Inverting the SAR Paradigm:

Applications of a Chemotype-Enrichment Approach within EPA's Computational Toxicology Programs

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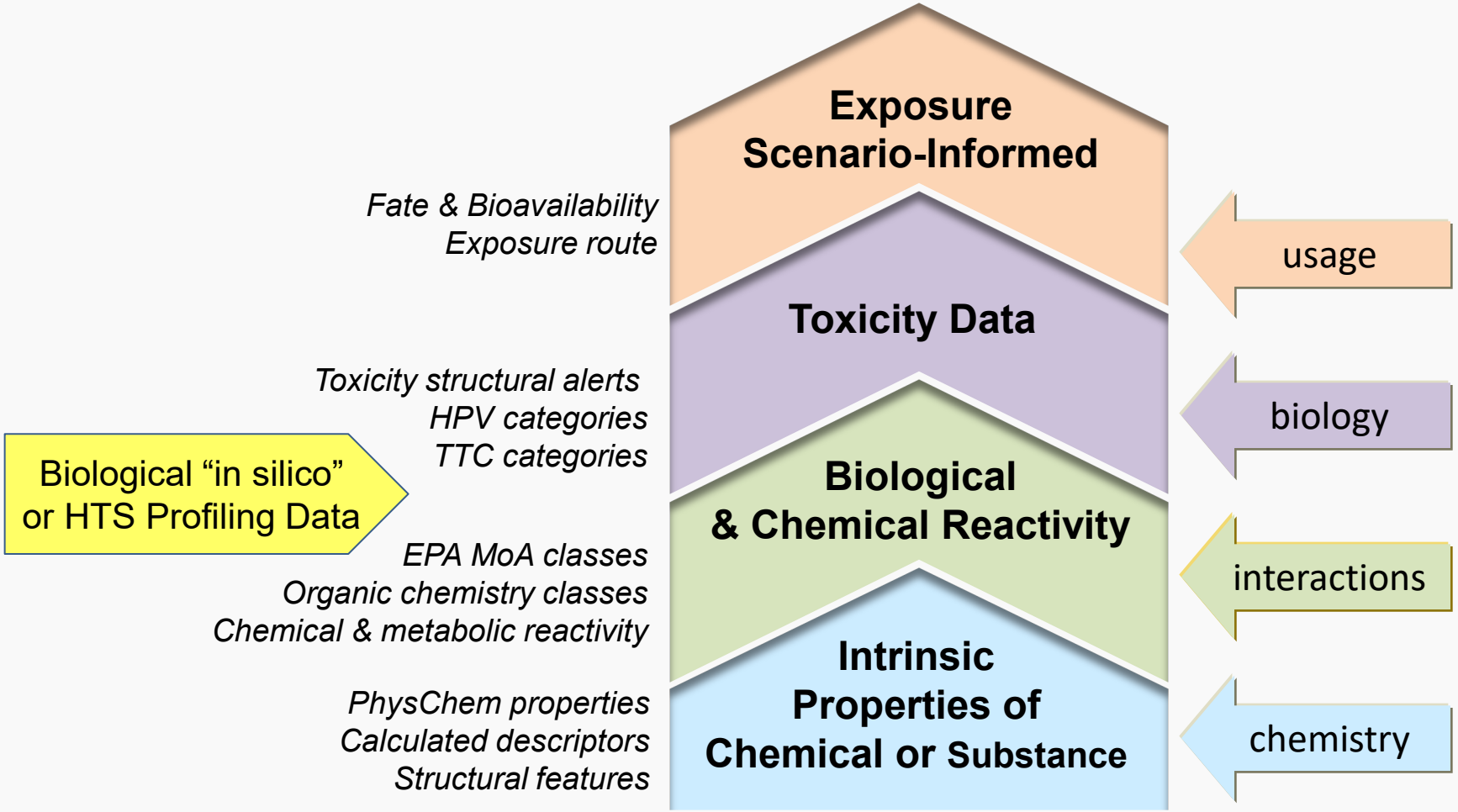
QSAR 2021 Virtual Meeting, June 7-9

Chemical/Substance representations for hazard & risk evaluation



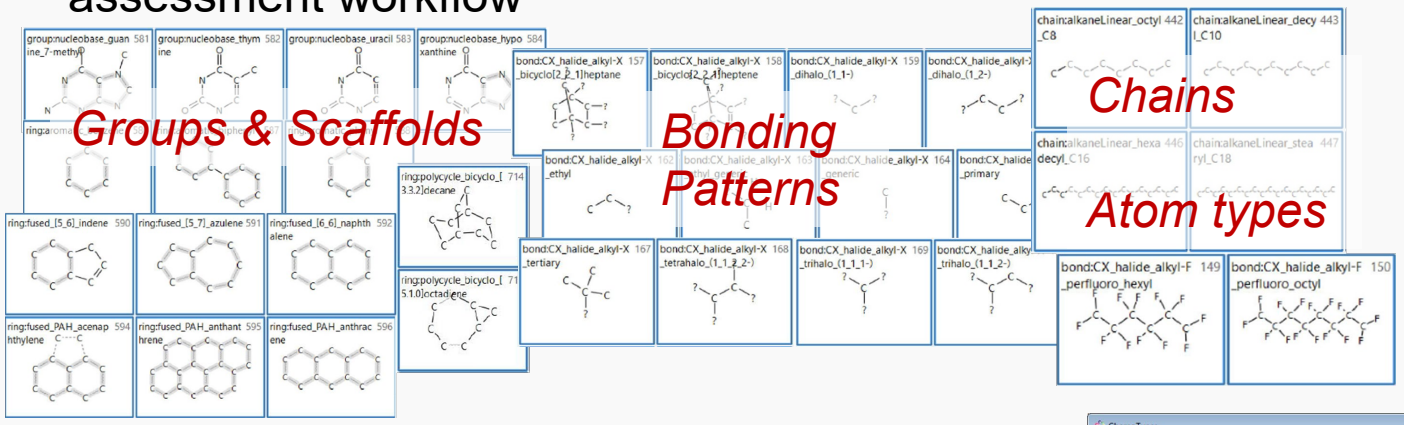
CSS workshop, April 2011

ToxPrints: A Public Set of Chemotypes

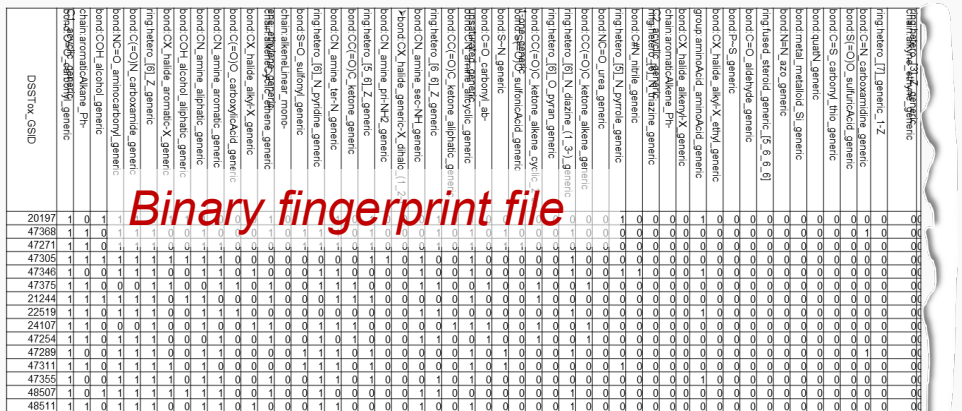
→ *Clear, reproducible means for defining & visualizing local chemistry*

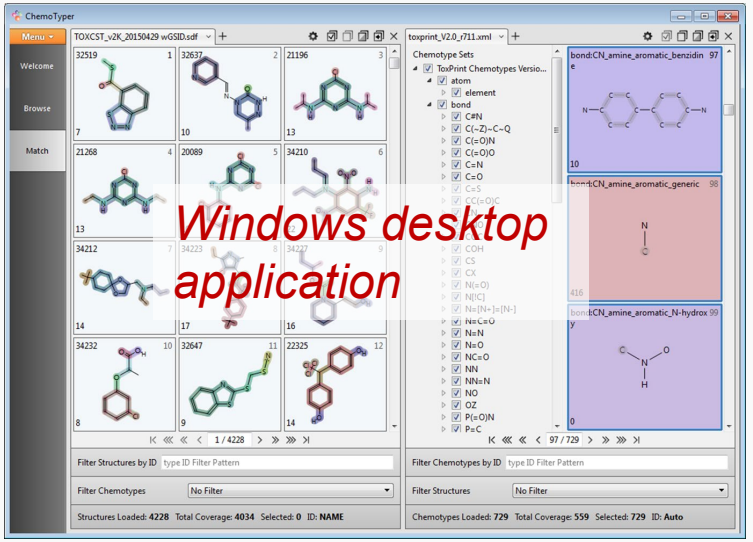
ToxPrints: http://www.toxprint.org

729 features important to EPA & FDA's "chemical exposure" landscape and safety assessment workflow



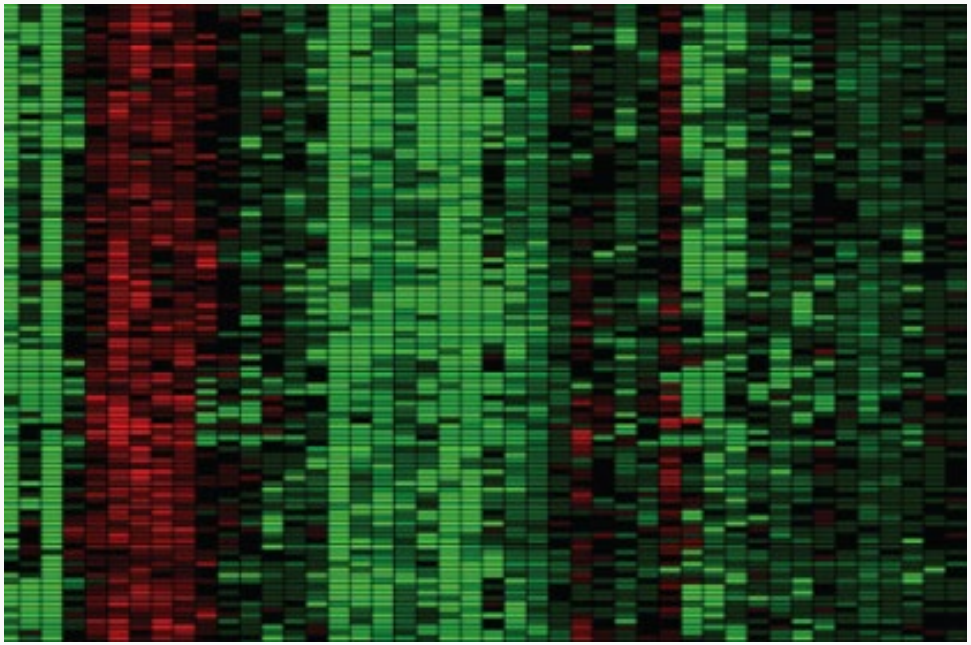
Chemotyper: http://www.chemotyper.org





Chemotype-Enrichment Approaches:

Finding chemical-activity patterns in the noise



Chemotype Enrichment,
e.g., *Flame Retardant (FR) Use Category*

42 chemicals labeled as "Flame Retardants" included in ToxCast & Tox21

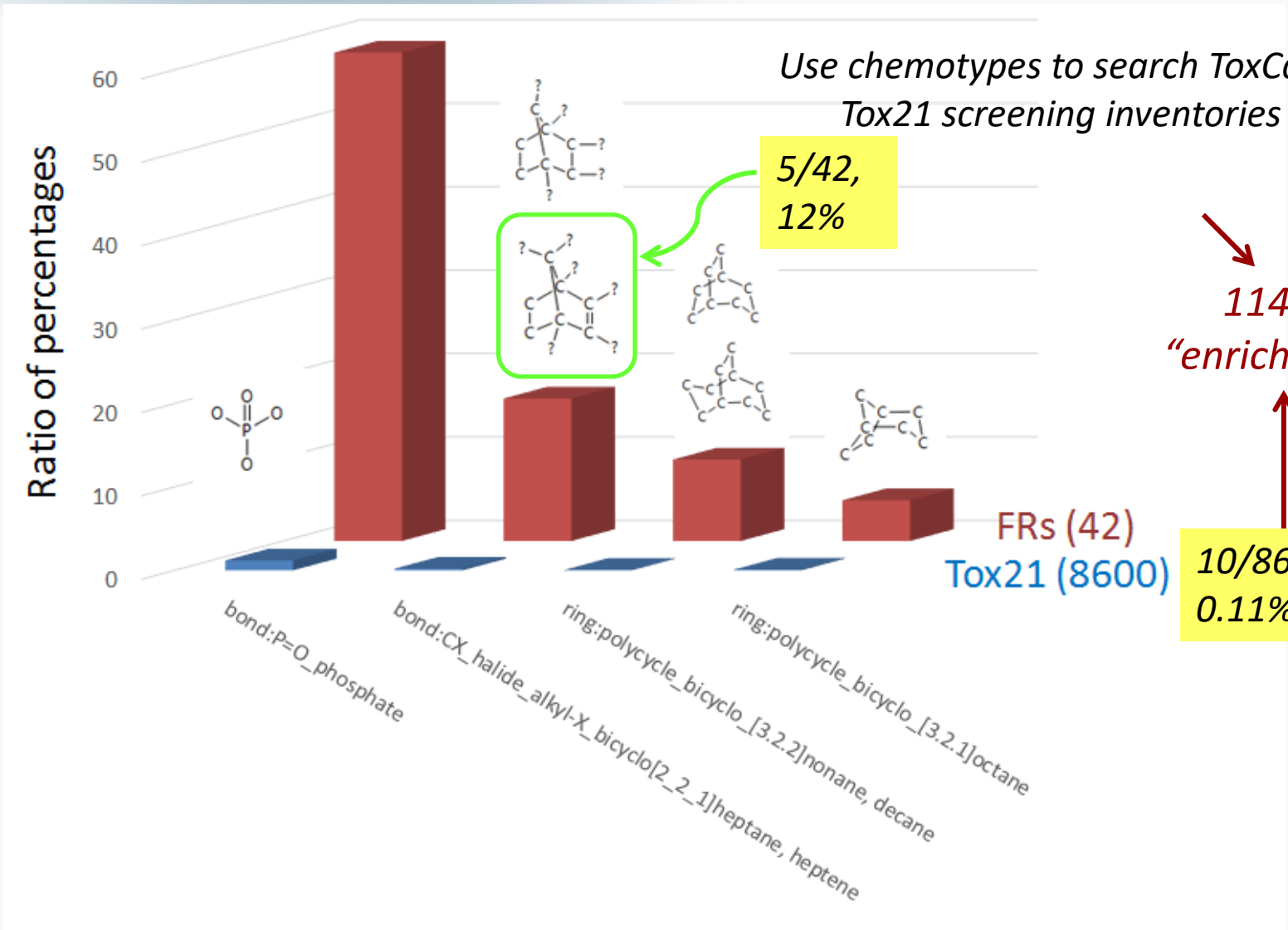
3296-90-0	2,2-Bis(bromomethyl)-1,3-propanediol
115-28-6	Chlorendic acid
2921-88-2	Chlorpyrifos
2385-85-5	Mirex
115-96-8	Tris(2-chloroethyl) phosphate
78-42-2	Tris(2-ethylhexyl) phosphate
115-86-6	Triphenyl phosphate
126-73-8	Tributyl phosphate
79-94-7	3,3',5,5'-Tetrabromobisphenol A
13674-87-8	Tris(1,3-dichloro-2-propyl)phosphate
1163-19-5	Decabromodiphenyl
19660-16-3	2,3-Dibromopropyl a
563-04-2	Tri-m-cresyl phosph
20120-33-6	Phosphonic acid, (3- dimethyl ester
868-85-9	Dimethyl hydrogen p
756-79-6	Dimethyl methylpho
124-64-1	Tetrakis(hydroxymethyl)phosphonium chloride
55566-30-8	Tetrakis(hydroxymethyl)phosphonium sulfate
1330-78-5	Tricresyl phosphate
512-56-1	Trimethyl phosphate

126-72-7	Tris(2,3-dibromopropyl) phosphate
78-51-3	Tris(2-butoxyethyl) phosphate
79-95-8	2,2',6,6'-Tetrachlorobisphenol A
118-79-6	2,4,6-Tribromophenol
56803-37-3	tert-Butylphenyl diphenyl phosphate
26444-49-5	Cresyl diphenyl phosphate
1241-94-7	2-Ethylhexyl diphenyl phosphate
29761-21-5	Isodecyl diphenyl phosphate
632-79-1	Tetrabromophthalic anhydride
117-08-8	Tetrachlorophthalic anhydride
68937-41-7	Phenol, isopropylated, phosphate (3:1)
2781-11-5	Phosphonic acid, [[bis(2-hydroxyethyl)amino]methyl]-, diethyl ester
78-30-8	Tri-o-cresyl phosphate
4162-45-2	Ethanol, 2,2'-((1-methylethylidene)bis((2,6-dibromo-4,1-phenylene)oxy))bis-

Are there enriched chemotypes within this FR subset relative to ToxCast & Tox21?

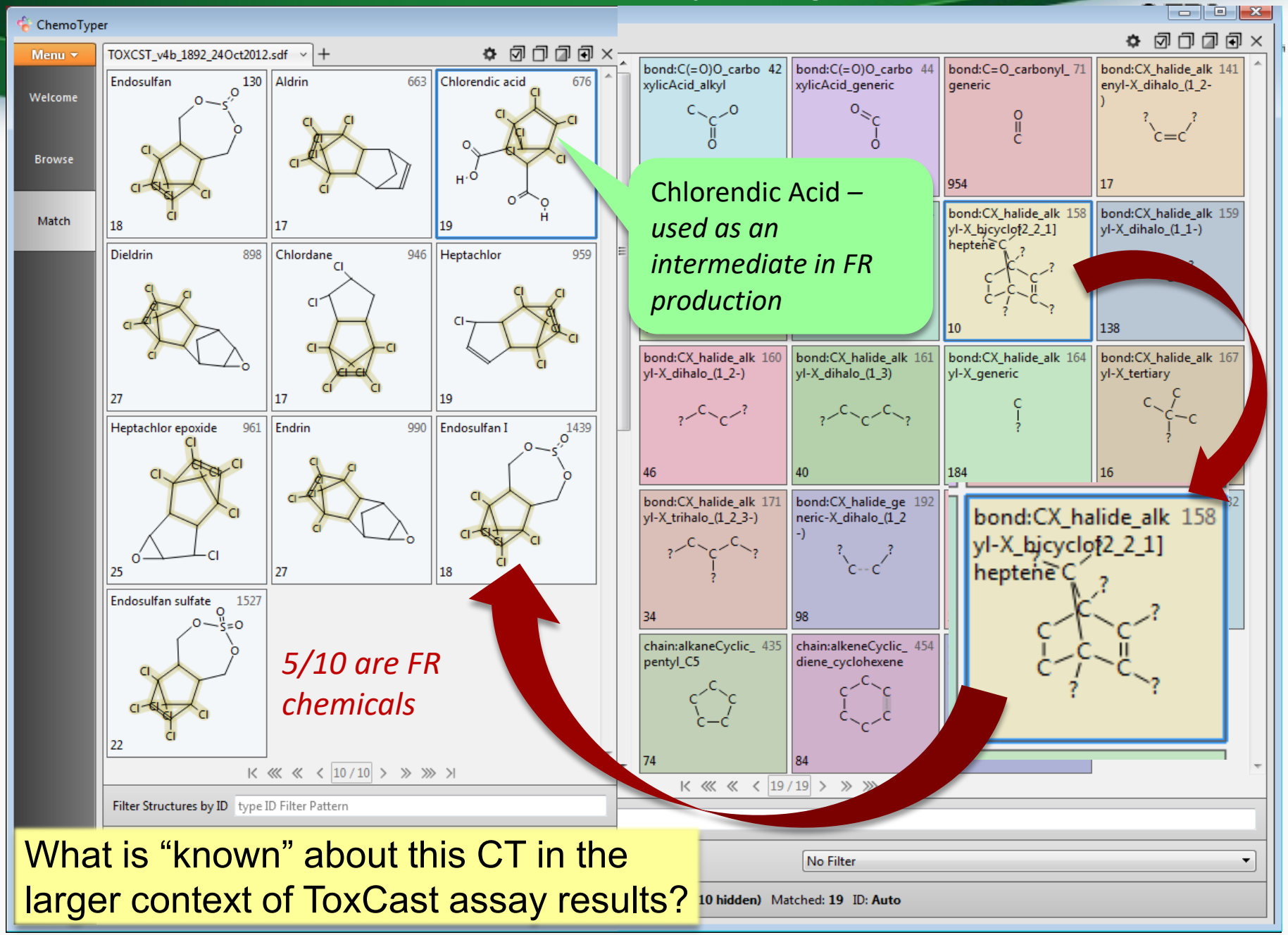
w/ Tom Knudsen (NCCT), Bhavesh Ahir, NCCT (ORISE)

Chemotype Enrichment,
e.g., *Flame Retardant (FR) Use Category*



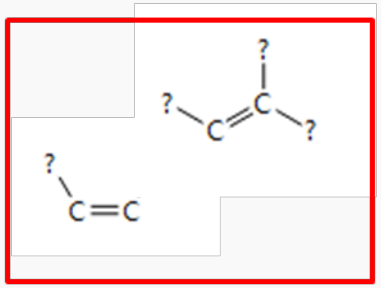
w/ Tom Knudsen and Bhavesh Ahir

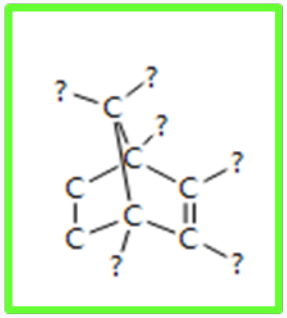
www.chemotyper.org

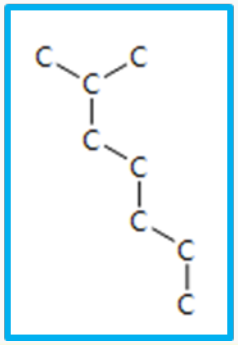


{CT-Assay}$_{ToxCast-FR}$

Assay	FR Chemotype (CT)	Odds Ratio	# CT TP	# CT total	# assay positive	Total cmpds
ATG_RARg_TRANS	bond:CX_halide_alkyl-X_dihalo_(1_2-)	6	8	46	71	1857
ATG_RARa_TRANS	bond:CX_halide_alkyl-X_dihalo_(1_2-)	5	8	46	77	1857
ATG_RARg_TRANS	bond:CX_halide_alkyl-X_dihalo_(1_3)	8	9	40	71	1857
ATG_RARg_TRANS	bond:CX_halide_alkyl-X_trihalo_(1_2_3-)	9	8	34	71	1857
ATG_RARa_TRANS	bond:CX_halide_alkyl-X_trihalo_(1_2_3-)	5	6	34	77	1857
ATG_RXRb_TRANS	chain:alkaneBranch_isooctyl_hexyl_2-methyl	6	8	17	261	1857
Tox21_TR_LUC_GH3_Antagonist	bond:CX_halide_alkyl-X_bicyclo[2_2_1]heptene	18	6	10	151	1858
Tox21_MitochondrialToxicity_ratio	bond:CX_halide_alkenyl-X_dihalo_(1_2-)	10	12	17	385	1858
Tox21_MitochondrialToxicity_ratio	bond:CX_halide_alkyl-X_bicyclo[2_2_1]heptene	35	9	10	385	1858
Tox21_MitochondrialToxicity_ratio	bond:CX_halide_alkyl-X_tertiary	8	10	15	385	1858





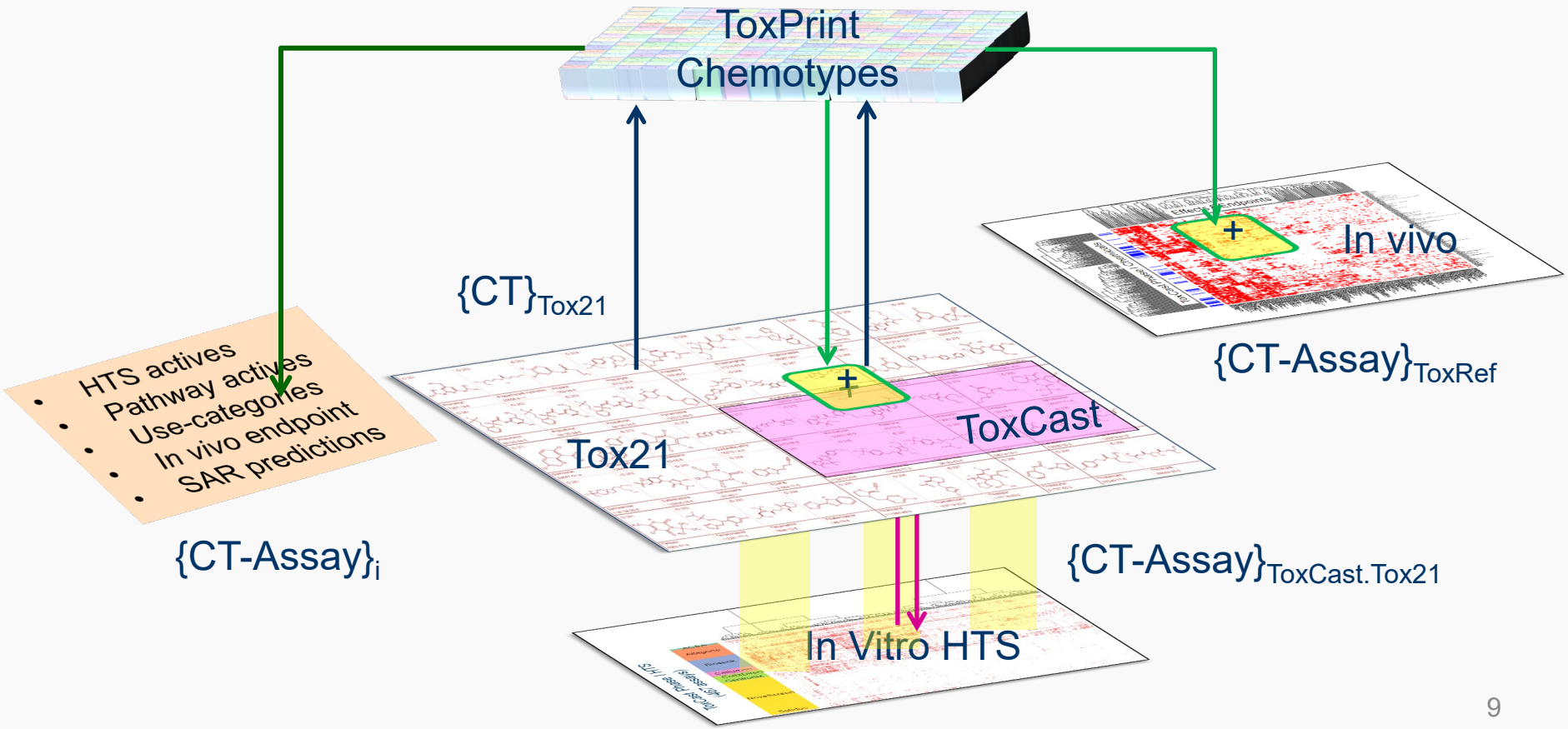


Significant {CT-Assay}$_{ToxCast-FR}$ associations potentially related to developmental outcomes

w/ Tom Knudsen and Bhavesh Ahir

Chemotype-Activity Enrichments

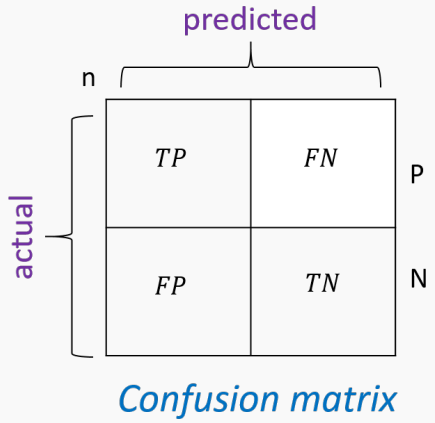
- ✓ Create {CT-Activity} enrichment profiles for any “active” subspace of a test set
- ✓ Focus studies in local CT domains
- ✓ Enhance HTS or structure-activity signal



Computing CT-Assay "Enrichments"

Simple statistical thresholds & filters of significance:

TP_ID	ToxPrint_CT_name[2]	CT_{Tot}	T_{pos}	F_{pos}	F_{neg}	T_{neg}	Odd's Ratio	Fischer's pval
423	chain:alkaneBranch_t-butyl_C4	41	24	17	294	693	**3.3**	**2.0E-04**
479	chain:aromaticAlkane_Ph-C1-Ph	39	27	12	291	698	**5.4**	**6.5E-07**
303	bond:X[any_!C]_halide_inorganic	28	17	11	301	699	**3.6**	**9.0E-04**



Confusion matrix

- Odds Ratio ≥ 3, *conveys simple fractional enrichment*
- Fischer's exact p value ≤ 0.05, *compensates for size of dataset*
- T_{pos} (TP) ≥ 3, *require at least 3 chemicals with CT in Positives*

Binary "Activity" Hit Call + Fingerprint = CT Enrichment Table

DTXSID	PREFERRED_NAME	Hit_Call	bond:CX_halide_alkenyl-Cl_dichloro_(1_1-)	bond:CX_halide_alkenyl-X_acyclic_generic	bond:CX_halide_alkenyl-X_dihalo_(1_1-)	bond:CX_halide_alkenyl-X_generic	bond:CX_halide_alkyl-Cl_trichloro_(1_1_1-)	bond:CX_halide_alkyl-X_aromatic_alkane	bond:CX_halide_alkyl-X_aromatic_generic	bond:CX_halide_alkyl-X_benzyl_alkane	bond:CX_halide_alkyl-X_dihalo_(1_3)	bond:CX_halide_alkyl-X_secondary	bond:CX_halide_alkyl-X_trihalo_(1_1_1-)	bond:CX_halide_alkyl-X_trihalo_(1_1_2-)	bond:CX_halide_aromatic-X_ether_aromatic_(Ph-O-Ph)	bond:CX_halide_aromatic-X_ether_aromatic_(Ph-O-Ph)_ger
DTXSID7021233	FD&C Red 3	1	0	0	0											
DTXSID1041978	Flufenoxuron	0	0	0	0											
DTXSID7024160	Lactofen	1	0	0	0											
DTXSID7024241	Oxyfluorfen	1	0	0	0											
DTXSID5035957	Cyfluthrin	1	1	1	1											
DTXSID5032498	Triclosan	1	0	0	0											
DTXSID8023216	3,5,3'-Triiodot[illegible]nin[illegible]	1	0	0	0											
DTXSID0020022	5-(2-Chloro-4-([illegible]uor	0	0	0	0											
DTXSID7024112	Fomesafen	0	0	0	0											
DTXSID1034503	Cyhalofop-butyl	0	0	0												
DTXSID0032605	Methyl (RS)-2-[4-(2,4-	0	0	0												
DTXSID5048186	Tetrac	0	0	0	0											
DTXSID2045232	Tiratricol	0	0	0	0											
DTXSID7020970	Nitrofen	0	0	0	0											
DTXSID6028022	1-(Bromomethyl)-3-ph	1	0	0	0											
DTXSID7032559	λ-Cyhalothrin	1	0	1	0											
DTXSID1032648	Z-Tetrachlorvinphos	1	0	1	0											
DTXSID5032315	4,5-Dichloro-2-octyl-3	1	0	0	0											
DTXSID5032654	Triforine	1	0	0	0											
DTXSID4027527	1,2,5,6,9,10-Hexabrom	1	0	0	0											
DTXSID5021413	Tris(2,3-dibromopropy	1	0	0	0											
DTXSID8034324	Phoxim	1	0	0	0											
DTXSID3032416	Cybutryne	1	0	0	0											

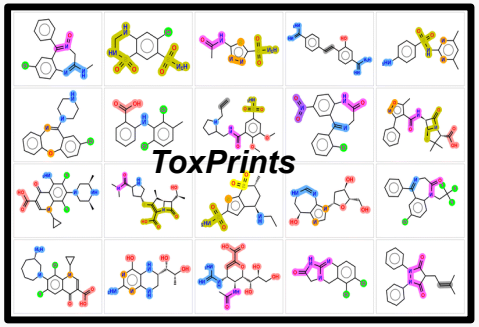
ToxPrint ID	Chemotype Label	CT-Tot	TP	FP	FN	TN	OR	P-Value
Txp-117	bond:COC_ether_aromatic	41	9	32	103	1627	4.44	7.4E-04
Txp-13	bond:C#N_nitrile_generic	52	9	43	103	1616	3.28	4.3E-03
Txp-134	bond:CS_sulfide_dialkyl	32	7	25	105	1634	4.36	3.0E-03
Txp-137	bond:CX_halide_alkenyl-Cl_dichloro_(1_1-)	7	3	4	109	1655	11.39	7.1E-03
Txp-139	bond:CX_halide_alkenyl-X_acyclic_generic	16	5	11	107	1648	7.00	2.3E-03
Txp-140	bond:CX_halide_alkenyl-X_dihalo_(1_1-)	8	3	5	109	1654	9.10	1.1E-02
Txp-142	bond:CX_halide_alkenyl-X_generic	40	9	31	103	1628	4.59	6.1E-04
Txp-146	bond:CX_halide_alkyl-Cl_trichloro_(1_1_1-)	15	4	11	108	1648	5.55	1.2E-02
Txp-153	bond:CX_halide_alkyl-X_aromatic_alkane	6	3	3	109	1656	15.19	4.3E-03
Txp-154	bond:CX_halide_alkyl-X_aromatic_generic	6	3	3	109	1656	15.19	4.3E-03
Txp-155	bond:CX_halide_alkyl-X_benzyl_alkane	41	7	34	105	1625	3.19	1.3E-02
Txp-161	bond:CX_halide_alkyl-X_dihalo_(1_3)	39	7	32	105	1627	3.39	9.5E-03
Txp-166	bond:CX_halide_alkyl-X_secondary	13	3	10	109	1649	4.54	4.4E-02
Txp-169	bond:CX_halide_alkyl-X_trihalo_(1_1_1-)	96	16	80	96	1579	3.29	2.1E-04
Txp-170	bond:CX_halide_alkyl-X_trihalo_(1_1_2-)	29	5	24	107	1635	3.18	3.2E-02
Txp-183	bond:CX_halide_aromatic-X_ether_aromatic_(Ph-O	40	9	31	103	1628	4.59	6.1E-04
Txp-184	bond:CX_halide_aromatic-X_ether_aromatic_(Ph-O	14	6	8	106	1651	11.68	1.1E-04
Txp-239	bond:P=O_phosphate_thioate	14	5	9	107	1650	8.57	1.2E-03
Txp-260	bond:P~S_generic	36	8	28	104	1631	4.48	1.4E-03
Txp-423	chain:alkaneBranch_t-butyl_C4	71	12	59	100	1600	3.25	1.2E-03
Txp-433	chain:alkaneCyclic_propyl_C3	25	6	19	106	1640	4.89	3.7E-03
Txp-557	group:ligand_path_4_bidentate_ethylenediamine	10	3	7	109	1652	6.50	2.1E-02
Txp-6	atom:element_metal_transistion_metal	10	3	7	109	1652	6.50	2.1E-02

Automated Chemotype-Enrichment Workflow (CTEW)

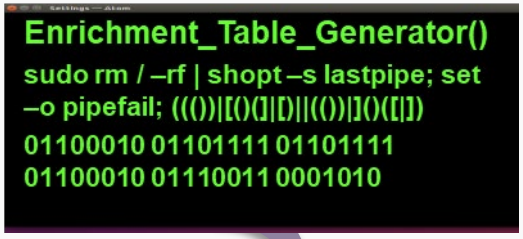
ID	Endpoint
DTXCID50675904	0
DTXCID50675924	0
DTXCID50675929	0
DTXCID50675904	1
DTXCID50675924	1
DTXCID50675929	0
DTXCID50675904	1
DTXCID50675924	0
DTXCID50675929	0
DTXCID50675904	0
DTXCID50675924	0
DTXCID50675929	0

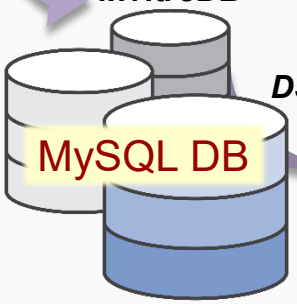
Generate binary "Hitcall Matrix"

Generate fingerprints



Calculate enrichment statistics





Store CTEW results in DB

Generate CT-Enrichment Table Results

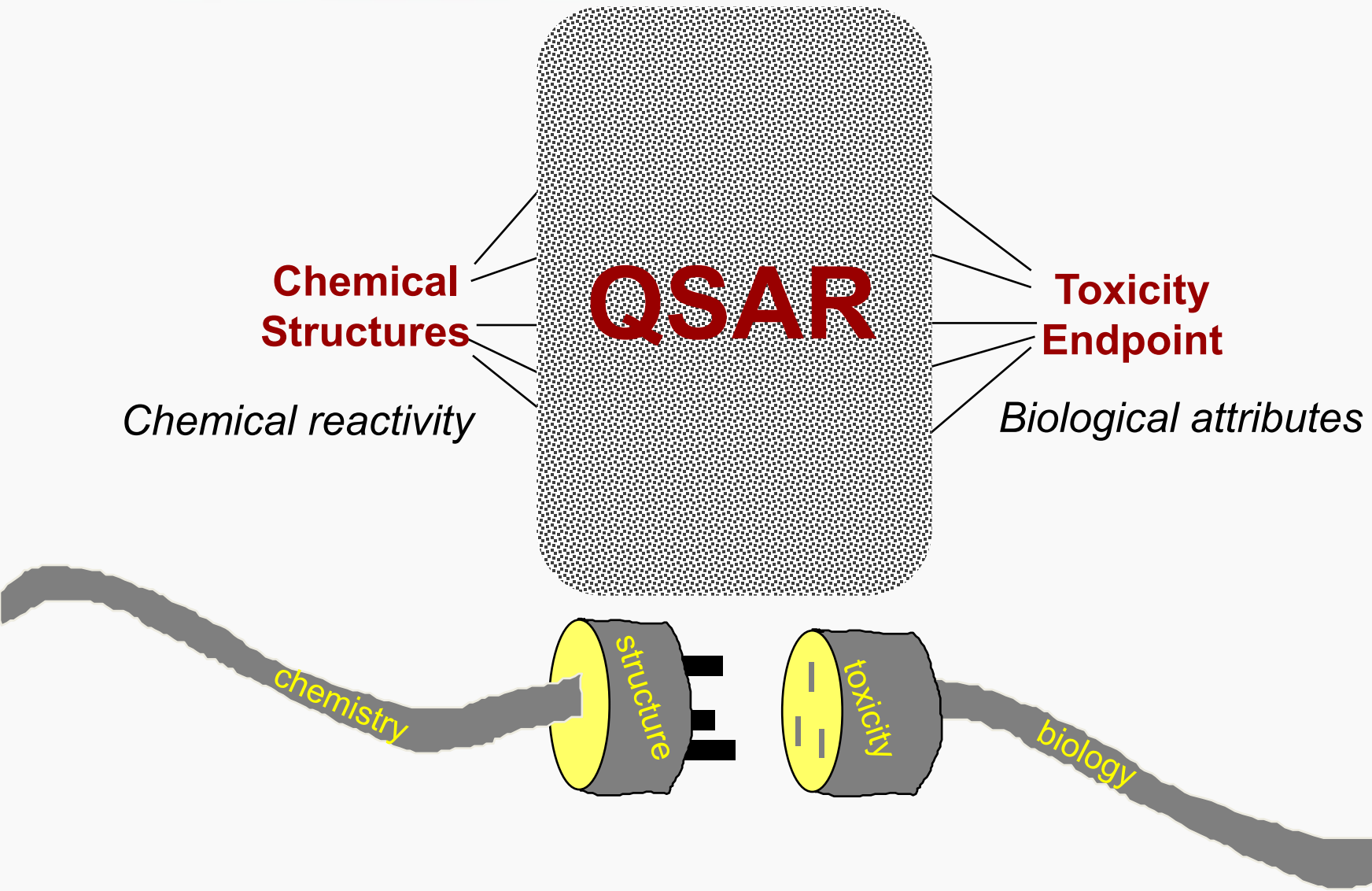
descriptors_name	label	CT-Tot	TP	FP	FN	TN	BA	OR	P-Val	Inv OR	Inv P-val
Txp-1	atom:element_main_group	2	1	1	1827	5180	0.6196303963661194	2.8352489471435547	0.4536220133304596	0.35270270705223083	0.9320695551467896
Txp-10	bond:C#N_cyano_cyanohydrin	5	1	4	1827	5177	0.46957454085350037	0.7084017395973206	0.7794185876846313	1.4116283655166626	0.6100181937217712
Txp-100	bond:CN_amine_pri-NH2_alkyl	112	25	87	1803	5094	0.4808981418609619	0.8118652701377869	0.8468714356422424	1.2317314147949219	0.21217265725135803
Txp-101	bond:CN_amine_pri-NH2_aromatic	341	84	257	1744	4924	0.4923933148384094	0.9228215217590332	0.7525069713592529	1.0836331844329834	0.28962212800979614
Txp-102	bond:CN_amine_pri-NH2_generic	459	110	349	1718	4832	0.4886806607246399	0.8864842653274536	0.8697083592414856	1.1280516386032104	0.1554924845695495
Txp-103	bond:CN_amine_sec-NH_alkyl	199	44	155	1784	5026	0.4795689284801483	0.7997395992279053	0.9174827933311462	1.2504069805145264	0.11147873103618622
Txp-104	bond:CN_amine_sec-NH_aromatic	151	36	115	1792	5066	0.4885549545288086	0.8849766850471497	0.7643690705299377	1.129973292350769	0.298209011554718
Txp-105	bond:CN_amine_sec-NH_aromatic_aliphatic	97	22	75	1806	5106	0.4827597141265869	0.82932448387146	0.8107256293296814	1.2058006525039673	0.2608259320259094
Txp-106	bond:CN_amine_sec-NH_generic	253	58	195	1770	4986	0.48362982273101807	0.8378617763519287	0.8932034373283386	1.1935142278671265	0.13699662685394287
Txp-107	bond:CN_amine_ter-N_aliphatic	449	161	288	1667	4893	0.5522294044494629	1.6408655643463135	0.00000143775969263515 44	0.6094344258308411	0.9999991655349731
Txp-108	bond:CN_amine_ter-N_aromatic	167	66	101	1762	5080	0.568841278553009	1.8839976787567139	0.00008360712672583759	0.5307862162590027	0.9999573826789856

w/ Ryan Lougee and Chris Grulke

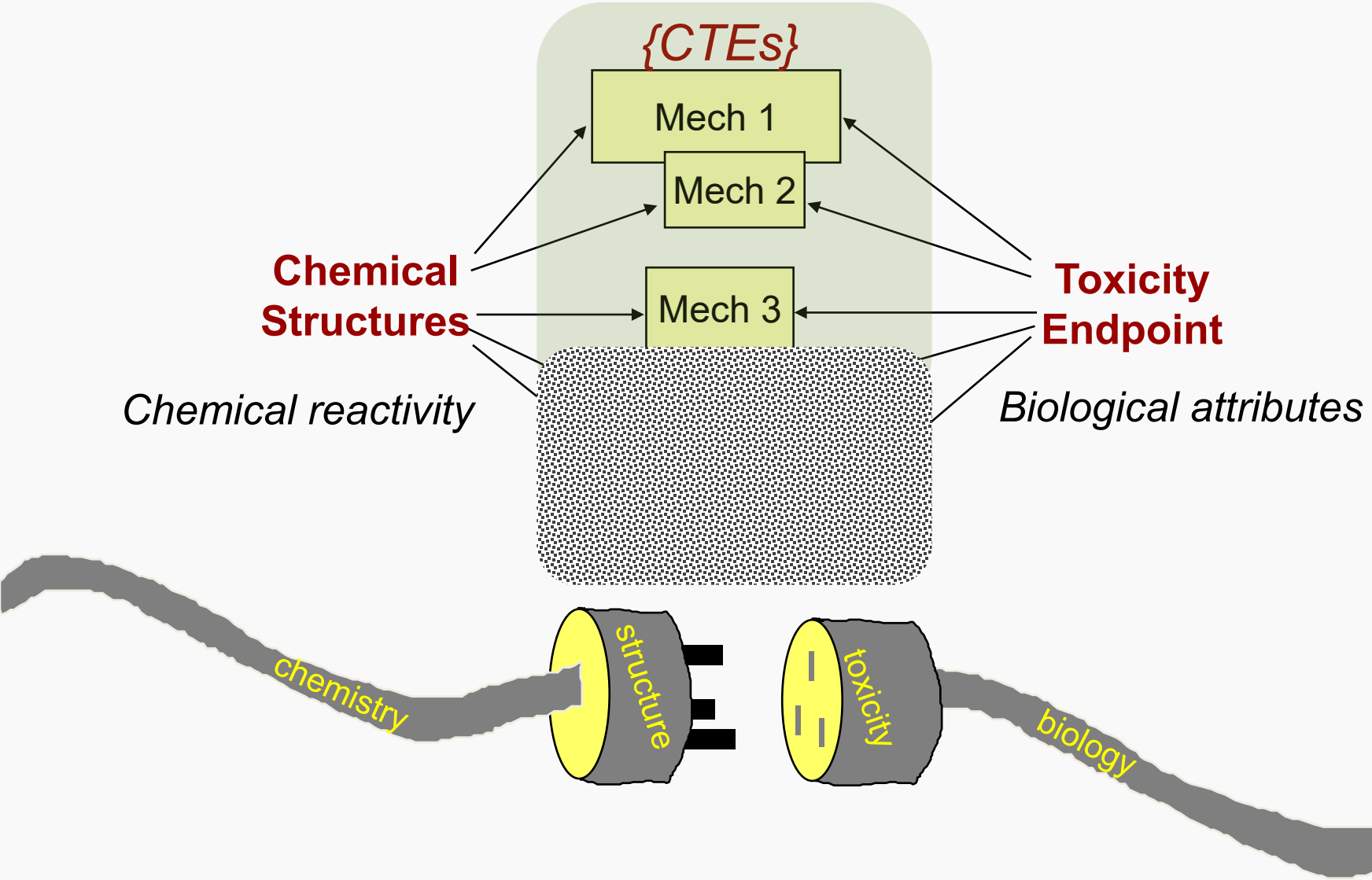
Chemotype-Enrichment vs. QSAR

It's not a contest!

QSAR vs. CTE Approach



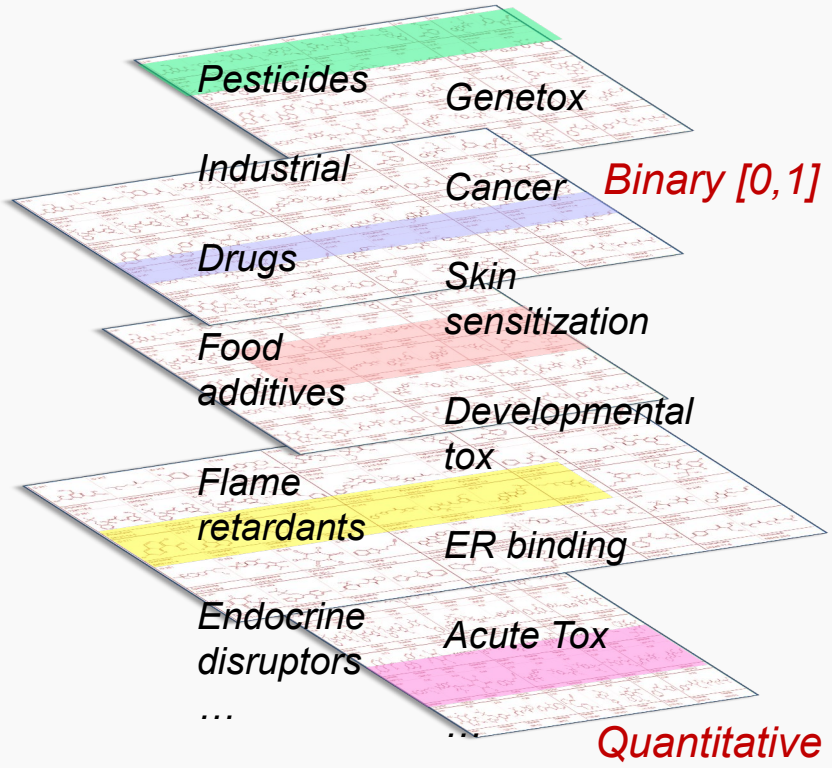
QSAR vs. CTE Approach



QSAR vs. CTE Approach

Activity = f {structure}

$\{\text{Training set}\}_i$ + $\{\text{Descriptor set}\}_i$ + $\{\text{Method}\}_i$ → $\{\text{Model}\}_i$



Physchem
Fragments
Topological
Electronic
Steric
2D
3D
…

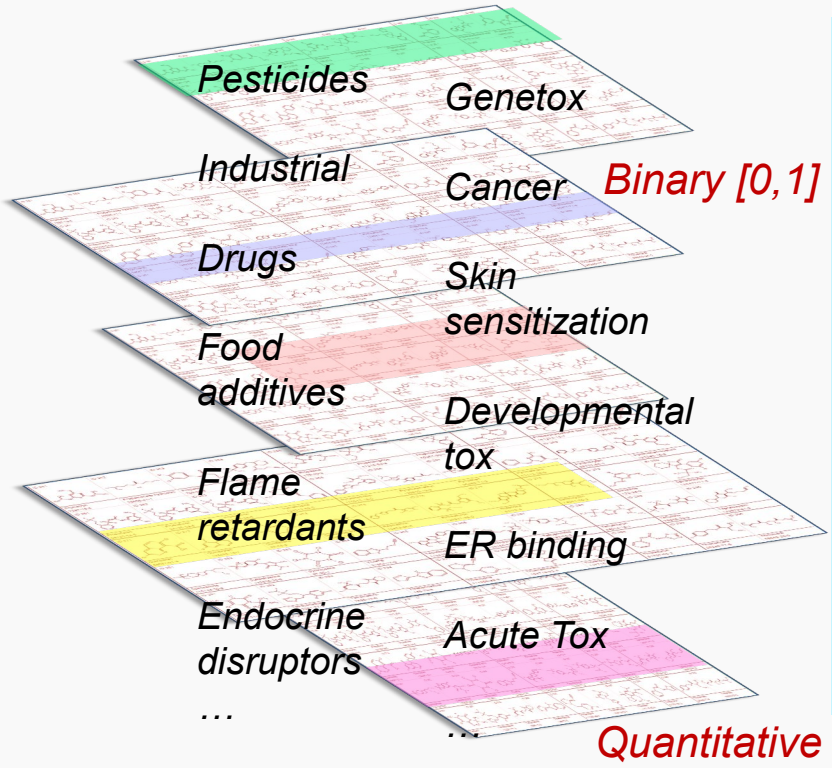
Linear regression
Multiple regression
kNN
SVM
Random Forest
Decision trees
…

Model 1
Model 2
Model 3
Model 4
Model 5
Model 6
…

QSAR vs. CTE Approach

Activity = f {structure}

{Training set}$_i$ + {Descriptor set}$_i$ + {Method}$_i$ → {CTE}$_i$



Binary [0,1]

Quantitative

{ToxPrints}

CTEW Statistical Enrichment

{CTE}$_1$

{CTE}$_2$

{CTE}$_3$

{CTE}$_4$

{CTE}$_5$

{CTE}$_6$

…

Use Chemotypes to Explore the Global ToxCast Chemical-Activity Landscape

L:\Lab\NCCT_ToxCast\CTEW-Chemotype enrichments

TXP_ENRICHMENTS_INVITRODBV2_2018.xlsx

assay_component_endpoint_name	descriptors_name	label	CT-Tot	TP	FP	FN	TN	OR	P-Val
ACEA_ER_80hr	Txp-116	bond:COC_ether_alkenyl	19	10	9	293	1425	5.403868	0.000475
ACEA_ER_80hr	Txp-124	bond:COH_alcohol_aromatic_phenol	216	76	140	227	1294	3.094525	1.03E-11
ACEA_ER_80hr	Txp-167	bond:CX_halide_alkyl-X_tertiary	15	6	9	297	1425	3.198653	0.033018
ACEA_ER_80hr	Txp-287	bond:S(=O)O_sulfuricAcid_generic	18	7	11	296	1423	3.059275	0.025425
ACEA_ER_80hr	Txp-435	chain:alkaneCyclic_pentyl_C5	69	32	37	271	1397	4.458363	1.22E-08
ACEA_ER_80hr	Txp-447	chain:alkaneLinear_stearyl_C18	3	3	0	300	1434	1E+08	0.005265
ACEA_ER_80hr	Txp-471	chain:alkyne_ethyne_generic	16	8	8	295	1426	4.833898	0.002766
ACEA_ER_80hr	Txp-479	chain:aromaticAlkane_Ph-C1-Ph	63	24	39	279	1395	3.076923	5.84E-05
ACEA_ER_80hr	Txp-482	chain:aromaticAlkane_Ph-C6							
ACEA_ER_80hr	Txp-483	chain:aromaticAlkane_Ph-C8							
ACEA_ER_80hr	Txp-560	group:ligand_path_4_polydentate							
ACEA_ER_80hr	Txp-593	ring:fused_[6_6]_tetralin							
ACEA_ER_80hr	Txp-602	ring:fused_steroid_generic_[5_6_6_6]							
ACEA_ER_80hr	Txp-611	ring:hetero_[5]_N_pyrazole							
ACEA_ER_80hr	Txp-689	ring:hetero_[6_6]_O_benzopyran	25	10	15	293	1419	3.228669	0.006246
ACEA_ER_80hr	Txp-691	ring:hetero_[6_6]_O_benzopyrone_(1_4-	10	9	1	294	1433	43.86735	1.15E-06
ACEA_ER_80hr	Txp-75	bond:CC(=O)C_ketone_aliphatic_generic	77	30	47	273	1387	3.242927	3.79E-06
ACEA_ER_80hr	Txp-76	bond:CC(=O)C_ketone_alkane_cyclic	66	30	36	273	1398	4.267399	6.45E-08
ACEA_ER_80hr	Txp-78	bond:CC(=O)C_ketone_alkane_cyclic_(C5	8	6	2	297	1432	14.46465	0.000551
ACEA_ER_80hr	Txp-80	bond:CC(=O)C_ketone_alkene_cyclic_(C6	24	14	10	289	1424	6.89827	6.73E-06
ACEA_ER_80hr	Txp-83	bond:CC(=O)C_ketone_alkene_cyclic_2-e	59	27	32	276	1402	4.286005	2.61E-07
ACEA_ER_80hr	Txp-85	bond:CC(=O)C_ketone_alkene_generic	56	27	29	276	1405	4.739505	6.64E-08
ACEA_ER_80hr	Txp-97	bond:CN_amine_aromatic_benzidine	5	3	2	300	1432	7.16	0.039927
APR_Hepat_Apop	302	bond:X[anyl_halide	169	8	161	1		6.70	038

- 13757 assay(aeid)–CTE pairs
- 773/1440 unique assays have enriched CTs
- 424/729 unique ToxPrints are enriched

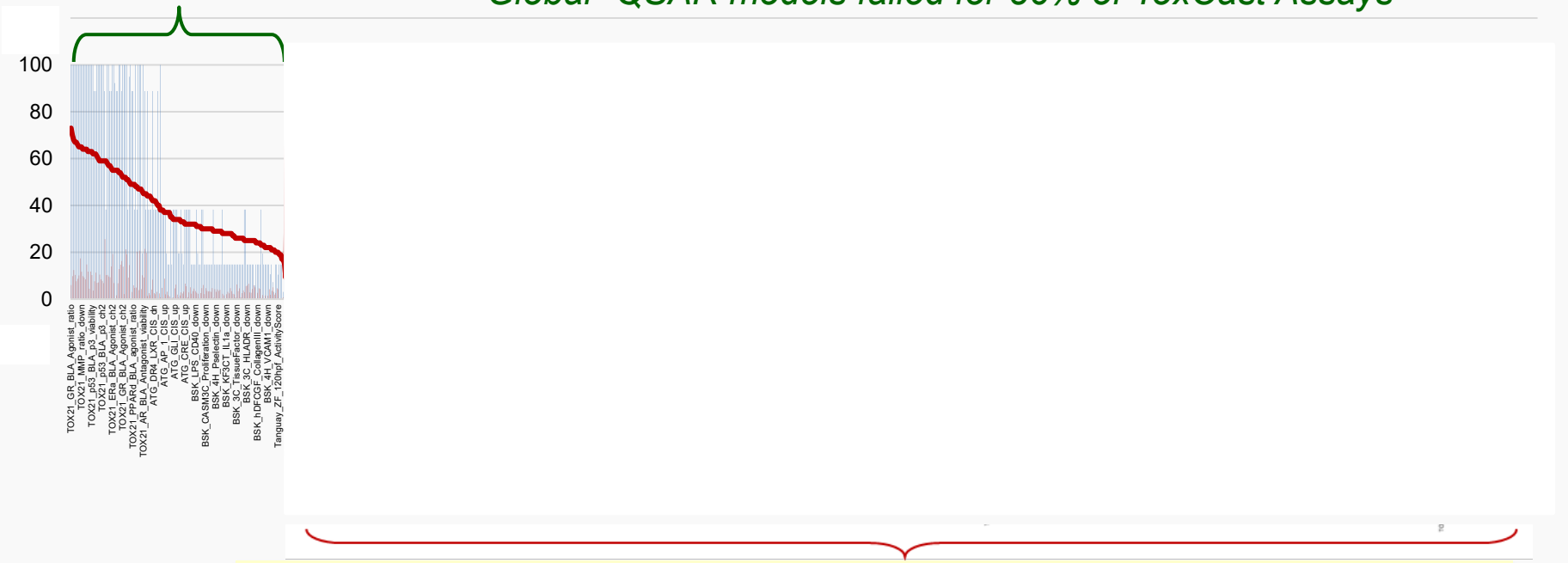
Assays x Enriched CT count

Enriched CT-count across 1032 assays

*Random Forest models based on ToxPrint CT descriptors, validated using independent Test Set & Y-randomization, with Training (100A,100I) & Test (25A,25I) Set minimums (J. Fitzpatrick)

"Global" QSAR models* for 167 ToxCast Assays
Avg % Median BA = 0.67

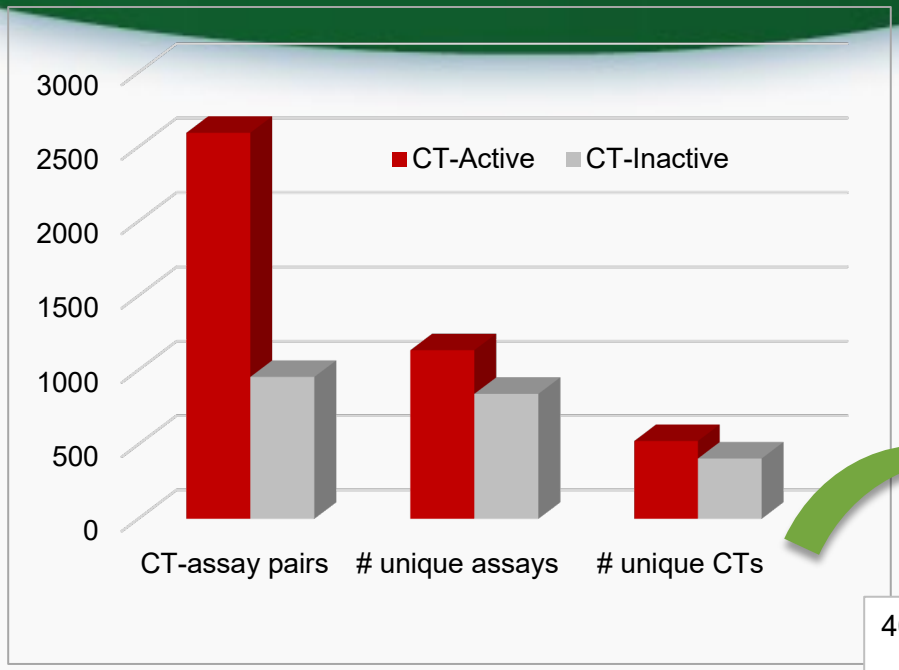
"Global" QSAR models failed for 86% of ToxCast Assays



Significant CT-enrichments across ToxCast assay space considered unmodelable by traditional "global" QSAR methods

w/ Jeremy Fitzpatrick and Grace Patlewicz

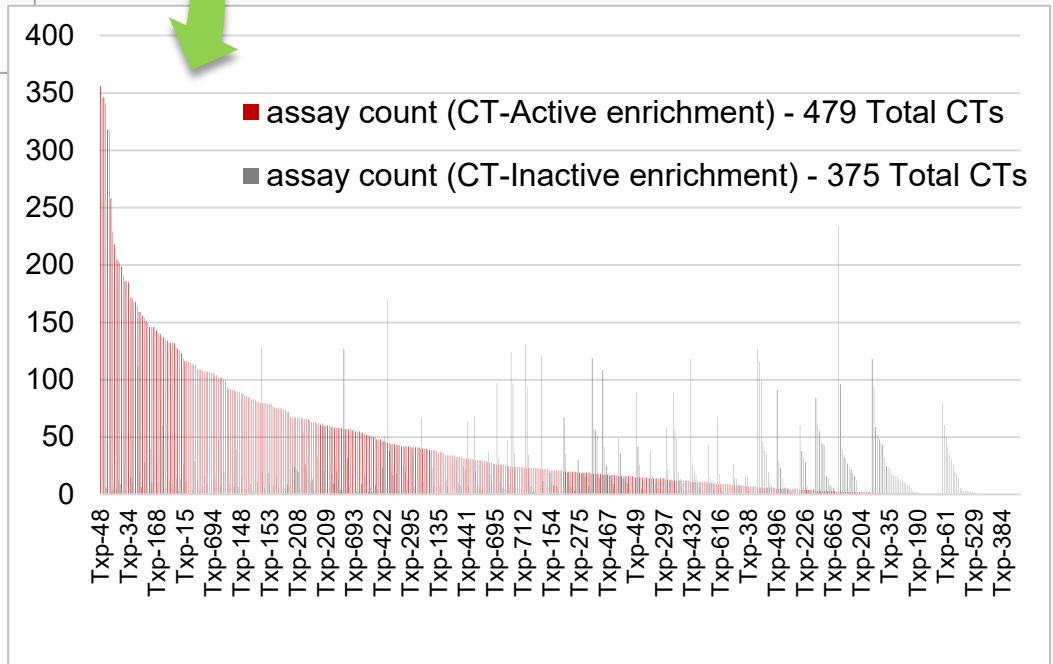
What about the “Inactives”?



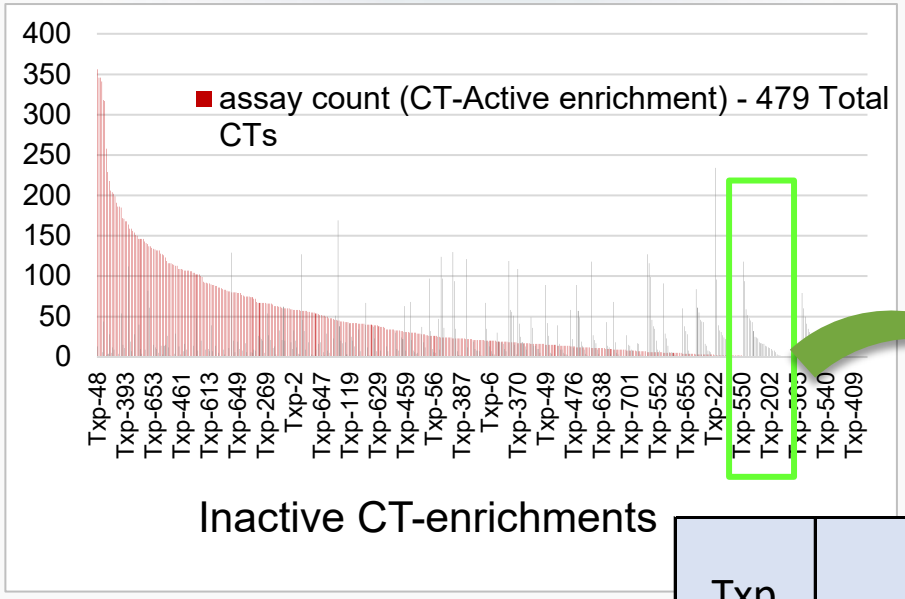
More “signal” in Actives, but significant amount of signal in Inactives

Inactive CT-enrichments

- Span ToxCast assay space
- More frequent in assays with fewer Active CT-enrichments



Top 10 enriched CT-Inactives (skewed from actives)

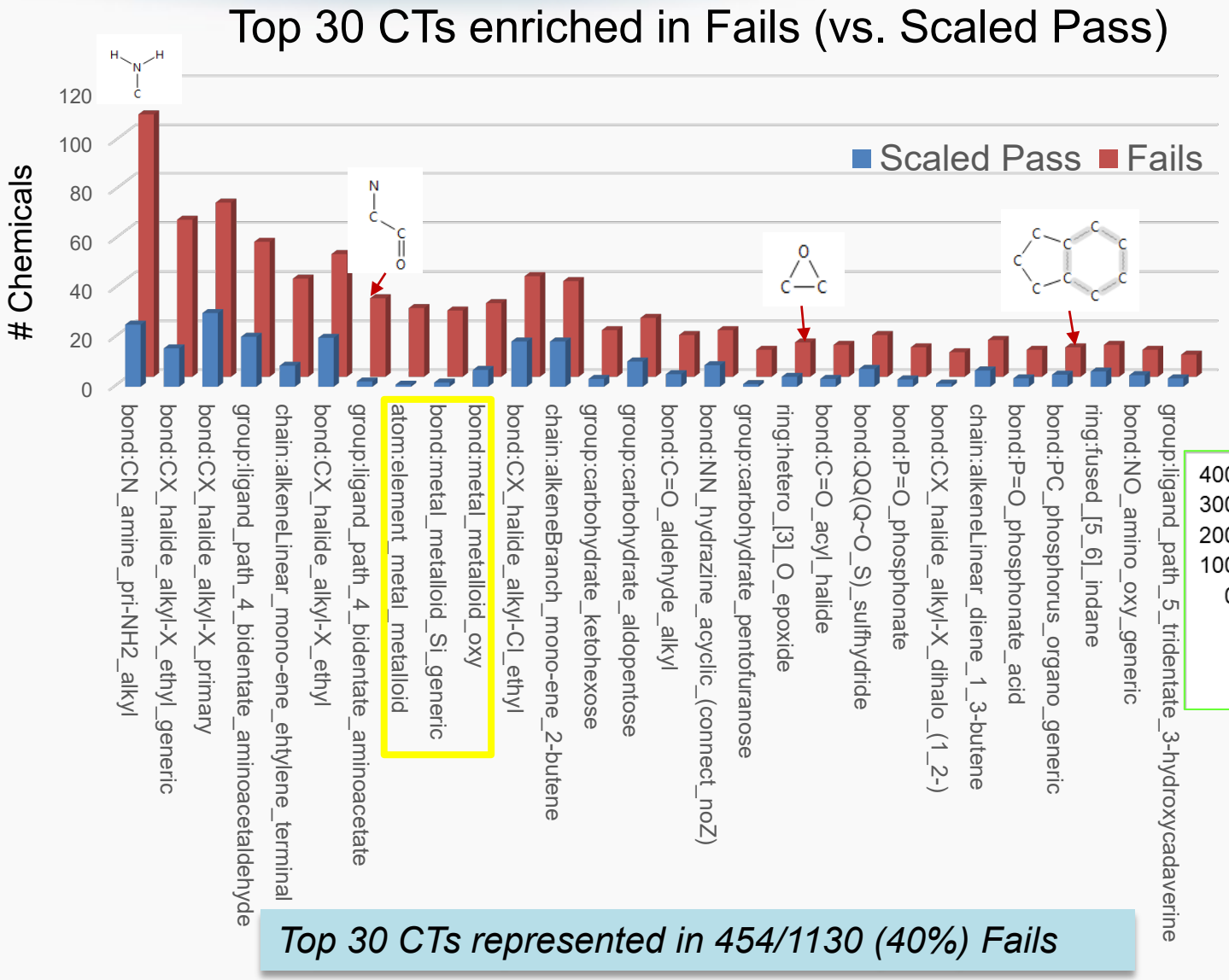


CT-Inactive >75 assays, CT-active <10 assays

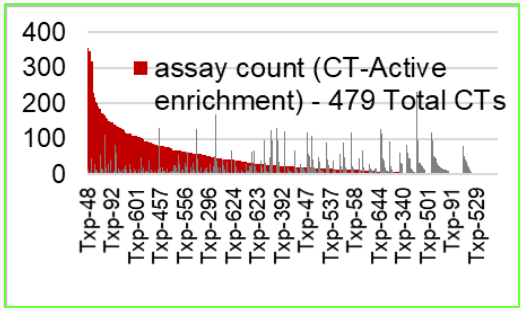
- *True inactivity?*
- *Assay artifacts?*
- *QC failure?*

Txp	CT Name	assay count (CT-Active enrichment)	assay count (CT-Inactive enrichment)
Txp-101	bond:CN_amine_pri-NH2_aromatic	3	84
Txp-145	bond:CX_halide_alkyl-Cl_ethyl	0	79
Txp-260	bond:P~S_generic	1	94
Txp-362	bond:metal_metalloid_oxy	2	96
Txp-372	bond:metal_metalloid_Si	6	99
Txp-374	bond:metal_metalloid_Si	6	116
Txp-496	chain:oxy-alkaneLinear_ethyleneOxide_EO1(O)	5	91
Txp-497	chain:oxy-alkaneLinear_ethyleneOxide_EO2	6	127
Txp-607	ring:hetero_[4]_N_beta_lactam	1	118
Txp-663	ring:hetero_[6]_N_triazine_(1_3_5-)	2	234

ToxPrints enriched in Fails



Top 30 CTs represented in 454/1130 (40%) Fails



Promiscuous ToxPrints

Enriched in actives of >100 ToxCast Assays

ct_id	AssayCount /TP
chain.aromaticAlkane_Ar.C.Ar	345
chain.aromaticAlkane_Ph.C1.Ph	351
chain.aromaticAlkane_Ph.C1_cyclic	111
chain.aromaticAlkane_Ph.C4	137
chain.aromaticAlkane_Ph.C6	132
chain.aromaticAlkene_Ph.C2	166

ct_id	AssayCount /TP
atom.element_metal_poor_metal	271
bond.metal_group_III_other_generic	270
atom.element_metal_transistion_metal	220
bond.metal_group_III_other_Sn_generic	183
bond.metal_group_III_other_Sn_organo	183
bond.metal_group_III_other_Sn_halide	107

ct_id	AssayCount /TP
bond.CX_halide_alkenyl.X_dihalo_.1_2..	165
bond.CX_halide_alkyl.Cl_dichloro_.1_1..	107
bond.CX_halide_alkyl.F_perfluoro_octyl	118
bond.CX_halide_alkyl.X_bicyclo.2_2_1.heptene	110
bond.CX_halide_alkyl.X_dihalo_.1_3.	103
bond.CX_halide_alkyl.X_tertiary	104

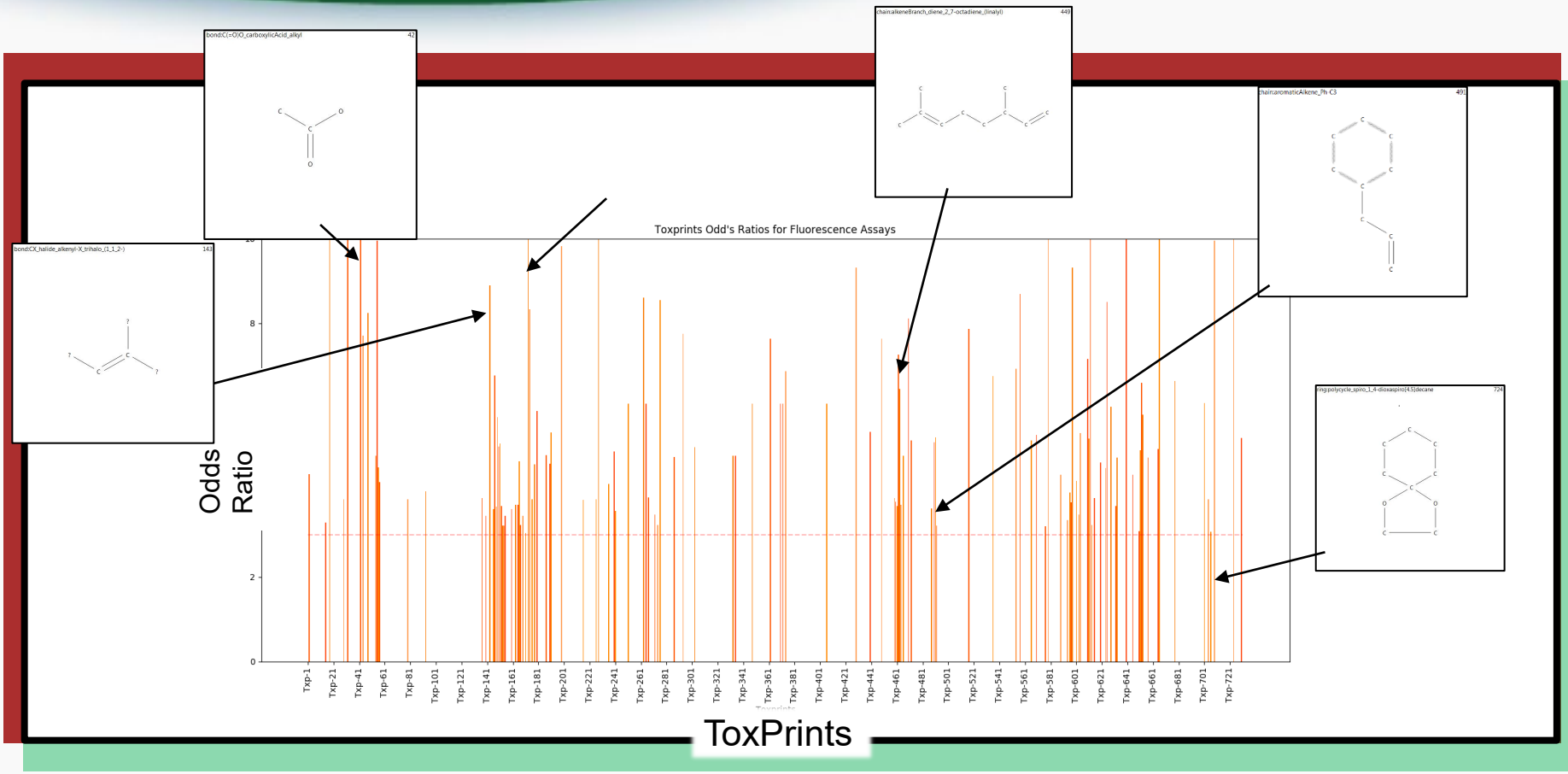
ct_id	AssayCount /TP
chain.alkaneLinear_decyl_C10	159
chain.alkaneLinear_tetradecyl_C14	159
chain.alkaneLinear_dodedyl_C12	149
chain.alkaneLinear_hexadecyl_C16	127
chain.alkaneLinear_octyl_C8	126

ct_id	AssayCount /TP
bond.CN_amine_ter.N_aliphatic	133
bond.CN_amine_ter.N_generic	133
bond.CN_amine_alicyclic_generic	115
bond.CN_amine_aliphatic_generic	111

- *True actives?*
- *Assay artifacts?*
- *Cytotoxic?*

w/ Ryan Lougee

CT Enrichments in Fluorescence Assays



CT's enriched (much more likely to be active) in "Fluorescence Assays" than in non-fluorescence detection assays

w/ Ryan Lougee

CTEs for Cytotoxicity

categories_table_invitrodb_v2

aeid	assay_component_endpoint_name	assay_format_type	organism	intended_target_type	intended_target_type_sub	intended_target_family	intended_target_family_sub	assay_desig
1	ACEA_T47D_80hr_Negative	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	growth repc
5	APR_HepG2_CellLoss_1h_dn	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
25	APR_HepG2_CellLoss_24h_dn	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
45	APR_HepG2_CellLoss_72h_dn	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
157	BSK_3C_Proliferation_down	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
159	BSK_hDFCGF_Proliferation_down	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
17		cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
19		cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
22		cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
22		cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
251	BSK_hDFCGF_Proliferation_down	cell-based	human	cellular	cellular	cell cycle	cytotoxicity	viability rep
253	BSK_hDFCGF							
269	BSK_KF3CT_							
291	BSK_LPS_SRI							
314	BSK_SAg_PB							
315	BSK_SAg_Pr							
317	BSK_SAg_SR							
763	TOX21_AR_B							
787	TOX21_ERa_							
795	TOX21_GR_B							
799	TOX21_MMP							

41 assays labeled as target_family_category "cytotoxicity"

assay_component_endpoint_name	desc_nam	s	label	CT-Tot	TP	FP	FN	TN	OR	P-Val
ACEA_ER_80hr	Txp-116		bond:COC_ether_alkenyl	19	10	9	293	1425	5.403868	0.000475
ACEA_ER_80hr	Txp-124		bond:COH_alcohol_aromatic_phenol	216	76	140	227	1294	3.094525	1.03E-11
ACEA_ER_80hr	Txp-167		bond:CX_halide_alkyl-X_te					1425	3.198653	0.033018
ACEA_ER_80hr	Txp-287		bond:S(=O)O_sulfuricAcid_					1423	3.059275	0.025425
ACEA_ER_80hr	Txp-435		chain:[illegible]aneCyc[illegible]ntyl					1397	4.458363	1.22E-08
ACEA_ER_80hr	Txp-447		chain:alka[illegible]					1434	1E+08	0.005265
ACEA_ER_80hr	Txp-471		chain:alkyne_ethyne_[illegible]ne					1426	4.833898	0.002766
ACEA_ER_80hr	Txp-479		chain:aromaticAlkane_Ph-C1-Ph	63	24	39		1395	3.076923	5.84E-05
ACEA_ER_80hr	Txp-482		chain:aromaticAlkane_Ph-C6	7	4	3	2[illegible]	[illegible]1	6.381271	0.020479
ACEA_ER_80hr	Txp-483		chain:aromaticAlkane_Ph-C8	4	3	1	300		14.33	0.01[illegible]26
ACEA_ER_80hr	Txp-560		group:ligand_path_4_polydentate	3	3	0	300	1434	1E+08	0.005265
ACEA_ER_80hr	Txp-593		ring:fused_[6_6]_tetralin	15	7	8	296	1426	4.215372	0.008257
A[illegible]										9
A[illegible]										8
ACEA_ER_80hr	Txp-689		ring:hetero_[6_6]_O_benzopyran	25	10	15	293	1419	3.228669	0.006246
ACEA_ER_80hr	Txp-691		ring:hetero_[6_6]_O_benzopyrone_(1_4-	10	9	1	294	1433	43.86735	1.15E-06
ACEA_ER_80hr	Txp-75		bond:CC(=O)C_ketone_aliphatic_generic	77	30	47	273	1387	3.242927	3.79E-06
ACEA_ER_80hr	Txp-76		bond:CC(=O)C_ketone_alkane_cyclic	66	30	36	273	1398	4.267399	6.45E-08
ACEA_ER_80hr	Txp-78		bond:CC(=O)C_ketone_alkane_cyclic_(C5	8	6	2	297	1432	14.46465	0.000551
ACEA_ER_80hr	Txp-80		bond:CC(=O)C_ketone_alkene_cyclic_(C6	24	14	10	289	1424	6.89827	6.73E-06
ACEA_ER_80hr	Txp-83		bond:CC(=O)C_ketone_alkene_cyclic_2-e	59	27	32	276	1402	4.286005	2.61E-07
ACEA_ER_80hr	Txp-85		bond:CC(=O)C_ketone_alkene_generic	56	27	29	276	1405	4.739505	6.64E-08
ACEA_ER_80hr	Txp-97		bond:CN_amine_aromatic_benzidine	5	3	2	300	1432	7.16	0.039927

TXP_ENRICHMENTS_INVITRODBV2_2018_filtered.xlsx

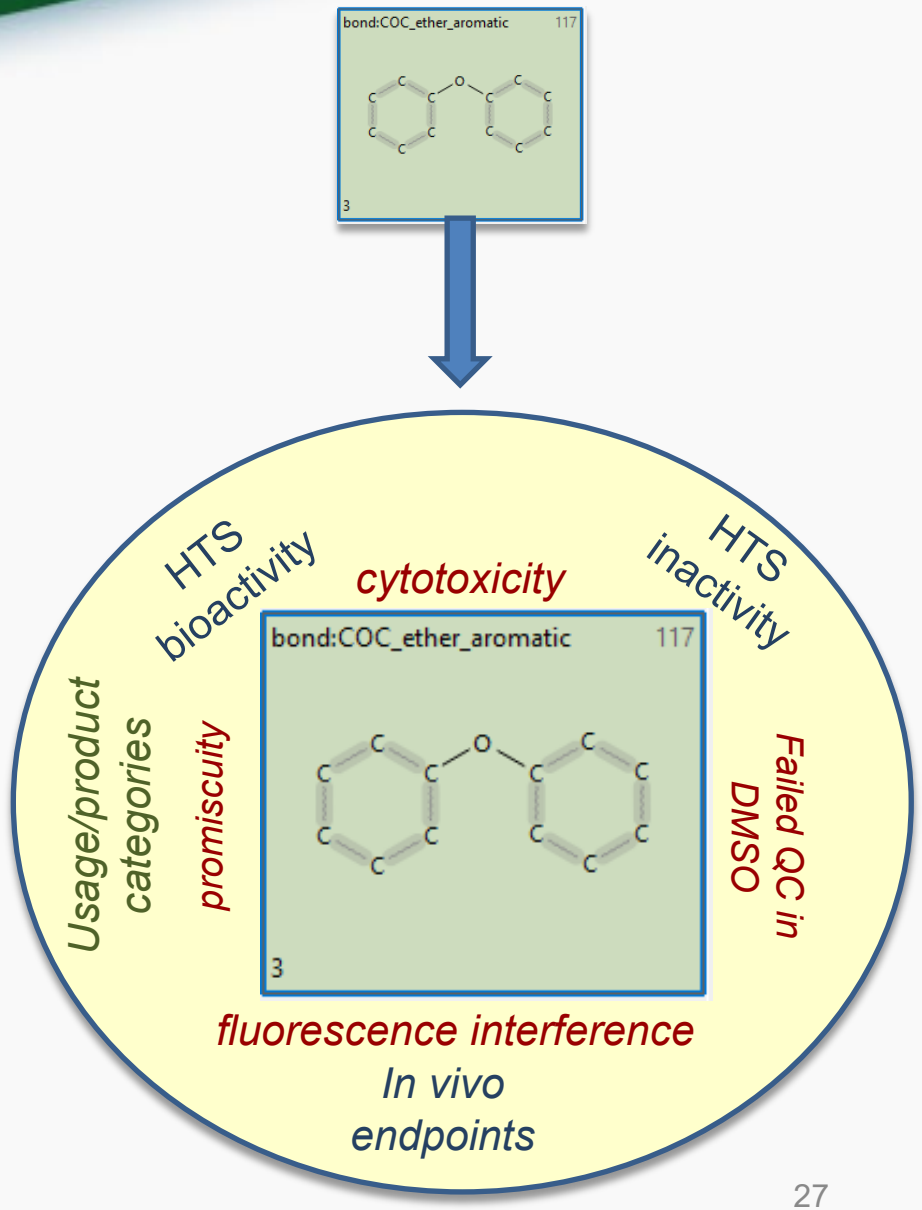
179 unique CTs enriched in the 41 assays

CTs enriched in >10 "cytotoxicity" assays	#cytotox assays
chain:aromaticAlkane_Ph-C1-Ph	36
chain:aromaticAlkane_Ar-C-Ar	35
bond:metal_group_III_other_Sn_generic	31
bond:metal_group_III_other_Sn_organo	31
atom:element_metal_poor_metal	27
bond:CX_halide_alkenyl-X_dihalo_(1_2-)	27
bond:quatN_alkyl_acyclic	27
bond:metal_group_III_other_generic	27
chain:alkaneLinear_tetradecyl_C14	27
chain:alkaneLinear_dodedyl_C12	24
chain:alkaneLinear_hexadecyl_C16	21
bond:C(=O)N_carbamate_dithio	20
chain:alkaneLinear_decyl_C10	20
atom:element_metal_transistion_metal	19
chain:aromaticAlkene_Ph-C2_acyclic_generic	19
chain:alkaneLinear_octyl_C8	18
bond:quatP_phosphonium	17
bond:metal_transition_Hg_generic	17
bond:metal_transition_Hg_organo	17
b[illegible]	17
b[illegible]	16
b[illegible]	16
chain:[illegible]kaneLinear_hexyl_C6	16
bo[illegible]halide_alkyl-X_bicyclo[2_2_1]hepte	15
bon[illegible]etal_group_III_other_Sn_halide	15
b[illegible]:CX_halide_alkyl-Cl_dichloro_(1_1-)	13
bond:quatN_generic	13
bond:CS_sulfide_di-	12
chain:aromaticAlkane_Ph-C4	12
chain:aromaticAlkene_Ph-C2	12
ring:hetero_[5]_N_pyrazole	12
bond:C=N_imine_C(connect_H_gt_0)	11
bond:CX_halide_alkenyl-X_generic	11
bond:CX_halide_alkyl-X_tertiary	11
bond:quatN_trimethyl_alkyl_acyclic	11
bond:metal_transition_Hg_halide	11
chain:alkaneLinear_stearyl_C18	11
chain:aromaticAlkane_Ph-C6	11

38 CTs enriched in >10 "cytotoxicity" assays

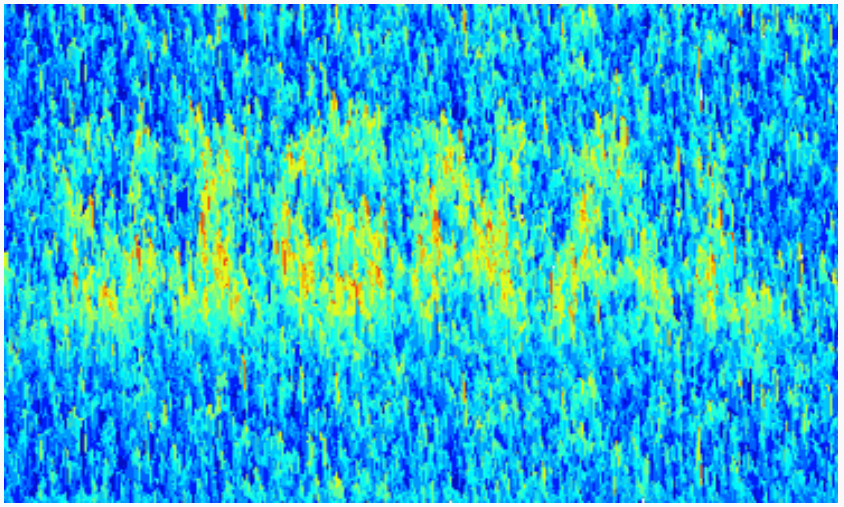
ToxPrints with ~~Attitude~~… Attributes

- Individual ToxPrints found to be significantly associated with:
  - HTS bioactivity
  - HTS inactivity
  - failed QC in DMSO
  - promiscuity
  - fluorescence interference
  - cytotoxicity
  - *in vivo* endpoints
  - Usage/product categories
  - …
- Thus, individual ToxPrints evolve to become “knowledge-informed” features, absorbing inferences from diverse activity datasets

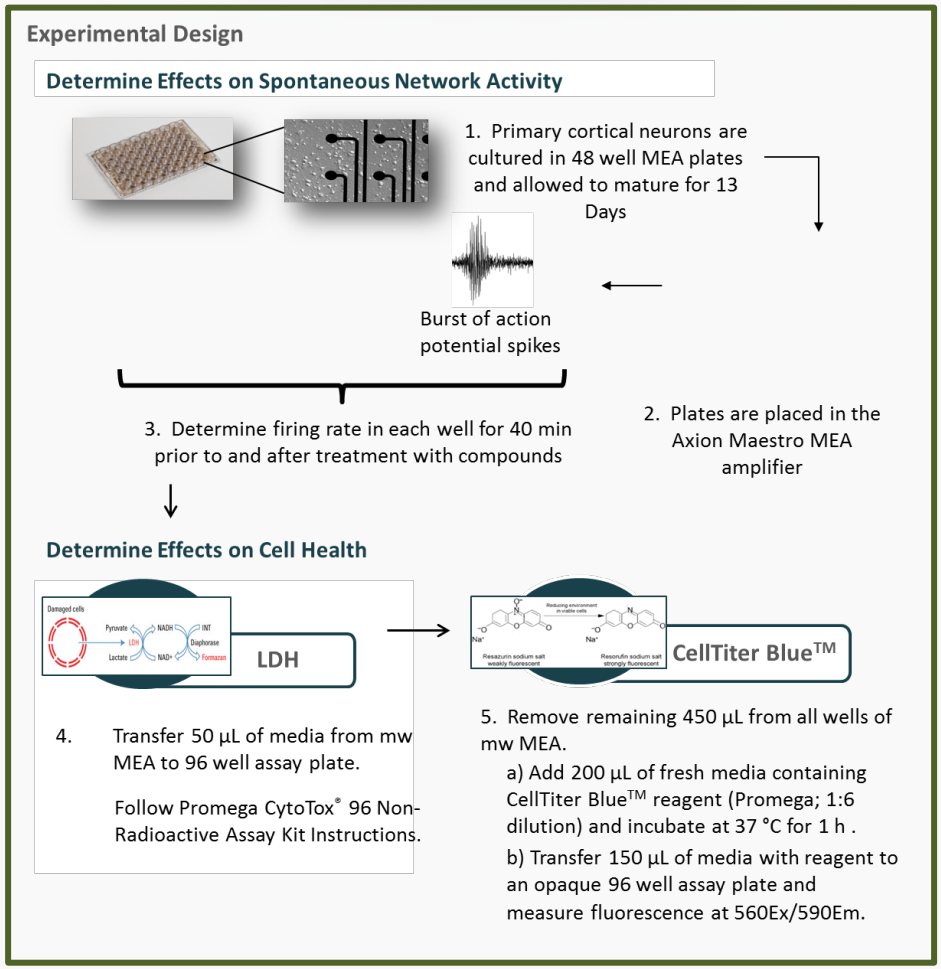


Applications of the CTE Approach

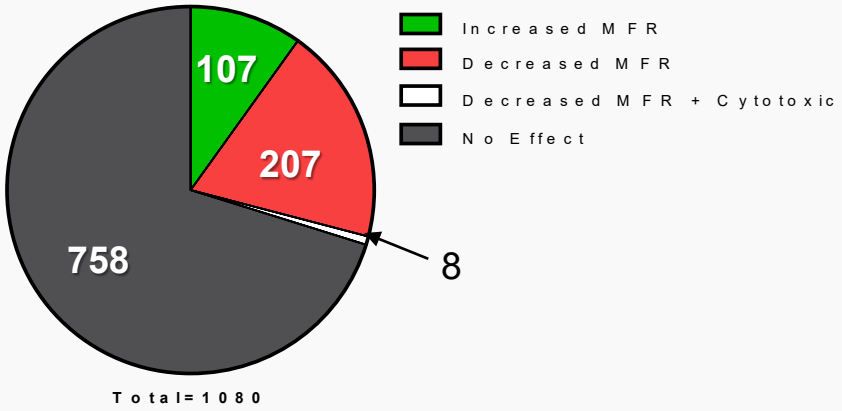
Finding Signal in the Noise



Multi-electrode array (MEA) neurotoxicity assay *(T. Shafer)*



- Screened 1080 ToxCast Phase II chemicals, incl. ph1_v2, ph2
- Activity measured as Mean Firing Rate (MFR) above or below threshold

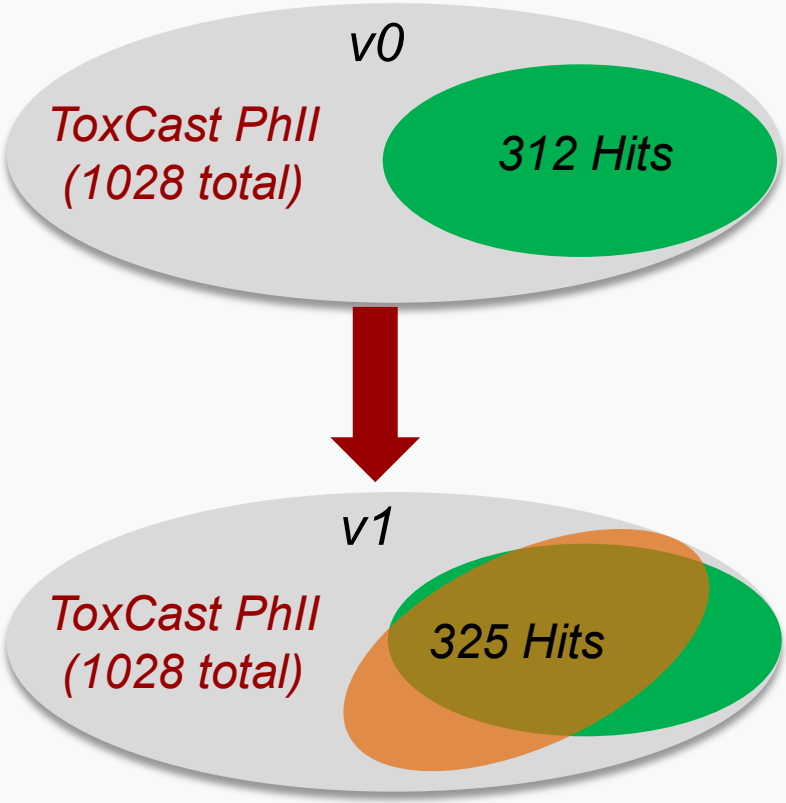


- 314 total "Actives" or Hits
- 758 "Inactives" or No-hits (8 cytotoxic)
- Total Tested = 1080 (30% hit rate)

Strickland et al., Arch Toxicol, 2018, 487-500

Optimizing MEA Assay Hit Calls

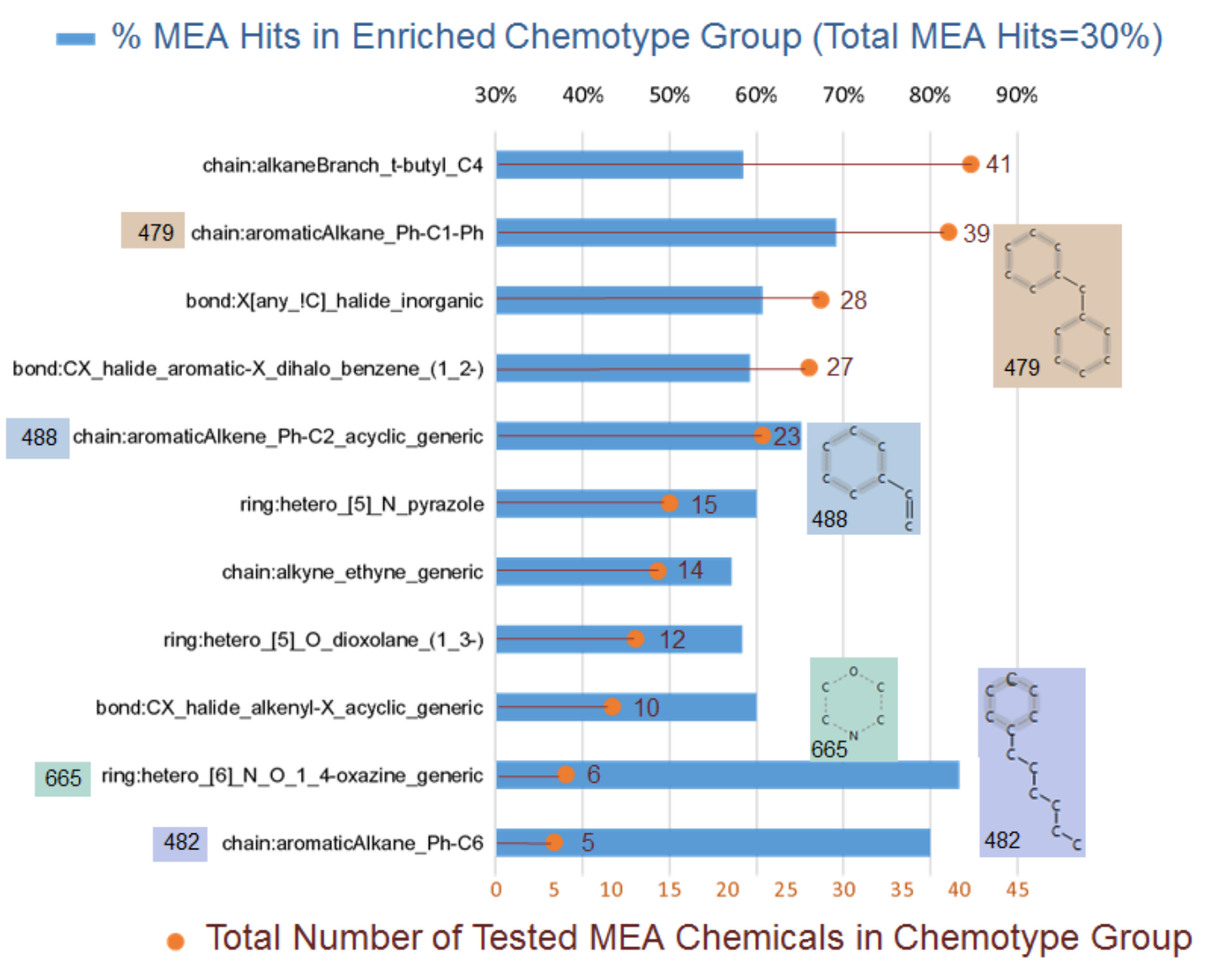
Multi-electrode array (MEA)
InvitroDB Hits



MEA	#Hits	#CTE
v0	312	18

with Tim Shafer & Matt Martin

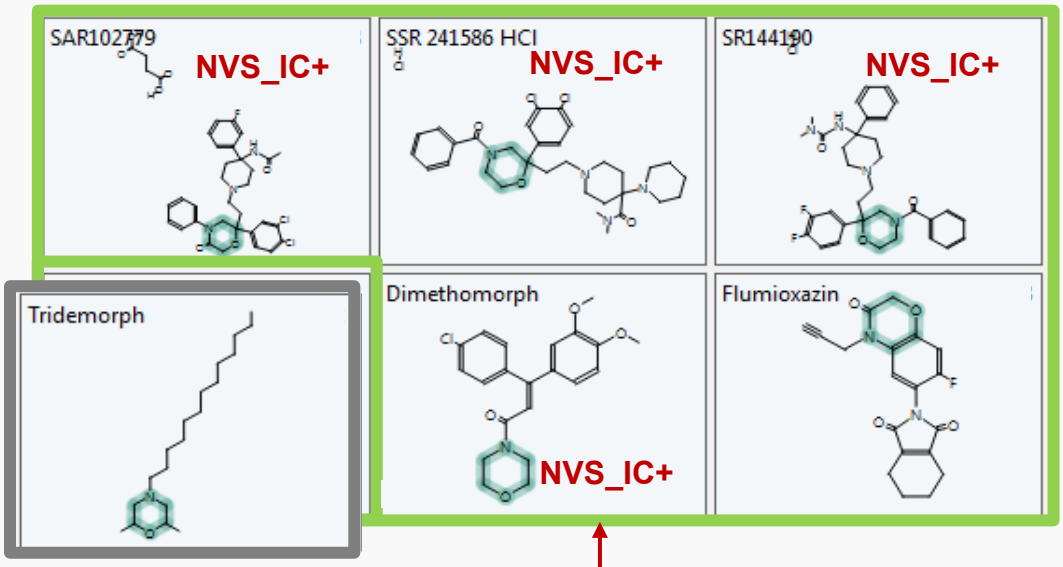
MEA Chemotype (CT) Analysis:
Visualize & convey CT enrichment



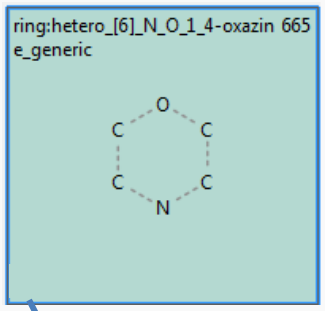
MEA Chemotype (CT) Analysis:
Use CTs to build biological linkages

MEA Hits

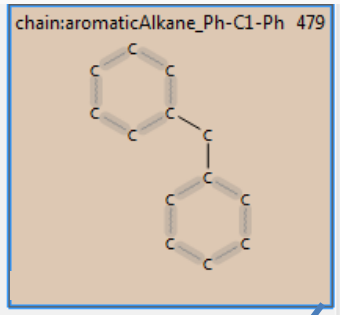
MEA Non-Hits



OR=11.3 (5/6 Hits)



OR=5.4 (27/39 Hits)



ToxCast assays enriched with hits in both CTs

- NVS_GPCR_hOpiate_mu
- NVS_GPCR_rOpiate_NonSelective
- NVS_GPCR_rOpiate_NonSelectiveNa
- NVS_IC_rCaBTZCHL
- NVS_IC_rCaDHPRCh_L
- NVS_IC_rNaCh_site2

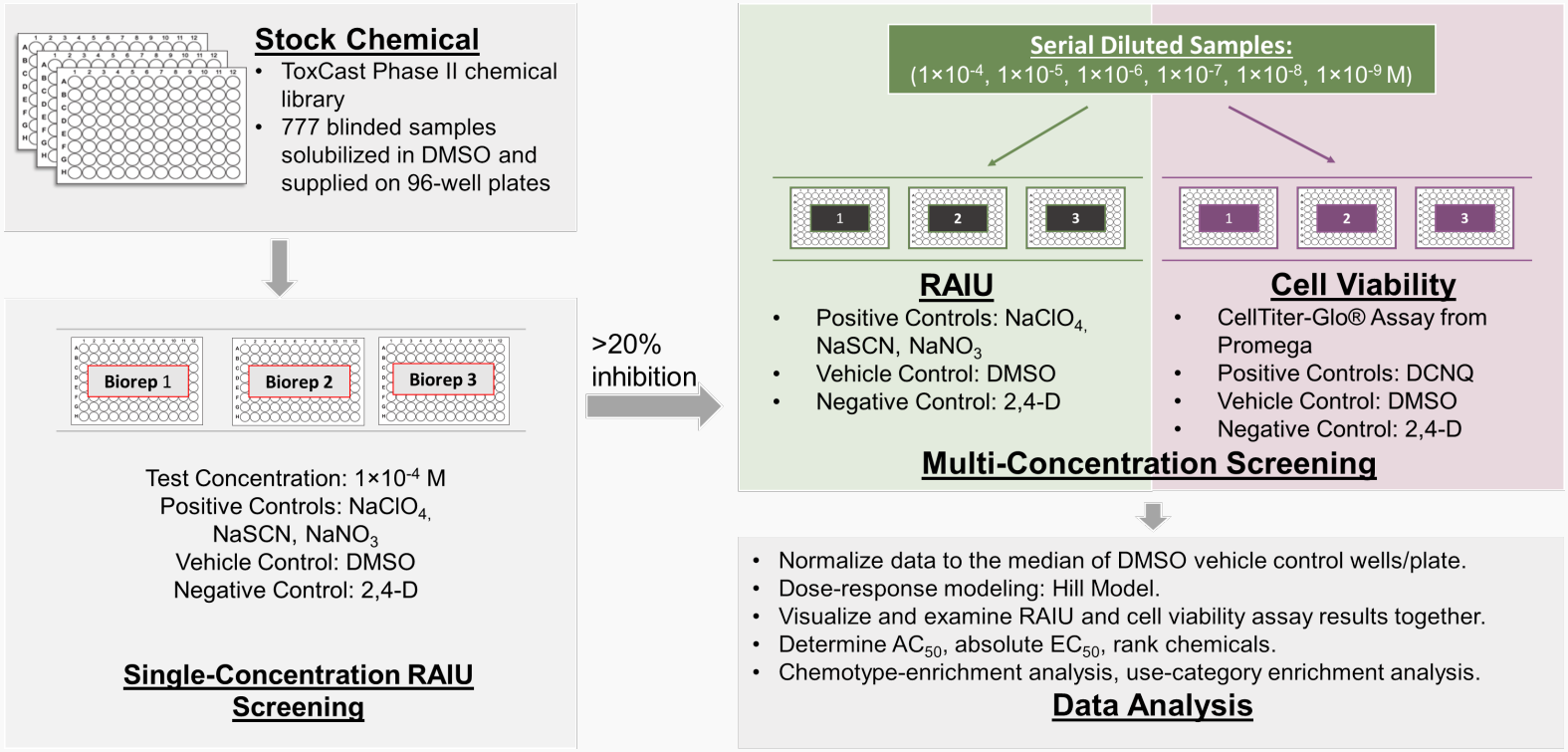
4/5 MEA hits with CT665 also MEA hits in Ion Channel (IC) assays

16/27 MEA hits in CT479 also hits in NVS_IC assays

Strickland et al., Arch Toxicol, 2018, 487-500

NIS Inhibition Assay

High-Throughput Screening and Chemotype-Enrichment Analysis of ToxCast Phase II Chemicals Evaluated for Human Sodium-Iodide Symporter (NIS) Inhibition



1028 total ToxCast Phase II chemicals screened

Wang et al., Environ. Int, 2019, 126:377-386

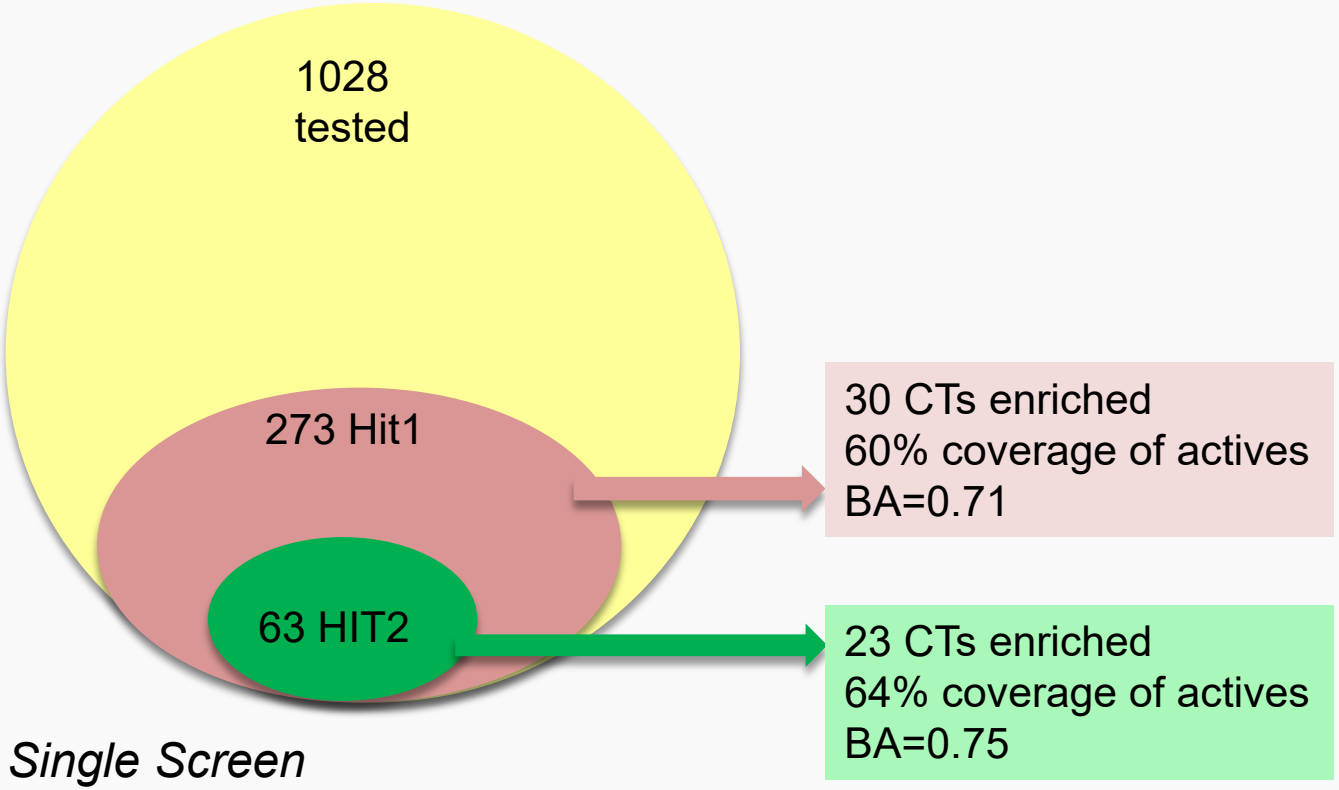
NIS Inhibition Assay

Single screen vs. Multiscreen results

1028 ToxCast Phase II chemicals screened

→ 27% positive at 20% threshold in single screen …**Hit1**

→ 6% positive in multiscreen, cell viability filtered results …**Hit2**

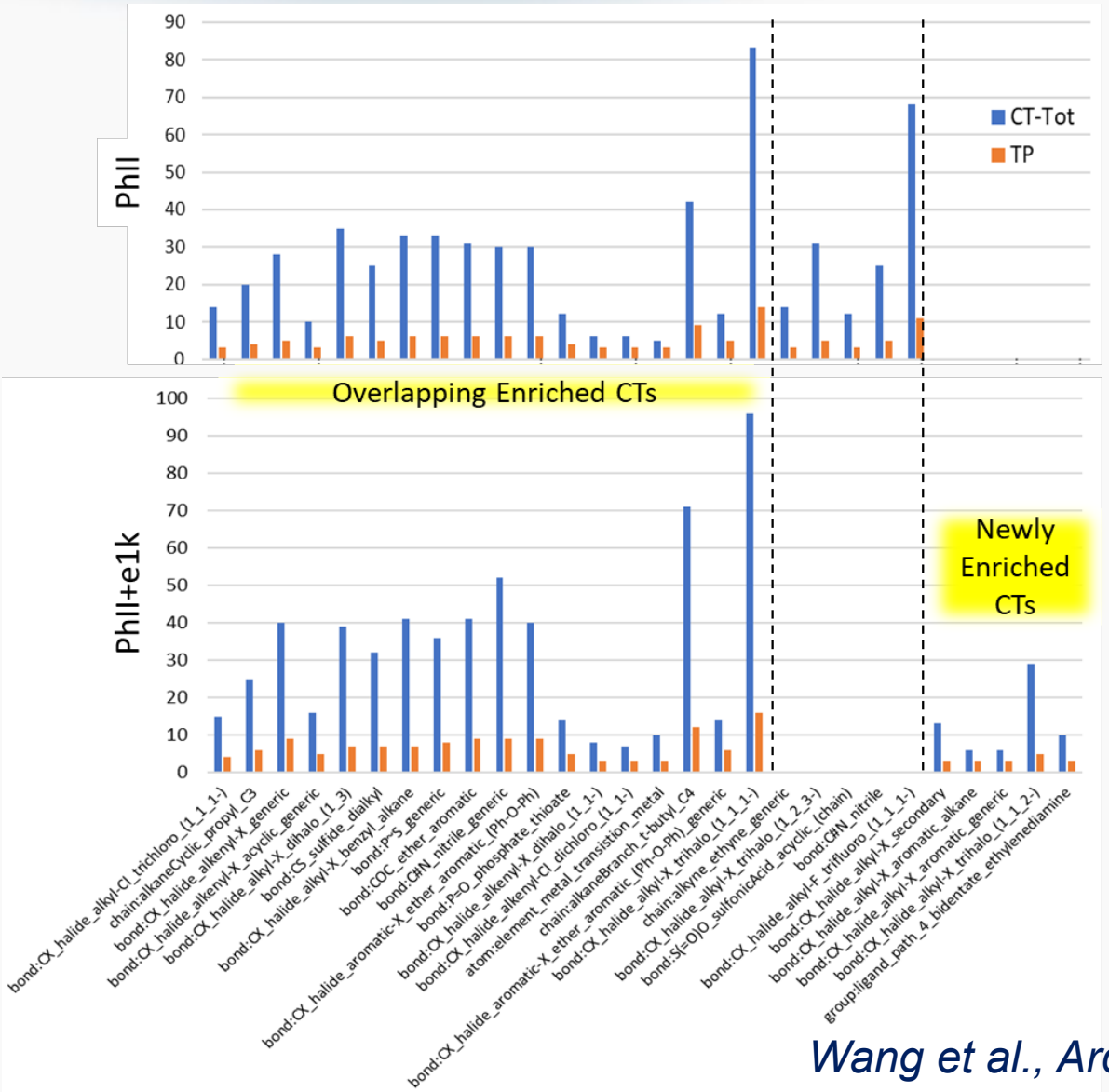


HIT1: Single Screen

HIT2: Multiscreen + cell viability filters

Wang et al., Environ. Int, 2019, 126:377-386

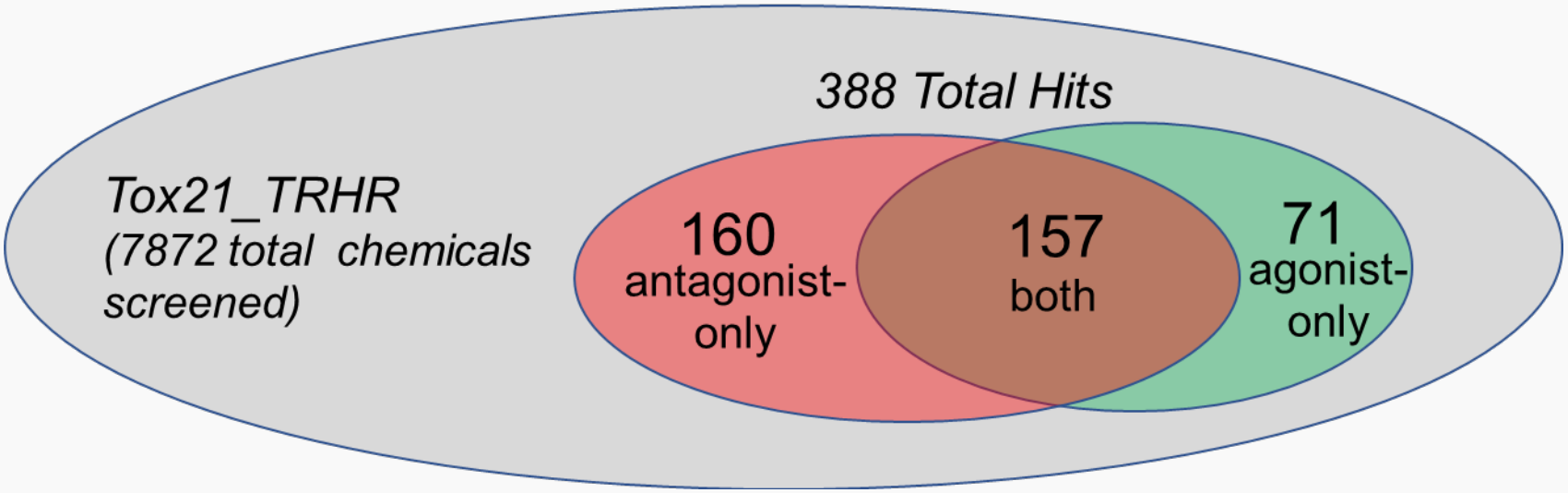
CT Enrichments (NIS Hit2) profiles from PhII to PhII+e1k



- *Majority of PhII CTEs validated for Hit2 Actives*
- *Small number of CTEs disappeared*
- *Equal number were added with e1k expanded inventory*

Wang et al., Arch Toxicol, 2021, 95:1723-37

Thyrotropin-Release Hormone Receptor Assay
Tox21_TRHR



Goal: Prioritize subset of actives (true hits) and inactives (potential false negatives) for follow-up testing using:

- *domain knowledge*
- *chemotype enrichments*
- *in silico computational chemistry models*

M. Shobair et al., Poster #252

Thyrotropin-Release Hormone Receptor Assay
Tox21_TRHR

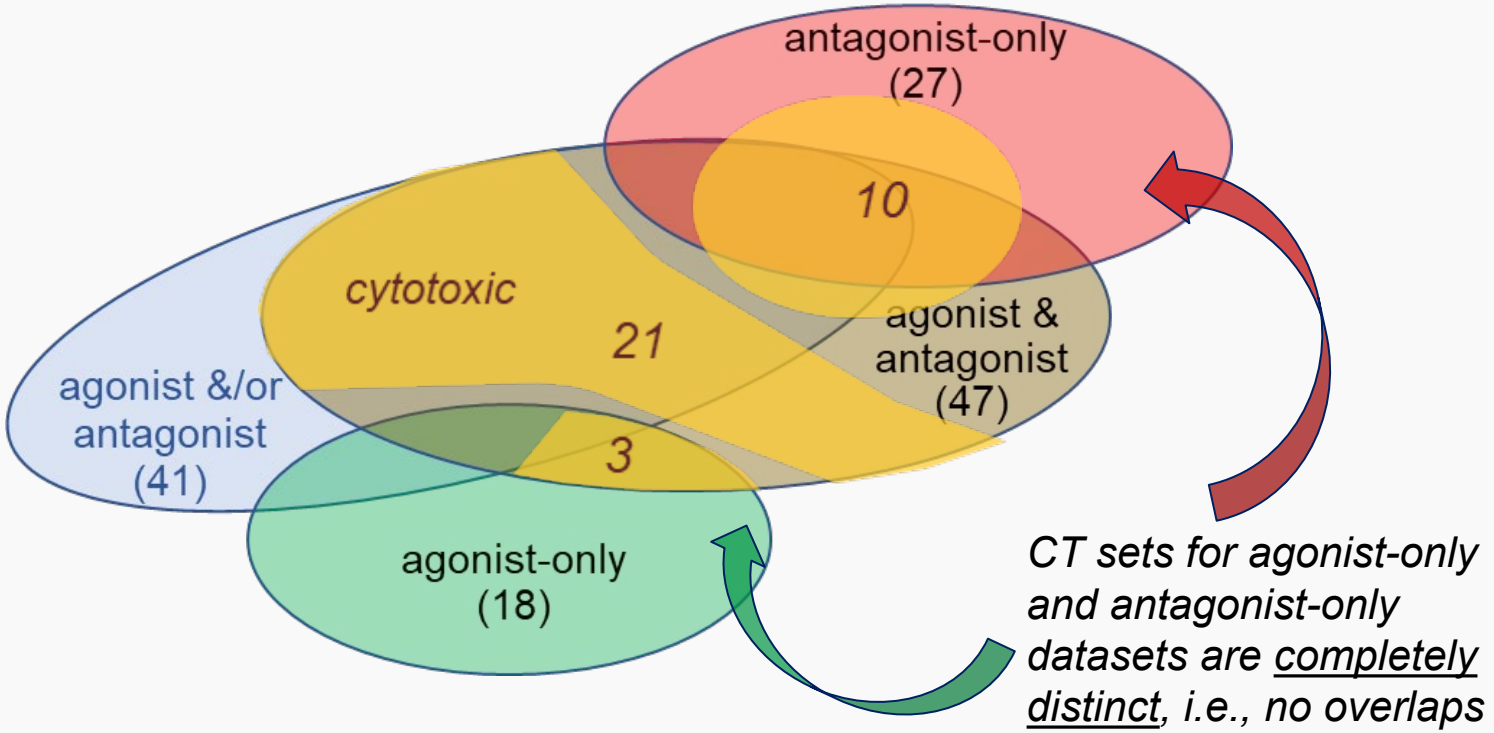
Unique chemotypes	Combined	Agonist_only	Antagonist_only	Agonist_and_antagonist
bond:CN_amine_aliphatic_generic		bond:CN_amine_aliphatic_generic		
bond:CN_amine_alkyl_ethanolamine		bond:CN_amine_alkyl_ethanolamine		
bond:CN_amine_ter-N_aliphatic		bond:CN_amine_ter-N_aliphatic		
bond:CN_amine_ter-N_aromatic		bond:CN_amine_ter-N_aromatic		
bond:CN_amine_ter-N_generic		bond:CN_amine_ter-N_generic		
bond:CS_sulfide_dialkyl		bond:CS_sulfide_dialkyl		
bond:quatN_alkyl_acyclic	bond:quatN_alkyl_acyclic	bond:quatN_alkyl_acyclic		bond:quatN_alkyl_acyclic
bond:quatN_generic		bond:quatN_generic		bond:quatN_generic
bond:quatN_trimethyl_alkyl_acyclic		bond:quatN_trimethyl_alkyl_acyclic		bond:quatN_trimethyl_alkyl_acyclic
bond:X[any_!C]_halide_inorganic	bond:quatN_trimethyl_alkyl_acyclic	bond:X[any_!C]_halide_inorganic		
group:ligand_path_4_bidentate_aminoethanol		group:ligand_path_4_bidentate_aminoethanol		
group:ligand_path_5_bidentate_propandiami	group:ligand_path_5_bidentate_pro	group:ligand_path_5_bidentate_propandiamine		
ring:hetero_[5]_N_pyrrole		ring:hetero_[5]_N_pyrrole		
ring:hetero_[6]_O_pyran_generic		ring:hetero_[6]_O_pyran_generic		ring:hetero_[6]_O_pyran_generic
ring:hetero_[6_6]_O_benzopyran	ring:hetero_[6_6]_O_benzopyran	ring:hetero_[6_6]_O_benzopyran		ring:hetero_[6_6]_O_benzopyran
ring:hetero_[6_6]_Z_generic		ring:hetero_[6_6]_Z_generic		
ring:hetero_[6_6_6]_N_S_phenothiazine	ring:hetero_[6_6_6]_N_S_phenothia	ring:hetero_[6_6_6]_N_S_phenothiazine		ring:hetero_[6_6_6]_N_S_phenothia
ring:hetero_[6_6_6]_O_benzopyran_dibenzo	ring:hetero_[6_6_6]_O_benzopyran	ring:hetero_[6_6_6]_O_benzopyran_dibenzo[b_e]		ring:hetero_[6_6_6]_O_benzopyran_
atom:element_metal_poor_metal	atom:element_metal_poor_metal		atom:element_metal_poor_metal	atom:element_metal_poor_metal
bond:C(=O)N_carbamate_dithio	bond:C(=O)N_carbamate_dithio		bond:C(=O)N_carbamate_dithio	
bond:C=N_imine_C(connect_H_gt_0)	bond:C=N_imine_N(connect_noZ)		bond:C=N_imine_C(connect_H_gt_0)	
bond:C=N_imine_N(connect_noZ)			bond:C=N_imine_N(connect_noZ)	
bond:C=S_carbonyl_thio_generic			bond:C=S_carbonyl_thio_generic	
bond:CC(=O)C_quinone_1_4-benzo	bond:CC(=O)C_quinone_1_4-benzo		bond:CC(=O)C_quinone_1_4-benzo	
bond:CC(=O)C_quinone_1_4-naphtho	bond:CC(=O)C_quinone_1_4-naphtho		bond:CC(=O)C_quinone_1_4-naphtho	
bond:COH_alcohol_aromatic			bond:COH_alcohol_aromatic	
bond:COH_alcohol_aromatic_phenol			bond:COH_alcohol_aromatic_phenol	
bond:CS_sulfide_di-			bond:CS_sulfide	
bond:CS_sulfide			bond:CS_sulfide_di-	
bond:CX_halide_alkenyl-X_dihalo_(1_2-)	bond:CX_halide_alkenyl-X_dihalo_(1_2-)		bond:CX_halide_alkenyl-X_dihalo	bond:CX_halide_alkenyl-X_dihalo_(1
bond:CX_halide_alkenyl-X_generic			bond:CX_halide_alkenyl-X_generic	
bond:CX_halide_alkyl-X_benzyl_alkane			bond:CX_halide_alkyl-X_benzyl_alkane	
bond:CX_halide_alkyl-X_trihalo_(1_1_1-)			bond:CX_halide_alkyl-X_trihalo_(1_1_1-)	
bond:CX_halide_aromatic-X_dihalo_benzene_(1_3-)			bond:CX_halide_aromatic-X_dihalo_benzene_(1_3-)	
bond:CX_halide_aromatic-X_halo_phenol_o	bond:CX_halide_aromatic-X_halo_phenol_ortho		bond:CX_halide_aromatic-X_halo	bond:CX_halide_aromatic-X_halo_p
bond:CX_halide_aromatic-X_halo_phenol_p	bond:CX_halide_aromatic-X_halo_phenol_para		bond:CX_halide_aromatic-X_halo	bond:CX_halide_aromatic-X_halo_p
bond:CX_halide_aromatic-X_trihalo_benzen	bond:CX_halide_aromatic-X_trihalo_benzene_(1_2_3-)		bond:CX_halide_aromatic-X_triha	bond:CX_halide_aromatic-X_trihalo_
bond:CX_halide_generic-X_dihalo_(1_2-)			bond:CX_halide_generic-X_dihalo_(1_2-)	
bond:metal_group_III_other_generic	bond:metal_group_III_other_generic		bond:metal_group_III_other_gener	
chain:aromaticAlkane_Ar-C-Ar	chain:aromaticAlkane_Ar-C-Ar		chain:aromaticAlkane_Ar-C-Ar	
chain:aromaticAlkane_Ph-C1-Ph	chain:aromaticAlkane_Ph-C1-Ph		chain:aromaticAlkane_Ph-C1-Ph	
chain:aromaticAlkene_Ph-C2_acyclic_generic			chain:aromaticAlkene_Ph-C2_acy	
ring:hetero_[5]_N_triazole_(1_2_4-)			ring:hetero_[5]_N_triazole_(1_2_4-	
ring:hetero_[5]_N_triazole_(1_3_4-)			ring:hetero_[5]_N_triazole_(1_3_4-	
ring:hetero_[5]_Z_1_2_4_1_3_4-Z			ring:hetero_[5]_Z_1_2_4_1_3_4-Z	
bond:CX_halide_alkyl-Cl_dichloro_(1_1-)				
bond:CX_halide_alkyl-X_bicyclo[2_2_1]hepte	bond:CX_halide_alkyl-X_bicyclo[2_2_1]heptene			
bond:CX_halide_alkyl-X_tertiary				
bond:metal_group_III_other_generic_oxy	bond:metal_group_III_other_generic_oxy			
bond:metal_group_III_other_Sn_generic	bond:metal_group_III_other_Sn_generic			
bond:metal_group_III_other_Sn_halide	bond:metal_group_III_other_Sn_halide			

Generate CT enrichments for the 4 activity subsets relative to the larger Tox21 test set

M. Shobair et al., Poster # 252

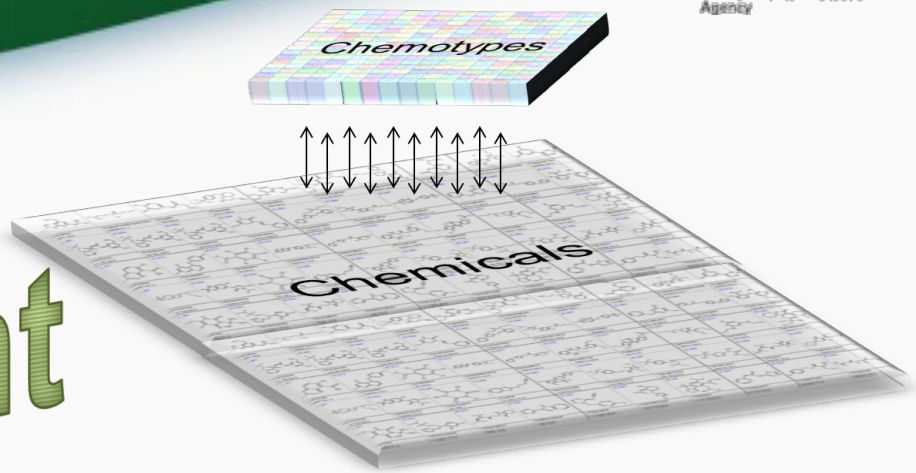
Thyrotropin-Release Hormone Receptor Assay
Tox21_TRHR

(#) of Enriched CTs per TRHR Activity Subset



CTs included in the "cytotoxic" regions are significantly enriched in >10/42 Tox21 "cytotoxicity"-labeled assays (invitrodb_v2)

M. Shobair et al., Poster # 252


Chemotypes
Chemicals

Enrichment


NO TAXATION
WITHOUT
REPRESENTATION

ToxPrint enrichments only possible with inclusion of NCATS partner library

Tox21 Library Profile

NCATS (Drugs)

vs.

EPA+NTP (Environmental)

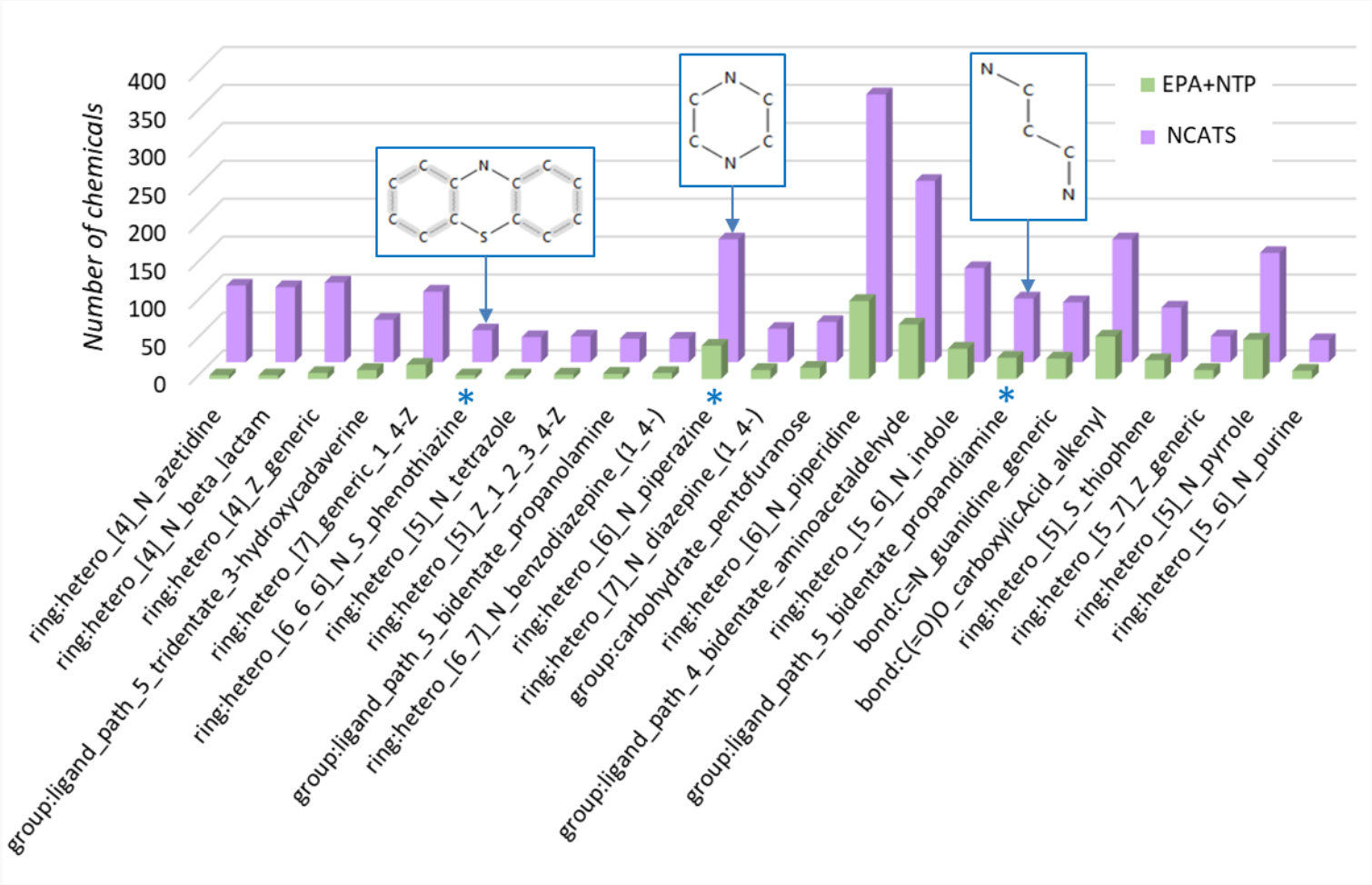
ToxPrint enrichments only possible with inclusion of EPA+NTP partner libraries

Richard et al., CRT, 2021, 34:189-216

CT Profile & Assay "Enrichments"

ToxPrint enrichments only possible with inclusion of NCATS partner library

Each ToxPrint below is enriched in 1 or more Tox21 assays

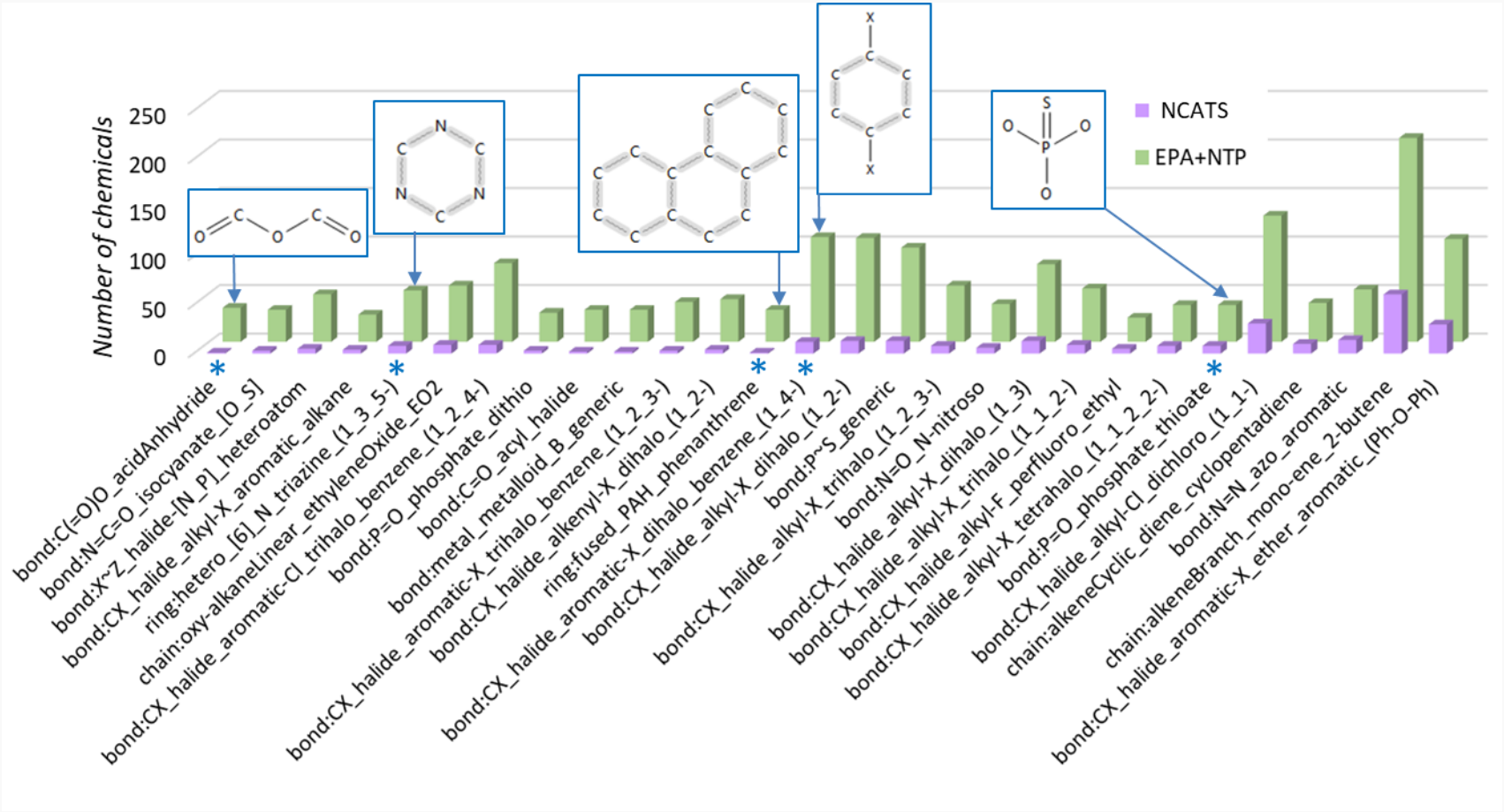


Richard et al., CRT, 2021, 34:189-216

CT Profile & Assay "Enrichments"

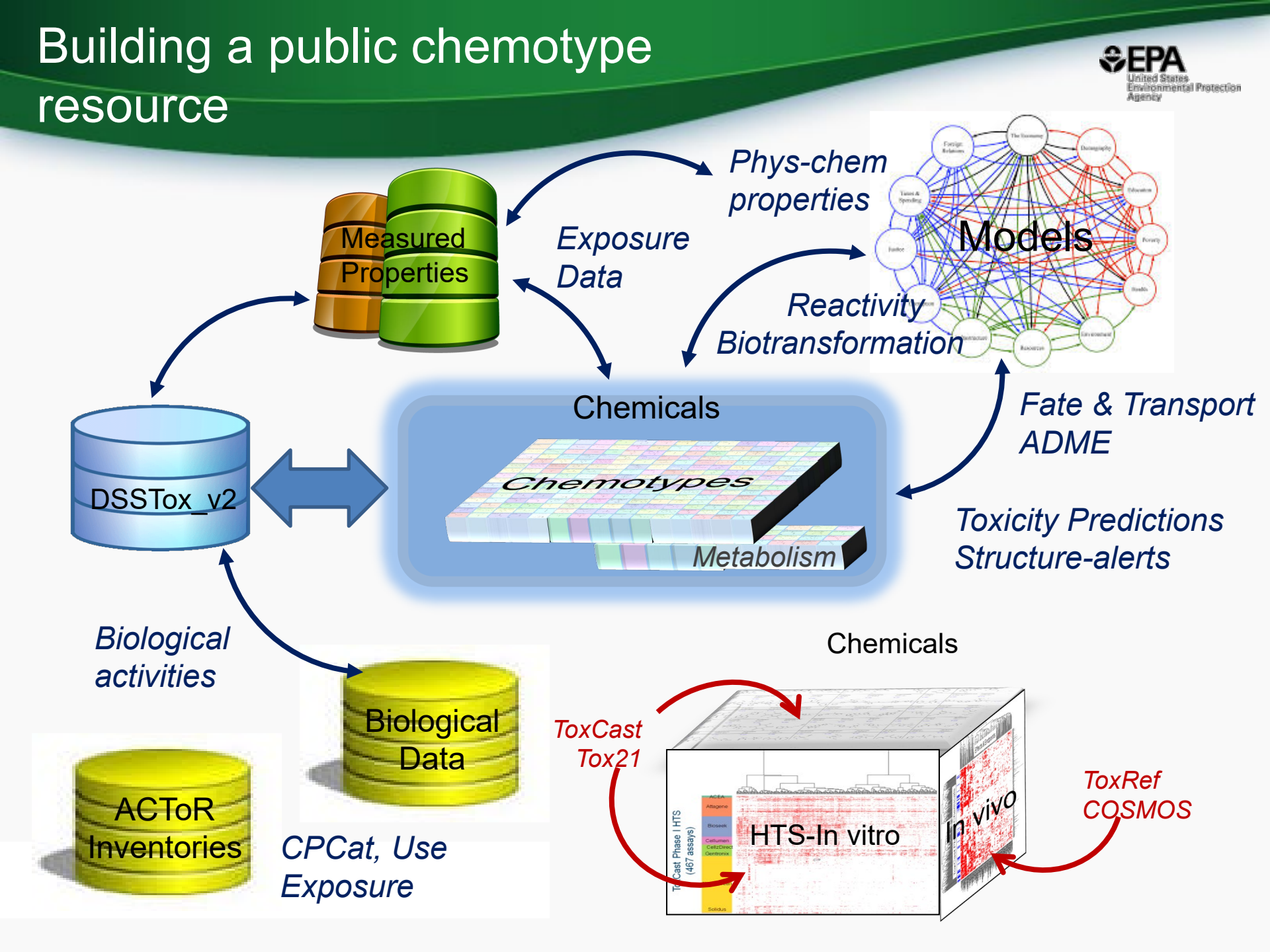
ToxPrint enrichments only possible with inclusion of EPA+NTP partner libraries

Each ToxPrint below is enriched in 1 or more Tox21 assays



Richard et al., CRT, 2021, 34:189-216

Building a public chemotype resource



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thank you
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Баярлалаа
merci