

Laying the Chemical Foundation for Tox21 & ToxCast

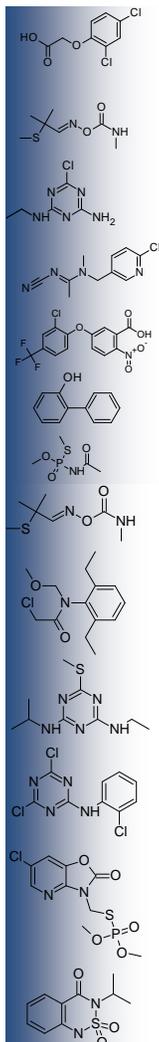
Chemical Prioritization Community of Practice, November 19, 2009

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY



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Outline



● Past

- ToxCast Phase I
- Analytical QC
- Building a chemical registry and sample tracking system
- Tox21 (& ToxCast Phase II) Nomination Process

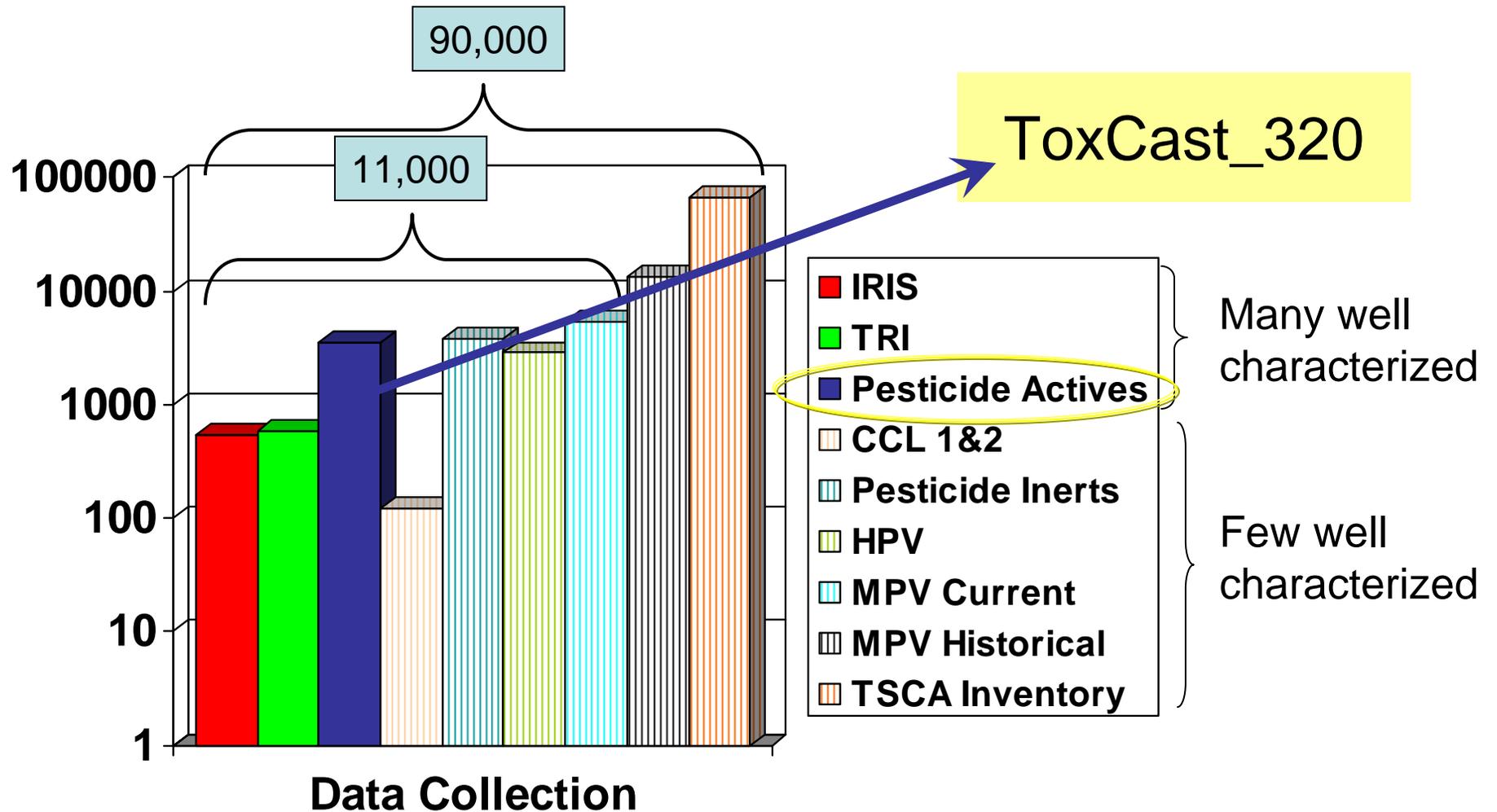
● Present

- Status of EPA nominations, procurements
- Analytical QC contract for Tox21 (& ToxCast Phase II)

● Future

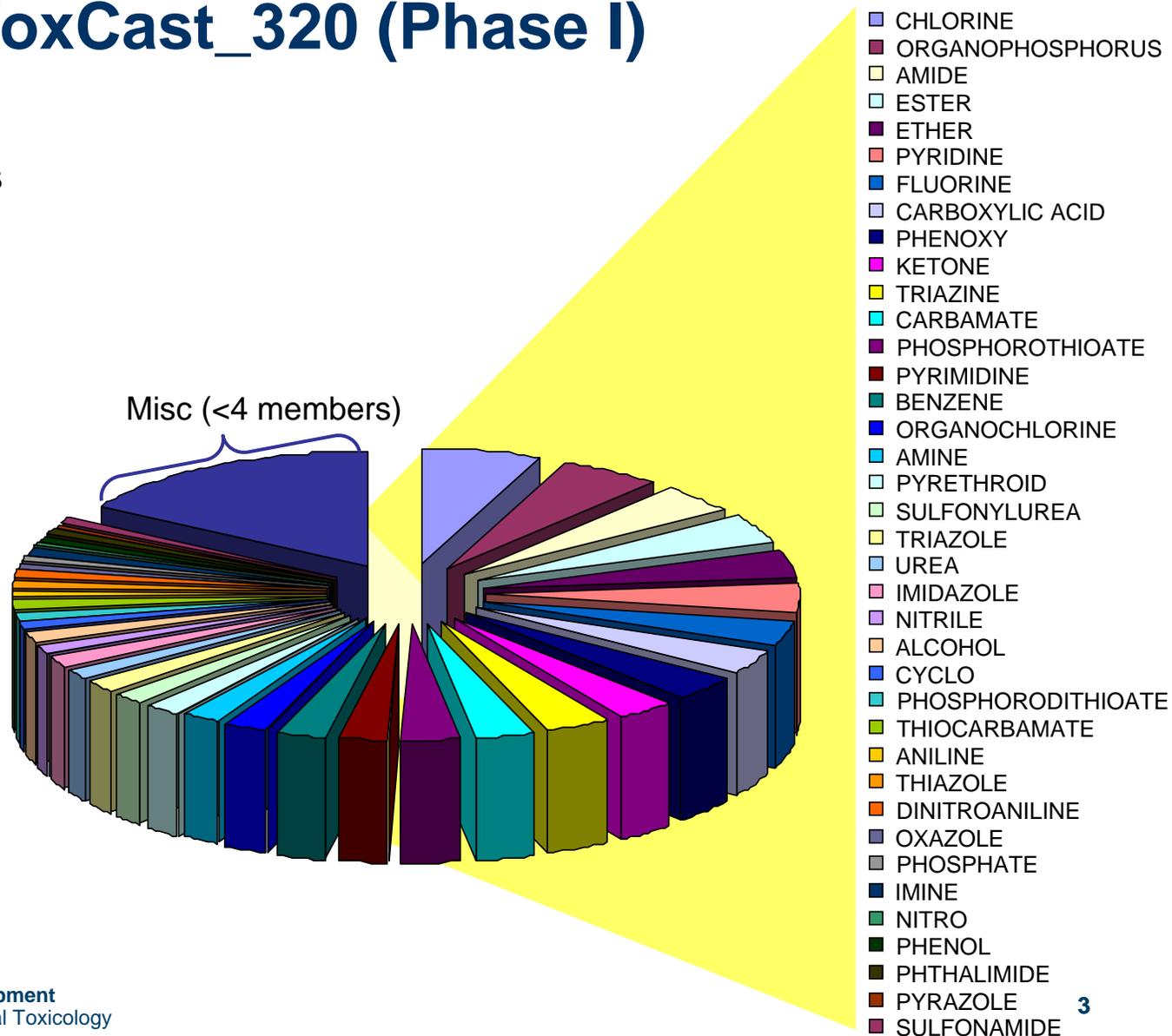
- Linkage of chemical registry to assay results
- Building a cheminformatics analysis capability
- Finalize & publish Tox21 chemical libraries

ToxCast Phase I Chemicals



Chemical Classes in ToxCast_320 (Phase I)

- 309 Unique Structures
- Replicates for QC
- 291 Pesticide Actives
- 9 Industrial Chemicals
- 13 Parent/Metabolite pairs
- 56/73 Proposed Tier 1 Endocrine Disruption Screening Program
- 14 High Production Volume Chemicals
- 11 HPV Challenge

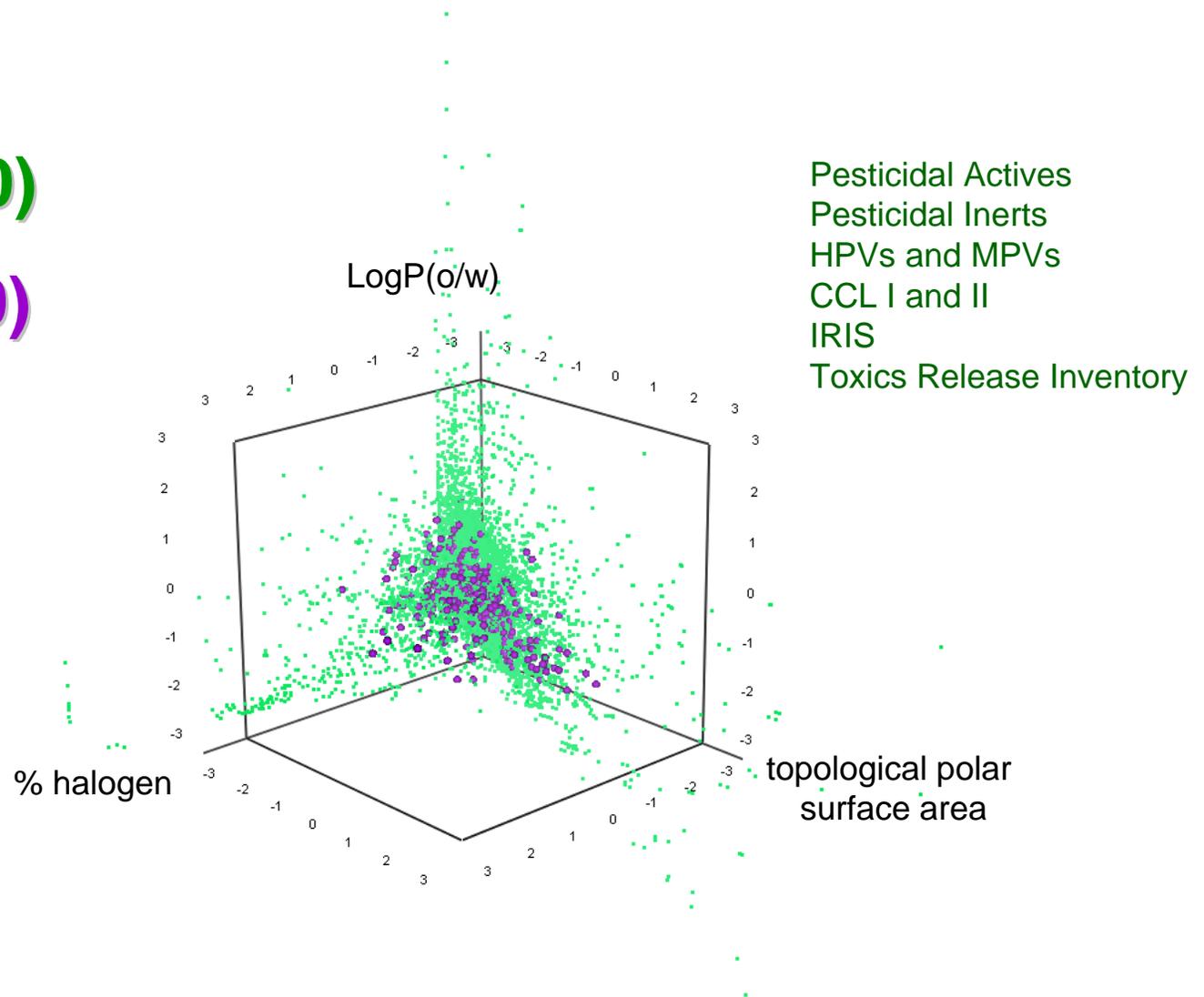


Chemical Diversity/Coverage

ACToR (8000)

ToxCast (320)

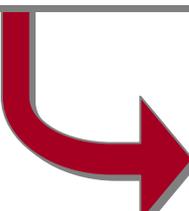
- Good representation of compounds across property space
- Few compounds with extreme property values



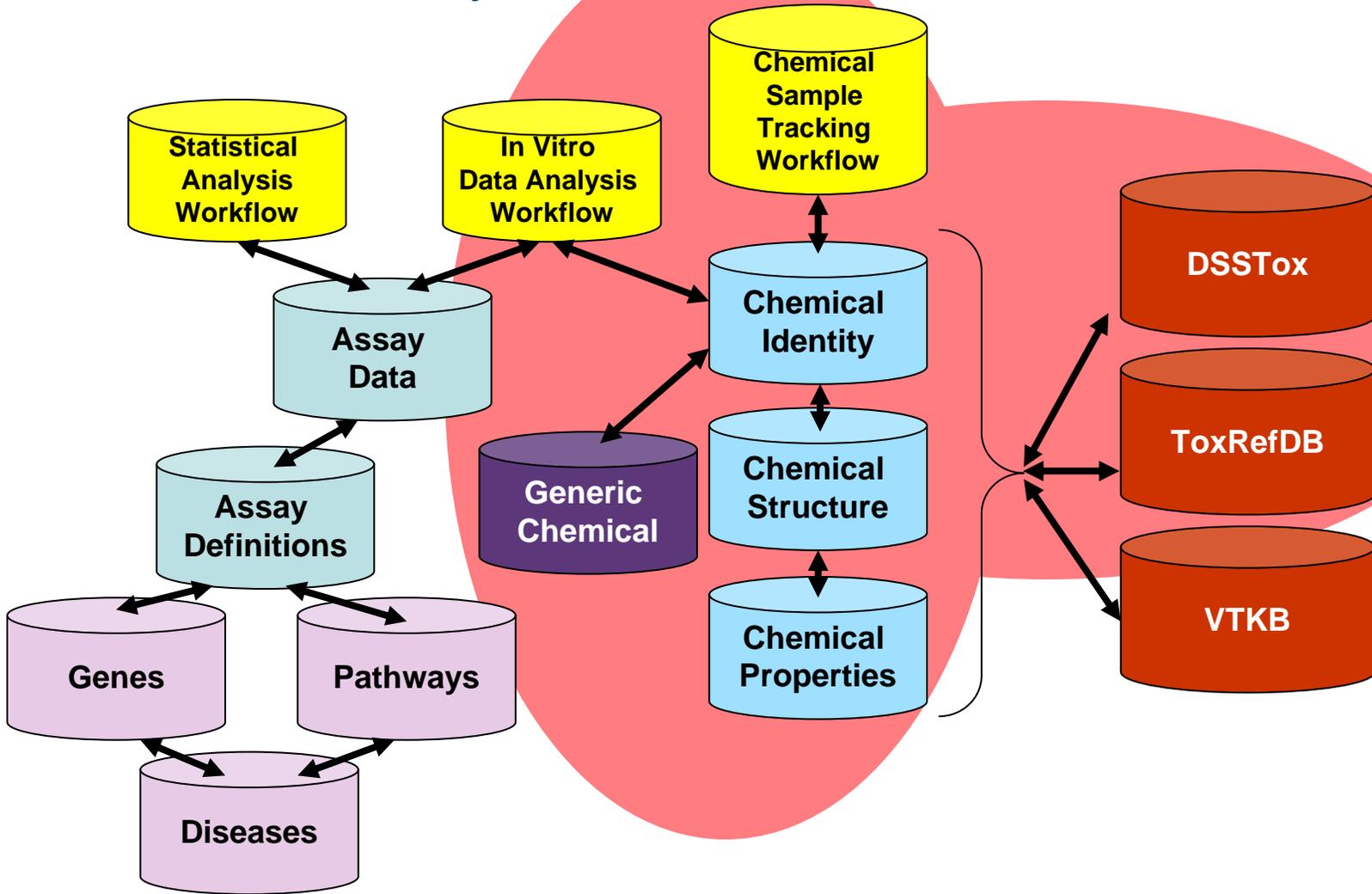
ToxCast Phase I: Analytical QC

- Initial solution plates analyzed subsequent to assay data generation
- PC/HPLC/UPLC automated methods used to test purity of samples
- Additional review of results against reference spectra; follow-up with GC-MS

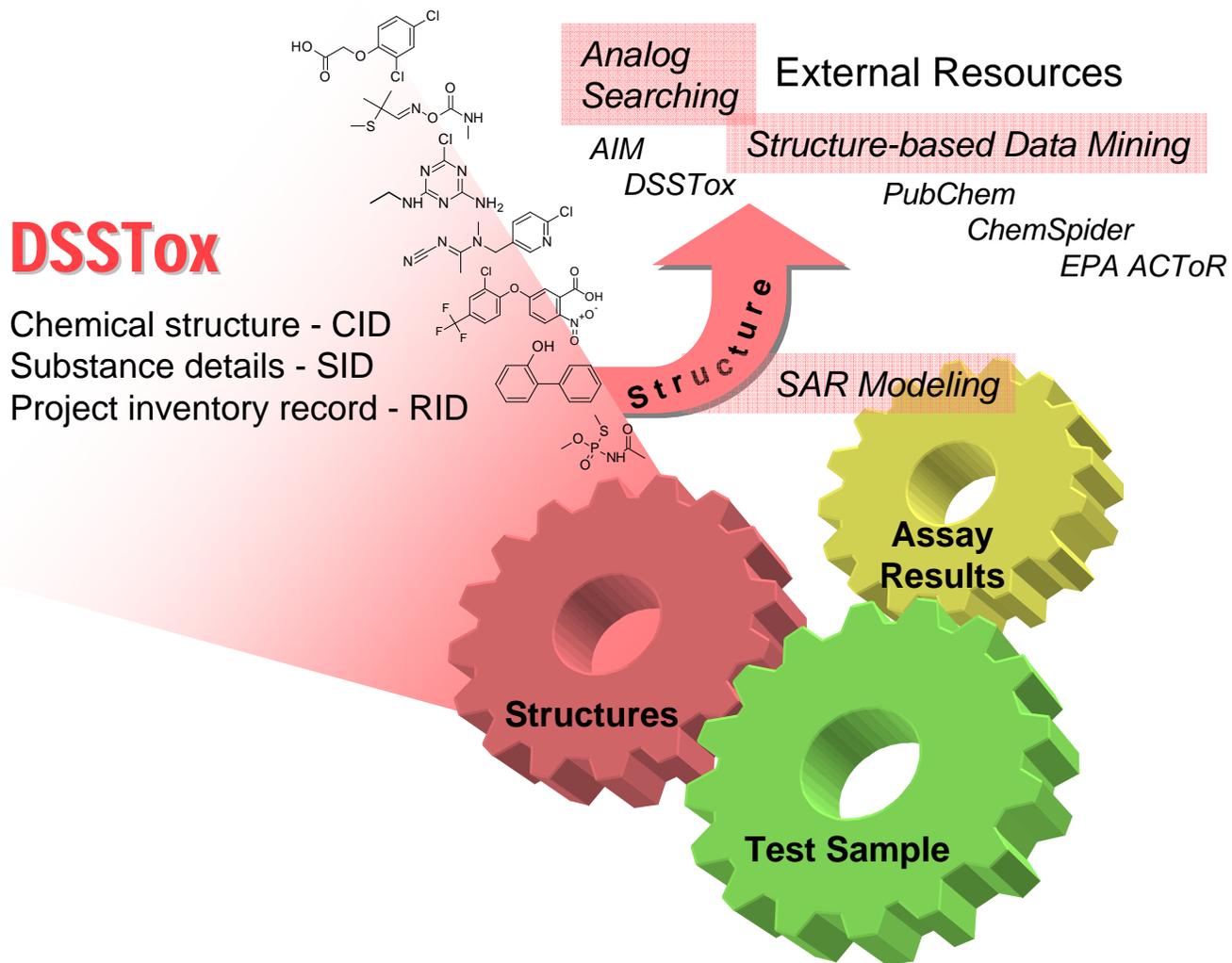
- 
- Results were able to confirm parent ion peak and >80% purity for 85% of Phase I solution samples
 - Some problems in stability observed for a class of compounds (sulfurones) with generally low *in vivo* and *in vitro* activity

- 
- Proposed summary QC score:
 - *PASS, Undetermined, FAIL*
 - Plan to publish chemical file with summary QC scores to inform use of corresponding assay results

ACToR: Aggregated Computational Toxicity Resource



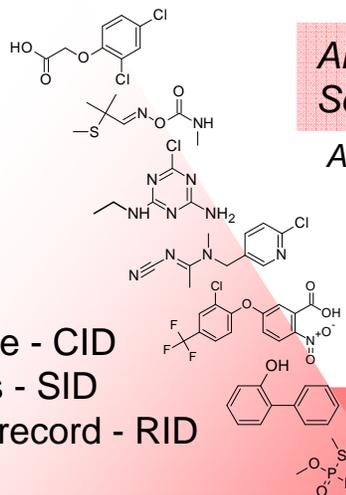
ToxCast/Tox21 Chemical Registry



ToxCast/Tox21 Chemical Registry

DSSTox

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



Analog Searching

External Resources

AIM

Structure-based Data Mining

DSSTox

PubChem

ChemSpider

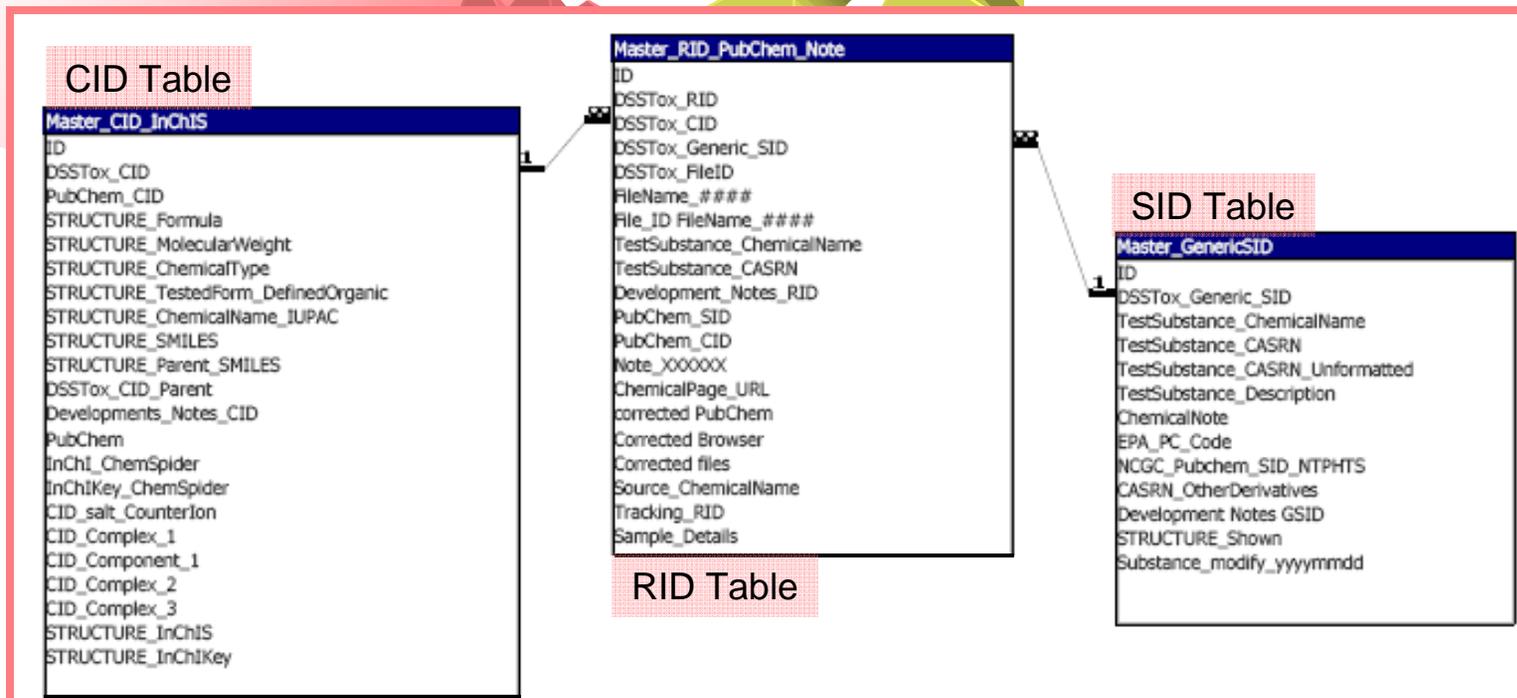
EPA ACToR

Structure

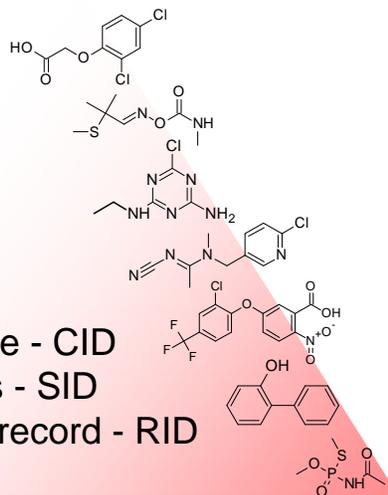
SAR Modeling



Tox21
 ToxCast
 ToxRefDB



ToxCast/Tox21 Chemical Registry

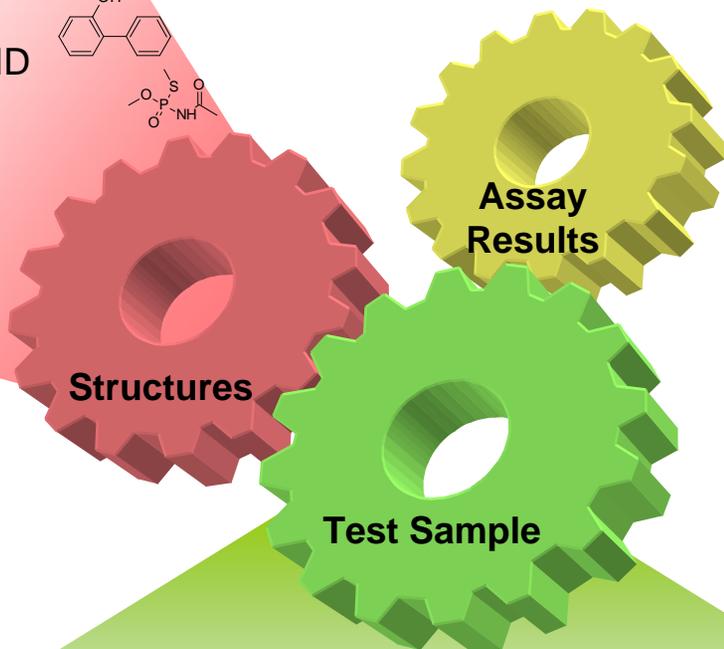


DSSTox

Chemical structure - CID

Substance details - SID

Project inventory record - RID



DSSTox RID

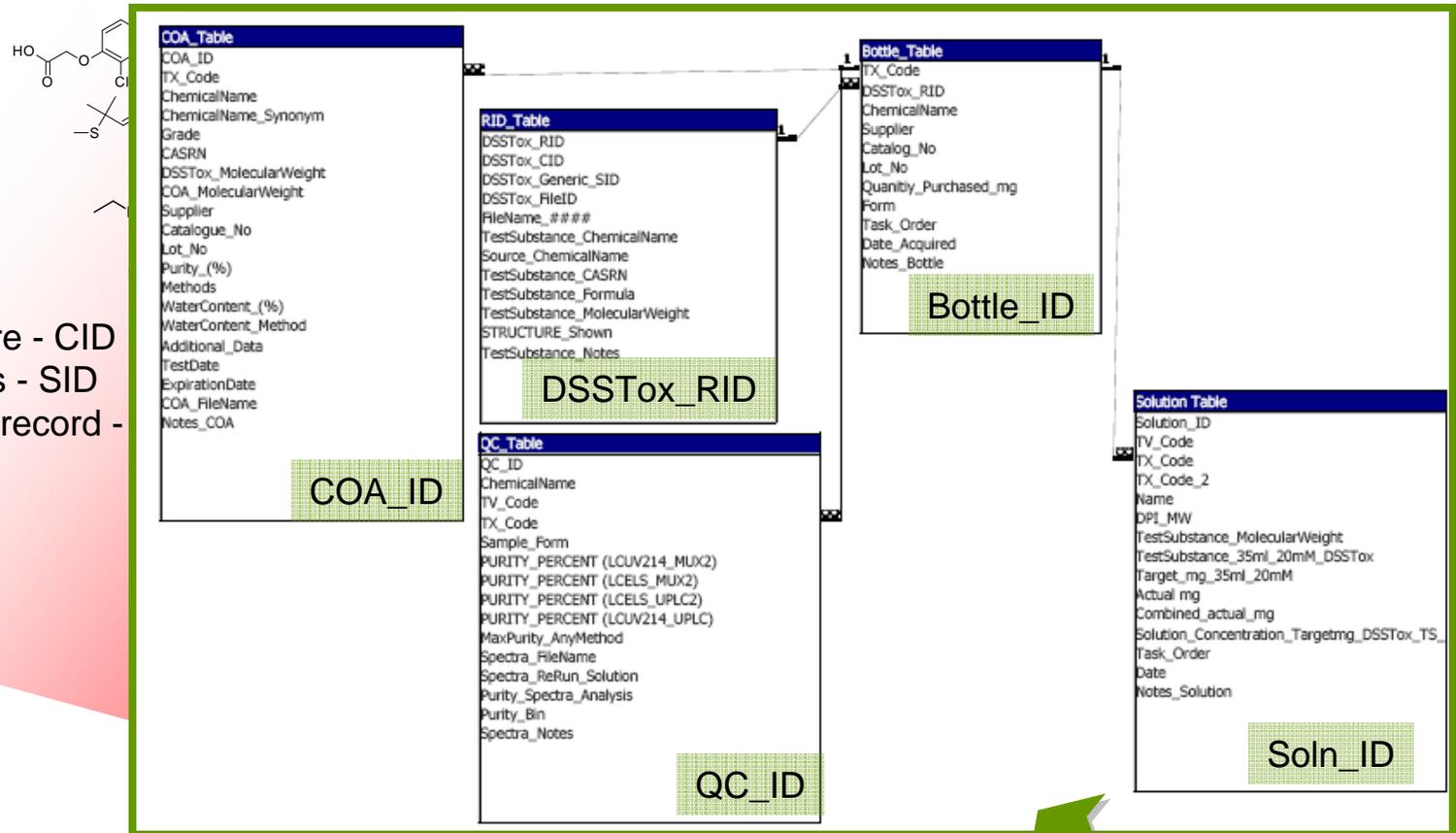
Bottle ID (→ COA ID)

Solution ID (→ QC ID)

Tox21 Sample Tracking Database

Past | Present | Future

ToxCast/Tox21 Chemical Registry



DSSTox

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

Test Sample

DSSTox RID

Bottle ID (→ COA ID)

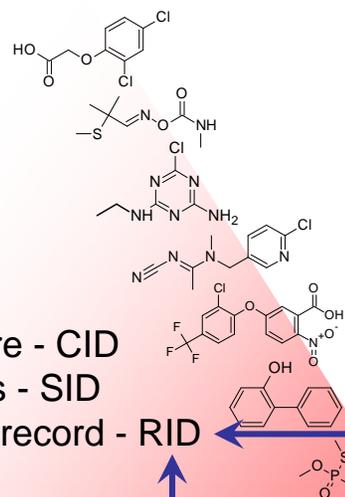
Solution ID (→ QC ID)

Tox21 Sample Tracking Database

ToxCast/Tox21 Chemical Registry

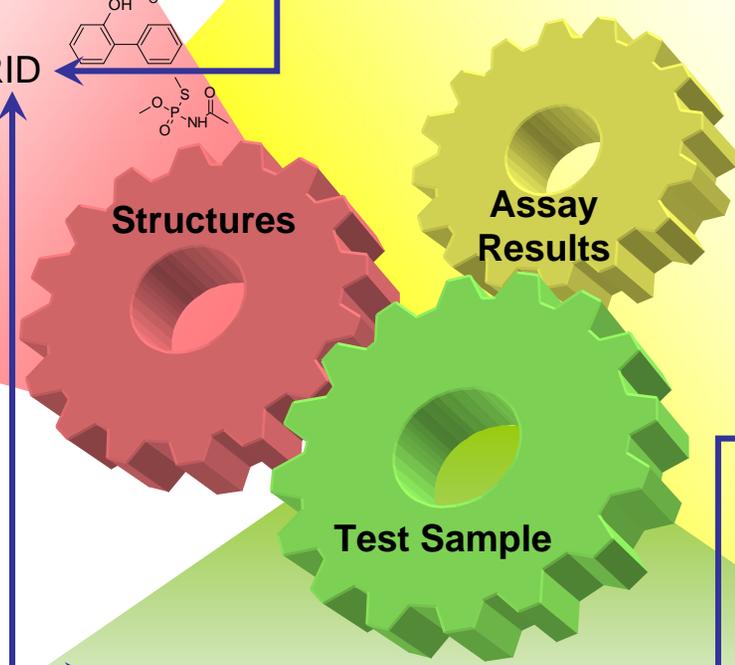
DSSTox

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



ACToR/ToxMiner

Assay name
Assay details
Assay outcome

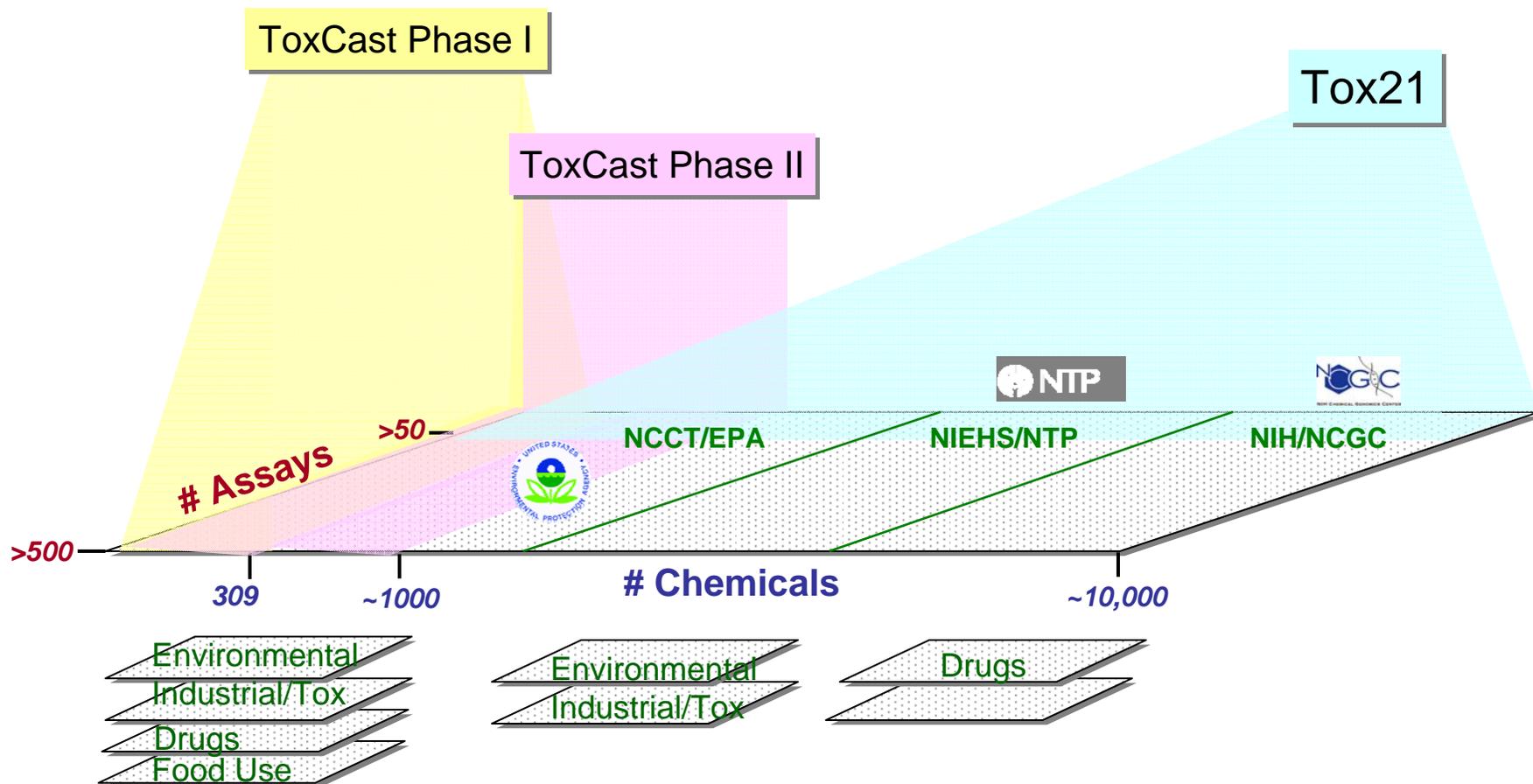


Solution ID
Plate ID
Plate Address ID

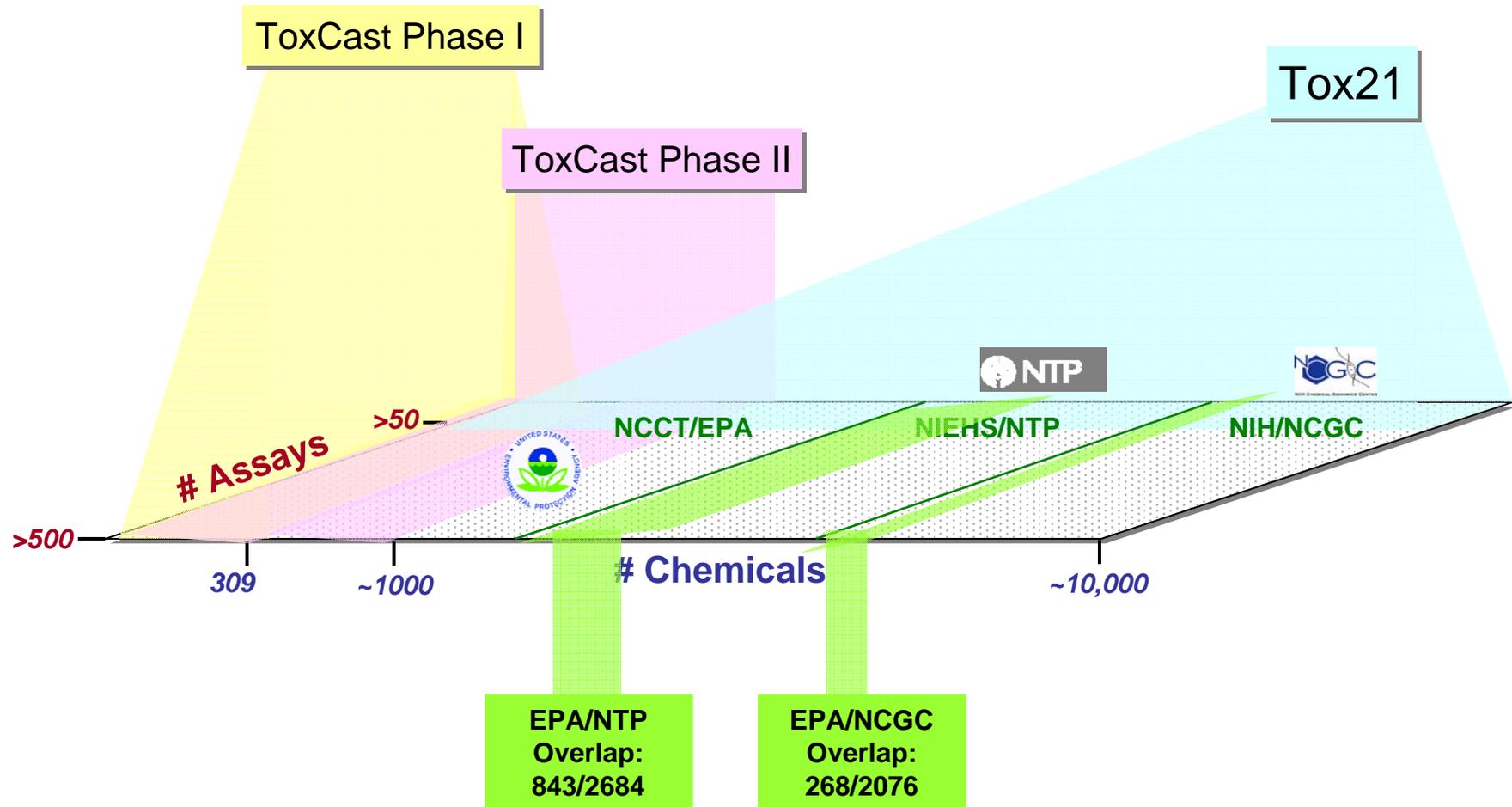
DSSTox RID
Bottle ID (→ COA ID)
Solution ID (→ QC ID)

Tox21 Sample Tracking Database

ToxCast/Tox21 Chemical Landscape

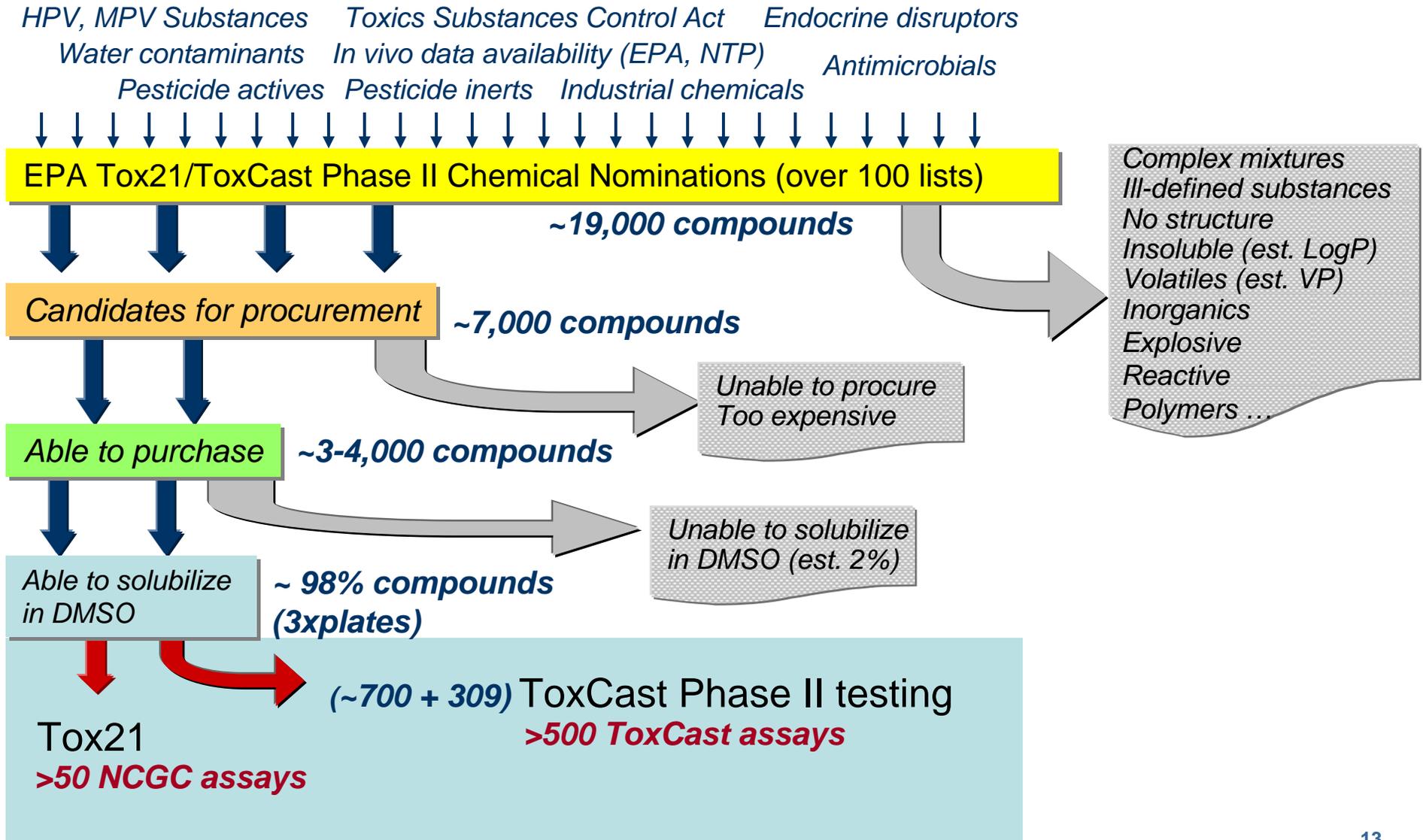


ToxCast/Tox21 Chemical Landscape



- NTP & NCGC overlaps for Phase II
- Need to weigh cost/benefits of additional overlap

EPA ToxCast/Tox21 Chemical Procurement



Procurement status of priority EPA Lists

Antimicrobials

Antimicrobials_359		
Total	359	% of total
Pass Phys Chem	191	53%
Requested	186	52%
# on NTP Plates	126	35%
# on ToxCast_309	33	9%
#Procured	113	31%
61% of requested were procured		

Chemical Contaminant List

CCL3_102		
Total	102	% of total
Pass Phys Chem	67	66%
Requested	45	44%
# on NTP Plates	66	65%
# on ToxCast_309	26	25%
#Procured	41	40%
91% of requested were procured		

HPV Challenge

OPPT_RAD_399		
Total	399	% of total
Pass Phys Chem	195	49%
Requested	255	64%
# on NTP Plates	263	66%
# on ToxCast_309	4	1%
#Procured	113	28%
44% of requested were procured		

Preliminary Chemical Contaminant List

PCCL_528		
Total	528	% of total
Pass Phys Chem	352	67%
Requested	346	65%
# on NTP Plates	410	77%
# ToxCast_309	73	14%
#Procured	235	45%
67% of requested were procured		

Pesticide Inerts

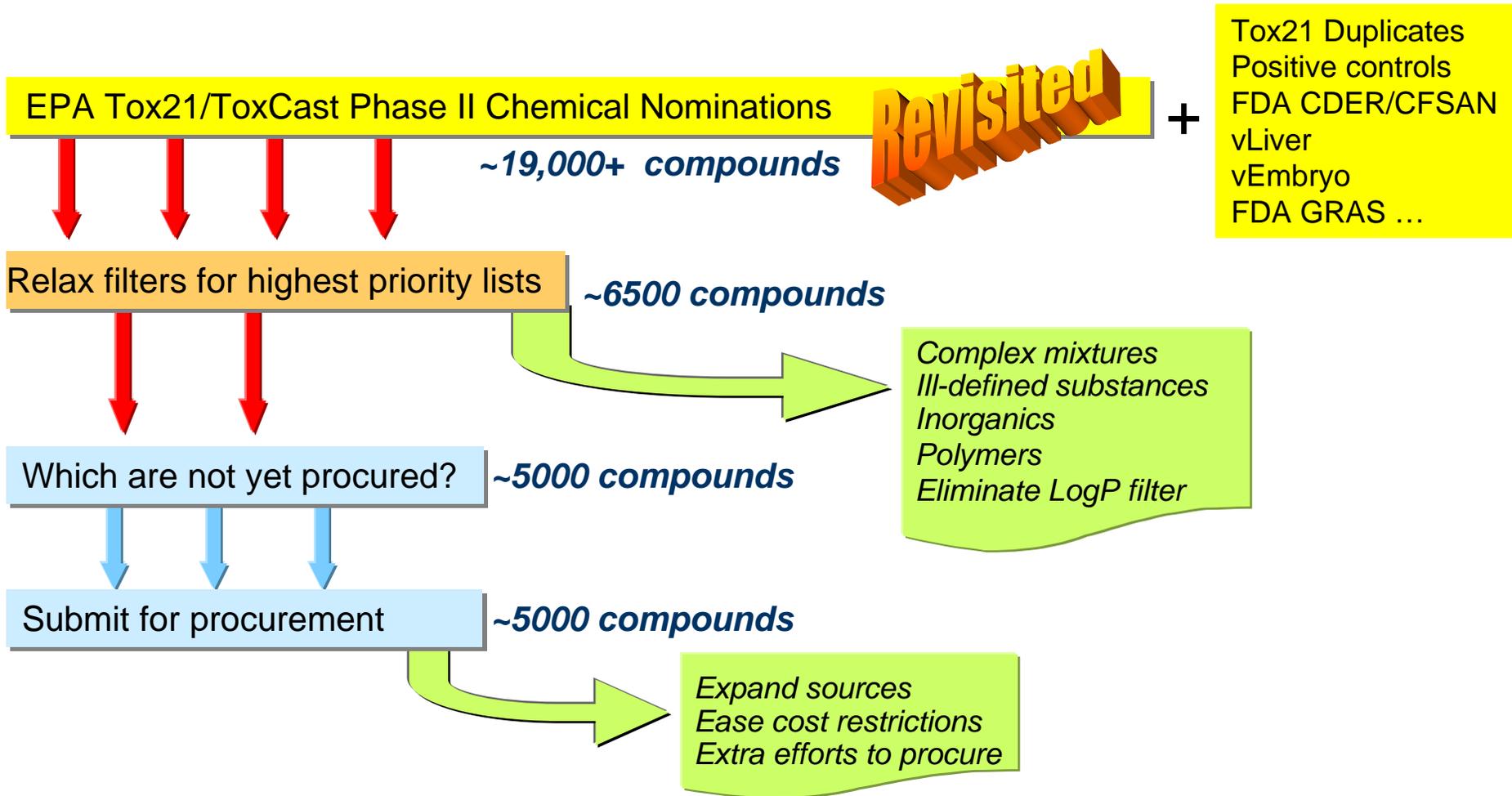
Inerts_3836		
Total	3836	% of total
Pass Phys Chem	519	14%
Requested	512	13%
# on NTP Plates	395	10%
# on ToxCast_309	50	1%
#Procured	222	6%
43% of requested were procured		

Green Chemistry

DfE_183		
Total	183	% of total
Pass Phys Chem	105	57%
Requested	132	72%
# on NTP Plates	115	63%
# on ToxCast_309	4	2%
#Procured	58	32%
44% of requested were procured		

- 14-67% of totals pass PhysChem filters
- Most of those were requested
- 43-91% of requested were procured

EPA ToxCast/Tox21 Chemical Procurement



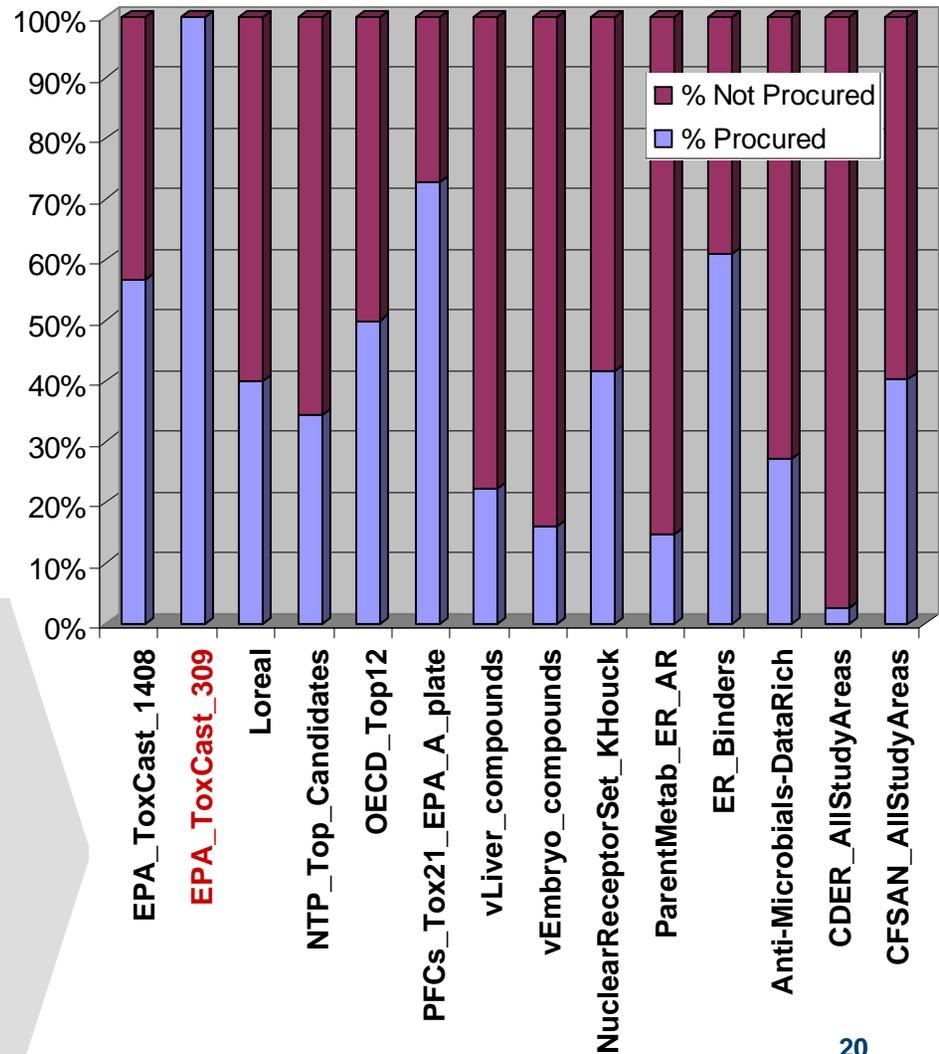
- Approx 40% of 5000 were already requested, unsuccessful at procuring
- Remaining 60% have not been requested previously
- Prior procurement success rate ~40%; estimate 1200-1500 additional chemicals

Priority 1 Overlap Table

1	Tox21_OrdeID_0	CASRN	ChemicalName	PhysChemPass	EPA_ToxCast_1408	EPA_ToxCast_320	Loreal	HTP_Top_Candidates	OECD_Top12	PFCS_in_Toxt21_EPA_A_plate	vLiver_compounds	vEmbryo_20091023	NuclearReceptorSet_KHouck	ParentMetab_ER_AR_Hormones	ER_Binders DD	Anti-Microbials-MM 0211ov	CDER_AllStudyAreas	CFSAIL_AllStudyAreas	Priority Incidences
2	Tox21_50022_0	50-02-2	Dexamethasone	1	1	0	0	1	0	0	0	1	1	1	0	0	0	0	5
3	Tox21_56531_0	56-53-1	Diethylstilbestrol (DES)	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	4
4	Tox21_50282_0	50-28-2	Estradiol 17B	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	4
5	Tox21_57830_0	57-83-0	Progesterone	1	1	0	0	1	0	0	0	0	1	1	0	0	0	0	4
6	Tox21_302794_0	302-79-4	All-trans retinoic acid	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	3
7	Tox21_99661_0	99-66-1	Valproate (Valproic acid)	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	3
8	Tox21_13311847_0	13311-84-7	Flutamide	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	3
9	Tox21_637070_0	637-07-0	Clofibrate	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	3
10	Tox21_97322877_0	97322-87-7	Troglitazone	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0	3
11	Tox21_427510_0	427-51-0	Cyproterone acetate	1	1	0	0	0	0	0	0	0	1	1	0	0	0	0	3
12	Tox21_54965241_0	54965-24-1	Tamoxifen citrate	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	3
13	Tox21_434071_0	434-07-1	Oxymetholone	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	3
14	Tox21_51525_0	51-52-5	Propylthiouracil	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	3
15	Tox21_446720_0	446-72-0	Genistein	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	3
16	Tox21_98953_0	98-95-3	Nitrobenzene	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	3
17	Tox21_58220_0	58-22-0	Testosterone and its esters	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	3
18	Tox21_64868_0	64-86-8	Colchicine	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	3
19	Tox21_57556_0	57-55-6	Propylene Glycol	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
20	Tox21_25265718_0	25265-71-8	Dipropylene glycol	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
21	Tox21_111308_0	111-30-8	Glutaraldehyde	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
22	Tox21_25155300_0	25155-30-0	Dodecylbenzenesulfonic acid, Na salt	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
23	Tox21_112276_0	112-27-6	Triethylene glycol	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
24	Tox21_27176870_0	27176-87-0	4-Dodecylbenzenesulfonic acid	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
25	Tox21_7758192_0	7758-19-2	Chlorite	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	2
26	Tox21_7647145_0	7647-14-5	Sodium chloride	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2
27	Tox21_26530201_0	26530-20-1	Octhilinone	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
28	Tox21_112050_0	112-05-0	Nonanoic acid	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
29	Tox21_107534963_0	107534-96-3	Tebuconazole	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
30	Tox21_20018091_0	20018-09-1	Diiodomethyl 4-methylphenyl sulfone	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
31	Tox21_142596_0	142-59-6	Nabam	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
32	Tox21_126114_0	126-11-4	2-(Hydroxymethyl)-2-nitro-1,3-propanediol	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
33	Tox21_137428_0	137-42-8	Metham sodium	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
34	Tox21_63449412_0	63449-41-2	C8-18-Alkyldimethylbenzyl ammonium chlorides	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
35	Tox21_828002_0	828-00-2	Dimethoxane	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
36	Tox21_5915413_0	5915-41-3	Terbutylazine	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2
37	Tox21_10543574_0	10543-57-4	Tetraacetythylenediamine	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	2

ToxCast/Tox21 Priority I Chemical Set

- 1804 Priority 1 chemicals for EPA Tox21/ToxCast Phase II procurement
- 867 (48%) procured
- 937 not yet procured; of these 73% pass PhysChem filters
- All 937 will be submitted for final round of procurement



- *ToxCast Phase I*
- *Tox21 plate A*
- *In vivo data*
- *Known target activities*
- *High EPA interest*

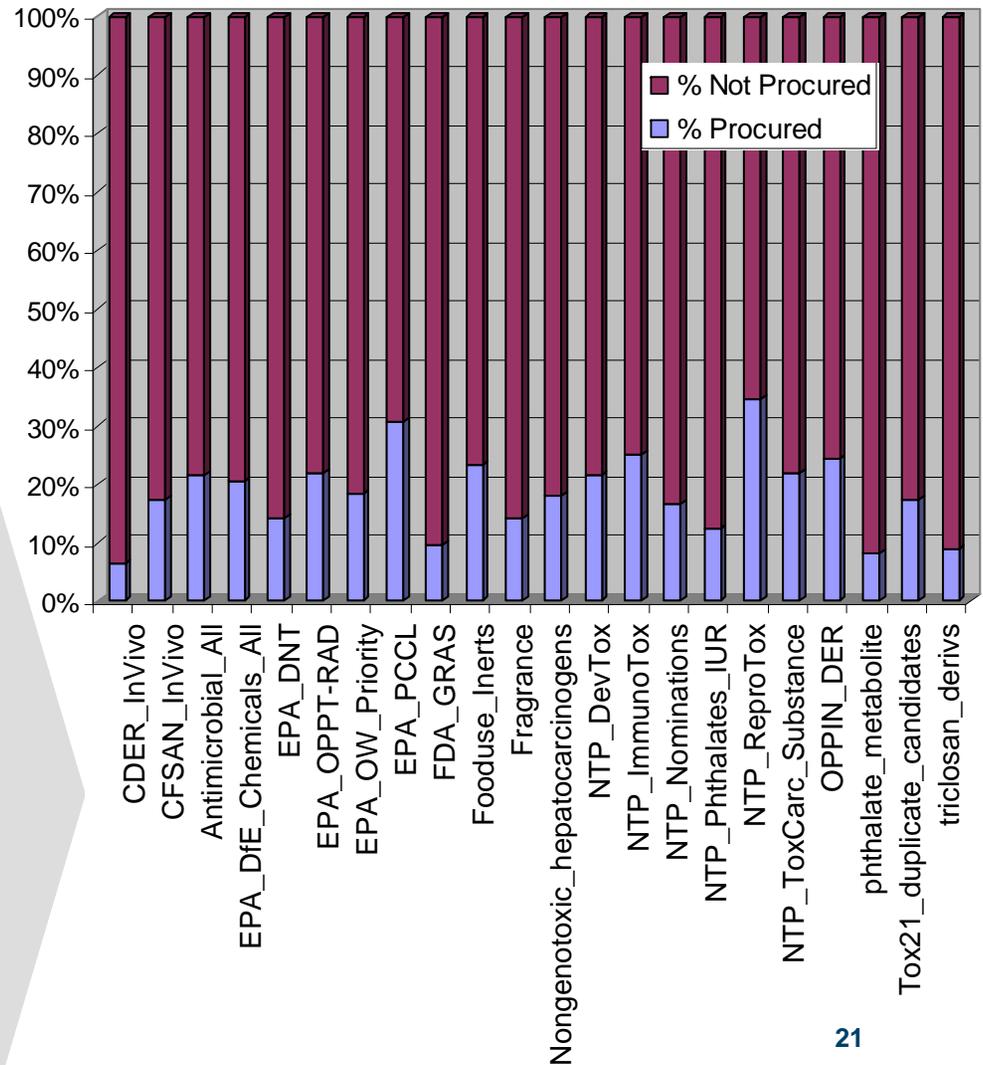
- EPA_ToxCast_1408
- EPA_ToxCast_309
- Loreal
- NTP_Top_Candidates
- OECD_Top12
- PFCs_Toxt21_EPA_A_plate
- vLiver_compounds
- vEmbryo_compounds
- NuclearReceptorSet_KHouck
- ParentMetab_ER_AR
- ER_Binders
- Anti-Microbials-DataRich
- CDER_AllStudyAreas
- CFSAN_AllStudyAreas

ToxCast/Tox21 Priority II Chemical Set

- 4776 Priority 2 chemicals for EPA Tox21/ToxCast Phase II procurement
- 826 (17%) procured
- 3950 not yet procured; of these 53% pass PhysChem filters
- Majority of 3950 will be submitted for final round of procurement

- *High/Med production chemicals*
- *Some in vivo data*
- *High EPA interest*
- *Tox21 duplicate candidates*
- *Metabolites*
- *Generally regarded as safe*

- CDER_InVivo
- CFSAN_InVivo
- Antimicrobial_All
- EPA_DfE_Chemicals_All
- EPA_DNT
- EPA_OPPT-RAD
- EPA_OW_Priority
- EPA_PCCL
- FDA_GRAS
- Fooduse_Inerts
- Fragrance
- Nongenotoxic_hepatocarcinogens
- NTP_DevTox
- NTP_ImmunoTox
- NTP_Nominations
- NTP_Phthalates_IUR
- NTP_ReproTox
- NTP_ToxCarc_Substance
- OPPIN_DER
- phthalate_metabolite
- Tox21_duplicate_candidates
- triclosan_derivs



NTP Chemicals Update for Tox21

REPLACEMENT PLATE

Procurement:

- ✓ 1112 procured
- ✓ Working on rest

Analysis:

- ✓ 1082 prepared
- ✓ 518 analyzed or analysis in review

NEW PLATE

Procurement:

- ✓ 1412 are received
- ✓ 120 being sourced/ordered
- ✓ Total so far is 1541

Analysis:

- ✓ 1112 of the compounds have been solubilized in DMSO
- ✓ 455 have been analyzed or analysis in review

TOTALS

- ✓ 2194 chemicals prepared
- ✓ 973 analyzed or analysis in review

OpAns Analytical QC for Tox21

- Contract issued, on-site QC audit completed 10/2/09
- NCGC Tox21 drug plates submitted for analysis
- OpAns will perform two analyses of solution test plates at t=0, and under assay conditions
- Excellent analytical capabilities, expertise, QC & reporting procedures
- All compounds that fail purity/identity checks will undergo follow-up review/analysis
- All results will be summarized and provided in final report to Tox21 partners
- NTP is performing analytical QC for neat compound; results will be available for OpAns analytical follow-up review/analysis

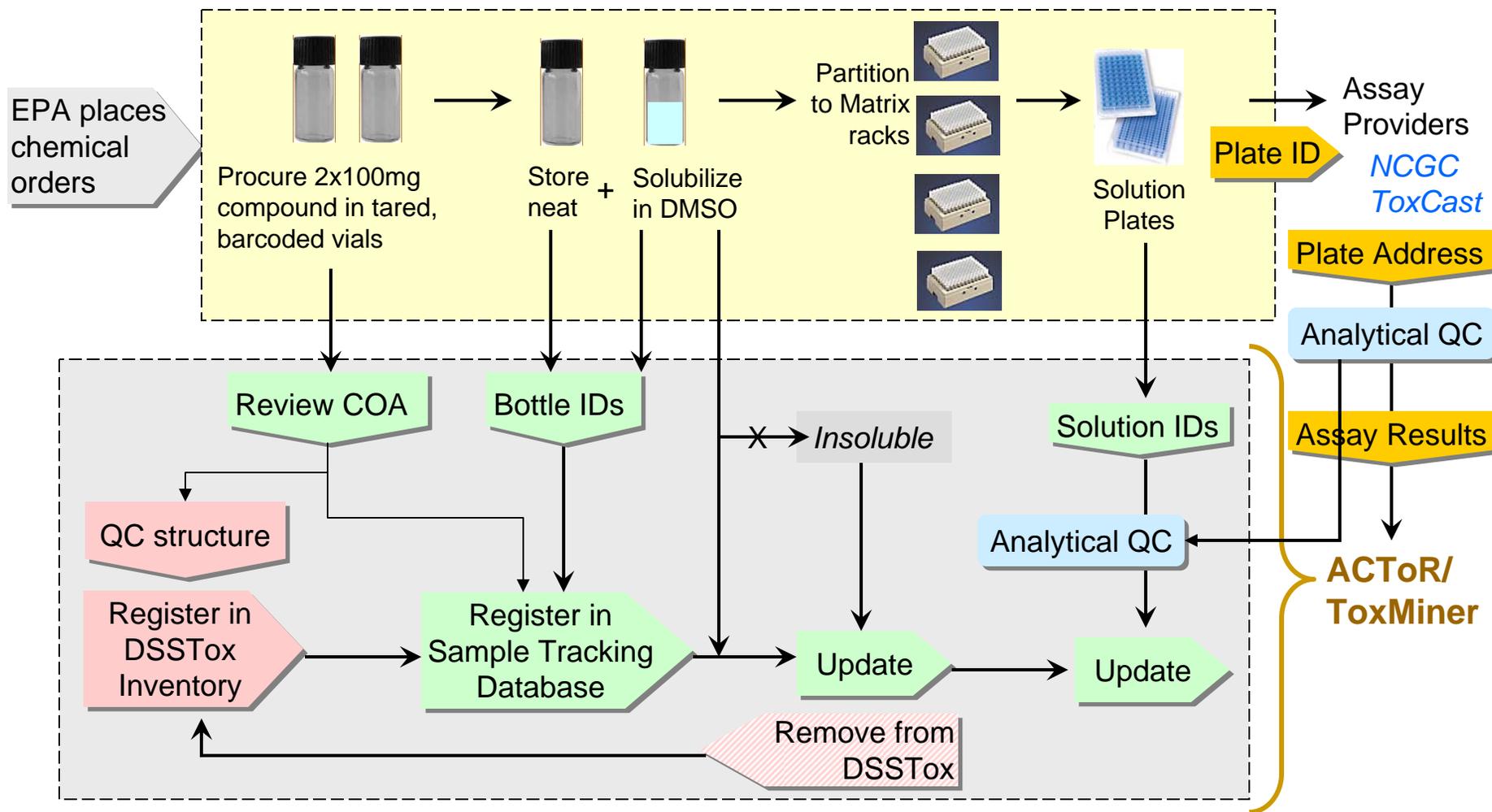
Remaining steps to finalizing EPA Tox21/Phase II Inventory

- Final round of high priority procurements
- QC of COAs, register in DSSTox, Sample Tracking Database
- Obtain external chemical sets from Glaxo, Pfizer, ...
- Consult with EPA colleagues to construct set of defined single or multi-MOA chemical mixtures
- Make decisions about duplicates (plates, chemicals, assays, ...)
- From finalized list of procured chemicals, will select subset for ToxCast Phase II chemical set (approx 700)

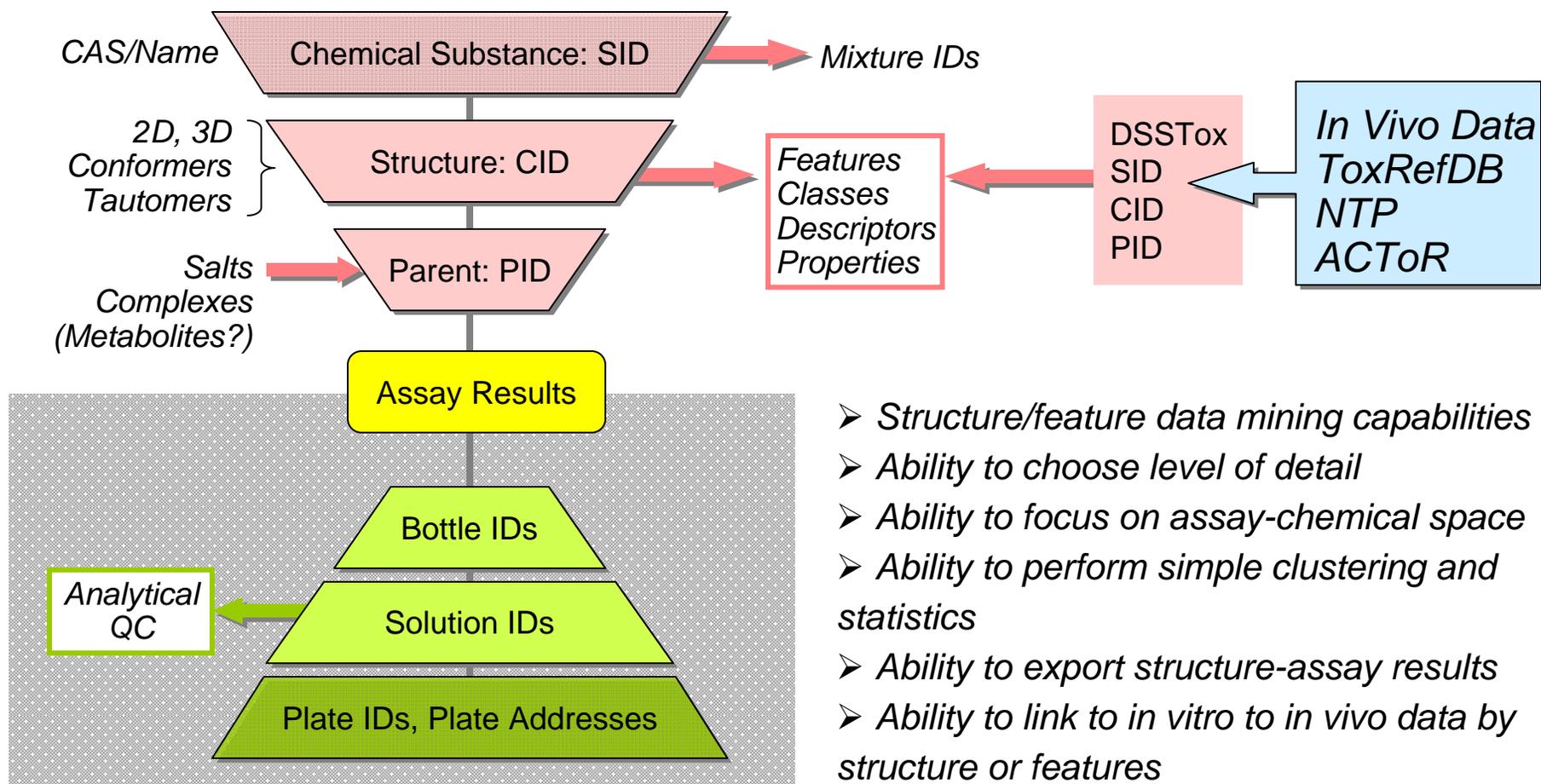
ToxCast Phase II

- Add approx 700 new chemicals to 309 Phase I set
 - Pfizer: ~ 100 failed drugs with pre-clinical/clinical tox data
 - Glaxo: liver toxicity data for approx 150 drugs
 - L'Oreal: sponsoring 10 chemicals for Phase II
 - FDA CFSAN/CDER chemicals if *in vivo* data shared
- Run new chemicals in ToxCast Phase I assays
- Run complete set in Tox21 assays
- Run complete set in new ToxCast assays
 - Model organisms: *c. elegans* (NTP), whole embryo zebrafish (EPA)
 - Selected genetox assays (Ames II, MN, GreenScreen)

Chemical Sample Registration Workflow



Building a Tox21 Cheminformatics Capability



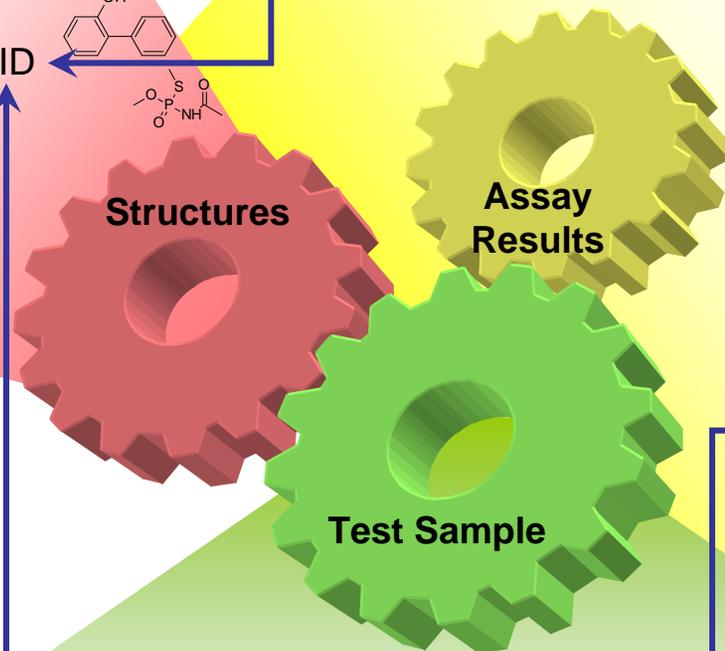
ToxCast/Tox21 Chemical Registry

DSSTox

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

ACToR/ToxMiner

Assay name
Assay details
Assay outcome

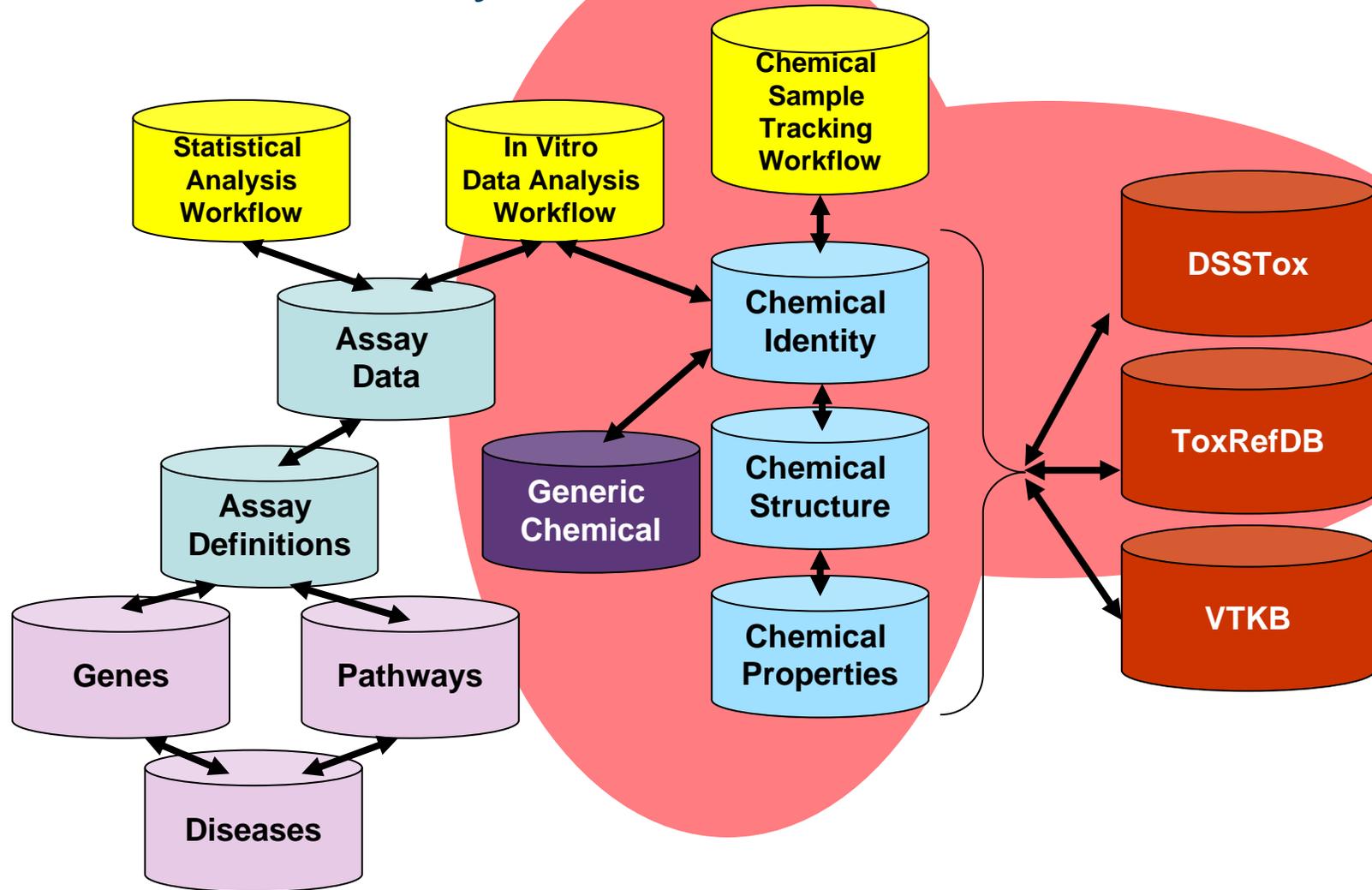


Solution ID
Plate ID
Plate Address ID

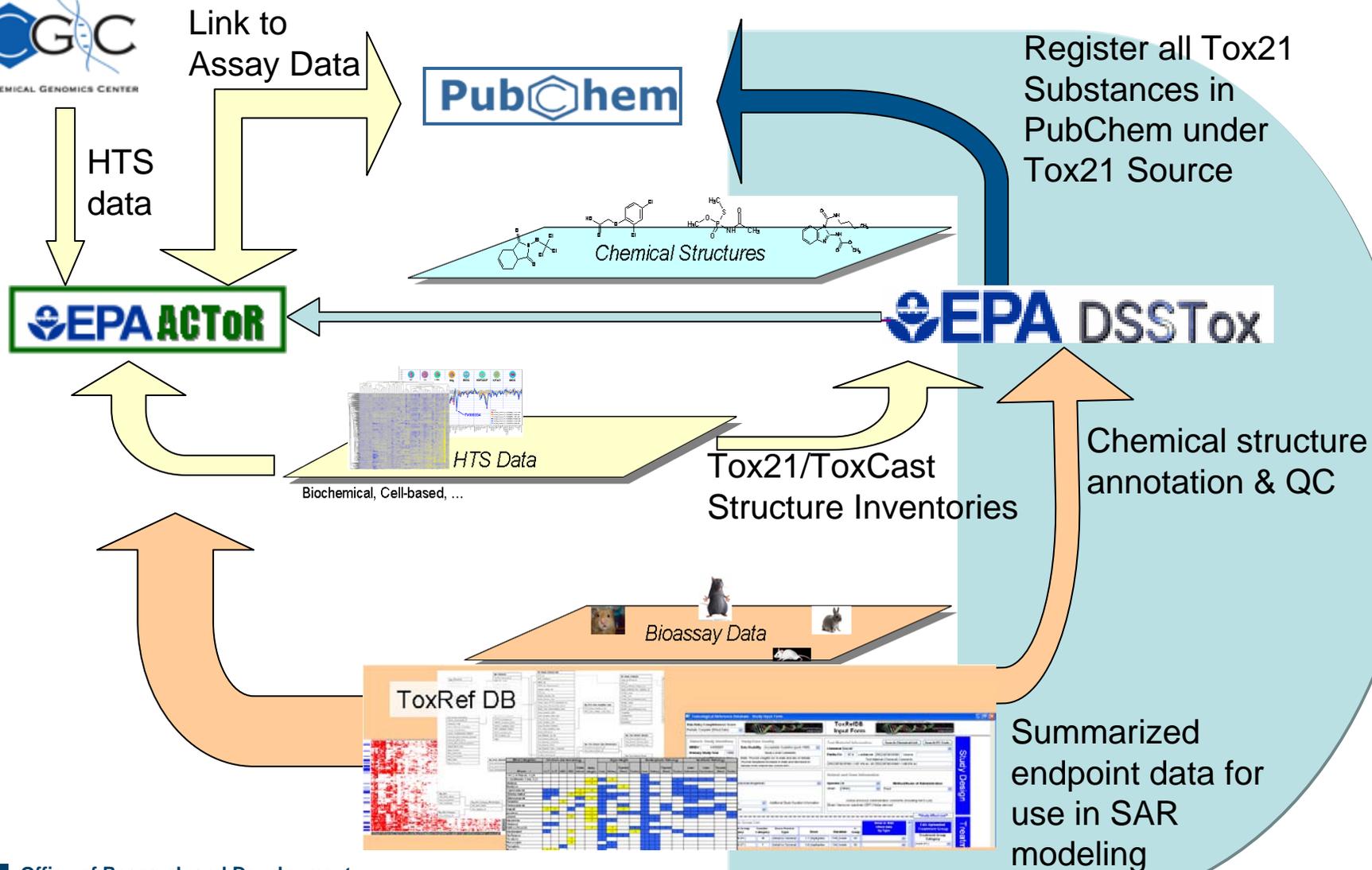
DSSTox RID
Bottle ID (→ COA ID)
Solution ID (→ QC ID)

Tox21 Sample Tracking Database

ACToR: Aggregated Computational Toxicity Resource



Tox21/ToxCast: Data Publication



Challenges for Tox21/ToxCast

Assays

- ❖ Reproducibility
- ❖ Sensitivity
- ❖ Biological relevance

- ✓ Plate replicates
- ✓ Dose response
- ✓ Assay replicates
- ✓ Positive controls

Chemicals

- ❖ Purity, Identity
- ❖ Stability
- ❖ Solubility
- ❖ Accuracy of representation

- ✓ Chemical filters & selection process
- ✓ Analytical QC
- ✓ Structure QC

Metabolism, ADME

- ❖ Many assays do not have metabolic capability
- ❖ ADME missing in *in vitro*

- ✓ Assays with metabolic capability
- ✓ Include known metabolites
- ✓ Active metabolite features represented
- ✓ Metabolic prediction models

Acknowledgements:

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Stephen Little

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Chris Austin & colleagues, NCGC/NIH
Ray Tice & colleagues, NTP/NIEHS

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