SYMPOSIUM: **New Frontiers in Developmental Toxicity Testing for Environmental Chemicals**

Society for Birth Defects Research & Prevention

EST. 1960 AS THE TERATOLOGY SOCIETY

Predictive developmental toxicity with pluripotent stem cell models and ToxCast/Tox21 assay batteries

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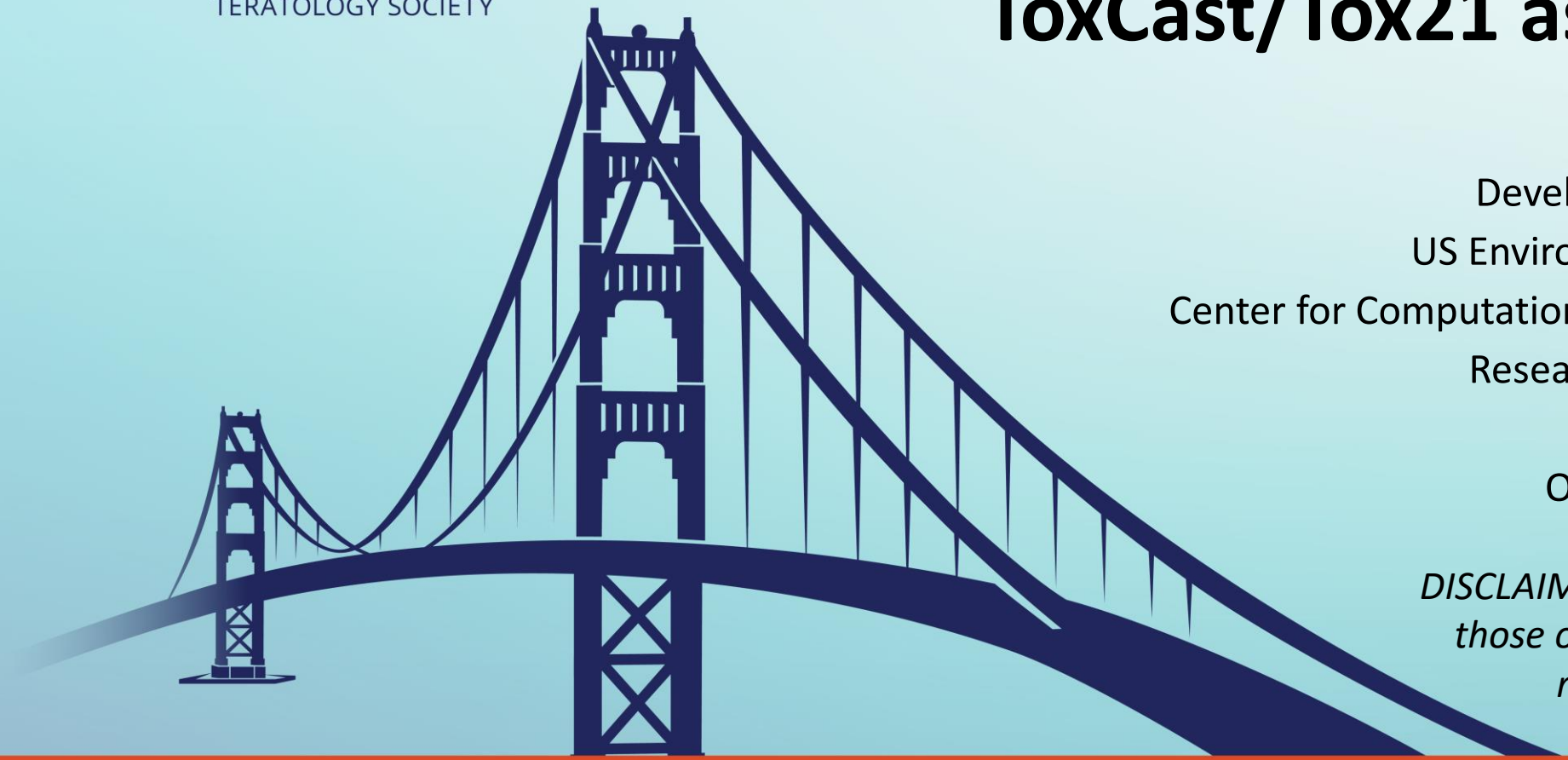
DISCLAIMER: The views expressed are those of the presenter and do not reflect Agency policy.



Society for Birth Defects Research and Prevention's **2021 Virtual 61st Annual Meeting**

Disclosures

https://stemcells.nih.gov/research/registry.htm

Funding: our research with human pluripotent stem cell lines (hPSCs) was performed under EPA's *Chemical Safety for Sustainability Research Program, Research Area 5 'Virtual Tissue Models' (VTMs).*

Compliance*:* CSS work involving established hPSC lines is compliant with Executive Order 13505 (issued 2009) to ensure that is ethically responsible, scientifically worthy, and conducted in accordance with applicable law.

Embryonic PSC lines are registered in the NIH Human Embryonic Stem Cell Registry: WA09 (H9) NIH Approval Number NIHhESC-10-0062 (EPA contract EP-D-13-055 with Stemina Biomarker Discovery) and RUES2, NIH Approval Number: NIHhESC-09-0013.

Other induced PSC lines: endodermal hPSC line from Allele Biotech #ABPSC-HDFAIPS (EPA contract EP-D-13-054 with Vala Sciences, Inc.).

Shifting toxicity testing to animal-free alternatives

- **June 2016:** *Lautenberg Chemical Safety Act* advances chemical safety evaluation with methods that reduce animal testing and are translatable to vulnerable populations & lifestages.
- **September 2019:** directive issued by USEPA Administrator Wheeler set a vision to reduce mammalian study requests 30% by the year 2025 and eliminate them by 2035.
- **June 2020:** USEPA work plan to accelerate scientifically valid *New Approach Methods* (NAMs) for assessing toxicity of large numbers of chemicals with less reliance on animal testing.

https://www.epa.gov

In vitro data and *in silico* models that reflect key aspects of embryo-fetal development will be indispensable for NAM-based detection of developmental hazard potential.

1. Pluripotent stem cell (hPSC) models

An active area of investigation and one of the most promising *in vitro* alternatives to pregnant animal testing for assessing developmental hazard potential; novel features:

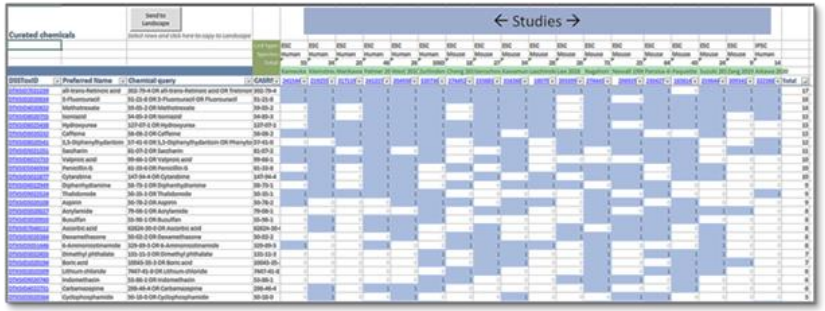
- **Self-renewal:** cells replicate themselves indefinitely when cultured under appropriate growth factor conditions.
- **Pluripotency:** cells have the potential to form most of the different cell types comprising the embryo/fetus.
- **Autopoiesis:** capacity to self-organize into rudimentary tissues and more complex organoid structures.

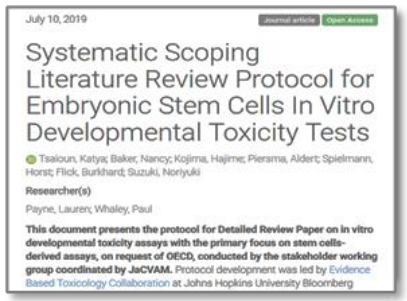
Established hPSC lines can recapitulate **some** of the biology driving embryogenesis during the period covered by guideline prenatal studies (e.g., OECD TG 414, OPPTS 870.3700).

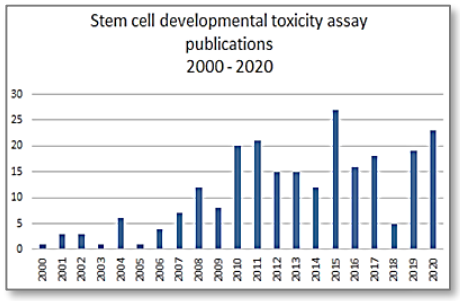
Systematic scoping review:

EST modalities for predictive developmental toxicity (1991-2021)

Detailed Review Paper (DRP) on EST platforms







Abstract Sifter, SWIFT, MeSH terms, Chemicals Dashboard, …

Writing team

Nancy Baker - Leidos
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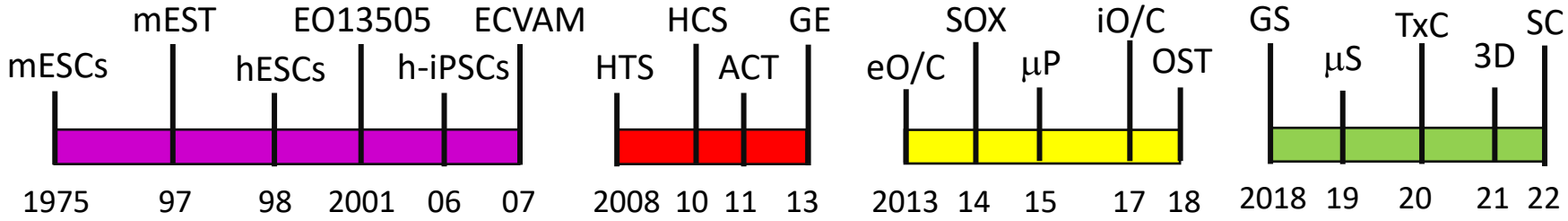
Thomas Knudsen – USEPA
Hajime Kojima – NIHS/JaCVAM (Japan - lead)
Aldert Piersma – RIVM (Netherlands)
Horst Spielmann – Berlin (retired)
Noriyuki Suzuki – Sumitomo Chemical Co. (Japan)
Katya Tsaioun – Johns Hopkins University

Piersma et al., manuscript in final preparation

PSC-based modalities for developmental toxicity



1997-07 traditional mEST [accuracy ~80%]

D3 mESCs, embryoid body (EB), cardiopoiesis (beating heart, MHC expression)
predictive capacity distinguishes strong from weak-/non-embryotoxicants (ECVAM)

2008-13 efforts to improve mEST sensitivity (non vs weak) and scalability (HTS/HCS) [accuracy 72-83%]

HTS (96-multiwell plates for EB formation), FACS sorting, adherent cell assay (ACT)
HCS (transcriptome, reporter assay (Wnt/β-catenin, Hand1), multi-gene expression (GE)

2013-18 targeted biomarker readouts in hPSCs [accuracy 77-87+%]

ORN:CYSS targeted biomarker in pluripotent H9 cells (eO/C) and h-iPSCs (iO/C)
HCI analysis of mesendoderm differentiation (SOX17, migratory rings), osteogenic (OST)

2018- recent [accuracy 77-87+%]

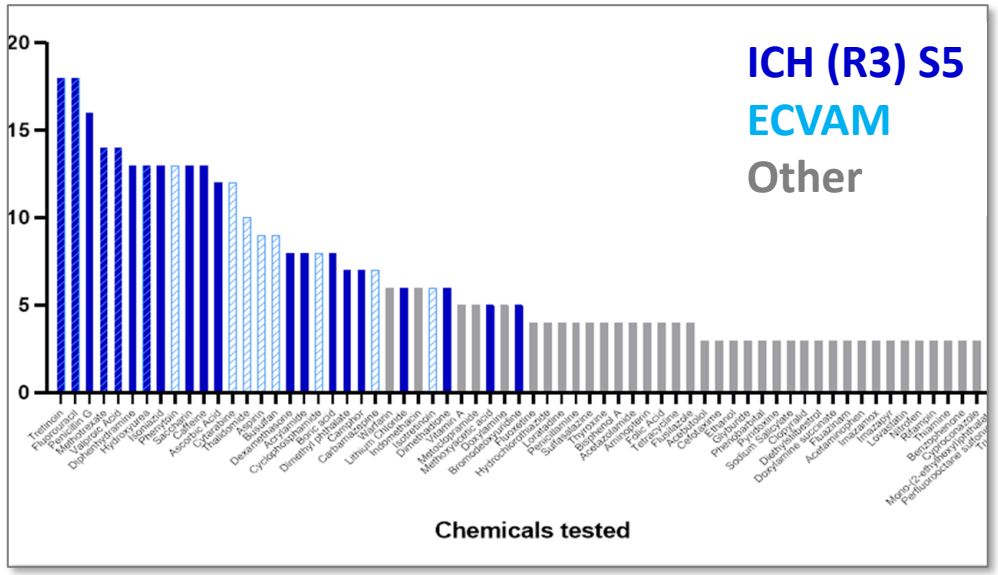
morphometrics (gastruloids, GS; microsystems, μS), 3D organoids
ToxCast profiling (TxC), scRNA-seq and germ layer scorecard (SC)

Conceptual and practical considerations

- **DRP:** survey of extant PSC assays used to classify developmental toxicants to evaluate:
  - chemical domain
  - biological domain
  - standardized protocols
  - reproducibility
  - biomarker readouts
  - predictive power.

1,533 records in PubMed reduced to 333 (AI for relevance) and 192 (manual curation).

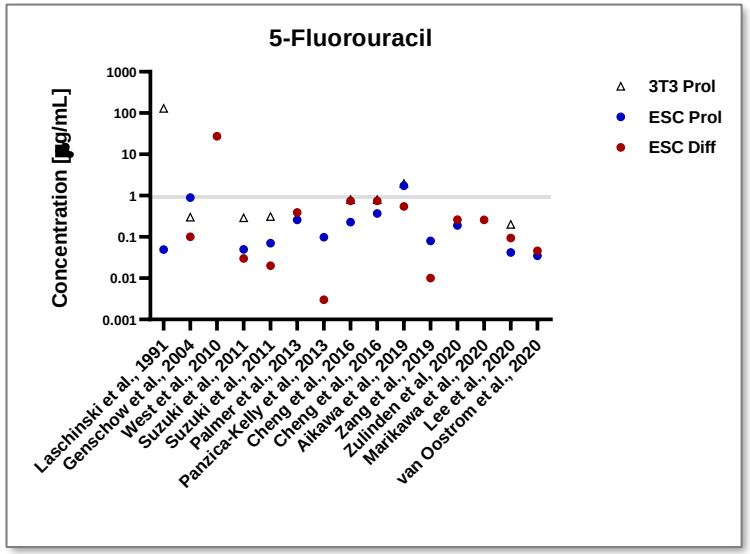
- **1,250 annotated chemicals (through 2020):**
  - 18 publications tested ≥ 10 compounds (primary)
  - 174 publications tested 1-9 (evidentiary support)
  - most frequently represented: ATRA, 5-FU, MTX.

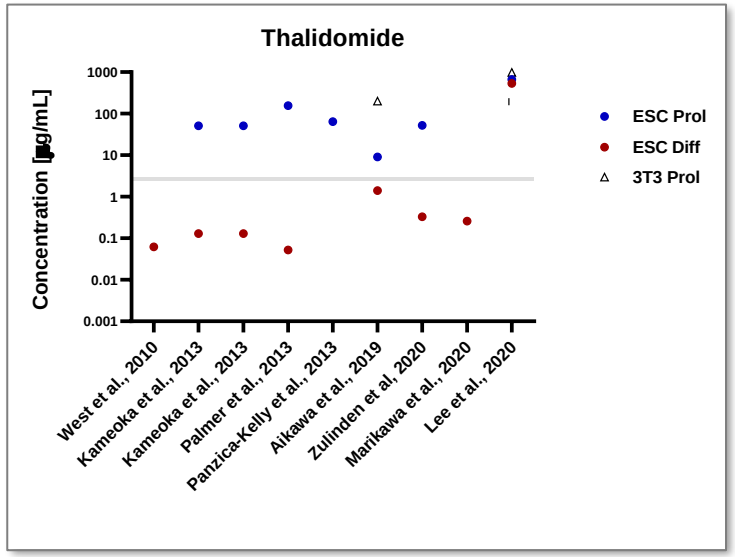


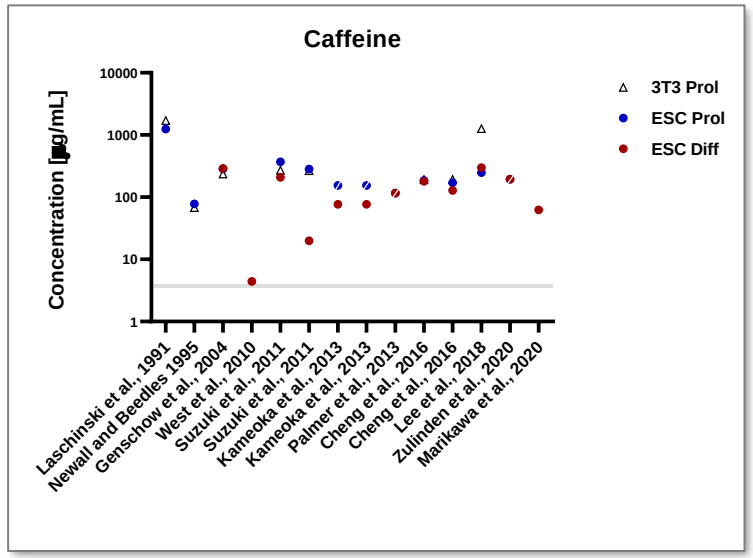
Number of studies investigating test compounds in relation to their listing as DevTox reference compounds

Piersma et al., manuscript in final preparation

Different EST modalities: *robust, reproducible, and relevant*







Selected case examples across EST platforms used in the DRP:

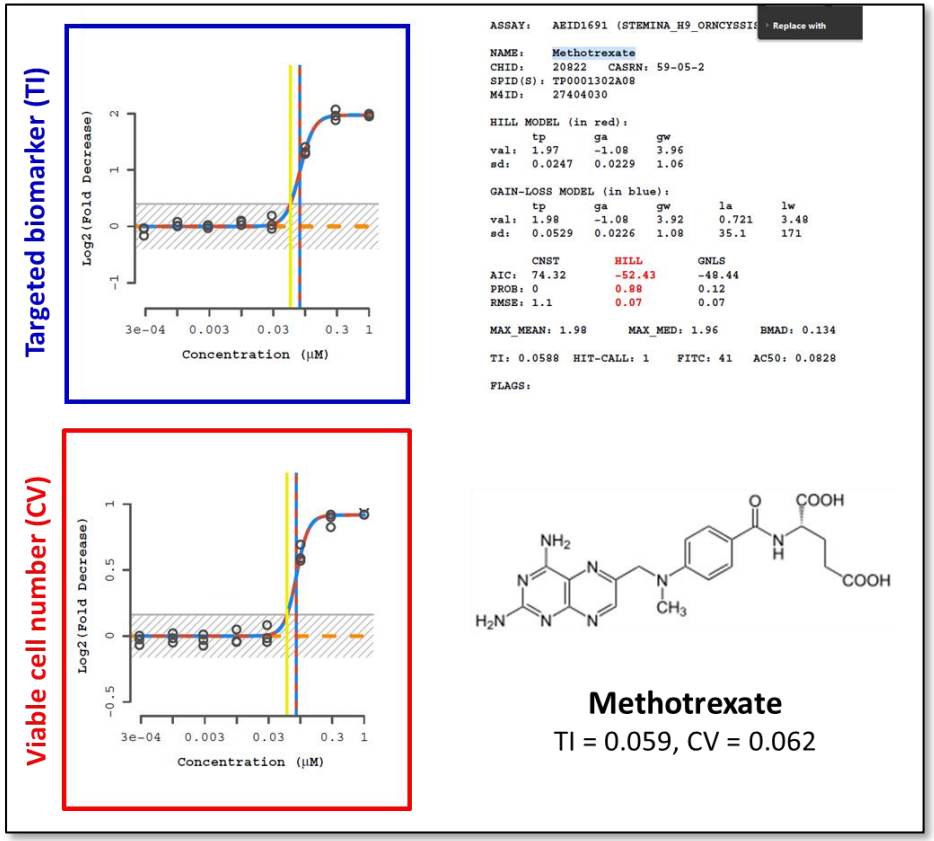
- 5-FU (teratogenic in virtually all species): consistent positivity in the 0.01-1 µg/ml range
- Thalidomide (species-specific): consistent positivity by hPSCs in the 0.1-1 µg/ml range
- Caffeine (equivocal): positivity in the 100 - 1000 µg/ml range

Analysis by B Flick (from Piersma et al., in preparation)

ToxCast_STM: *devTOX^{qp} assay contracted from Stemina Biomarker Discovery*

- 1065 ToxCast Ph I/II chemicals at single-conc. or multi-conc.;
- ToxCast_STM dataset includes controls for data quality;
- data pipelined to *in vitro-db_v3* database (>1125 features);
- dataset available in EPA's CompTox Chemicals Dashboard.

https://comptox.epa.gov/dashboard

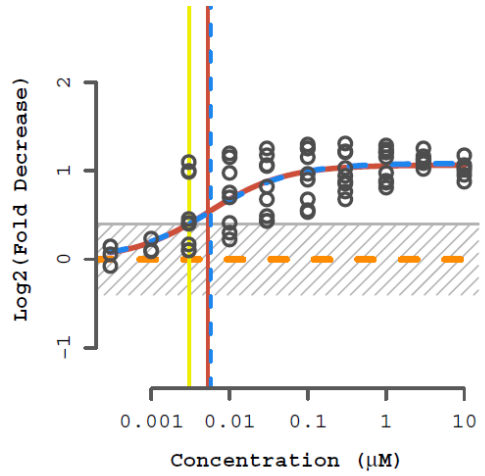


Zurlinden et al. (2020) Toxicol Sci

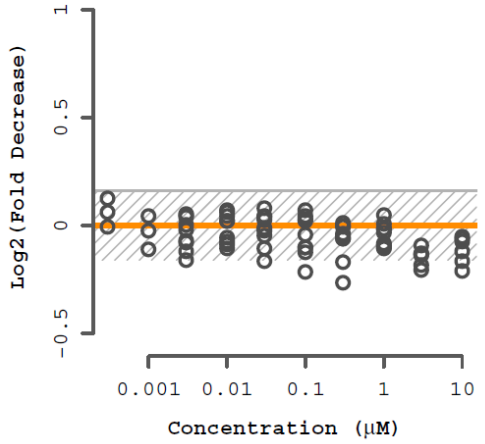
19.2% positivity rate indicative of teratogenic potential

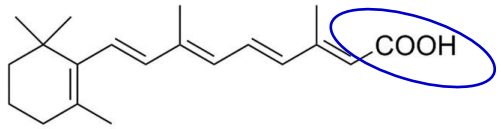
Example 1: *vitamin-A and its morphogenetic metabolite (all-trans Retinoic acid)*

Targeted biomarker (TI)



Viable cell number (CV)



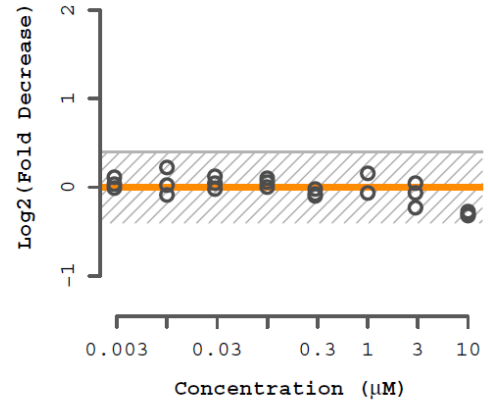


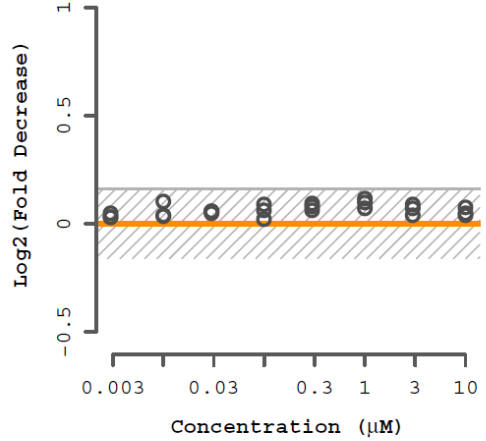
all trans Retinoic acid

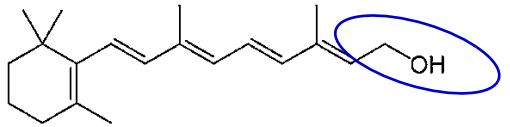
TI = 0.003 µM, CV = NA

dLEL rat = 2.5 mg/kg/day

dLEL rabbit = 0.5 mg/kg/day







Retinol (vitamin-A)

TI = NA, CV = NA

(True Negative)

ATRA thresholds: *teratogenesis and morphogenetic signaling*

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Retinoid signaling in skeletal development: Scoping the system for predictive toxicology

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ARTICLE INFO

ABSTRACT

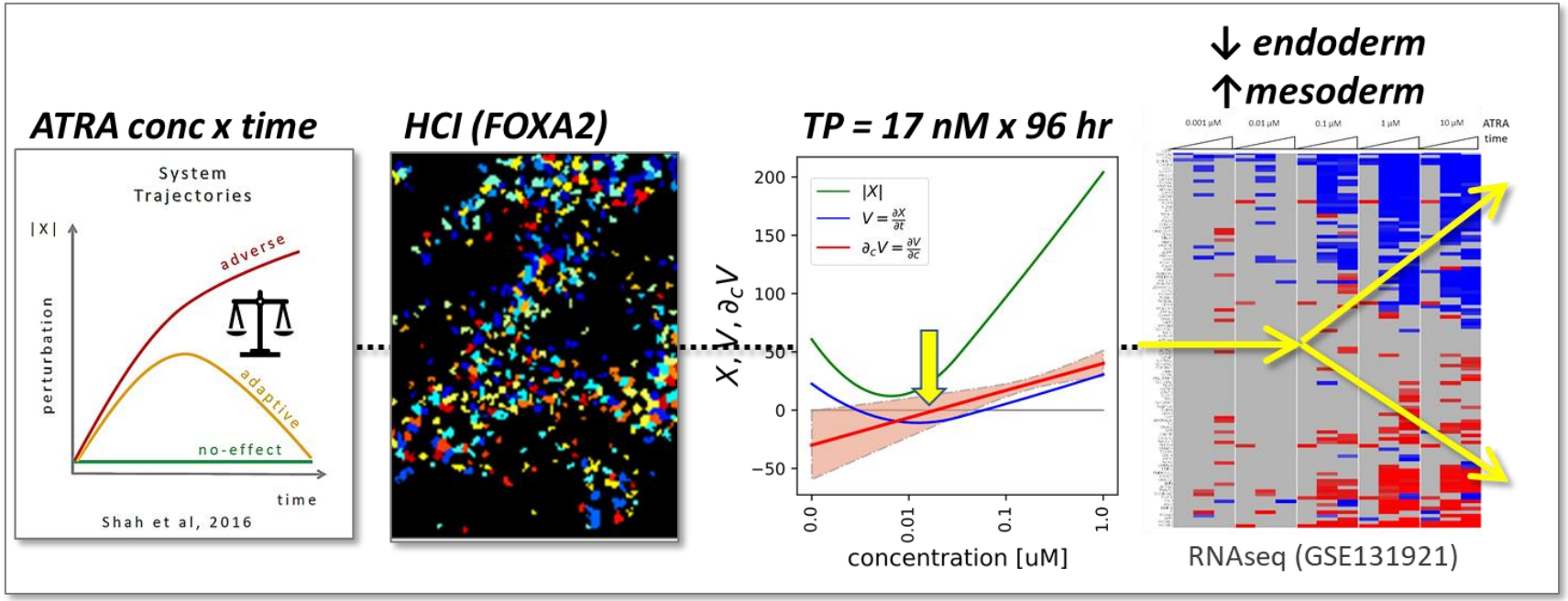
Preface

Dosimetric	Conc.	Indication	Reference
baseline ATRA (5 somite zebrafish embryo)	< 1 nM	non-morphogenetic	(Shimozono, Iimura et al. 2013)
maternal serum (animal study)	1.7 nM	non-teratogenic	(Daston, Beyer et al. 2014)
devTOX^{qp} assay (pluripotent hESC)	3.0 nM	teratogenic threshold	(Zurlinden, Saili et al. 2020)
normal plasma concentration	5.0 nM	physiological (adult)	(Napoli, Posch et al. 1991)
axial gradient (5 somite zebrafish embryo)	6.0 nM	morphogenetic signal	(Shimozono, Iimura et al. 2013)
endodermal differentiation (h-iPSC)	17 nM	toxicological tipping point	(Saili, Antonijevic et al. 2019)
devTOX^{qp} assay (pluripotent h-iPSC)	19 nM	DevTox potential	(Palmer, Smith et al. 2017)
genetic perturbation (mouse)	30 nM	altered homeostasis	(Helms, Thaller et al. 1994)
maternal serum (animal study)	30 nM	teratogenic potential	(Daston, Beyer et al. 2014)
limb-bud (GD 10.5 mouse embryo)	30 nM	physiological (embryo)	(Horton and Maden 1995)
pharmacological kinetics	1,000 nM	efficacious (therapeutic)	(Helms, Thaller et al. 1994)
limb-bud (GD 11 mouse embryo)	1,500 nM	weakly teratogenic dose	(Satre and Kochhar 1989)
limb-bud (GD 10.5 mouse embryo)	12,500 nM	fully teratogenic dose	(Horton and Maden 1995)

Knudsen et al., Reprod Toxicol (2021) – special issue devoted to retinoid signaling (Guest Editor: H Håkansson).

Molecular characterization of a toxicological tipping point:

transcriptomic (RNAseq) signature of adaptation versus adversity

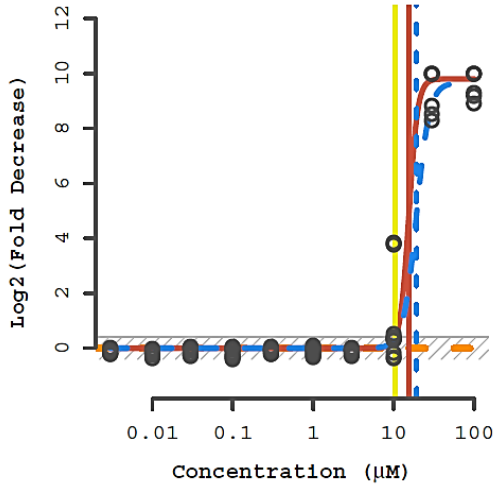


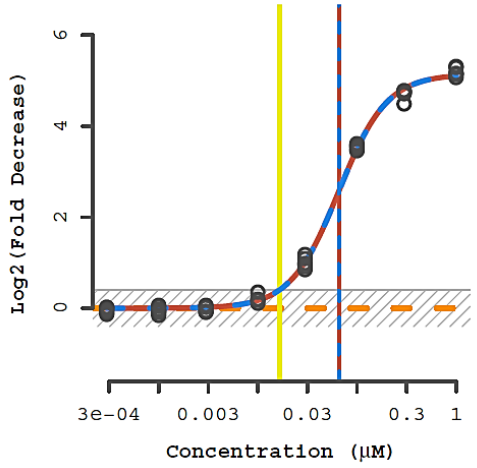
- What would a 'toxicological tipping point' on hPSC differentiation look like at the molecular level? Model = all trans retinoic acid (ATRA).
- ATRA is an endogenous signal (< 10 nM) and human teratogen (> 30 nM); tipping point computed at 17 nM @ 96 hr by imaging FOXA2 biomarker.
- RNAseq showed dysregulation by *EOMES* that normally drives endodermal specification and mesodermal delamination during gastrulation.

Saili et al. (2020) Reprod Tox

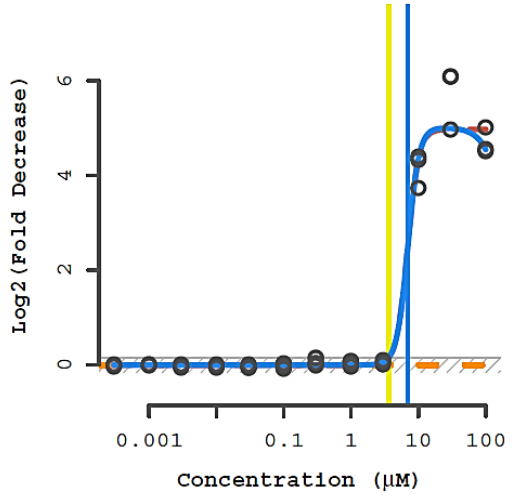
Example 2: *pharmacological angiogenesis inhibitors*

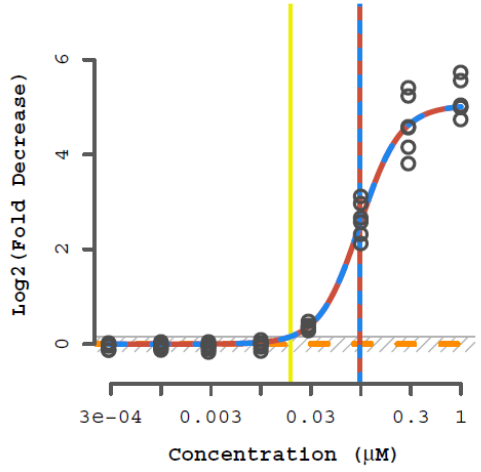
Targeted biomarker (TI)

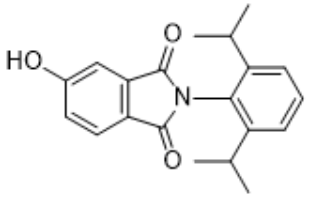




Viable cell number (CV)





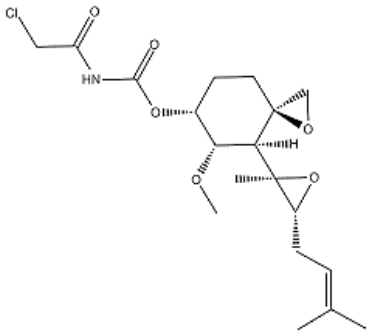


synthetic thalidomide analog

5HPP-33

TI = 10.5, CV = 16.4

(no rat or rabbit data)



synthetic fumagillin analog

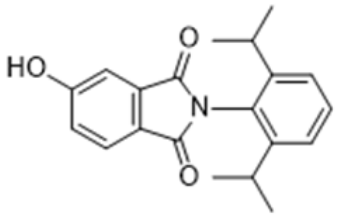
TNP-470

TI = 0.017, CV = 0.020

(no rat or rabbit data)

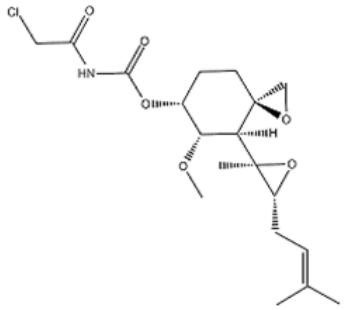
Case study: *checking forward predictivity of the hPSC biomarker assay*

Colleagues at Dow Chemical, led by Ed Carney, tested T.I. predictions for two structurally diverse potential vascular disrupters (pVDCs) in rat whole embryo culture (WEC):



5HPP-33: *synthetic thalidomide analog*

- T.I. predicted by hESC 10.5 µM
- AC50 observed in WEC 21.2 µM (embryo viability)



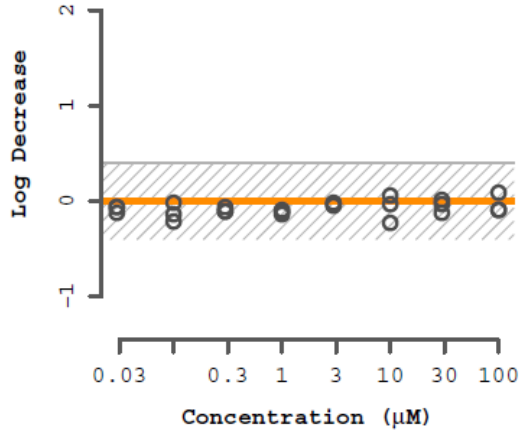
TNP-470: *synthetic fumagillin analog*

- T.I. predicted by hESC 0.02 µM
- AC50 observed in WEC 0.04 µM (dysmorphogenesis)

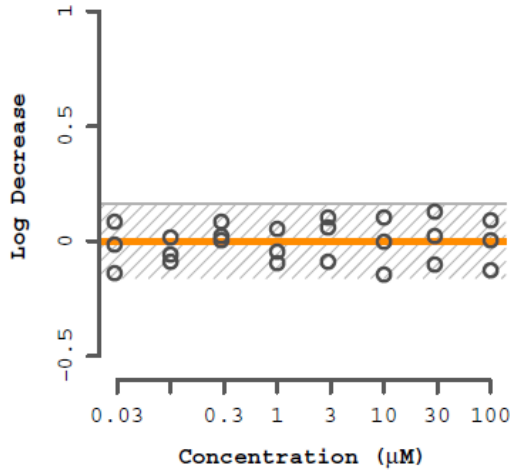
Ellis-Hutchings et al. (2017) Reprod Toxicol

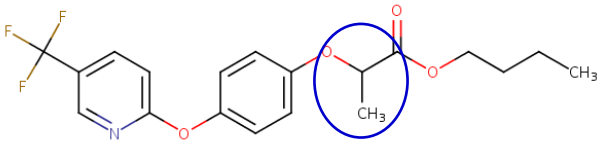
Example 3: *R-enantiomer (Fluazifop-P-butyl) is the active herbicide*

Targeted biomarker (TI)



Viable cell number (CV)



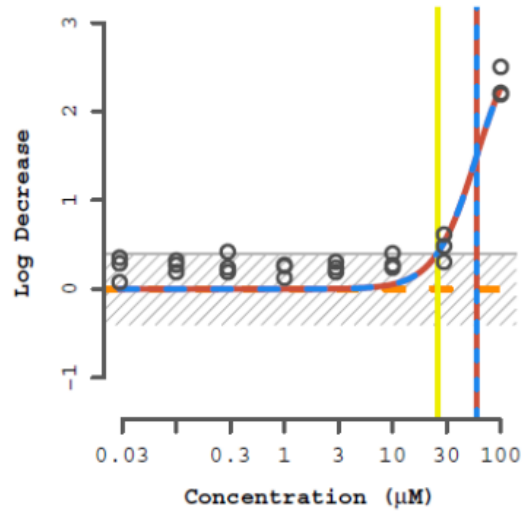


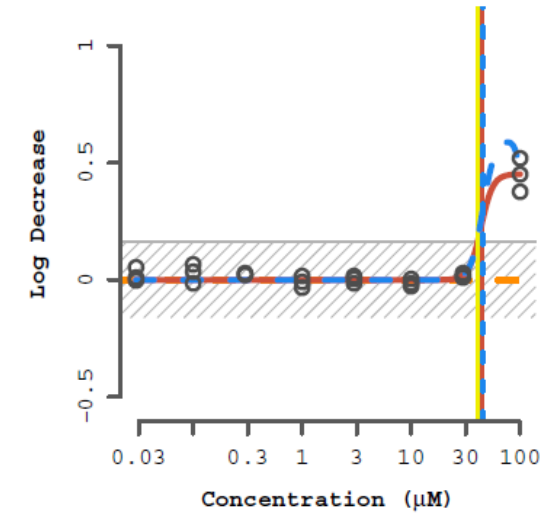
Fluazifop butyl

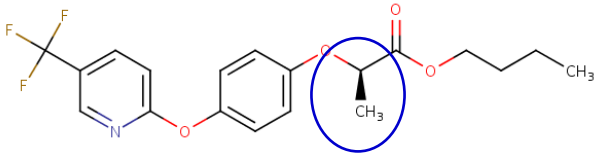
TI = not active, CV = no effect

dLEL rat = 10 mg/kg/day (< mLEL)

dLEL rabbit = 90 mg/kg/day (mLEL)







Fluazifop-P-butyl

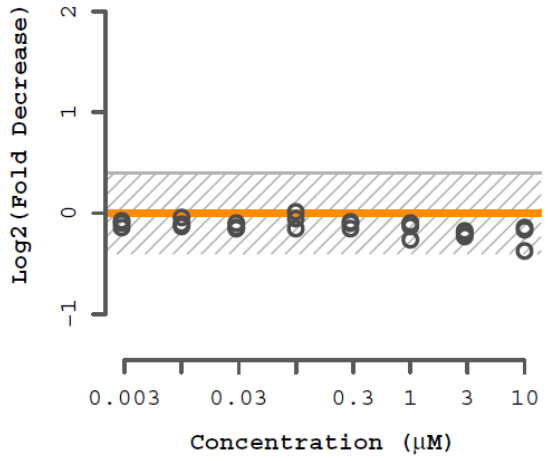
TI = 26 µM, CV = 40.8 µM

dLEL rat = 5 mg/kg/day (< mLEL)

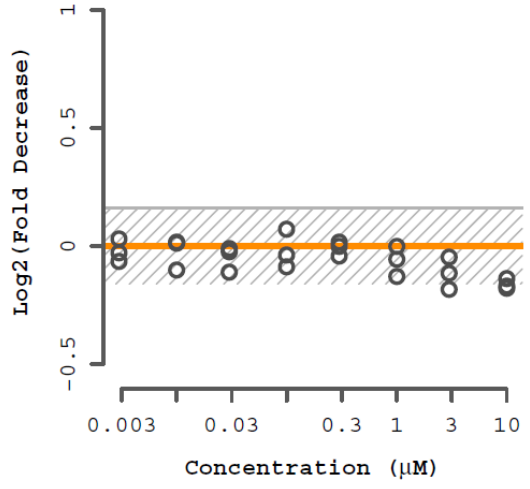
dLEL rabbit = 50 mg/kg/day (mLEL)

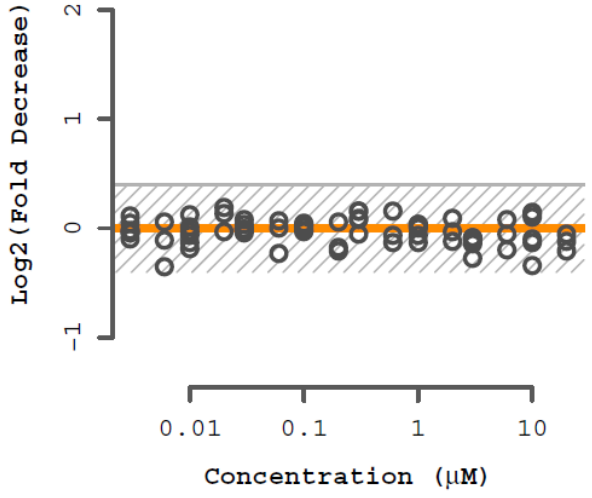
Example 4: *false negatives (not detected in ToxCast_STM)*

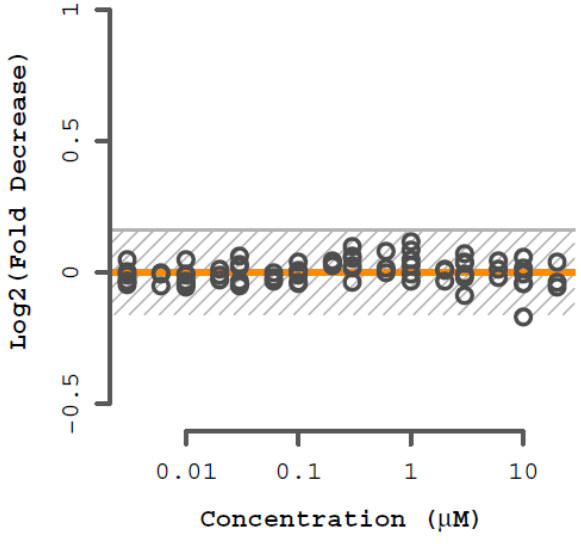
Targeted biomarker (TI)

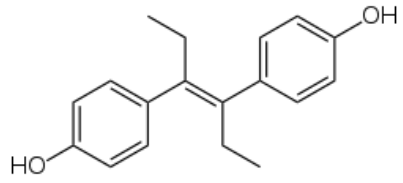


Viable cell number (CV)







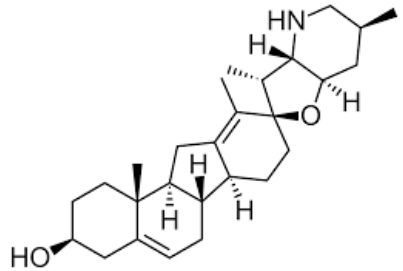


Diethylstilbestrol (DES)

TI = NA, CV = NA

dLEL rat = 0.03 mg/kg/day (= mLEL)

(no rabbit data in ToxRefDB)

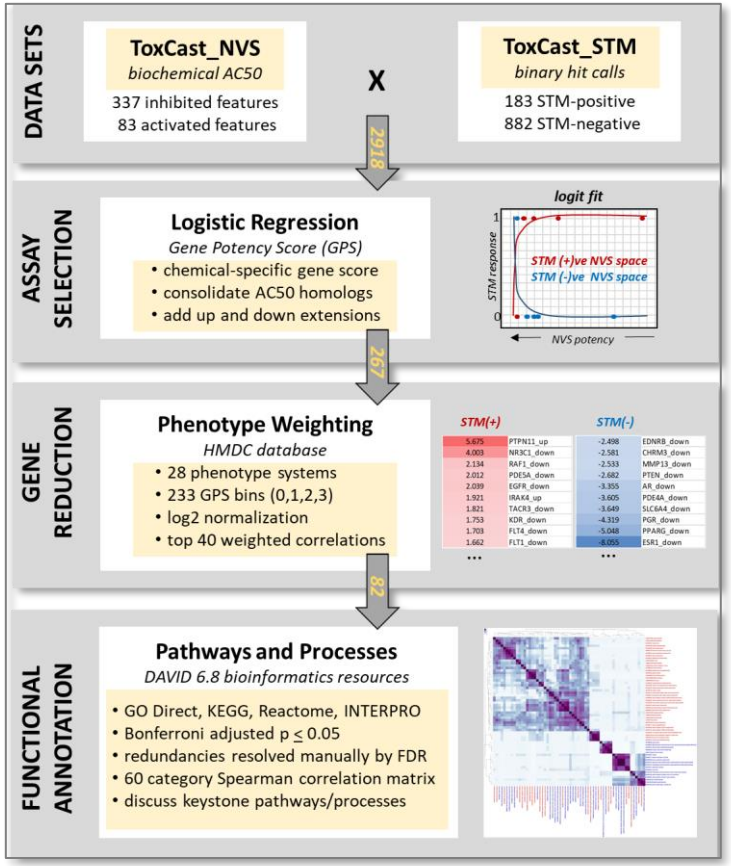


Cyclopamine

TI = NA, CV = NA

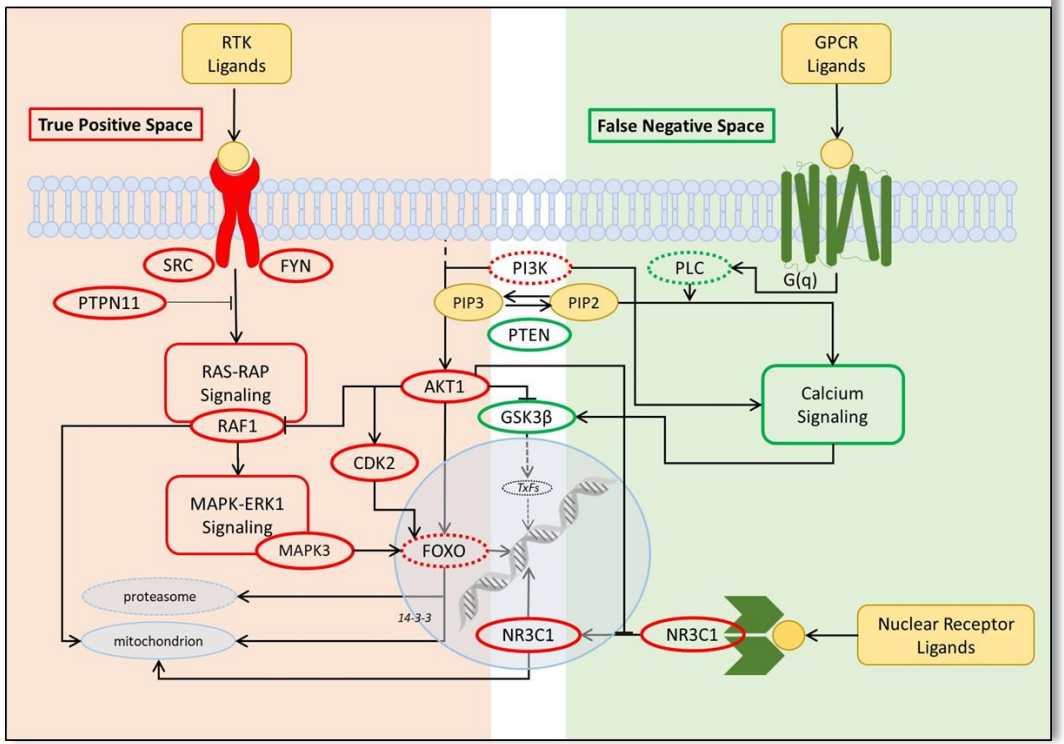
What human relevant pathways are detected or missed?

Workflow to mine the hPSC model against 337 biochemical assays in the ToxCast_NVS platform



Sensitive Domain **Insensitive Domain**

Annotation System	Keystone Pathway / Process	# MIEs	Class
GOTERM_BP_DIRECT	GO:0014066~regulation of phosphatidylinositol 3-kinase signaling	6	TP
KEGG_PATHWAY	hsa04068:FoxO signaling pathway	8	TP
KEGG_PATHWAY	hsa04510:Focal adhesion	13	TP
GOTERM_BP_DIRECT	GO:0007200~phospholipase C-activating G-protein coupled receptor signaling pathway	10	FN
INTERPRO	IPR001723:Steroid hormone receptor	7	FN
GOTERM_MF_DIRECT	GO:0005496~steroid binding	5	FN



Zurlinden et al. (2020) Toxicol Sci

Performance check for hPSC-based classification of DevTox

- Qualification on 42 well-curated reference compounds often used to validate alternative DevTox platforms[1].
- Balanced Accuracy (BAC) = 82% (0.65 sensitivity, 1.00 specificity) for these reference chemicals.
- Metrics are consistent with the original pharma-trained model [Palmer et al. 2013].

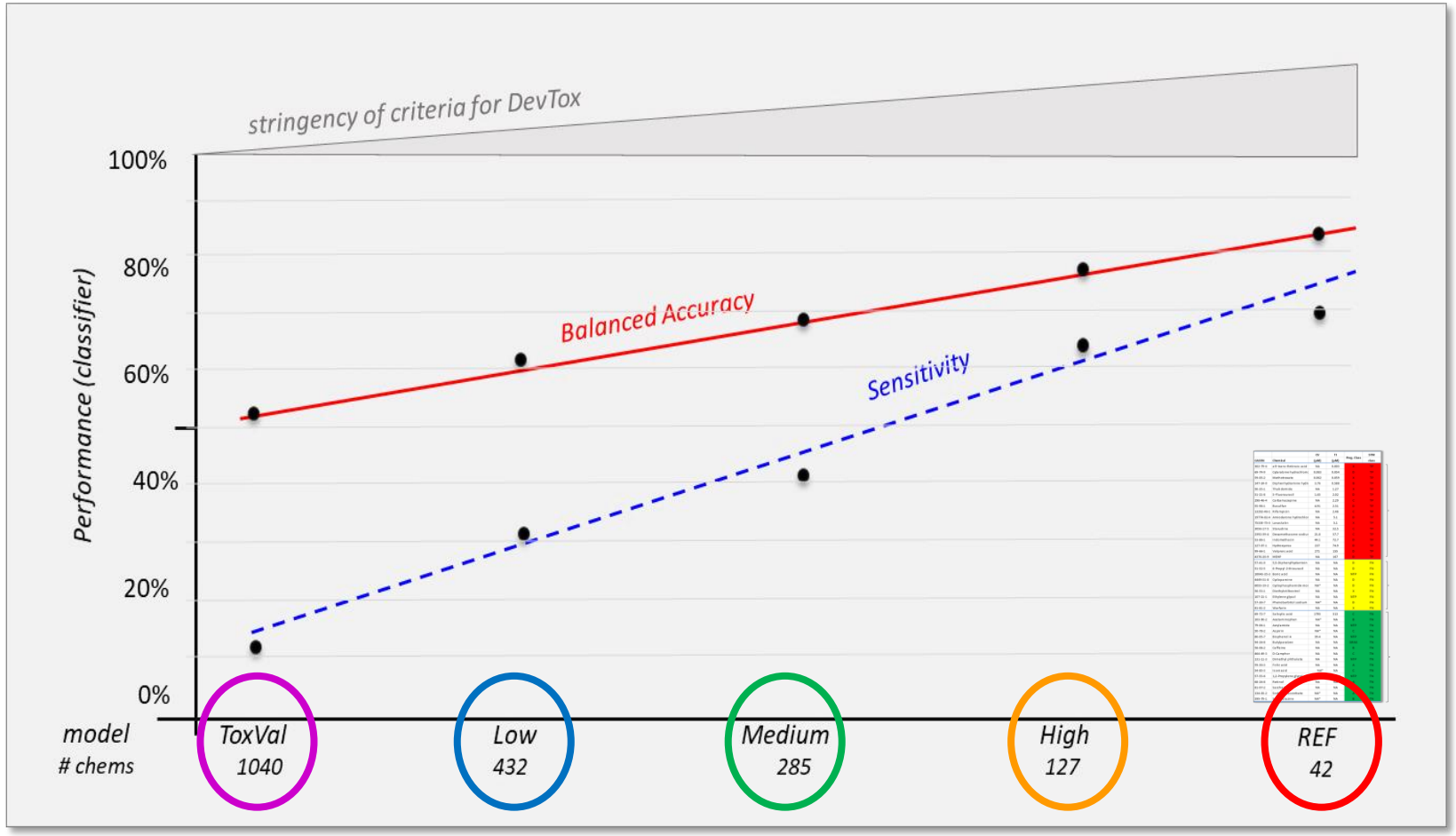
Many alternative assays have been validated with a limited set of data-rich chemicals, inflating predictive capacity of >80%; this has hampered regulatory acceptance.

CASRN	Chemical	CV (µM)	TI (µM)	Preg. Class	STM class	
302-79-4	all-trans-Retinoic acid	NA	0.003	X	TP	True Positive
69-74-9	Cytarabine hydrochloric	0.083	0.054	D	TP	
59-05-2	Methotrexate	0.062	0.059	X	TP	
147-24-0	Diphenhydramine hydr	3.76	0.588	B	TP	
50-35-1	Thalidomide	NA	1.27	X	TP	
51-21-8	5-Fluorouracil	1.45	2.02	D	TP	
298-46-4	Carbamazepine	NA	2.29	C	TP	
55-98-1	Busulfan	4.91	2.31	D	TP	
13292-46-1	Rifampicin	NA	2.46	C	TP	
19774-82-4	Amiodarone hydrochlor	NA	5.1	D	TP	
75330-75-5	Lovastatin	NA	5.1	X	TP	
3056-17-5	Stavudine	NA	32.5	C	TP	
2392-39-4	Dexamethasone sodiur	21.8	37.7	C	TP	
53-86-1	Indomethacin	44.1	72.7	D	TP	
127-07-1	Hydroxyurea	237	74.9	D	TP	
99-66-1	Valproic acid	271	155	D	TP	
4376-20-9	MEHP	NA	167	D	TP	
57-41-0	5,5-Diphenylhydantoin	NA	NA	D	FN	False Negative
51-52-5	6-Propyl-2-thiouracil	NA	NA	D	FN	
10043-35-3	Boric acid	NA	NA	NTP	FN	
4449-51-8	Cyclopamine	NA	NA	D	FN	
6055-19-2	Cyclophosphamide mor	NA*	NA	D	FN	
56-53-1	Diethylstilbestrol	NA	NA	X	FN	
107-21-1	Ethylene glycol	NA	NA	NTP	FN	
57-30-7	Phenobarbitol sodium	NA*	NA	D	FN	
81-81-2	Warfarin	NA	NA	X	FN	
69-72-7	Salicylic acid	1795	513	C	TN	True Negative
103-90-2	Acetaminophen	NA*	NA	B	TN	
79-06-1	Acrylamide	NA	NA	NTP	TN	
50-78-2	Aspirin	NA*	NA	C	TN	
80-05-7	Bisphenol A	39.4	NA	NTP	TN	
94-26-8	Butylparaben	NA	NA	GRAS	TN	
58-08-2	Caffeine	NA	NA	B	TN	
464-49-3	D-Camphor	NA	NA	C	TN	
131-11-3	Dimethyl phthalate	NA	NA	NTP	TN	
59-30-3	Folic acid	NA	NA	A	TN	
54-85-3	Isoniazid	NA*	NA	C	TN	
57-55-6	1,2-Propylene glycol	327552	246664	NTP	TN	
68-26-8	Retinol	NA	NA	A	TN	
81-07-2	Saccharin	NA	NA	A	TN	
134-03-2	Sodium L-ascorbate	NA*	NA	A	TN	
599-79-1	Sulfasalazine	NA*	NA	B	TN	

Zurlinden et al. (2020), Toxicol Sci

[1] Genschow et al. 2002; West et al. 2010; Daston et al. 2014; Augustine-Rauch et al. 2016; Wise et al. 2016

Chemical landscape: *hPSC biomarker (in vitro) and ToxRefDB (in vivo)*



Scaling Criteria (ToxRefDB)

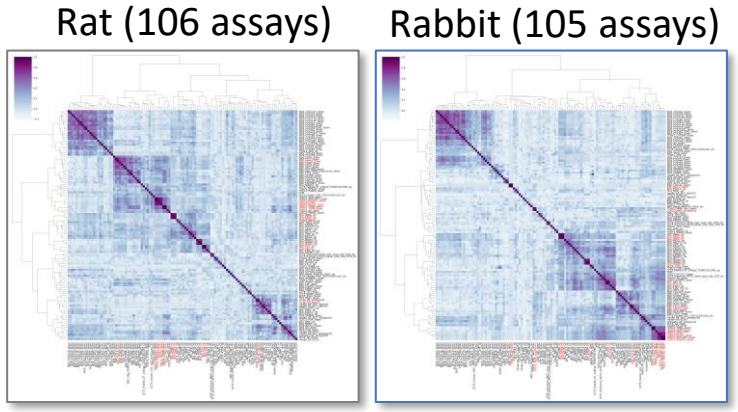
- **BM-42 reference**
- **concordant, rat AND rabbit**
- **dLEL < mLEL, rat OR rabbit**
- **dLEL ≤ 200 mg/kg/day**
- **LEL for any study type**

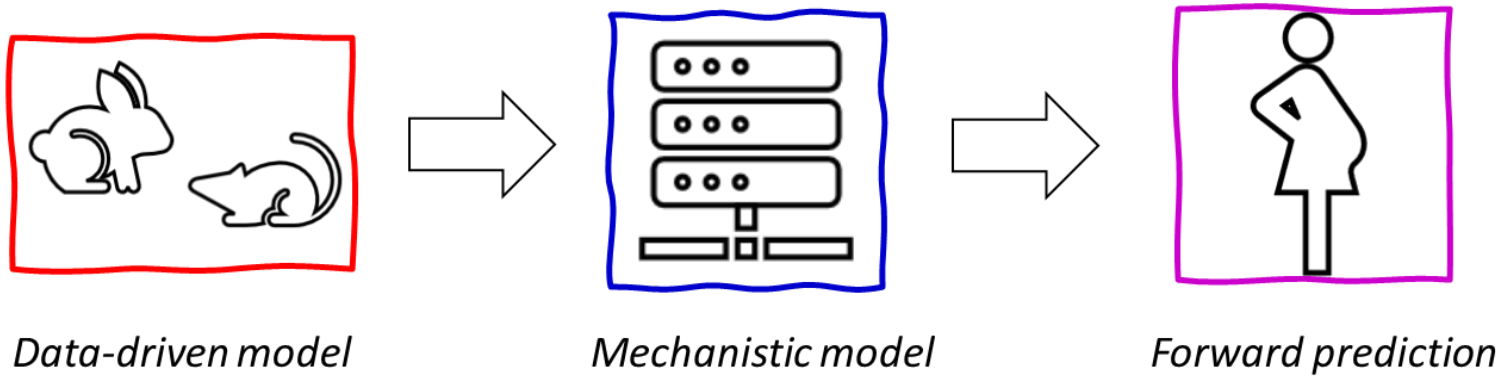
Predictivity of the hPSC biomarker declined as fetal outcome gained less concordance between rat-rabbit and concurrent maternal toxicity.

Zurlinden et al. (2020), Toxcol Sci

Bridging animal-human studies

- Although positivity rate (19.2%) of the hPSC assay was similar to concordant rat-rabbit studies (18.7%), only a subset of positives was detected by both platforms.
- Challenge for holistic understanding of the applicability domain and blind spots of *in vitro* platforms, as well as mechanisms against which bioactivity data may be qualified.
- AI models built from *in vitro* readouts having strongest correlation to univariate features for chemicals with stringent correlations to DEV outcomes in rat (n=218) or rabbit (n=244).

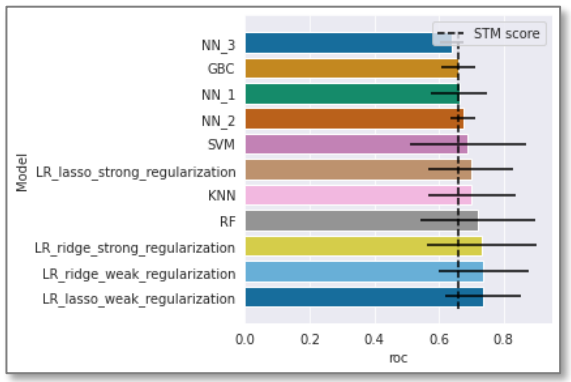


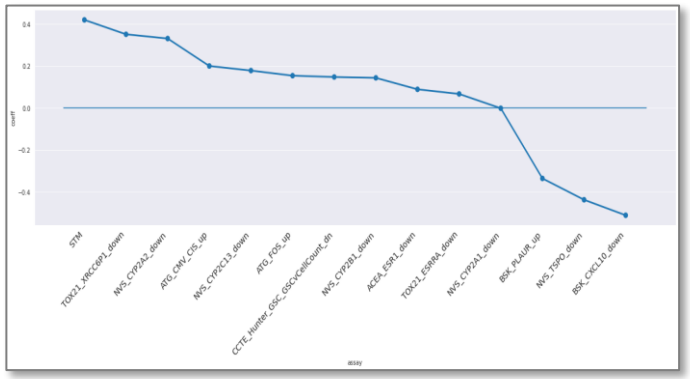


Zurlinden et al., work in progress

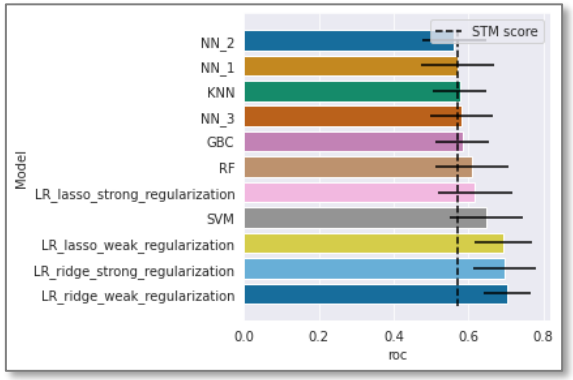
Bridging animal-human studies

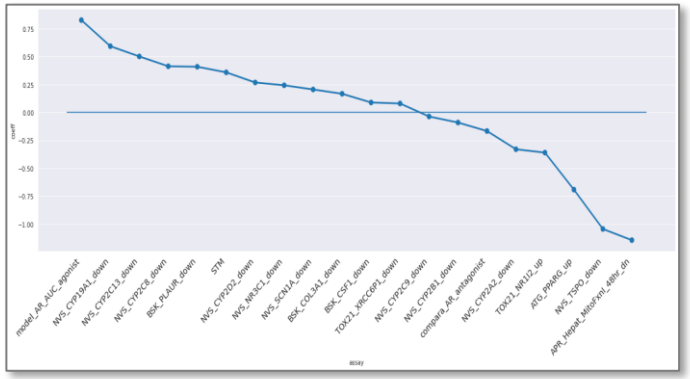
rat



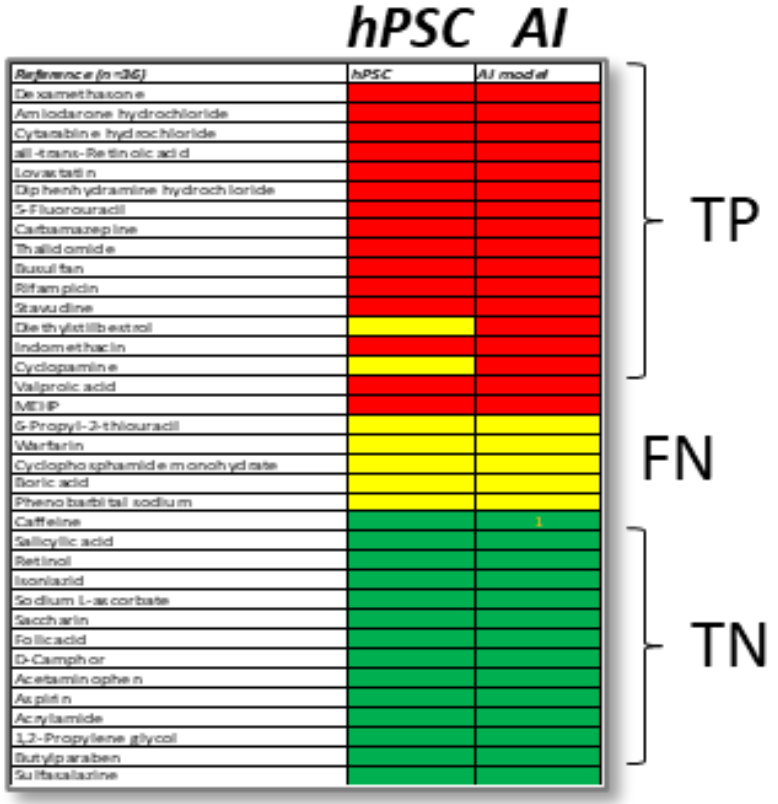


rabbit





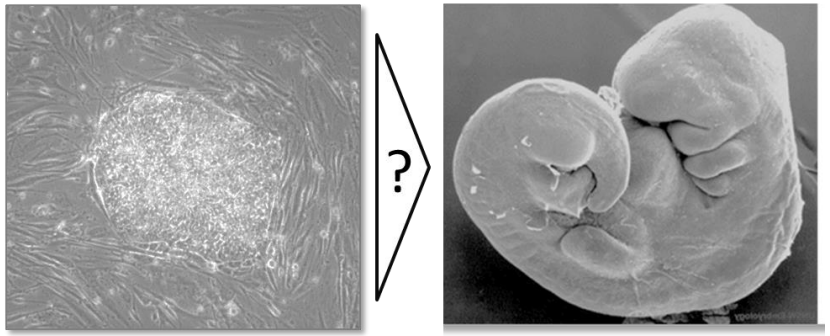
consensus



Preliminary: Top features discovered for training AI model improves sensitivity over the hPSC biomarker alone (BAC = 86.8% vs 83.3%), but still misses a few.

Zurlinden et al., work in progress

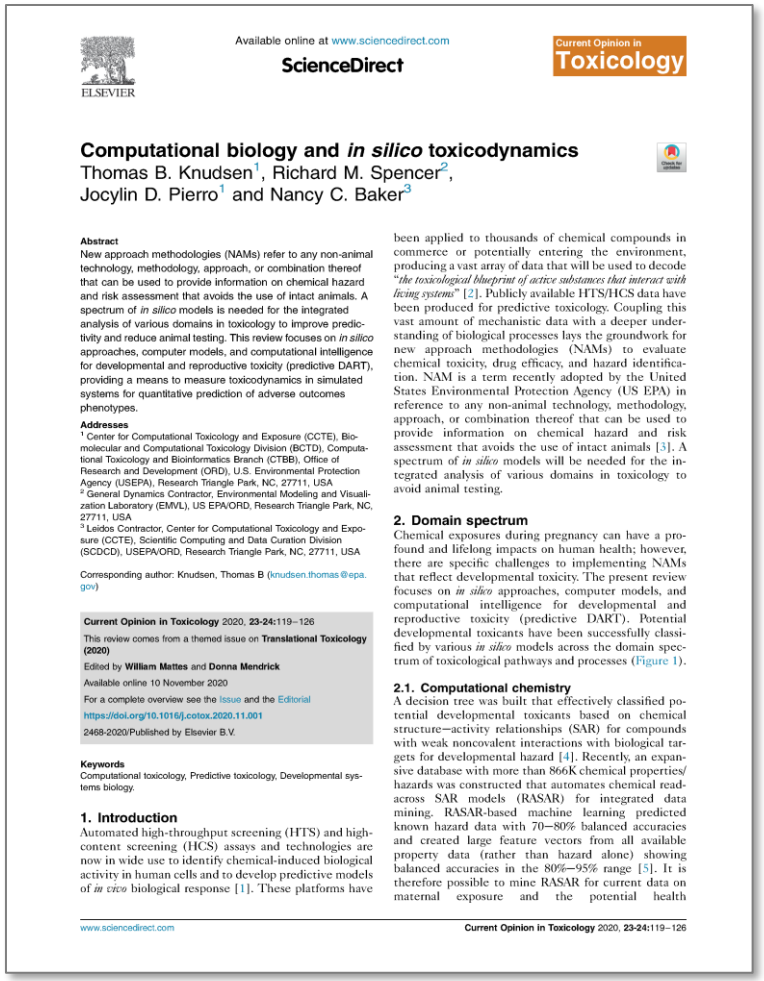
Can a hPSC assay live up to the NAM challenge?



Motivation for a building a more synoptic view to improve mechanistic understanding of developmental processes and toxicities around hPSCs.

- does not encompass the full complexity of anatomical development;
- blind to the precise spatial-temporal control of cell-cell interactions *in vivo* ;
- misses developmental effects secondary to maternal or placental toxicity;
- uncertainty of post-organogenesis vulnerability and post-natal manifestations;
- cross-species extrapolation (mESC to human, hPSC to animals);
- limited xenobiotic metabolism and other ADME considerations (toxicokinetics);
- uncertainties in translatability to the intact embryo (toxicodynamics).

A more synoptic view …



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Current Opinion in Toxicology

ELSEVIER

Computational biology and *in silico* toxicodynamics

Thomas B. Knudsen[1], Richard M. Spencer[2], Jocylin D. Pierro[1] and Nancy C. Baker[3]

Abstract

New approach methodologies (NAMs) refer to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals. A spectrum of *in silico* models is needed for the integrated analysis of various domains in toxicology to improve predictivity and reduce animal testing. This review focuses on *in silico* approaches, computer models, and computational intelligence for developmental and reproductive toxicity (predictive DART), providing a means to measure toxicodynamics in simulated systems for quantitative prediction of adverse outcomes phenotypes.

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Keywords

Computational toxicology, Predictive toxicology, Developmental systems biology.

1. Introduction

Automated high-throughput screening (HTS) and high-content screening (HCS) assays and technologies are now in wide use to identify chemical-induced biological activity in human cells and to develop predictive models of *in vivo* biological response [1]. These platforms have been applied to thousands of chemical compounds in commerce or potentially entering the environment, producing a vast array of data that will be used to decode "*the toxicological blueprint of active substances that interact with living systems*" [2]. Publicly available HTS/HCS data have been produced for predictive toxicology. Coupling this vast amount of mechanistic data with a deeper understanding of biological processes lays the groundwork for new approach methodologies (NAMs) to evaluate chemical toxicity, drug efficacy, and hazard identification. NAM is a term recently adopted by the United States Environmental Protection Agency (US EPA) in reference to any non-animal technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals [3]. A spectrum of *in silico* models will be needed for the integrated analysis of various domains in toxicology to avoid animal testing.

2. Domain spectrum

Chemical exposures during pregnancy can have a profound and lifelong impacts on human health; however, there are specific challenges to implementing NAMs that reflect developmental toxicity. The present review focuses on *in silico* approaches, computer models, and computational intelligence for developmental and reproductive toxicity (predictive DART). Potential developmental toxicants have been successfully classified by various *in silico* models across the domain spectrum of toxicological pathways and processes (Figure 1).

2.1. Computational chemistry

A decision tree was built that effectively classified potential developmental toxicants based on chemical structure–activity relationships (SAR) for compounds with weak noncovalent interactions with biological targets for developmental hazard [4]. Recently, an expansive database with more than 866K chemical properties/hazards was constructed that automates chemical read-across SAR models (RASAR) for integrated data mining. RASAR-based machine learning predicted known hazard data with 70–80% balanced accuracies and created large feature vectors from all available property data (rather than hazard alone) showing balanced accuracies in the 80%–95% range [5]. It is therefore possible to mine RASAR for current data on maternal exposure and the potential health

Knudsen et al. (2020) Curr Opin Toxicol

- Computational biology and computer simulation can extend data-driven models for mechanistic prediction.
- Enablers of virtual tissue models (VTMs):

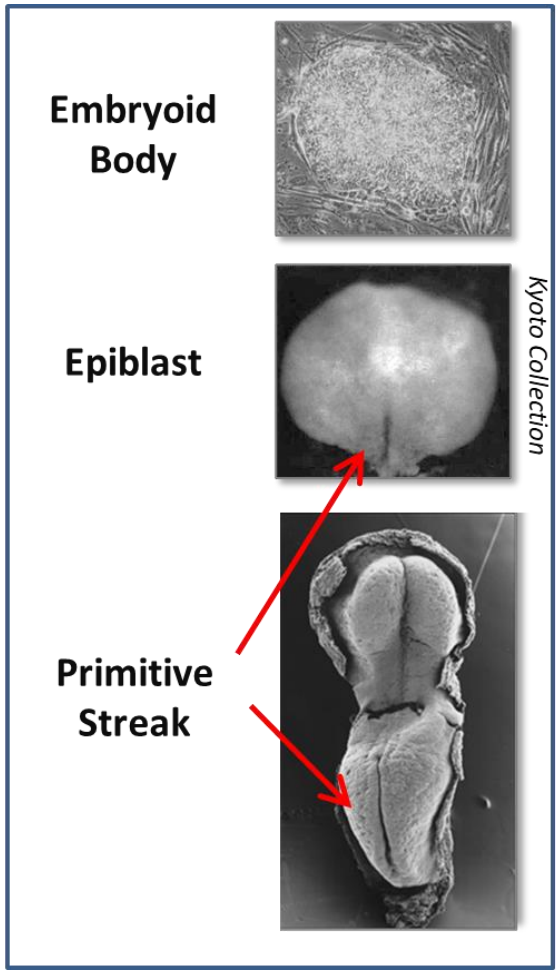
  - **synthetic microsystems:** recapitulate the microphysiology, cellular behaviors and spatial dynamics of the physical system.

  - **computational intelligence:** biology-inspired algorithms use fuzzy logic to fill in missing or incomplete information.

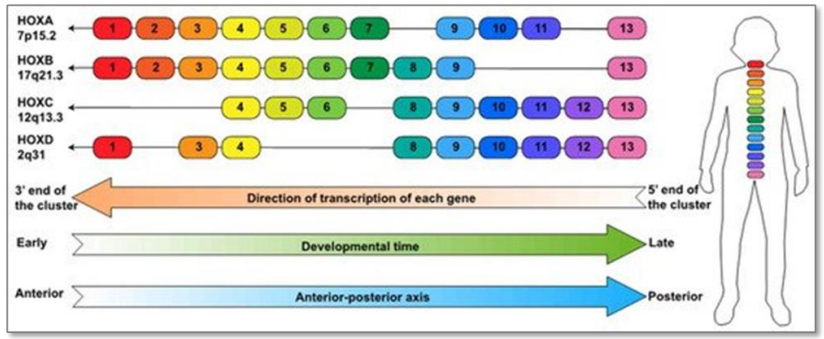
  - **artificial life:** computer simulation of biological processes evolved through automation, control networks.

Gastrulating embryo:

remarkable example of a self-organizing system



- The molecular biology and behavior of hPSCs in culture most closely resembles the epiblast of an early embryo during 'gastrulation'.
- Gastrulation '*decodes the genomic blueprint of the fetal body plan* ' through complex signaling pathways (e.g, FOX, SOX, HOX).
- Cell migration through the primitive streak is essential for regional organization but cultured hPSCs lack this positional information.

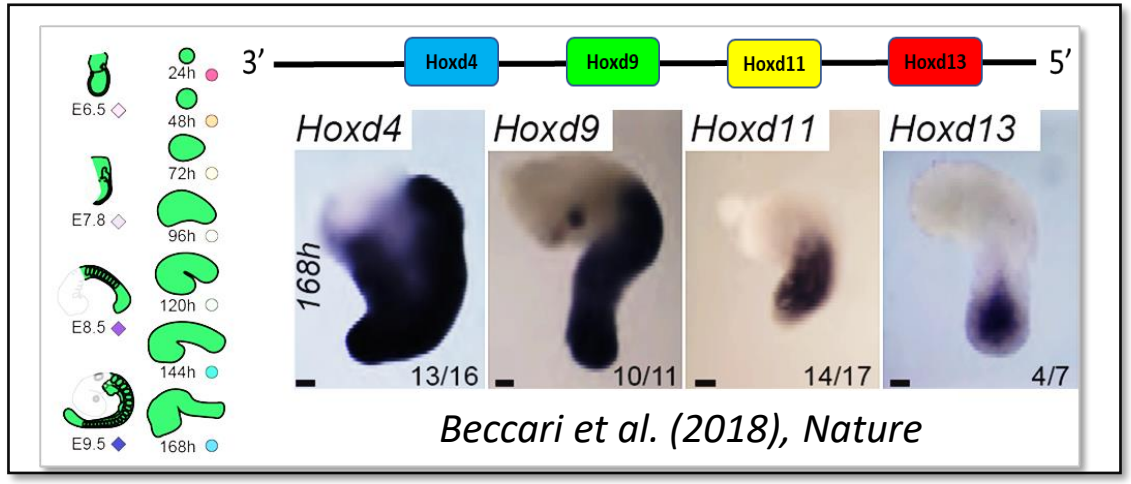


Luo et al. (2019)

"It is not birth, marriage, or death, but **gastrulation** which is truly the most important time in your life."

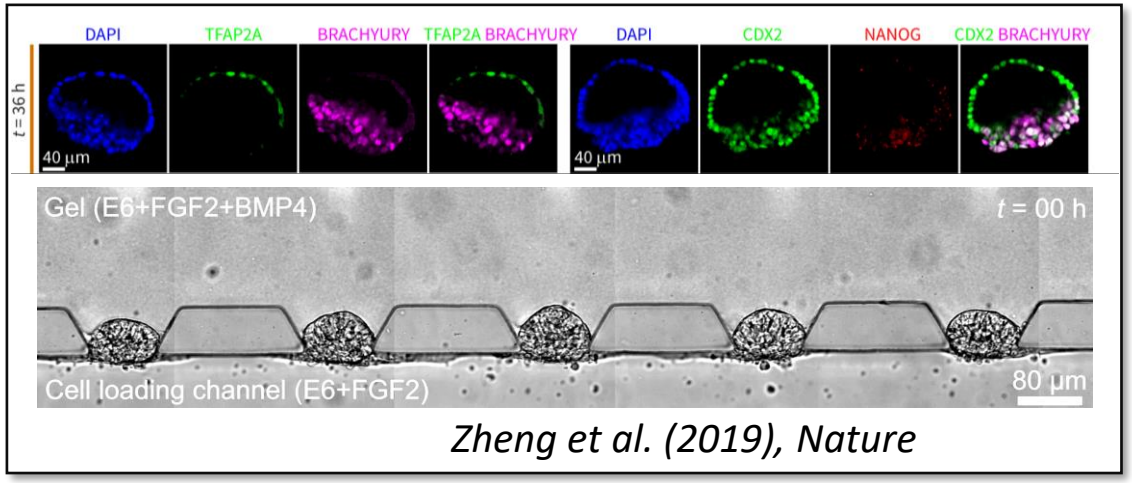
- Lewis Wolpert

Engineered *in vitro* microsystems



Beccari et al. (2018), Nature

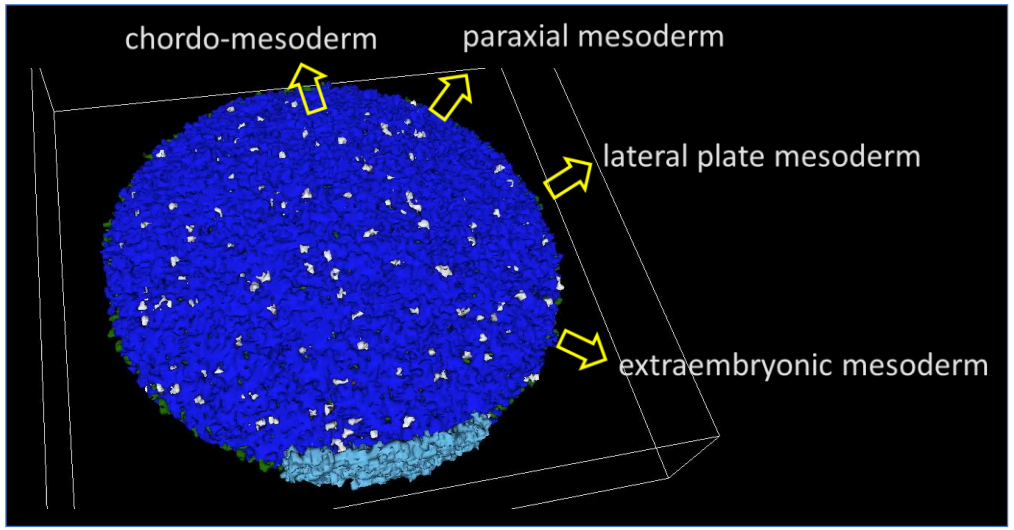
- iPSC-derived microsystems can self-organize at least some positional information.
- **Example**: colinear *Hox* expression in 'gastruloids' forming from mESC-aggregate.



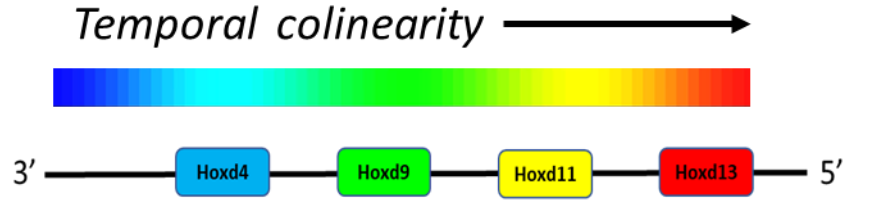
Zheng et al. (2019), Nature

- Properties come naturally to the epiblast via positional cell-cell signaling.
- **Example:** restoring FGF2-BMP4 signaling polarizes a synthetic epiblast from hPSCs.

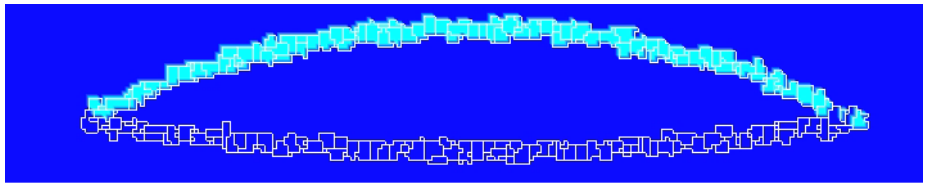
Computational (*in silico*) microsystems



- *CompuCell3D* cell agent-based model of the epiblast to restore positional information.
- Goal to '*recode the genomic blueprint of the fetal body plan*' for chemical effects data.
- Starting point is regulation of the 'Hox clock' by FGF and ATRA signaling.



Transverse slice at the organizer node
(4_9_11_13 @5000 MCS)



↓FGF signaling slows the Hox clock
(4_9_11_13 @5000 MCS)

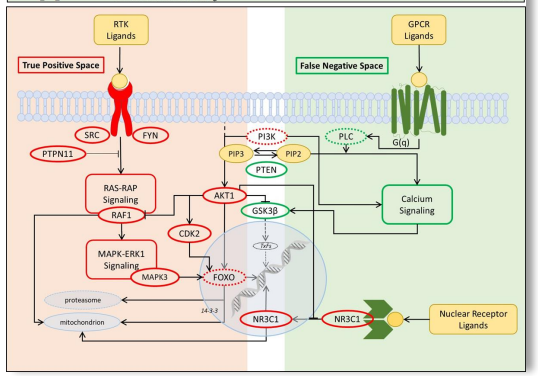
Deletions in the HOXD cluster are associated with severe limb and genital defects.

R Spencer, EMVL (work in progress

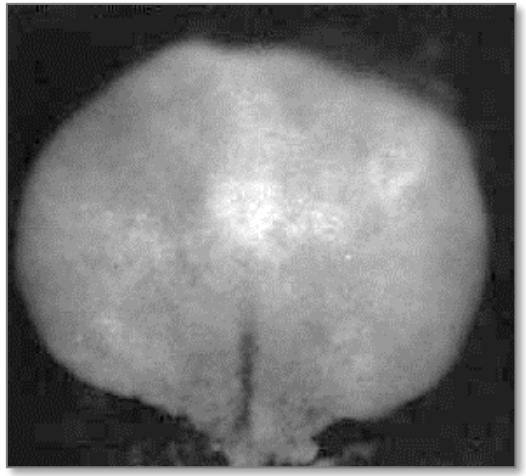
Synoptic manifold for toxicodynamics

hPSC profiling

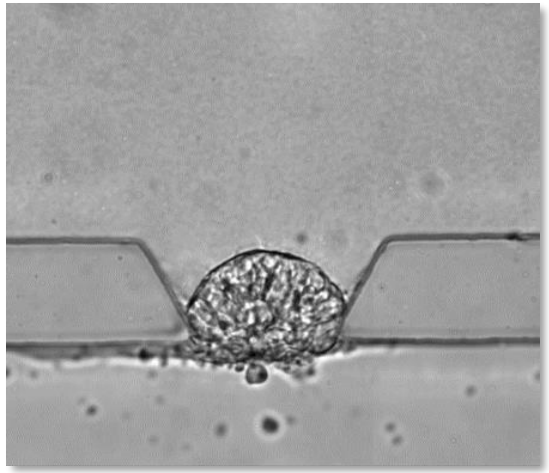
Annotation System	Keystone Pathway / Process	# MIEs	Class
GOTERM_BP_DIRECT	GO:0014066~regulation of phosphatidylinositol 3-kinase signaling	6	TP
KEGG_PATHWAY	hsa04068:FoxO signaling pathway	8	TP
KEGG_PATHWAY	hsa04510:Focal adhesion	13	TP
GOTERM_BP_DIRECT	GO:0007200~phospholipase C-activating G-protein coupled receptor signaling pathway	10	FN
INTERPRO	IPR001723:Steroid hormone receptor	7	FN
GOTERM_MF_DIRECT	GO:0005496~steroid binding	5	FN



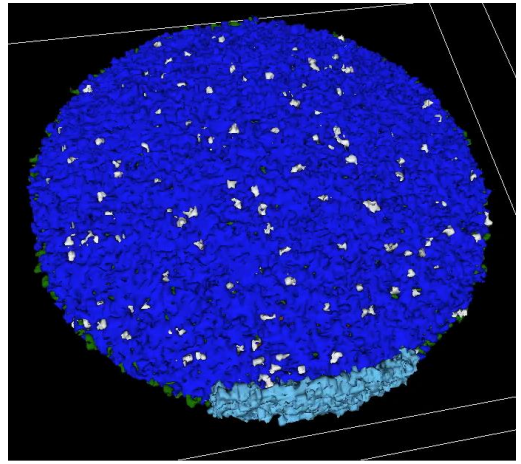
In vivo - knowledge



In vitro - MPS



In silico - ABM



- **Bioactivity profiling:** high-throughput screening of hPSCs (e.g., ToxCast/Tox21)
- **Synthetic microsystems:** recapitulate microphysiology of the physical system.
- **Computational intelligence:** fuzzy logic to fill in missing or incomplete information.
- **Artificial life:** biological plausibility evolved through control networks.

Virtual Tissue Models

The VTM Research Area will provide physical models and mathematical simulations of specific organ systems and developmental outcomes informing risk-based assessments of new and existing chemicals. This research area expands understanding of chemical effects on developmental and reproductive toxicology.

Outputs

CSS 5.1 (C Deisenroth)
CSS 5.2 (S Hunter)
CSS 5.3 (T Knudsen)

CSS 5.3– Computational VTMs

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Tox21 CPP #6 and CPP #13
OECD-WNT-TGP