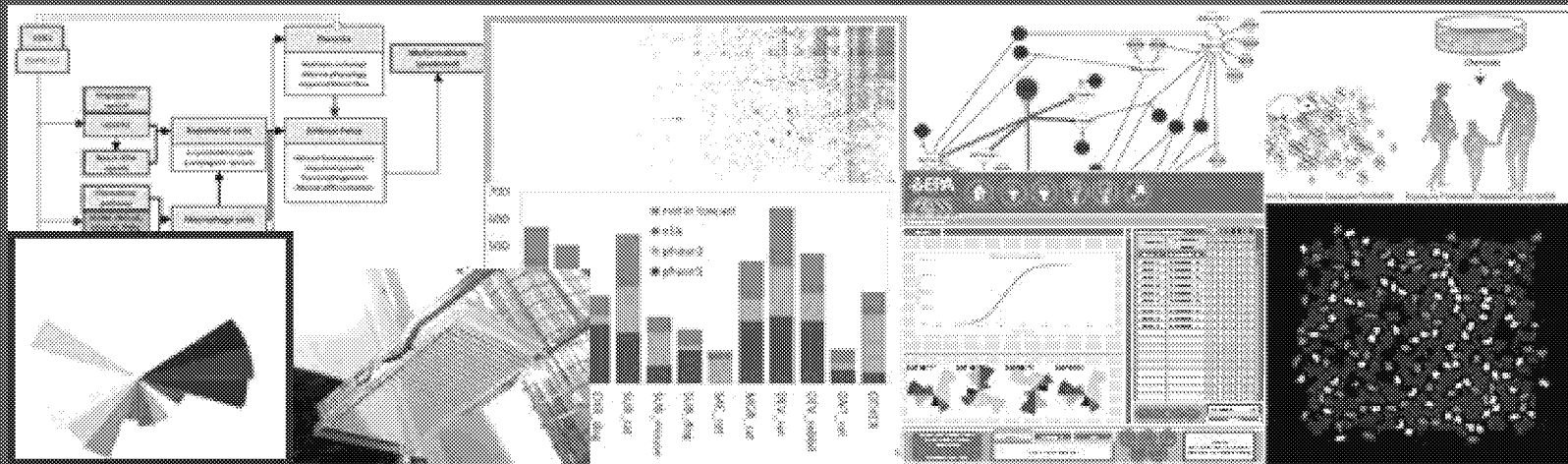


US EPA ORD Ongoing Research for the Toxicity of PFAS



ECOS-EPA PFAS Call

February 23, 2018

Reeder Sams

*US EPA Office of Research and Development
National Center for Computational Toxicology*

Tiered Toxicity & Toxicokinetic Testing

- Problem formulation:
 - Hundreds to thousands of untested PFAS in the environment
 - Traditional single-chemical toxicity testing not practical or efficient
 - Toxicity and toxicokinetic information needed to inform decision making
- How do we generate informative toxicity and kinetic information quickly?
 - EPA ORD, National Toxicology Program (NTP) and partners have the capacity to generate toxicity and toxicokinetic data through high throughput methods (HTT) assays

Tiered Testing Overview



- Procure a library of ~300-400 PFAS (PFAS Screening Library) to support development of analytical methods, environmental monitoring, and toxicity testing
- Define a reference subset of ~75 PFAS based on:
 - Conduct tiered toxicity and toxicokinetic testing chemicals within the PFAS landscape
 - Data will support multiple outputs, making use of the integrated dataset (in vivo, in vitro, etc.) to inform toxicity and kinetics across the PFAS landscape

PFAS Library and Chemical Selection

<https://comptox.epa.gov/dashboard>

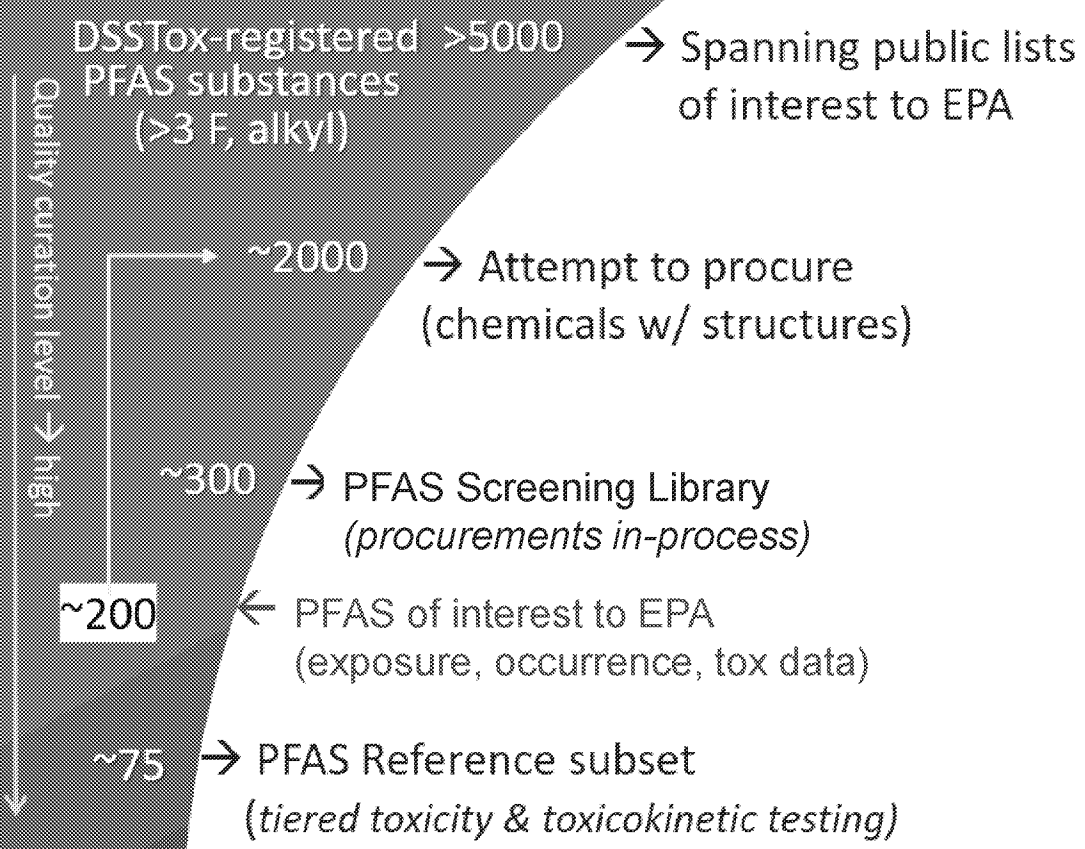
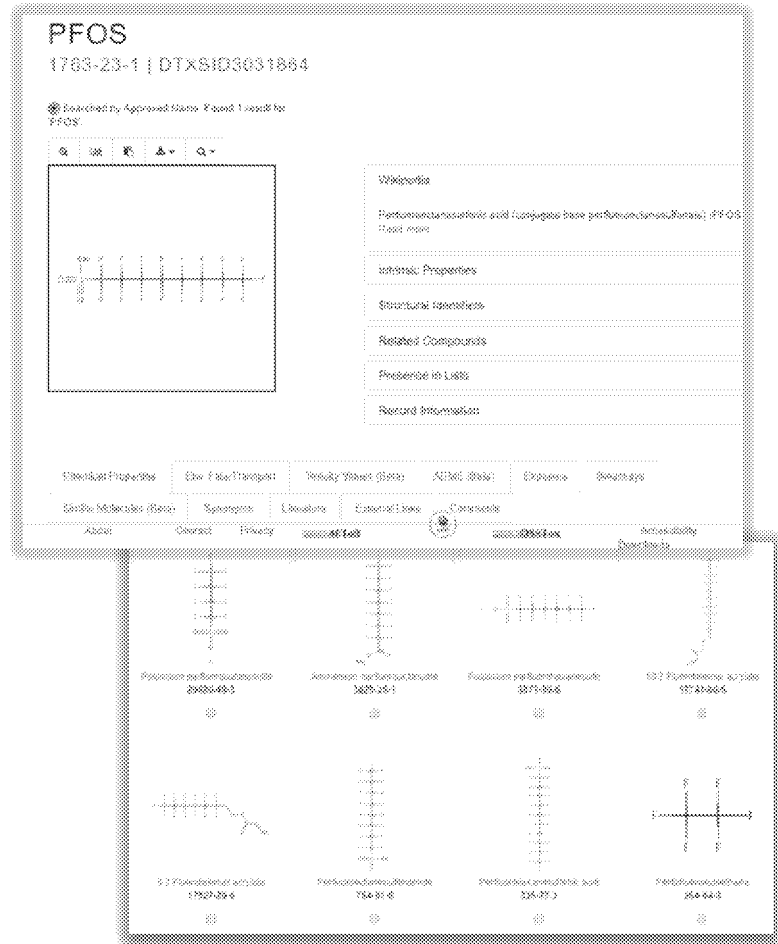
EPA DSSTox Database: > 758K chemicals



- Chemical structures, downloadable files
- Predicted phys-chem properties
- External links & list overlaps

- Per(poly)-fluorinated alkyl substances (PFAS)
- PFAS chemical names, acronyms, synonyms
- PFAS chemical structure categories

PFAS Chemical Landscape:

PFOS
1783-23-1 | DTXSID3031864

Coarctically Approved Name Found 1 result for PFOS

Webpage: Perfluorooctanesulfonic acid (long-chain base perfluorooctanesulfonate) (PFOS) (view more)

Internal Properties

Structural isomers

Related Compounds

Present in Lists

Record Information

Standard InChIKey: EWVLEFTHPGT-UBWJLDUBVS-NS

Chemical Molecular Weight: 368.0463

SMILES: [O-]S(=O)(=O)CCCC(F)(F)F

Abstract | General | Toxicity | **Associated Lists** | **Associated Files** | **Download** | **Share**

Perfluorooctanesulfonic acid 368.0463

Perfluorooctanesulfonate 368.0463

Perfluorooctanesulfonate 368.0463

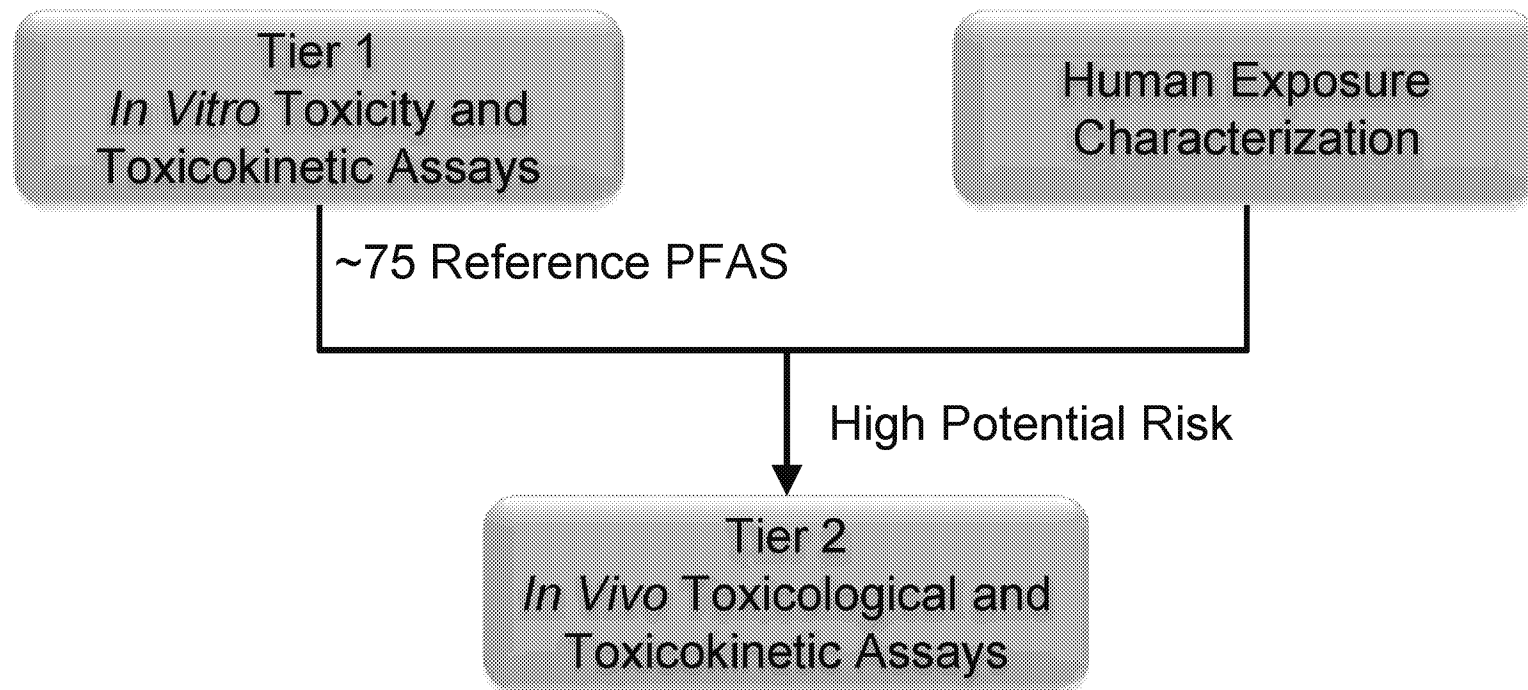
1,1,1-Trifluoro-2,2,2-trifluoroethane 1783-23-1

Perfluorooctanesulfonate 368.0463

Perfluorooctanesulfonate 368.0463

Perfluorooctanesulfonate 368.0463

Tiered Toxicity and Toxicokinetic Testing Strategy



*Collaboration between EPA and NTP

Not for Release

Considerations for use of Tiered Toxicity Testing Outputs



- PFAS potency estimations: Primary outputs will be administered dose equivalents (ADE) and Bioactivity-to-exposure ratio (BER)
 - *Characterization of uncertainty is critical to decision context*
- Communicate characteristics for Tier I data to partners and stakeholders in advance for use-considerations in different decision making contexts
- Prioritize PFAS for Tier 2 *in vivo* testing

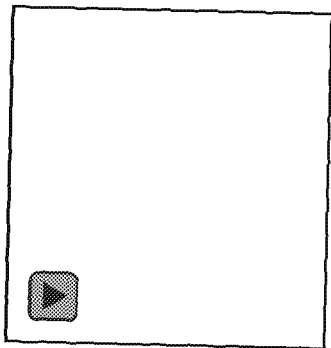
ORD Medium Throughput Research Studies

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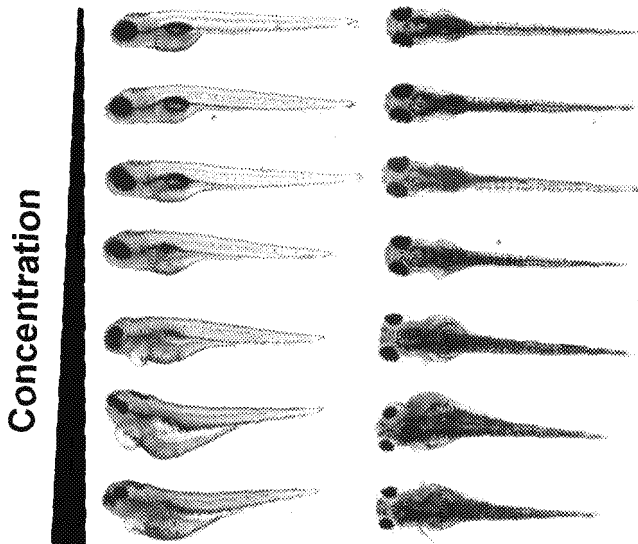
Using zebrafish for developmental toxicity (DevTox) assessments

- Established model of vertebrate embryonic development
- Rapid, external development
- ~75% of genes have human homologs
- Transparent
 - Transgenics, mutants
 - Amenable to chemical screening

Not for Release



0-24 hpf; Karlstrom
et al. Dev. 1996.

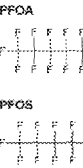


Tal et al. Repro Tox.
2015.

Tamara Tal, U.S. EPA

Using zebrafish to assess PFAS t

- One embryo seeded per well of a 96 well plate
- Exposed daily (days 0-5) to 8 PFAS compounds
- On day 6:
 - **DevTox:** Malformations and lethality
 - **Dev Neurotoxicity (DNT):** Behavioral activity as a *functional readout* of DNT
 - **Toxicokinetics:** Determine parent and chemical metabolite tissue dose using targeted analytical chemistry and non-targeted analysis



Tamara Tal, U.S. EPA

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- Study on the effects of GenX in the fetal and pregnant Charles River Laboratories (CRSD) rat
- Pilot study on the effects of *in utero* GenX in the F1 male and female rat offspring using a one generation screening protocol
- In vitro studies of potential agonist and antagonist interactions with the following steroid receptors
 - Androgen receptor (Chimpanzee)
 - Estrogen receptor (ER α)
 - Glucocorticoid receptor (hGR)

Summary

- Toxicity and toxicokinetic testing
- Ongoing research
- Integration with risk assessment efforts

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- Ann Richard
- Bruce Rodan
- Tim Shafer
- Mark Strynar
- Tamara Tal
- Barbara Wetmore

and many others....

***Disclaimer: The views expressed in this presentation are that of the presenters and do not necessarily represent the views and/or policies of the U.S. Environmental Protection Agency.**

Additional Slides

Not for Release

Tier 1 *In Vitro* Toxicity Assay

Endpoint of Interest	NTP
Hepatotoxicity	2D HepaRG cytotoxicity; 3D HepaRG (spheroid) transcriptomics
Developmental Toxicity	
Immunotoxicity	Bioseek Immune panel
Mitochondrial Toxicity	2D HepaRG mitochondrial dye and Seahorse assay
Developmental Neurotoxicity	

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EPA	
National Center for Computational Toxicology (NCCT)	National Health and Environmental Effects Research Laboratory (NHEERL)
Attagene cis- and trans- Factorial assay (HepG2)	
	Zebrafish embryo assay
	Neurodevelopmental microelectrode assay

Tier 1 *In Vitro* Toxicokinetic

Endpoint of Interest	NTP
Hepatic Clearance	Human primary hepatocyte clearance
Plasma Protein Binding	
Renal Transport	Renal proximal tubule permeability assay
Enterohepatic Recirculation	
<i>In Vitro</i> Disposition	Cell vs. nominal concentration (Tox21 joint project)

Not for Release

EPA	
National Center for Computational Toxicology (NCCT)	National Health and Environmental Effects Research Laboratory (NHEERL)
	Ultracentrifugation assay
Piloting hepatic transporter assay	
Cell vs. nominal concentration (Tox21 joint project)	