EPA National Center for Computational Toxicology UPDATE

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National Center for Computational Toxicology

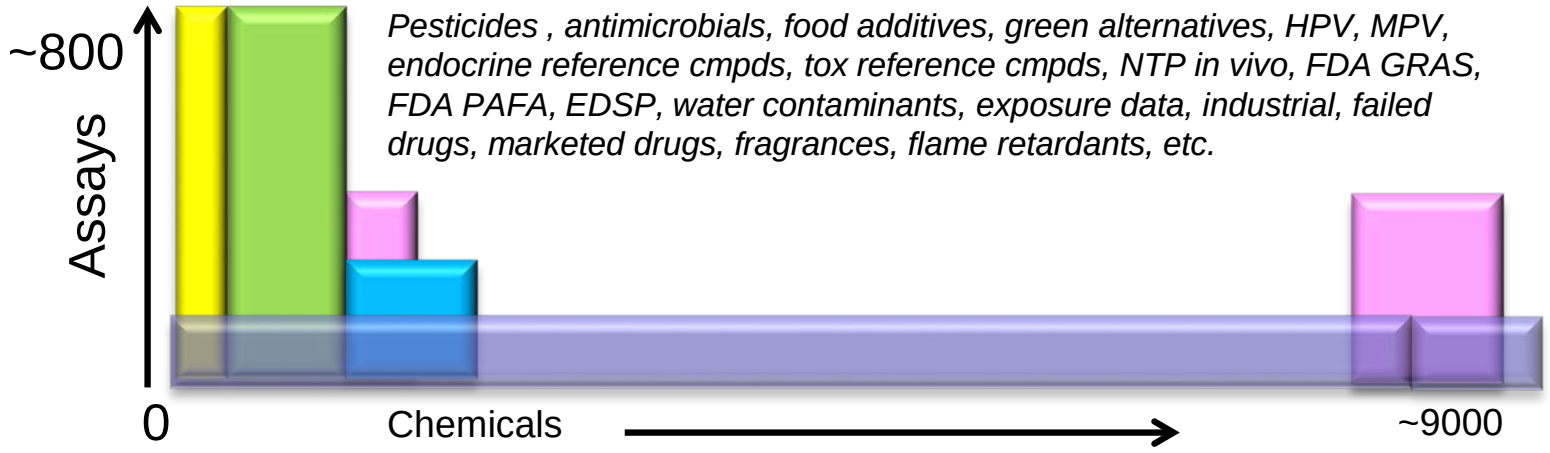
ICCVAM Public Forum
25 May 2016

A couple of new and exciting activities at NCCT

1. Chemical library update
2. Chemistry Dashboard
3. Retrofitting in vitro assays with metabolic competence
4. In vitro PK
5. Summer Surprise

ToxCast & Tox21
Chemicals, Data and Release 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
ToxCast Phase III	~900	~300	~300	In progress	2016
Tox21	~9000	~80	~150	In progress	ongoing



Pesticides , antimicrobials, food additives, green alternatives, HPV, MPV, endocrine reference cmpds, tox reference cmpds, NTP in vivo, FDA GRAS, FDA PAFA, EDSP, water contaminants, exposure data, industrial, failed drugs, marketed drugs, fragrances, flame retardants, etc.

Chemical Library Update

1) Filling in holes

- Completing testing of all Phase 1-3 chemicals in Attagene assays

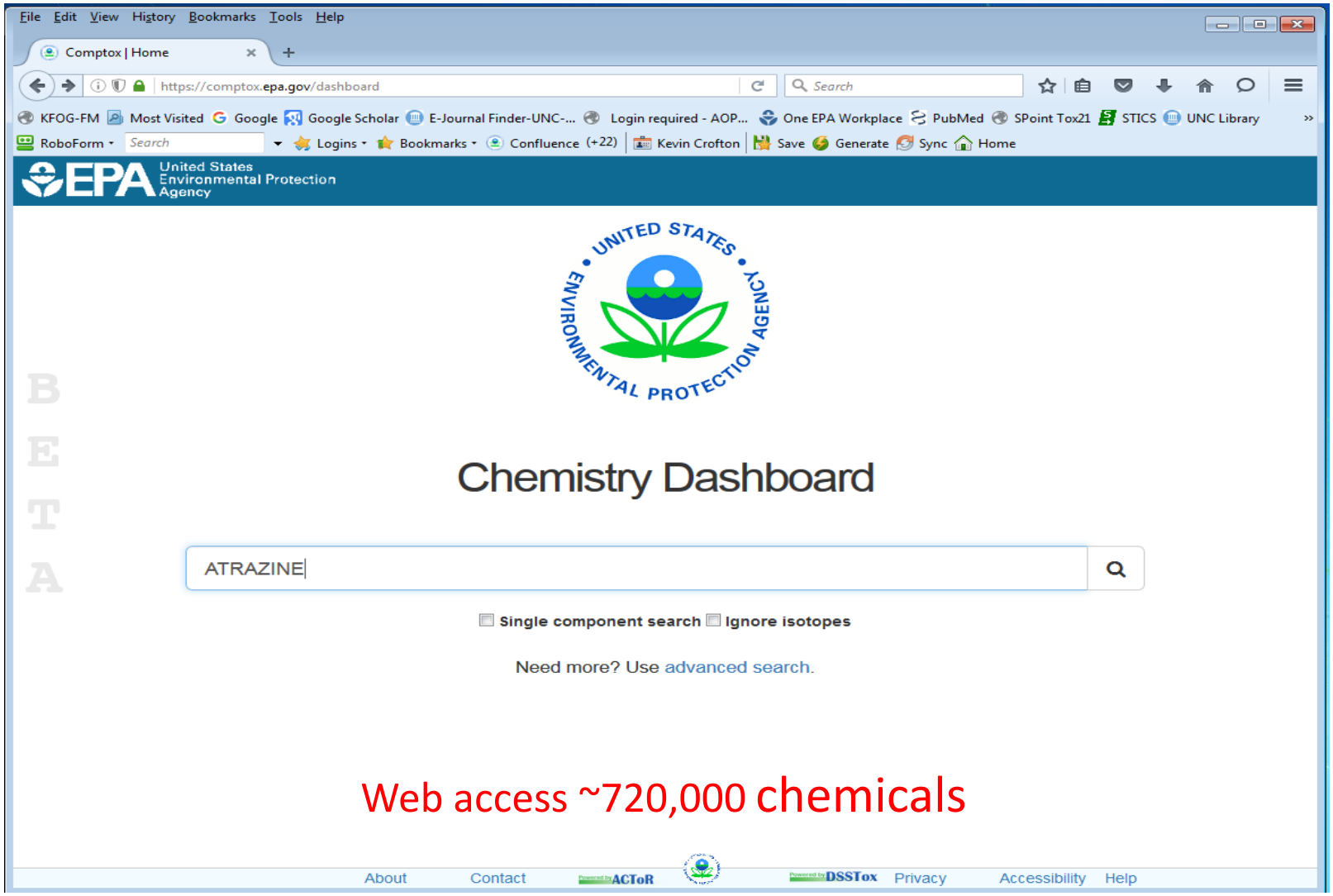
2) Water Soluble Chemicals

- About 650 chemicals were not tested as part of ToxCast due to lack of solubility in DMSO (e.g., glyphosate)
- Currently developing a ‘water-soluble’ chemical library

3) Volatile Chemicals

- Current assays do not allow for accurate testing of many VOCs/SVOCs
- Working with NHEERL to develop medium throughput assay methods for volatile chemicals.

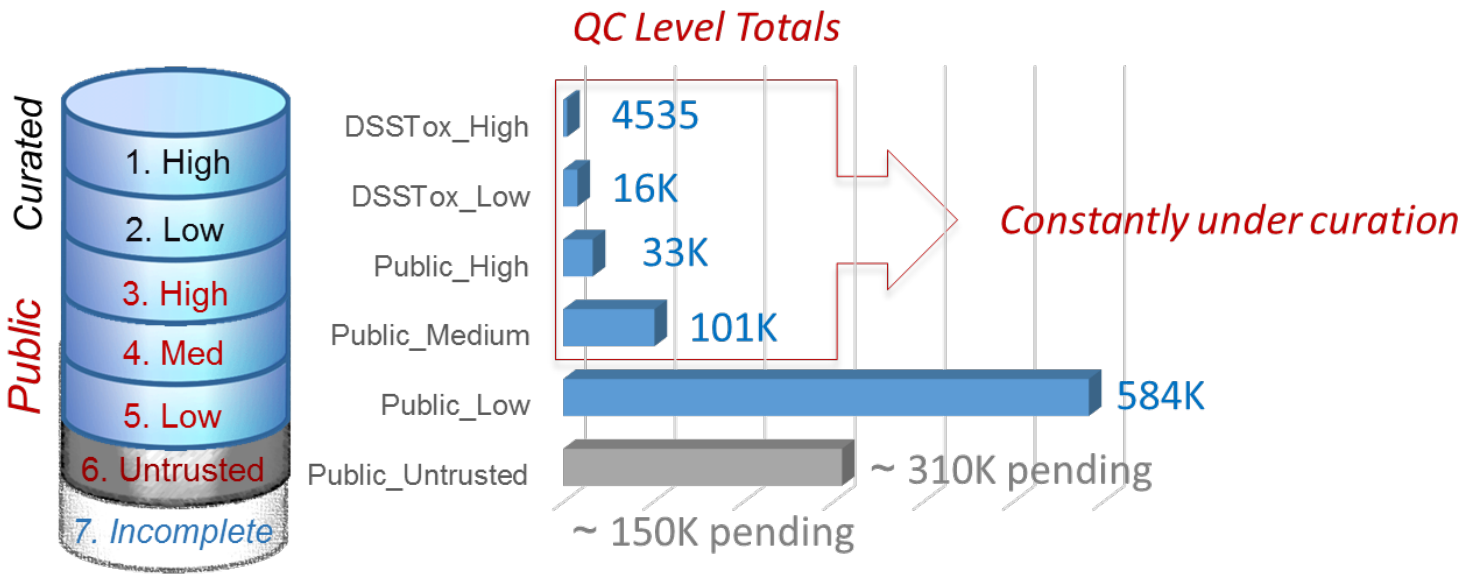
iCSS Chemistry Dashboard



Recently Released:
https://comptox.epa.gov/dashboard

Chemistry Dashboard
DSSTox Chemistry Content

Establishing confidence levels for content



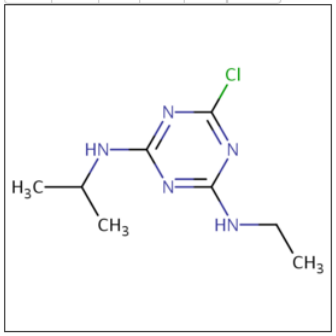
QC Levels	
DSSTox_High:	Hand curated and validated
DSSTox_Low:	Hand curated and confirmed using multiple public sources
Public_High:	Extracted from EPA SRS and confirmed to have no conflicts in ChemID and PubChem
Public_Medium:	Extracted from ChemID and confirmed to have no conflicts in PubChem
Public_Low:	Extracted from ACToR or PubChem
Public_Untrusted:	Postulated, but found to have conflicts in public sources

Expt'l and Predicted PhysChem Data

Search: atrazine

Searched by Synonym: Found 1 result for 'atrazine'.

2D | 3D



Intrinsic Properties

Molecular Formula: C8H14ClN5 — Search in DSSTox

Average Mass: 215.690002 g/mol

Monoisotopic Mass: 215.093773 g/mol

Structural Identifiers

Citation

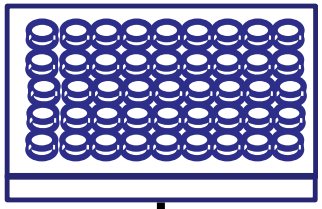
Chemical Properties | External Links | Synonyms | PubChem Biological Activities | PubChem Articles | PubChem Patents | Comments

Property	Average (Exp.)	Range (Exp.)	Average (Pred.)	Range (Pred.)
Solubility	0.0 (1)	0.0001298 to 0.0001298	0.525 (2)	0.05716 to 0.9926
Melting Point	174.25 (6)	172.5 to 177.0	150.55 (2)	113.9 to 187.2
Boiling Point	N/A	N/A	326.0 (2)	313.0 to 339.0
LogP	2.617 (3)	2.61 to 2.632	2.721 (3)	2.67 to 2.82
Atmospheric Hydroxylation Rate	N/A	N/A	0.0 (1)	1.711e-11 to 1.711e-11
LogBCF	0.9 (1)	0.9 to 0.9	0.936 (1)	0.936 to 0.936
Biodegradation Half-life	N/A	N/A	4.921 (1)	4.921 to 4.921
Henry's Law Constant	N/A	N/A	0.0 (1)	4.2e-10 to 4.2e-10
Fish Biotransformation Half-life	0.089 (1)	0.08913 to 0.08913	0.136 (1)	0.1359 to 0.1359
LogKOA	N/A	N/A	8.395 (1)	8.395 to 8.395
LogKOC	2.24 (1)	2.24 to 2.24	2.305 (1)	2.305 to 2.305
Vapor Pressure	0.0 (1)	7.209e-11 to 7.209e-11	0.0 (1)	2.025e-07 to 2.025e-07

Strategy for Retrofitting *In Vitro* Assays with Metabolic Competence

"Extracellular" Strategy

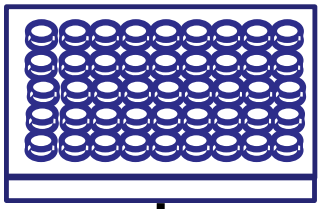
Chemicals metabolism in the media or buffer of cell-based and cell-free assays



More closely models effects of hepatic metabolism and generation of circulating metabolites

"Intracellular" Strategy

Capable of metabolizing chemicals inside the cell in cell-based assays



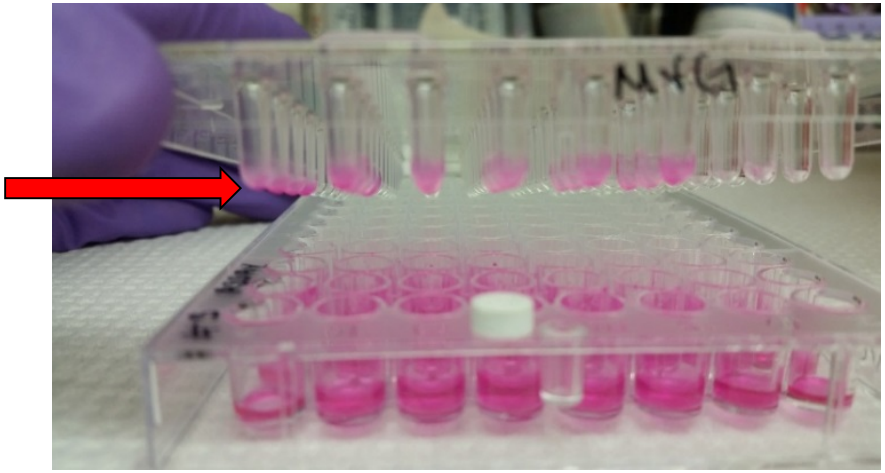
More closely models effects of target tissue metabolism

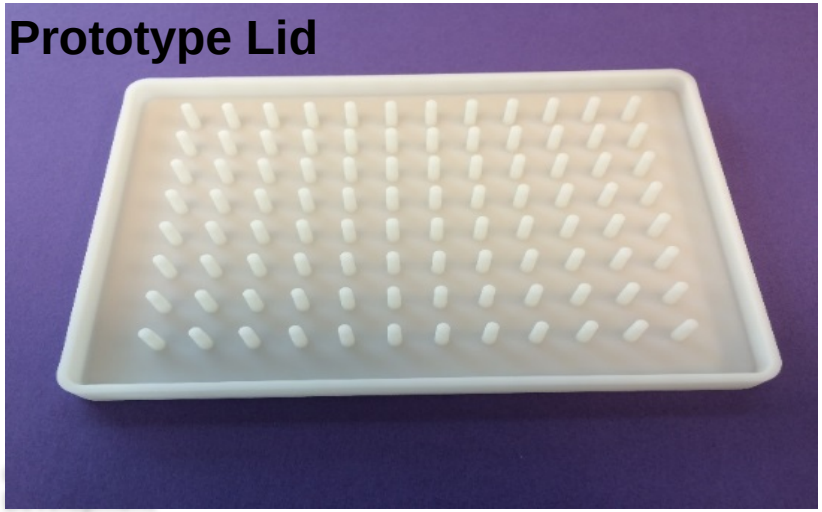
Integrated approach to model *in vivo* metabolic bioactivation and detoxification

NCCT Metabolic Competence Project Group:
Steve Simmons (PI)
Danica DeGroot (Postdoc)

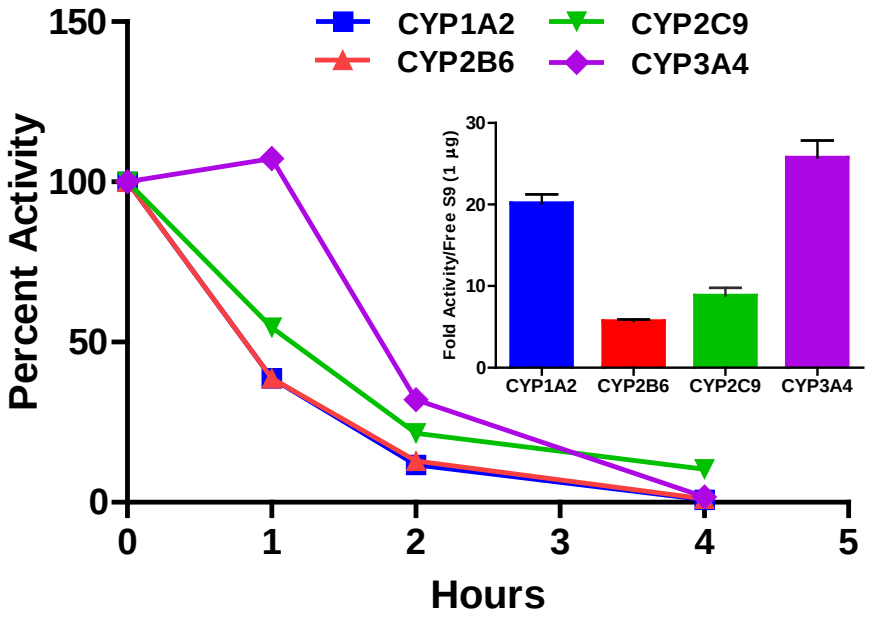
1. Retrofitting Assays for Metabolic Competence – Extracellular Approach

Alginate Immobilization of Metabolic Enzymes (AIME)



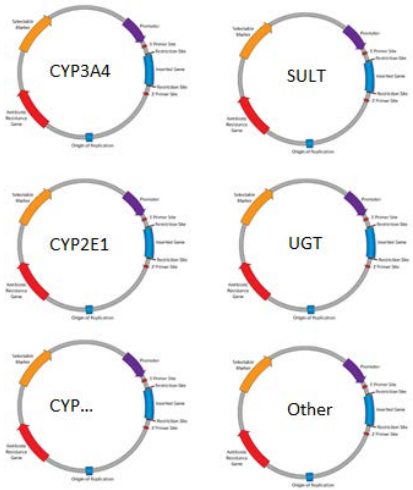


Amount of XME Activity in Microspheres

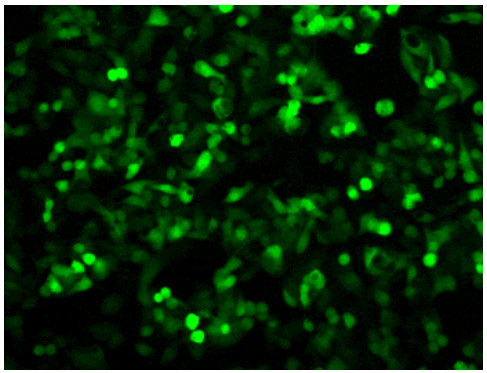


DeGroot et al. 2016 SOT poster #3757

2. Retrofitting Assays for Metabolic Competence – mRNA Intracellular Strategy

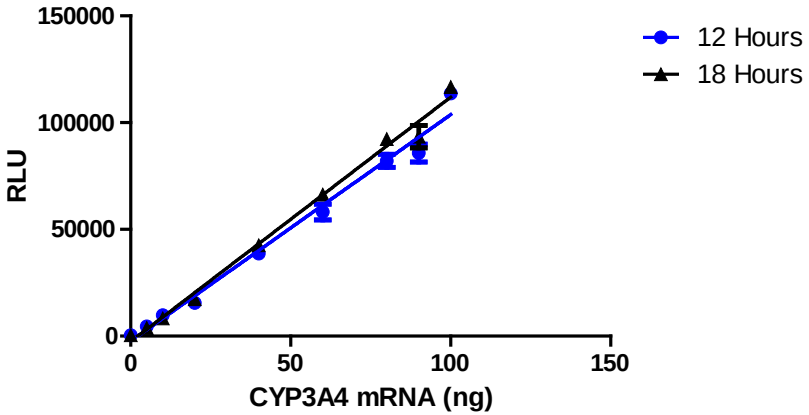


Pool in vitro transcribed mRNAs chemically modified with pseudouridine ad 5-methylcytidine to reduce immune stimulation

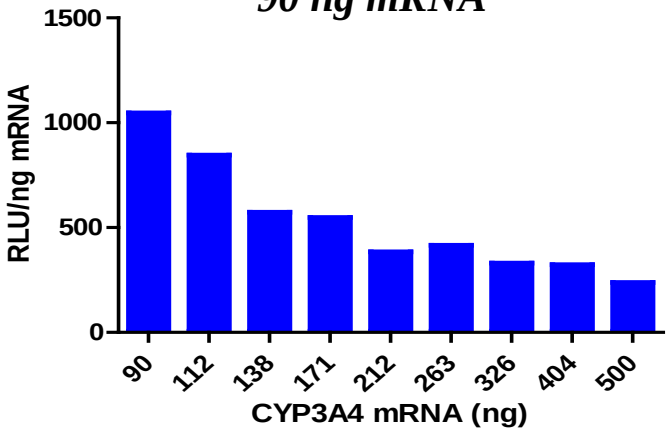


293T cells 21.5 h post transfection with 90 ng of EGFP mRNA using TransIT reagent

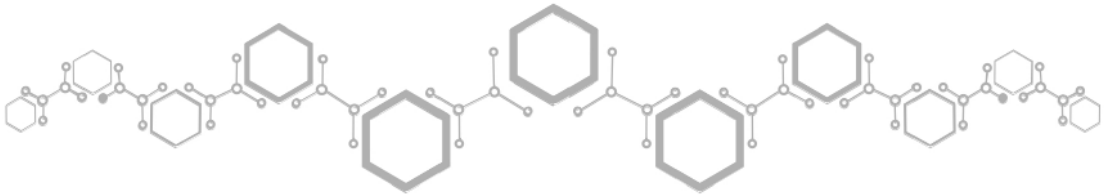
***Linear Response* of CYP3A4 Activity in HepG2 Cells with Increasing CYP3A4 mRNA**



Efficiency of CYP3A4 Transfection in HepG2 Cells Begins to Decline Above *90 ng mRNA*



Advantage of transfecting with mRNA: Titrate different CYPs to match different ratios in different tissues



TRANSFORM TOX TESTING CHALLENGE

INNOVATING FOR METABOLISM

- **NCCT, NTP, NCATS joint sponsored challenge to retrofit HTS assays with xenobiotic metabolic competence**

- **Stage 1**
  - Deadline was 4/8/16
  - ~25 proposals – 10 were selected for Phase 2
  - Each gets $10k to develop method and provide proof of principal
- **Stage 2**
  - Semi-finalists start-up workshop RTP 7/8/16
  - Up to five applicants selected as finalists, awarded a prize of up to $100,000 each, and invited to participate in the final stage of the competition.
- **Stage 3**
  - Based on results one winner may be selected and awarded $400k

http://www.transformtoxtesting.com/

In vitro PK

(or how to make sense of an IC50 of 23.2 uM in terms of human exposures)

Toxicokinetics (TK) provides a bridge between toxicity and exposure assessment by predicting tissue concentrations due to exposure

- Traditional TK methods are very resource intensive

NCCT recently released an R package called "httk"

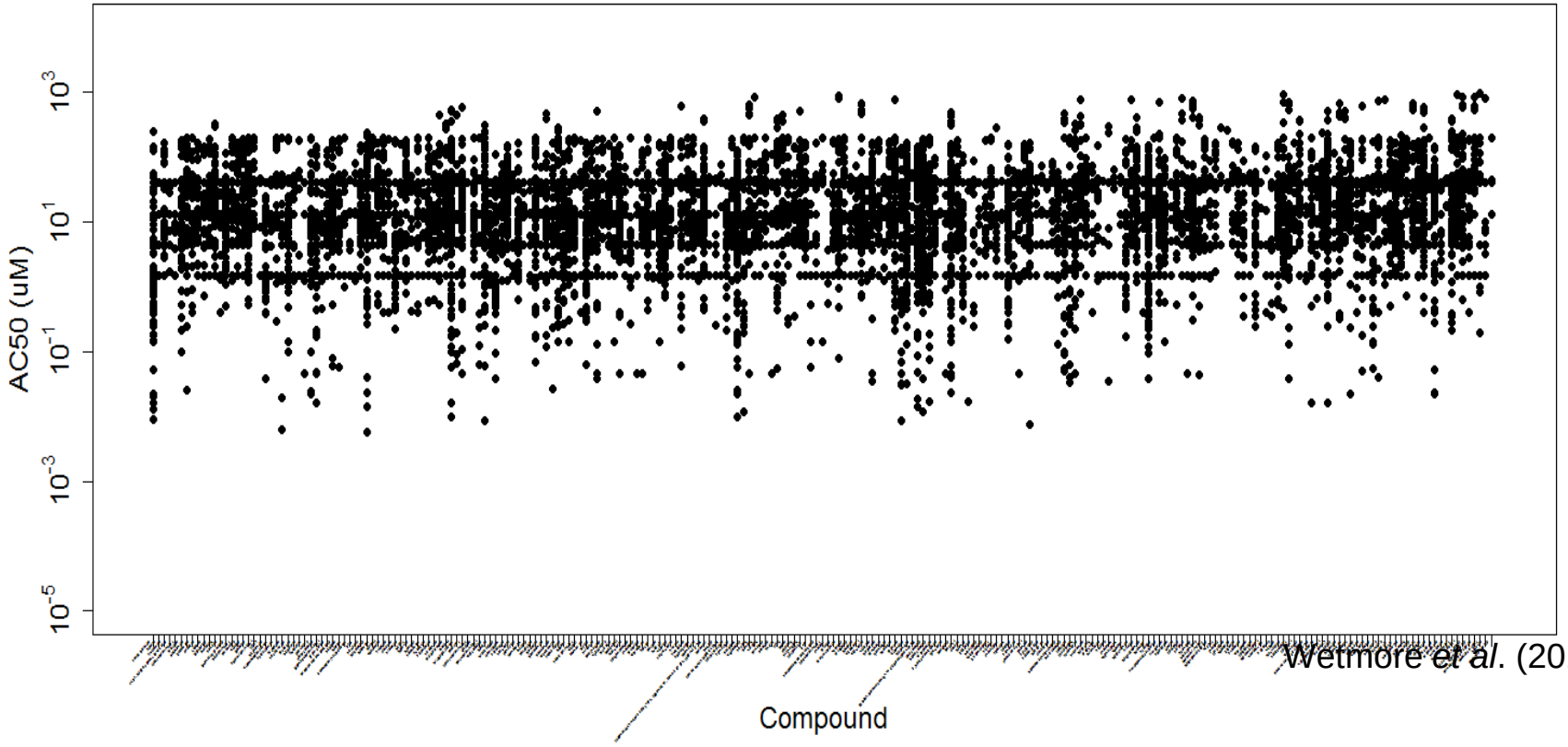
- Uses "reverse dosimetry" (Reverse TK or RTK) converts *in vitro* HTS conc to human daily dose
- Freely available on CRAN
- *Allows RTK for 543 chemicals (more coming)*

New R package called 'httk-pop" package

- estimates exposures for susceptible populations
- *Ring et al. Refining high-throughput prioritization of environmental chemicals to include inter-individual variability across subpopulations. (submitted)*

Access httk from the R GUI: "Packages" then "Install Packages"

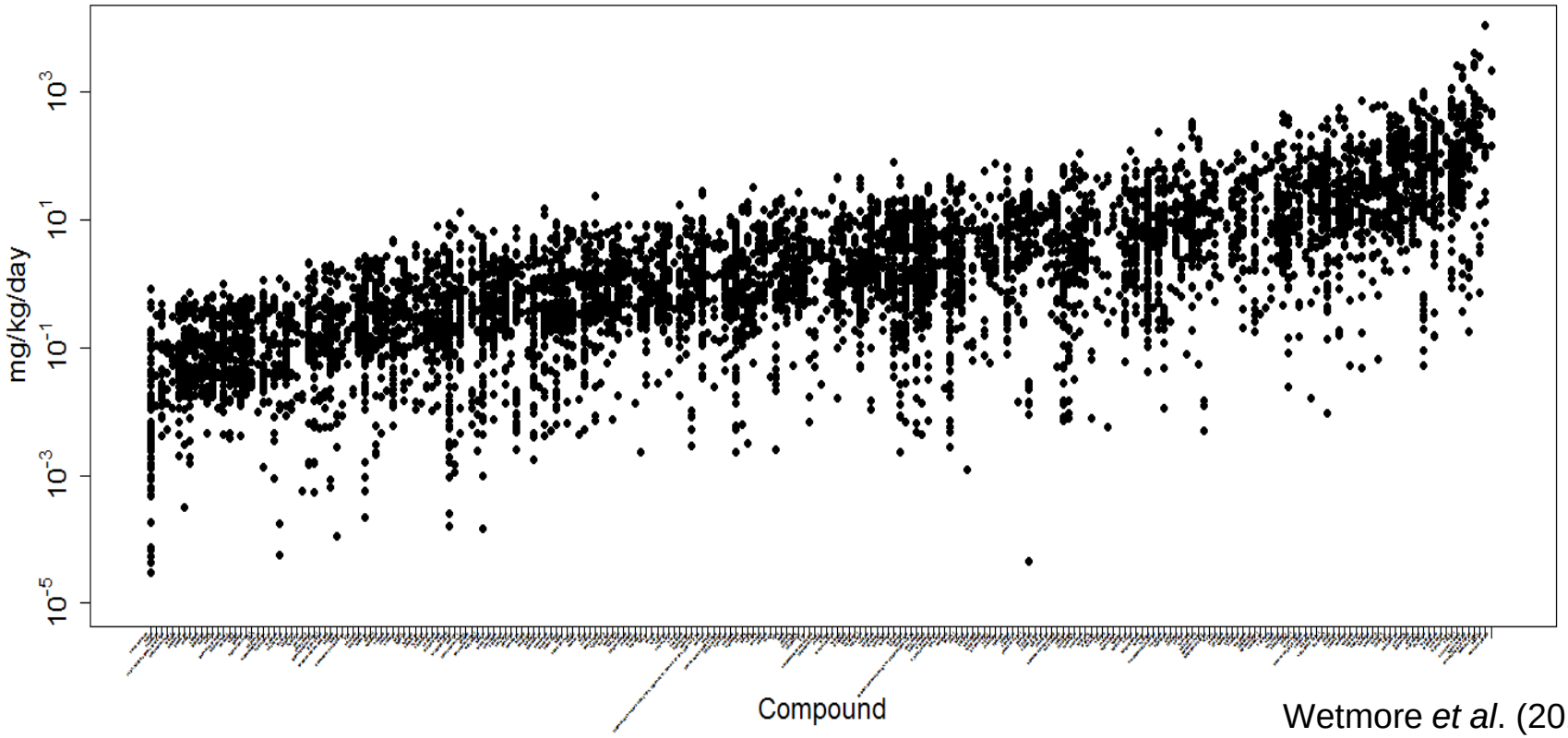
ToxCast *in vitro*



Wetmore *et al*. (2012)

- ToxCast/Tox21 bioactive concentrations alone make it hard to prioritize chemicals

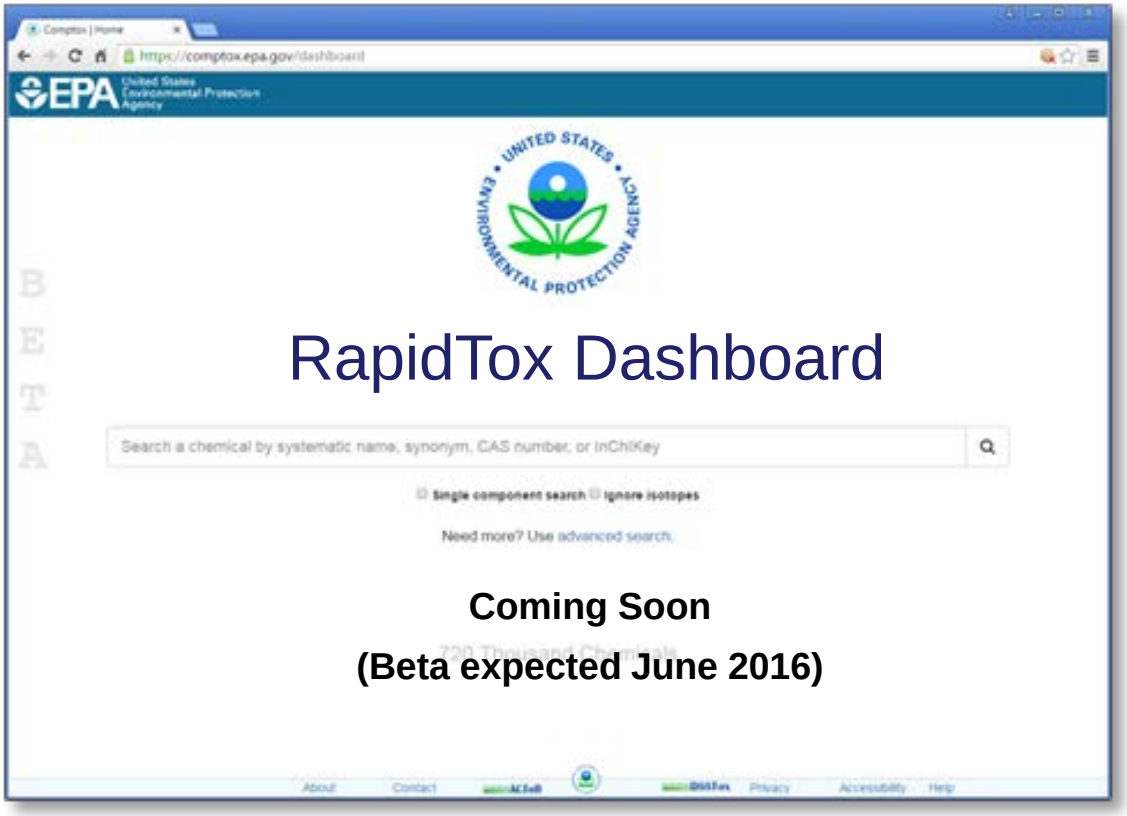
ToxCast In Vitro



Wetmore *et al*. (2012)

- Translation from *in vitro* to steady-state oral equivalent doses allow greater discrimination between effective chemical potencies

Coming Soon
RapidTox Dashboard



Provides a place to find and integrate all available chemistry, exposure, and bioactivity information

Goals of the Project

- Development of a screening level decision support tool for hundreds to thousands of data poor chemicals
- Integrate a range of information related to chemical properties, fate and transport, hazard, and exposure through an interactive on-line dashboard, including…
  - Traditional data (as available)
  - New ORD data streams such as automated read-across methods, ToxCast data, AOPs, ExpoCast, and high-throughput toxicokinetic models
- Deliver quantitative toxicity values with associated estimates of uncertainty
- Initial prototype expected end of FY16

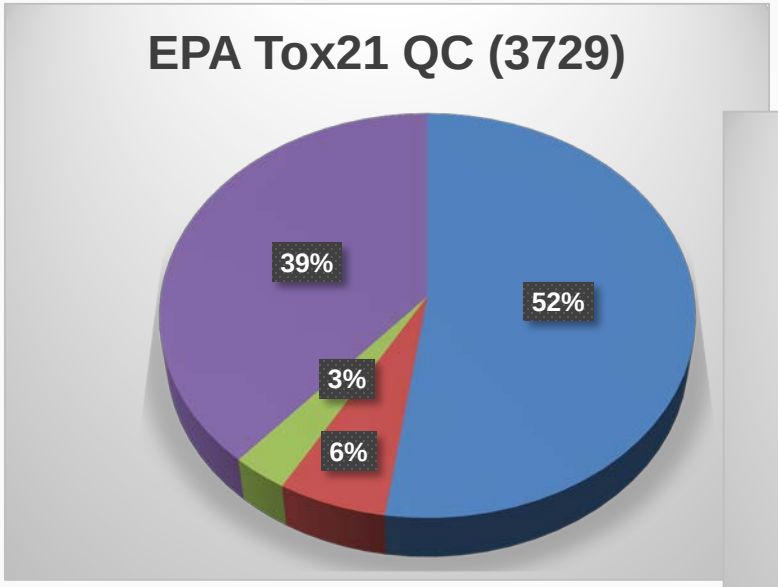
Thanks for Listening

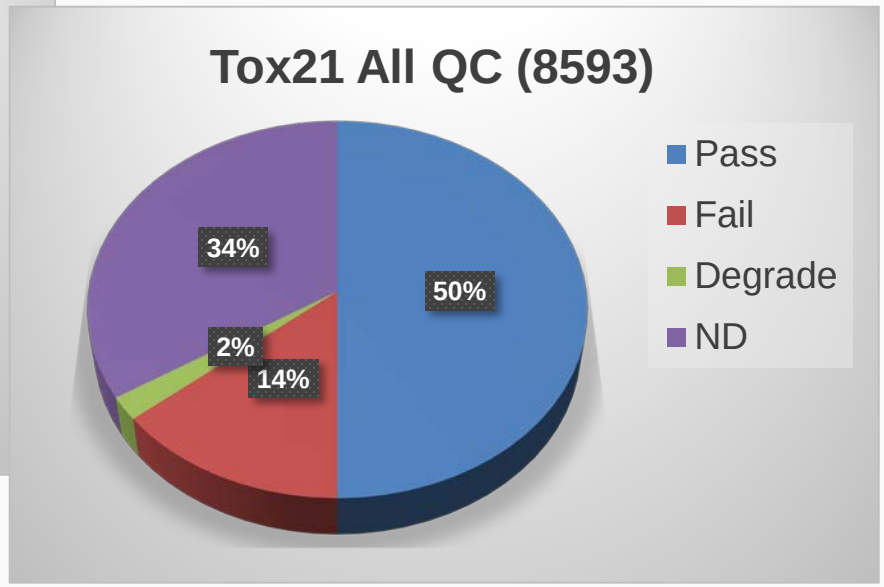
EPA's National Center for Computational Toxicology

EXTRAS

Tox21 & ToxCast Analytical QC

- Process, summarize & store results in database for surfacing in dashboards
- For what types of chemicals do methods (LC, GC) work? Not work?
- Can chemicals in different QC categories be characterized structurally?
- Why larger failure rate in Tox21 All library?





Pass = C (75%) or greater
Fail = D, F, Ac, Bc, Cc

Two Case Studies

OSWER-Region Case Study

Decision Context: Estimate toxicity values with associated uncertainty for data poor chemicals at Superfund sites

Desired Components:

- Phys-Chem properties with environ fate and transport
- Hazard profile – GL and GL-like studies, RA, and QSAR
    - Acute and chronic tox endpoints
- ToxCast data in AOP context
- Toxicokinetic data (*in vivo* and *in vitro*)
- Bioavailability (sediment and Caco-2)
- Consumer and industrial use
- Screening level estimates with defined exposure scenarios
- Available analytical chemistry methods

OPP Case Study

Decision Context: Prioritize non-food use inert ingredients for additional study

Desired Components:

- Phys-Chem properties with environ fate and transport
- Hazard profile – GL and GL-like studies, RA, and QSAR
    - Chronic tox endpoints
- ToxCast data in AOP context
- Toxicokinetic data (*in vivo* and *in vitro*)
- Consumer and industrial use