A Knowledge-Informed Chemotype Approach to Mining the ToxCast/Tox21 Chemical-Data Landscape

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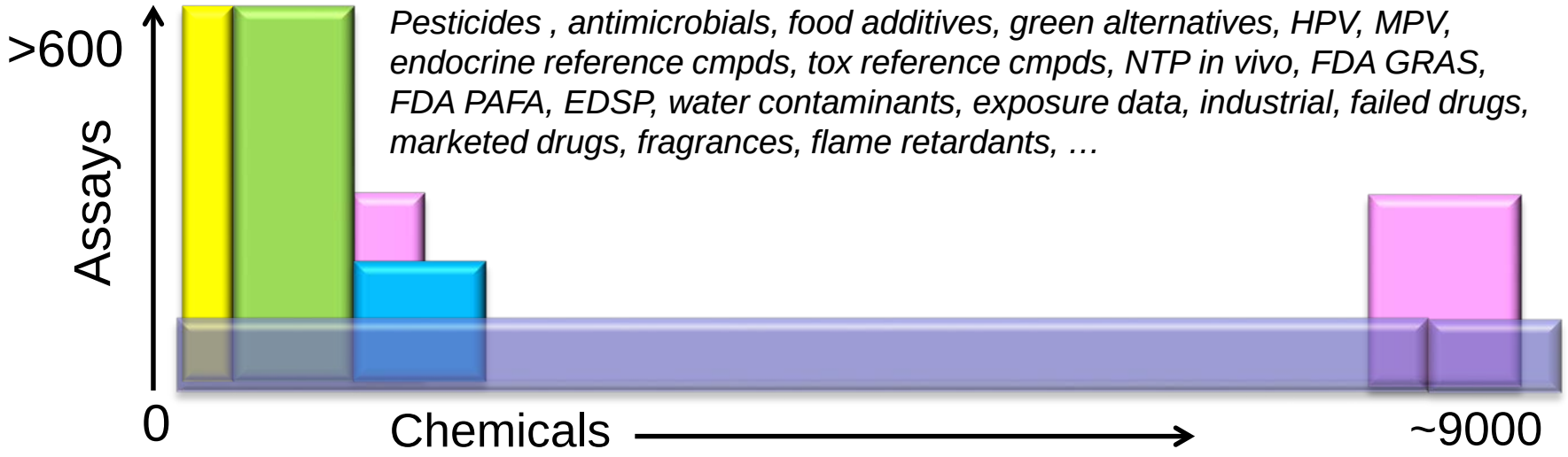
ToxCast & Tox21 Inventories: Chemicals, Data & Timelines

Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~700	2011	Now
ToxCast Phase II	767	~600	~700	03/2013	Now
ToxCast E1K	800	~50	~120	03/2013	Now
Tox21	~9000	~80	~150	Ongoing	Ongoing
ToxCast Phase III	~900	~300	~300	Just starting	2014-2015



Toxicity Prediction Challenge:

Bringing all knowledge & data to bear on problem

Data-mining

Biologically-based QSAR & Cheminformatics

Aggregation

Adverse Outcomes:
> Pathways
> Genes
> Assays
+ Statistical associations

Reactivity & toxicity-informed features & classes

Mechanistically well-defined toxicity endpoint

Structures

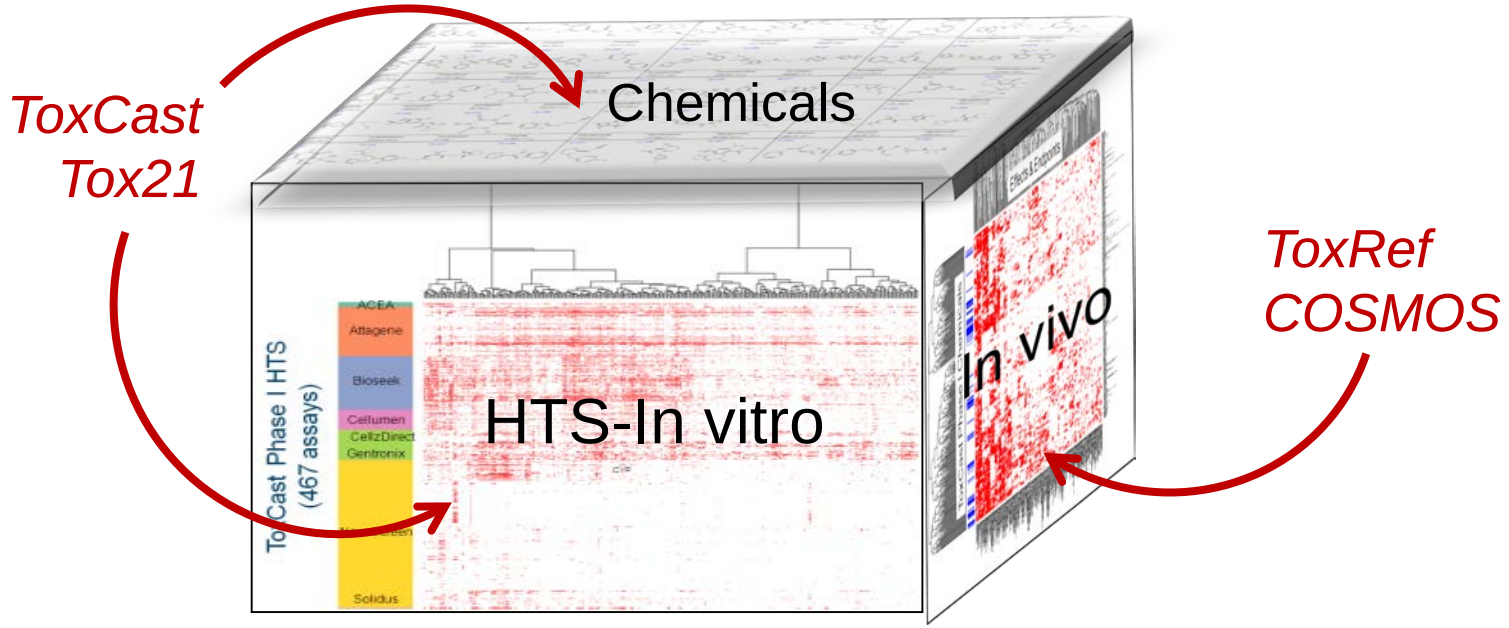
In Vivo

In Vitro/HTS

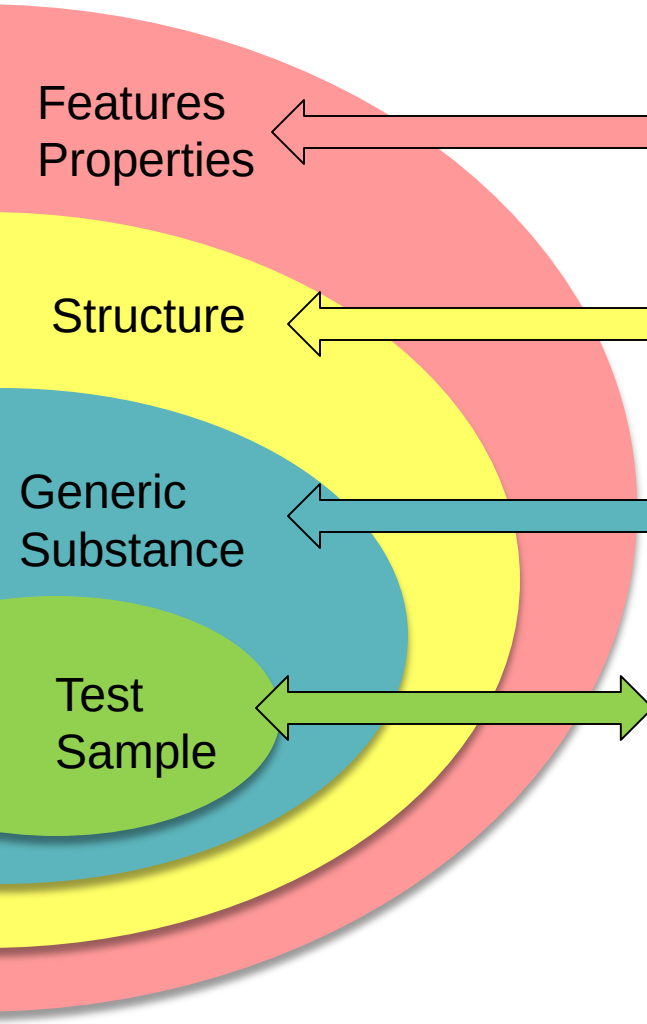
Existing knowledge

Chemical “probes” of biological activity

- Use existing knowledge & SAR to mine HTS data
- Use HTS data to inform & refine SAR models & approaches
- Use all of these data to improve ability to model toxicity

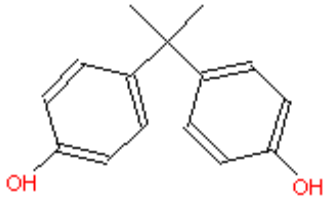


Chemical Elements to Data Integration: *Chemical representations → Uses*



Chemotypes, fingerprints, phys-chem properties, ...

Chemical analogs, Read-across, SAR modeling



SMILES
InChI

Structure searching & modeling

Chemical Name
CASRN

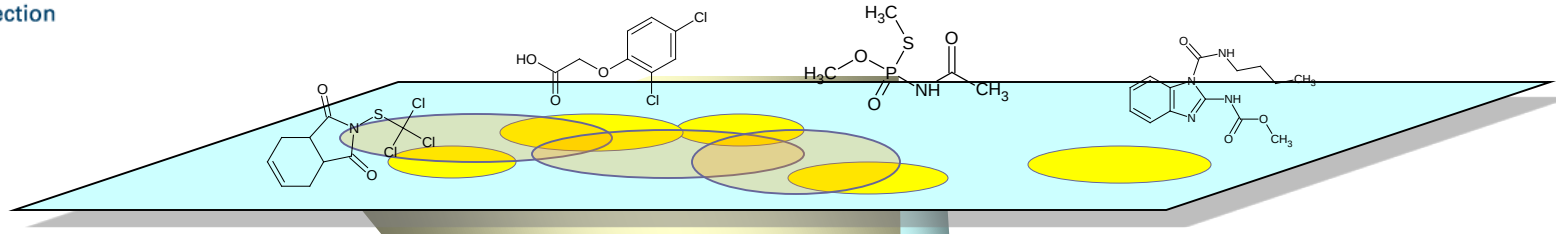
Public toxicity datasets

Supplier, Lot/Batch, physical description

Experimental Endpoint Data

Structure vs. Bioactivity Similarity

United States Environmental Protection Agency

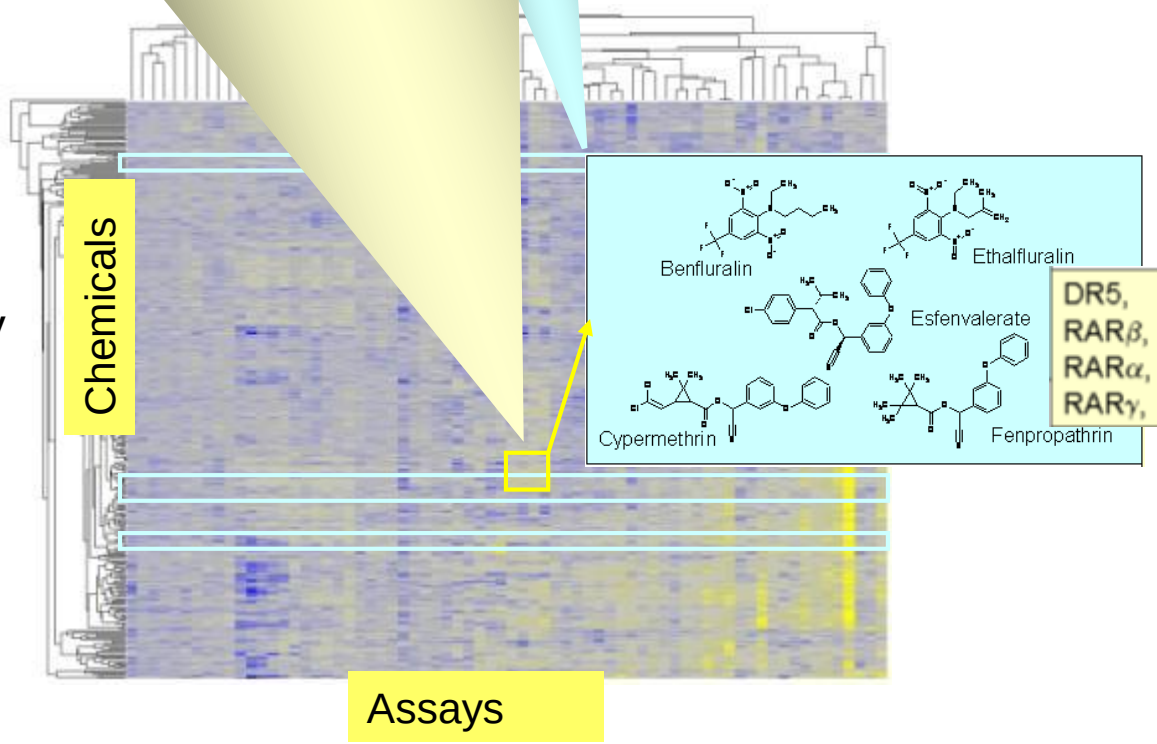


Structure similarity:

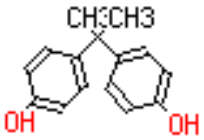
- implies biological similarity
- limited to local chemistry
- subject to “activity cliffs”

HTS bioactivity similarity:

- implies mechanistic similarity
- can link diverse local chemistries to common biological activities
- noisy data, difficult to extract clear signal



Similarity – in the "eye" of the beholder



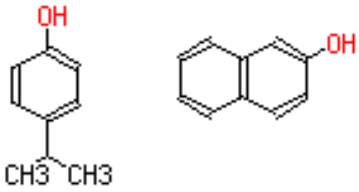
Chemical Similarity

Structural fingerprint

100011010100000110
(all parts considered equal)

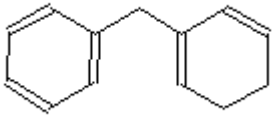
Tanimoto Coeff

1=exact match
0.9 = pretty good match
0.5 = who knows, depends



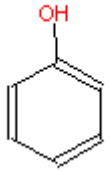
Chemotypes

Pre-defined features or scaffolds informed by SAR, public inventories, and FDA safety assessment workflows



biphenyl

phenol

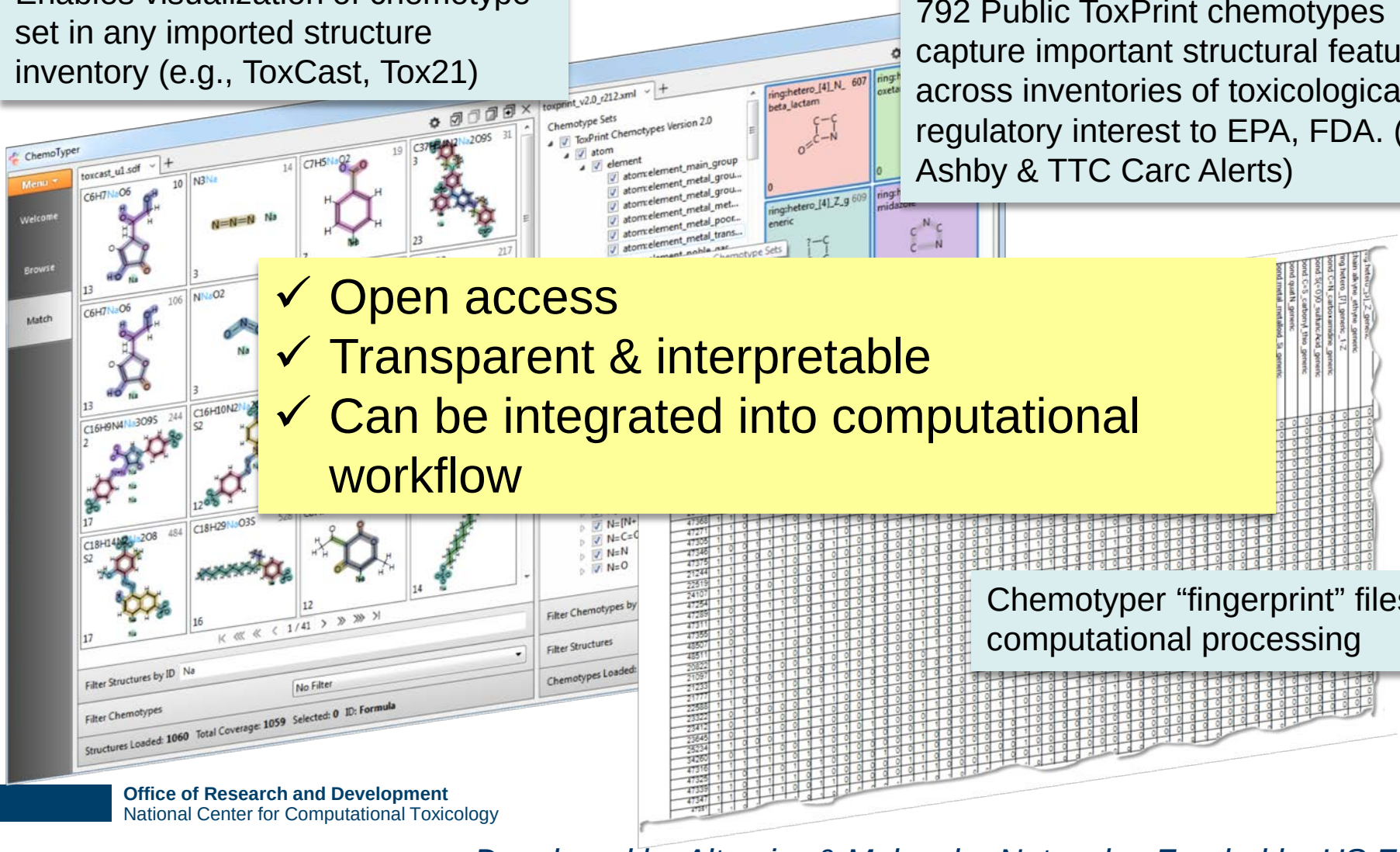


Rules for judging similarity are clearly defined, interpretable, computable, and transferable.

Public Resources: Chemotyper & ToxPrint Chemotypes

Enables visualization of chemotype set in any imported structure inventory (e.g., ToxCast, Tox21)

792 Public ToxPrint chemotypes capture important structural features across inventories of toxicological & regulatory interest to EPA, FDA. (incl. Ashby & TTC Carc Alerts)



- ✓ Open access
- ✓ Transparent & interpretable
- ✓ Can be integrated into computational workflow

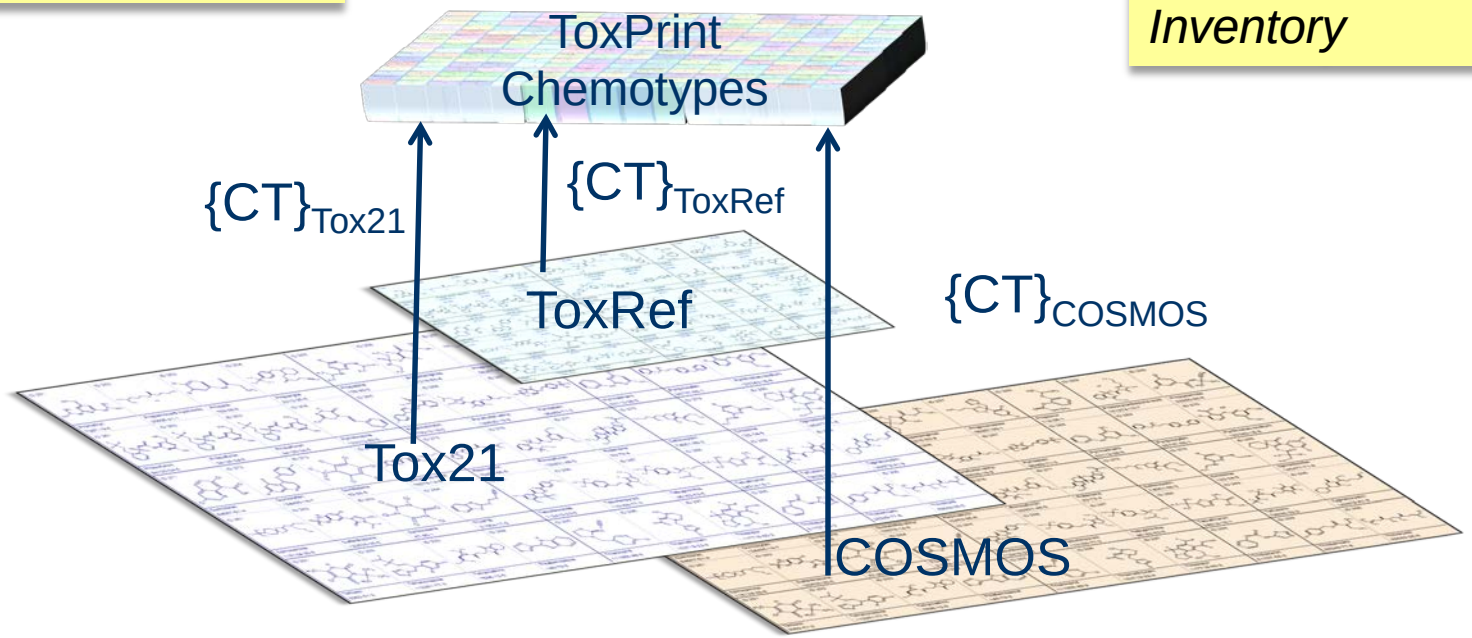
Chemotyper “fingerprint” files for computational processing

Developed by Altamira & Molecular Networks, Funded by US FDA

Chemotype (CT) inventory profiling

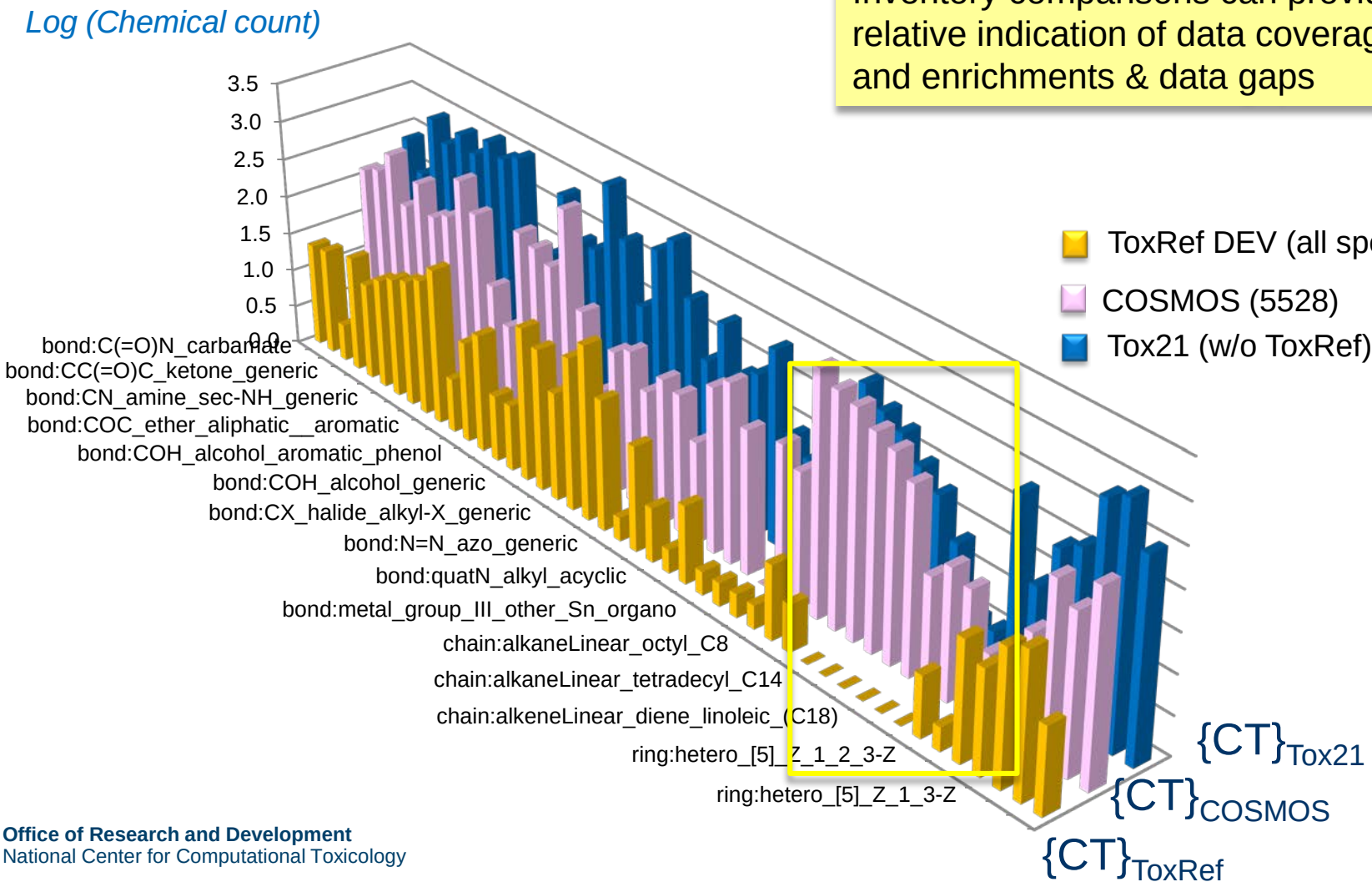
CT fingerprint provide a common structural basis for Inventory profiling & comparison

{CT} profile is a property of the Inventory

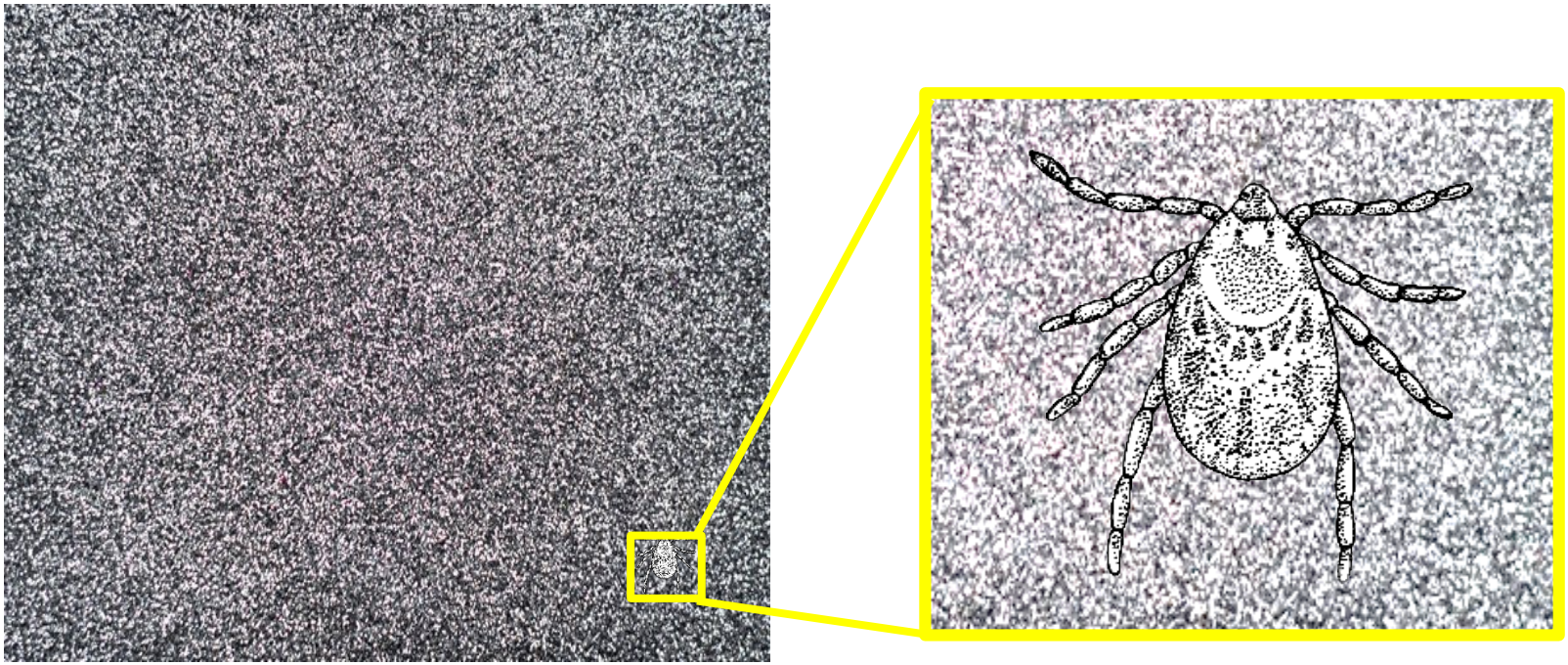


Chemotype inventory profiling

Inventory comparisons can provide relative indication of data coverage, and enrichments & data gaps



Concept of Enrichment: *Focus & Amplify to See*



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

- 42 FRs amenable to HTS included in ToxCast Ph3 & Tox21

$\{CT\}_{FR}$

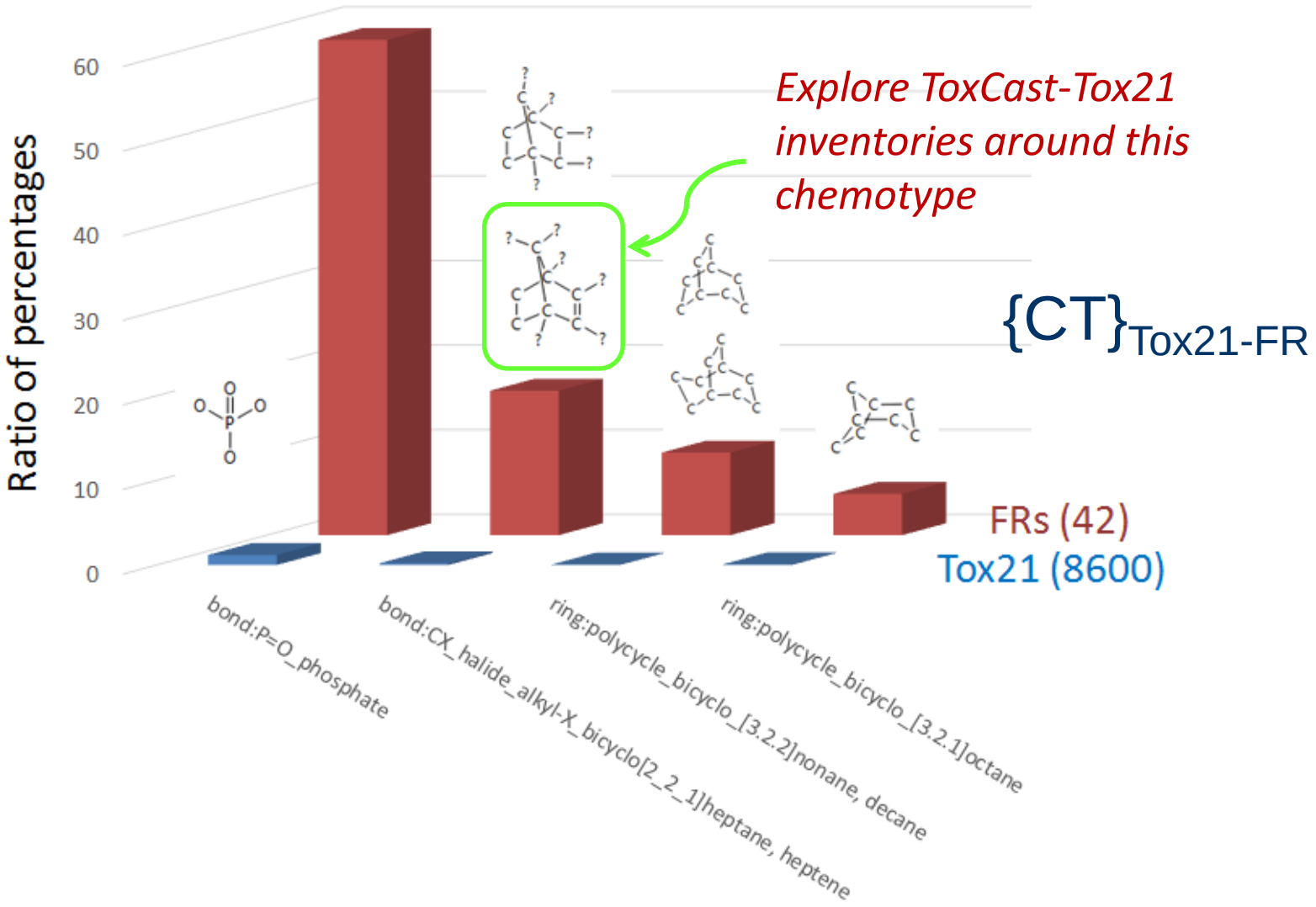
3296-90-0	2,2-Bis(bromomethyl)-1,3-propanediol
115-28-6	Chlorendic acid
2921-88-2	Chlorpyrifos
2385-85-5	Mirex
115-96-8	Tris(2-chloroethyl) phosphate
78-42-2	Tris(2-ethylhexyl) phosphate
115-86-6	Triphenyl phosphate
126-73-8	Tributyl phospha
79-94-7	3,3',5,5'-Tetrabro
13674-87-8	Tris(1,3-dichloro
1163-19-5	Decabromodiph
19660-16-3	2,3-Dibromoprop
563-04-2	Tri-m-cresyl pho
20120-33-6	Phosphonic acid dimethyl ester
868-85-9	Dimethyl hydrogen phosphite
756-79-6	Dimethyl methylphosphonate
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
	te
	Tris(2-chloroisopropyl)phosphate
25155-23-1	TXP
598-72-1	2-Bromopropionic acid
3194-55-6	1,2,5,6,9,10-Hexabromocyclododecane
6145-73-9	Tris(2-chloropropyl) phosphate
26040-51-7	Bis(2-ethylhexyl) tetrabromophthalate
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-

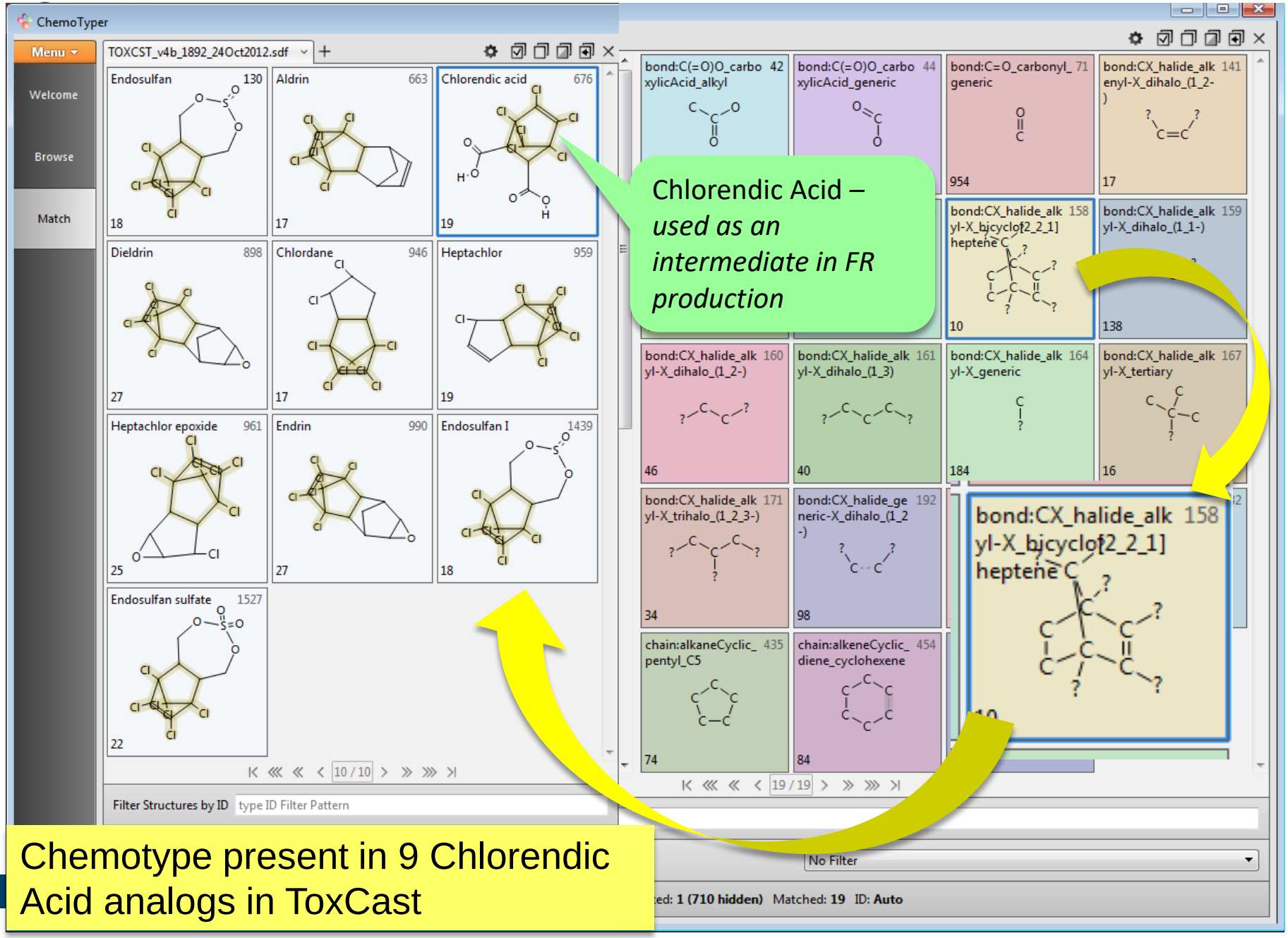
Are there enriched chemotypes within this FR subset relative to ToxCast & Tox21?

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

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



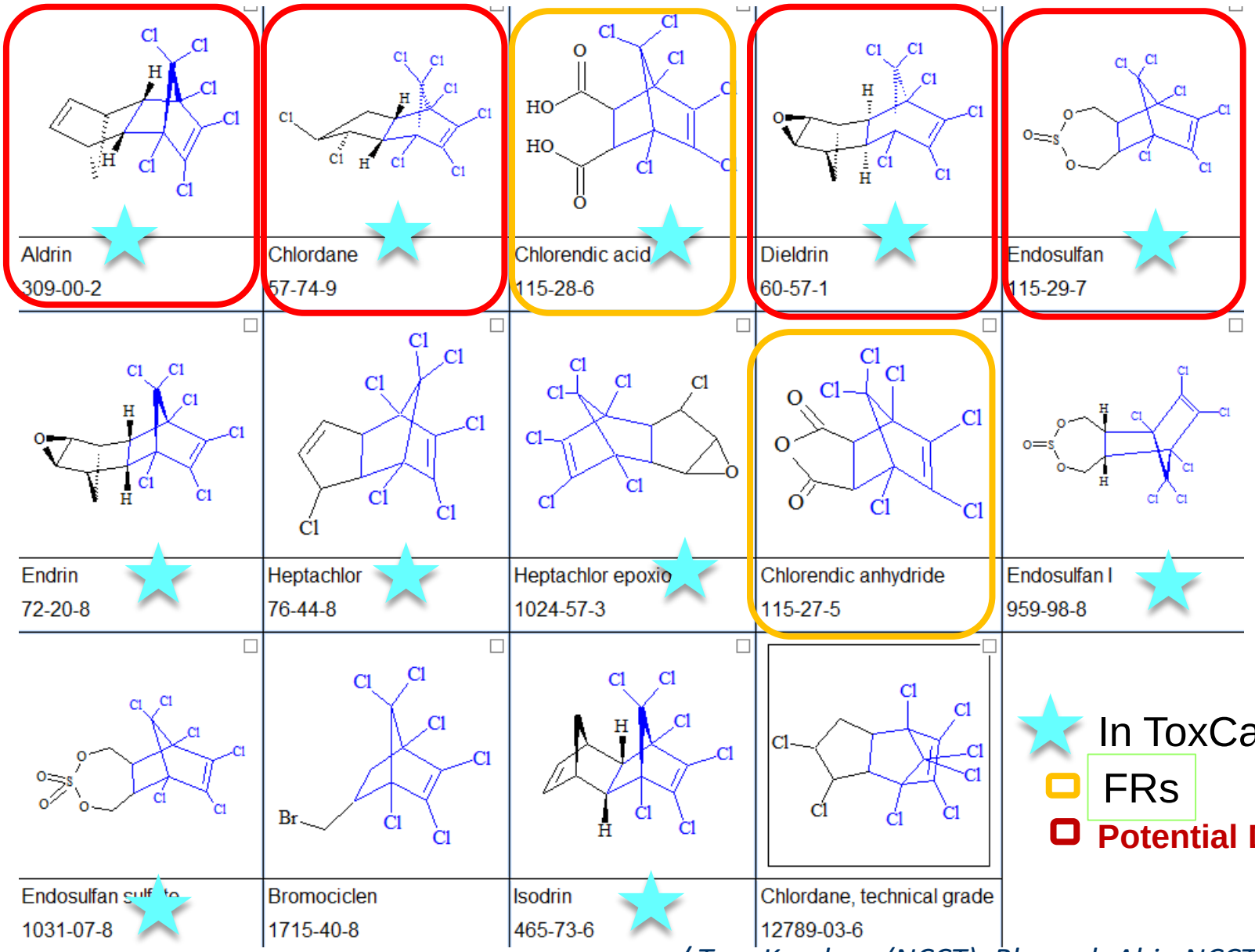
w/ Tom Knudsen (NCCT), Bhavesh Ahir, NCCT (ORISE)



Chlorendic Acid – *used as an intermediate in FR production*

Chemotype present in 9 Chlorendic Acid analogs in ToxCast

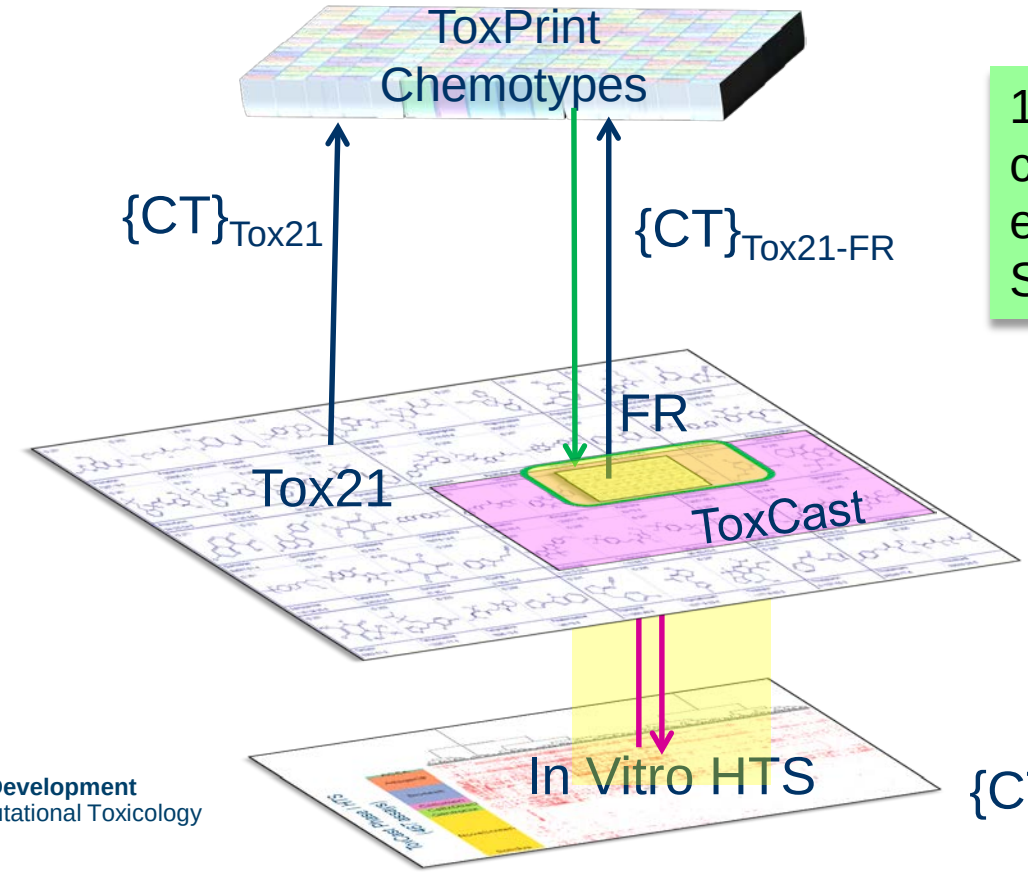
14 Tox21 Chemotype Analogs of Chlorendic Acid



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

Chemotype-Assay Enrichments {CT-Assay}: Mining the data

Use FR-chemotypes to explore possible ToxCast assay associations relevant to this dataset



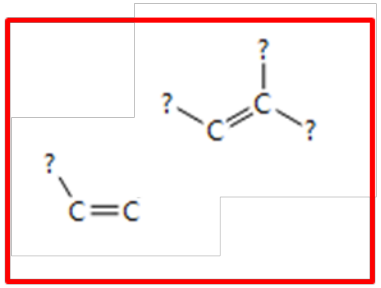
1. Identify all Tox21 chemicals containing enriched CTs for SAR follow-up

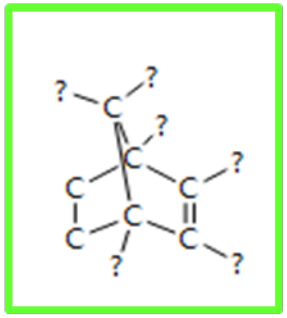
2. Identify ToxCast CT-Assay enrichments

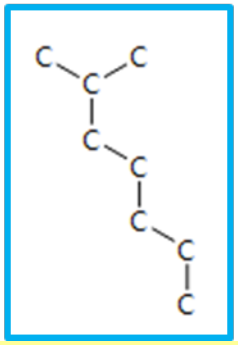
{CT-Assay}ToxCast-FR

Preliminary Results: {CT-Assay}$_{ToxCast\text{-}FR}$

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







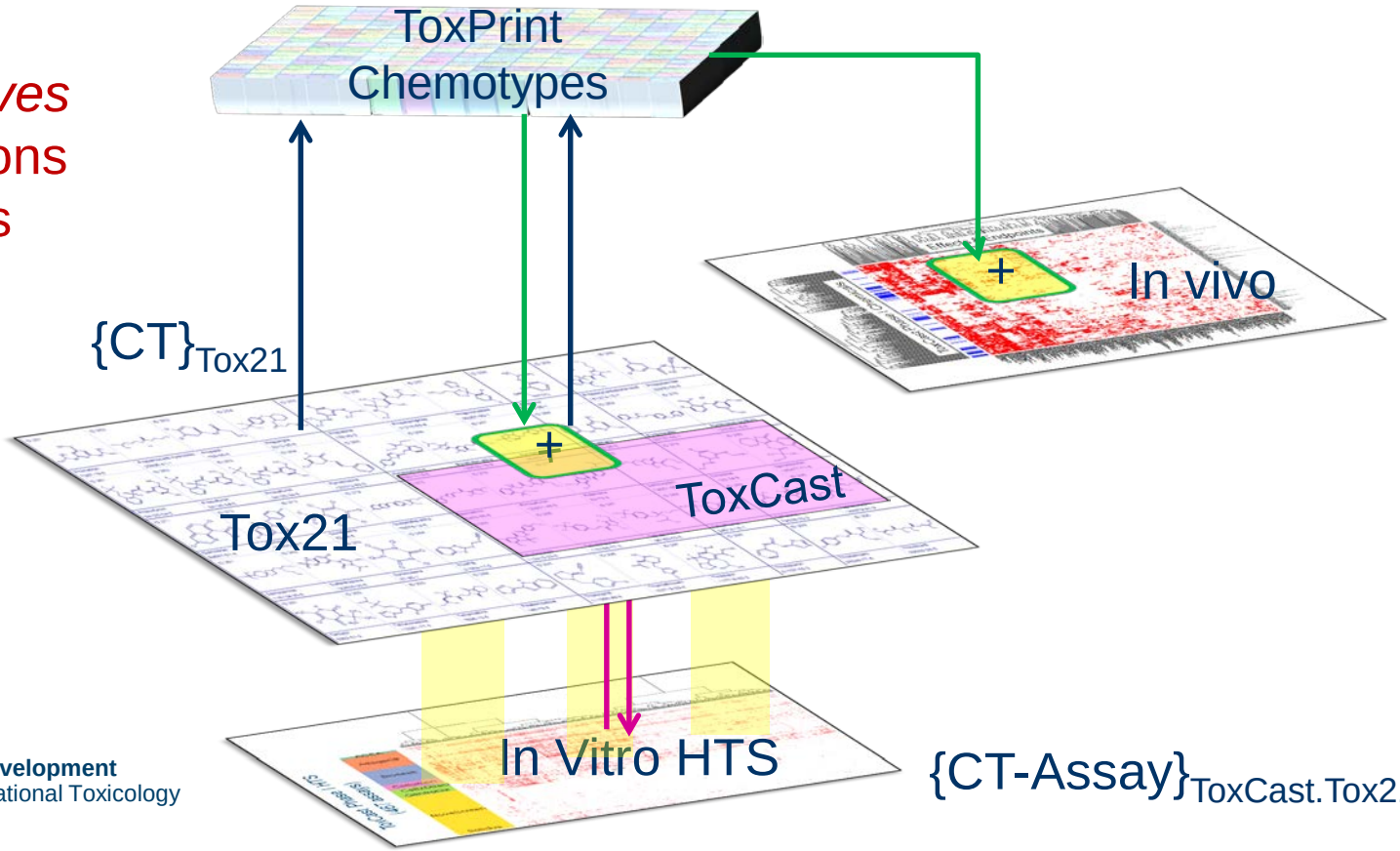
Significant {CT-Assay}$_{ToxCast\text{-}FR}$ associations potentially related to developmental outcomes

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

Chemotype-Activity Enrichments

Create {CT-Activity} profiles for any biological activity subset to focus investigations into local CT domains and enhance HTS or structure-activity signal

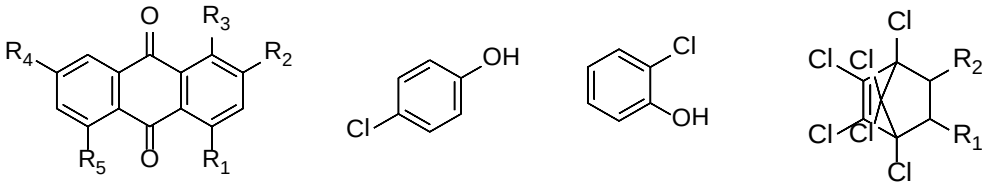
- *HTS actives*
- *Pathway actives*
- SAR predictions
- *In vivo* results



Tox21 Mitochondrial Membrane Potential (MMP) Disruption Assay Study

OBJECTIVES: To identify individual chemicals and general structural features associated with the decrease of mitochondrial membrane potential (MMP) in HepG2 cells.

- 913/8300 MMP “actives”
- Precompute 651 structural similarity clusters for Tox21 Inventory using ChemAxon proprietary fingerprints
  - ➢ 76 of 651 clusters significantly enriched for actives
  - ➢ representative features extracted from active clusters by visual inspection



(http://www.ncbi.nlm.nih.gov/pcsubstance; search term “tox21”).

Profiling of the Tox21 Chemical Collection for Mitochondrial Function I. Compounds that Decrease Mitochondrial Membrane Potential
Attene-Ramos MS, Huang R, Michael S, Witt KL, Richard A, Tice R, Simeonov A, Austin CP, Xia M, (*Submitted to EHP)*

Tox21 Mitochondrial Membrane Potential (MMP) Disruption Assay

United States Environmental Protection Agency

1. Compute $\{CT\}_{Tox21\text{-}MMP}$

	OR
chain:aromaticAlkane_Ph-C6	∞
bond:CX_halide_alkenyl-X_dihalo_(1_2-)	19.7
ring:hetero_[6_6]_O_benzopyrone_(1_4-)	19.0
ring:fused_PAH_phenanthrene	18.4
bond:metal_group_III_other_Sn_organo	17.5
bond:P=O_phosphate_thioate	14.6
bond:metal_group_III_other_Sn_generic	13.1
bond:COH_alcohol_aromatic_phenol	10.0

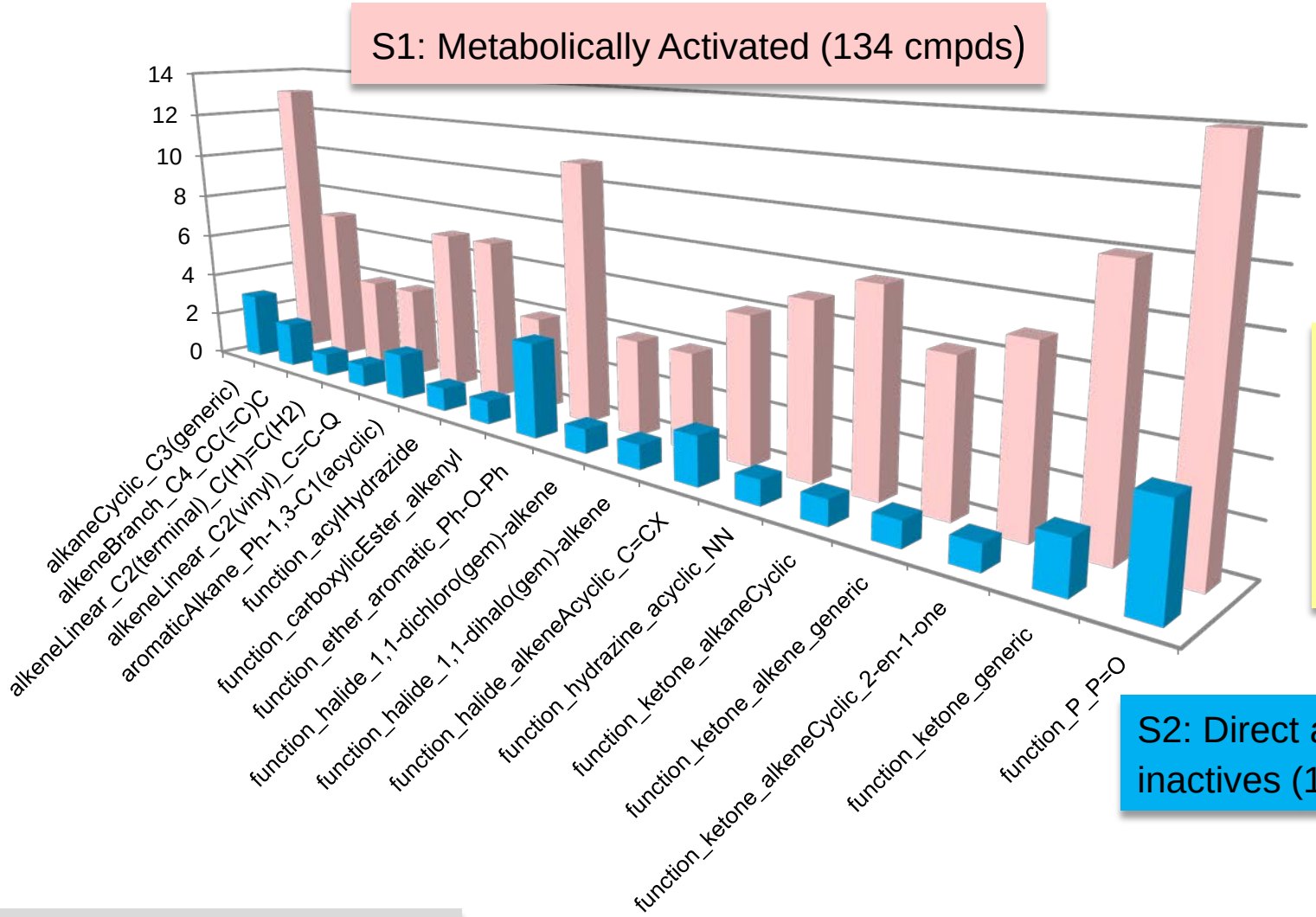
Most significantly enriched CTs in MMP active subset

Which Tox21 & ToxCast assays are enriched w/ positives in these {CT} subsets?

2. Compute $\{CT\text{-}Assay\}_{ToxCast\text{-}MMP}$

ToxCast/Tox21 Assays	{CT}MMP	OR	# TP (CT & Assay)	# CT Positive	# Assay positive	Total # Cmpds
APR_CellLoss_72h_dn	bond:C(=O)N_carbamate_dithio	∞	8	8	305	958
ATG_PXRE_CIS	bond:CX_halide_aromatic-X_ether_aromatic_(Ph-O-Ph)_generic	∞	13	13	708	1857
ATG_NRF2_ARE_CIS	bond:metal_group_III_other_Sn_generic	∞	8	8	635	1857
NVS_GPCR_hORL1	bond:C(=O)N_carbamate_dithio	94.6	7	9	45	1067
Tox21_ERa_BLA_Agonist_ratio	ring:hetero_[6_6]_O_benzopyrone_(1_4-)	67.7	9	10	227	1858
ACEA_T47D_80hr_Positive	ring:hetero_[6_6]_O_benzopyrone_(1_4-)	61.7	9	10	240	1815
NVS_ENZ_rCOMT	bond:COH_alcohol_aromatic_phenol	50.4	11	115	13	1059
Tox21_PPARg_BLA_Agonist_ratio	bond:metal_group_III_other_Sn_generic	50.0	6	8	111	1858
OT_ER_ERaERb_1440	chain:aromaticAlkane_Ph-C6	43.7	9	11	181	1846
Tox21_AhR	bond:P=O_phosphate_thioate	43.5	12	14	237	1858
Tox21_MitochondrialToxicity_viability	bond:metal_group_III_other_Sn_generic	43.4	6	8	126	1858
APR_p53Act_24h_up	bond:C(=O)N_carbamate_dithio	36.8	7	8	160	958
Tox21_MitochondrialToxicity_ratio	ring:hetero_[6_6]_O_benzopyrone_(1_4-)	35.4	9	10	385	1858
OT_ER_ERaERb_0480	chain:aromaticAlkane_Ph-C6	35.4	9	11	218	1853
Tox21_AhR	ring:hetero_[6_6]_O_benzopyrone_(1_4-)	28.4	8	10	237	1858

Chemotype Activity Profiling: e.g. Modeling *in vivo* activity subsets



Significantly different {CT} profiles in S1 vs S2 subsets

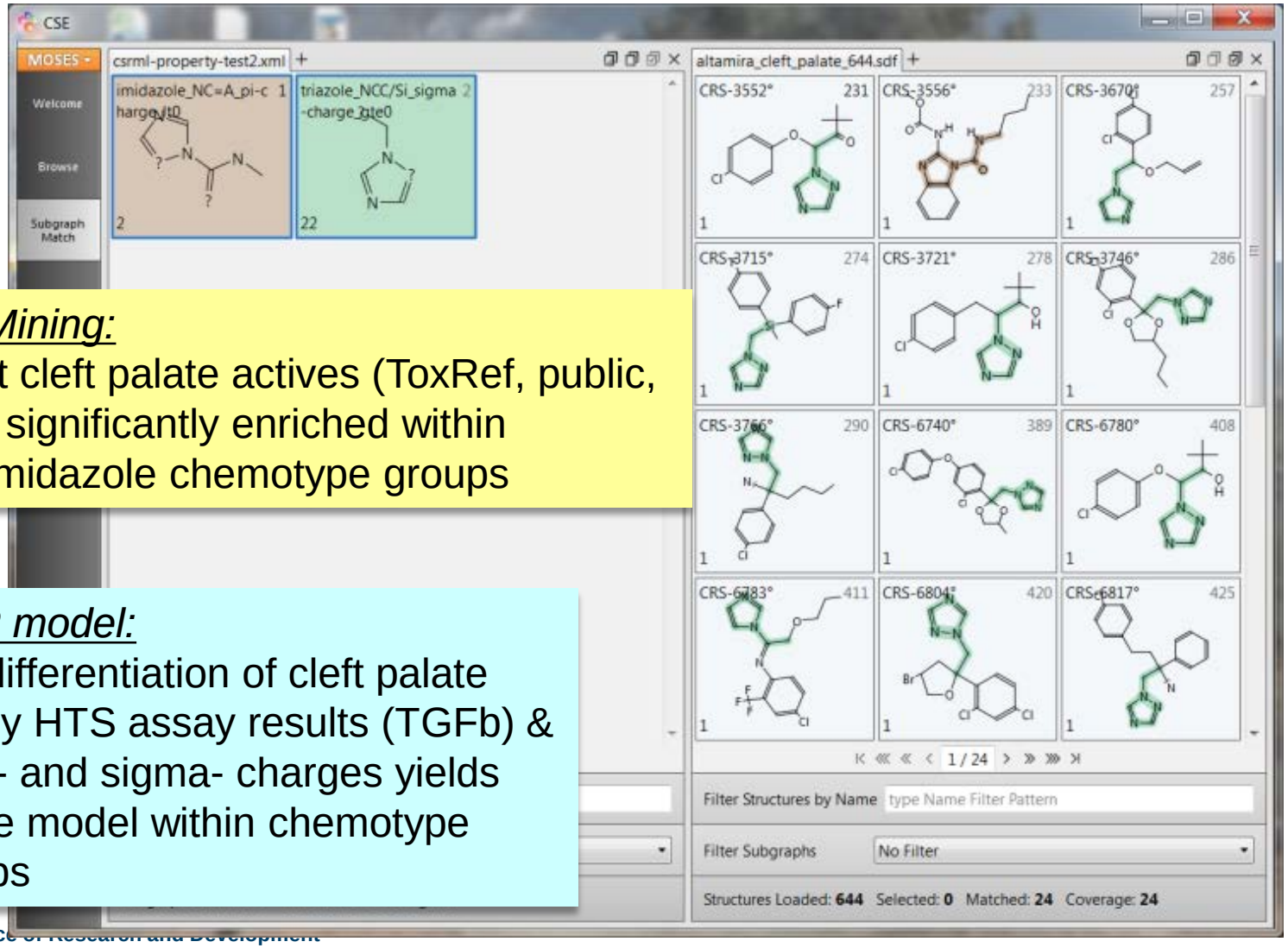
Use S1 {CT} set to predict chemical space likely to require metabolic activation for Rat Carcinogenicity

S2: Direct acting & inactives (157 cmpds)

**Altamira beta version of ToxPrint Chemotypes*

ToxCast Phase I (291 total) Rat Carcinogenicity Study using ToxRefDB & Meteor:Derek workflow, *Volarath et al.*

Chemotype Activity Profiling: e.g. Data mining & QSAR models



1. Data Mining:

Tox21 rat cleft palate actives (ToxRef, public, CERES) significantly enriched within triazole/imidazole chemotype groups

2. QSAR model:

Further differentiation of cleft palate actives by HTS assay results (TGFb) & partial pi- and sigma- charges yields predictive model within chemotype subgroups

C Yang et al., Altamira

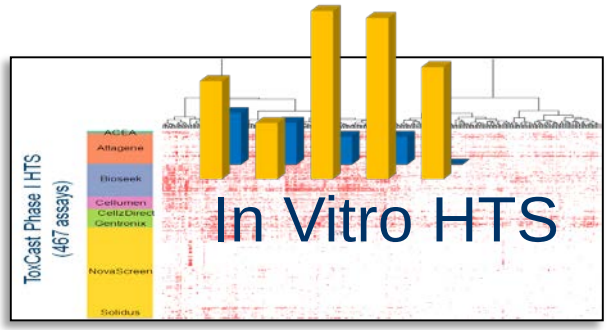
Building a public chemotype {CT} "knowledge- base"

3. Compute relative {CT} enrichments for chemical sets of interest

- *Use categories (exposure), e.g. Flame Retardants*
- *Activity subsets*
- *Metabolically activated toxicants*
- *Assay-assay associations to inform pathways*

2. Precompute CT- Assay enrichments for data inventories of interest

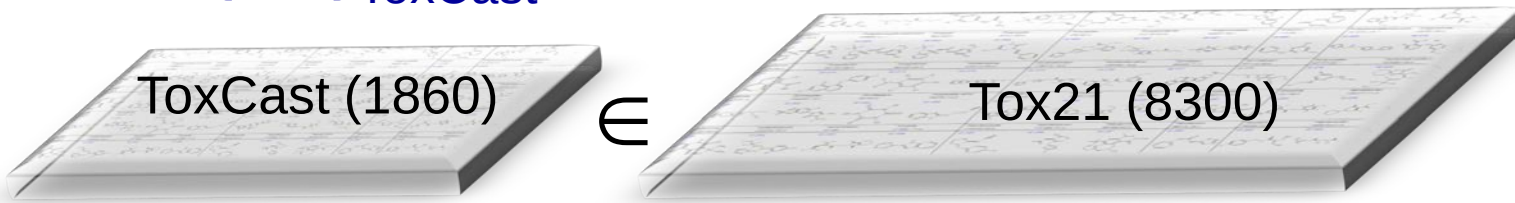
{CT-Assay}$_{ToxCast,Tox21}$



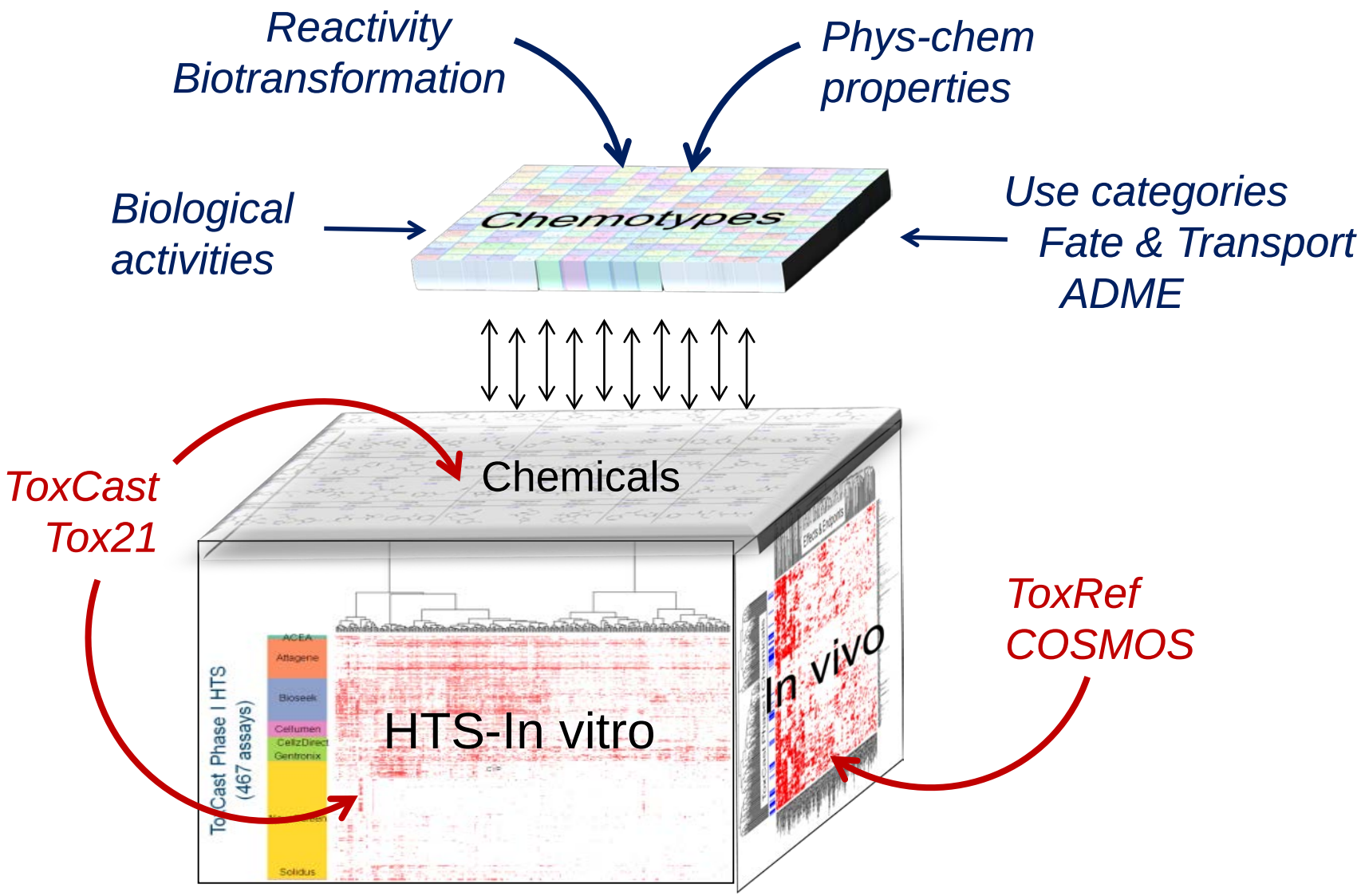
1. Precompute CT profiles for chemical inventories of interest

{CT}$_{ToxCast}$

{CT}$_{Tox21}$



Building a public chemotype "knowledge- base"



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  - Chris Grulke (DSSTox, Chemotypes)
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- Flame Retardants
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  - Altamira: Jim Rathman
  - U.S. FDA: Kirk Arvidson, Patra Volarath

This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.