



Roundtable – “Reproductive and Developmental Toxicology”
XX Brazilian Congress of Toxicology – Goiania, Brazil Oct 8-11, 2017

Computational Modeling of Developmental Toxicity: *modeling with human pluripotent stem cells*

Thomas B. Knudsen, PhD
Developmental Systems Biologist
US EPA, National Center for Computational Toxicology
Chemical Safety for Sustainability Research Program

knudsen.thomas@epa.gov
ORCID 0000-0002-5036-596x

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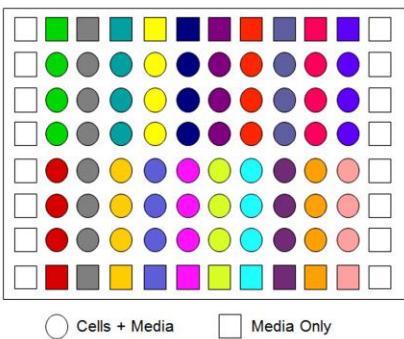
Prenatal Developmental Toxicity



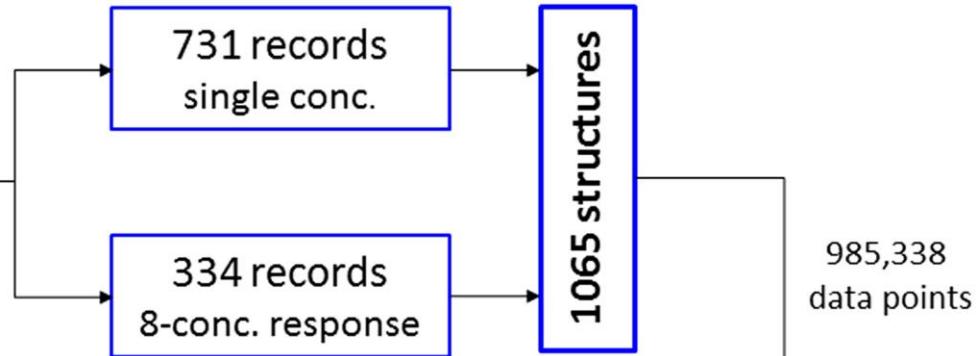
- An *in vivo* protocol commonly used to test for prenatal developmental toxicity (OECD TG 414) assesses litters of pregnant rats or rabbits exposed during organogenesis.
- Guideline protocols designed for health-protective assessment based on fetal effects at relatively high dosing scenarios, but are 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

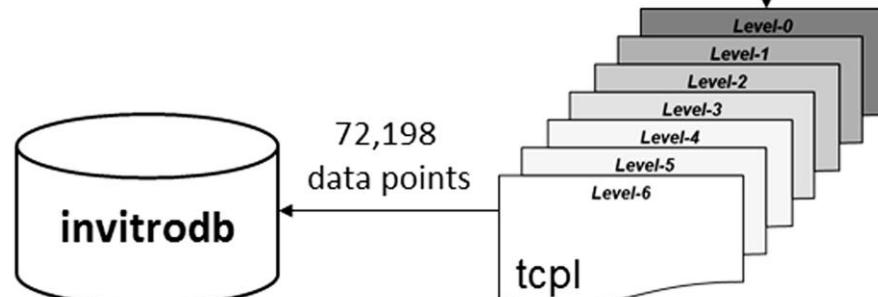
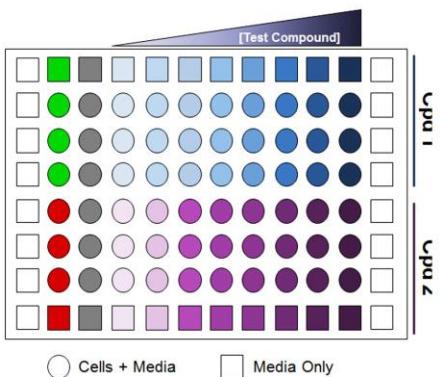
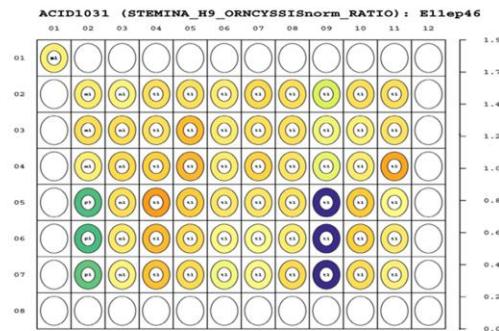
* Plate Maps



1323 samples



Virtual Plates



biochemistry

- urea agmatine gabaculine
- glutaminase arginase
- transcarbamylase 3.5.3.1 carbamoyltransferase
- arginase delta-1-pyrroline-5-carboxylate
- 3.5.3.1 carbamoyltransferase arg development
- retina

2.6.1.13

- medicine 1-pyrroline-5-carboxylate
- arginosuccinate hyperornithinemia
- choroid hyperammonemias
- citrulline polyamines putrescine
- retina

Ornithine release
urea cycle, polyamine &
pyrimidine synthesis.



$$TI = ORN/CYSS$$

Cystine utilization
glutathione synthesis,
redox cycling.

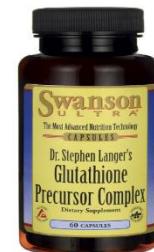
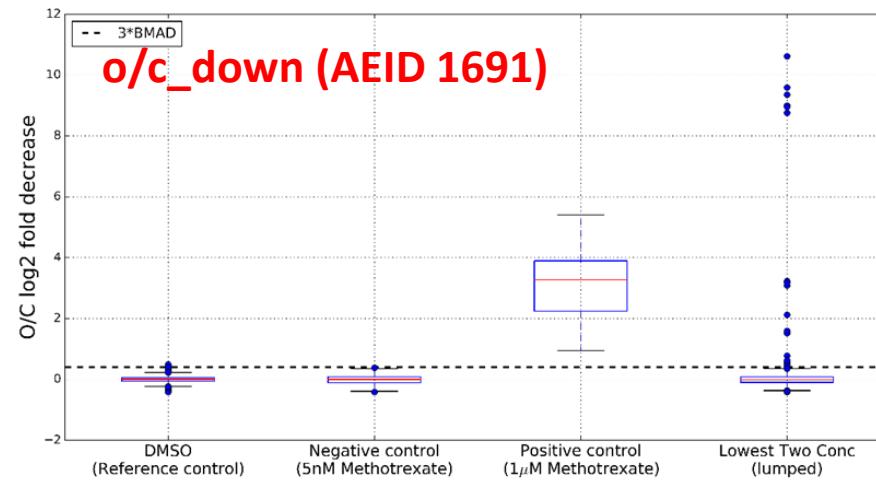
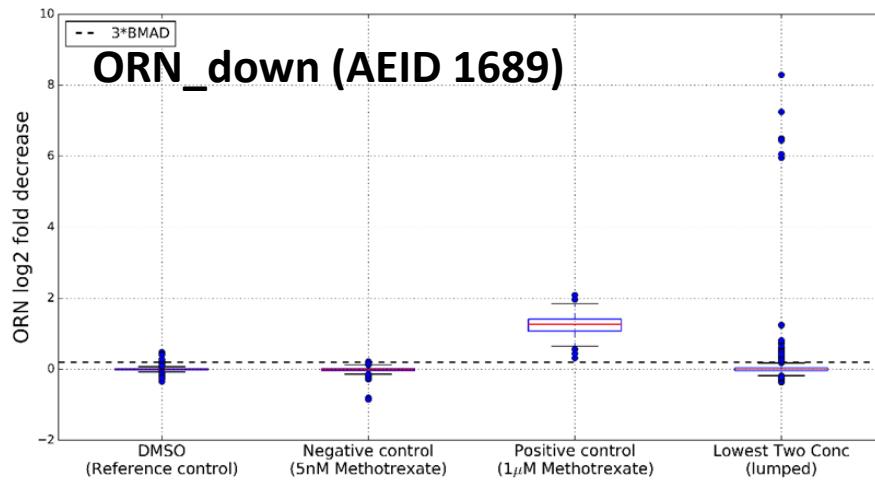


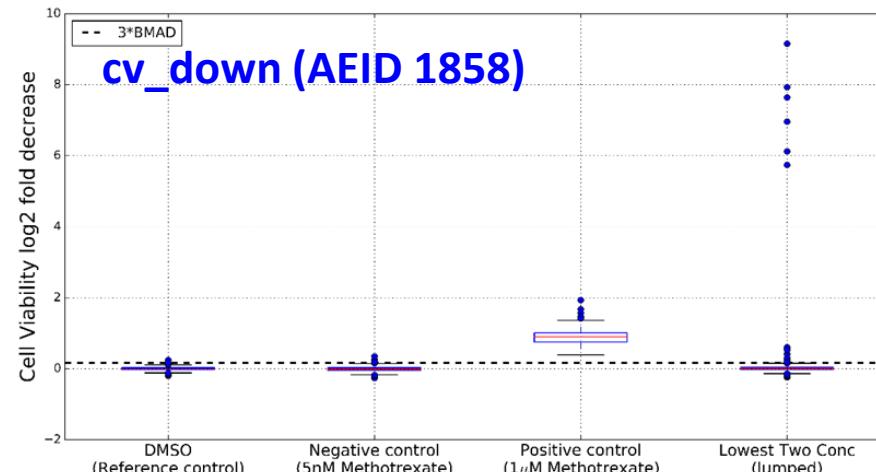
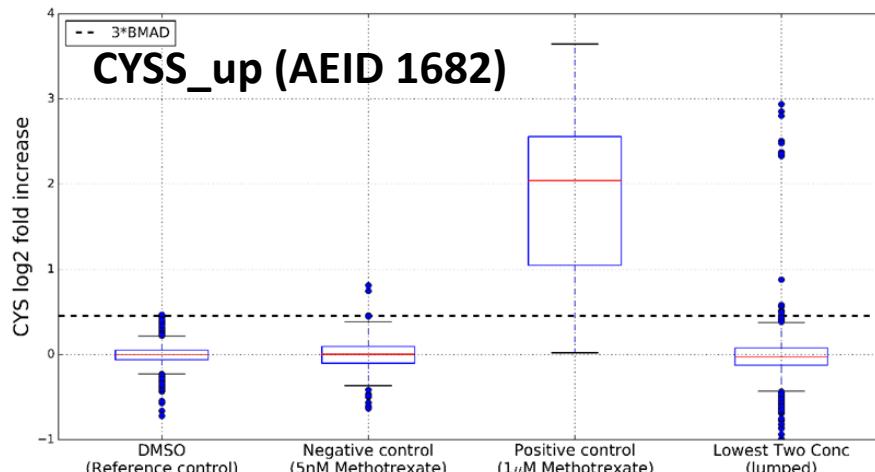
Plate-level Controls

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



Targeted Biomarker

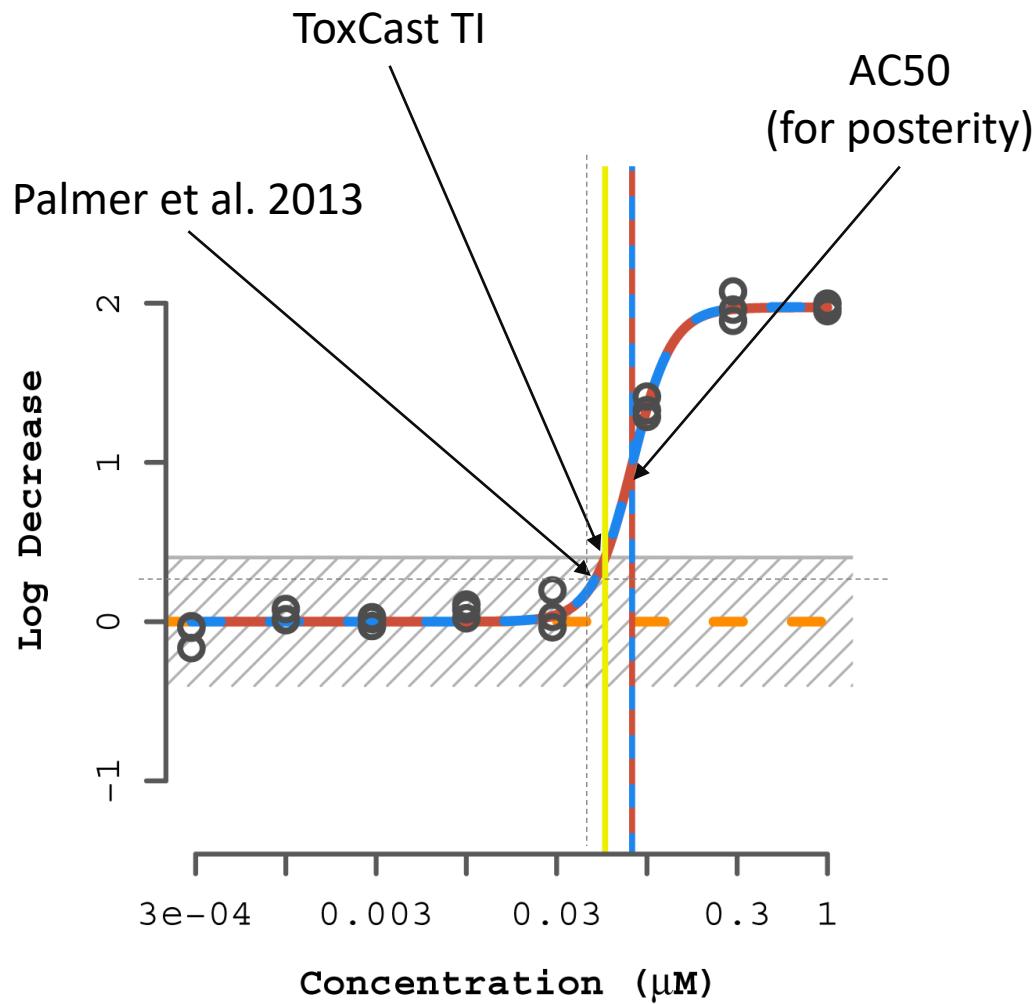
tcpl default at 3*BMAD
($o/c < 0.76$) accurately
classifies negative/positive
reference controls.



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)



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

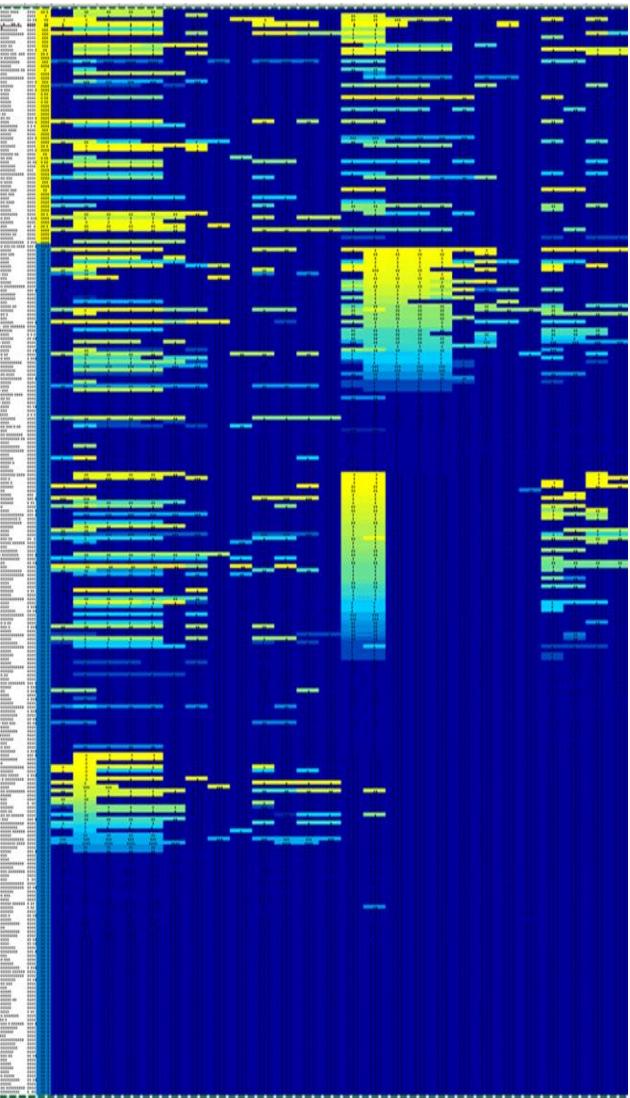
- 181 of 1065 chems (17%) gave a positive response for developmental toxicity prediction.
- Point of departure for biomarker (acb ~0.76) usually before loss of cell viability (acb ~11%).

		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 \leq 125 mg/kg/d for fetal endpoints
- Negatives = no dLEL \geq 1000 mg/kg/day

ToxRefDB_DEV

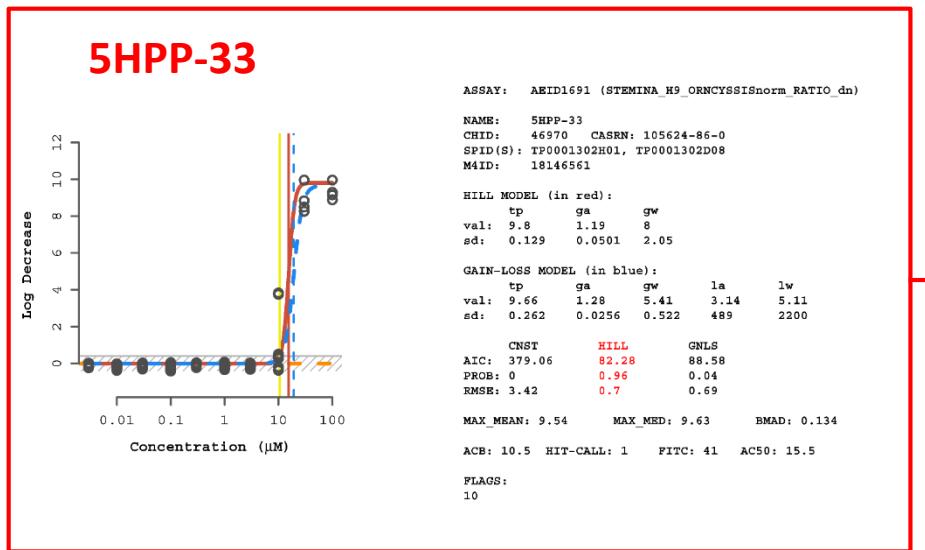
TP	9
FP	19
FN	15
<u>TN</u>	<u>103</u>
n	146
Sensitivity	0.375
Specificity	0.844
Accuracy	76.7%
Mathew's cc	0.206
F1 score	0.005

Combined Model

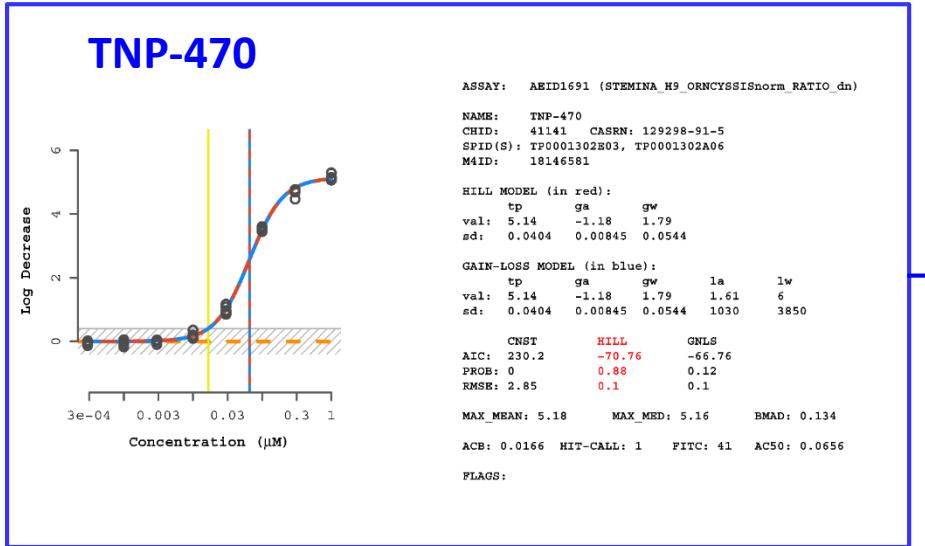
TP	27
FP	19
FN	24
<u>TN</u>	<u>118</u>
n	188
Sensitivity	0.529
Specificity	0.861
Accuracy	77.1%
Mathew's cc	0.404
F1 score	0.006

Example 1: anti-angiogenic compounds confirmed in rat WEC

AC50 (embryolethal) = 21.2 μM



TI = 10.48 μM



TI = 0.017 μM



AC50 (dysmorphogenesis) = 0.038 μM

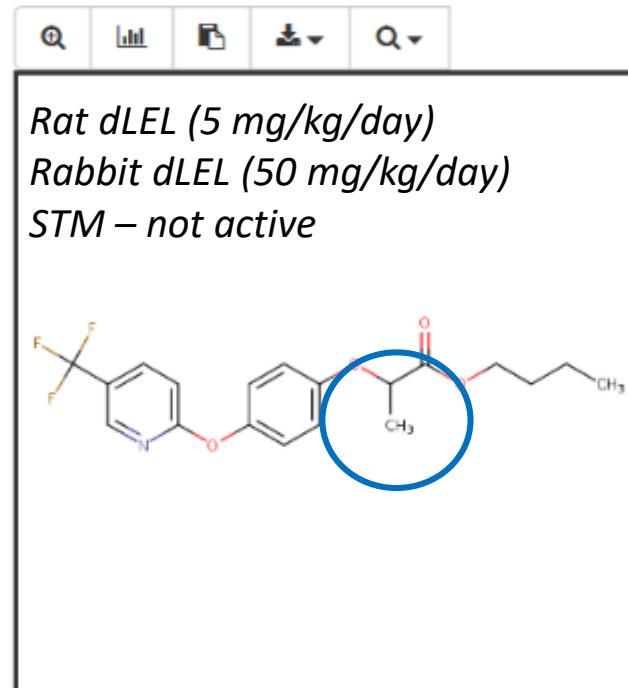
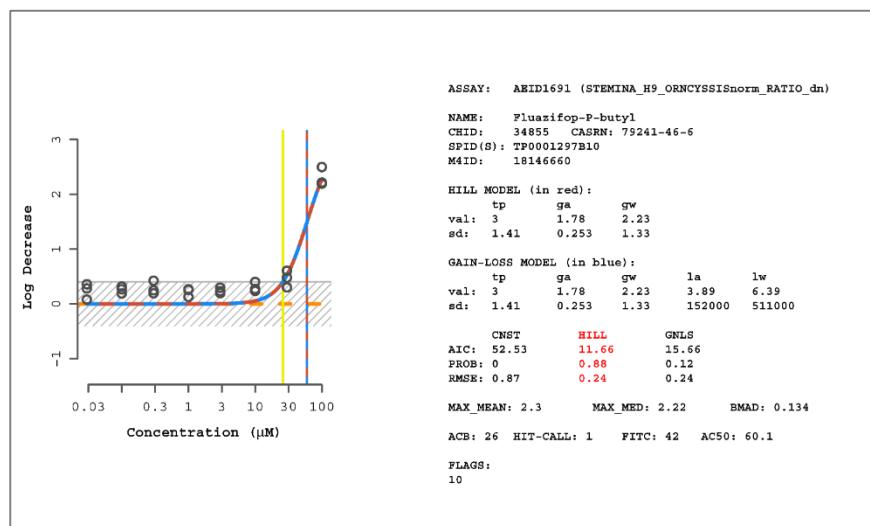
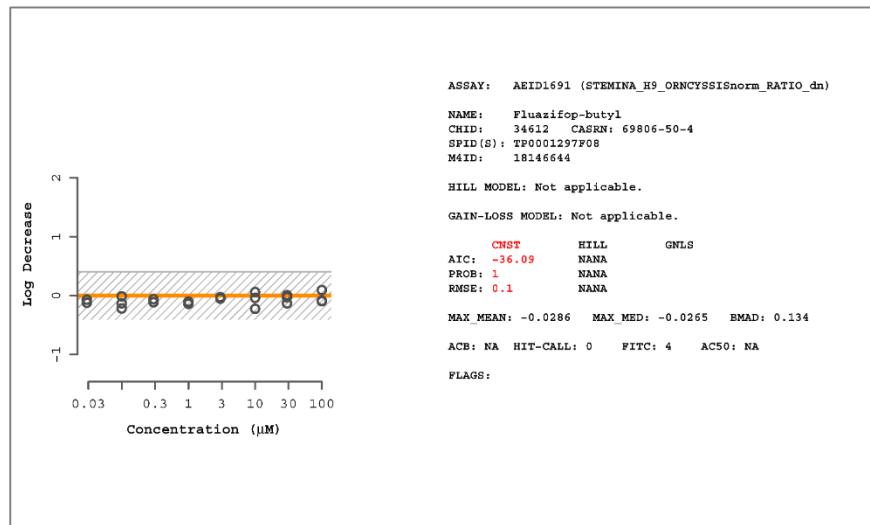
Example 2: stereoisomer pair resolved by STM but not animal studies

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

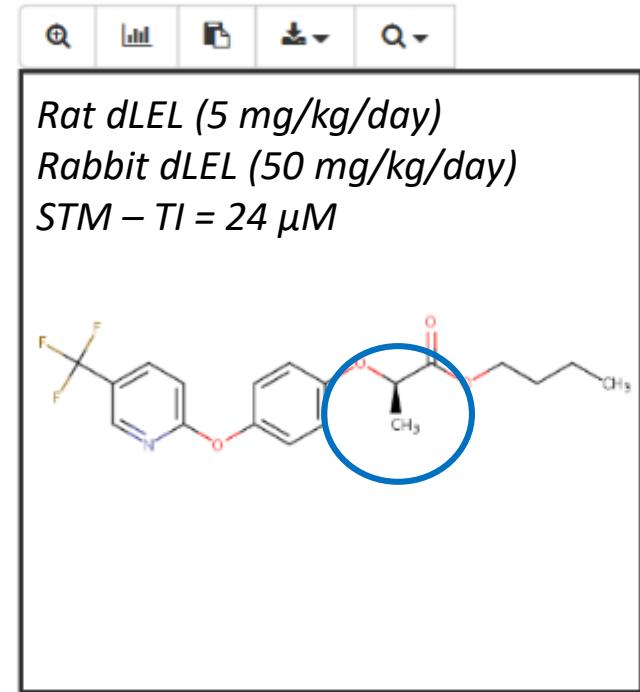
CC(=O)C(C)C(OCCCC)C[C@H](C(F)(F)c1ccc(Oc2ccc(F)c(F)c2)cc1)C(F)(F)F

Fluazifop-P-butyl

79241-46-6 | DTXSID0034855 ⓘ

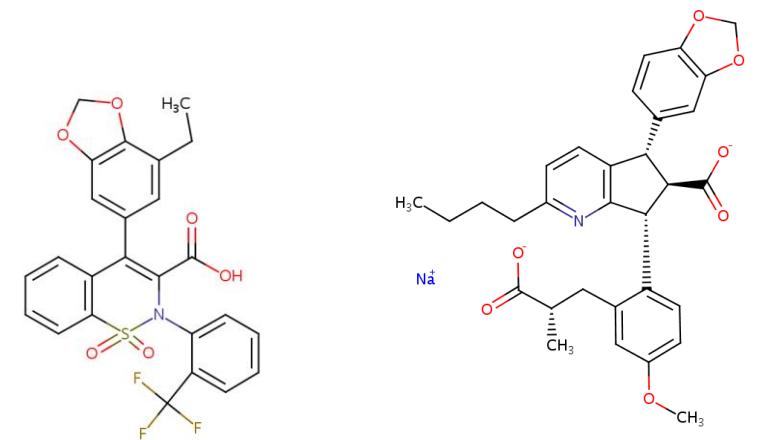
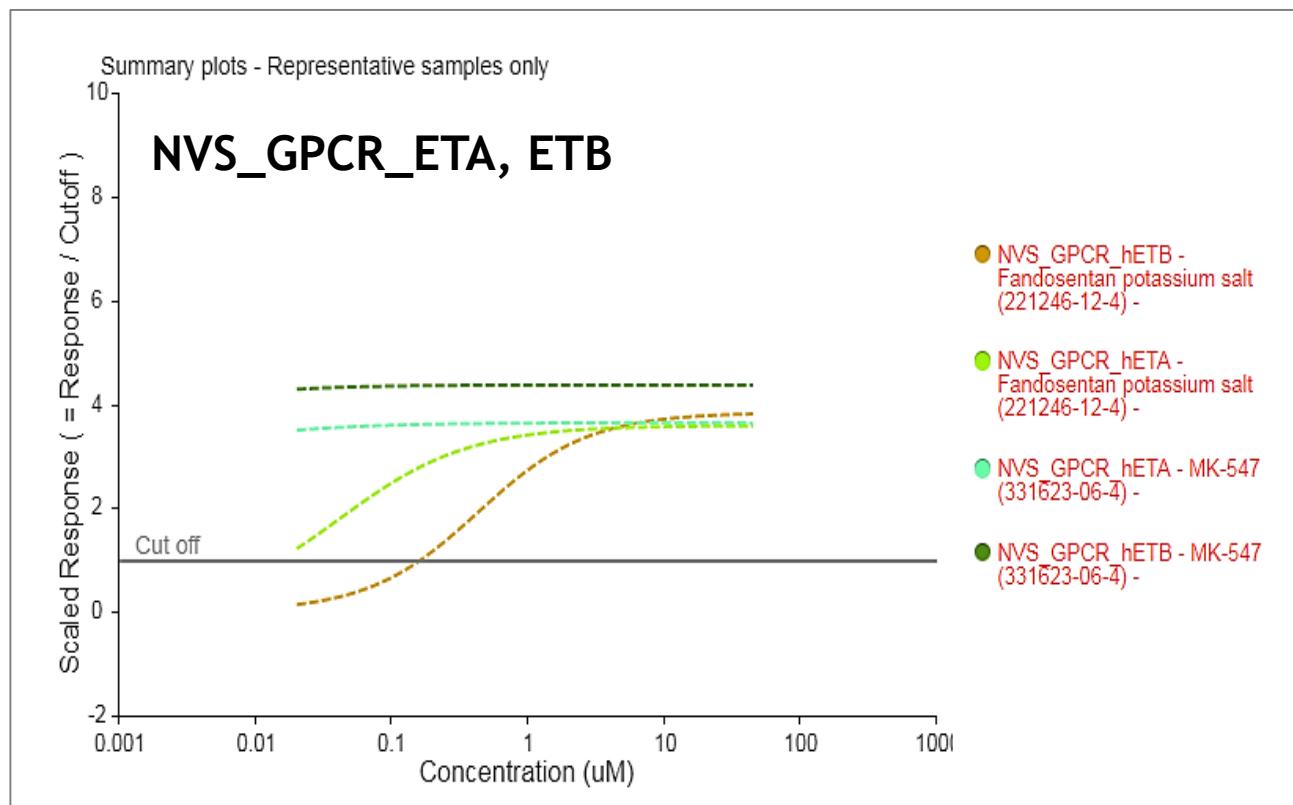
ⓘ Searched by Approved Name: Found 1 result for 'fluazifop-p-butyl'.

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

CC(=O)C(C)C(OCCCC)C[C@H](C(F)(F)c1ccc(Oc2ccc(F)c(F)c2)cc1)C(F)(F)F

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
 $h\text{ETA} = 0.042 \mu\text{M}$
 $STM = \text{inactive} (10 \mu\text{M})$

MK-547
 $h\text{ETB} = 0.0002 \mu\text{M}$
 $STM = \text{inactive} (20 \mu\text{M})$

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

NVS ASIDs selected from invitroDB v2 (June, 2017)

AD score (weighted NVS potency and discretized targeted biomarker)

NIH_DAVID annotation:
functional clusters

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

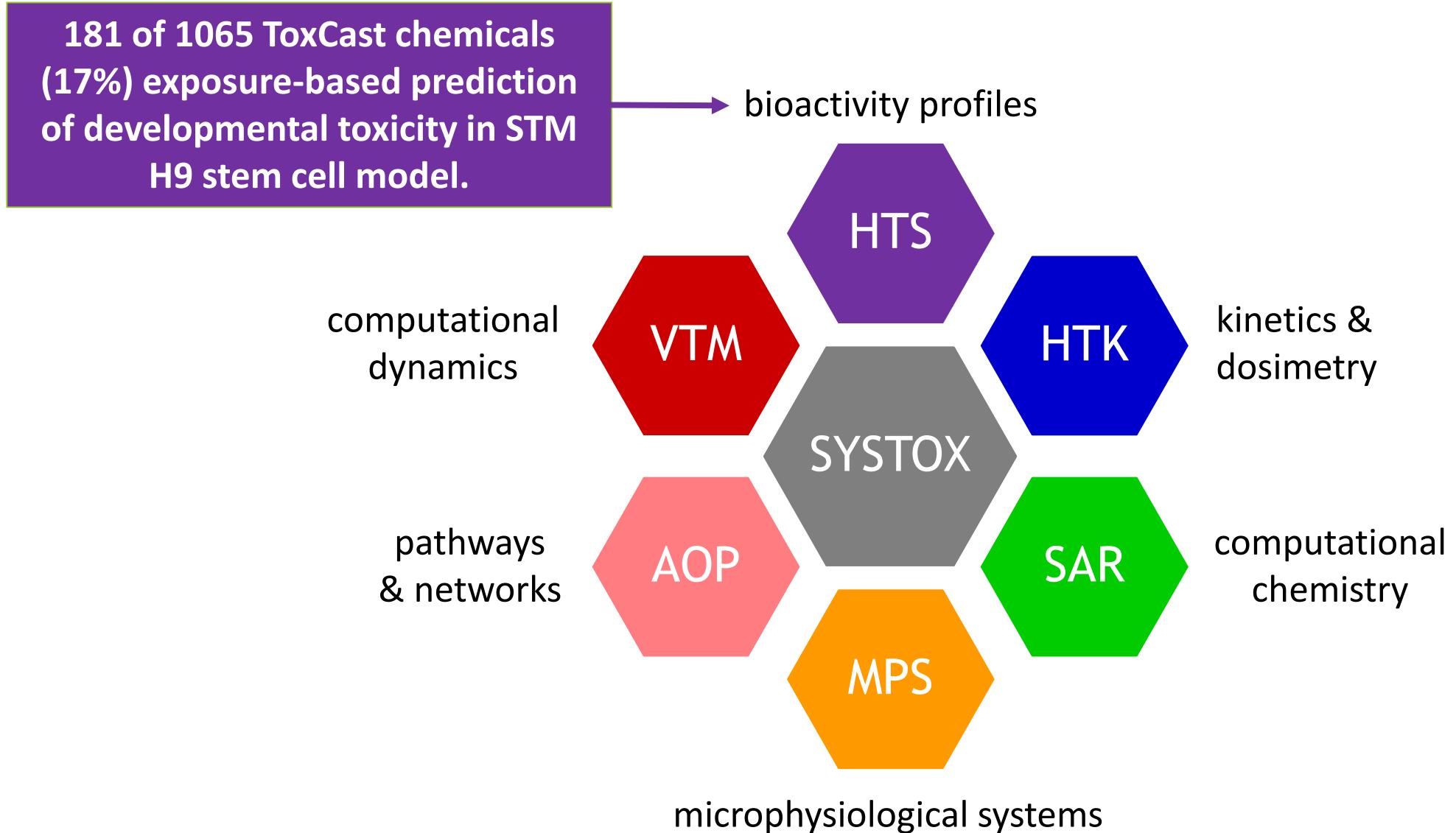
Feature selection with Scikit
(Python) classification
ANOVA 40th percentile

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

GPRC_AdrA2A	Adra2a	adrenergic receptor, alpha 2A	0.008	0.031	2.00	0.117	0.029	0.022	0	1.096	2.00	0.24
ENZ_hhM2	Mmp9	matrix metalloproteinase 9	0.008	0.045	2.00	0.117	0.029	0.022	0	1.096	2.00	0.24
ENZ_hhK2	Cdkn2	Ser/Thr kinase	0.008	0.045	1.14	0.114	0.029	0.022	0	1.096	1.001	1.14
ENZ_hhA1	Abl1	non-receptor tyrosine kinase	0.005	0.038	1.53	0.055	1.004	1.048	1.088	1.176	1.53	4.53
MP_PBP	Tspn	TSPN	0.001	0.035	1.459	0.055	1.004	1.089	1.047	1.278	1.459	4.49
ENZ_hhR3	Hmgb1	histone	0.001	0.035	1.459	0.055	1.004	1.089	1.047	1.278	1.459	4.49
OR_x50MA NonSelective	Smar1	2nd messager	0	0	1.344	0.047	1.014	1.016	1.016	1.271	1.344	4.25
GPRC_hhX2	Tbx2a2	2nd messager	0.009	0.046	1.121	0.057	1.004	1.077	1.055	1.096	1.121	4.03
ENZ_hhDAG Activator	Hdac6	deacetylase	0.009	0.046	1.121	0.057	1.004	1.077	1.055	1.096	1.121	3.96
ADM_hhP29	Cyp2c19	CYP inhibition	0.008	0.046	1.041	0.057	1.004	1.088	1.078	1.096	1.096	3.96
GPRC_nmK3	Tgfb3	neurokinin	0.032	0.046	1.287	0	0.111	0.112	1.287	0	1.344	2.04
NR_hhR Antagonist	Nr3d1	corticotropin	0.043	0.047	0.979	0.057	1.004	1.072	1.053	1.096	1.121	4.27
NR_hhX Antagonist	Nr3d4	RXR	0.043	0.047	0.979	0.057	1.004	1.072	1.053	1.096	1.121	4.27
ADM_hhCP5A1	Cyp2f1	CYP inhibition	0.021	0.045	1.113	0.057	1.004	1.072	1.053	1.096	1.121	3.96
ADM_hhGFR	Nr3d5	corticotropin	0.121	0.045	0.945	0.113	0.049	0.091	0.13	0.39	0.545	2.3
NR_hhA Antagonist	Nr3d3	RXR	0.045	0.046	0.945	0.121	0.059	0.143	0.252	0.367	0.445	1.58
ENZ_hhA1	Jak2	non-receptor tyrosine kinase	0.025	0.026	0.422	0.258	0.369	0.468	0.334	0.465	0.422	1.44
ENZ_hhA1	Ras4l	Ser/Thr kinase	0.076	0.025	0.382	0.258	0.369	0.485	0.334	0.465	0.422	1.41
ENZ_hhP2C	Htr4c	seroton	0.013	0.028	0.2	0.071	0.081	0.123	0.263	0.368	0.318	1.39
GPRC_hhT2A	Nrk2	Ser/Thr kinase	0.024	0.032	0.341	0.222	0.303	0.431	0.301	0.371	0.41	1.30
GPRC_hhT2A	Htr2a	serotonin	0.014	0.024	0.168	0.079	0.081	0.168	0.171	0.234	0.218	1.18
ADM_hhR	Serpr	steroid hormone receptor	0.008	0.024	0.168	0.079	0.081	0.168	0.171	0.234	0.218	1.15
ENZ_hhGFR	Fgrf	proto-oncogene fibroblast growth factor receptor	0	0.032	0.442	0.052	0.056	0.331	0.216	0.342	0.342	0.24
ENZ_hhYn	Fyrf	non-receptor tyrosine kinase	0	0.037	0.341	0.055	0	0.070	0.383	0	0.346	0.91
GPRC_hhR	Cyp1a1	CYP inhibition	0.021	0.031	0.669	0.116	0	0	0	0	0	0.80
ADM_hhA1	Hrh1	histamine	0.021	0.031	0.669	0.116	0	0	0	0	0	0.80
ENZ_hhDAG Activator	Hdac3	deacetylase	0.021	0.031	0.669	0.116	0	0	0	0	0	0.80
ENZ_hhP2A2	Htr2a	Ser/Thr kinase	0.024	0.031	0.669	0.116	0	0	0	0	0	0.80
ENZ_hhT2 Activator	Tek	receptor tyrosine kinase	0.024	0.031	0.669	0.116	0	0	0	0	0	0.80
ENZ_hhPAK4	Pak4	Ser/Thr kinase	0	0	0.358	0	0	0.035	0.504	0	0.358	0.74
ENZ_hhART2	Akt2	Ser/Thr kinase	0	0	0.358	0	0	0	0	0	0	0.74
ENZ_hhLCA	Prraca	Ser/Thr kinase	0	0	0.358	0	0	0	0	0	0	0.74
ENZ_hhRnR	Insr	receptor tyrosine kinase	0.001	0.027	0.34	0.054	0	0.187	0.132	0	0.34	0.73
ENZ_hhPTPN13 Activator	Ptpn13	non-receptor tyrosine phosphatase	0.048	0	0	0	0	0	0	0	0	0.65
GPRC_hhD2	Aldobz	adrenergic	0	0.029	0.354	0.059	0	0	0.349	0	0	0.64
ADM_hhP2A2	Cyp2d2	CYP inhibition	0.021	0.029	0.354	0.059	0	0	0.349	0	0	0.64
NR_hhPARA	Ppara	RXR	0	0.028	0.255	0	0.157	0	0.18	0.286	0.225	0.63
ENZ_hhSRT3	Sirt3	deacetylase	0	0	0.354	0	0	0	0	0	0	0.56
ENZ_hhMAPK3 Activator	Mapk3	Ser/Thr kinase	0.021	0.025	0.354	0.059	0	0	0.349	0	0	0.55
ADM_hhC12	Cyp2d2	CYP inhibition	0.021	0.025	0.354	0.059	0	0	0.349	0	0	0.55
ENZ_hhPTPN14	Ptpn14	non-receptor tyrosine phosphatase	0.021	0	0	0.048	0	0	0.042	0	0	0.53
ENZ_hhPTPN2	Ptpn2	non-receptor tyrosine phosphatase	0.015	0.025	0.353	0.059	0	0.023	0	0.295	0.46	0.40
ADM_hhC24	Cyp2d6	CYP inhibition	0.026	0	0.354	0.059	0	0	0.341	0	0	0.53
ENZ_hhBACE1 Activator	Bace1	beta-secretase	0.049	0.049	0.354	0.059	0	0	0.349	0	0	0.53
ADM_hhCP5A1	Cyp2c18	CYP inhibition	0.021	0.025	0.354	0.059	0	0	0.349	0	0	0.53
ADM_hhVGF3	Flt4	receptor tyrosine kinase	0.082	0.027	0.357	0.059	0	0	0.331	0	0.126	0.34
ENZ_hhFGR1	Fgrf1	receptor tyrosine kinase	0.045	0.025	0.357	0.059	0	0	0.301	0	0.127	0.32
ENZ_hhPTPN2	Ptpn2	non-receptor tyrosine phosphatase	0.003	0.034	0.357	0.059	0	0.007	0.322	0	0.344	0.33
GPRC_hhNT1 NonSelective	Htr1a	serotonin	0	0.027	0	0	0	0	0	0	0	0.35
GPRC_gppA	Nppa	guanosine peptide	0	0.027	0	0	0	0	0	0	0	0.36
ENZ_hhMMP1	Mmp1	protease	0.054	0	0	0	0	0.229	0	0	0.399	0.36
ADM_hhC24A	Cyp2d6	CYP inhibition	0.078	0	0.357	0.059	0	0	0.347	0	0.137	0.33
GPRC_gdh4	Htr4	serotonin	0.013	0	0.357	0.059	0	0	0.347	0	0.137	0.33
ENZ_hhSRT2	Sirt2	deacetylase	0.051	0.04	0.357	0.059	0	0.105	0	0.31	0	0.38
ENZ_hhBACE1 Activator	Bace1	beta-secretase	0.052	0	0.357	0.059	0	0.222	0	0	0.39	0
ENZ_hhRnR	Adrb2b	steroid hormone receptor	0.078	0	0.357	0.059	0	0.222	0	0	0.39	0.37
ENZ_hhC24	Cyp2d2	adrenergic	0.004	0.028	0.357	0.059	0	0.234	0.244	0.17	0.208	0.64
IC_CaCN3	Cannab1	channel	0.062	-0.041	0	0.234	0	0	0.493	-0.401	0	0.68
GPRC_hhSRT7	Htr7	serotonin	0	0	0.357	0.059	0	0	0	0	0	0.88
ENZ_hhAT1	Art1	Ser/Thr kinase	0.021	0	0.357	0.059	0	0.037	0.359	0.211	0.107	0.78
ENZ_hhNSK2	Gsk3b	Ser/Thr kinase	0	0	0.4	-0.114	0.056	0.239	-0.14	-0.102	-0.4	0.93
ENZ_hhSRT1	Sirt1	deacetylase	0.091	-0.049	0	0.357	0.059	0	0.663	0.589	0	0.99
ENZ_hhMAPK3	Mapk3	Ser/Thr kinase	0.043	0.046	0.357	0.059	0	0.246	0.195	0.18	0.446	0.123
ENZ_hhPTPN11 Activator	Ptpn11	non-receptor tyrosine phosphatase	0.018	0.031	0.357	0.059	0	0.246	0.195	0.18	0.446	0.123
ENZ_hhPTPN20 Activator	Ptpn20	non-receptor tyrosine phosphatase	0.056	-0.058	0.357	0.059	0	0.222	0	0.662	0.58	0.52
GPRC_hhEta	Endorin	endothelin	0.077	-0.044	0.357	0.059	0	0.222	0	0.384	0.569	0.52
ENZ_hhPTPN9 Activator	Ptpn9	non-receptor tyrosine phosphatase	0.077	-0.058	0.357	0.059	0	0.222	0	0.341	0.401	0.50
ENZ_hhIRAK4 Activator	Ira4a	Ser/Thr kinase	0.032	-0.067	0	0.357	0.059	0	0.311	0.399	0	0.66
ENZ_hhPDE4A1	Pde4a	2nd messenger	0.021	-0.042	0	0.357	0.059	0	0.243	0.195	0.18	0.446
ENZ_hhNES	Pde5	steroid hormone receptor	0.017	-0.068	0.357	0.059	0	0.227	0	0	0.397	0.09
ENZ_hhMmp9	Mmp9	protease	0.078	-0.055	0.357	0.059	0	0.222	0	0.303	0.386	0.220
ENZ_hhRnR Antagonist	Rara	RXR	0.079	-0.067	0.357	0.059	0	0.228	0	0.363	0.386	0.211
ENZ_hhD60	D60	dopamine	0.008	0.038	0.357	0.059	0	0	0	0	0	0.78
ENZ_hhN132	Rxx	RXR	0.013	0.037	0.357	0.059	0	0.222	0	0.313	0.349	0.20
ADM_hhCP246	Cyp246	CYP inhibition	0.093	-0.067	0.357	0.059	0	0.228	0	0.368	0.387	0.203

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



National Center for Computational Toxicology

Special Thanks

Todd Zurlinden – NCCT

Kate Sali – NCCT

Richard Judson - NCCT

Nathan Rush –NCCT

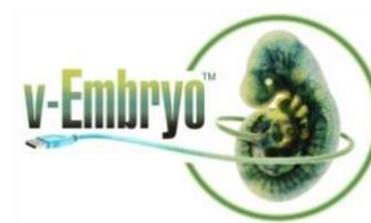
Parth Kothiya – NCCT

Nancy Baker – Leidos / NCCT

Richard Spencer – ARA / EMVL

Keith Houck -NCCT

Jessica Palmer – Stemina



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http://hexwebz.net/gosho/production/files/2015/08/documents/virtual_tissue_models_fact_sheet_final.pdf