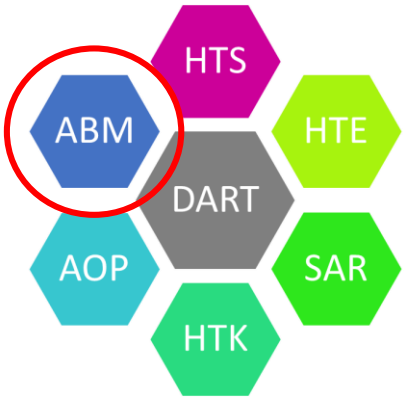
Hutson et al. (2017) Chem Res Toxicol

Imputing ToxCast data

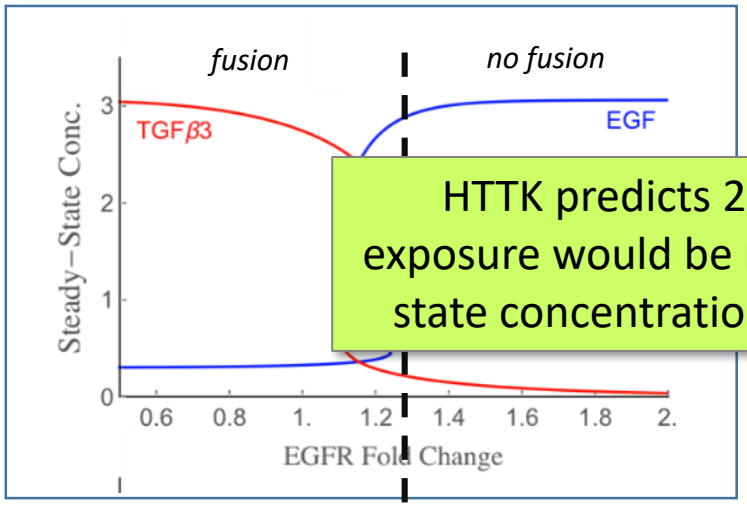


Captan → cleft palate

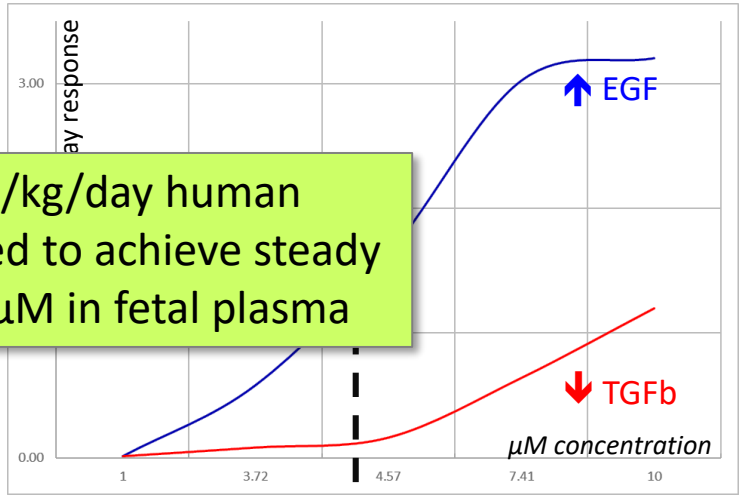
ToxRefDB NOAEL = 10 mg/kg/day
(rabbit) LOAEL = 30 mg/kg/day



INPUT: ToxCast HTS data



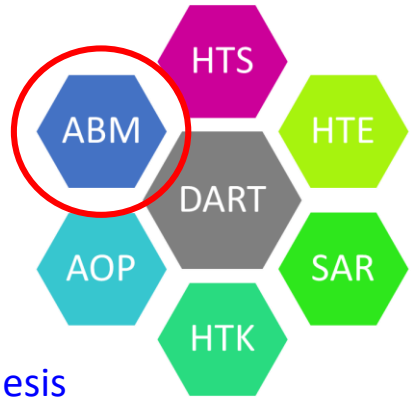
HTTK predicts 2.39 mg/kg/day human exposure would be required to achieve steady state concentration of 4 µM in fetal plasma



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

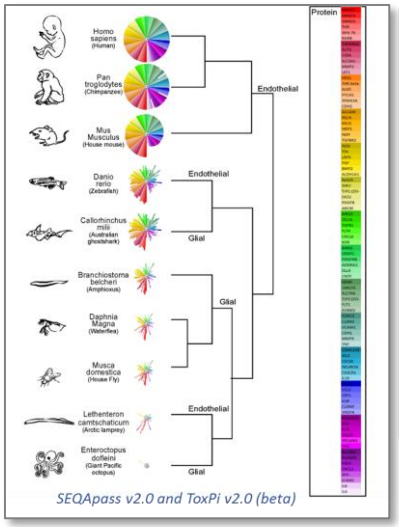
OUTPUT: tipping point mapped to ToxCast concentration response (4 µM for Captan)

In silico framework for hypothesis-based testing

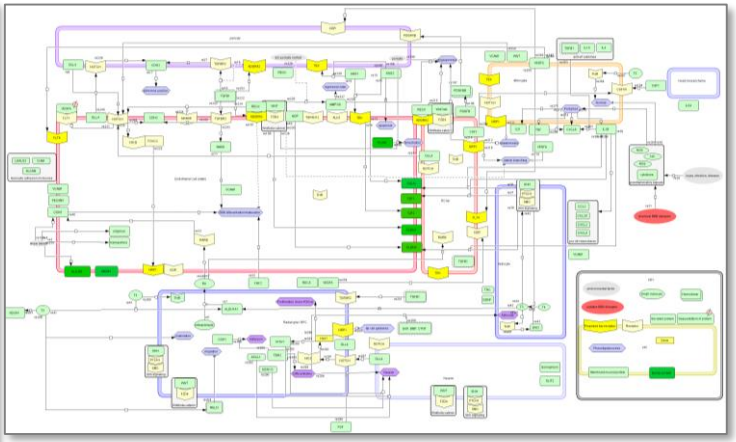


- Blood-brain-barrier (BBB) development
- driven by >90 genes and > 5 cell types

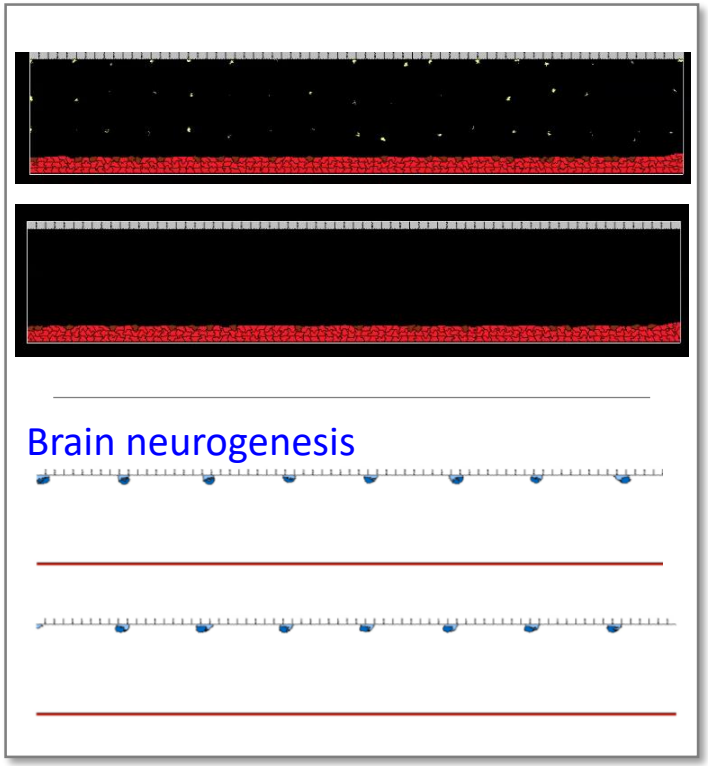
BBB Phylogeny



BBB Ontogeny



Brain angiogenesis



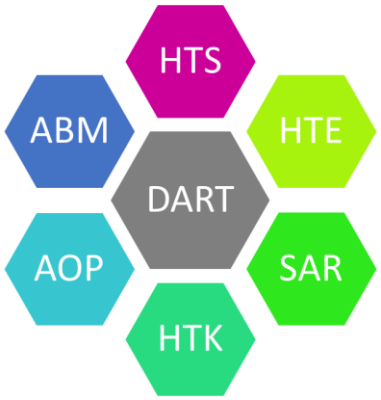
Brain neurogenesis

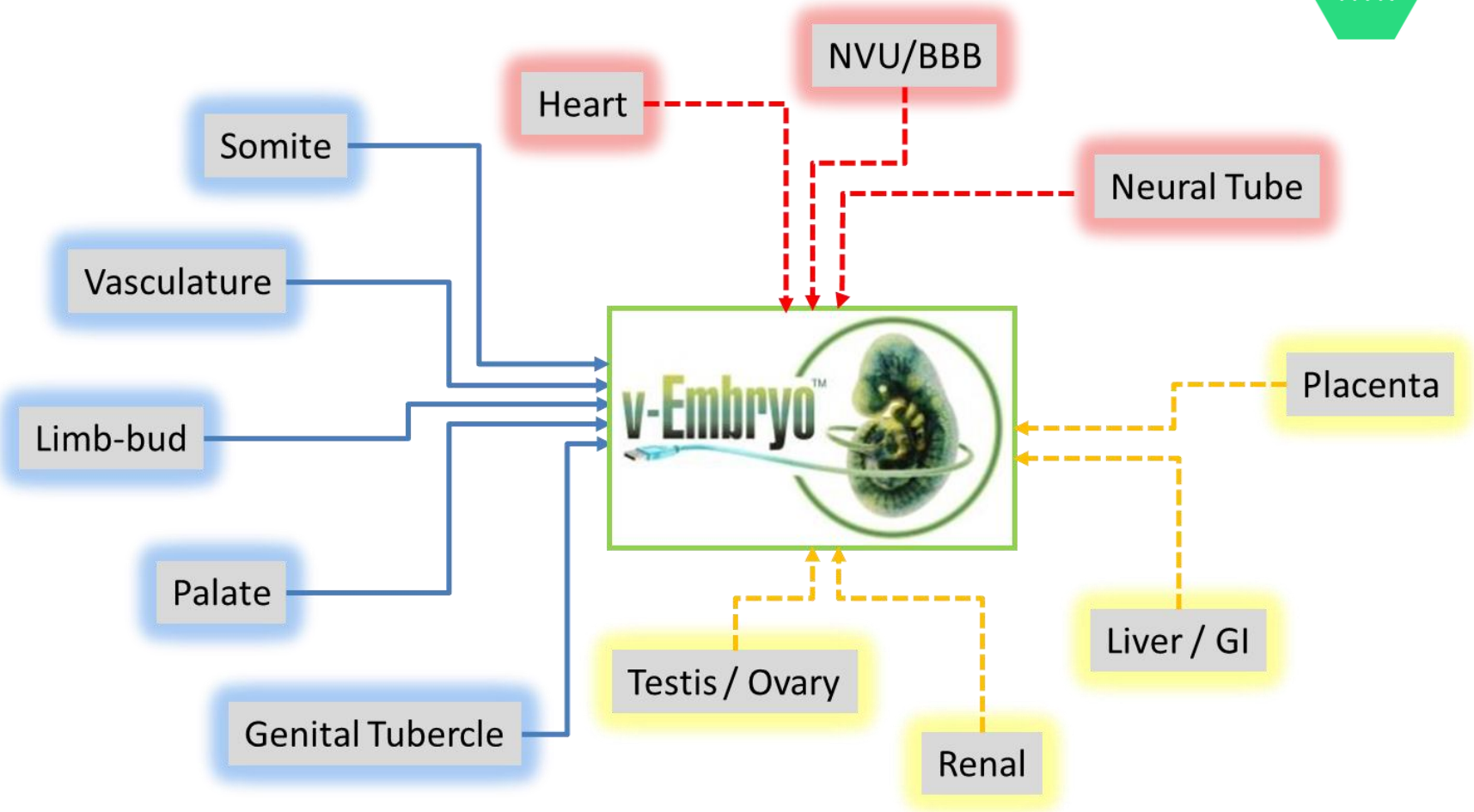
We are building and testing computer models formulated around novel hypotheses such as '*chemical disruption of microglia perturb neurovascular development*'.

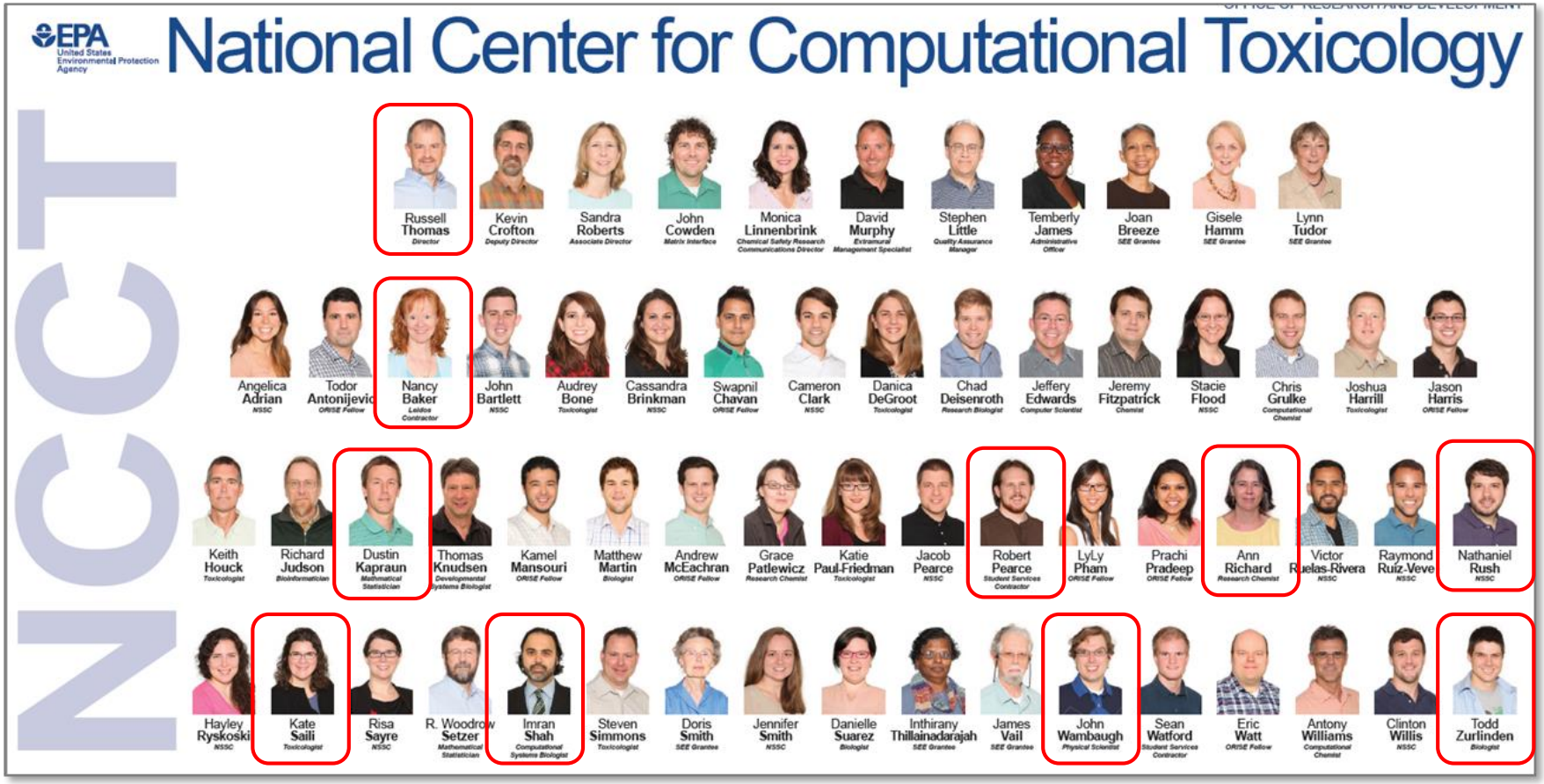
Saili et al. (2017) manuscript in preparation; Zurlinden et al. (2017) in preparation

Grand Challenge: a predictive virtual embryo

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







http://www2.epa.gov/sites/production/files/2015-06/documents/virtual_tissue_models_fact_sheet_final.pdf