CPP#6

Tox21 Face to Face Meeting
December 4-5, 2017
College Park MD

Predictive Modeling of Developmental Toxicity with Human Pluripotent Stem Cells

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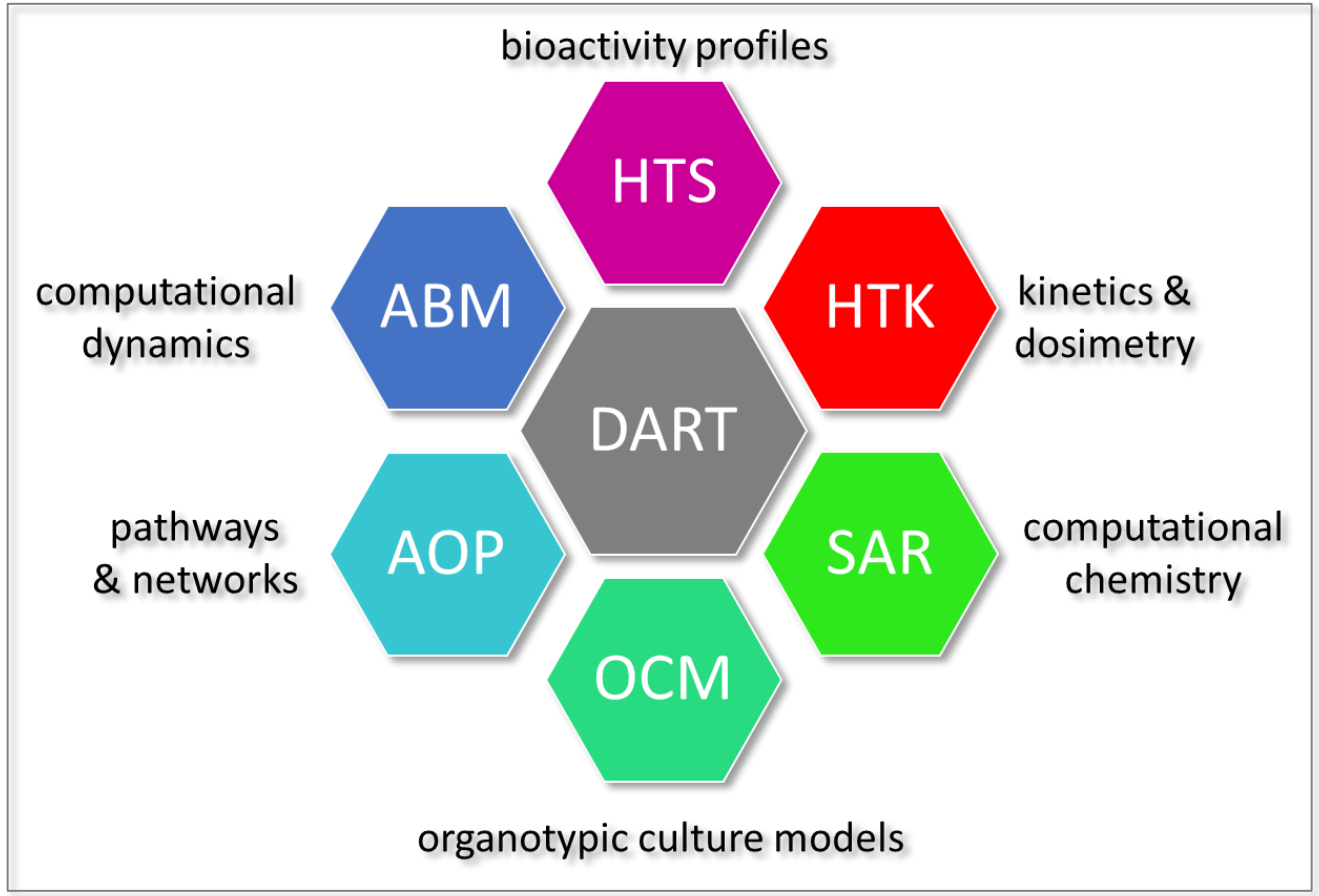
DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

1. Background



- Goal is computational modeling of ToxCast/Tox21 chemicals for ***predictive DART*** (prenatal developmental toxicity).

- Project focuses on applying fundamental principles of teratogenesis (birth defects) to human embryonic stem cells:
  - initiating mechanisms
  - dosimetry & bioavailability
  - stage vulnerability
  - chemistry
  - genetic susceptibility

2. Hypothesis and Specific Aims

Hypothesis: systematic integration of human pluripotent stem cell data into spatially-dynamic computer models of embryonic development will recapitulate the critical determinants of developmental toxicity based on the fundamental principles of teratology.

1. Data-mining: chemical-assay specific correlations to identify relevant mechanisms.
2. Validation: use in a prioritization schema for human teratological outcome.
3. Kinetics: reverse dosimetry for exposure-based hazard prediction in the human fetus.
4. Dynamics: system-specific vulnerabilities of the developing embryo.

3. Description of the overall project

Birth Defects Research Part B | Developmental and Reproductive Toxicology

Explore this journal >

Original Article

Establishment and Assessment of a New Human Embryonic Stem Cell-Based Biomarker Assay for Developmental Toxicity Screening

Jessica A. Palmer, Alan M. Smith, Laura A. Egnash, Kevin R. Conard, Paul R. West, Robert E. Burrier, Elizabeth L.R. Donley, Fred R. Kirchner

First published: August 2013 Full publication history

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Cited by (CrossRef): 18 articles Check for updates | Citation tools

View issue TOC
Volume 98, Issue 4
August 2013
Pages 343–363

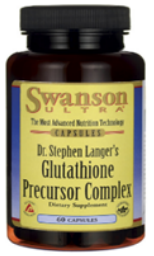
DevTOX^{qP} platform (Stemina Biomarker Discovery) Palmer et al. 2013

Pluripotent H9 human embryonic stem cell metabolomics assay that "… identified the potential developmental toxicants in the test set with 77% accuracy (57% sensitivity, 100% specificity)."

Ornithine release
urea cycle, polyamine & pyrimidine synthesis.

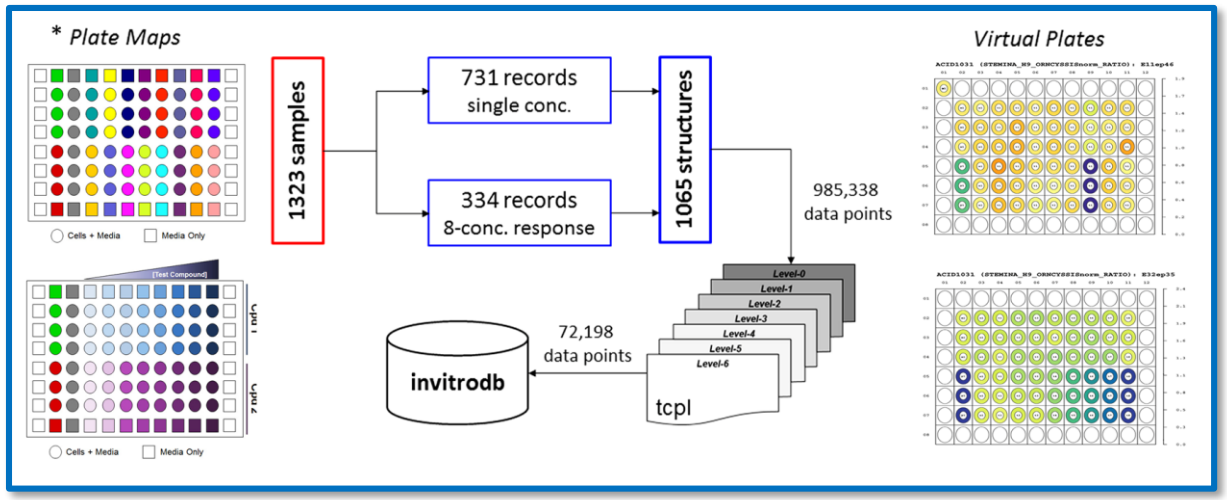
TI = ORN/CYSS

Cystine utilization
glutathione synthesis, redox cycling.



ToxCast Phase I/II library tested

(EPA contract EP-D-13-055)



Main STM assay component identifiers (*acid*):

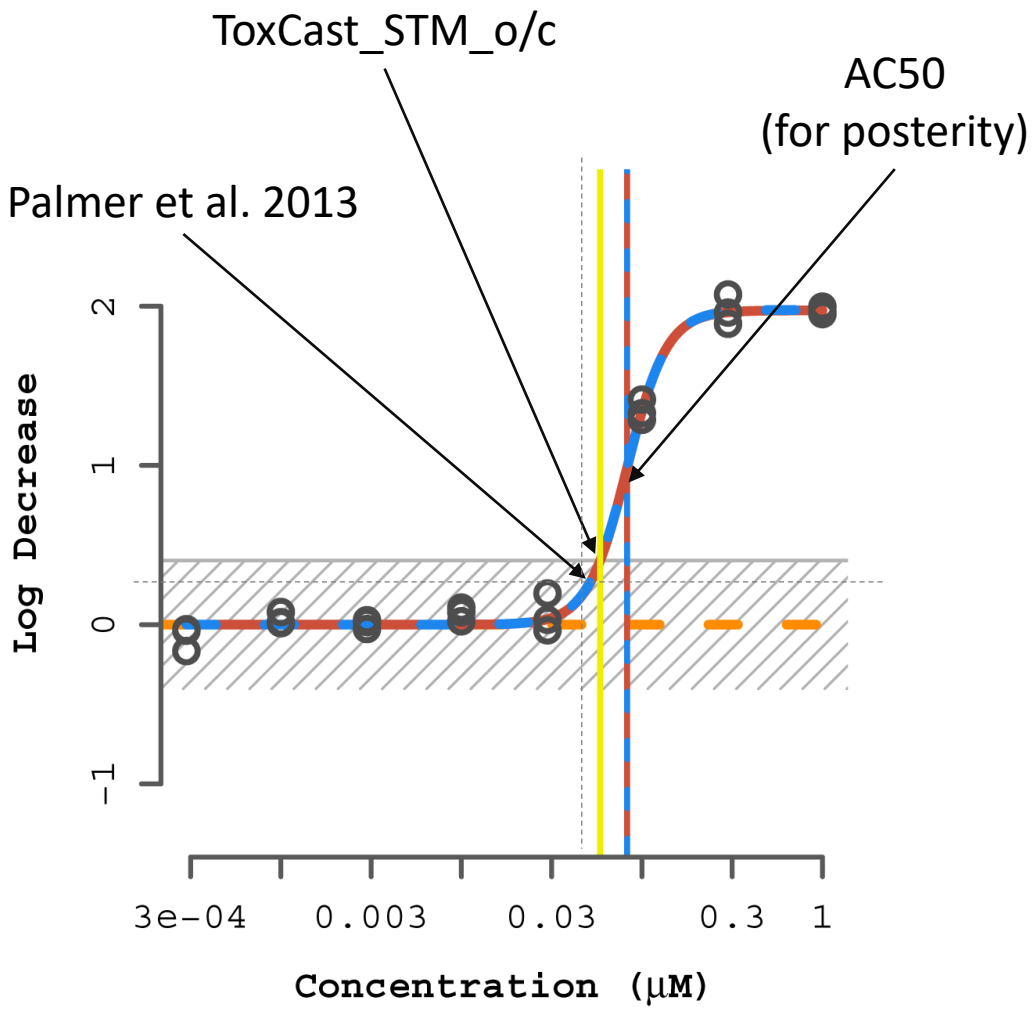
- ORN in the secretome normalized to control (*acid 1029*)
- CYSS in the secretome normalized to control (*acid 1026*)
- ORN:CYSS ratio (*acid 1031*)
- Cell viability normalized to control (*acid 1114*)

Invitrodb currently holds results for the STM dataset on 1065 chemicals tested to date:

- concentration-response profiles for 334 chemicals pushed through the ToxCast pipeline (tcpl)
- single-concentration screens for 731 chemicals (mostly negative under the specific test condition)
- dataset has been manually curated and essentially locked-in for data release upon publication (FY18 product)
- positive response on the targeted biomarker translates to a micromolar threshold predicting teratogenicity
- 181 of 1065 (17%) chemicals generated a STM-positive signal <200 µM

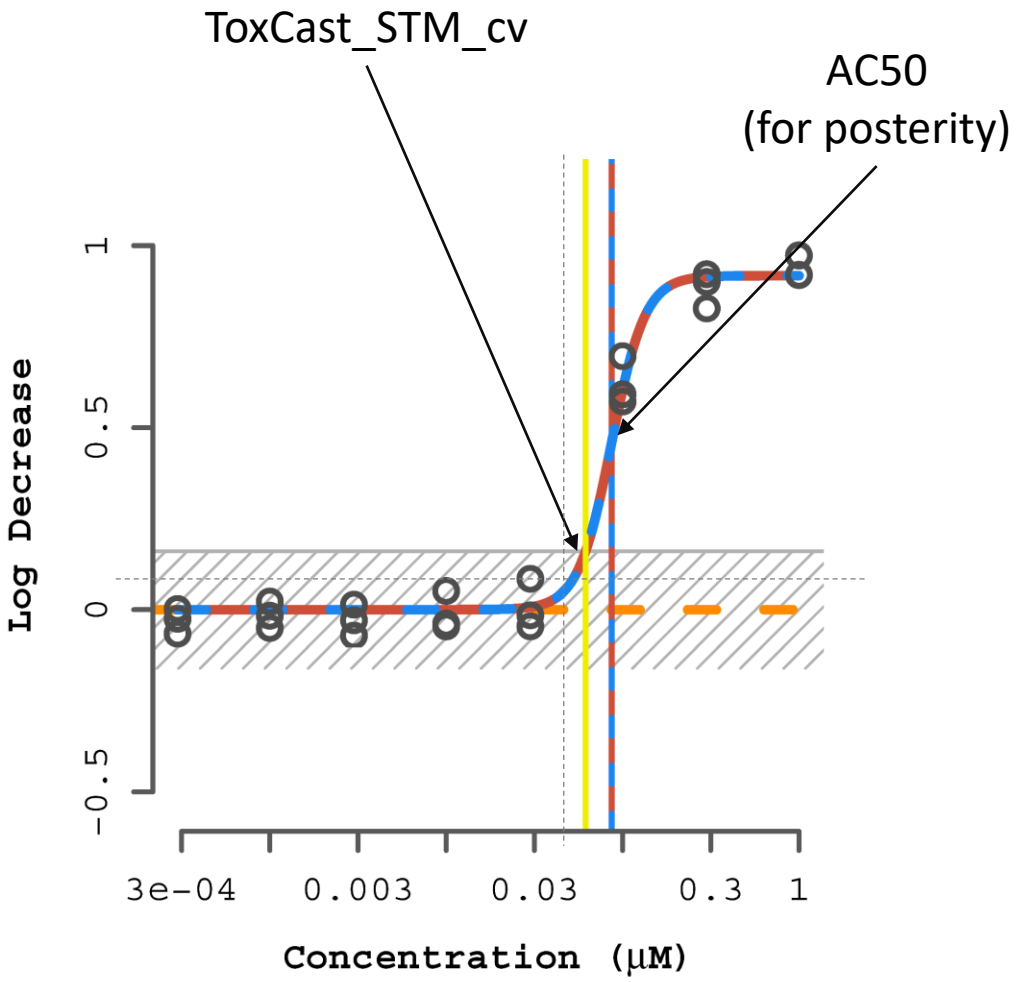
Knudsen et al. (MS in preparation)

Example tcpl readout for the targeted biomarker (o/c ratio)



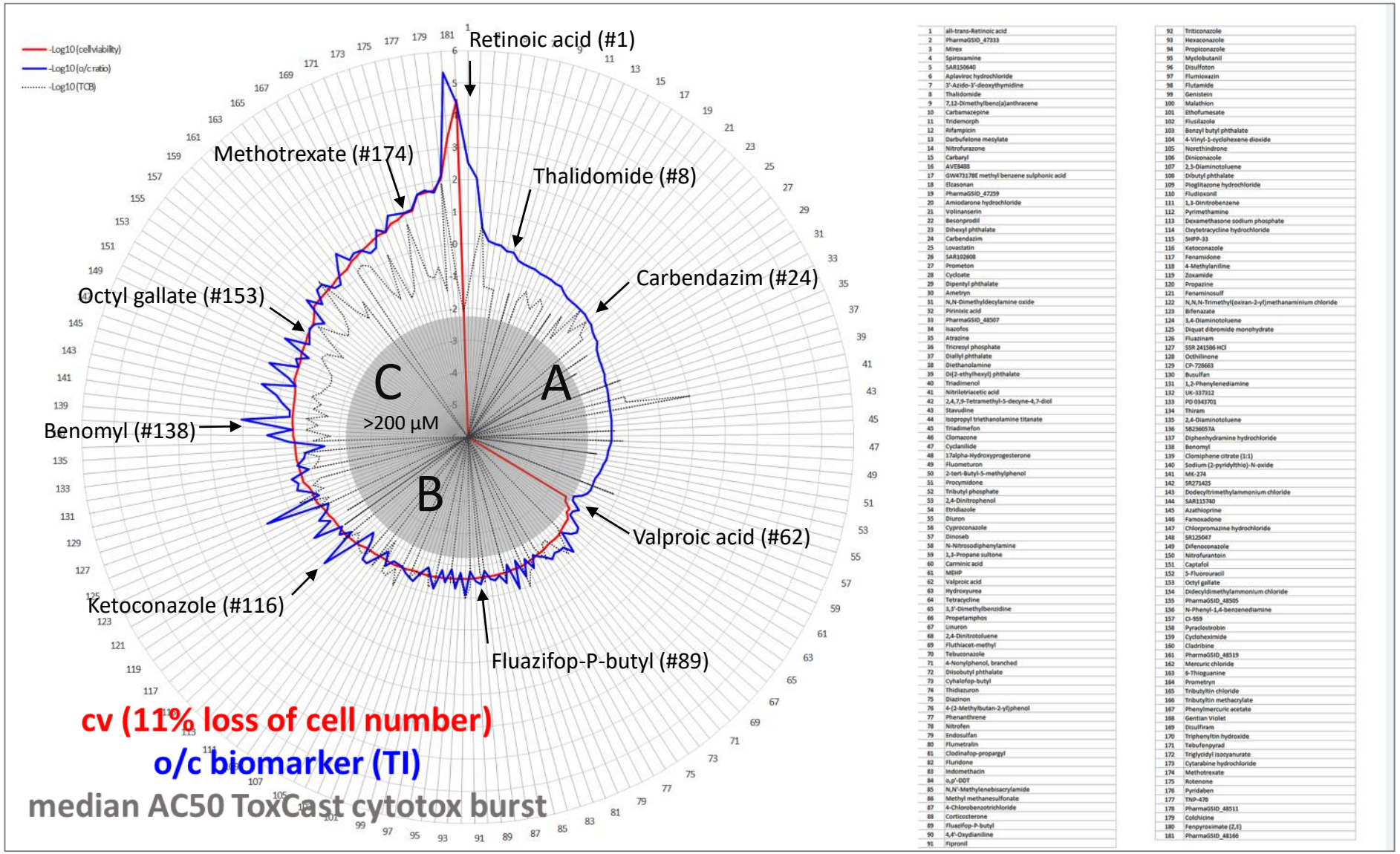

```
ASSAY:    AEID1691 (STEMINA_H9_ORNCYSSISnorm_RATIO_dn)

NAME:     Methotrexate
CHID:     20822    CASRN: 59-05-2
SPID(S): TP0001302A08
M4ID:     18146613

HILL MODEL (in red):
          tp        ga        gw
val:   1.97      -1.08     3.96
sd:    0.0247    0.0229    1.06

GAIN-LOSS MODEL (in blue):
          tp        ga        gw        la        lw
val:   1.98      -1.08     3.92      0.721     3.48
sd:    0.0529    0.0226    1.08      35.1      171

          CNST         HILL         GNLS
AIC:   74.32        -52.43       -48.44
PROB:  0            0.88         0.12
RMSE:  1.1          0.07         0.07

MAX_MEAN: 1.98      MAX_MED: 1.96      BMAD: 0.134

ACB: 0.0588  HIT-CALL: 1    FITC: 41    AC50: 0.0828

FLAGS:
```

Example tcpl readout for cell viability: calculated point of departure reflects an 11% loss




```
ASSAY:    AEID1858 (STEMINA_H9_Viability_Norm)

NAME:     Methotrexate
CHID:     20822    CASRN: 59-05-2
SPID(S): TP0001302A08
M4ID:     15456100

HILL MODEL (in red):
          tp        ga        gw
val:   0.917     -1.06     4.56
sd:    0.0185    0.0404    3.05

GAIN-LOSS MODEL (in blue):
          tp        ga        gw        la        lw
val:   0.917     -1.06     4.56      1.79      5.05
sd:    0.0185    0.0404    3.05      1650      4670

          CNST         HILL         GNLS
AIC:   37.29        -69.83       -65.83
PROB:  0            0.88         0.12
RMSE:  0.51         0.05         0.05

MAX_MEAN: 0.938      MAX_MED: 0.921      BMAD: 0.0537

ACB: 0.0617   HIT-CALL: 1    FITC: 41    AC50: 0.0867

FLAGS:
```

Stratification of 181 STM-positives



Performance Check

- ToxCast_STM anchored to DevTox benchmark compounds aimed at assessing alternative models[1] and having information on human pregnancy risk.
- Overall accuracy 82.1% (71% sensitivity, 100% specificity, MCC = 0.695, n=39).
- Compares well to the Palmer et al. (2013) who reported an overall accuracy of 77% (57% sensitivity, 100% specificity).

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

NAME	CASRN	Benchmark	Pregnancy Risk Category	TI (uM)	Class
all-trans-Retinoic acid	302-79-4	teratogen	X	0.003	TP
Cytarabine hydrochloride	69-74-9	teratogen	D	0.054	TP
Methotrexate	59-05-2	teratogen	X	0.059	TP
Diphenhydramine hydrochloride	147-24-0	teratogen	B	0.588	TP
Thalidomide	50-35-1	teratogen	X	1.27	TP
5-Fluorouracil	51-21-8	teratogen	D	2.02	TP
Carbamazepine	298-46-4	teratogen	C	2.29	TP
Busulfan	55-98-1	teratogen	D	2.31	TP
Rifampicin	13292-46-1	teratogen	C	2.46	TP
Amiodarone hydrochloride	19774-82-4	teratogen	D	5.10	TP
Lovastatin	75330-75-5	teratogen	X	6.67	TP
Stavudine	3056-17-5	teratogen	C	32.5	TP
Dexamethasone sodium phosphate	2392-39-4	teratogen	C	37.7	TP
Indomethacin	53-86-1	teratogen	D	72.7	TP
Hydroxyurea	127-07-1	teratogen	D	74.9	TP
Valproic acid	99-66-1	teratogen	D	155	TP
MEHP	4376-20-9	teratogen	D	167	TP
5,5-Diphenylhydantoin	57-41-0	teratogen	D	1000	FN
6-Propyl-2-thiouracil	51-52-5	teratogen	D	1000	FN
Boric acid	10043-35-3	teratogen	N	1000	FN
Cyclophosphamide monohydrate	6055-19-2	teratogen	D	1000	FN
Diethylstilbestrol	56-53-1	teratogen	X	1000	FN
Phenobarbitol sodium	57-30-7	teratogen	D	1000	FN
Warfarin	81-81-2	teratogen	X	1000	FN
Salicylic acid	69-72-7	non-teratogen	C	513	TN
Acetaminophen	103-90-2	non-teratogen	B	1000	TN
Acrylamide	79-06-1	non-teratogen	NTP	1000	TN
Aspirin	50-78-2	non-teratogen	C	1000	TN
Bisphenol A	80-05-7	non-teratogen	NTP	1000	TN
Butylparaben	94-26-8	non-teratogen	GRAS	1000	TN
Caffeine	58-08-2	non-teratogen	B	1000	TN
D-Camphor	464-49-3	non-teratogen	C	1000	TN
Dimethyl phthalate	131-11-3	non-teratogen	NTP	1000	TN
Folic acid	59-30-3	non-teratogen	A	1000	TN
Isoniazid	54-85-3	non-teratogen	C	1000	TN
Retinol	68-26-8	non-teratogen	A	1000	TN
Saccharin	81-07-2	non-teratogen	A	1000	TN
Sodium L-ascorbate	134-03-2	non-teratogen	A	1000	TN
Sulfasalazine	599-79-1	non-teratogen	B	1000	TN

4. Timeline and Deliverables

✓ **Year 0.5**

- Issue task order 85-90 NTP/NICEATM chemicals for iPSC data generation (Aim 3)
- Build literature mining database for chemical-assay connectivity (Aim 1)

Year 1.0

- Integrate results from iPSC data with extant H9 (ToxCast) data in tcpl (Aim 1)
- Run additional assays needed to strengthen concordance between iPSC and H9 (Aim 1)

Year 1.5

- Comprehensive correlation analysis to ToxRefDB and NTP animal studies (Aim 3)
- Implement the human fetal PBPK exposure model (Aim 2)

Year 2.0

- Quality Control (QC) and manual curation plan to evaluate the dataset (Aim 1)
- Draft relevant manuscripts for publication (Aims 1 &2)

Year 2.5

- Compendium of sensitivity-specificity predictors based on adverse outcomes (Aim 3)
- Develop 3 case studies for computational dynamics at windows of vulnerability (Aim 4)

Year 3.0

- Virtual embryo agent-based models to simulate spatial dynamics (Aim 4)
- Draft relevant manuscripts for publication (Aims 3 & 4).

5. Progress to date (Year 0.5)

Toward issuing a task order 85-90 NTP/NICEATM chemicals for iPSC data generation (Aim 3):

- Nicole learned of a draft document "*ICH Reference Compounds for Qualifying Alternative Assays*" candidate list of 84 DevTox reference compounds with detailed dosimetry. She inquired whether the Expert Working Group of ICHS5(R3) could share kinetic and toxicity information, but it was not ready until Q1-2018.

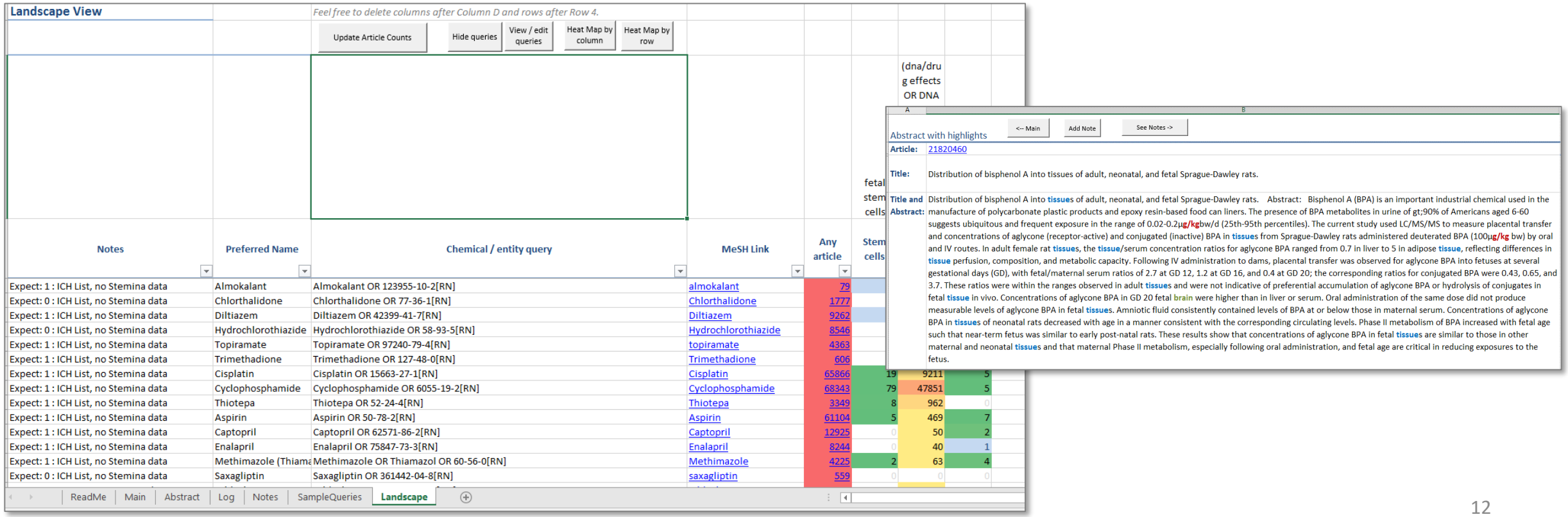
- Finalized 81 chemicals based on : (a) NTP prenatal animal studies; (b) potential for human exposure and suspected developmental effects; (c) high priority to NTP, EPA, or other ICCVAM member agencies; and (d) inclusion in the ICH guidance: "*Detection of toxicity to reproduction for human pharmaceuticals.*"

- Almost ½ of the list shares overlap with ToxCast_STM (H9 cells). Stemina kindly shared the list of compounds they already compared between H9 and iPSC versions of the platform.

5. Progress to date (Year 0.5)

Toward building a literature mining database for chemical-assay connectivity (Aim 1):

- Nancy Baker (NCCT) developed a sifter/literature miner knowledge mapping tool for the test compounds.



Landscape View

Feel free to delete columns after Column D and rows after Row 4.

Update Article Counts | Hide queries | View / edit queries | Heat Map by column | Heat Map by row

Notes	Preferred Name	Chemical / entity query	MeSH Link	Any article	Stem cells	(dna/drug effects OR DNA	fetal stem cells
Expect: 1 : ICH List, no Stemina data	Almokalant	Almokalant OR 123955-10-2[RN]	almokalant	79			
Expect: 0 : ICH List, no Stemina data	Chlorthalidone	Chlorthalidone OR 77-36-1[RN]	Chlorthalidone	1777			
Expect: 1 : ICH List, no Stemina data	Diltiazem	Diltiazem OR 42399-41-7[RN]	Diltiazem	9262			
Expect: 0 : ICH List, no Stemina data	Hydrochlorothiazide	Hydrochlorothiazide OR 58-93-5[RN]	Hydrochlorothiazide	8546			
Expect: 1 : ICH List, no Stemina data	Topiramate	Topiramate OR 97240-79-4[RN]	topiramate	4363			
Expect: 1 : ICH List, no Stemina data	Trimethadione	Trimethadione OR 127-48-0[RN]	Trimethadione	606			
Expect: 1 : ICH List, no Stemina data	Cisplatin	Cisplatin OR 15663-27-1[RN]	Cisplatin	65866	19	9211	5
Expect: 1 : ICH List, no Stemina data	Cyclophosphamide	Cyclophosphamide OR 6055-19-2[RN]	Cyclophosphamide	68343	79	47851	5
Expect: 1 : ICH List, no Stemina data	Thiotepa	Thiotepa OR 52-24-4[RN]	Thiotepa	3349	8	962	0
Expect: 1 : ICH List, no Stemina data	Aspirin	Aspirin OR 50-78-2[RN]	Aspirin	61104	5	469	7
Expect: 1 : ICH List, no Stemina data	Captopril	Captopril OR 62571-86-2[RN]	Captopril	12925	0	50	2
Expect: 1 : ICH List, no Stemina data	Enalapril	Enalapril OR 75847-73-3[RN]	Enalapril	8244	0	40	1
Expect: 1 : ICH List, no Stemina data	Methimazole (Thiama	Methimazole OR Thiamazol OR 60-56-0[RN]	Methimazole	4225	2	63	4
Expect: 0 : ICH List, no Stemina data	Saxagliptin	Saxagliptin OR 361442-04-8[RN]	saxagliptin	559	0	0	0

ReadMe | Main | Abstract | Log | Notes | SampleQueries | Landscape

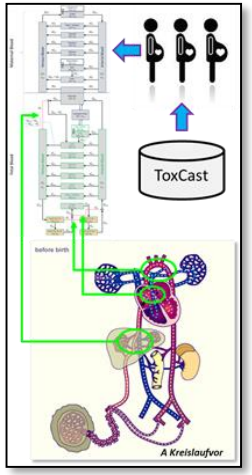
Abstract with highlights | <-- Main | Add Note | See Notes ->

Article: 21820460

Title: Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague-Dawley rats.

Title and Abstract: Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague-Dawley rats. Abstract: Bisphenol A (BPA) is an important industrial chemical used in the manufacture of polycarbonate plastic products and epoxy resin-based food can liners. The presence of BPA metabolites in urine of gt;90% of Americans aged 6-60 suggests ubiquitous and frequent exposure in the range of 0.02-0.2µg/kgbw/d (25th-95th percentiles). The current study used LC/MS/MS to measure placental transfer and concentrations of aglycone (receptor-active) and conjugated (inactive) BPA in tissues from Sprague-Dawley rats administered deuterated BPA (100µg/kg bw) by oral and IV routes. In adult female rat tissues, the tissue/serum concentration ratios for aglycone BPA ranged from 0.7 in liver to 5 in adipose tissue, reflecting differences in tissue perfusion, composition, and metabolic capacity. Following IV administration to dams, placental transfer was observed for aglycone BPA into fetuses at several gestational days (GD), with fetal/maternal serum ratios of 2.7 at GD 12, 1.2 at GD 16, and 0.4 at GD 20; the corresponding ratios for conjugated BPA were 0.43, 0.65, and 3.7. These ratios were within the ranges observed in adult tissues and were not indicative of preferential accumulation of aglycone BPA or hydrolysis of conjugates in fetal tissue in vivo. Concentrations of aglycone BPA in GD 20 fetal brain were higher than in liver or serum. Oral administration of the same dose did not produce measurable levels of aglycone BPA in fetal tissues. Amniotic fluid consistently contained levels of BPA at or below those in maternal serum. Concentrations of aglycone BPA in tissues of neonatal rats decreased with age in a manner consistent with the corresponding circulating levels. Phase II metabolism of BPA increased with fetal age such that near-term fetus was similar to early post-natal rats. These results show that concentrations of aglycone BPA in fetal tissues are similar to those in other maternal and neonatal tissues and that maternal Phase II metabolism, especially following oral administration, and fetal age are critical in reducing exposures to the fetus.

5. Progress to date (Year 0.5)



Toward HTTK modeling (Aim 2):

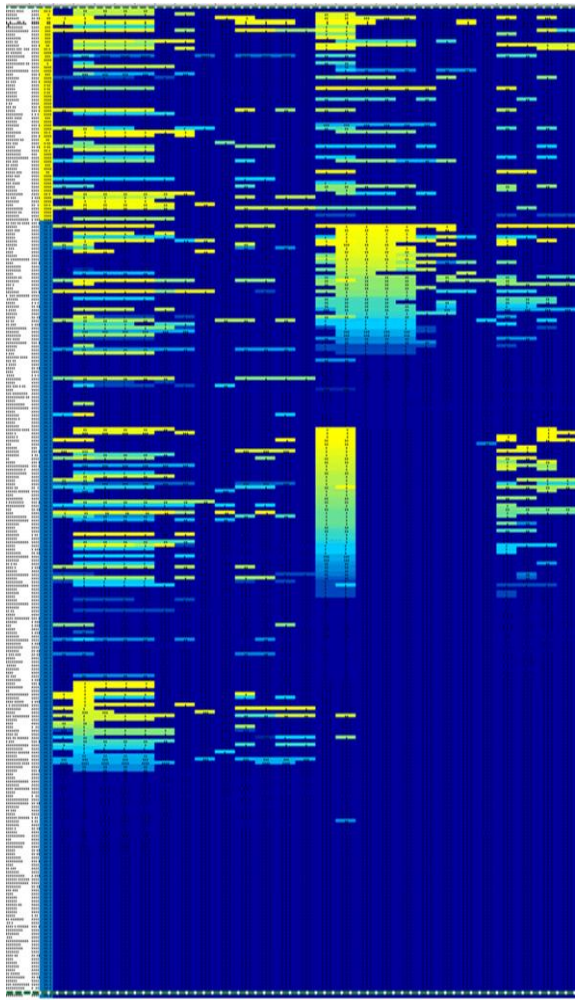
- Since STM outputs an exposure-based hazard prediction, we can translate TI to human pregnancy using a fetal PBPK adaptation of the HTTK model. Annie installed fetal httk package from NCCT and is running initial trials on maternal serum levels for retinoic acid (RA) utilizing parameters provided by NTP.

- RA has a well-defined role in human development and teratogenesis, and was the most potent compound of the 181 positives in ToxCast_STM. Palmer et al. 2017 just published the bioactivity of several retinoids in the iPSC-version of the assay to address RA homeostasis.

- Annie compiled a list of the compounds (n=271) for which the httk package can be used and a slightly longer list of chemicals (n=349) for which a 3 compartment steady state model can be built. Since the STM prediction has **high specificity**, we can begin to make inferences on margins of exposure.

5. Progress to date (Year 0.5)

Toward computational modeling with the stem cell data (Aim 3):



Concordance model (rat & rabbit):

- 272 chemicals tested for prenatal DevTox in both species
- Positives = dLEL ≤ 125 mg/kg/d for fetal endpoints
- Negatives = no dLEL ≥ 1000 mg/kg/day

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

Low **sensitivity** raises a question of what is missing biologically or metabolically in the H9 cell platform that animal studies pick up.

5. Progress to date (Year 0.5)

Toward computational modeling with the stem cell data (Aim 3):

- Todd Zurlinden (NCCT) built a logistic regression model to mine the strongest positive and negative correlates to 331 enzymatic and receptor signaling assays in the ToxCast_NVS dataset. Initial machine-learning defined several functional annotation groups and pathways.

- Top sensitive pathways were kinase signaling, some neuroactive GPCRs, and corticotrophs. In contrast, top negative correlations were observed with other neuroactive GPCRs (dopamine, serotonin, endothelins), estrogen signaling, and RAR antagonism.

- These findings point to molecular processes that potentially account for the sensitivity of the STM model and set the stage for AOP integration and mechanistic *in silico* multiscale agent-based models (Aim 4).

Pathway sensitivity against 337 biochemical targets in ToxCast

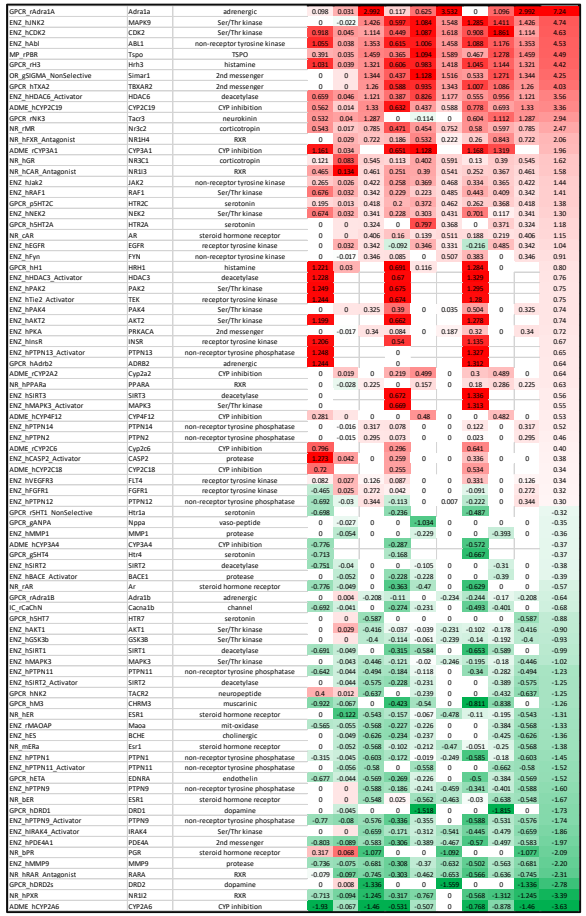
NVS ASIDs selected from invitroDB v2 (June, 2017)

Filter NVS+ by AC50 cutoffs (50 nM, 20 nM, 10 nM)

Feature selection with Scikit (Python) classification ANOVA 40th percentile

Build logistic regression model based on STM+ (1) and STM- (0) calls

AD score (weighted NVS potency and discretized targeted biomarker)



NIH_DAVID annotation: functional clusters

Strongest (-) enrichment clusters

DRD2	dopamine receptor D2(DRD2)
DRD1	dopamine receptor D1(DRD1)
Cacna1b	calcium voltage-gated channel subunit alpha1 B(CACNA1B)
Htr1a	5-hydroxytryptamine receptor 1A(HTR1A)
TACR2	tachykinin receptor 2(TACR2)
Adra1b	adrenoceptor alpha 1B(ADRA1B)
HTR7	5-hydroxytryptamine receptor 7(HTR7)
CHRM3	cholinergic receptor muscarinic 3(CHRM3)
Htr4	5-hydroxytryptamine receptor 4(HTR4)
BACE1	beta-secretase 1(BACE1)
EDNRA	endothelin receptor type A(EDNRA)
ESR1	estrogen receptor 1(ESR1)
RARA	retinoic acid receptor alpha(RARA)
PGR	progesterone receptor(PGR)
Ar	androgen receptor(AR)
NR1I2	nuclear receptor subfamily 1 group I member 2(NR1I2)

6. Science challenges

- The **high specificity** of the STM assay provides confidence in predictivity; however, it's **low sensitivity** raises the science challenge of making the assay more health protective.
    - Understanding the dynamics that potentially account for sensitivity of the STM model set the stage for AOP integration and mechanistic *in silico* agent-based models (Aim 4).
    - Reducing uncertainty via computational synthesis and integration to stratify connections between the most relevant pathways and link DSSTox structural descriptors with the biology.
    - This cycles back to the principles of teratogenesis (initiating mechanisms, dosimetry & bioavailability, stage vulnerability, chemistry, and genetic susceptibility).
- Next steps: (a) NCCT will continue pushing the ToxCast_STM dataset forward on H9 cells and could expand chemicals to meet needs for amended TSCA; (b) NTP will pilot the iPSC version of the assay on 81 chemicals and focus on data processing and integration; (c) NCTR will continue with exposure reconstruction on RA as a case study for the fetal httk.