Roundtable – "Reproductive and Developmental Toxicology"
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Computational Modeling of Developmental Toxicity:
modeling with human pluripotent stem cells

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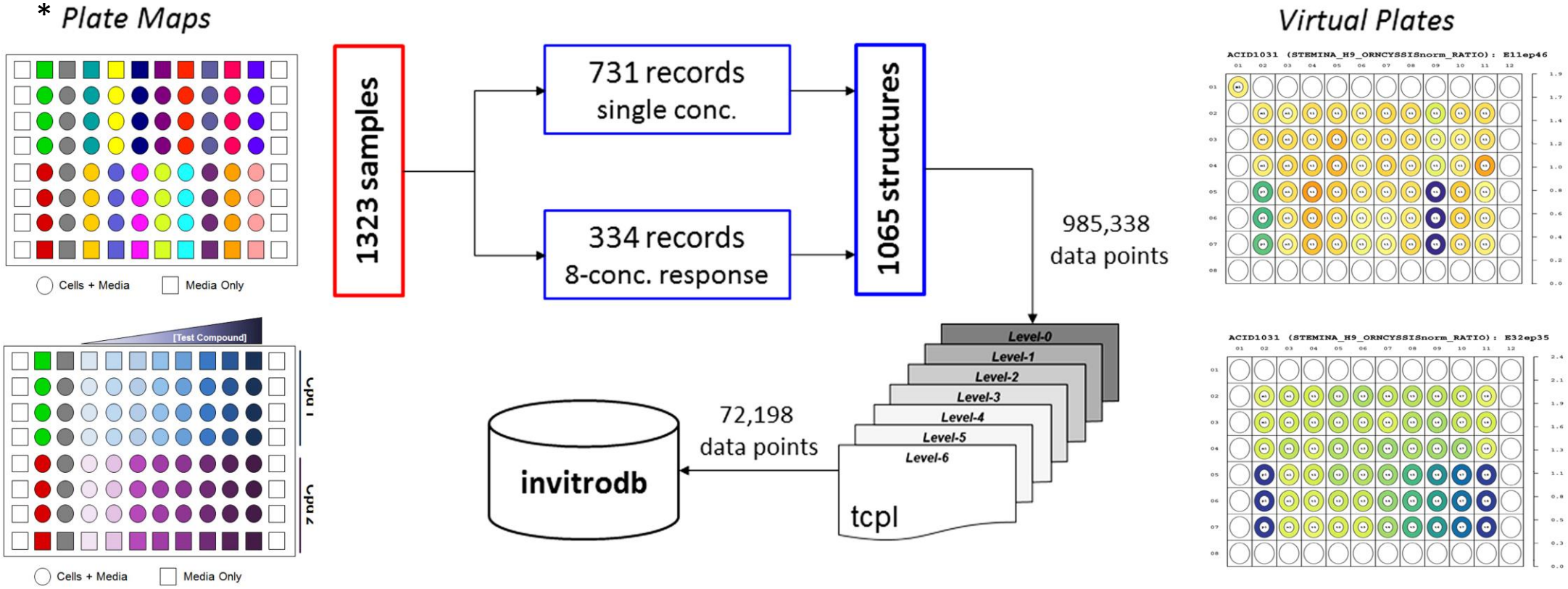
Prenatal Developmental Toxicity

- An *in vivo* protocol commonly used to test for prenatal developmental toxicity (OECD TG 414) assesses litters of pregnant rats or rabbits exposed during organogenesis.
- Guideline protocols designed for health-protective assessment based on fetal effects at relatively high dosing scenarios, but are animal-intensive and low-throughput.
- HTS *in vitro* bioactivity (ToxCast/Tox21) provide alternative testing paradigm although low diversity of assays for assessing prenatal effects **https://actor.epa.gov/dashboard/#Chemicals**
- ToxCast library (1065 chemicals) tested in a pluripotent H9 human embryonic stem cell metabolomics assay, EPA/NCCT contract EP-D-13-055 with Stemina Biomarker Discovery.

Workflow: 1065 chemicals from ToxCast phase I/II library



Ornithine release
urea cycle, polyamine & pyrimidine synthesis.

TI = ORN/CYSS

Cystine utilization
glutathione synthesis, redox cycling.

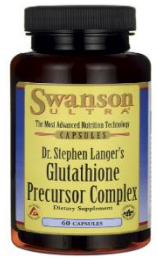
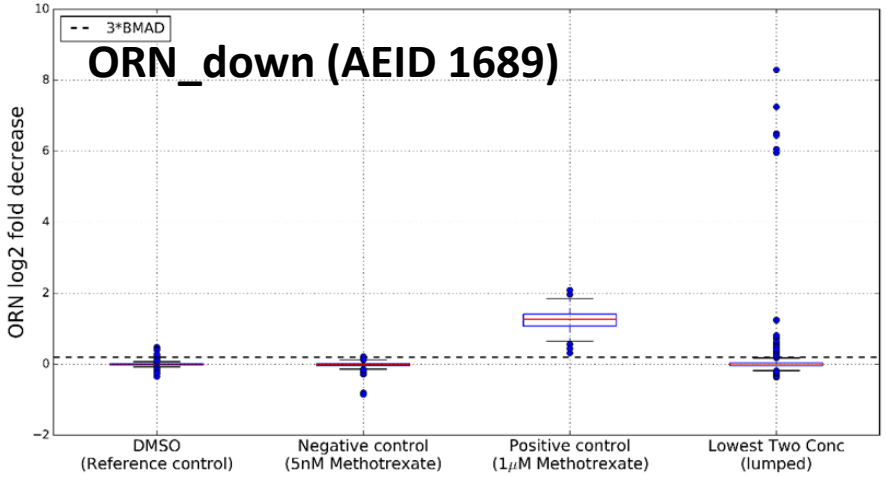
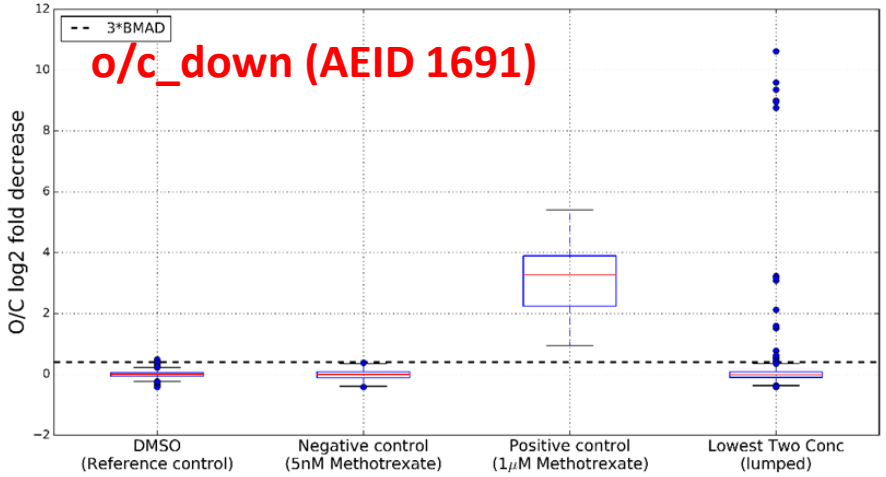


Plate-level Controls

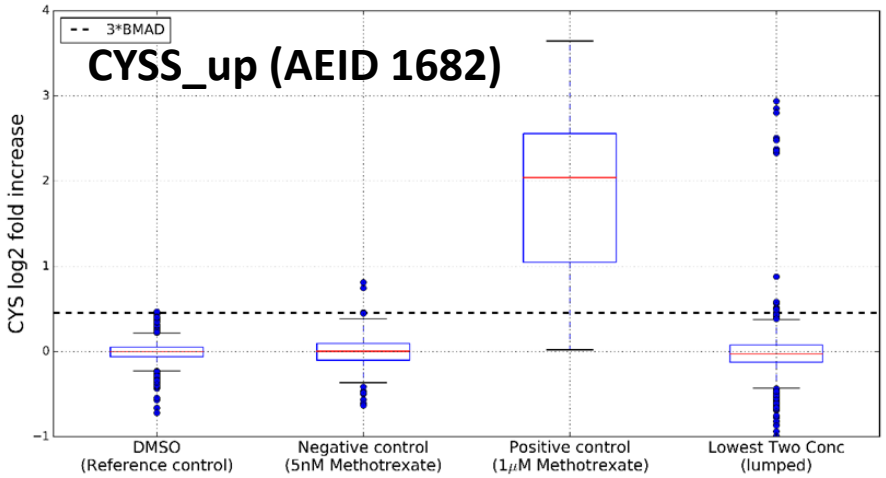
n=1158 DMSO, 581 MTX-, 580 MTX+ (P <0.05 Mann-Whitney), 2069 two-lowest two concentrations for ToxCast

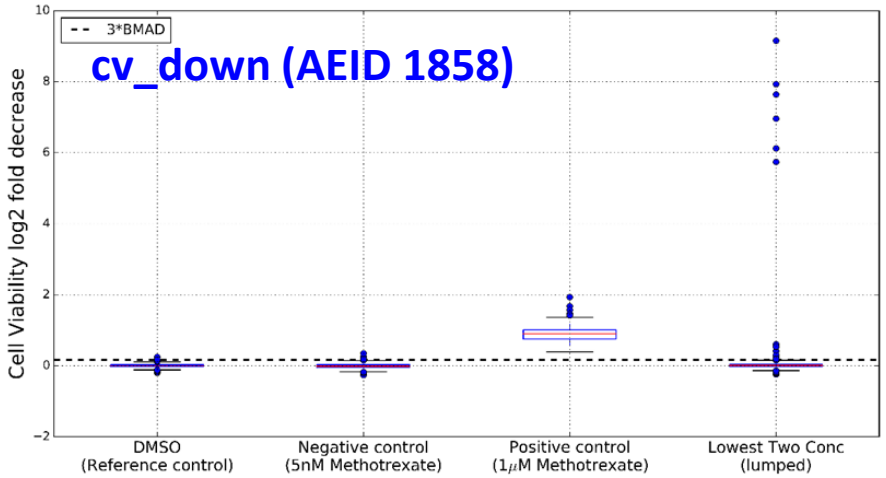




Targeted Biomarker

tcpl default at 3*BMAD (**o/c < 0.76**) accurately classifies negative/positive reference controls.

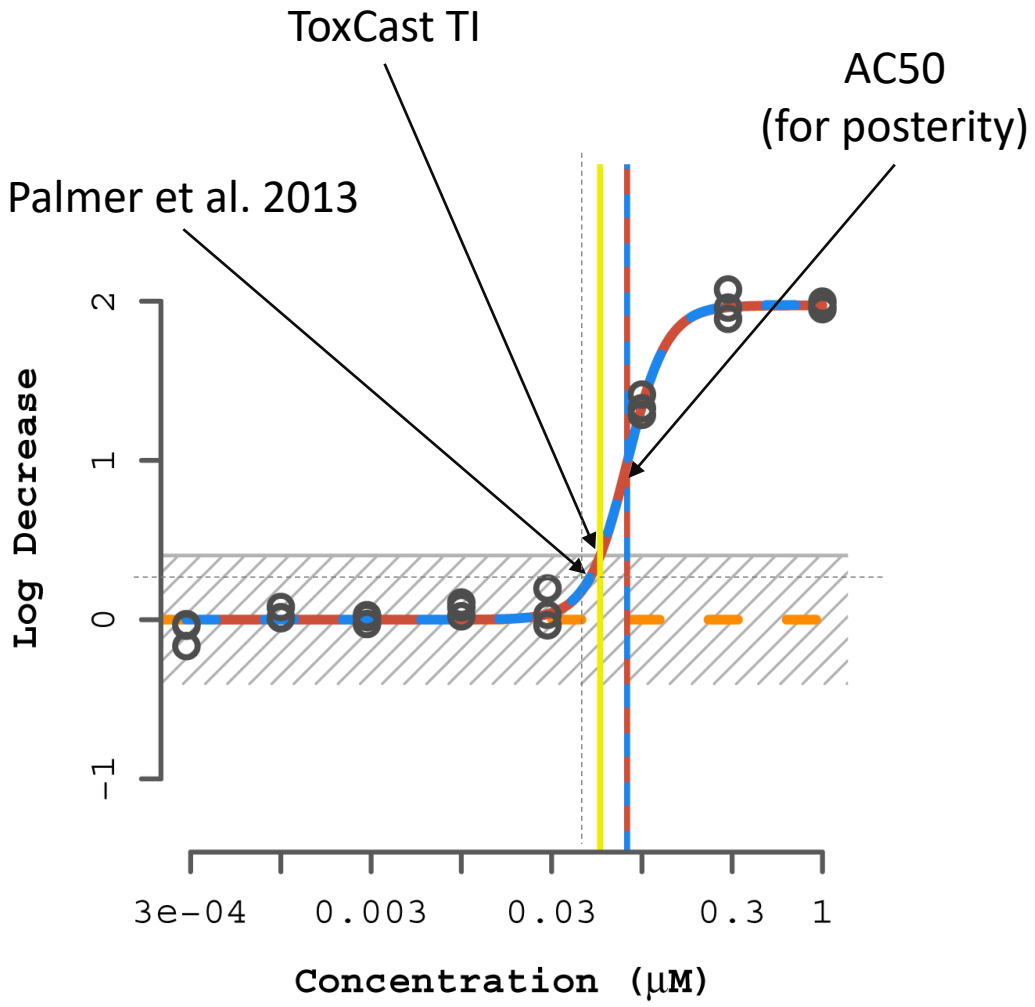




Cell Viability

3*BMAD for (acb = 0.894) indicates a hit for **>10.6%** cell loss.

Methotrexate: example of tcpl readout for the targeted biomarker (o/c ratio)

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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:
```

Performance vs DevTox Anchor

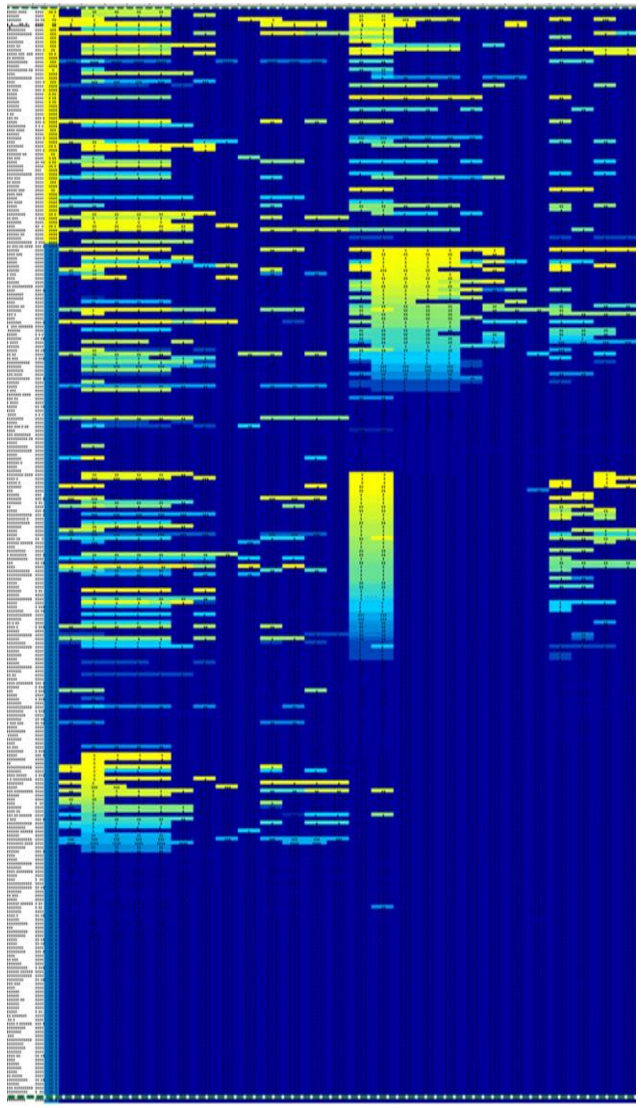
- 181 of 1065 chems (17%) gave a positive response for developmental toxicity prediction.
- Point of departure for biomarker (acb ~0.76) usually before loss of cell viability (acb ~11%).

	ANCHOR	
TEST	TP	FP
	FN	TN

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

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

Performance vs ToxRefDB fetal endpoints



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

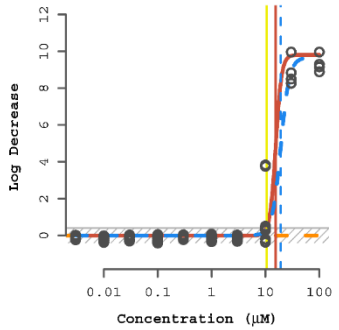
ToxRefDB_DEV	
TP	9
FP	19
FN	15
TN	103
n	146
Sensitivity	0.375
Specificity	0.844
Accuracy	76.7%
Mathew's cc	0.206
F1 score	0.005

Combined Model	
TP	27
FP	19
FN	24
TN	118
n	188
Sensitivity	0.529
Specificity	0.861
Accuracy	77.1%
Mathew's cc	0.404
F1 score	0.006

Example 1: anti-angiogenic compounds confirmed in rat WEC

AC50 (embryolethal) = 21.2 µM

5HPP-33

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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
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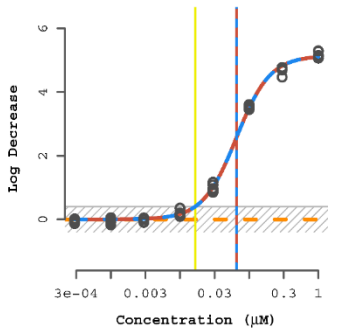
TI = 10.48 µM

TNP-470

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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:
```
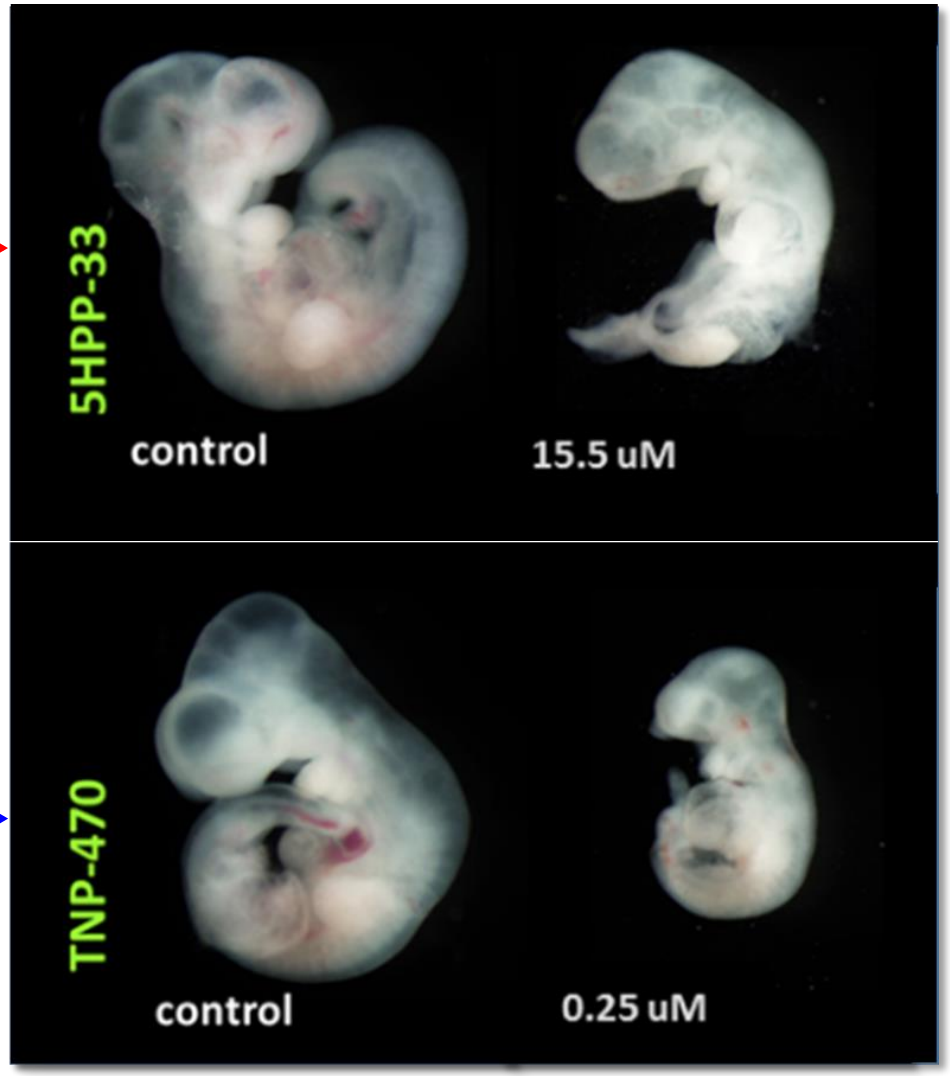

TI = 0.017 µM



Ellis-Hutchings (2017) Reprod Toxicol.

AC50 (dysmorphogenesis) = 0.038 µM

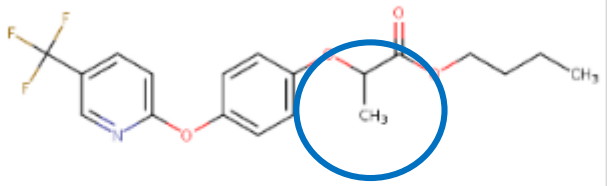
Example 2: stereoisomer pair resolved by STM but not animal studies

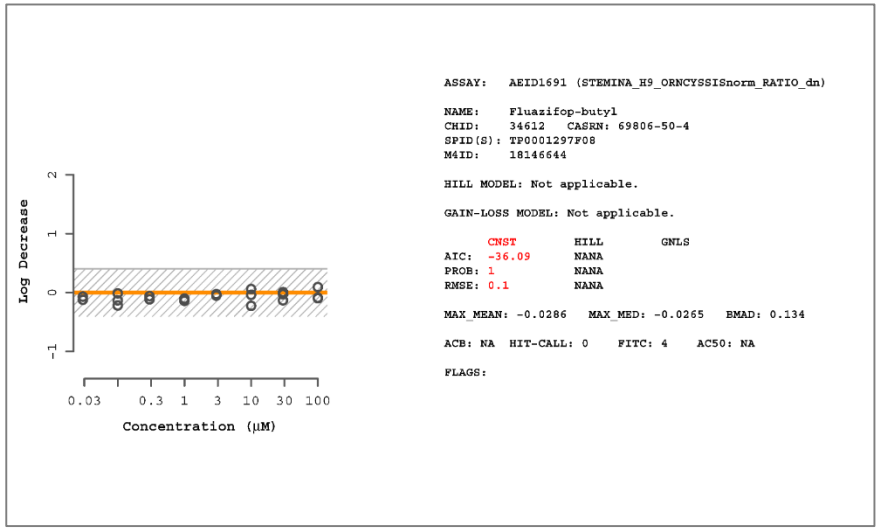
Fluazifop-butyl

69806-50-4 | DTXSID3034612

Searched by Integrated Source Name: Found 1 result for 'fluazifop butyl'.

Rat dLEL (5 mg/kg/day)
Rabbit dLEL (50 mg/kg/day)
STM – not active





ASSAY: AEID1691 (STEMINA_H9_ORNCYSSISnorm_RATIO_dn)

NAME: Fluazifop-butyl
CHID: 34612 CASRN: 69806-50-4
SPID(S): TP0001297F08
M4ID: 18146644

HILL MODEL: Not applicable.

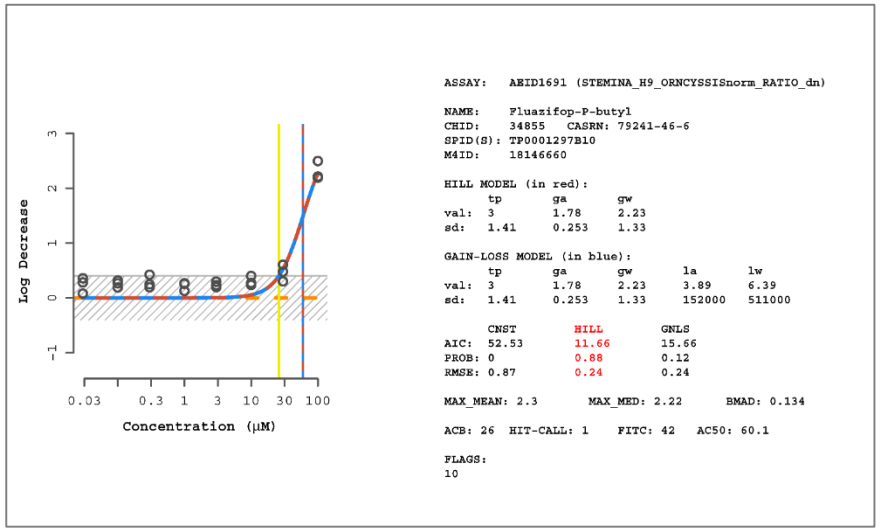
GAIN-LOSS MODEL: Not applicable.

	CNST	HILL	GNLS
AIC:	-36.09	NANA	
PROB:	1	NANA	
RMSE:	0.1	NANA	

MAX_MEAN: -0.0286 MAX_MED: -0.0265 BMAD: 0.134

ACB: NA HIT-CALL: 0 FITC: 4 AC50: NA

FLAGS:



ASSAY: AEID1691 (STEMINA_H9_ORNCYSSISnorm_RATIO_dn)

NAME: Fluazifop-P-butyl
CHID: 34855 CASRN: 79241-46-6
SPID(S): TP0001297B10
M4ID: 18146660

HILL MODEL (in red):

	tp	ga	gw
val:	3	1.78	2.23
sd:	1.41	0.253	1.33

GAIN-LOSS MODEL (in blue):

	tp	ga	gw	la	lw
val:	3	1.78	2.23	3.89	6.39
sd:	1.41	0.253	1.33	152000	511000

	CNST	HILL	GNLS
AIC:	52.53	11.66	15.66
PROB:	0	0.88	0.12
RMSE:	0.87	0.24	0.24

MAX_MEAN: 2.3 MAX_MED: 2.22 BMAD: 0.134

ACB: 26 HIT-CALL: 1 FITC: 42 AC50: 60.1

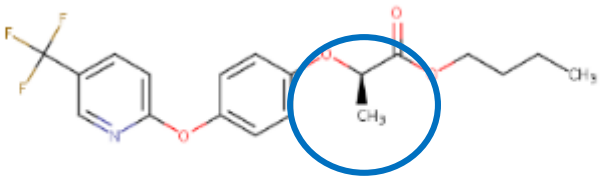
FLAGS:
10

Fluazifop-P-butyl

79241-46-6 | DTXSID0034855

Searched by Approved Name: Found 1 result for 'fluazifop-p-butyl'.

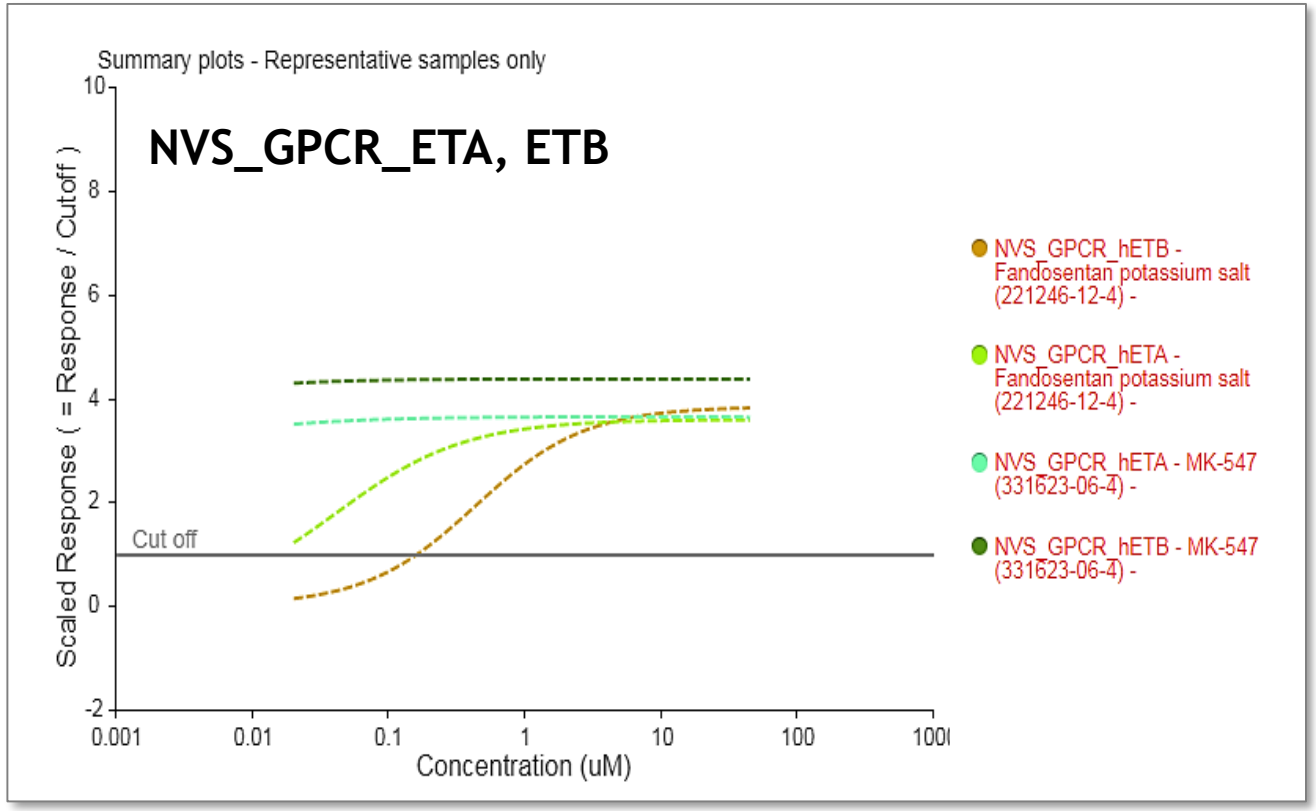
Rat dLEL (5 mg/kg/day)
Rabbit dLEL (50 mg/kg/day)
STM – TI = 24 μM

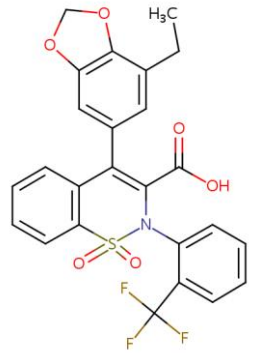


Example 3: endothelin receptor system (EDNR) is missed

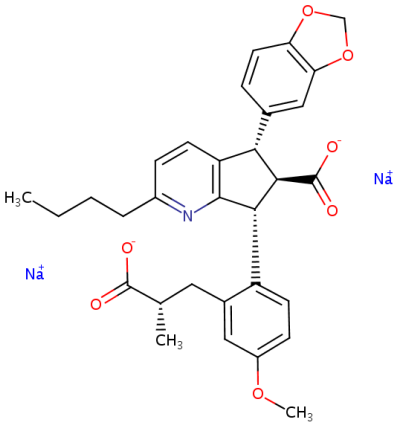
- Several STM-positives hit EDNRs with submicromolar AC50s; strongest were Fandosentan (ETA antagonist) and MK-547 (probably ETB antagonist) but neither were STM-positive.

- Pharmacologic antagonists phenocopy *Ednra*-nullizygous (craniofacial/cardiac neural crest) and *Ednrb*-nullizygous (enteric neural crest) mice [Clouthier et al. 1998; Puffenberger et al. 1994; Spence et al. 1999].





Fandosentan
hETA = 0.042 µM
STM = inactive (10 µM)



MK-547
hETB = 0.0002 µM
STM = inactive (20 µM)

Machine-learning: pathway sensitivity against 337 biochemical targets in ToxCast

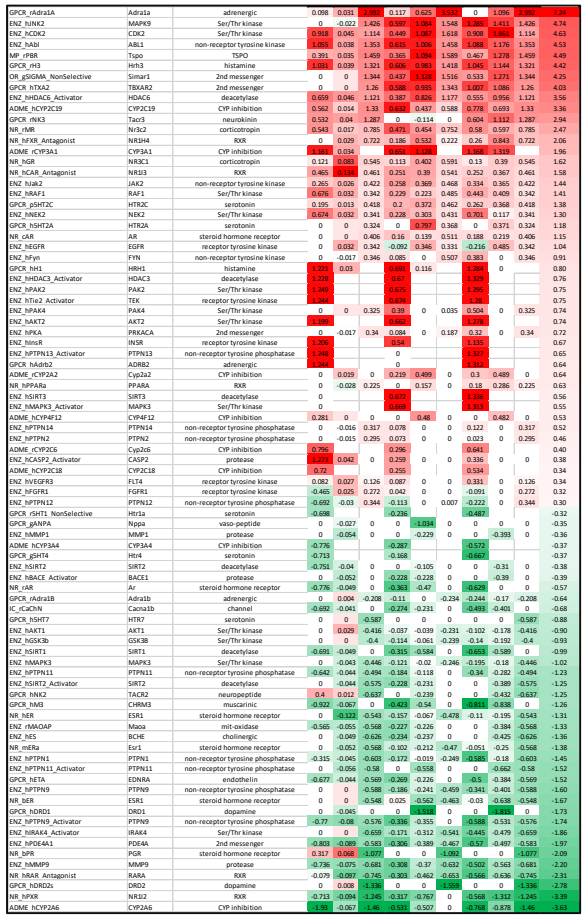
NVS ASIDs selected from invitroDB v2 (June, 2017)

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

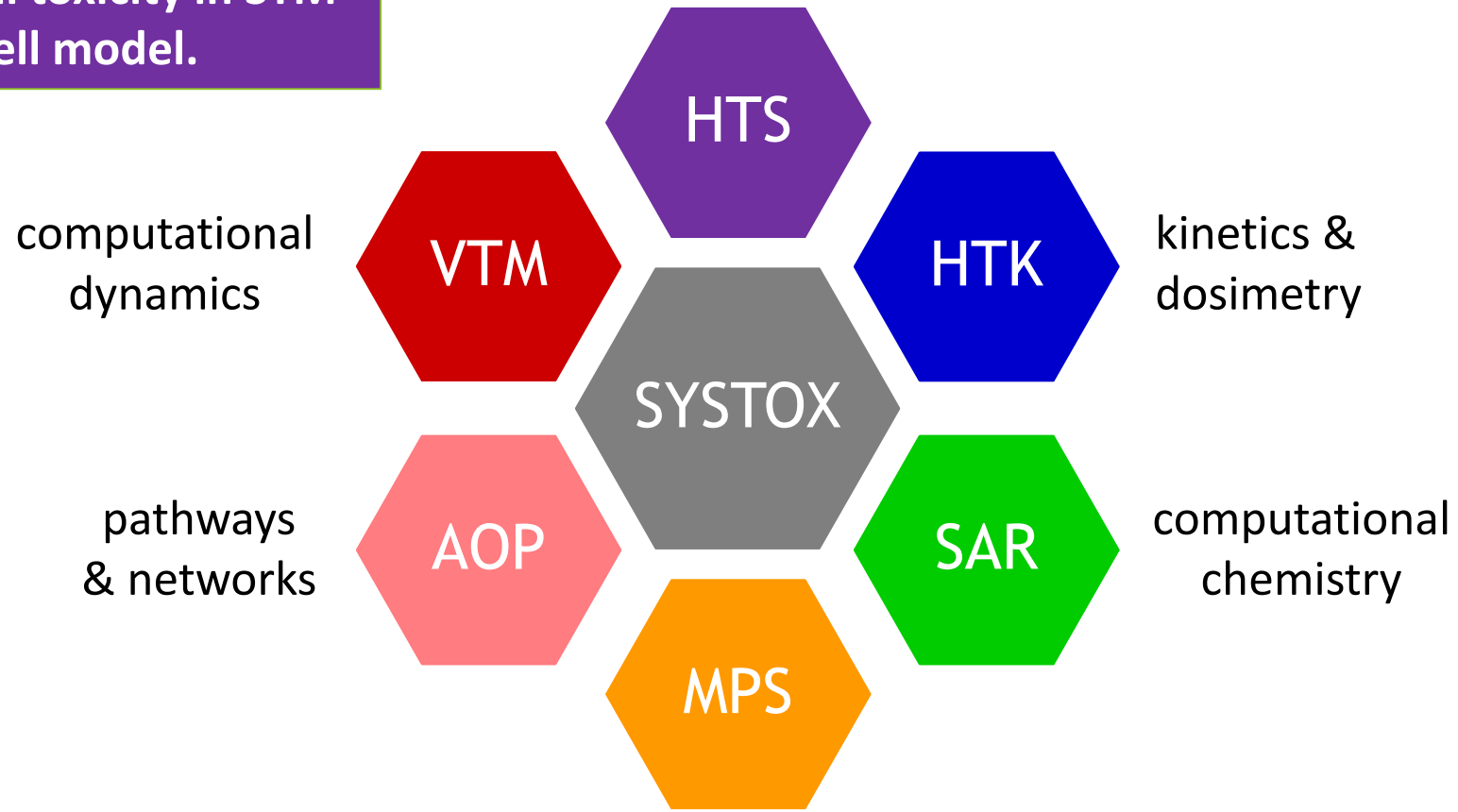
Strongest (-) enrichment clusters

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)

Developmental Systems Biology & Systems Toxicology

181 of 1065 ToxCast chemicals (17%) exposure-based prediction of developmental toxicity in STM H9 stem cell model.

bioactivity profiles



computational dynamics

kinetics & dosimetry

pathways & networks

computational chemistry

microphysiological systems

Special Thanks

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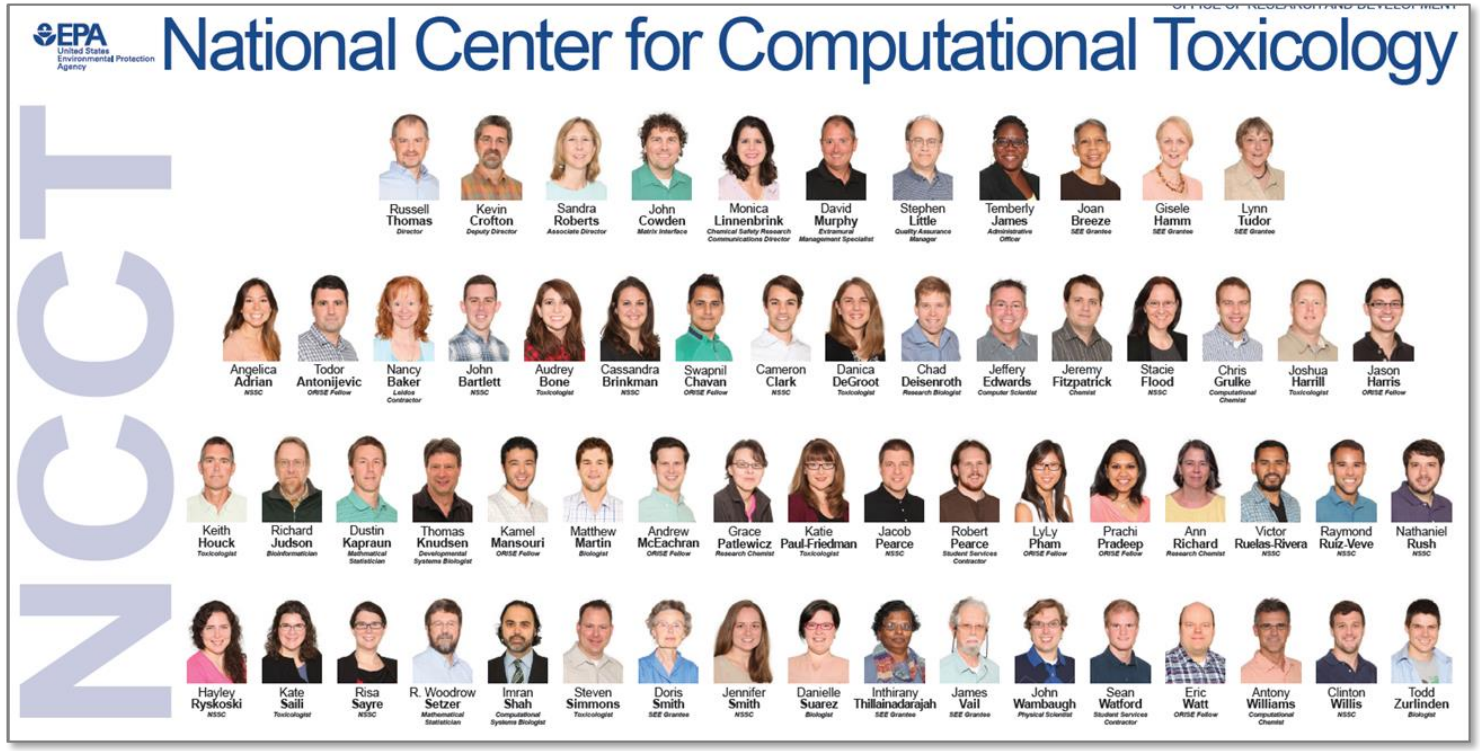
Parth Kothiya – NCCT
Nancy Baker – Leidos / NCCT
Richard Spencer – ARA / EMVL
Keith Houck -NCCT
Jessica Palmer – Stemina



Tox21 cross-partner project with NIH/NTP (N Kleinstreuer) and FDA/NCTR (A Lumen)