

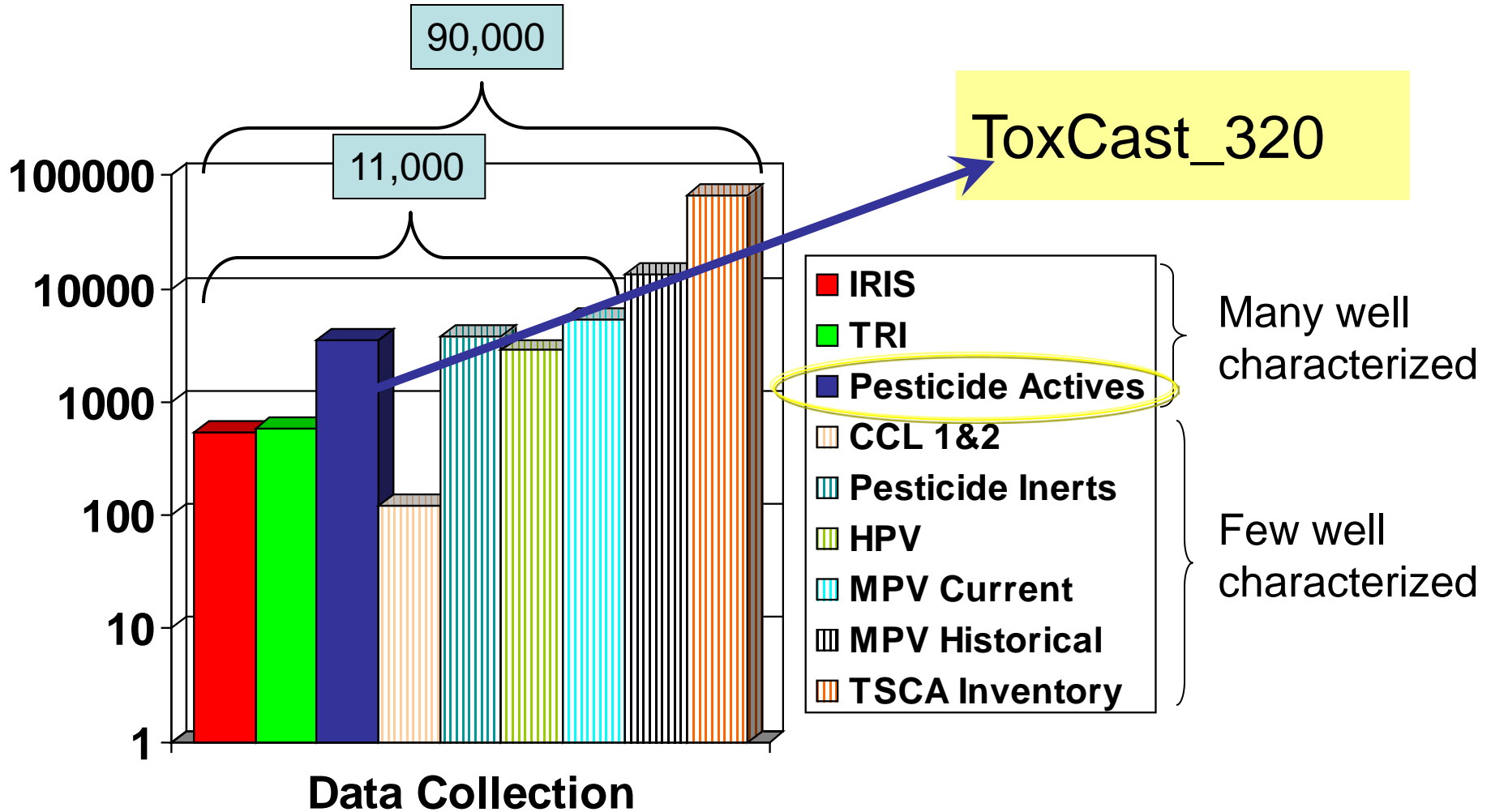
Overview of ToxCast Phase II Chemicals

December 16, 2010

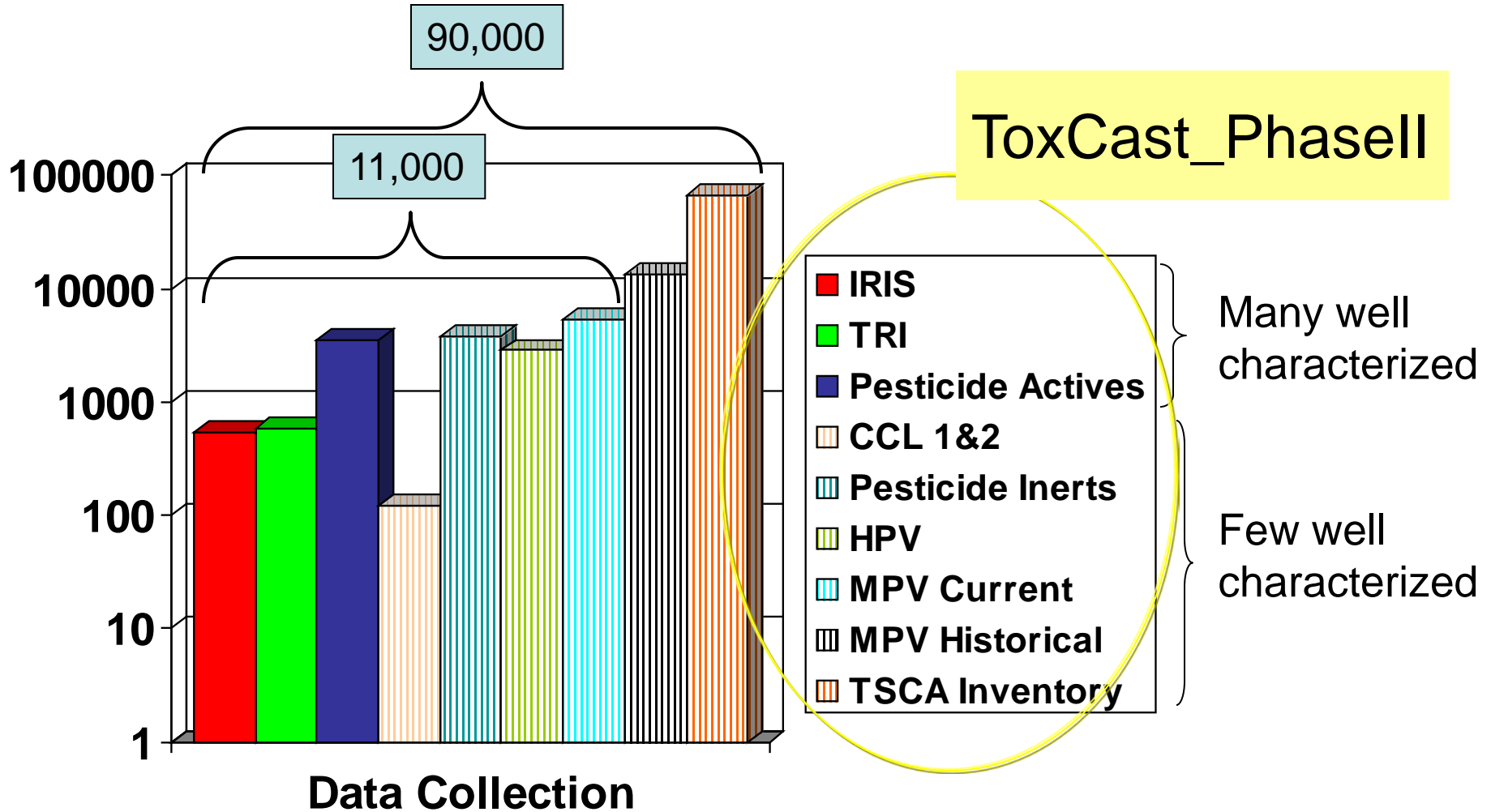


*Computational Toxicology Research Program
Communities of Practice*

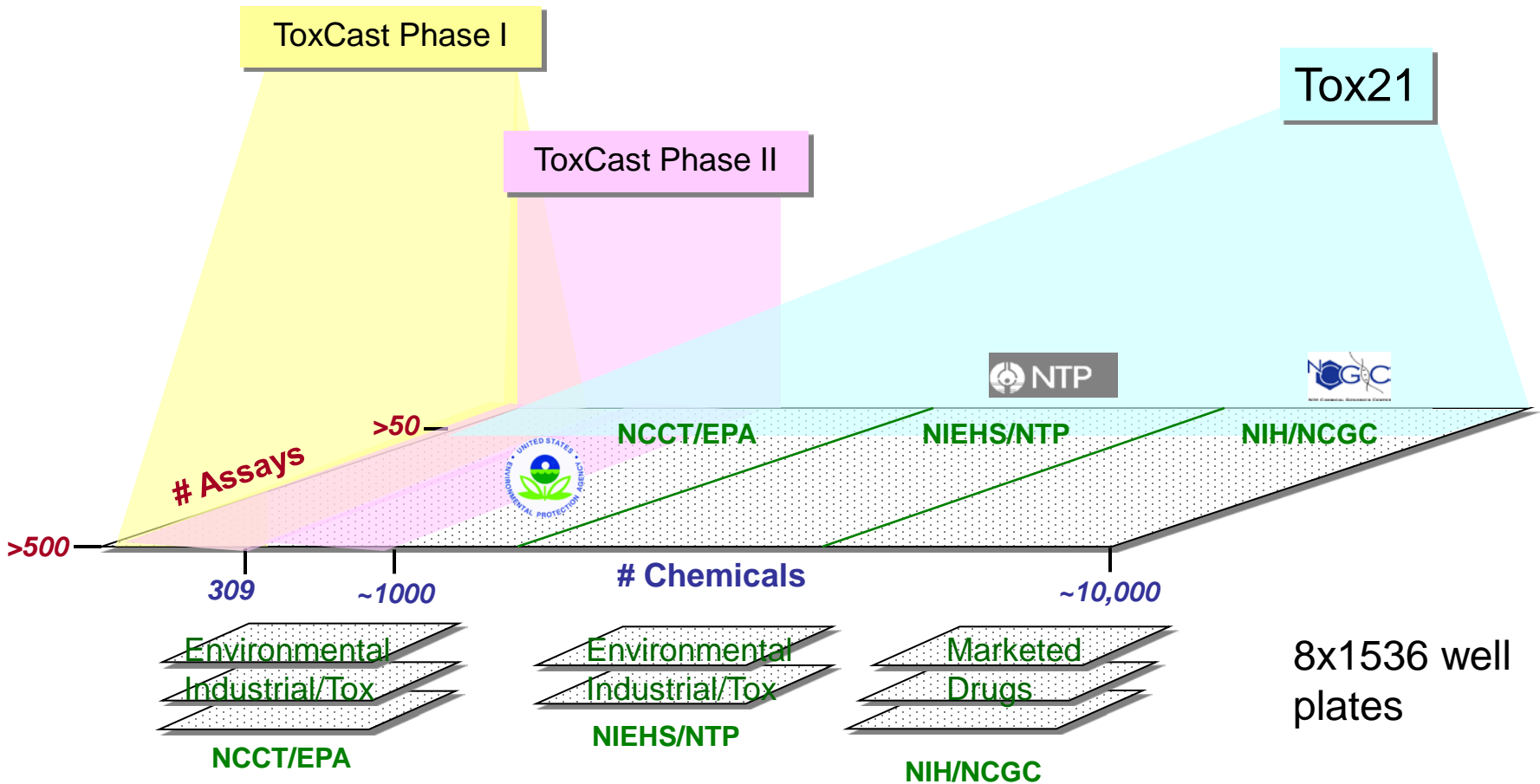
ToxCast Chemicals



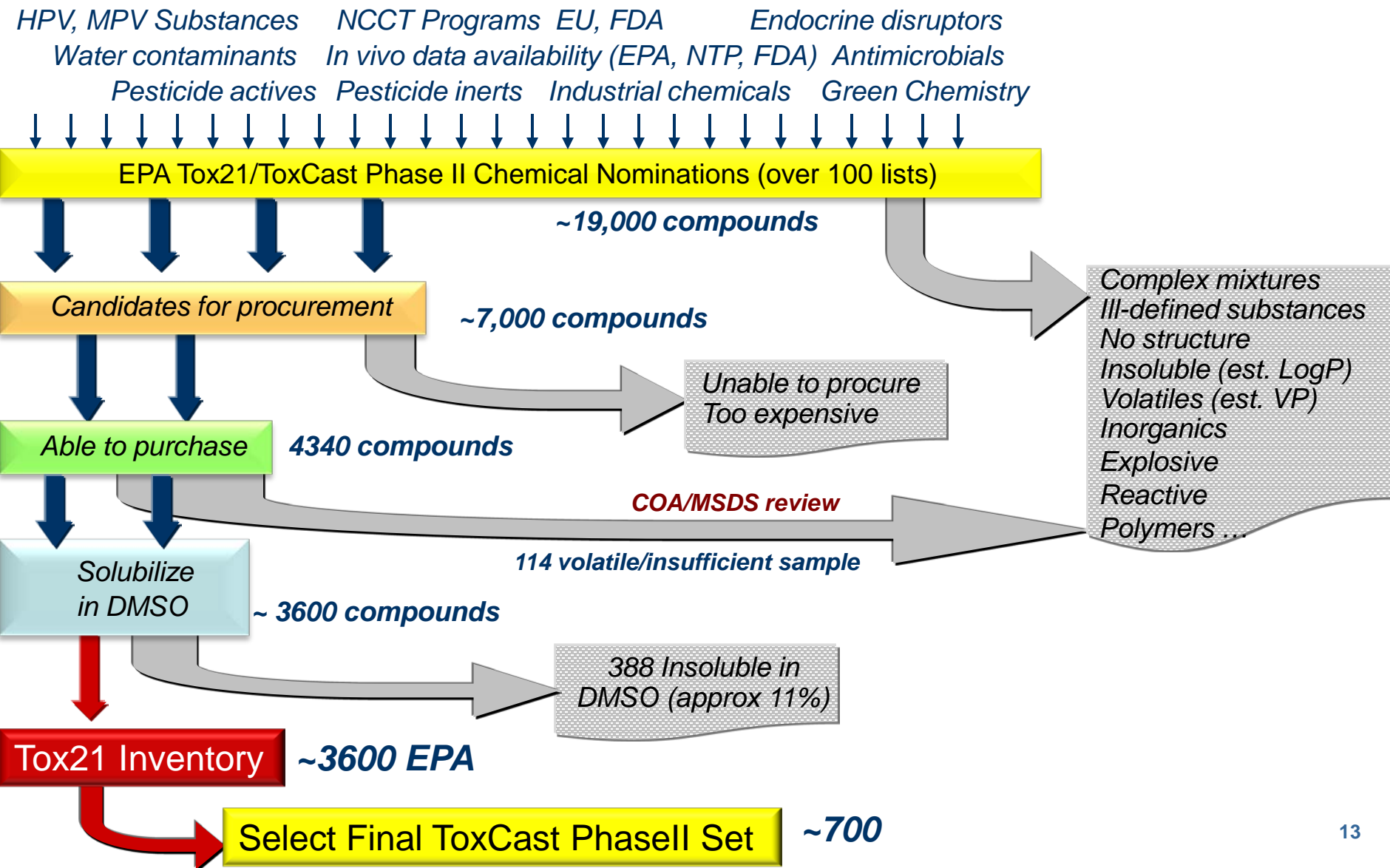
ToxCast Chemicals



ToxCast/Tox21 Testing Landscape



EPA ToxCast/Tox21 Chemical Procurement

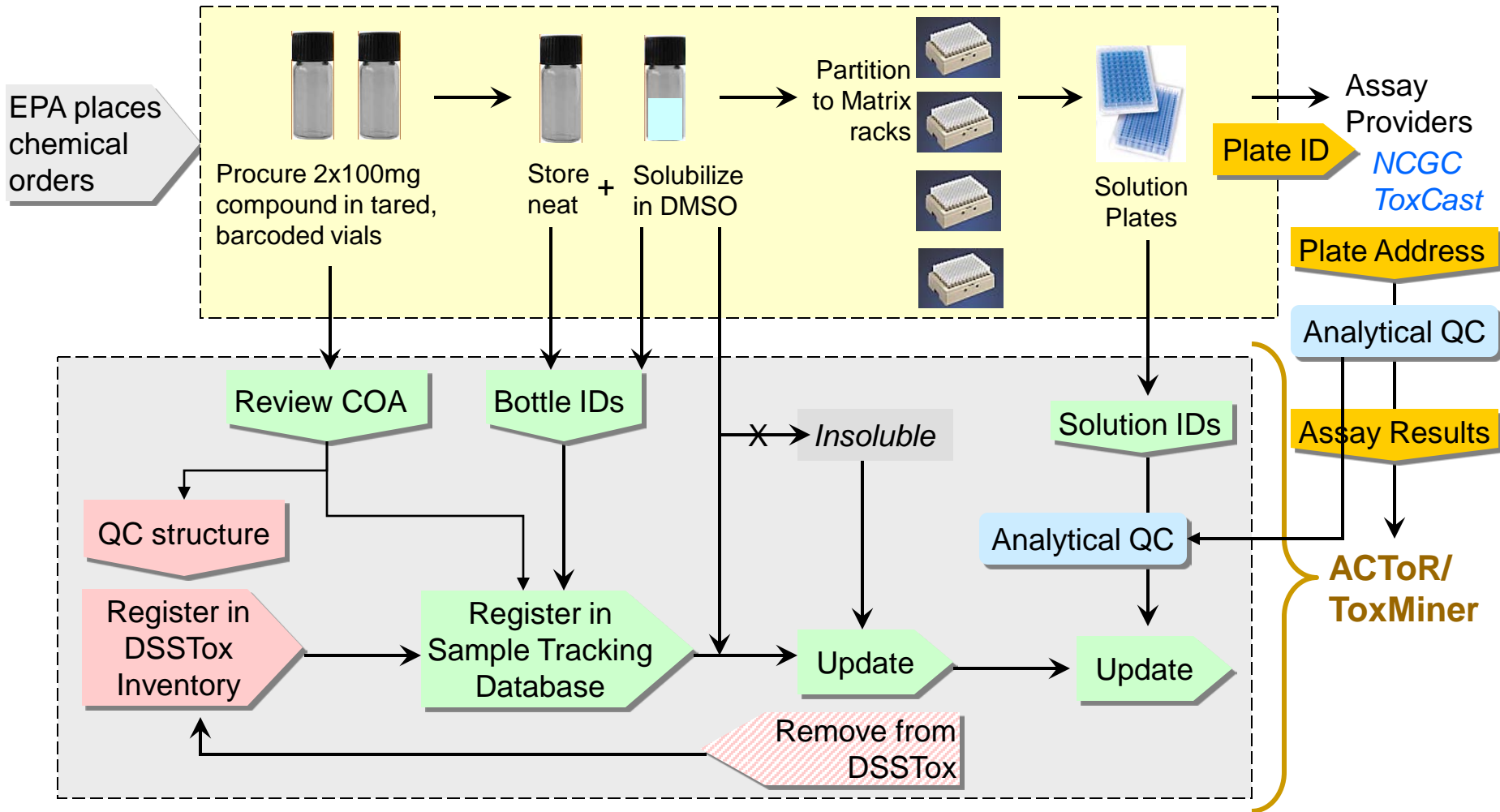


Tox21 & ToxCast Phase II Chemicals

Row #	CAS #	Chemical Name	Phase II Tox21	Phase II ToxCast	Inventory	Procurement	DMSO	NCCT	MW/Phys-Chem	MSDS	QC	Overlaps	Phasell
1	101-01-7	Acetone	42	112	14								
2	101-01-7	Acetone	42	112	14								
3	101-01-7	Acetone	42	112	14								
4	101-01-7	Acetone	42	112	14								
5	101-01-7	Acetone	42	112	14								
6	101-01-7	Acetone	42	112	14								
7	101-01-7	Acetone	42	112	14								
8	101-01-7	Acetone	42	112	14								
9	101-01-7	Acetone	42	112	14								
10	101-01-7	Acetone	42	112	14								
11	101-01-7	Acetone	42	112	14								
12	101-01-7	Acetone	42	112	14								
13	101-01-7	Acetone	42	112	14								
14	101-01-7	Acetone	42	112	14								
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29	101-01-7	Acetone	42	112	14								
30	101-01-7	Acetone	42	112	14								
31	101-01-7	Acetone	42	112	14								
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80	101-01-7	Acetone	42	112	14								
81	101-01-7	Acetone	42	112	14								
82	101-01-7	Acetone	42	112	14								

- Tracking of inventory overlaps by CAS
- Prioritize EPA & outside inventories for procurement
- Track procurements and received compounds
- Track DMSO solubilization orders & results
- Track special requests (NCCT)
- Track MW, phys-chem, volatility, MSDS cautions
- Incorporate past analytical QC results (Phi_v1)
- Track overlaps w/ Tox21 partners (NTP, NCGC, FDA)
- Prioritize solubilized compounds for Phasell

Chemical Sample Registration Workflow



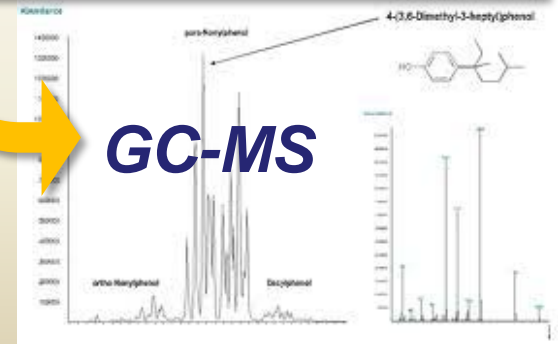


A copy of each parent Tox21 assay plate (352 cmpds/plate) will be subjected to analytical QC for assessing purity, identity, stability

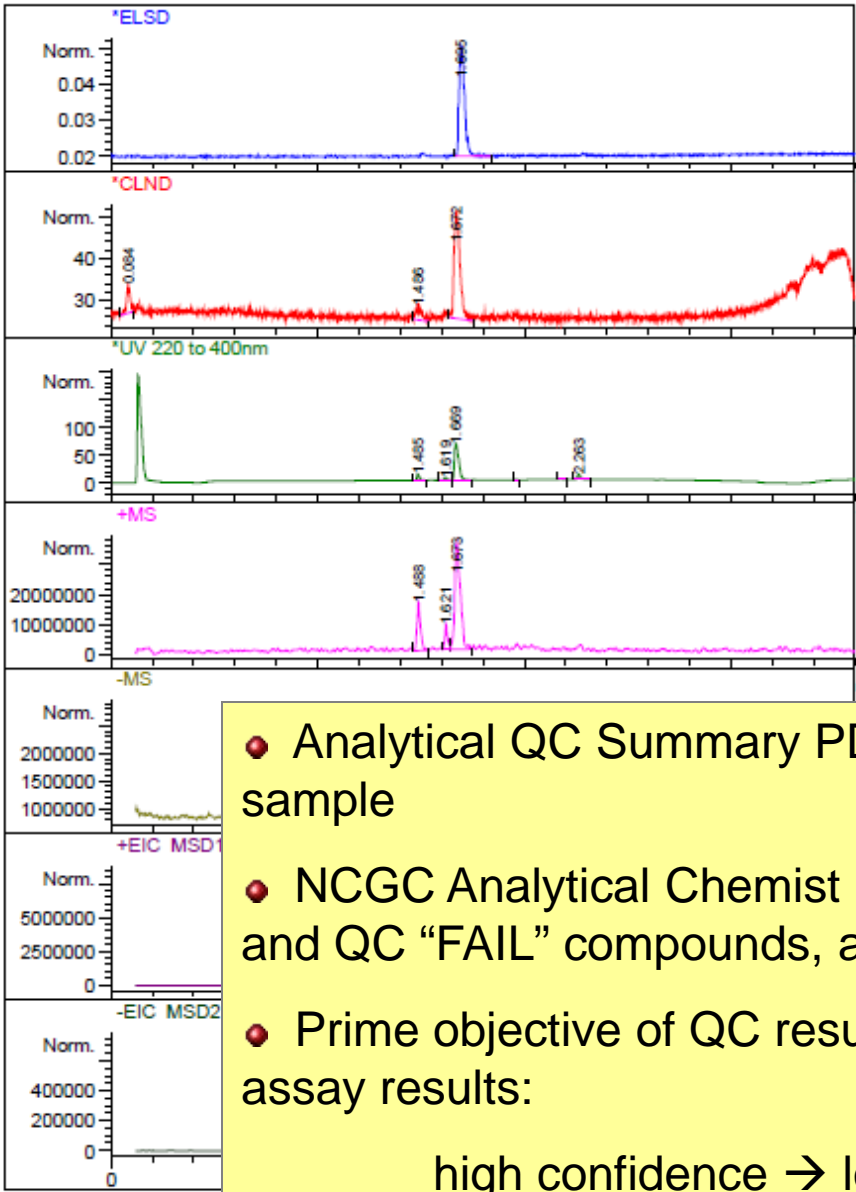
PASS = Confirm parent ion peak and >90% purity

Fail, inconclusive or analytical method inappropriate

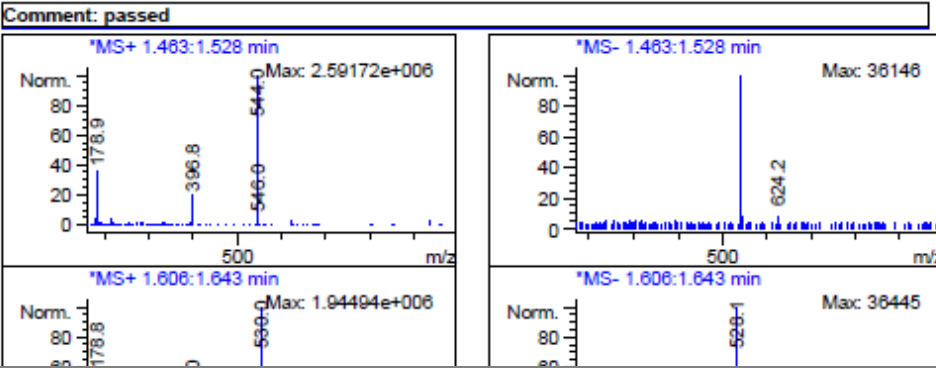
Retest at later time point under assay conditions for stability



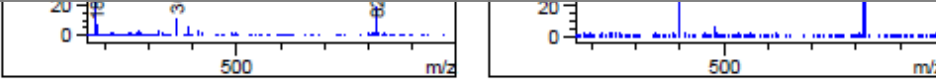
Publish QC summary results in association with assay data



RT	Found	ELS%	UV %	ELS[mg/mL]	Adj [ELS]	[N mM]	Adj [CLN]	#N
0.08		0.0	0.0			0.27 mM		1.0
1.48		0.0	8.2			0.24 mM		1.0
1.62		0.0	3.7					1.0
1.67	Yes	100.0	77.7	1.5	2.85 mM	1.71 mM	1.71 mM	1.0
1.96		0.0	0.4					1.0
2.17		0.0	1.3					1.0
2.26		0.0	8.7					1.0



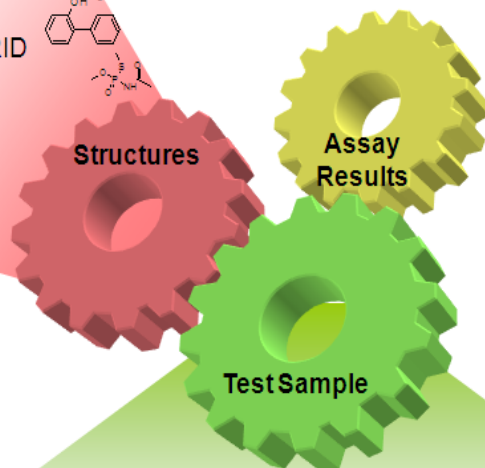
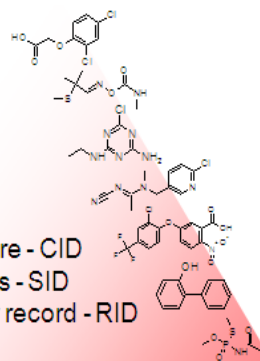
- Analytical QC Summary PDF results will be available for each analyzed sample
- NCGC Analytical Chemist (B. Leister) will review all preliminary LC results and QC "FAIL" compounds, and supervise all follow-up testing
- Prime objective of QC results is to inform analysis and interpretation of assay results:
 high confidence → low confidence → fail



Tox21/ToxCast Test Sample Registry

DSSTox

Chemical structure - CID
Substance details - SID
Project/inventory record - RID

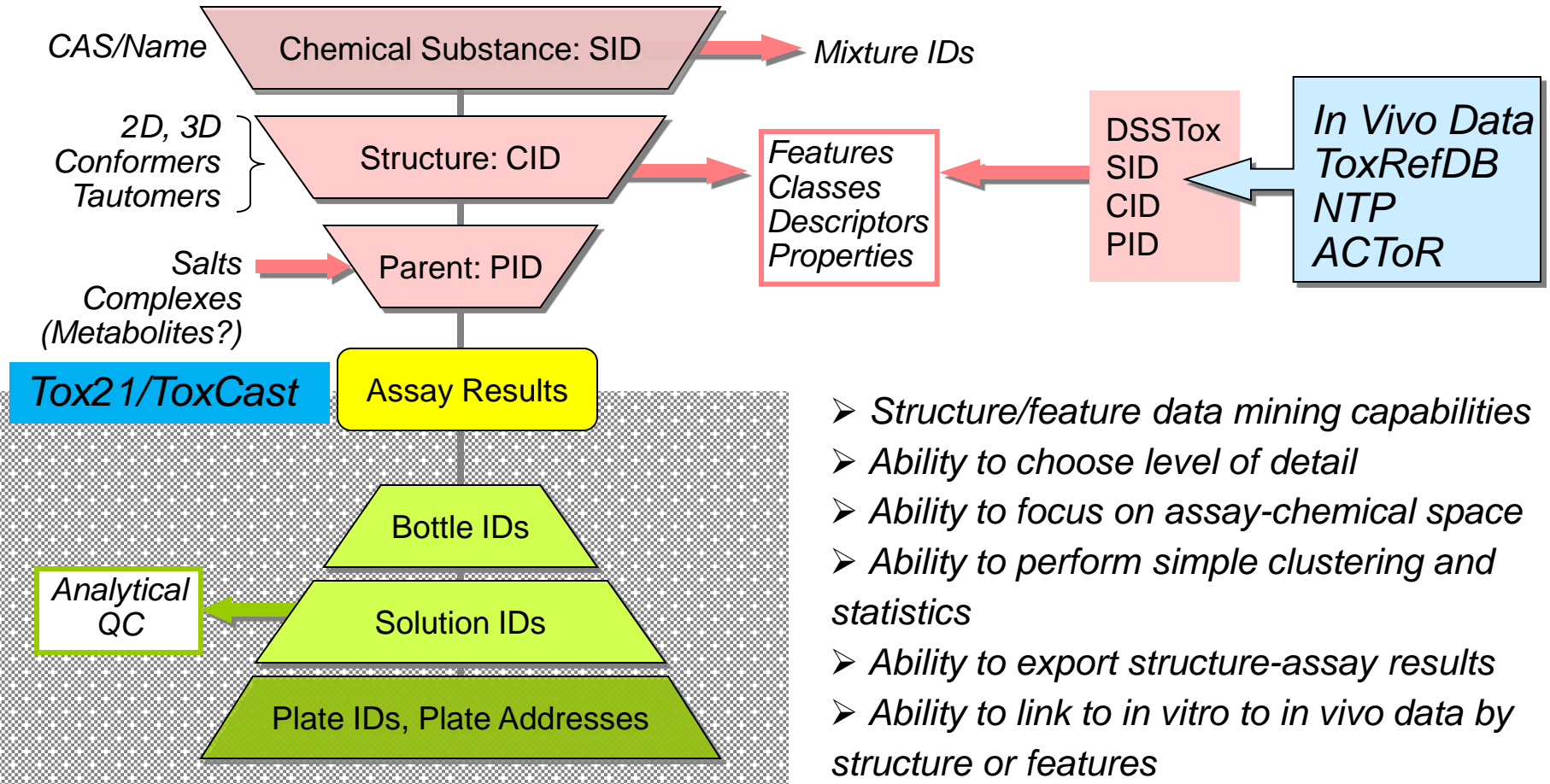


DSSTox RID
Bottle ID (→ COA ID)
Solution ID (→ QC ID)

Tox21 Sample Tracking Database

- Tracks all procured chemicals
- *Source, Lot/Batch, Bottle code*
- Test Sample identity QC, extract info from CoA/MSDS
- *Accurate name, CAS, MW*
- Track Test Sample to level of unique chemical stock solution used in plating
- Analytical QC of Test Sample solution
- Chemical information QC – substance/structure-annotation and registration in DSSTox ⁹

Building a Tox21 Cheminformatics Capability



- Structure/feature data mining capabilities
- Ability to choose level of detail
- Ability to focus on assay-chemical space
- Ability to perform simple clustering and statistics
- Ability to export structure-assay results
- Ability to link to in vitro to in vivo data by structure or features

Status of EPA Tox21/ToxCast Chemicals

- Approx 4300 chemicals procured, 3570 soluble (388 insoluble; 114 volatile/insufficient sample)
- Review & registration of sample information into EPA Tox21/ToxCast test substance database (CoA, MSDS, chemical identity) complete
- Phase I_v2_311 shipped Aug 2010
- Phase IIa_350 plates shipped Sept 2010
- Phase IIb_350 plates shipped Nov 2010
- QC of structure annotation for ToxCast Phase I&II complete
- First two Tox21 plates (352x2) plates containing ToxCast Phase II compounds prepared and will ship mid-Dec
- Analytical QC will begin in Jan; Tox21 testing in Feb-Mar 2011

Reprocurement of ToxCast_Phase1: v2

- Original 309 unique cmpds
- 14 Sulfurons excluded due to confirmed decomposition in DMSO
- 2 reprocured compounds insoluble in DMSO (Metiram, Prohexadione-calcium)
- 2 compounds procured in slightly different form (different CAS than v1)
- Final Phase1_v1 contains 293 unique compounds
- Final Phase1_v1 test set plates contain 9 cmpds (richest HTS hit profile) in triplicate, or 311 total

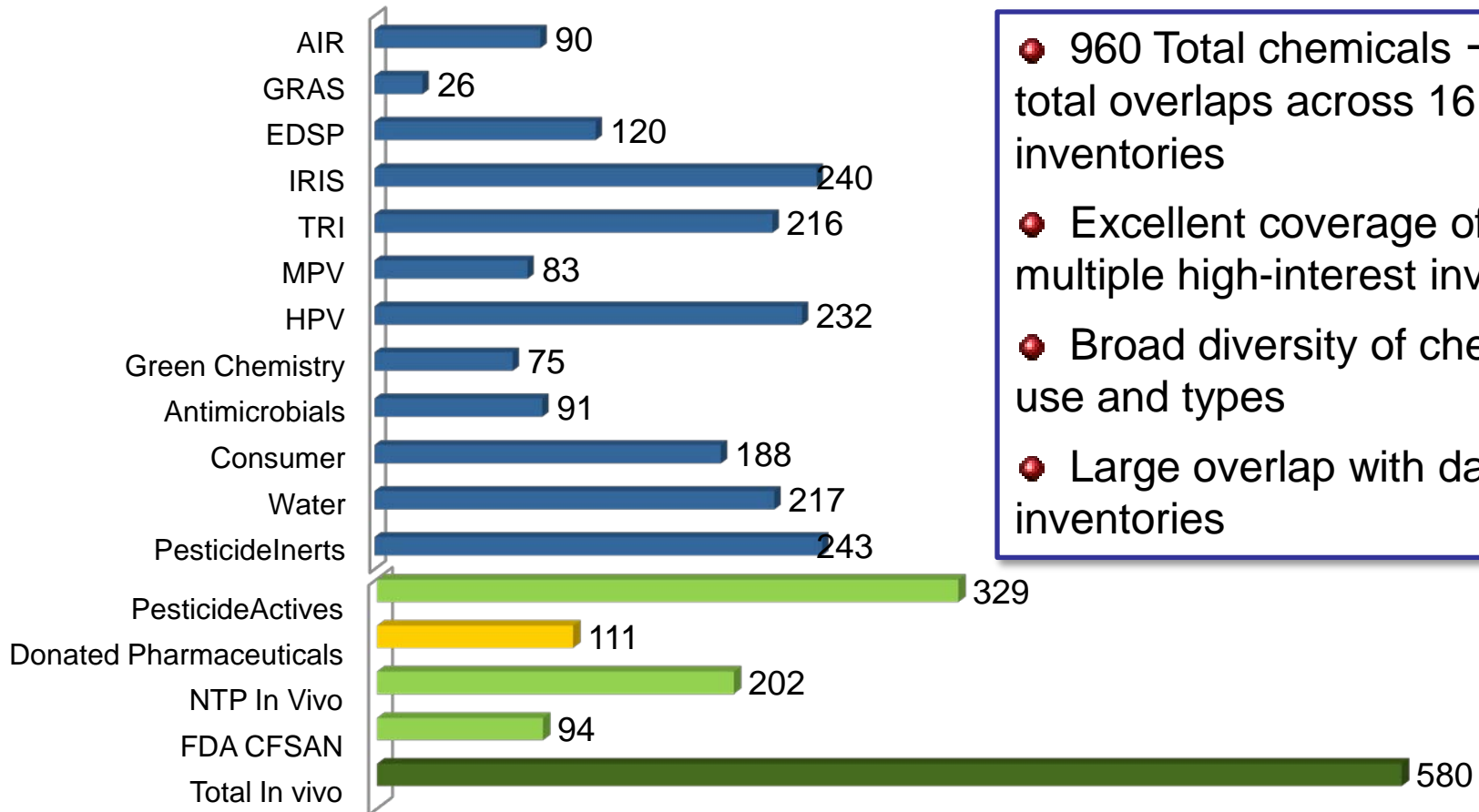
ToxCast Phase II

- Augment Phase I (309) with 700 environmentally relevant, diverse chemicals to expand chemical-assay space for model development:
 - *EPA pesticides, high interest EPA and stakeholder inventories, data rich chemicals (EDSP, OPPT, Antimicrobials, Inerts, ...)*
 - *EPA HPV classes, metabolite/parent pairs, Green chemistry (toxic, safe)*
 - *NCCT projects: vLiver, vEmbryo, alternative plasticizers, ...*
- Pharma-donated failed drugs with pre-clinical/clinical tox data (Pfizer, GSK, Sanofi-Aventis, Merck)
- L'Oreal: sponsoring 10 chemicals for Phase II testing
- NTP sponsoring 50 immunotoxic chemicals in Phase II
- FDA NCTR LTKB (Liver Tox Knowledge Base)-donated chemicals); FDA CFSAN data-rich chemicals included
- Supplier donations of 7 “green plasticizers”, alternatives to BPA

ToxCast Phase II ...

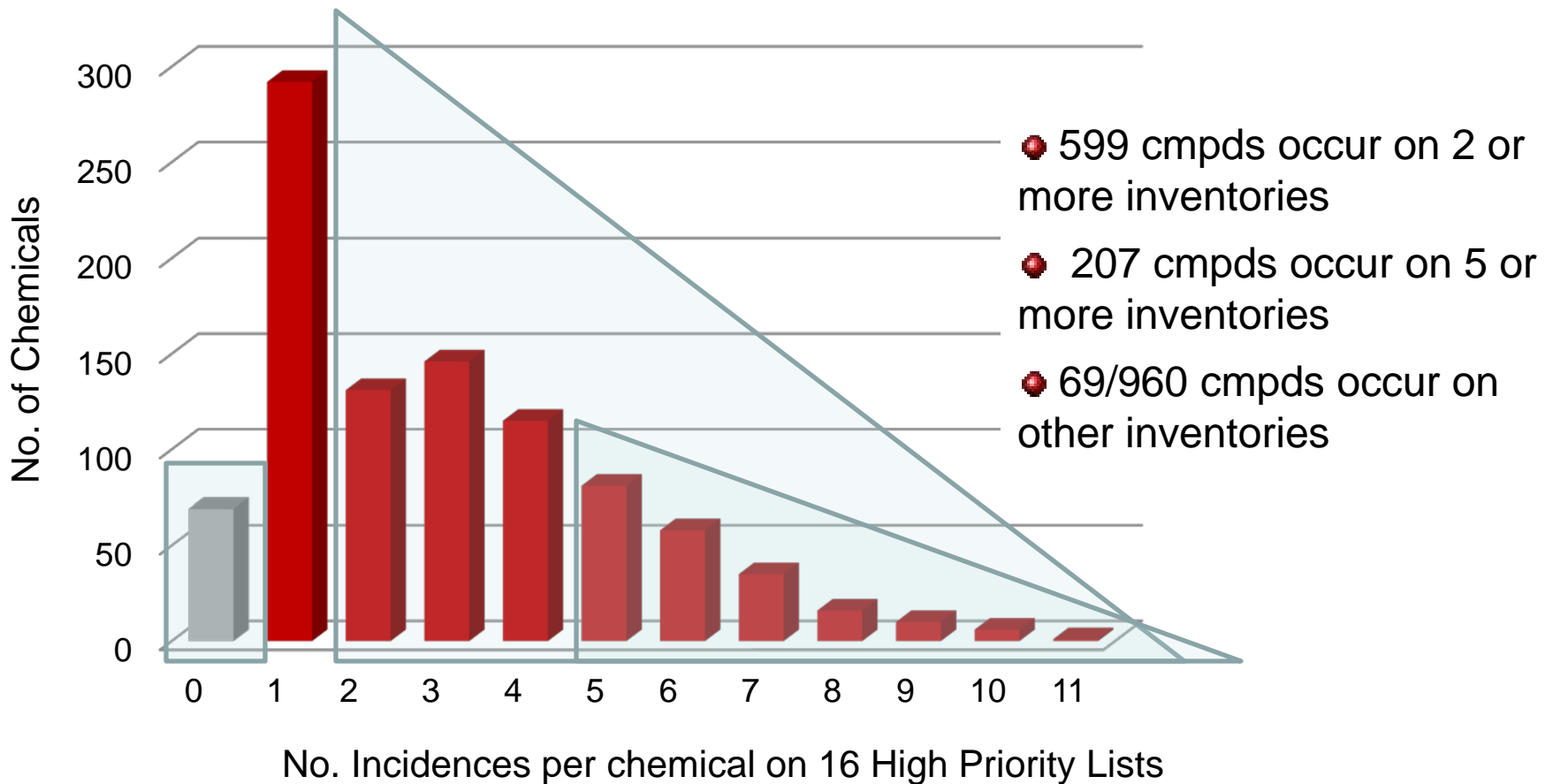
- 676 unique compounds
- 111 donated pharmaceuticals
- 7 additional donated compounds (green plasticizers)
- 9 PhaseI_v2 (triplicate) compounds serve as plate replicates – a total of 33 incidences of the 9 in total
- PhaseII test set plates include a total of 700 compounds

ToxCast PhI&PhII 960: # Compounds per Inventory (960 x 16)



- 960 Total chemicals → 2740 total overlaps across 16 diverse inventories
- Excellent coverage of multiple high-interest inventories
- Broad diversity of chemical-use and types
- Large overlap with data-rich inventories

ToxCast PhI&PhII 960: # Inventories per Compound (16 x 960)

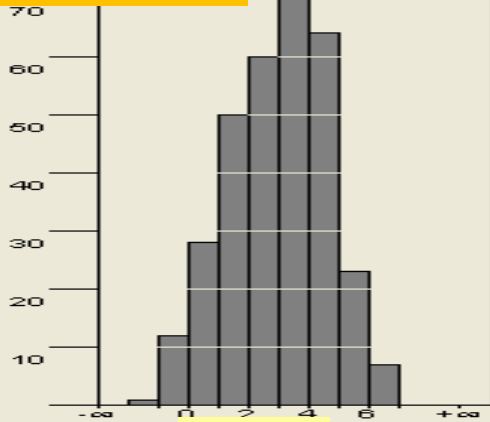


ToxCast PhI&PhII 960 Landscape

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103-90-2	50594-66-6	79-06-1	309-00-2	117-79-3	97-56-3	4180-23-8	101-05-3	134-03-2	50-78-2	
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1912-24-9	446-86-6	26628-2	<div style="background-color: yellow; padding: 10px; text-align: center;"> <p>Property distributions</p> <p>Feature incidences</p> <p>Analog series</p> <p>Alerting features</p> </div>						271-89-6	65-85-0
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31 (ID:31) <input type="checkbox"/> 	32 (ID:32) <input type="checkbox"/> 	33 (ID:33) <input type="checkbox"/> 							39 (ID:39) <input type="checkbox"/> 	40 (ID:40) <input type="checkbox"/>
85-68-7	88-85-7	94-26-6	1948-33-0	98-54-4	96-48-0	58-08-2	105-60-2	6/1/2425	133-06-2	
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63-25-2	1563-66-2	120-80-9	133-90-4	57-74-9	115-28-6	100-00-5	95-83-0	50892-23-4	532-27-4	
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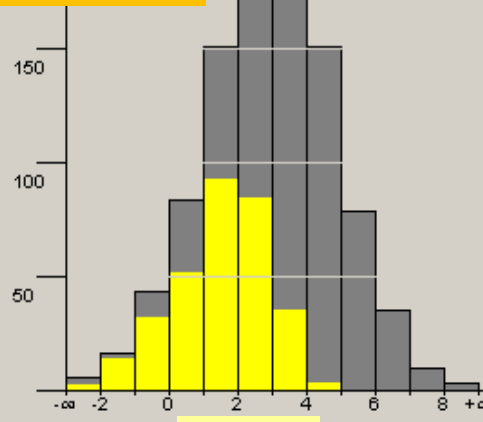
ToxCast PhI&PhII Property Distributions

PhaseI_v2

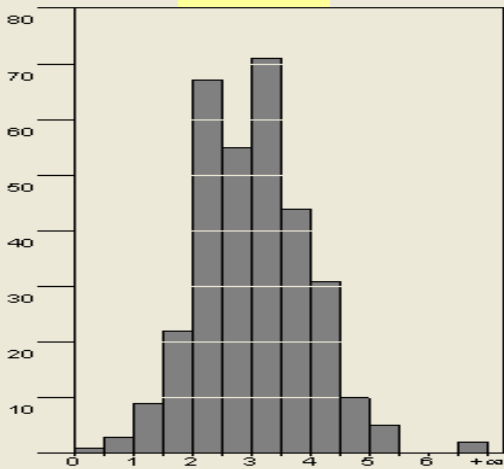


ALogP

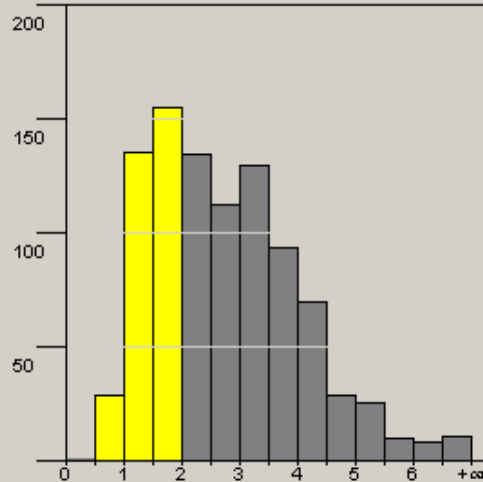
PhaseII



ALogP



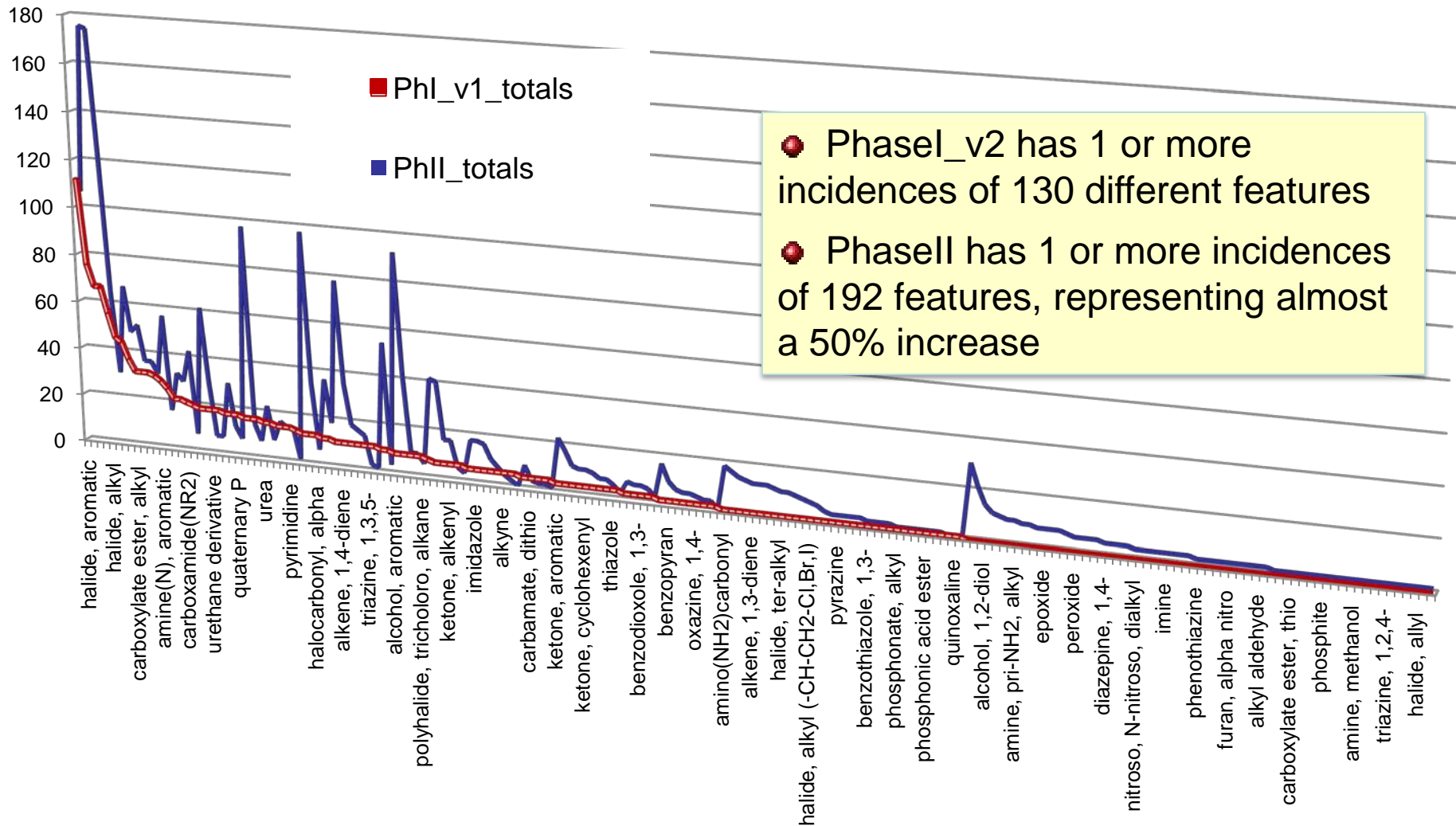
Molecular Wt (100's)



Molecular Wt (100's)

- Much larger proportion of low MW compounds
- LogP distribution broader
- Pesticides vs broad diversity of categories
- Property extremes pose testing challenges
- Property profile categories may prove useful in analysis

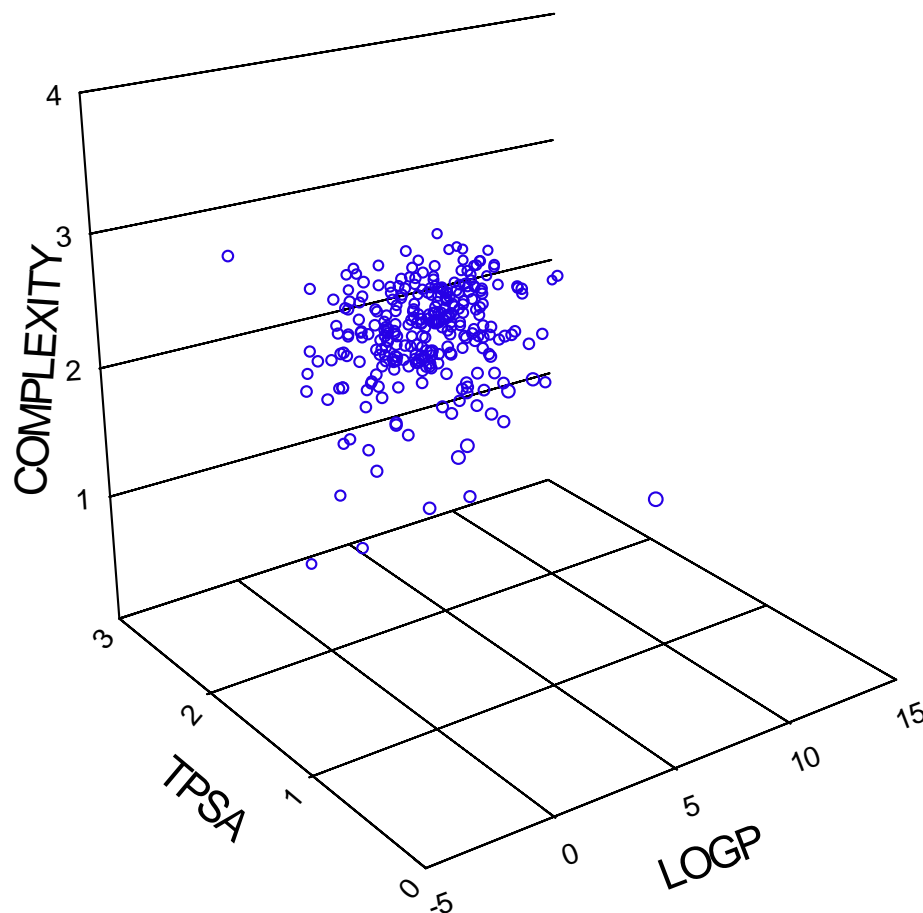
ToxCast PhaseI_v2 vs Phase II: Feature incidence comparison



ToxCast/Tox21 property distributions

ToxCast_PhaseI_v2_293

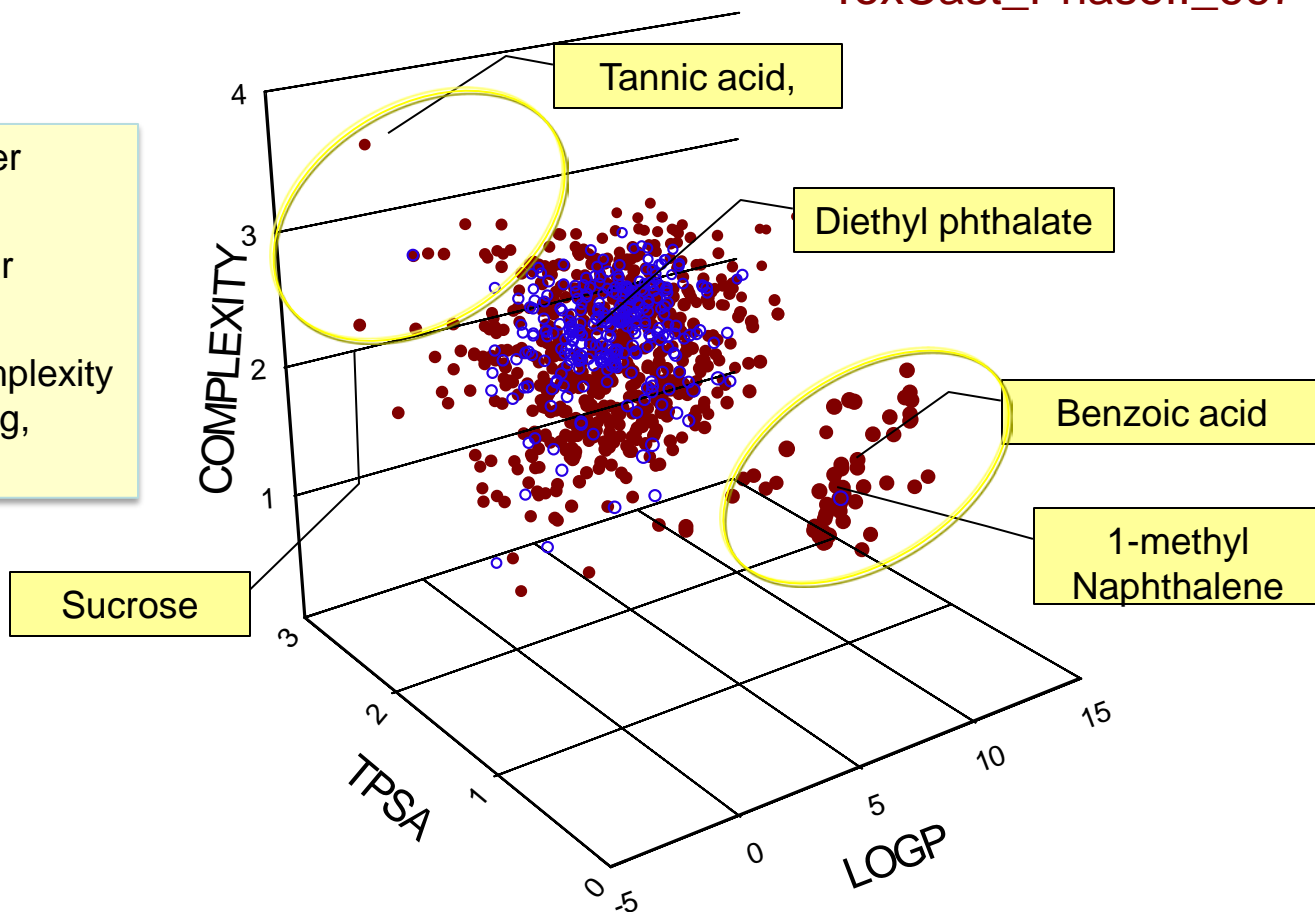
- LOG P = Octanol/Water partition coefficient
- TPSA = log (Total Polar Surface Area)
- Complexity = log (complexity based on paths, branching, atoms)



ToxCast/Tox21 property distributions

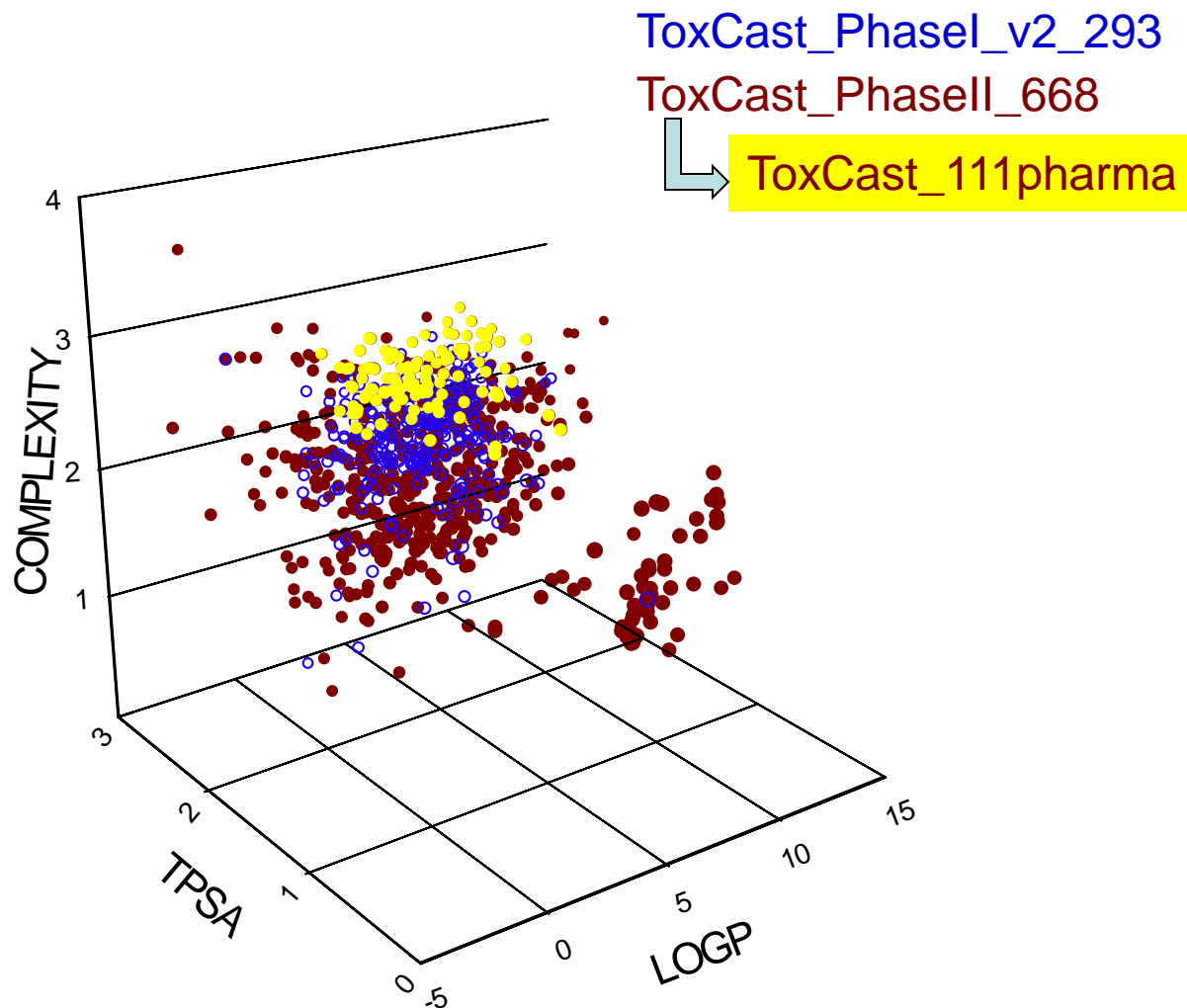
ToxCast_PhaseI_v2_293
ToxCast_PhaseII_667

- LOG P = Octanol/Water partition coefficient
- TPSA = log (Total Polar Surface Area)
- Complexity = log (complexity based on paths, branching, atoms)



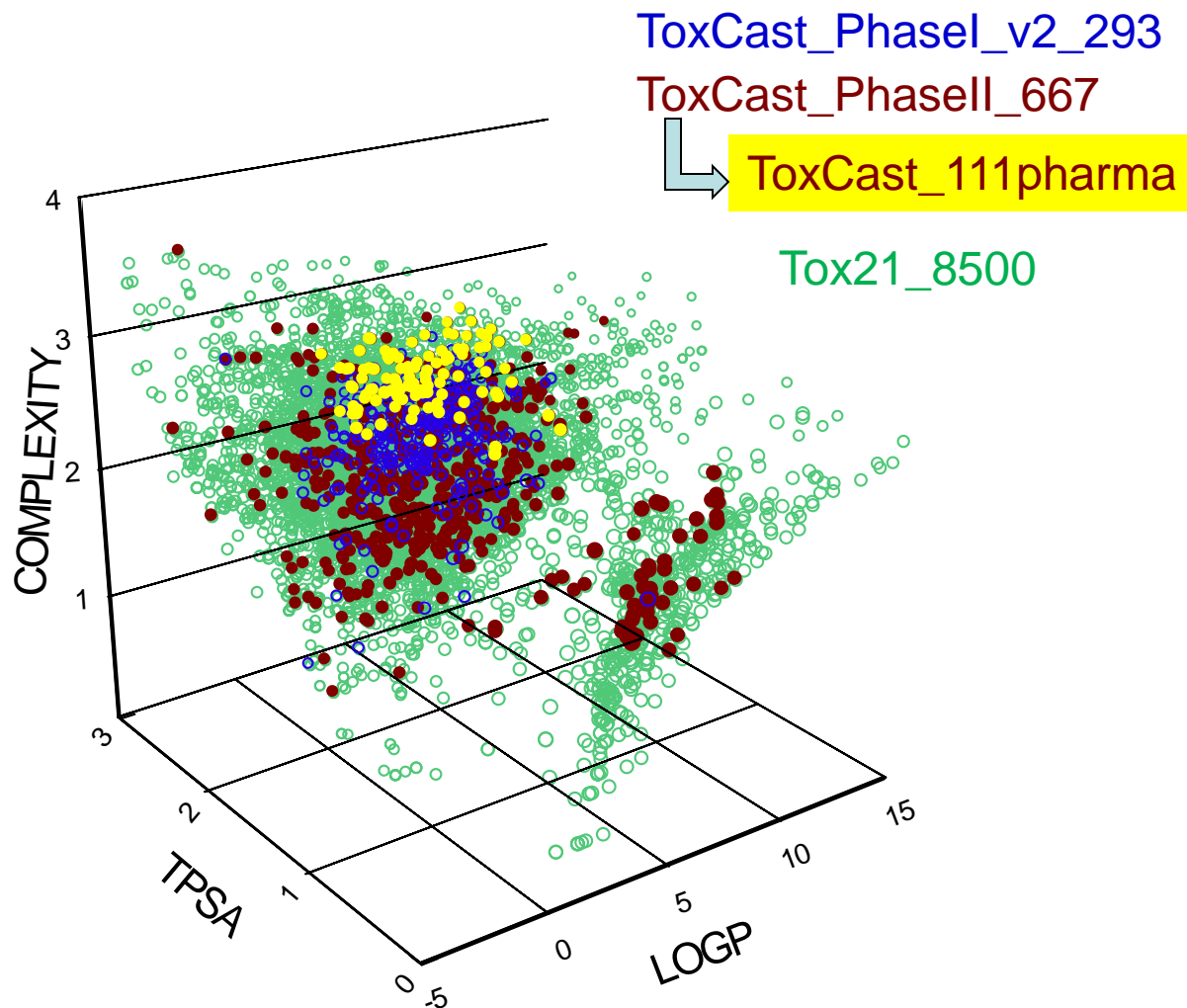
ToxCast/Tox21 property distributions

- LOG P = Octanol/Water partition coefficient
- TPSA = log (Total Polar Surface Area)
- Complexity = log (complexity based on paths, branching, atoms)



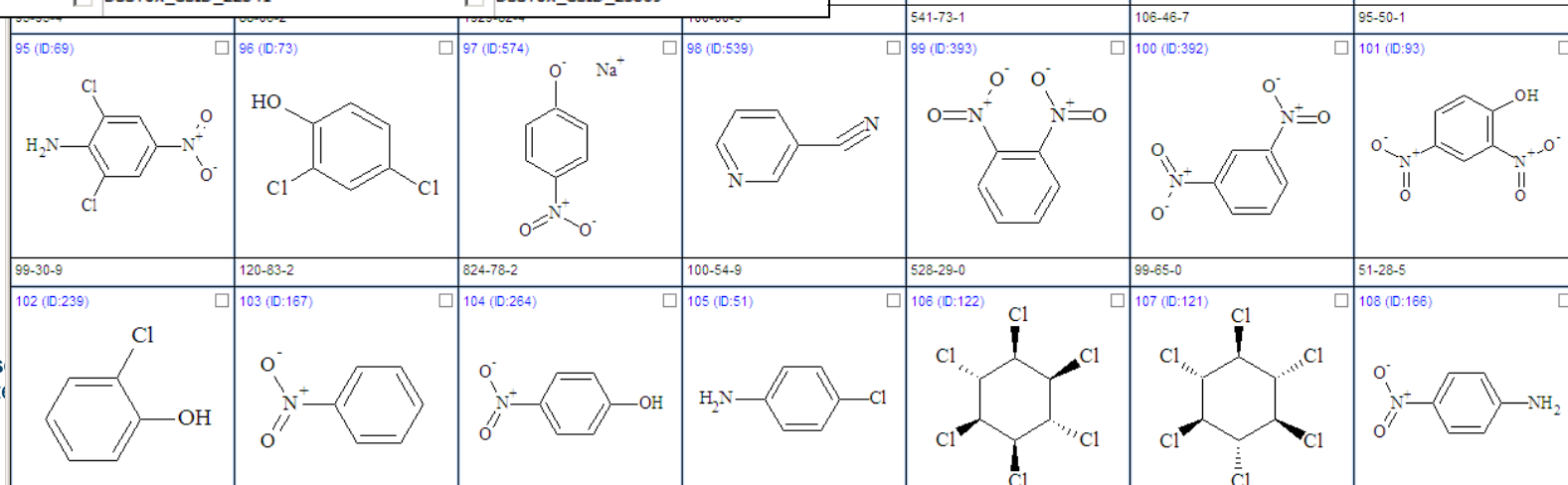
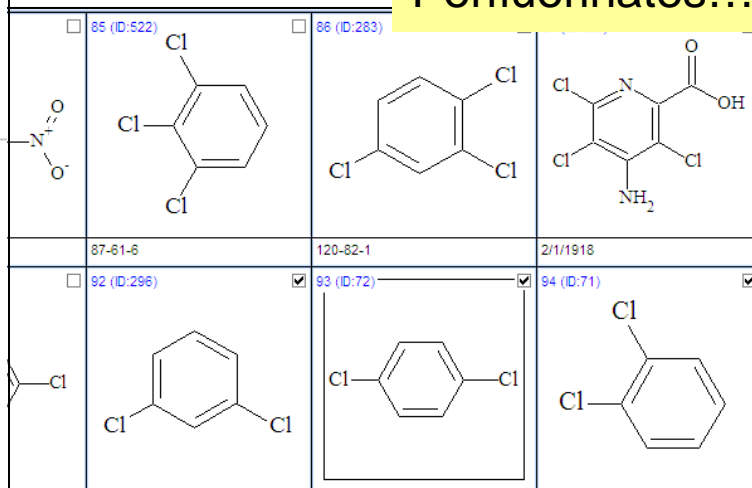
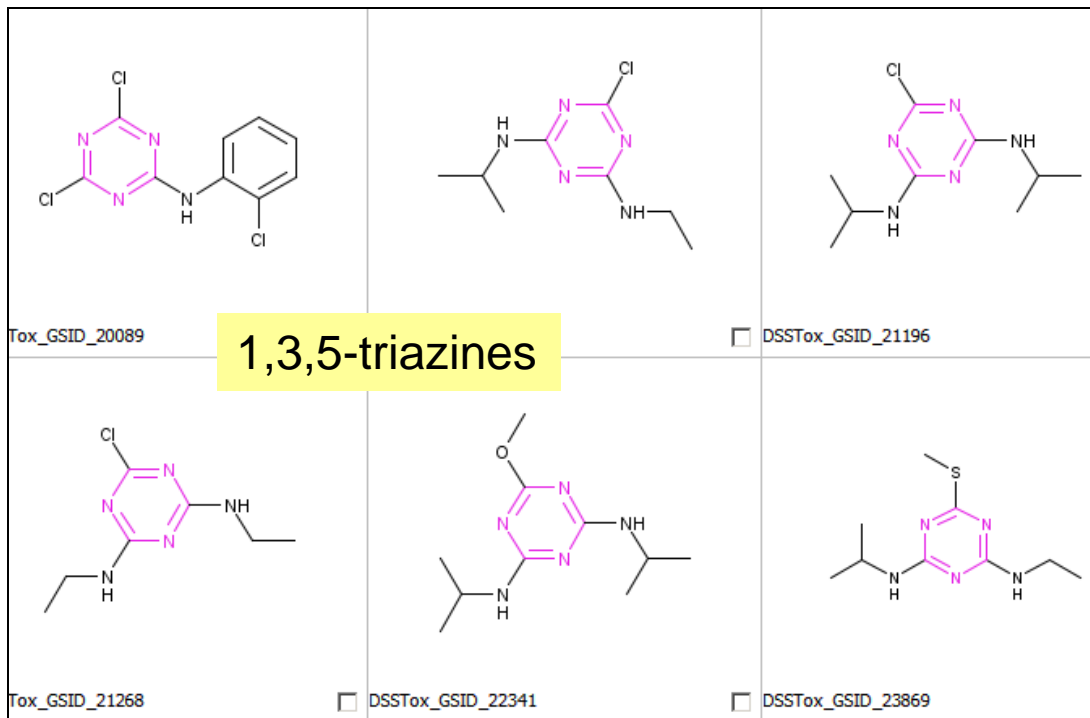
ToxCast/Tox21 property distributions

- LOG P = Octanol/Water partition coefficient
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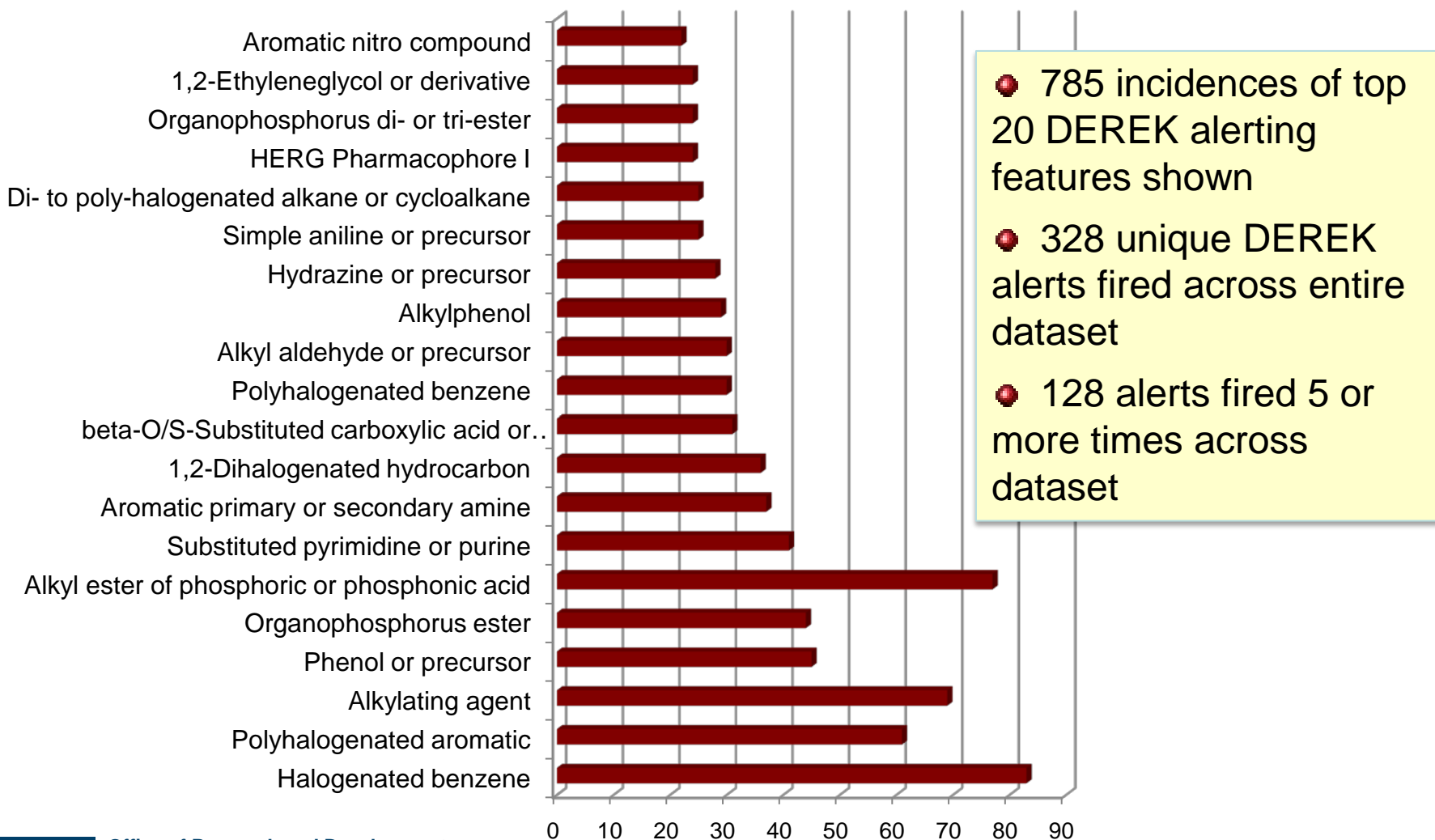


ToxCast_960 Analog Sets

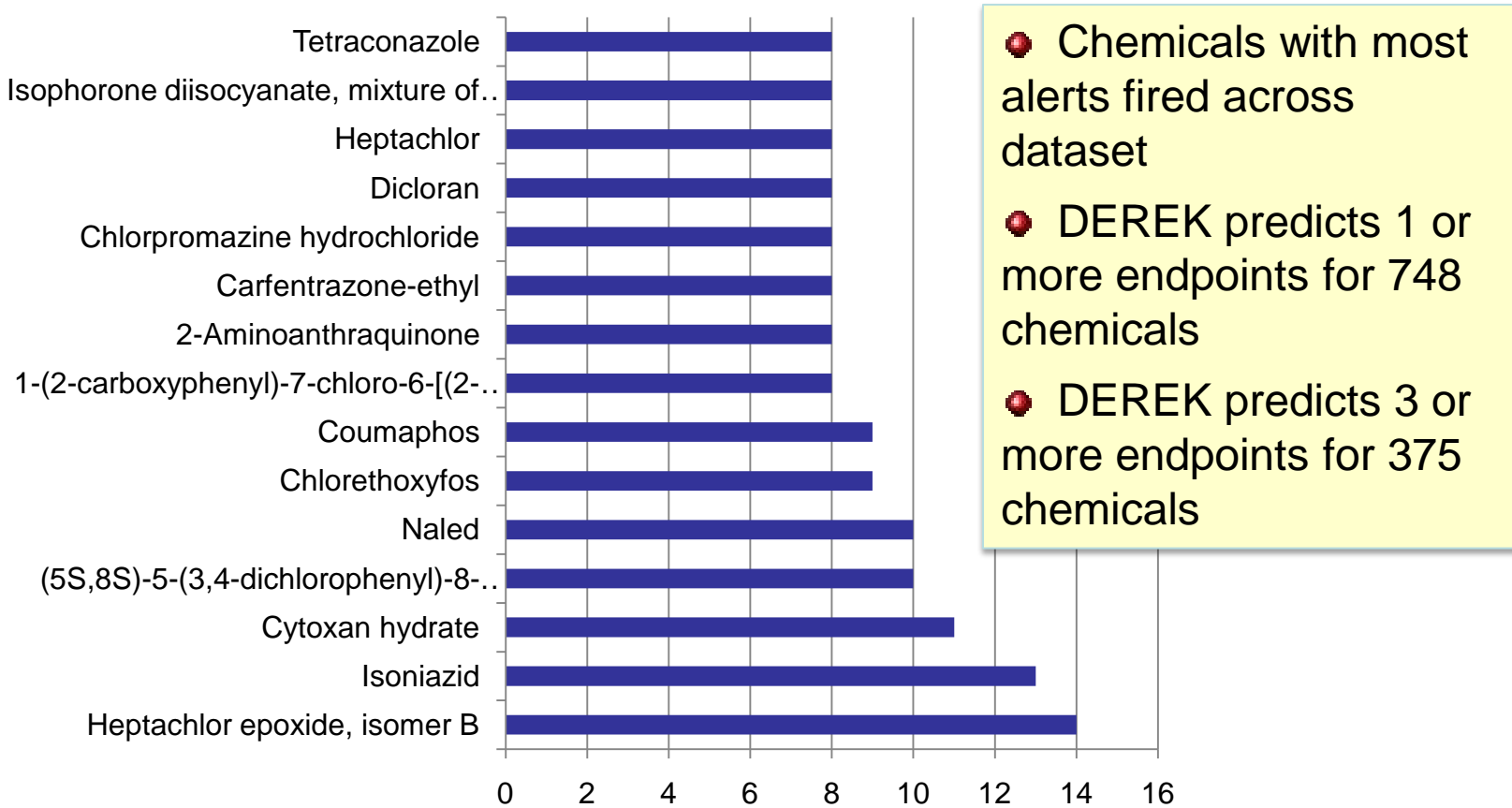
Chlorobenzenes
Phenols
Nitrobenzenes
Aromatic amines
Phthalates
Perfluorinates...



DEREK Predictions for ToxCast_960



DEREK Predictions for ToxCast_960



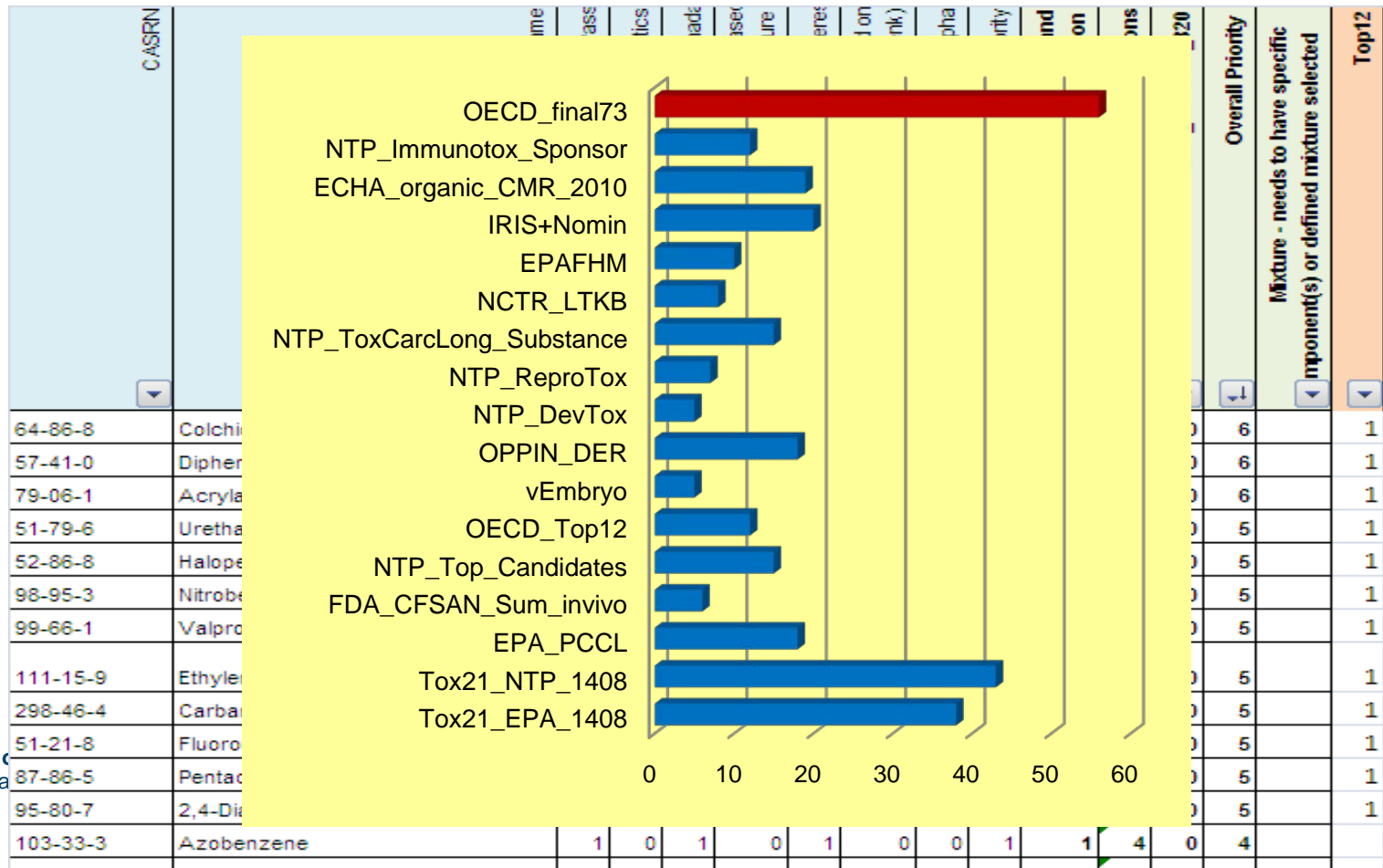
DEREK Predictions for ToxCast_960

- 41 Endpoints predicted across dataset
- 31 Endpoints predicted for 6 or more compounds (1925 total predictions)
- Top endpoints shown

	Carcinogenicity	Cholinesterase inhibition	Chromosome damage	Genotoxicity	Mutagenicity	Neurotoxicity	Peroxisome proliferation	Nephrotoxicity	Skin sensitisation	Teratogenicity	Total No. Compounds
Carcinogenicity	1.00										217
Cholinesterase inhibition	0.52	1.00									45
Chromosome damage	0.52	0.58	1.00								178
Genotoxicity	0.55	0.86	0.64	1.00							12
Mutagenicity	0.60	0.69	0.61	0.68	1.00						155
Neurotoxicity	0.53	0.85	0.61	0.93	0.65	1.00					17
Peroxisome proliferation	0.49	0.76	0.54	0.83	0.56	0.82	1.00				60
Nephrotoxicity	0.45	0.52	0.41	0.54	0.41	0.54	0.49	1.00			220
Skin sensitisation	0.42	0.49	0.47	0.46	0.55	0.45	0.40	0.37	1.00		289
Teratogenicity	0.52	0.75	0.57	0.82	0.59	0.84	0.77	0.49	0.42	1.00	63

OECD Priority Chemicals in ToxCast

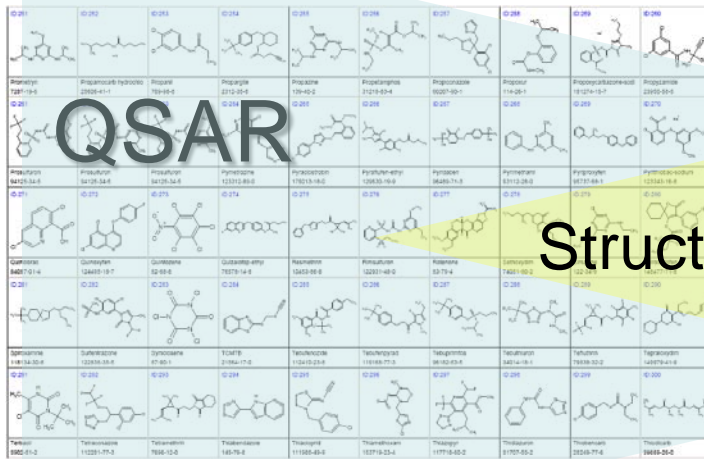
- 59 of 73 nominated compounds procured
- 56 of these in PhaseII, including top 12 (*volatiles, mixtures*)



Next Steps...

- Publish EPA Tox21 & ToxCast chemical library files (ACToR, DSSTox, PubChem, Leadscope)
- Facilitate creation of cheminformatics capabilities; generate suite of properties for chemical library:
 - > **Molecular Networks (ADRIANNA, MOSES); DEREK, etc**
- Integrate chemical-assay-in vivo databases
- Examine chemical analog sets, pairs for assay correspondence, metabolic surrogates, read-across...
- Engage QSAR community
- Phase III challenges: DMSO solubility, volatility, water solubility, procurement, ...

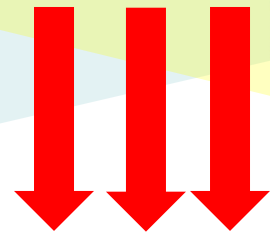
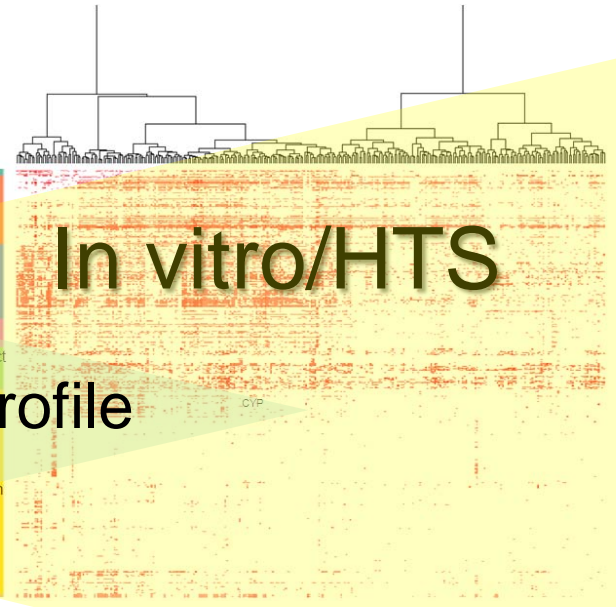
Combined Approaches



Structure + Biological profile

ToxCast Phase I HTS
(40 assays)

- ACEA
- Attagene
- Bioseek
- Cellumen
- CellzDirect
- Centronix
- NovaScreen
- Solidus



Acknowledgements:

- ✦ EPA NCCT ToxCast Team:
 - Robert Kavlock - Director
 - David Dix
 - Keith Houck
 - Matt Martin (ToxRefDB)
 - Richard Judson (ACToR)
 - Thomas Knudsen
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