

# *Analytical QC Challenges in EPA's Computational Toxicology Program: ToxCast data to environmental samples*

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*August 13-15, 2018*

*Research Triangle Park, NC*

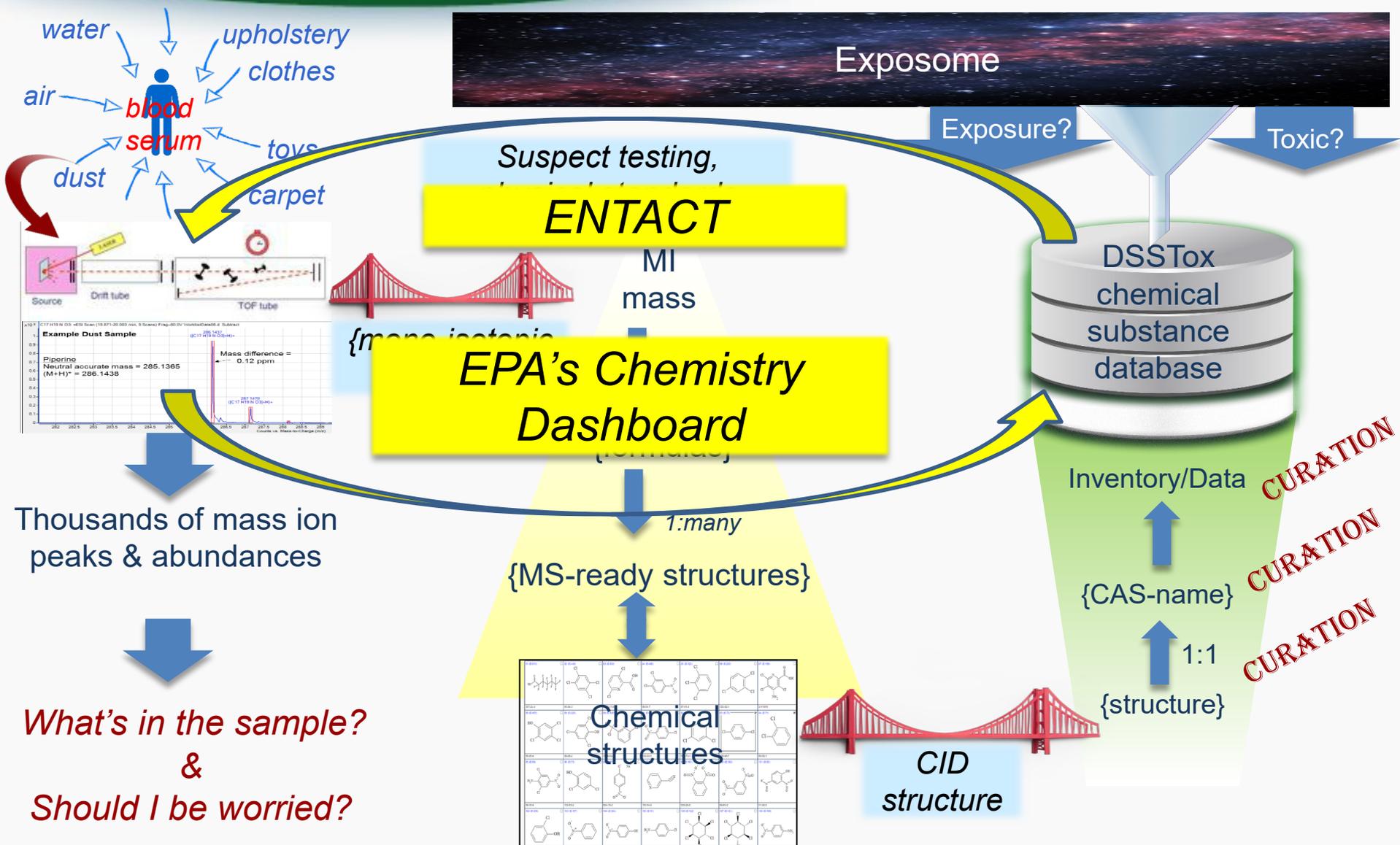
# Lots of questions ...

Tuesday, August 14, 2018

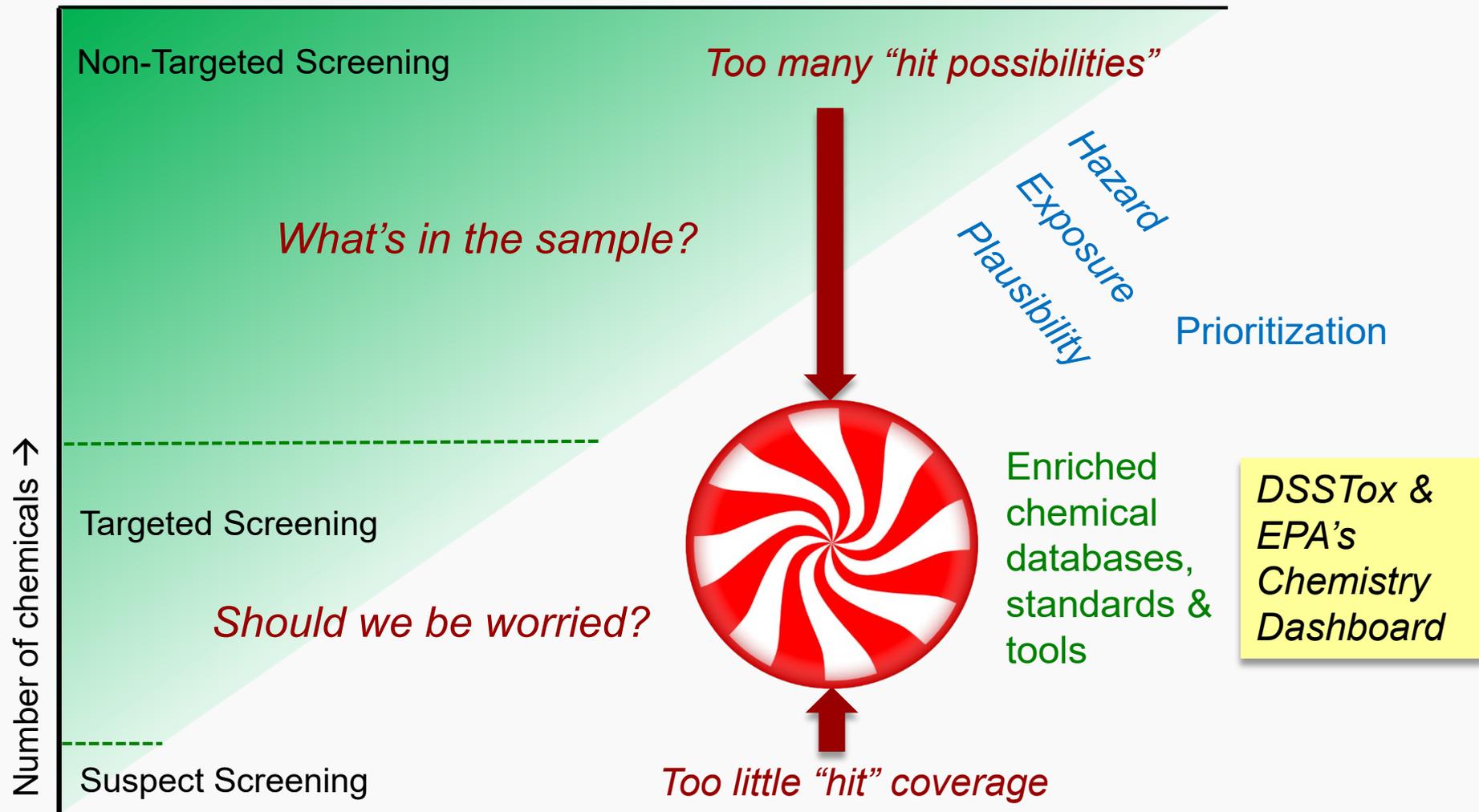
Judge a **person** by **their**  
questions rather than  
**their** answers.

— Voltaire **(post-enlightenment)**

# Cheminformatics view of non-targeted testing problem

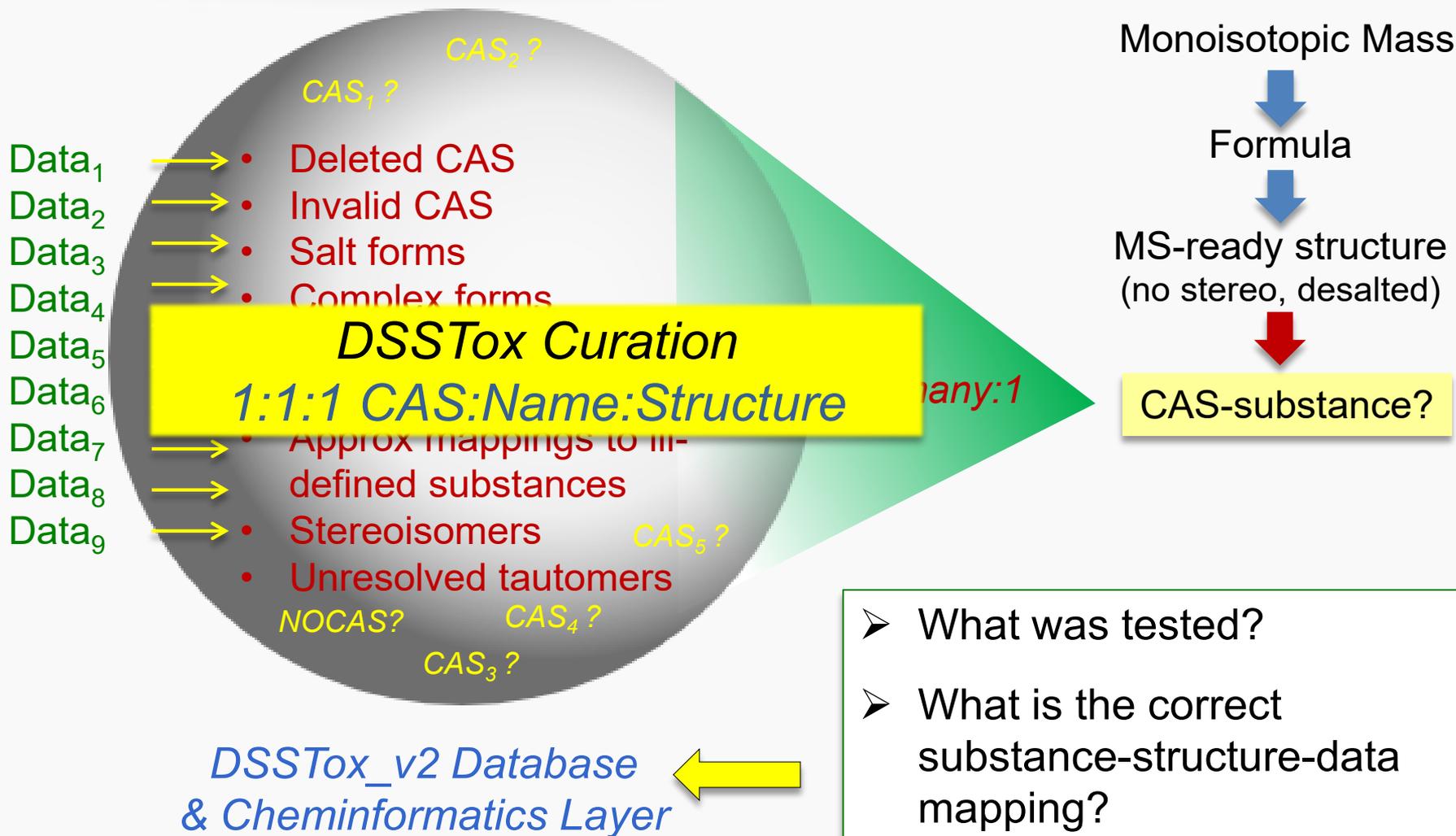


# Cheminformatics view of non-targeted testing problem

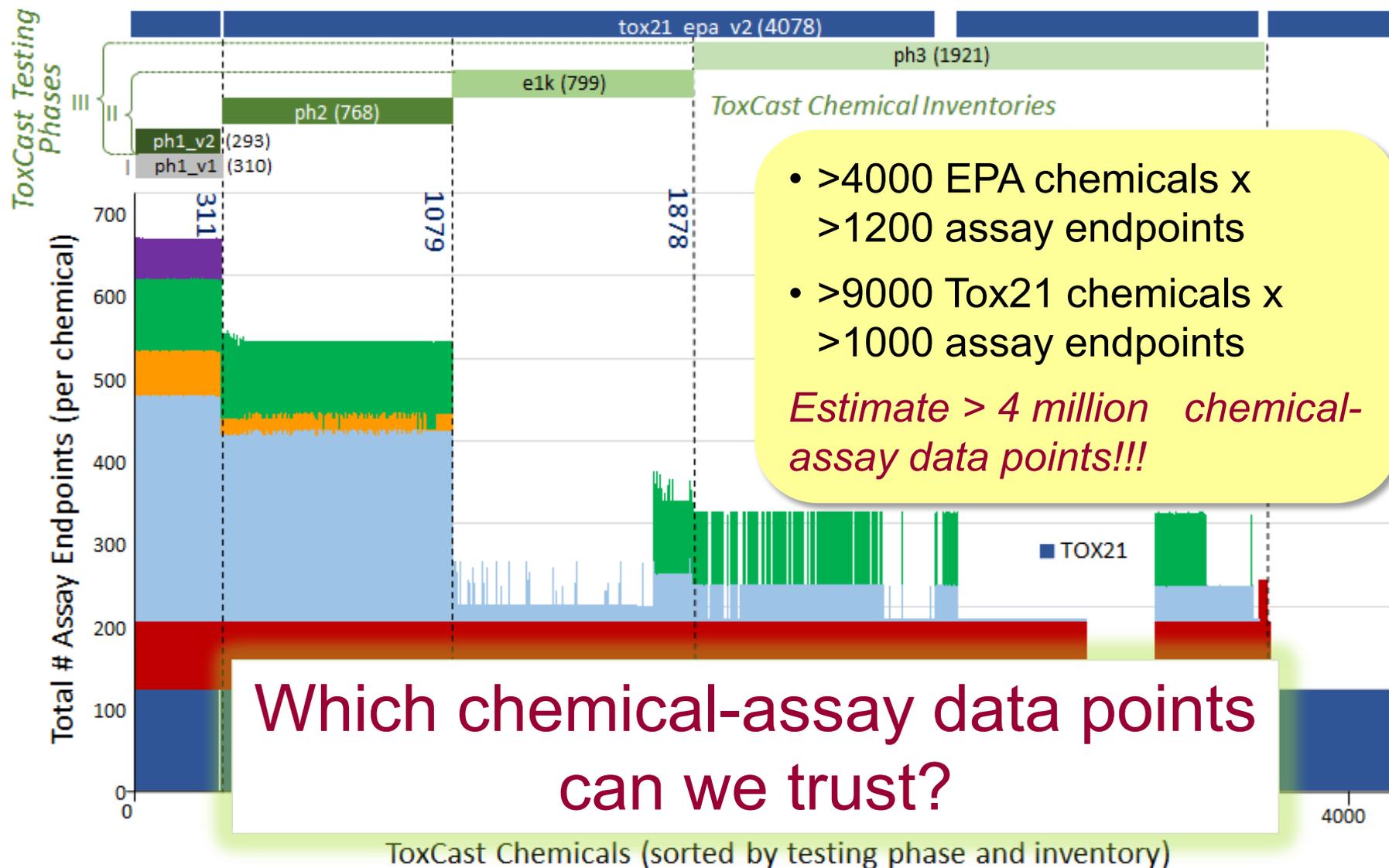


# CAS-Structure

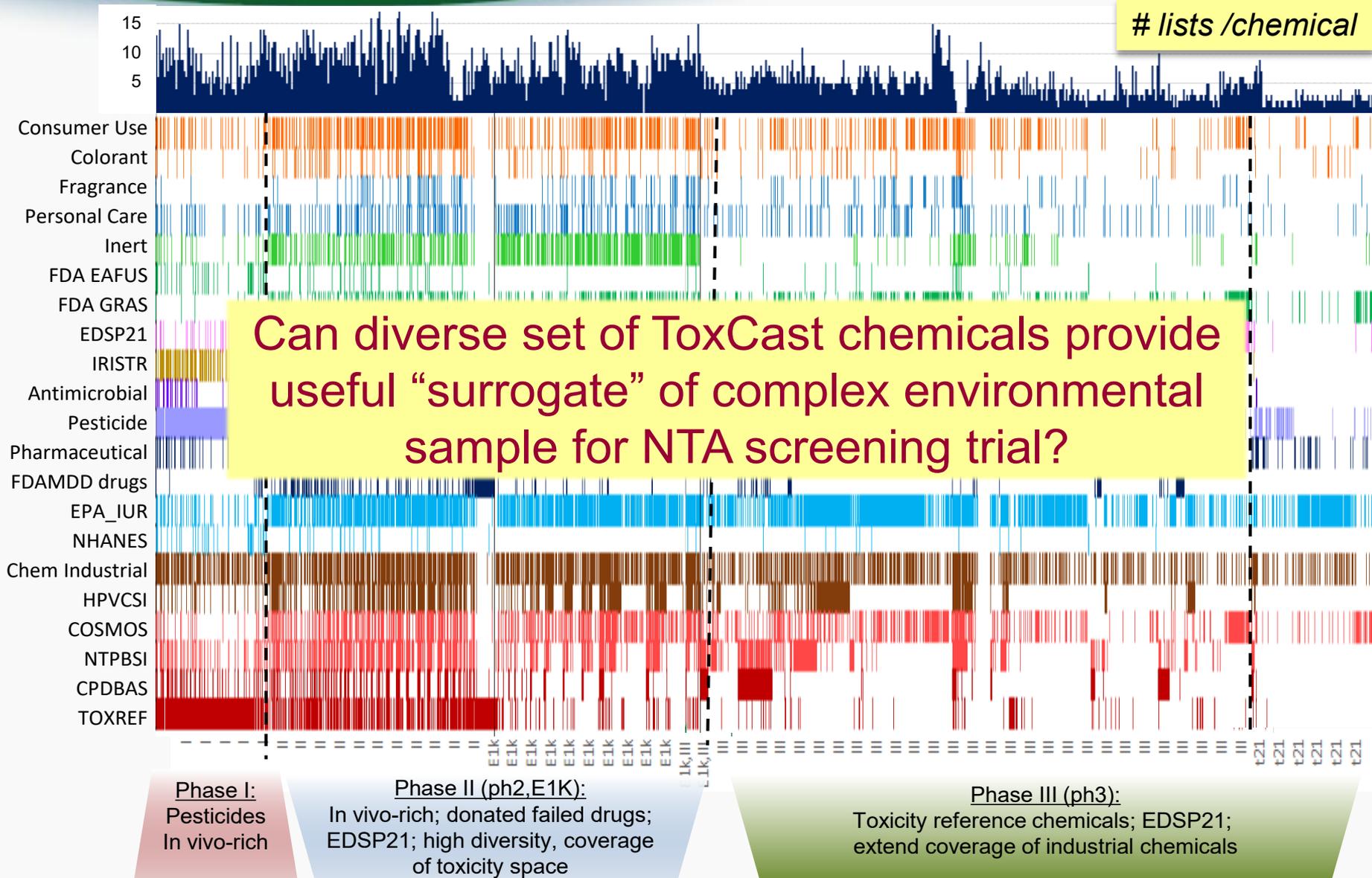
## “Sphere of Confusion”



# ToxCast HTS data



# ToxCast Chemical Coverage: Use, Exposure, Toxicity



# lists /chemical

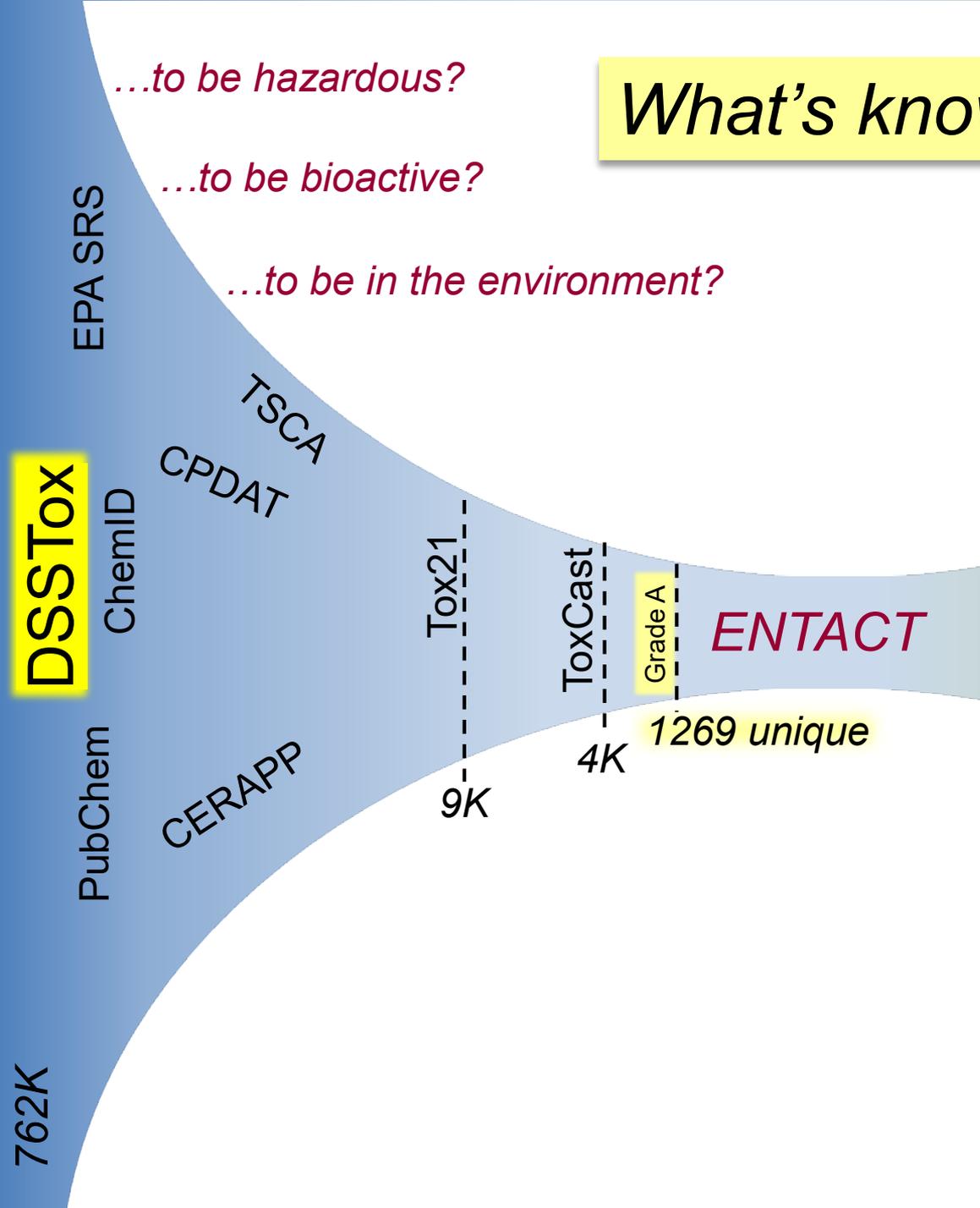
Can diverse set of ToxCast chemicals provide useful “surrogate” of complex environmental sample for NTA screening trial?

**Phase I:**  
Pesticides  
In vivo-rich

**Phase II (ph2, E1K):**  
In vivo-rich; donated failed drugs;  
EDSP21; high diversity, coverage  
of toxicity space

**Phase III (ph3):**  
Toxicity reference chemicals; EDSP21;  
extend coverage of industrial chemicals

# What's known?



*...to be hazardous?*

*...to be bioactive?*

*...to be in the environment?*

**DSSTox**

EPA SRS

TSCA

CPDAT

ChemID

Tox21

ToxCast

Grade A

**ENTACT**

PubChem

CERAPP

9K

4K

1269 unique

762K

# What's known?

...to be hazardous?

...to be bioactive?

...to be in the environment?

...to be detectable by  
LCMS?  
GCMS?

...to require special  
methods or  
analysis?

**DSSTox**

EPA SRS

TSCA

CPDAT

ChemID

Tox21

ToxCast

Grade A

**ENTACT**

1269 unique

?  
?

m/z cloud

NIST MS library

**NTA**

**Exposome**

GCMS LCMS

MetFrag

PubChem

CERAPP

9K

4K

8K

15K

What's in our samples?

What Dashboard capabilities are available?

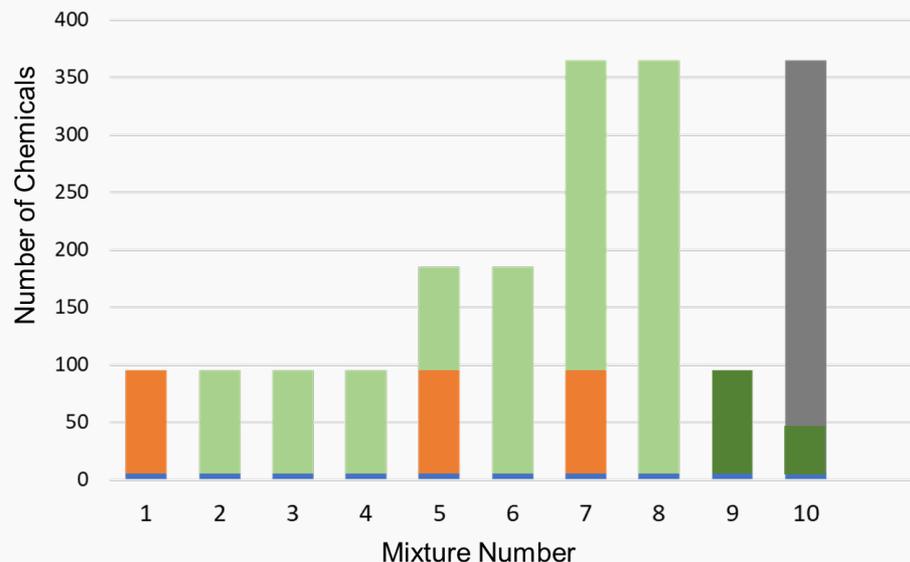
What happens to cmpds when we create mixtures?

What are our analytical methods capable of detecting?

?  
?

762K

# ENTACT Mixture Trial



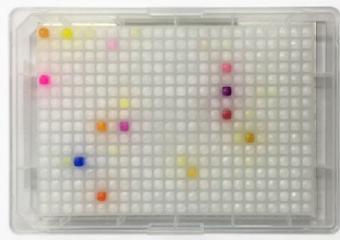
1269 unique ToxCast chemicals used to construct 10 mixtures

Chemicals in Mix 1-8 (amenable):

- *“Grade A” analytical QC results (LC/GC only)*
- *single DSSTox structure*
- *contain no metals*
- *chosen to avoid identification issues*
- *broad range of logP values*

Chemicals in Mix 9,10 (challenging):

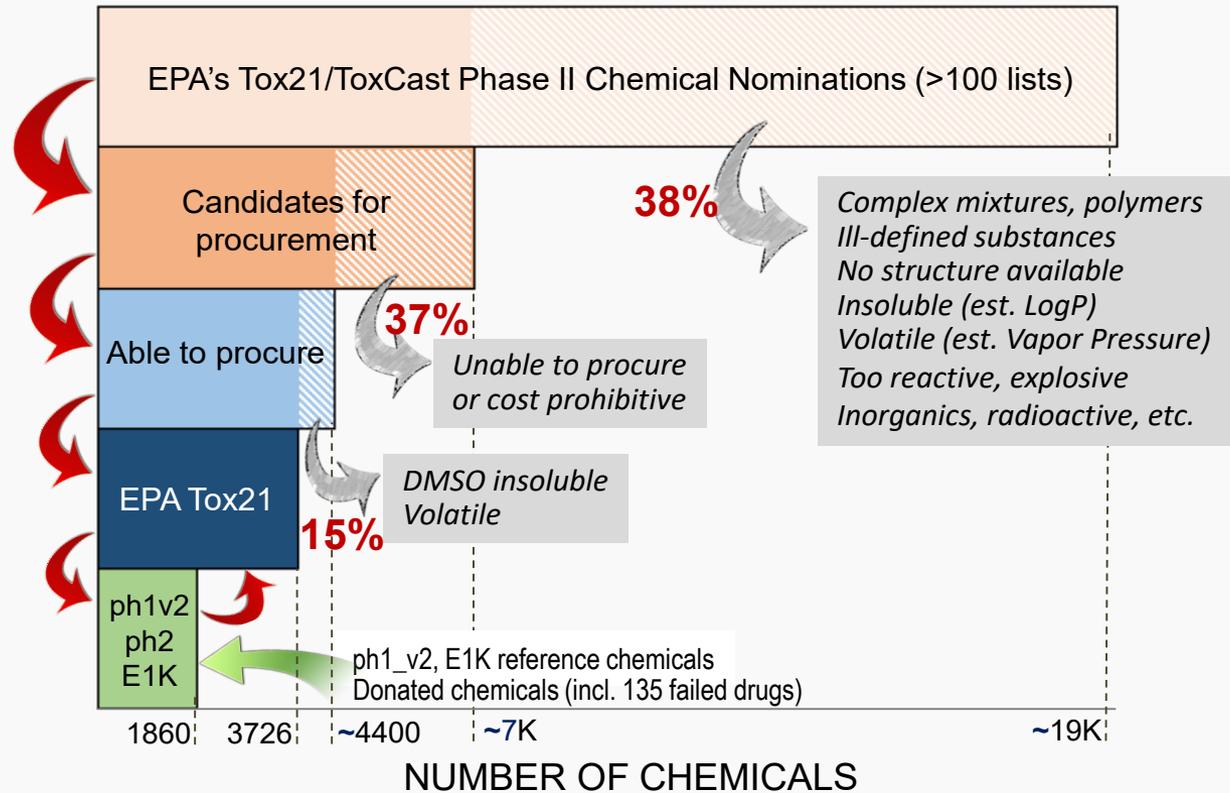
- *contain isomeric & isobaric cmpds*
- *contain cmpds graded as <80% purity*
- *contain a few organometallics*

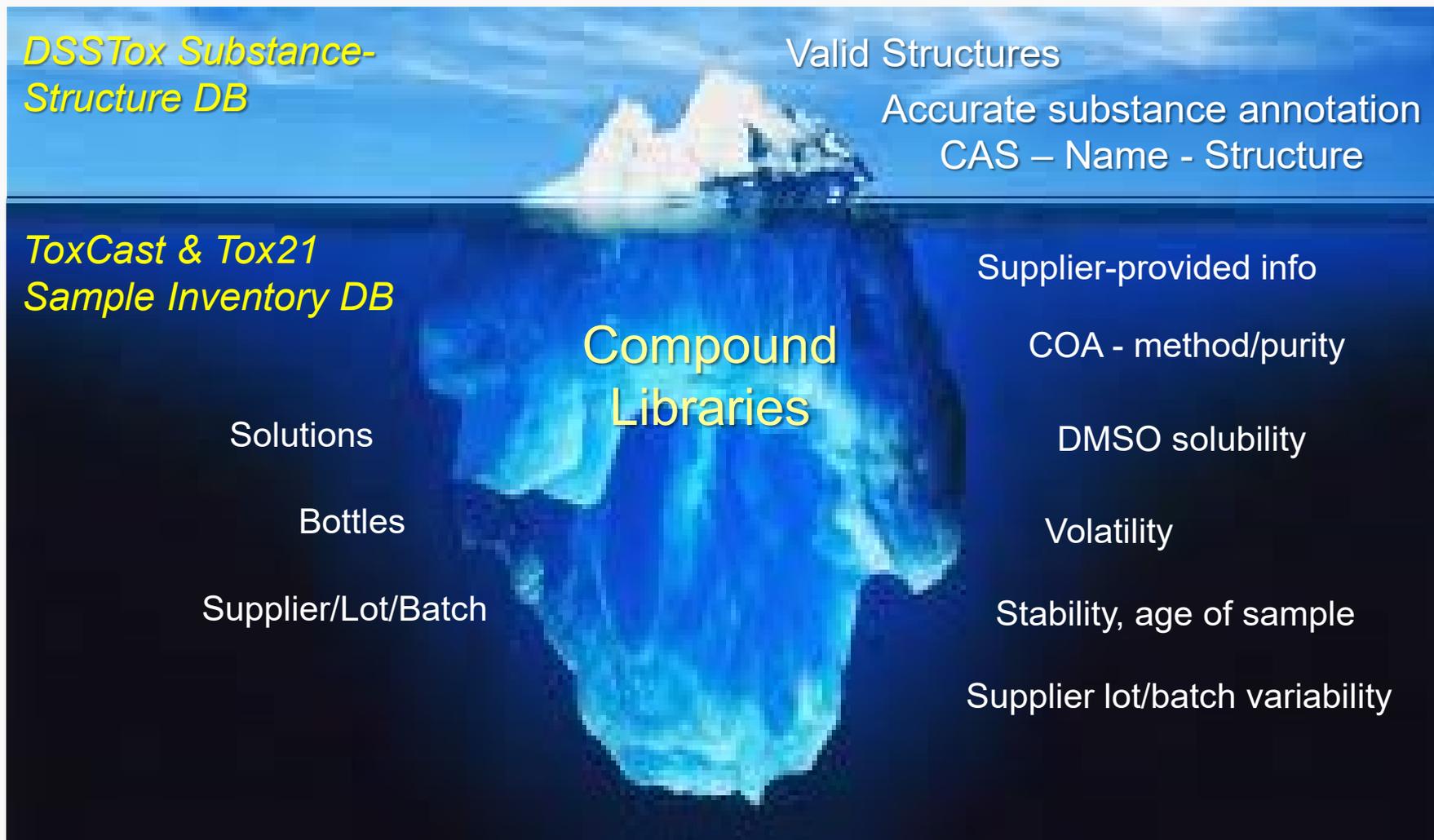


# Bias in ToxCast Library Build

EPA ACToR  
EPA DSSTox  
EPA Program Offices  
OECD, EU  
Stakeholder Nominations

Endocrine disruptor lists  
FDA in vivo data sets  
Plasticizers  
Flame retardants  
Drugs, Failed Drugs  
Fragrances  
FDA Food Additives  
High production volume  
Industrial chemicals  
Reference chemicals  
Water contaminants  
Antimicrobials  
Pesticides





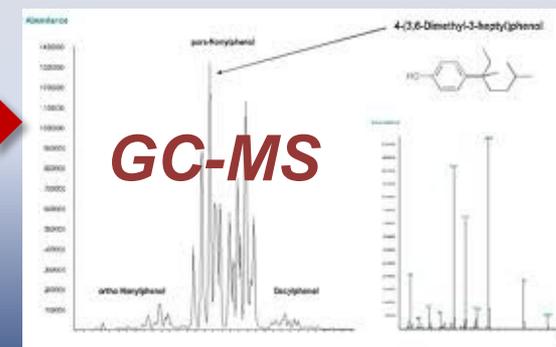


Confirm parent ion peak  
and >90% purity

A copy of each parent Tox21 384 well plate is  
subjected to analytical QC for assessing purity,  
identity, concentration, stability

Fail, inconclusive or analytical  
method inappropriate

Retest at 4 mo. time point under  
assay conditions for stability



Publish QC summary results in  
association with assay data

*Work performed under  
NIH Contract with  
OpAns, Durham, NC*

# What can analytical QC data tell us?

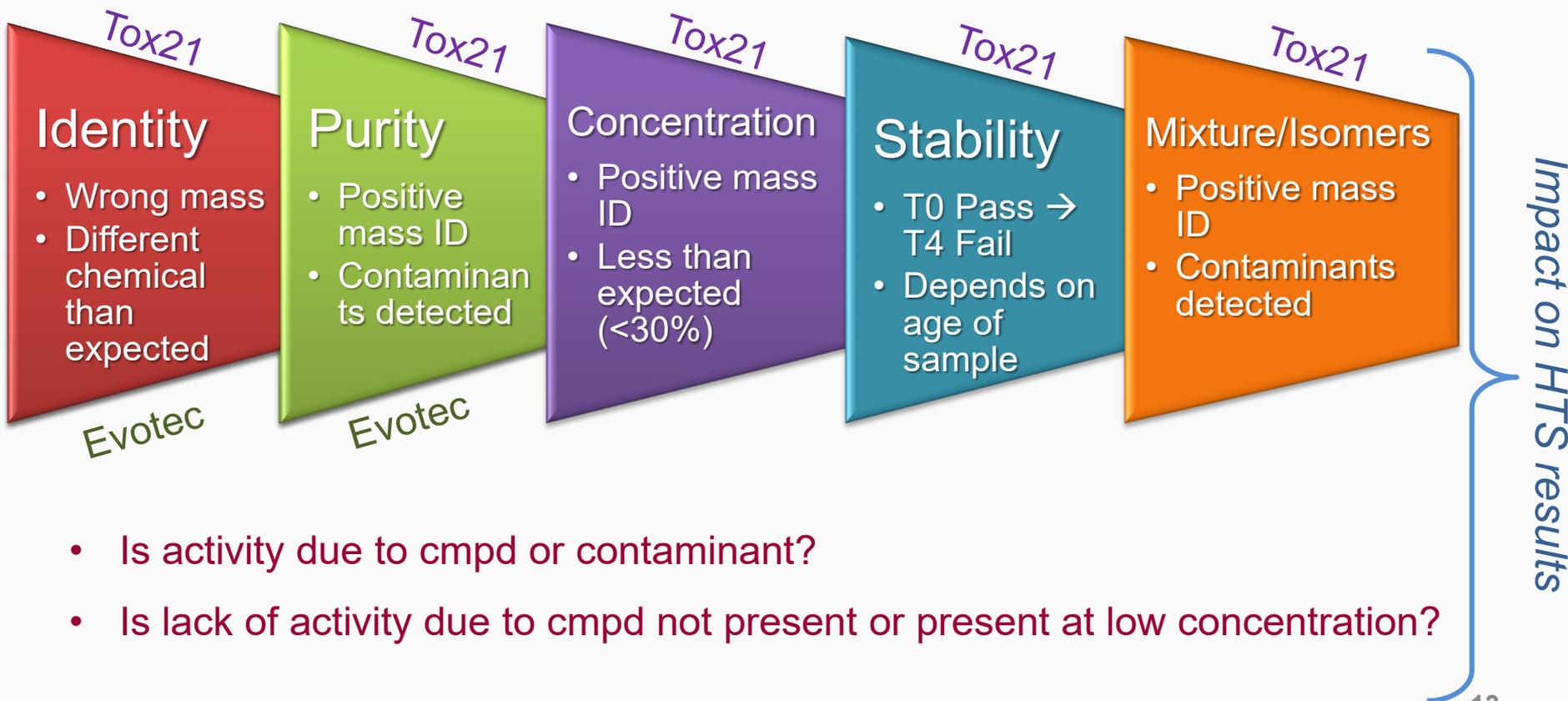
## Sample-specific

- purity
- age of sample
- supplier variability
- plating errors



## Compound - specific

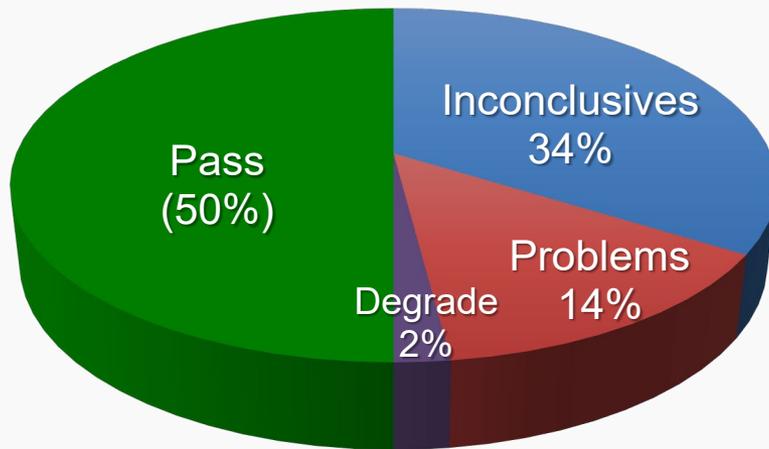
- volatility
- reactivity
- limited solubility in DMSO
- adheres to plastic



- Is activity due to cmpd or contaminant?
- Is lack of activity due to cmpd not present or present at low concentration?

# Tox21 and ToxCast Chemical Library Analytical QC Results (8/2015)

Tox21\_QC\_Sum-GSID (8593 total)



- 50% pass purity/ID/concentration checks
- A third(34%) of library pose analytical QC challenges (LCMS and GCMS only)
- 2% degrade after 4 months under testing conditions
- 14% problems - purity (<75%), ID and/or low concentration (<30% of expected [C])

- *Which chemicals have QC issues? (e.g., SVOCs?)*
- *Which chemicals were not analyzed? (e.g., mixtures, inorganics, etc.)*
- *How are HTS activity profiles linked to QC?*

# Tox21 Analytical QC Data

<https://tripod.nih.gov/tox21/>

QC Grade T0

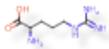
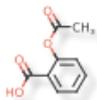
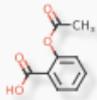
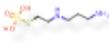
12495 Tox21 IDs

... 8 9 10 11 ... 1249 1250 »

- A** 6372
- ND** 4230
- Fns** 403
- D** 272
- Ac** 254
- B** 154
- M** 135
- Fc** 131
- Z** 122

QC Grade T4

- ND** 6989
- A** 4385
- B** 207
- D** 139
- M** 134
- Z** 133
- C** 127
- Ac** 115
- I** 77

Structure	Tox21 ID	Name	QC Grade T0	QC Grade T4
	Tox21_113046	L-Arginine	<b>Z</b> MW Confirmed, No Purity Info	<b>Z</b> MW Confirmed, No Purity Info
	Tox21_110076	Aspirin	<b>Fc</b> CAUTION, Very Low Concentration Concentration <5% of expected value Biological Activity Unreliable	<b>ND</b> Not Determined Analytical analysis is in progress
	Tox21_300146	Aspirin	<b>A</b> MW Confirmed, Purity > 90%	<b>ND</b> Not Determined Analytical analysis is in progress
	Tox21_202117	Aspirin	<b>A</b> MW Confirmed, Purity > 90%	<b>ND</b> Not Determined Analytical analysis is in progress
	Tox21_110077	Amifostine	<b>Fns</b> CAUTION, No Sample Detected	<b>Fc</b> CAUTION, Very Low Concentration Concentration <5% of expected

# Chemistry Dashboard

## Bisphenol A

## Bisphenol A

80-05-7 | DTXSID7020182

Searched by DSSTox Substance Id.

### DETAILS

EXECUTIVE SUMMARY

PROPERTIES

ENV. FATE/TRANSPORT

HAZARD

▶ ADME

▶ EXPOSURE

▶ BIOACTIVITY

SIMILAR COMPOUNDS

GENRA (BETA)

RELATED SUBSTANCES

SYNONYMS

▶ LITERATURE

LINKS

COMMENTS

### General

- EPA Substance Registry Service
- Household Products Database
- Chemical Entities of Biological Interest (ChEBI)
- PubChem
- Chempider
- CPCat
- DrugBank
- HMDB
- Wikipedia
- MSDS Lookup
- ChEMBL
- Chemical Vendors
- CalEPA Office of Environmental Health Hazard Assessment
- NIOSH Chemical Safety Cards
- ToxPlanet
- ACS Reagent Chemicals
- Wikidata
- ChemHat: Hazards and Alternatives Toolbox
- Wolfram Alpha
- ScrubChem
- ECHA Brief Profile
- ECHA Infocard
- ChemAgora
- Consumer Product Information Database (CPID)
- ChEBI
- Sigma-Aldrich Chemicals
- NIST Chemistry Webbook

### Toxicology

- ACToR
- DrugPortal
- CCRIS
- ChemView
- CTD
- eChemPortal
- Gene-Tox
- HSDB
- ToxCast Dashboard 2
- LactMed
- International Toxicity Estimates for Risk
- ATSDR Toxic Substances Portal
- Superfund Chemical Data matrix
- NIOSH IDLH Values
- ACToR PDF Report
- Toxics Release Inventory
- CREST
- National Air Toxics Assessment

### Publications

- Toxline
- Environmental Health Perspectives
- NIEHS
- National Toxicology Program
- Google Books
- Google Scholar
- Google Patents
- PPRTVWEB
- PubMed
- IRIS Assessments
- EPA HERO
- NIOSH Skin Notation Profiles
- NIOSH Pocket Guide
- RSC Publications
- BioCaddie DataMed
- Springer Materials
- Federal Register
- Regulations.gov
- Bielefeld Academic Search Engine
- CORE Literature Search

### Analytical

- FOR-IDENT
- NEMI: National Environmental Methods Index
- RSC Analytical Abstracts
- MONA: MassBank North America
- mzCloud
- NIST IR Spectrum
- NIST MS Spectrum

### Prediction

- 2D NMR HSQC/HMBC Prediction
- Carbon-13 NMR Prediction
- Proton NMR Prediction
- ChemRTP Predictor

Tox21 analytical data

# Chemistry Dashboard

Secure | <https://tripod.nih.gov/tox21/samples/80-05-7>

Apps | Chemistry Dashboard | CompTox (zn) Dashbo | ChemReg\_v0.9.6 | ChemTrack | Confluence - NCCT KI | JIRA | Other bookmark

**Tox21**

Home / Tox21 Samples / Tox

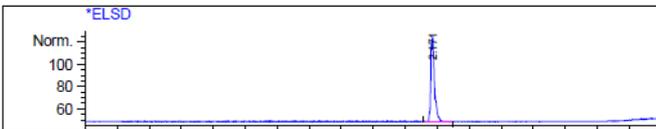
## Bisphenol A

ID Tox21\_400088 Plate Batch2-SP109801 Well P1-A-03 File SP109801\_A03.D Inj Date: 9 Sep 11 3:21 pm - MF C15H16O2 MW 228.1 Expected Conc: 3.00 mM

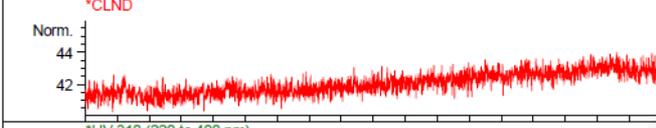
RT	Found	ELS%	UV %	ELS [mg/mL]	Adj [ELS]	[N mM]	Adj [CLN]	#N
2.17	Yes	100.0	100.0	1.16	5.09 mM			0.0
3.58		0.0	0.0					0.0

Comment: Passed

**ELSD**



**CLND**



**UV 310 (220 to 400 nm)**



**MS- 2.124:2.247 min**



**MS- 2.124:2.247 min**



**MS- 3.552:3.799 min**



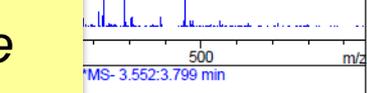
**MS- 3.552:3.799 min**



**MS- 3.552:3.799 min**



**MS- 3.552:3.799 min**



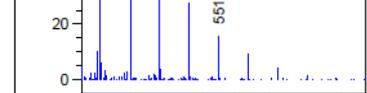
**MS- 3.552:3.799 min**



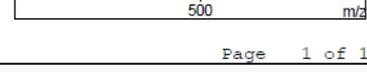
**MS- 3.552:3.799 min**



**MS- 3.552:3.799 min**



**MS- 3.552:3.799 min**

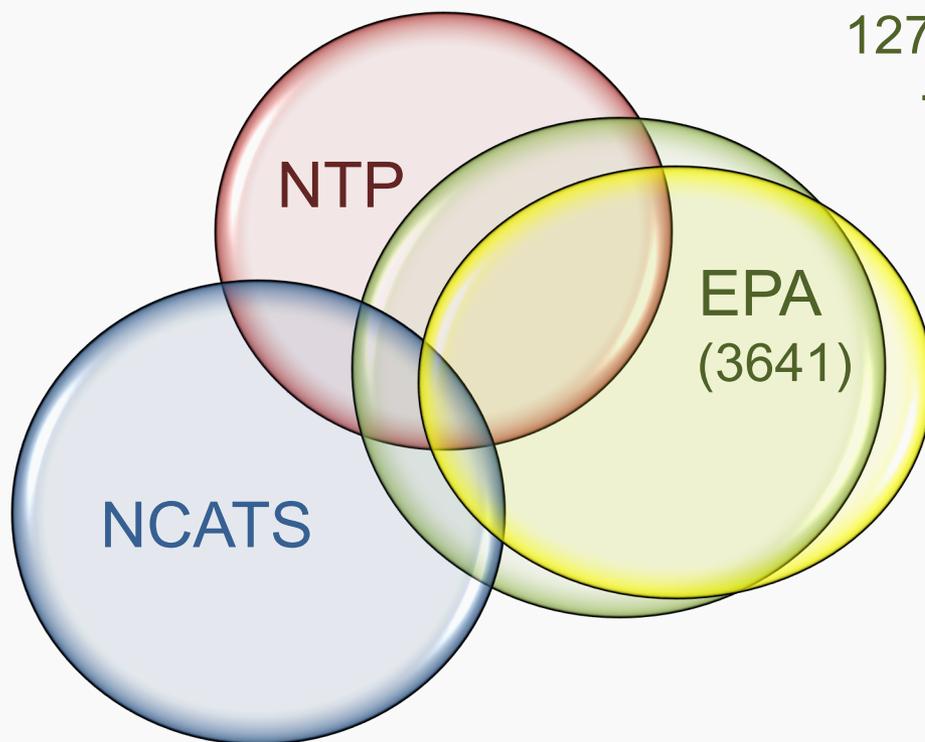


**MS- 3.552:3.799 min**



**MS- 3.552:3.799 min**

## **Tox21 NCATS (OpAns)** *LCMS, GCMS, NMR analyses*



12776 samples

→ 8598 unique CAS

→ 6681 (78%) graded

## **ToxCast (Evotec)** *HTP LCMS analysis*

3112 samples

→ 2857 unique CAS

→ 1589 (56%) graded

**1724/3641 EPA\_ToX21 chems graded (47%)**

# Evotec Analytical QC Data

gsid	20006	20014		
preferred_name	Acetaminophen	Dehydroacetic acid		
casrn	103-90-2	Purity_UV214 ESI POS MS+	96.24	100
Container TX	TX002867	Compound found (1=yes 0=no)	1	0
FileName SAMPLE	01129195	State	OK	Diversity fail
Date	15-Apr-15	Target MS+	152.0711	169.0501
Exact MF	C8H9NO2	Observed MS+	152.0573	
Exact MW	151.0633	Purity_UV214 ESI NEG MS-	96.24	100
Real MW(salted Average)	151.1626	Compound found	1	0
Salted MF				
Smiles				

How do we store, process, interpret, deliver & make use of all these data?

LCMS run in  
 4 detection  
 modes for  
 3125  
 samples

Compoundfound	1	0
State	OK	Diversity fail
Target MS+	152.0711	169.0501
Observed MS+	152.0573	
Purity_ELS ESI NEG MS-	100	52.86
Compound found	1	0
State	OK	Diversity fail
Target_MS-	150.0555	167.0345
Observed_MS-	150.0819	
Fail 90	no	yes
EPA_PDF_Reference File	SAM002476551_100.00perc.pdf	SAM002696017_0.00perc.pdf
Comments		

# Tox21 Grades: Confidence Levels

Grades	%Purity	Conc	QC-Detailed grade	QC-Detailed grade	
A	>90		Pass	Pass	
B	75-90				
C	50-75%				
Z	?				
T0-T4 fail					
D	<50%		Pass_NoPurityInfo	Caution	
Ac	90	5-30% of expected	Pass_degrades		
Bc	80		Caution_LowPurity		
Cc	75		Caution_LowConc		
M	?		Mixture		Caution_Mixture
Fc	?	<5% of expected	Caution_VeryLowConc		
F	wrong ID		Caution_WrongID		
Fns	no sample		Caution_NotDetected		
ND	Not determined		Tox21_ND		Tox21_ND

Positive MW identification

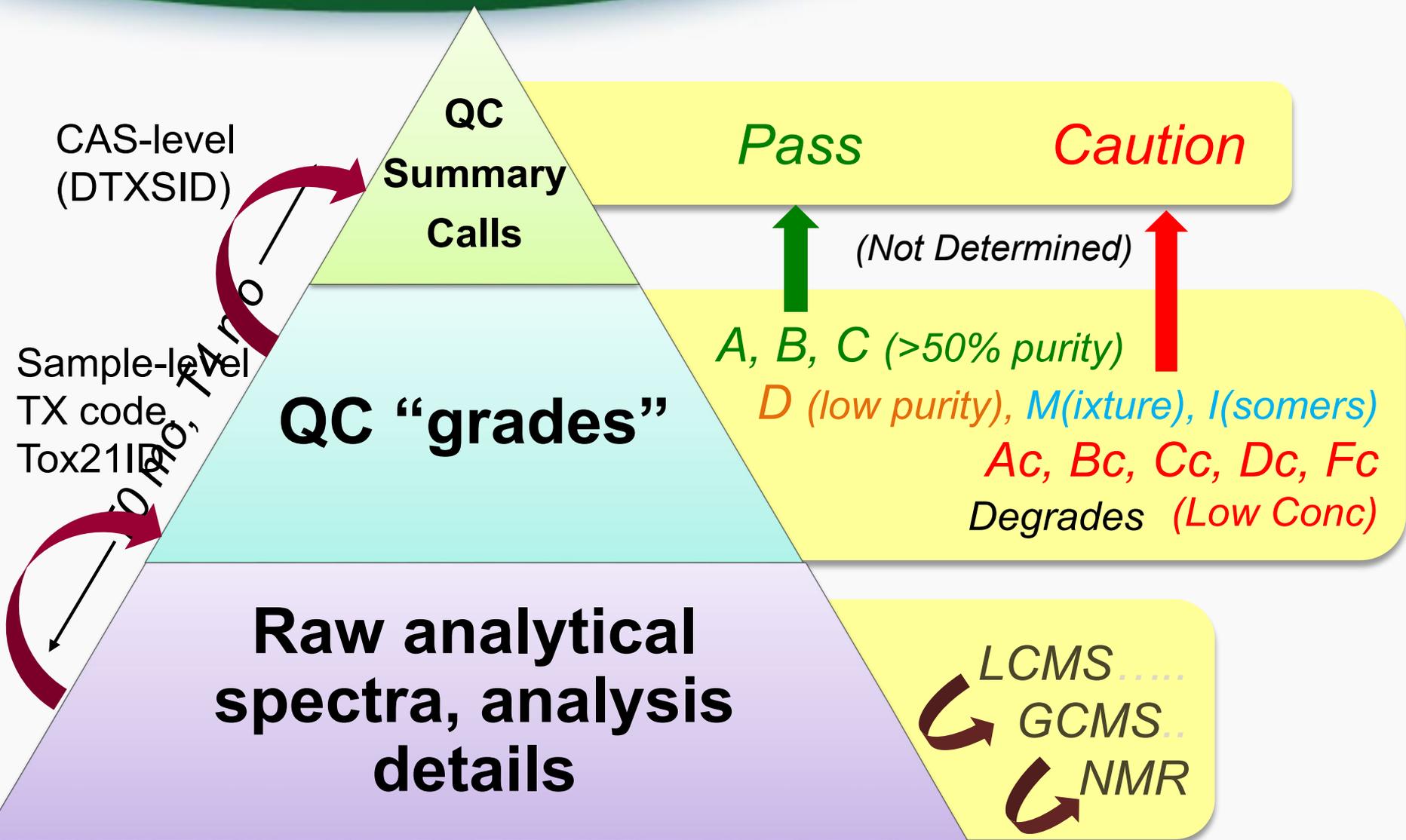
Decreasing confidence in sample

Increasing confidence in ToxCast HTS results

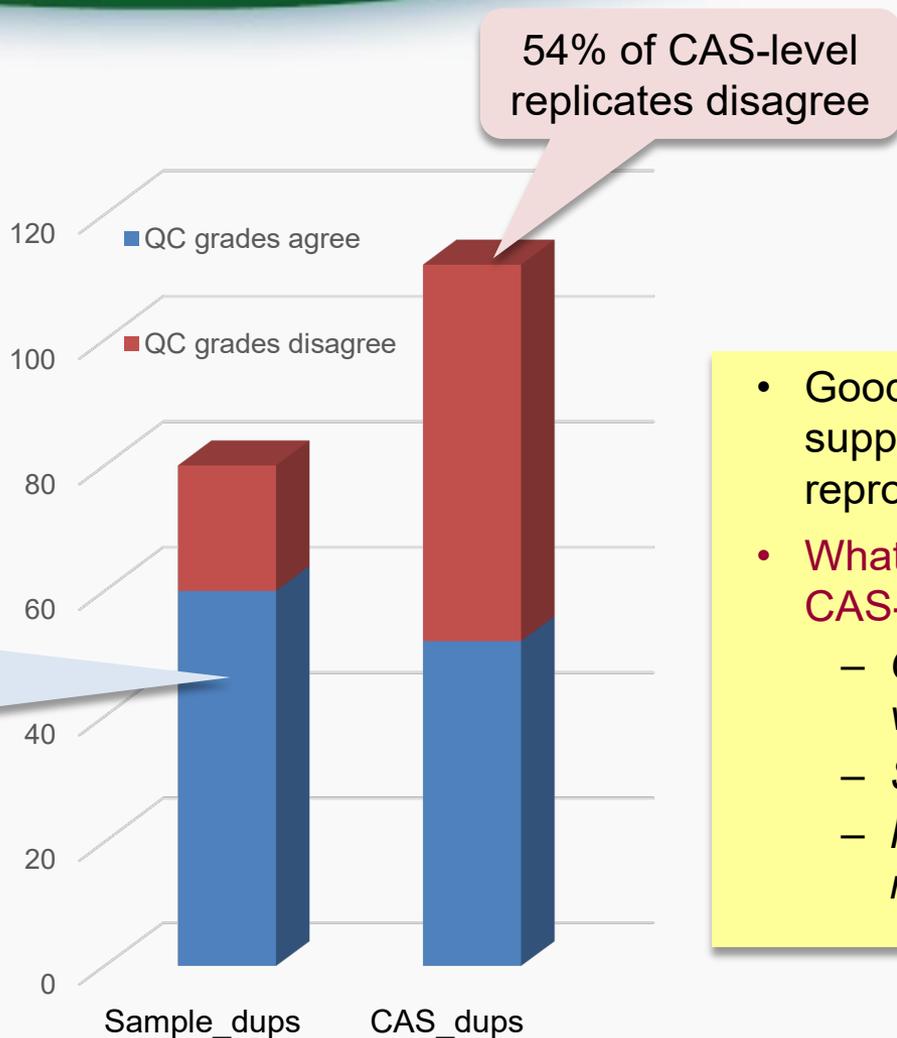


What level of reporting conveys “useful” guidance & level of confidence in QC results along with assay results?

# Processing Tox21 QC Data



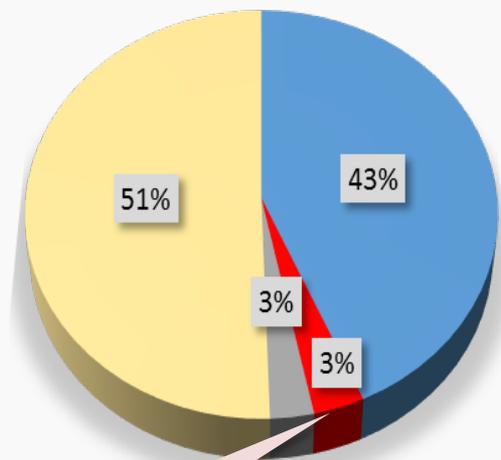
# Evotec QC Results: Reproducibility



- Good sample-level QC reproducibility supports analytical method reproducibility
- What accounts for poor CAS-level QC reproducibility?
  - Commercial source-sample variability
  - Sample age or history variability
  - More “rolling up” of multiple results

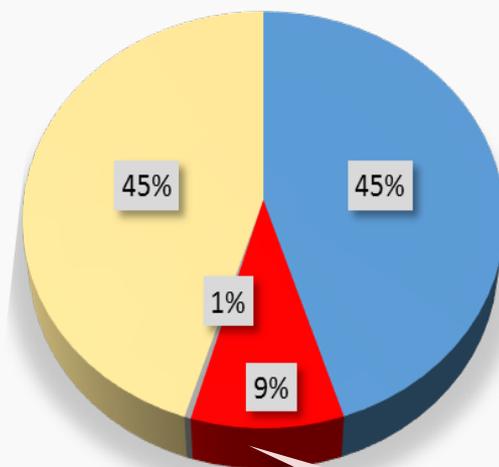
# Evotec QC (LCMS) Results

(1765 CAS)  
Phase II



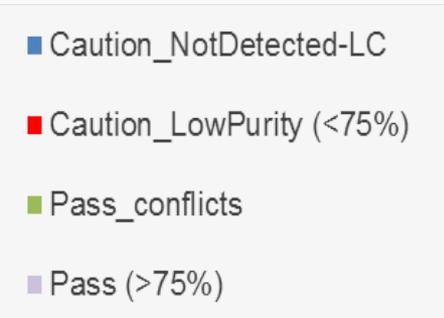
PhII - Few  
"Cautions"  
(Fails)

(1013 CAS)  
toxcast:ph3



ph3 - 3x as  
many "Cautions"  
(Fails)

Approx 45% Not detected by LCMS



# Use-cases benefiting from access to detailed QC data

- 1) ExpoCast projects attempting to detect ToxCast/Tox21 chemicals in consumer products or environmental samples
  - *knowledge of appropriate analytical method*
- 2) Use of ToxCast data to support safety assessments of individual chemicals with questionable QC (*e.g., 2,4,6-Triazole*)
- 3) Evaluate ToxCast HTS models (ER, Vascular) for sensitivity to results for chemicals with low QC (*e.g., TNP-40*)
- 4) Non-targeted screening projects of environmental & biological media (NERL)
  - *Reference spectra & knowledge of successful method for detecting chemical to guide analyses*
- 5) EPA chemical library management
  - *Prioritize reprocurments, solubilizations, platings*
  - *Propose QC analysis schedule for all/portions of library*

# Ongoing/future work with QC Data

(w/ Tony Williams & Andrew McEachran)

- ❑ Process & load all “raw” QC data into database to support structure-based modeling of analytical methods:
  - Ionization mode, chromatography/retention time, detector used

- ❑ Goals of analysis:

- Explain “Not Determined” and “Failed” QC labels

*Provide guidance for use of QC data to improve our science moving forward!*

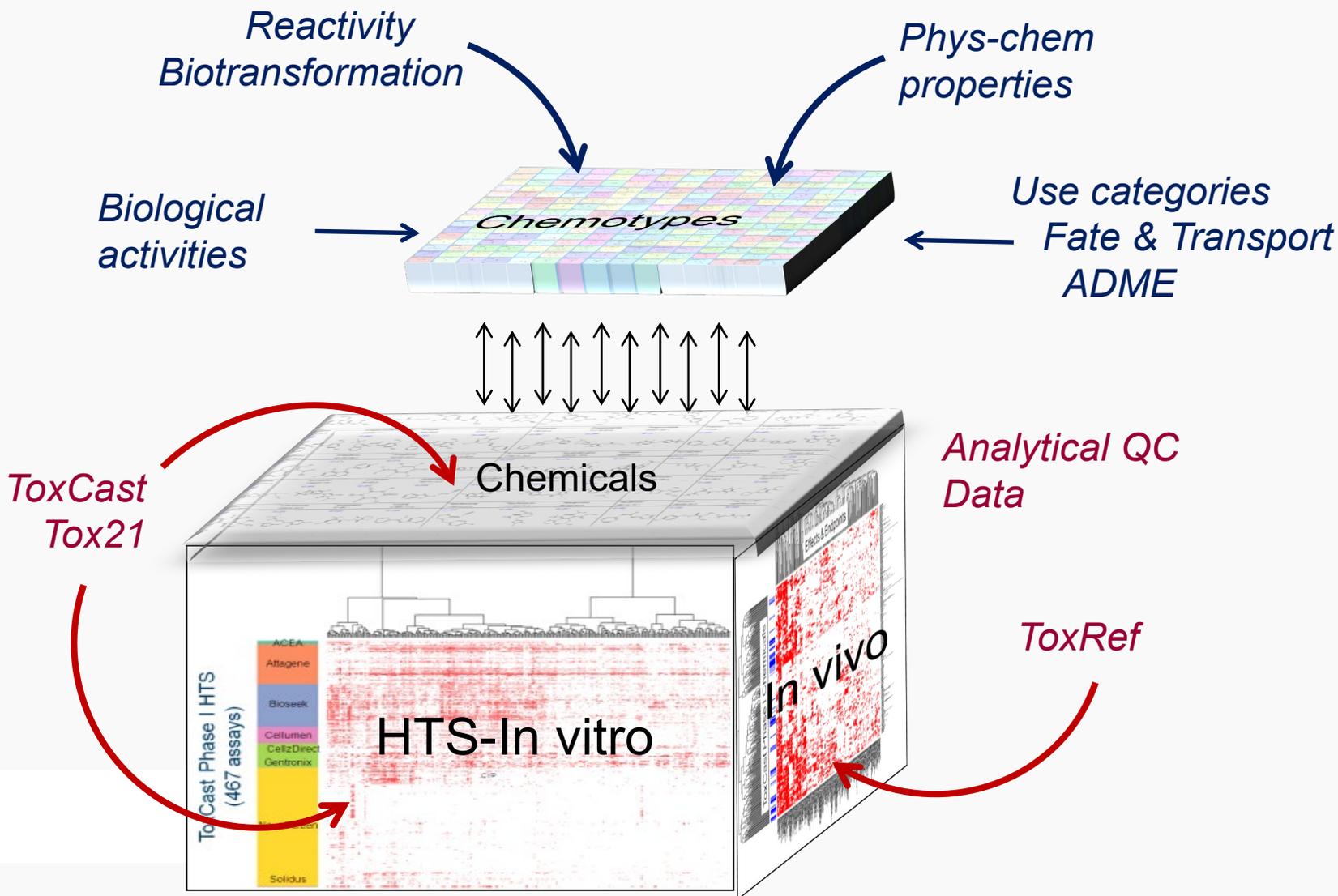
*And instrument conditions most suitable for analyzing chemical(s) of interest*

- *Reducing number of “Failed” QC labels and uncertainty in QC grades*

- ❑ EPA Chemistry Dashboard

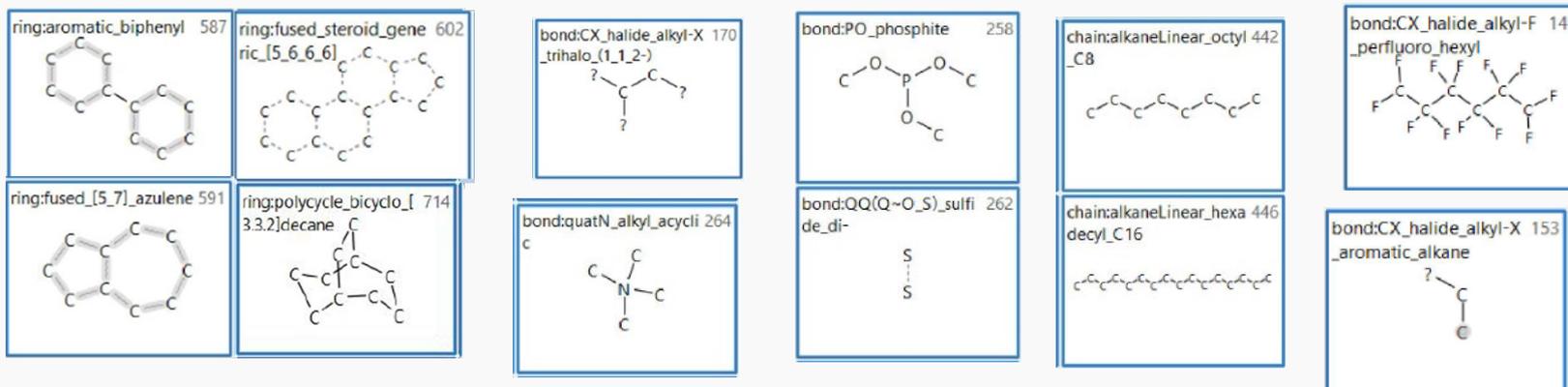
- Provide access to all available analytical QC results from CAS level summary grades to sample-level results
- Serve up predicted & actual MS spectra for >700K DSSTox chemicals for use in wide ranging environmental research projects

# Building a chemical “feature” knowledge-base



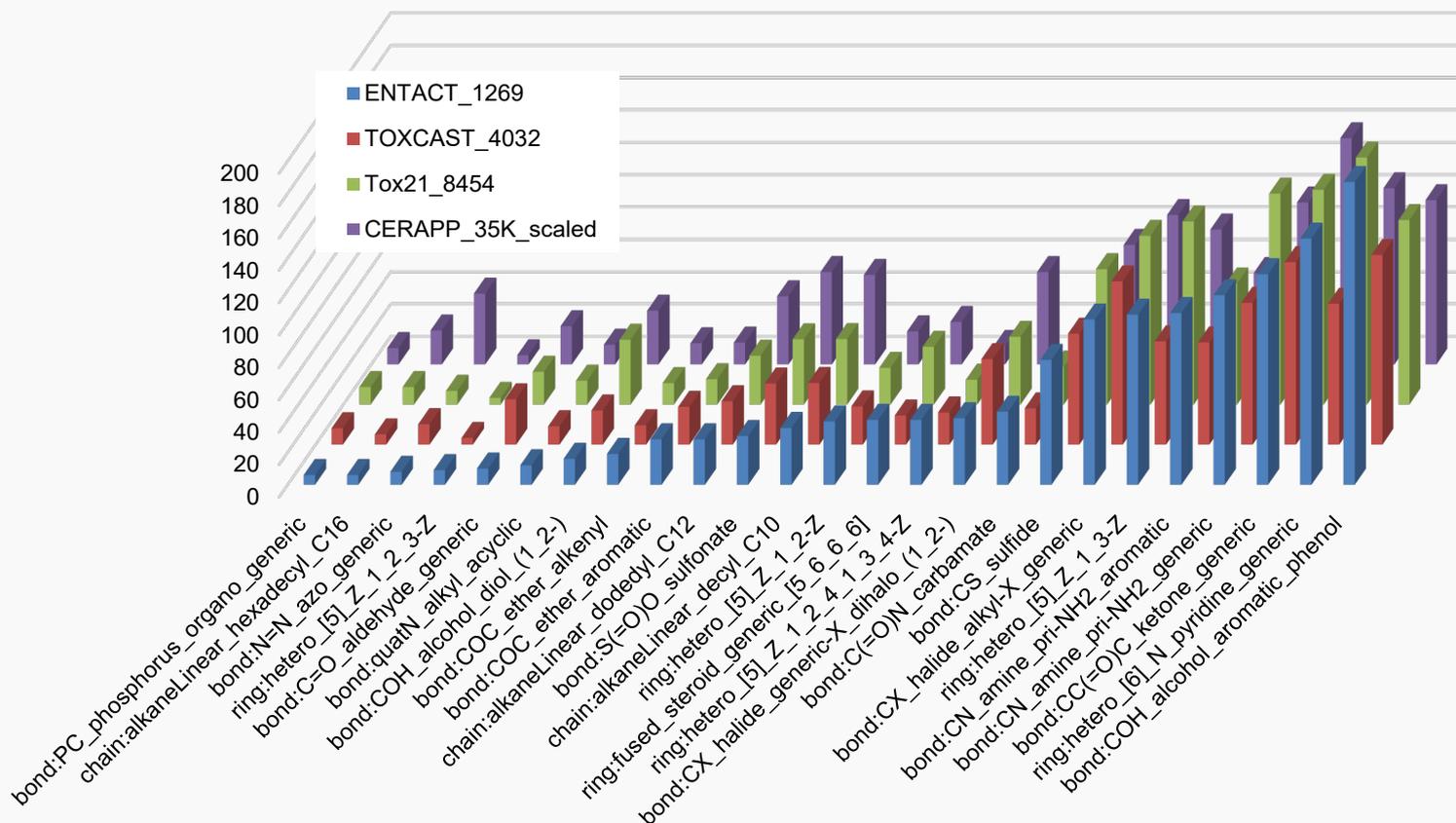
- 1) Publicly available Chemotyper application <http://www.chemotyper.org> & ToxPrint CSRML file <http://www.toxprint.org> for computing ToxPrint “fingerprints”, as implemented within EPA’s Chemistry Dashboard using Molecular Networks CORINA command-line code (Yang *et al.*, 2016)
- 2) ToxPrints include well-defined chemical features relevant to environmental datasets & toxicity (e.g., structure-alerts), and are computational, visualizable, and interpretable

*729 chemical features, capturing diverse chemistry:  
scaffolds, functional groups, chains, rings, bonding patterns, atom-types*



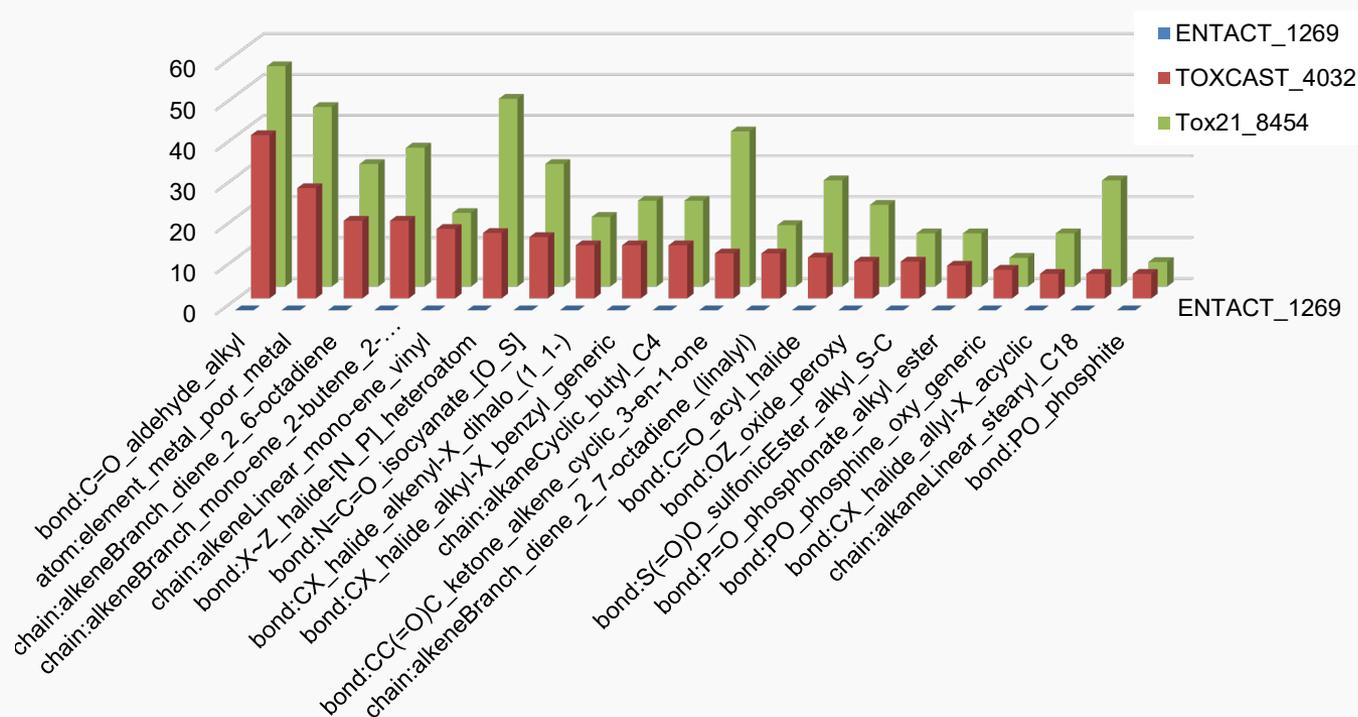
*→ structure-based means for defining regions of local chemistry.*

# ToxPrint inventory profile comparisons



*ENTACT chemical set has similar feature profile to larger ToxCast and DSSTox datasets, but ...*

## Top 20 ToxPrints missing in ENTACT Mixtures

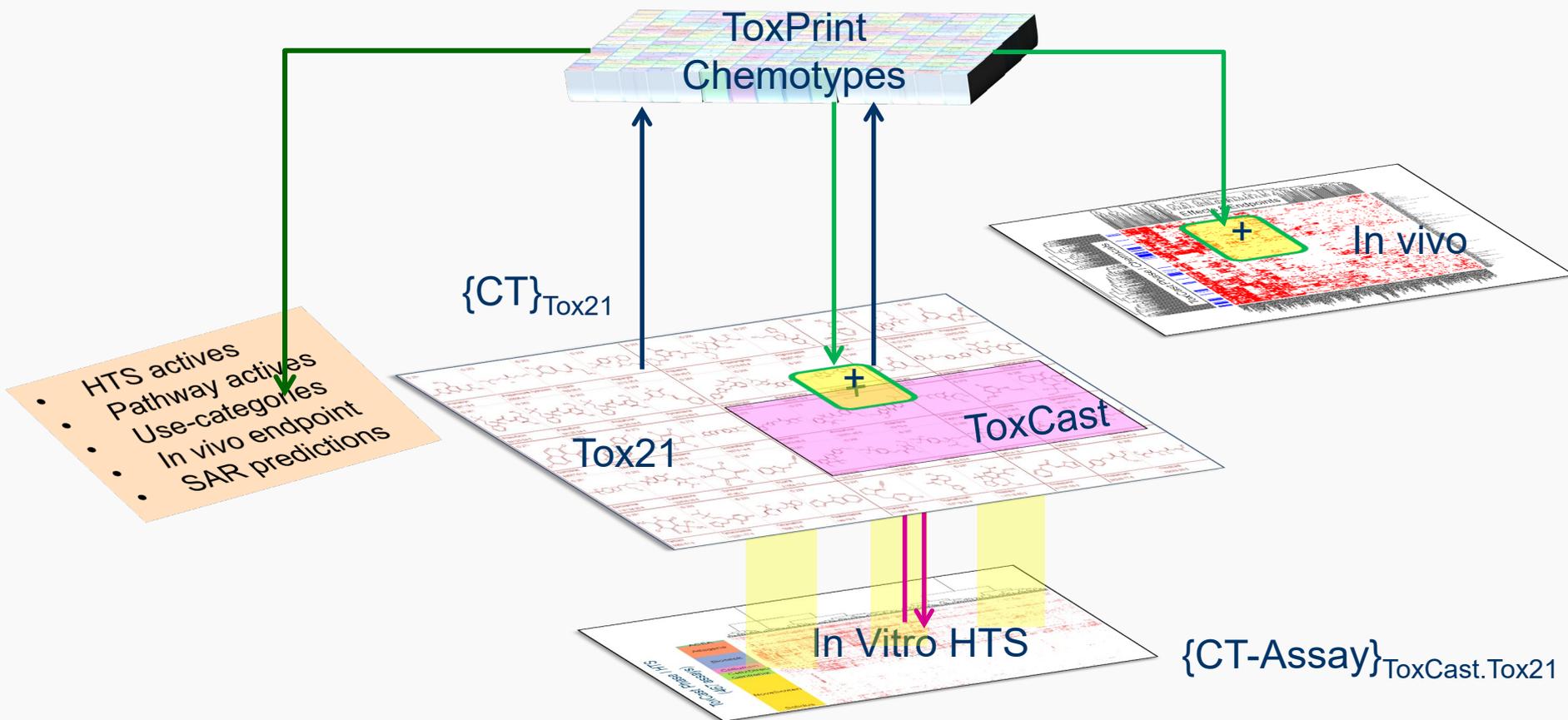


**Features in ToxCast & Tox21 missing in ENTACT either due to:**

- *ND (not detected) by LC/GC method*
- *Less than “Grade A” QC score*
- *Metal-containing*

# Chemotype-“Activity” Enrichments

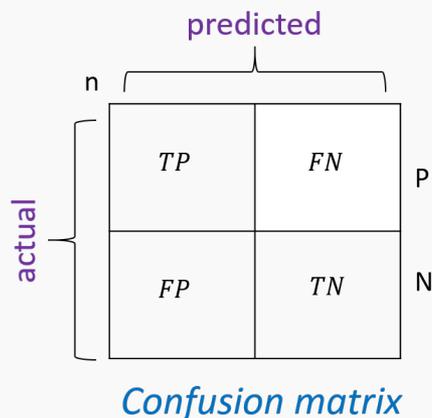
Create {CT-Activity} enrichment profiles for binary “activity” datasets



# Computing CT-Assay “Enrichments”

Set statistical thresholds & filters for significance to support data-mining objectives:

TP_ID	ToxPrint_CT_name <sup>2</sup>	CT <sub>Tot</sub>	T <sub>pos</sub>	F <sub>pos</sub>	F <sub>neg</sub>	T <sub>neg</sub>	Odds 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



TestSet = # Pos + # Neg = # chems tested

CT<sub>Tot</sub> = total # chems in TestSet w/ CT (Pos or Neg)  
TP (T<sub>pos</sub>) = # Pos in TestSet w/ CT  
FP (F<sub>pos</sub>) = # Neg in TestSet w/ CT  
FN (F<sub>neg</sub>) = # Pos in TestSet w/o CT  
TN (T<sub>neg</sub>) = # Neg in TestSet w/o CT

- Odds Ratio  $\geq 3$ , *conveys simple fractional enrichment*
- Fischer's exact p value  $\leq 0.05$ , *takes into account size of dataset*
- T<sub>pos</sub> (TP)  $\geq 3$ , *require at least 3 chemicals with CT in Positives*

# Finding chemical signal in HTS assay

## “noise”

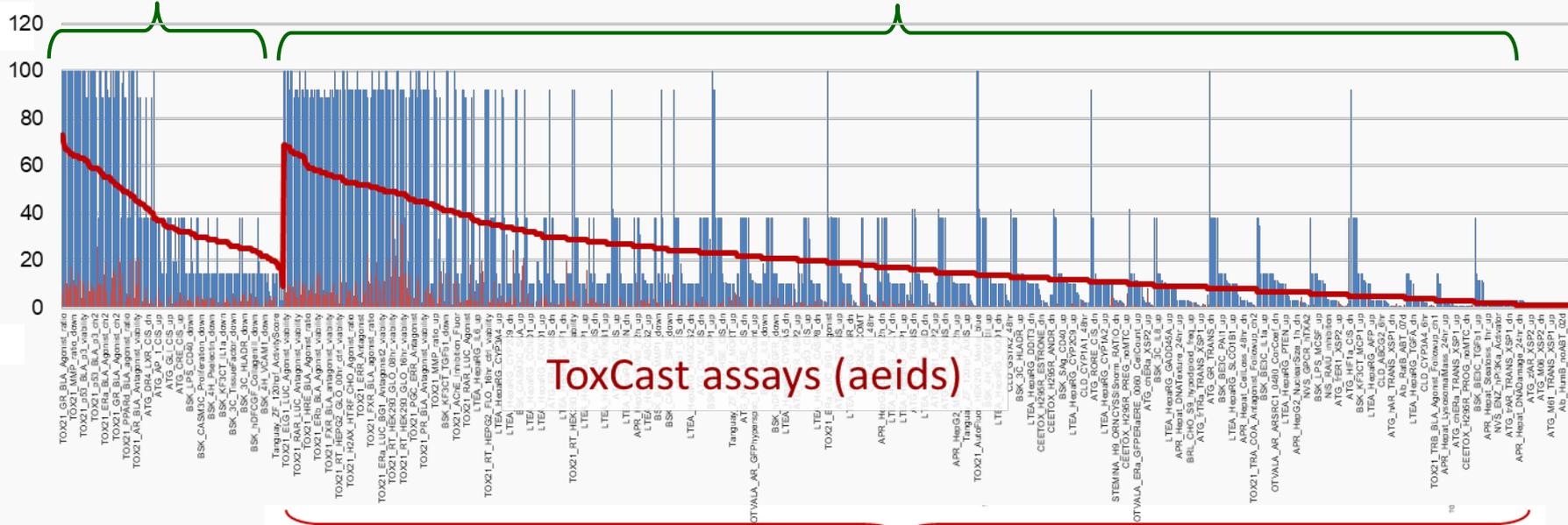
## Enriched CT-count across 1032 ToxCast assays

\*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\*  
Avg % Median BA = 0.67

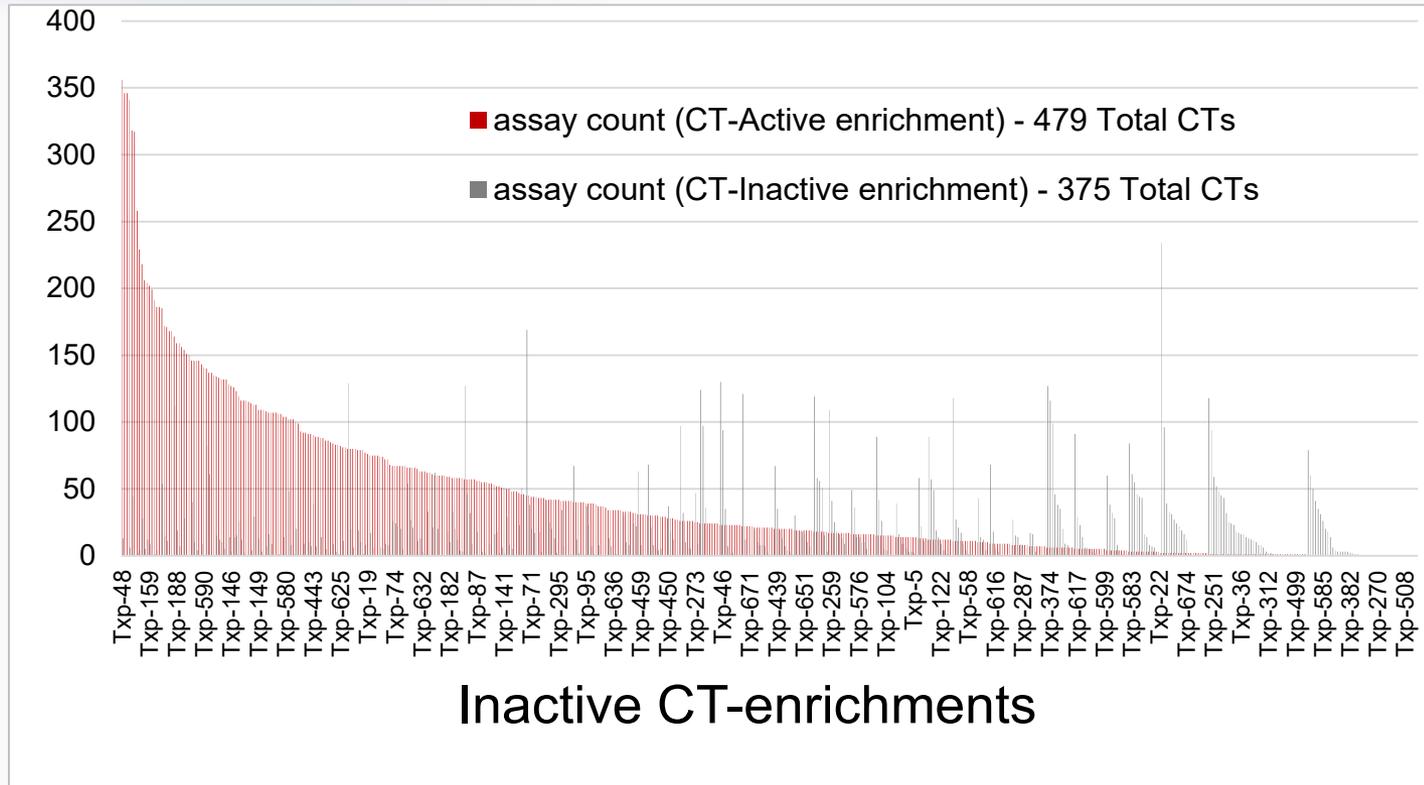
“Global” QSAR models failed for 86% of ToxCast Assays

Number of Tested Chems (scaled)



Significant CT-enrichments detected across ToxCast assay space

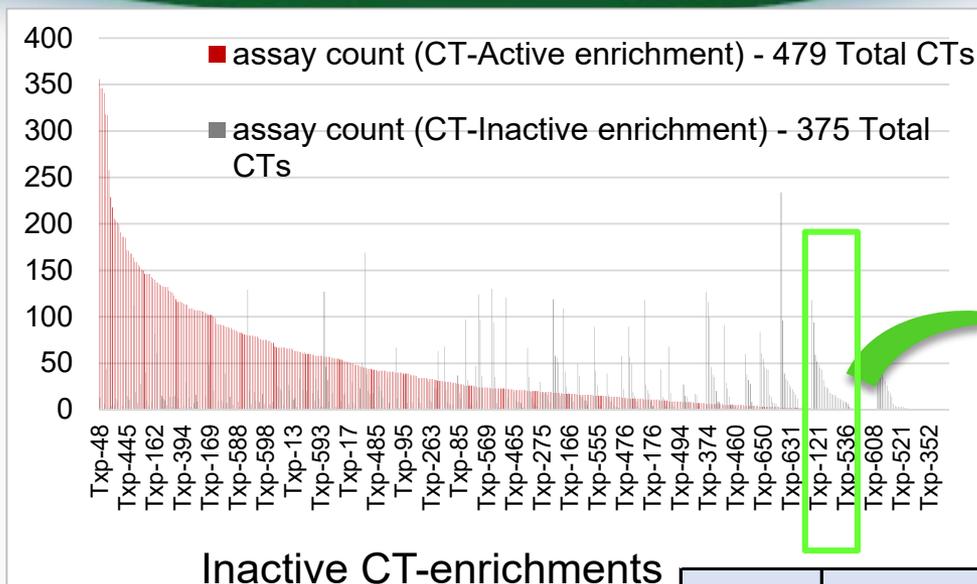
# What about the “Inactives”?



Are CT-enrichments in “negative” assay space due to:

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

# Top 10 enriched CT-Inactives (skewed from actives)

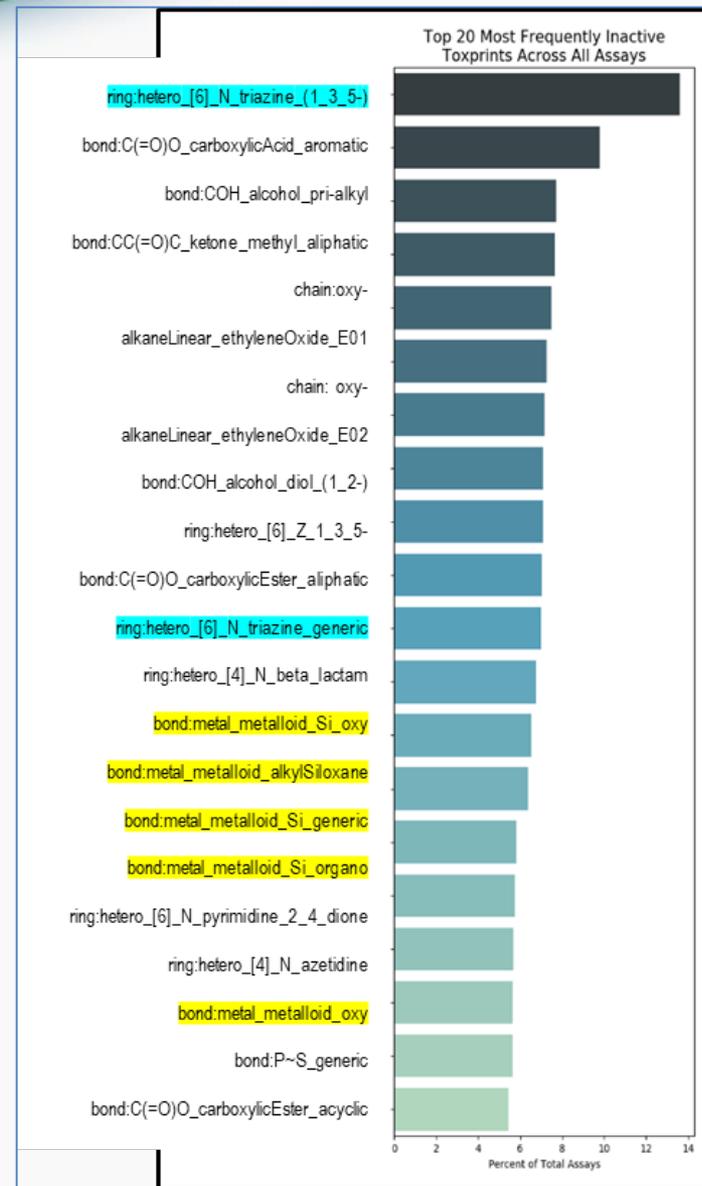
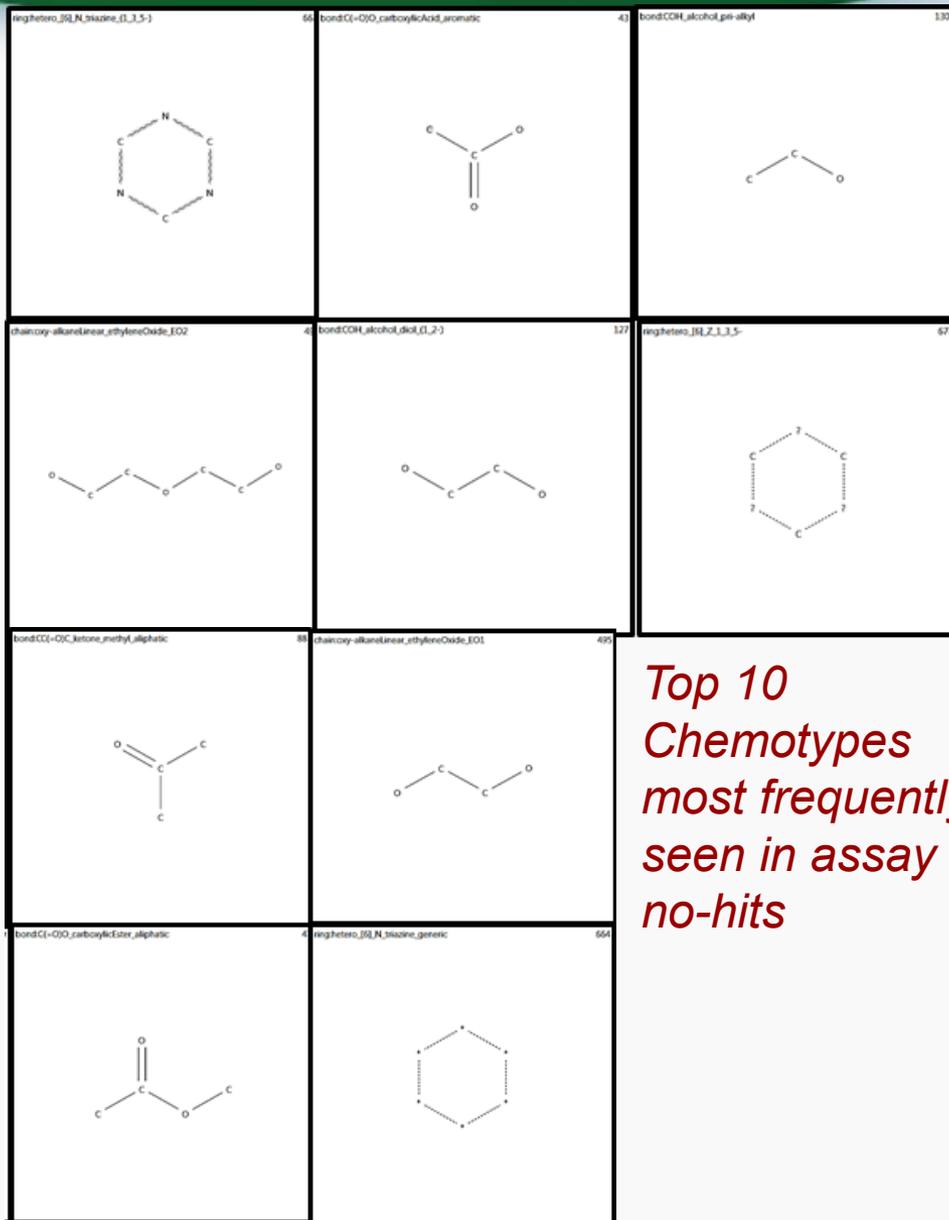


Features highly enriched in “inactive” space across large number of ToxCast assays (rarely enriched in “active” space)

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

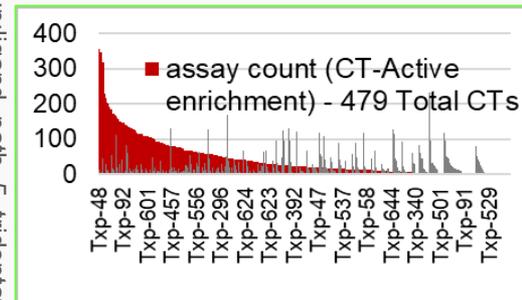
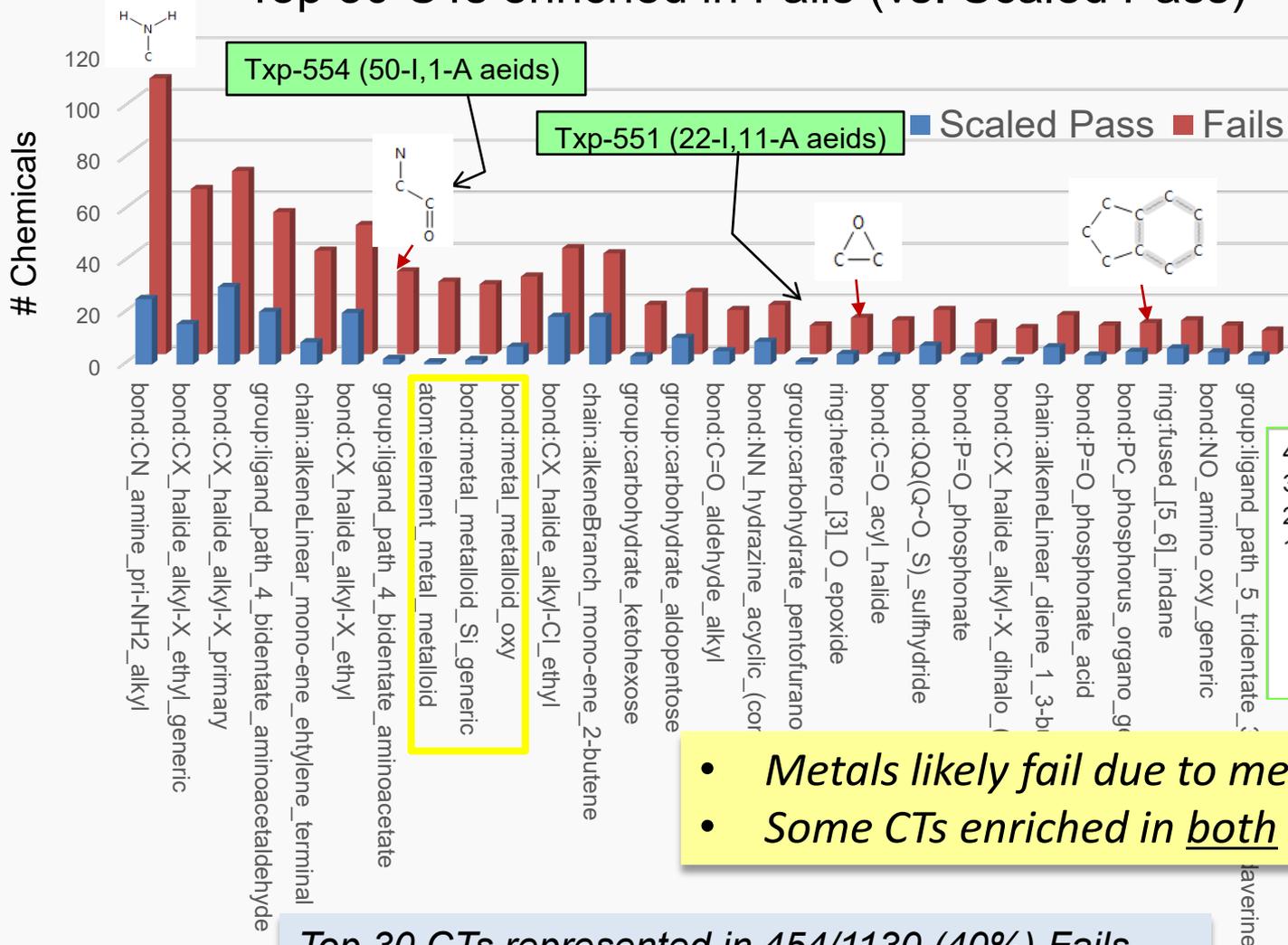
Txp	CT Name	assay count (CT-Active enrichment)	assay count (CT-Inactive enrichment)
Txp-101	bond:CN_amine_pri-NH2_aromatic	3	84
Txp-145	bond:CX_halide_alkyl-Cl_ethyl	0	79
Txp-260	bond:P~S_generic	1	94
Txp-362	bond:metal_metalloid_oxy	2	96
Txp-372	bond:metal_metalloid_Si_organo	6	99
Txp-374	bond:metal_metalloid_Si_oxy	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

# Inverse Promiscuity



# ToxPrints enriched in Tox21 QC Fails

## Top 30 CTs enriched in Fails (vs. Scaled Pass)



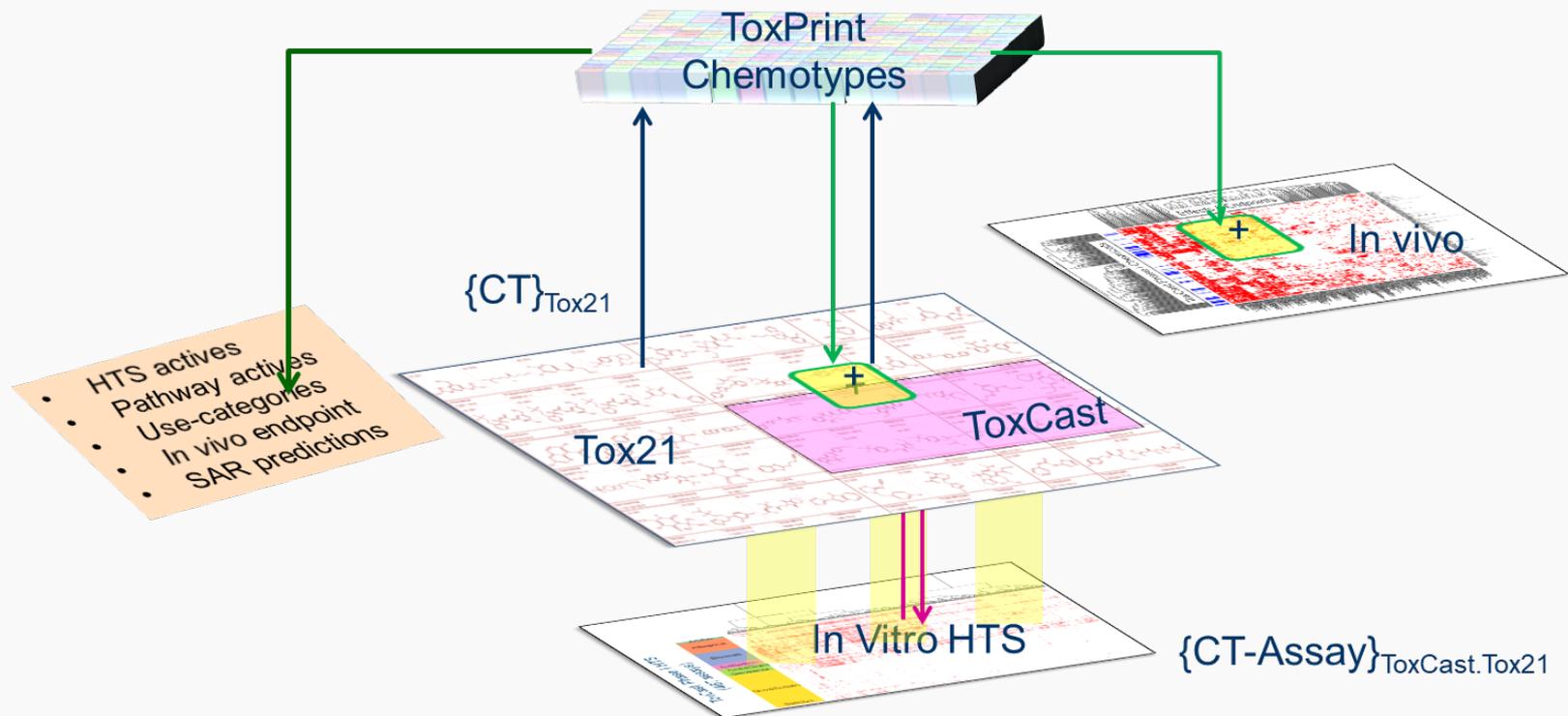
- Metals likely fail due to method-inadequacies
- Some CTs enriched in both Inactives & QC Fails

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

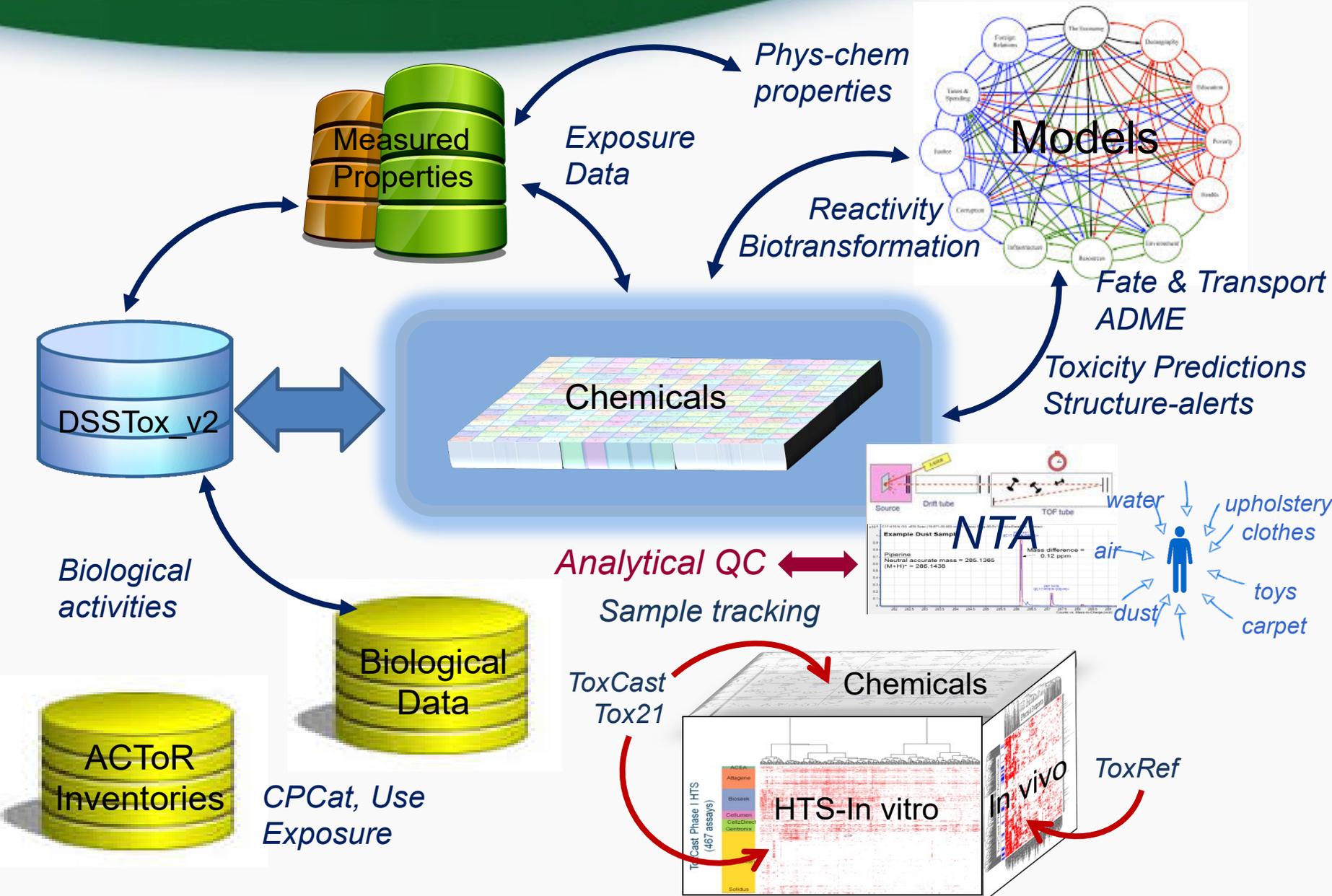
# Chemotype-“Activity” Enrichments

Create {CT-Activity} enrichment profiles for any binary “activity” dataset

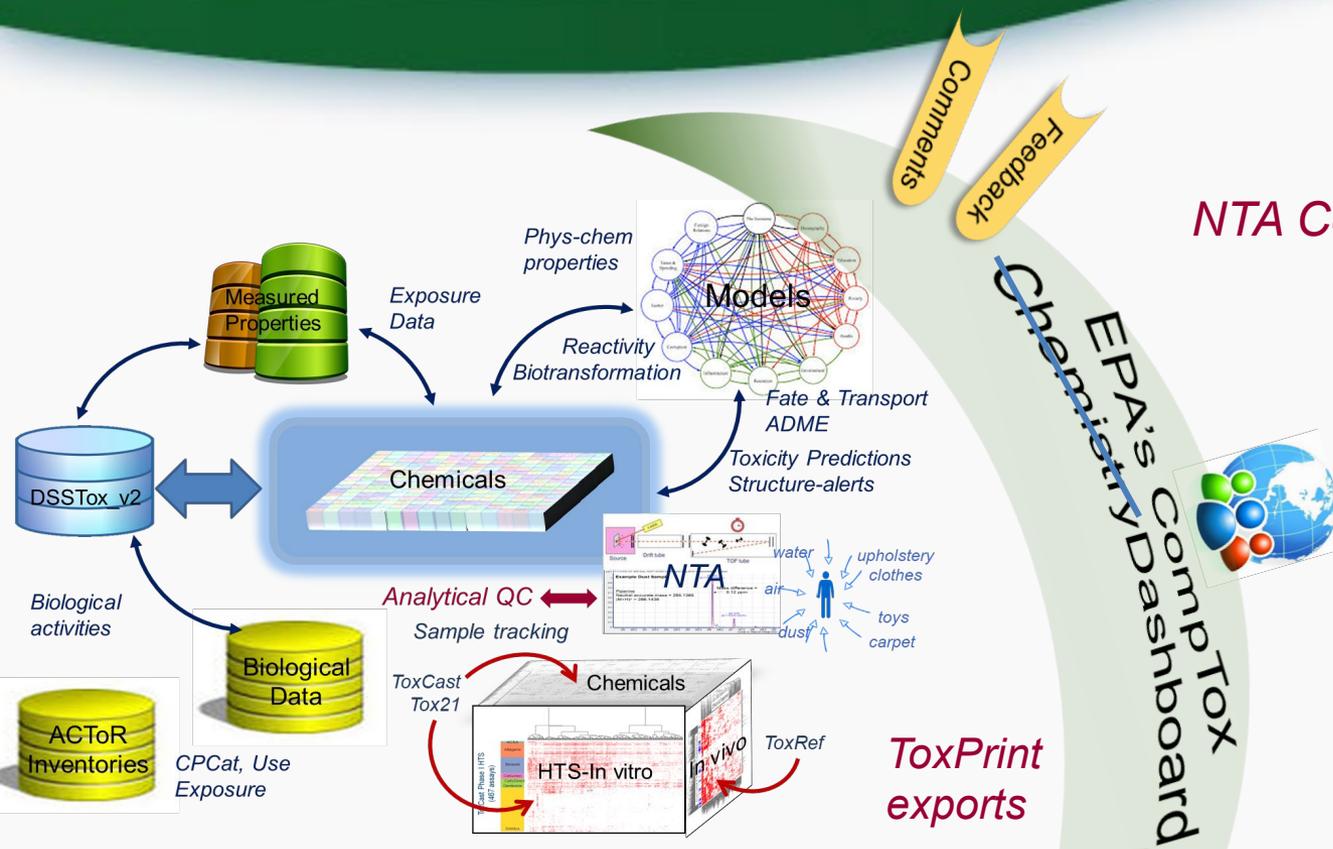
- Bioassay activity (active, inactive)
- Assay type artifacts (e.g., fluorescence detection method)
- QC “Fail” (yes, no)
- Sample degrades over time (yes, no)
- LCMS method suitability (yes, no)
- GCMS method suitability (yes, no)



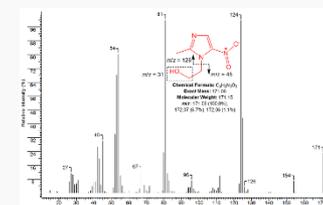
# EPA's Chemical Informatics Infrastructure & Linkages



# EPA's Chemical Informatics Infrastructure & Linkages



## NTA Community Collaboration



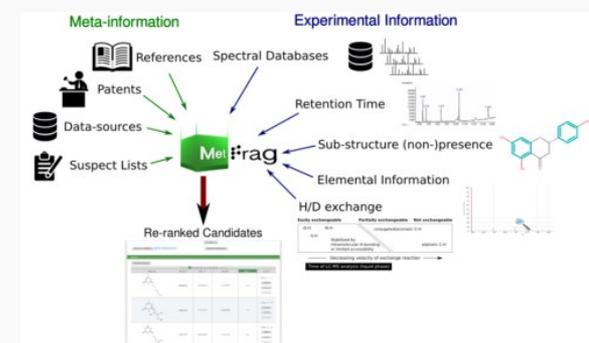
### Advanced Search MS-support tools:

- ✓ Monoisotopic mass
- ✓ Empirical formula
- ✓ Map to MS-ready structures
- ✓ Candidate substance mappings
- ✓ Data source rankings

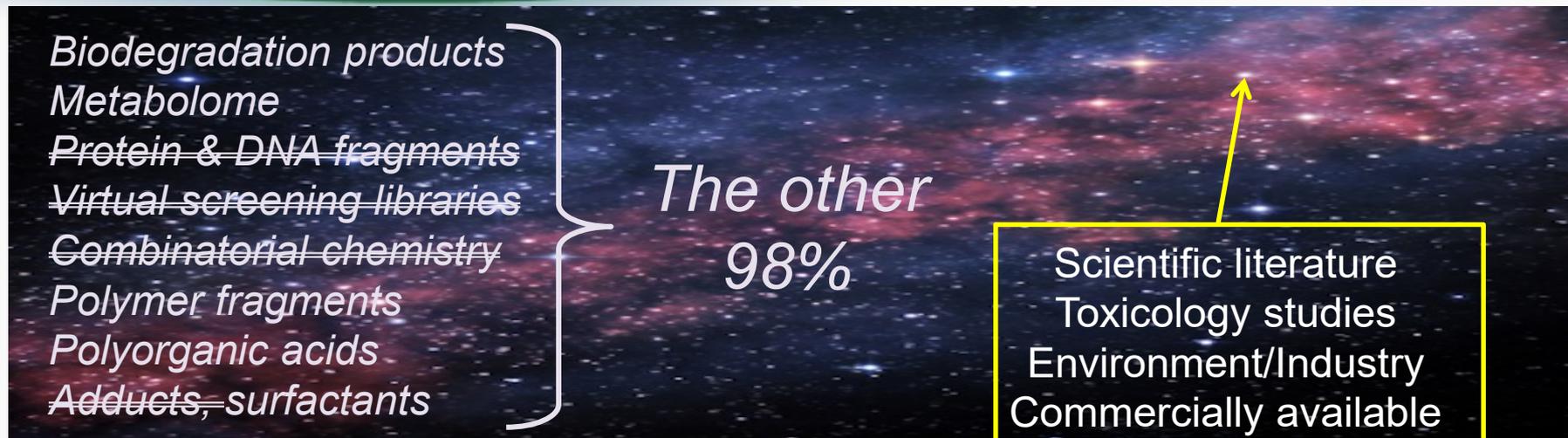
ToxPrint exports

EPA's Chemistry Dashboard

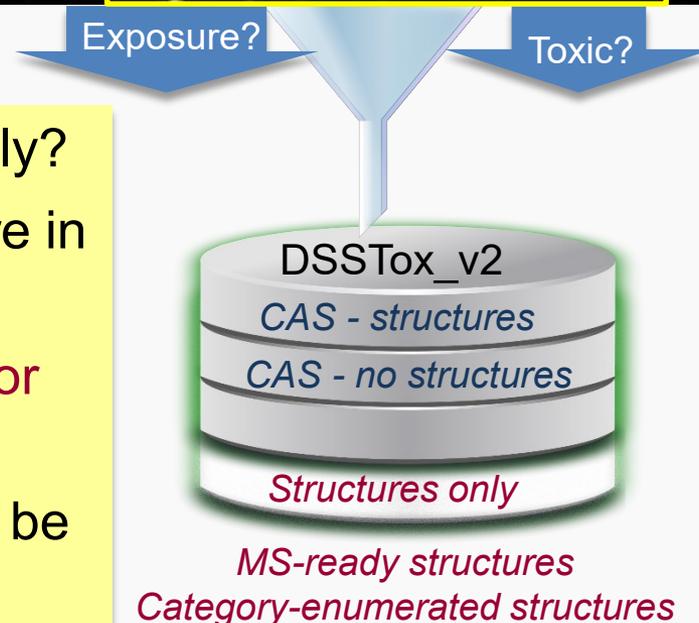
- Help
- Navigation
- External Links



# Chemical “Universe” problem



- ✓ Where should DSSTox expand chemically?
- ✓ What part of the universe should we store in DSSTox?
- ✓ How can the ToxCast library be shared for greatest gain?
- ✓ What cheminformatics “plumbing” would be most useful to this community?



# Chemical “Universe” problem

## EPAHFR - EPA Chemicals associated with hydraulic fracturing



1640

Substring search

### List Details

**Description:** Chemicals used in hydraulic fracturing fluids and/or identified in produced water from 2005-2013, corresponding to chemicals listed in Appendix H of EPA's Hydraulic Fracking Drinking Water Assessment Final Report (Dec 2016). Citation: U.S. EPA, Hydraulic Fracturing for Oil and Gas: Impacts from the Hydraulic Fracturing Water Cycle on Drinking Water Resources in the United States (Final Report). U.S. Environmental Protection Agency, Washington, D.C. EPA/600/R-16/236F, 2016. <https://www.epa.gov/hfstudy>

\*Note that Appendix H chemical listings in Tables H-2 and H-4 were mapped to current DSSTox content, which has undergone additional curation since the publication of the original EPA HF Report (Dec 2016). In the few cases where a Chemical Name and CASRN from the original report map to distinct substances (as of Jan 2018), both were included in the current EPAHFR chemical listing for completeness; additionally, 34 previously unmapped chemicals in Table H-5 are now registered in DSSTox (all but 2 assigned CASRN) and, thus, have been added to the current EPAHFR listing.

Number of Chemicals: 1640

## Endocrine Disruption Screening Program (EDSP) Universe of Chemicals



9411

Substring search

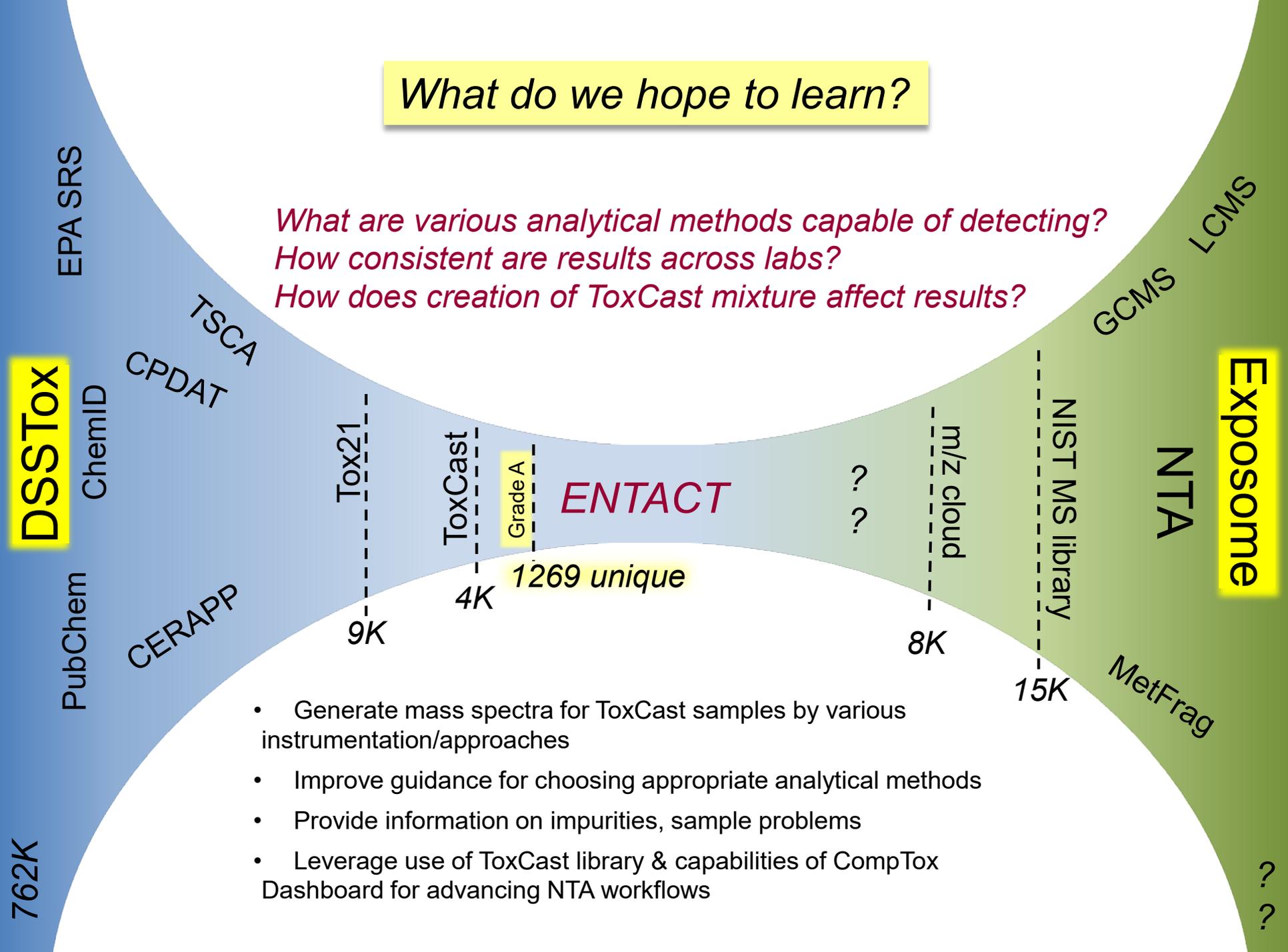
### List Details

**Description:** This list of Endocrine Disruption Screening Program (EDSP) related chemicals on the EPA CompTox Dashboard is not a complete listing from the EDSP Universe of Chemicals. Only those substances in the EDSP Universe of Chemicals that mapped uniquely to a substance in the CompTox Dashboard are included. The List also contains substances that are not part of the EDSP's statutory authority, but are part of the EPA's ongoing work on the validation of the endocrine and androgen bioactivity models.

Number of Chemicals: 9411

# What do we hope to learn?

*What are various analytical methods capable of detecting?  
How consistent are results across labs?  
How does creation of ToxCast mixture affect results?*



- Generate mass spectra for ToxCast samples by various instrumentation/approaches
- Improve guidance for choosing appropriate analytical methods
- Provide information on impurities, sample problems
- Leverage use of ToxCast library & capabilities of CompTox Dashboard for advancing NTA workflows

# Lots of questions, few clear answers ... YET!

Tuesday, August 14, 2018

Judge **ENTACT** by the  
posed & lessons learned  
questions <sup>^</sup> rather than  
by getting all of what we think may be the right  
answers.

- **Anonymous**

# Acknowledgements:

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- ✦ EPA NCCT Chemistry Team & DSSTox Curators  
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Altamira: Jim Rathman

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