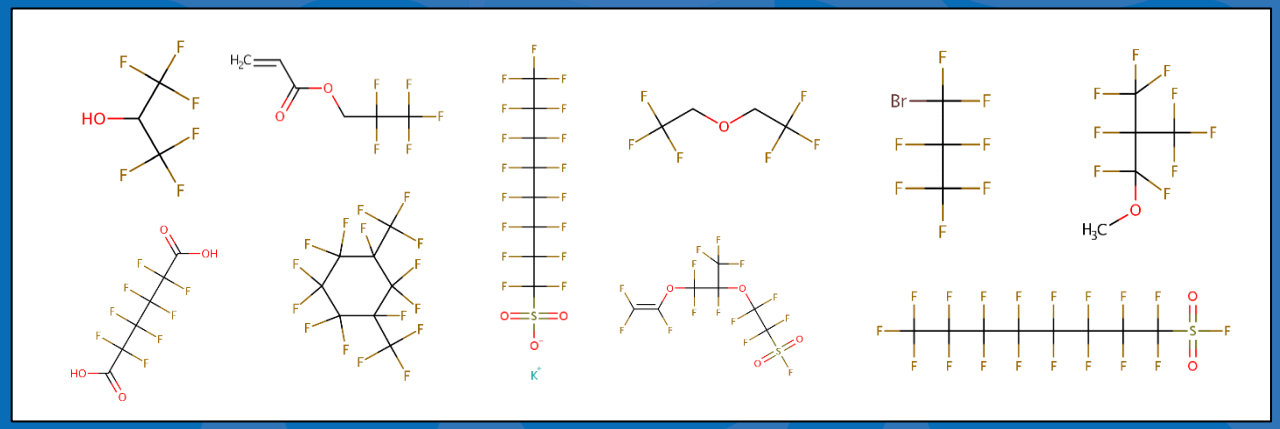
United States Environmental Protection Agency

A Chemical Category-Based Approach for Selecting and Screening PFAS for Toxicity and Toxicokinetic Testing

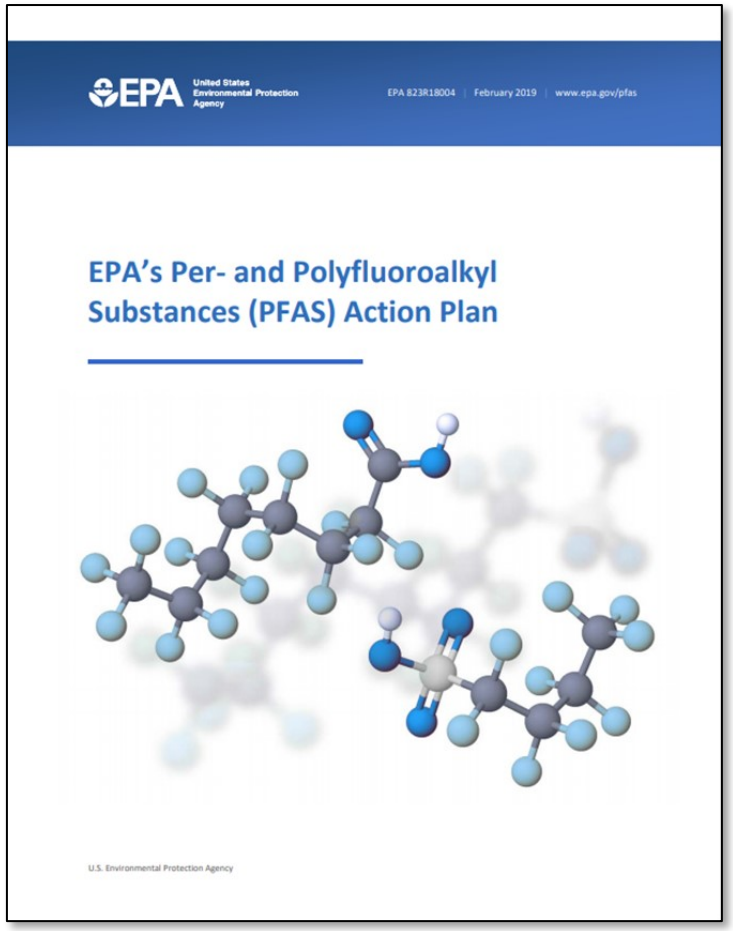


Grace Patlewicz
Center for Computational Toxicology & Exposure (CCTE), US EPA

Background and Importance of the Problem

Bottom line is that we cannot readily dig our way out using only traditional testing approaches…

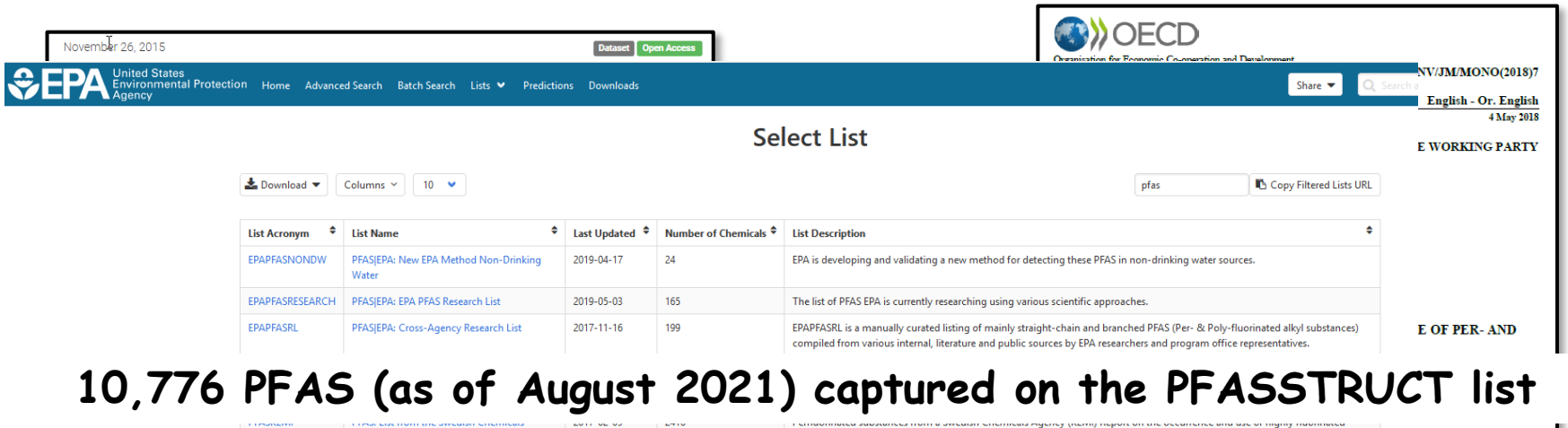
EPA is Using New Approach Methods (NAMs) to Help Fill Information Gaps



Research Area 1: *What are the human health and ecological effects of exposure to PFAS?*

- **Using computational toxicology approaches to fill in gaps.** For the many PFAS for which published peer-reviewed data are not currently available, the EPA plans to use new approaches such as high throughput and computational approaches to explore different chemical categories of PFAS, to inform hazard effects characterization, and to promote prioritization of chemicals for further testing. These data will be useful for filling gaps in understanding the toxicity of those PFAS with little to no available data. ***In the near term,*** the EPA intends to complete assays for a representative set of 150 PFAS chemicals, load the data into the CompTox Chemicals Dashboard for access, and provide peer-reviewed guidance for stakeholders on the use and application of the information. ***In the long term,*** the EPA will continue research on methods for using these data to support risk assessments using New Approach Methods (NAMs) such as read-across and transcriptomics, and to make inferences about the toxicity of PFAS mixtures which commonly occur in real world exposures. The EPA plans to collaborate with NIEHS and universities to lead the science in this area and work with universities, industry, and other government agencies to develop the technology and chemical standards needed to conduct this research.

But, It All Starts With Chemistry… Curating Names, Structures, and Identifiers

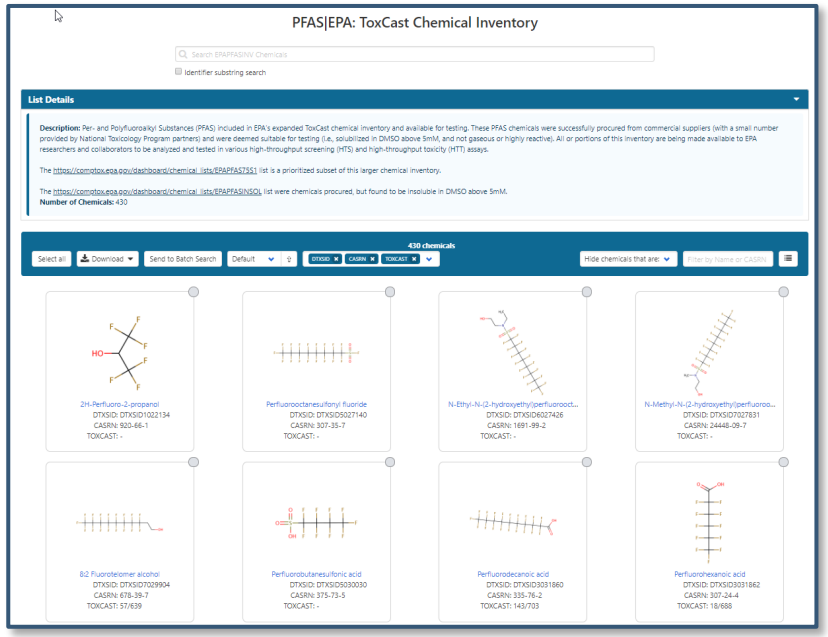


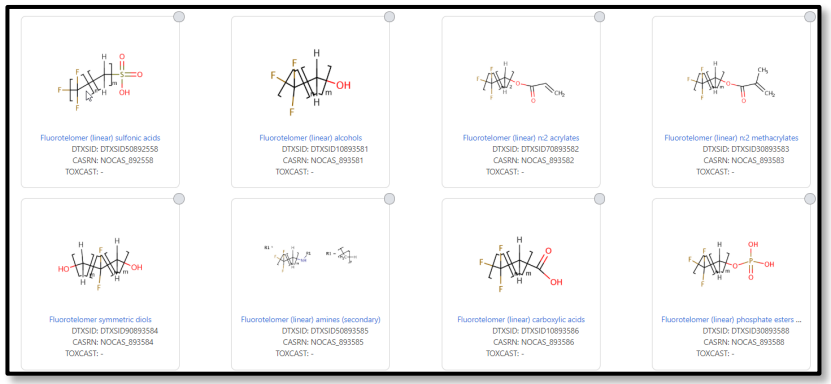
10,776 PFAS (as of August 2021) captured on the PFASSTRUCT list

Offices and Regions

Antony Williams, Ann Richard, Chris Grulke, and Chemical Curation Team

Assembled a PFAS Chemical Library for Research and Methods Development

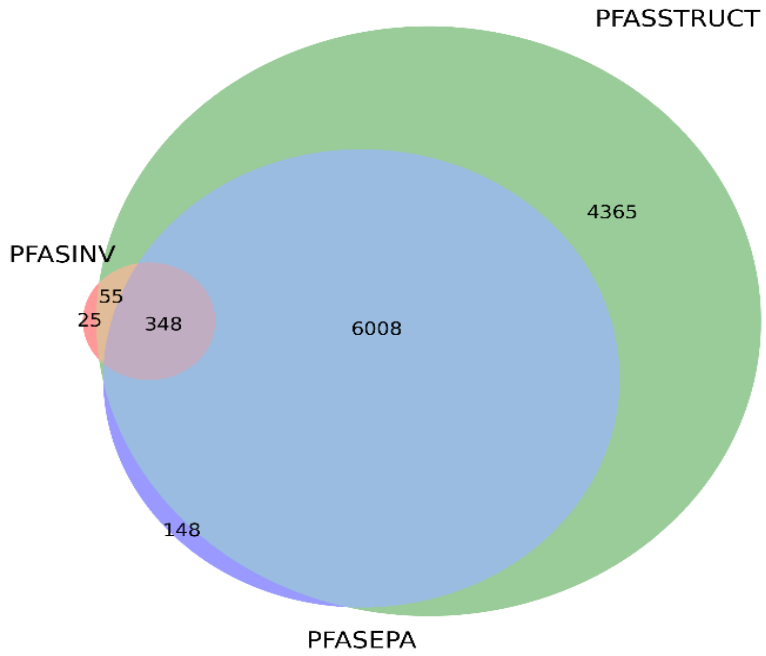




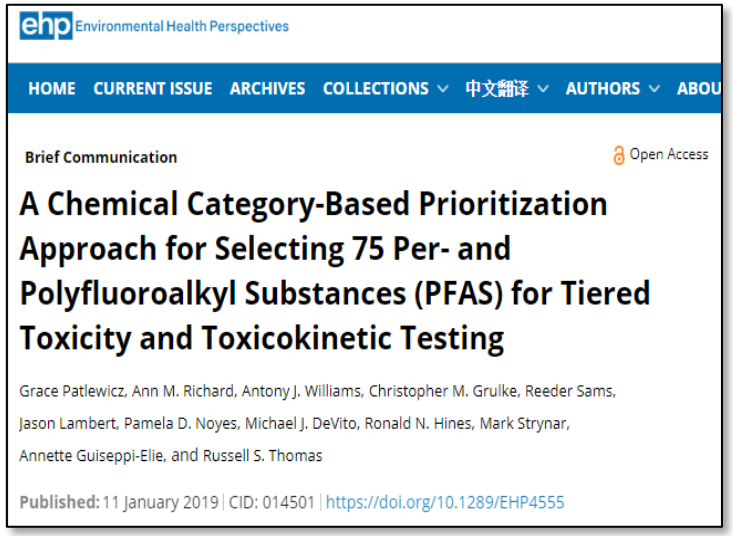
- Attempted to procure ~3,000 based on chemical diversity, Agency priorities, and other considerations
- Obtained 480 total unique chemicals
  - 430/480 soluble in DMSO (90%)
  - 54/75 soluble in water (72%) (incl. only 3 DMSO insolubles)
- Issues with sample stability and volatility
- Categories initially assigned based on three approaches
  - Buck et al., 2011 categories
  - Markush categories
  - OECD categories

PFAS List Overlap

	OECD	PFAS STRUCT	PFAS 430INV	PFAS150
OECD	4729			
PFASSTRUCT	3723	10776		
PFAS430INV	310	407	428	
PFAS150	119	139	146	146



Selecting a Subset of PFAS for Tiered Toxicity and Toxicokinetic Testing



Environmental Health Perspectives

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Brief Communication

Open Access

A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing

Grace Patlewicz, Ann M. Richard, Antony J. Williams, Christopher M. Grulke, Reeder Sams, Jason Lambert, Pamela D. Noyes, Michael J. DeVito, Ronald N. Hines, Mark Strynar, Annette Guiseppi-Elie, and Russell S. Thomas

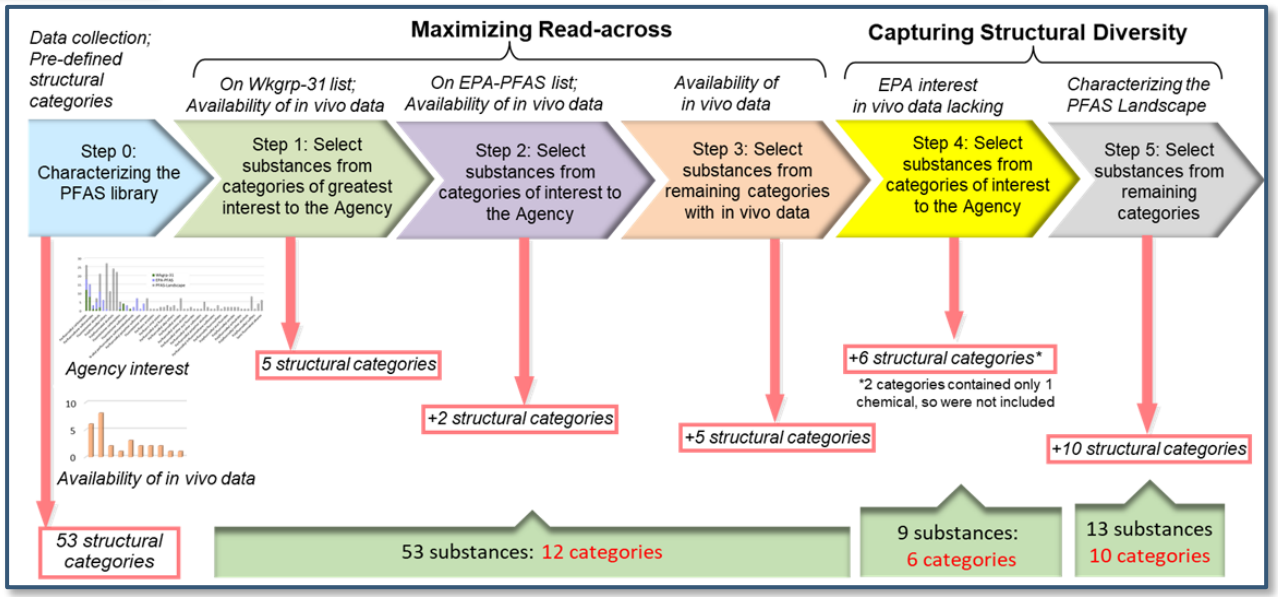
Published: 11 January 2019 | CID: 014501 | https://doi.org/10.1289/EHP4555

Goals:

- Generate data to support development and refinement of categories and read-across evaluation
- Incorporate substances of interest to Agency
- Characterise mechanistic and toxicokinetic properties of the broader PFAS landscape

Selected 150 PFAS in two phases representing 83 different categories

- 9 categories with > 3 members
- Lots of singletons



In Vitro Toxicity and Toxicokinetic Testing

Toxicological Response	Assay	Assay Endpoints	Purpose
Developmental Toxicity	Zebrafish embryo assay	Fertilisation, lethality, and structural defects	Assess potential teratogenicity
Immunotoxicity	Bioseek Diversity Plus	Protein biomarkers across multiple primary cell types	Measure potential disease and immune responses
Mitochondrial Toxicity	Mitochondrial membrane potential (HepaRG)	Mitochondrial membrane potential	Measure mitochondrial health and function
Developmental Neurotoxicity	Microelectrode array assay (rat primary neurons)	Neuronal electrical activity	Impacts on neuron function
Endocrine Disruption	ACEA real-time cell proliferation assay (T47D)	Cell proliferation	Measure ER activity
General Toxicity	Attagene cis- and trans-Factorial assay (HepG2)	Nuclear receptor and transcription factor activation	Activation of key receptors and transcription factors involved in hepatotoxicity
	High-throughput transcriptomic assay (multiple cell types)	Cellular mRNA	Measures changes in important biological pathways
	High-throughput phenotypic profiling (multiple cell types)	Nuclear, endoplasmic reticulum, nucleoli, golgi, plasma membrane, cytoskeleton, and mitochondria morphology	Changes in cellular organelles and general morphology

Toxicokinetic Parameter	Assay	Assay Endpoints	Purpose
Intrinsic hepatic clearance	Hepatocyte stability assay (primary human hepatocytes)	Time course metabolism of parent chemical	Measure metabolic breakdown by the liver
Plasma protein binding	Ultracentrifugation assay	Fraction of chemical not bound to plasma protein	Measure amount of free chemical in the blood

Objectives

- To inform
  - Chemical Category and Read-across approaches
  - Bioactive Dose Level (BDL) Approach (*in vitro* to *in vivo* extrapolation to define administered dose equivalent (ADE) values)

  In order to:

  Translate learnings to make inferences for a broader landscape of PFAS

Initially use structural categories to evaluate the degree of concordance in NAM results (per technology) within categories and across categories as a means to qualitatively and quantitatively infer *in vivo* toxicity

Characterising PFAS using structural categories

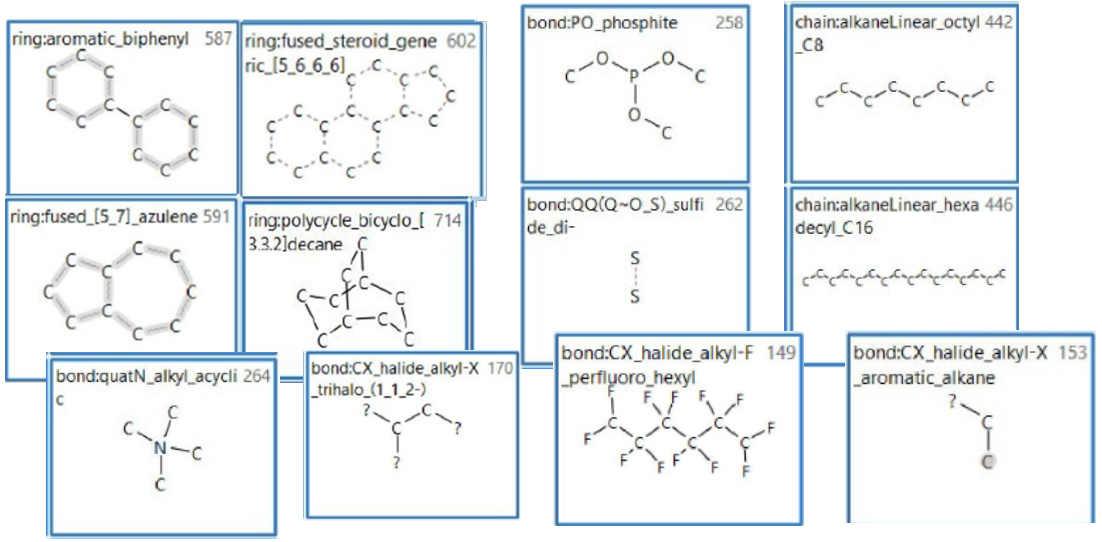
- Structural categories were assigned by visual inspection and whilst nominally consistent since only one individual was making the assignments, the approach was prone to error and not easily reproducible.
- The assignments provided by OECD were similar in their genesis – they were manually assigned by the same person.
- Indeed, authors of many of the published literature studies on PFAS have often end up deriving bespoke naming conventions for categories which leads to the generation of a lot of parallel nomenclature that differ, creating unintended barriers to effective communication among scientists
- Urgent need exists to develop a reproducible & objective means of developing structure-based categories

PFAS Structure-based Categorisation

- Reconcile the different structural categories schemes initially used by creating a harmonised set of structure-based categories
- Category assignments should be computationally generated from structure only → reproducible, transferable, standardised, extendable
- Permits nested & overlapping categories such that categories can be tailored to different datasets (i.e. the various NAM data streams being generated) and decision contexts

PFAS Structure-based Categorisation: ToxPrints

- Publicly available tools exist to generate & download ToxPrints e.g. ChemoTyper, CompTox Chemicals Dashboard
- Provides excellent coverage of PFAS chemical space
- Nested, hierarchical nature lends itself to creating flexible categories tailored to problem at hand, i.e., "fit for purpose"
- Can augment with computed structure properties (s.a., MW, size, etc.)
- Intuitive, easy to work with



ToxPrints:

- ✓ *729 chemical features*
- ✓ *Chemically interpretable*
- ✓ *Coverage of diverse chemistry*
- ✓ *Includes scaffolds, functional groups, chains, rings, bonding patterns, atom-types*

→ Clear, reproducible means for defining regions of local chemistry, i.e. categories!!

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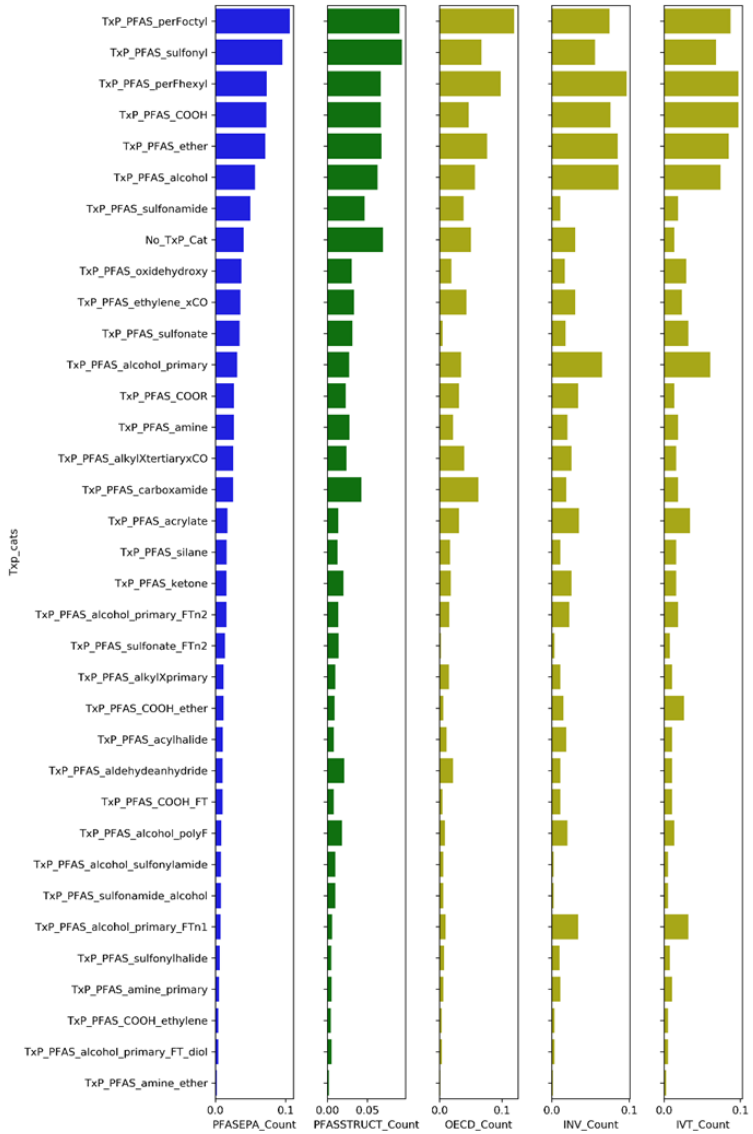
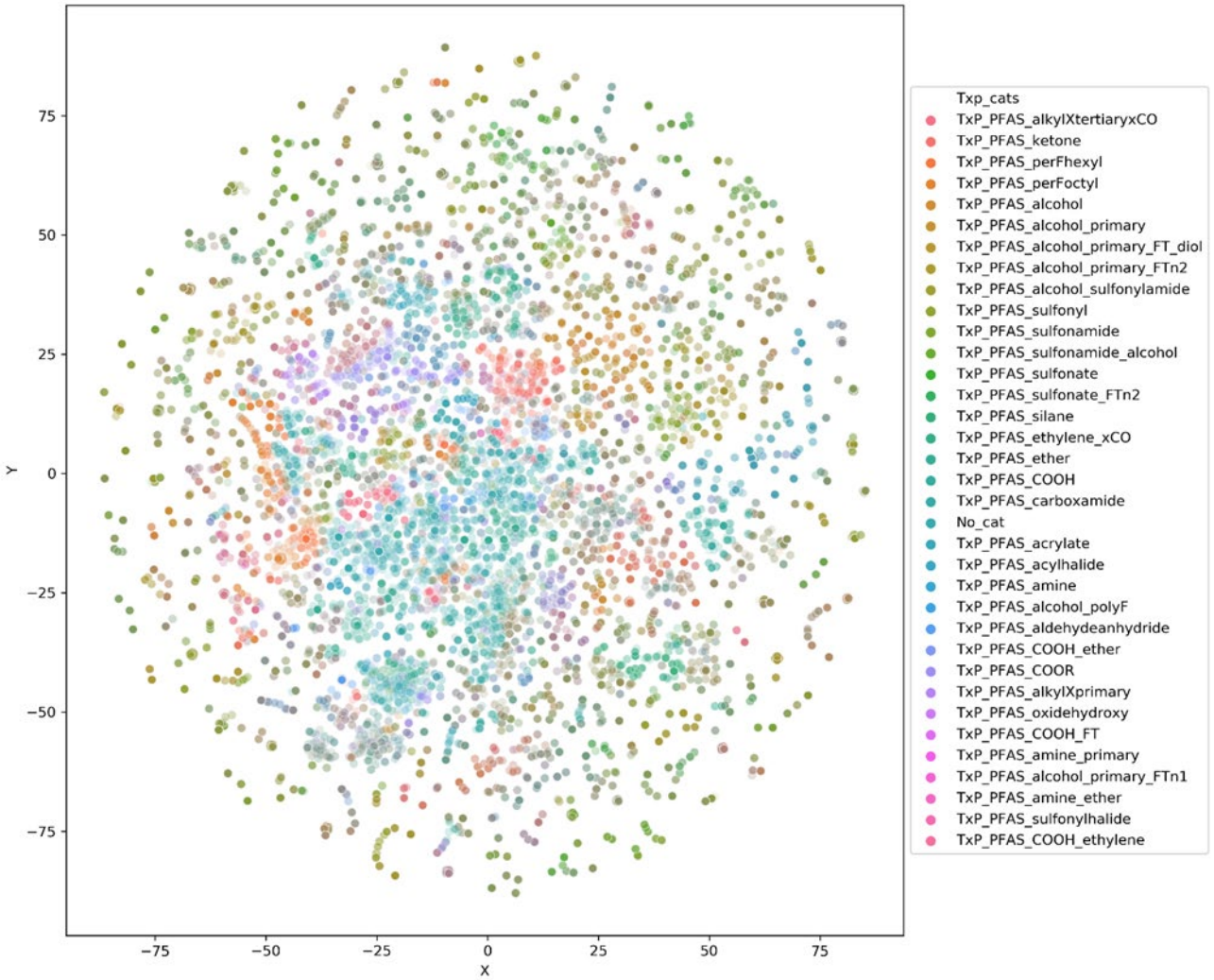
- Category assignments should be computationally generated from structure only → reproducible, transferable, standardised, extendable
- Permits nested & overlapping categories such that categories can be tailored to different datasets and decision contexts
- ToxPrints were used to develop 34 structural categories (TxP Categories) which cover >90% of the different PFAS inventories

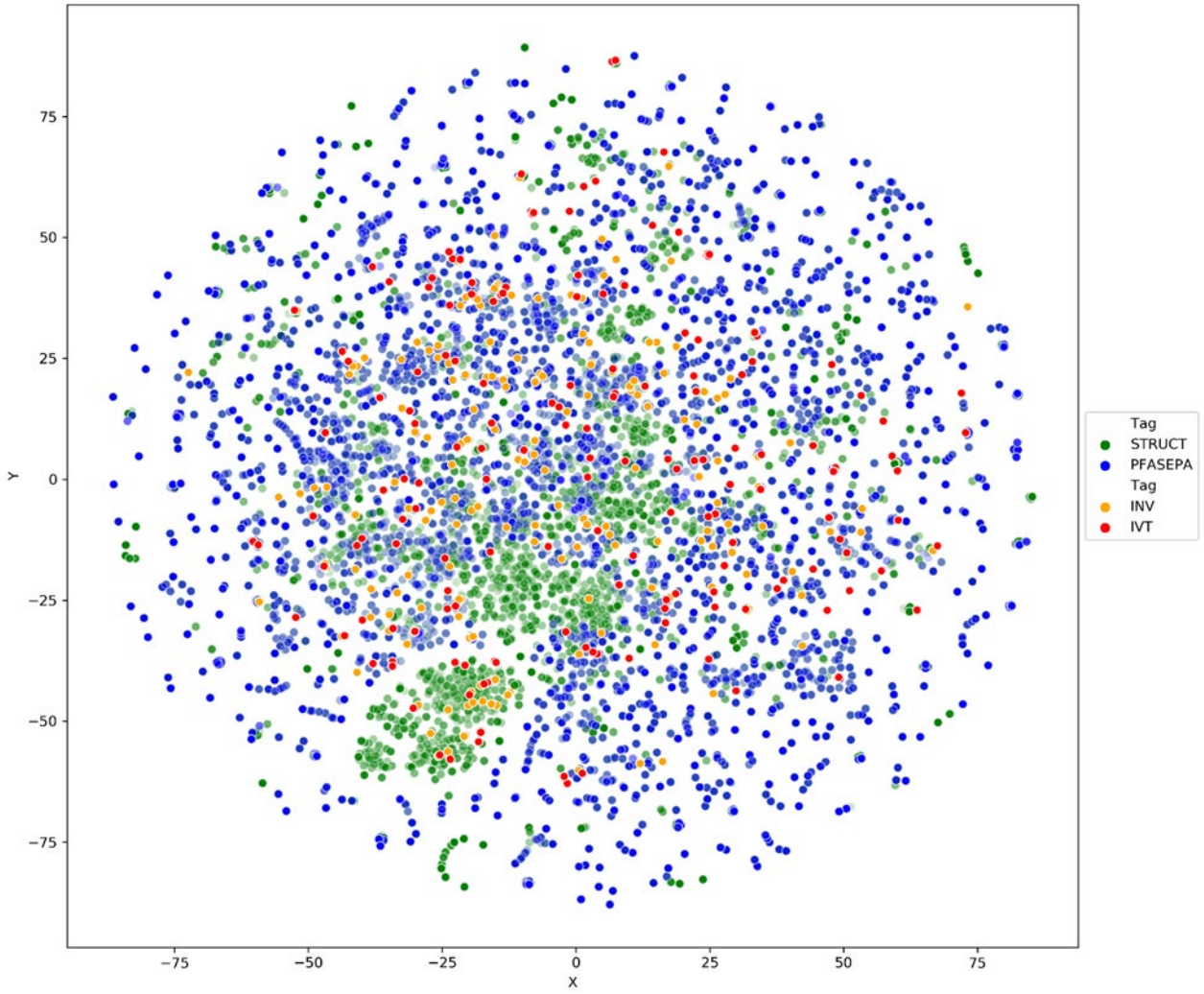
PFAS Structure-based Categorisation



Comparison of different inventories (PFASSTRUCT, OECD & the PFAS430INV) using the TxP Categories

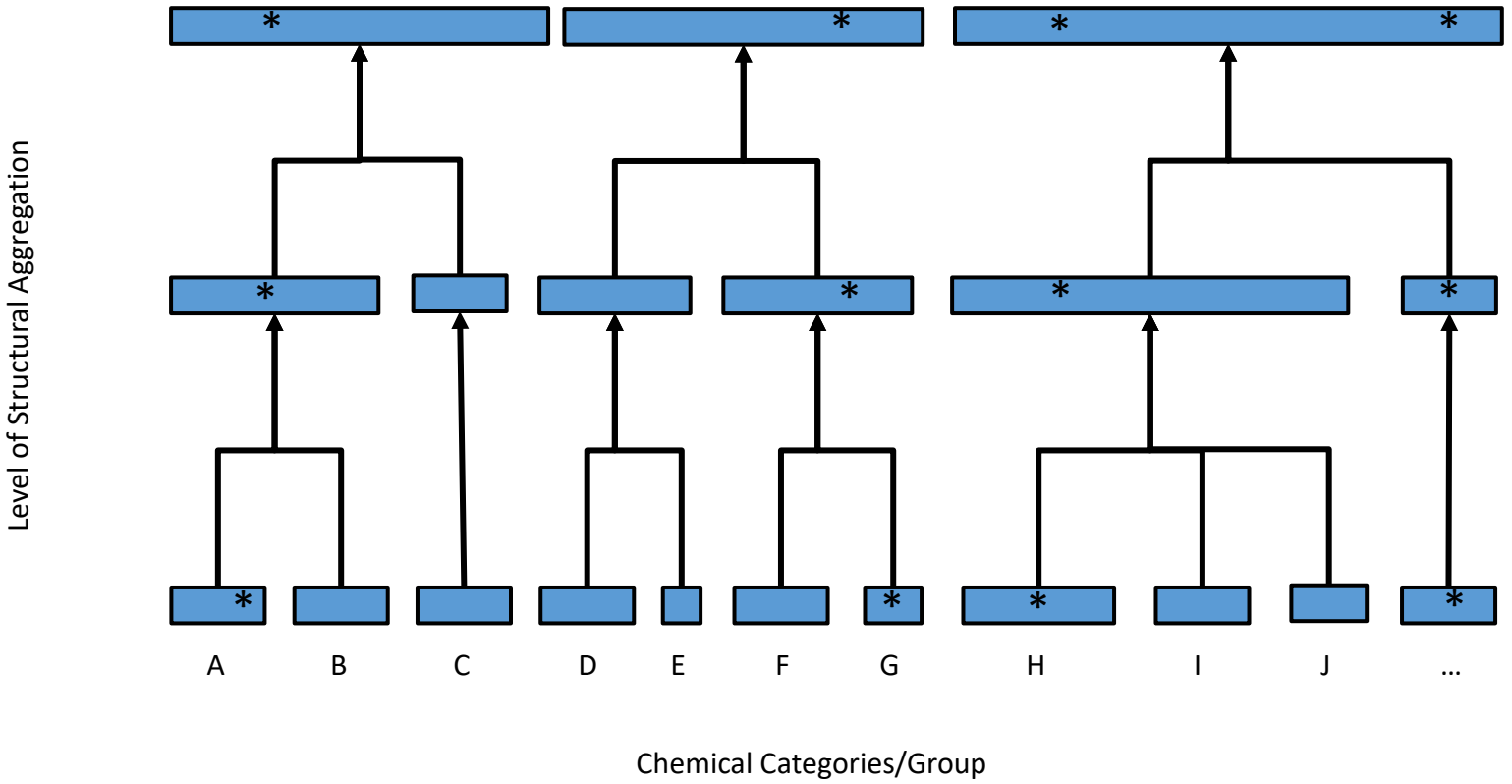


PFAS Coverage based on structure



- A 2D representation constructed using t-Distributed Stochastic Neighbour Embedding (t-SNE) based on 729 ToxPrints as chemical fingerprints
- PFAS430 inventory well distributed across the PFASSTRUCT inventory

Current PFAS Structural Grouping Approaches Use Different Levels of Aggregation



*Available source *in vivo* tox study

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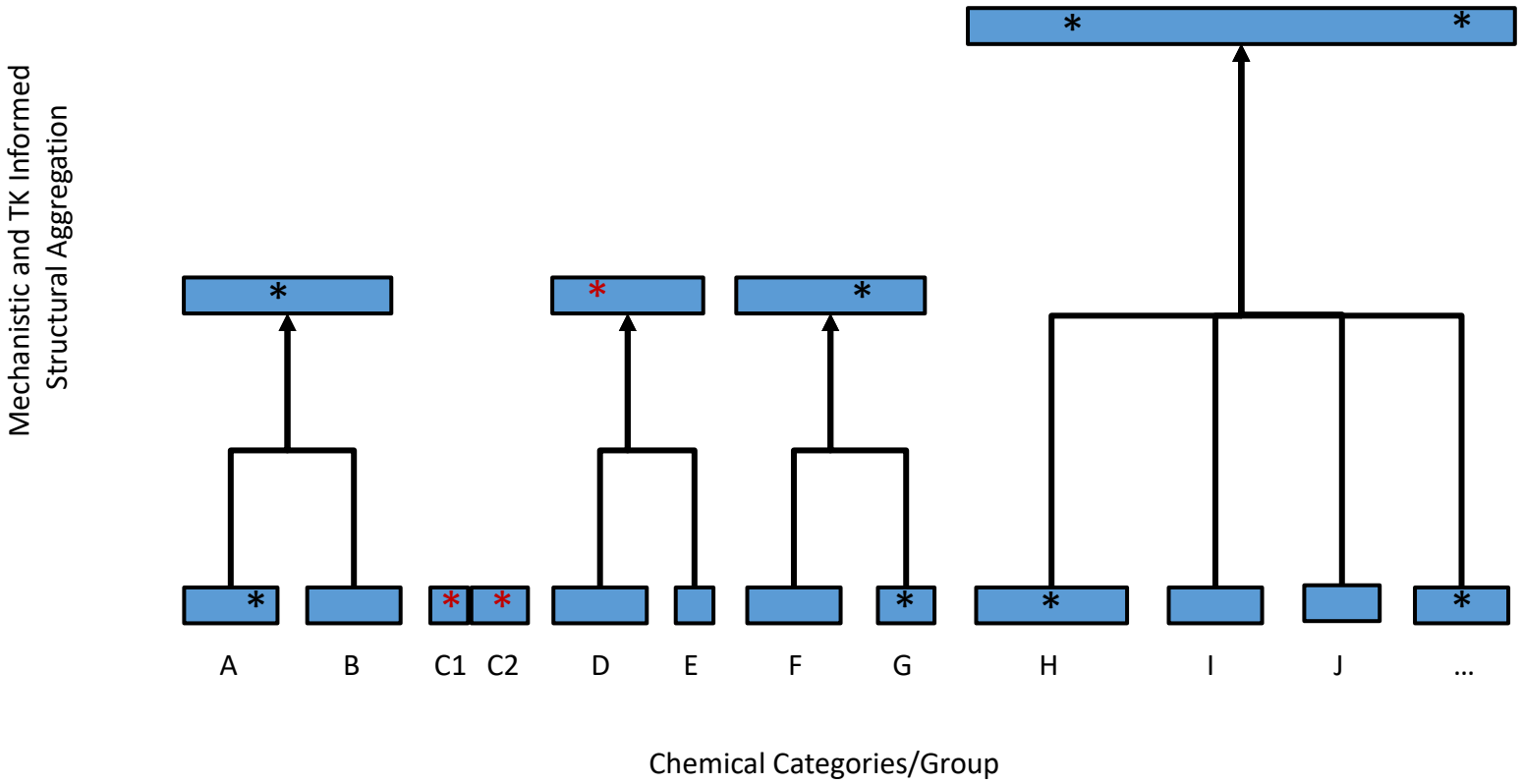
PFAS Category Aggregation that incorporates Structural, Mechanistic and Toxicokinetic Data



*Needed *in vivo* tox study

*Available source *in vivo* tox study

Targeted screening for nuclear receptor activation and cell stress

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Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/toxicol

Bioactivity profiling of per- and polyfluoroalkyl substances (PFAS) identifies potential toxicity pathways related to molecular structure

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[a] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA
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ARTICLE INFO

Keywords:
Perfluoroalkyl substances
PFAS
Transcription factors
Chemical safety
Nuclear receptors

ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) are a broad class of hundreds of fluorinated chemicals with environmental health concerns due to their widespread presence and persistence in the environment. Several of these chemicals have been comprehensively studied for experimental toxicity, environmental fate and exposure, and human epidemiology; however, most chemicals have limited or no data available. To inform methods for prioritizing these data-poor chemicals for detailed toxicity studies, we evaluated 142 PFAS using an *in vitro* screening platform consisting of two multiplexed transactivation assays encompassing 81 diverse transcription factor activities and tested in concentration-response format ranging from 137 nM to 300 μM. Results showed activity for various nuclear receptors, including three known PFAS targets–specifically estrogen receptor alpha and peroxisome proliferator receptors alpha and gamma. We also report activity against the retinoid X receptor beta, the key heterodimeric partner of type II, non-steroidal nuclear receptors. Additional activities were found against the pregnane X receptor, nuclear receptor related-1 protein, and nuclear factor erythroid 2-related factor 2, a sensor of oxidative stress. Using orthogonal assay approaches, we confirmed activity of representative PFAS against several of these targets. Finally, we identified key PFAS structural features associated with nuclear receptor activity that can inform future predictive models for use in prioritizing chemicals for risk assessment and in the design of new structures devoid of biological activity.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemicals that have been in use since the 1940s and are found in a broad array of industrial and consumer products (Glüge et al., 2020). Their common usage as non-stick surface repellants, in fire-fighting foams, in fluoropolymer manufacturing, and in other applications, coupled with a tendency of some members of the class to bioaccumulate and be resistant to biodegradation, has led to a high level of concern for their contamination of the environment (Wang et al., 2017). There are well documented, widespread, human and wildlife exposure to some of these chemicals, the best known being perfluorooctanoic acid (PFOA; DTXSID8031865) and perfluorooctane sulfonic acid (PFOS; DTXSID3031864) (Kelly et al., 2009; Poothong et al., 2020; Hansen et al., 2002; Noorlander et al., 2011). These two chemicals are no longer manufactured in the U.S. and their international manufacturing has declined, but other PFAS chemicals have been developed to replace their commercial utility (REACH, 2014; OECD, 2015; Stockholm Convention, 2017; EPA, 2000; EPA, 2017). While the toxicities of PFOA and PFOS have been extensively studied by many researchers, numerous other PFAS have little to no toxicity or environmental fate information available. The lack of data and potential environmental impact of this class of chemicals led the U.S. Environmental Protection Agency (EPA) and the National Institute of Health's National Toxicology Program (NTP) to collaborate on conducting PFAS toxicity testing to facilitate PFAS human health assessments (Patlewicz et al., 2019). A targeted selection of 430 PFAS (https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASINV) designed to be representative of the range of

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Houck et al. 2020

Gathering information on nuclear receptor and cell stress pathways via transcription factor activity profiling (TFAP)

- >3800 ToxCast chemicals have been screened in concentration response in the Attagene transcription factor profiling system
  - HepG2 HG19 subclone for elevated xenobiotic metabolic capacity
  - "CIS" assays: endogenous transcription factors that regulated transfected reporters (nuclear receptor promoter elements, cell stress)
  - "TRANS" assays: exogenous receptor-reporter system is transfected in (xenobiotic nuclear receptors)
  - Used for environmental mixtures and single chemical screening

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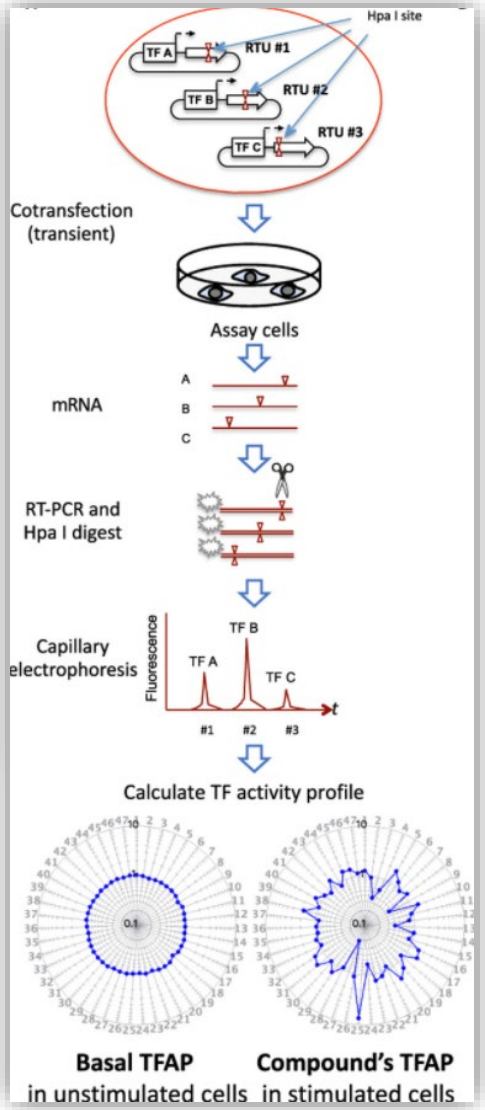


Number	Endpoint	Go Process
1	GAL4_TRANS	control
2	M_06_CIS	
3	M_06_TRANS	
4	M_19_CIS	
5	M_19_TRANS	
6	M_32_CIS	
7	M_32_TRANS	
8	M_61_CIS	
9	M_61_TRANS	
10	TA_CIS	
11	TAL_CIS	
12	CMV_CIS	
13	E_Box_CIS	cell proliferation
14	E2F_CIS	
15	EGR_CIS	
16	Ets_CIS	
17	Pax6_CIS	
18	AR_TRANS	reproduction
19	ERa_TRANS	
20	ERE_CIS	
21	THRa1_TRANS	
22	VDR_TRANS	
23	VDRE_CIS	
24	ISRE_CIS	immune system process
25	NF_kB_CIS	
26	IR1_CIS	lipid metabolic process
27	FXR_TRANS	
28	DR4_LXR_CIS	
29	LXRa_TRANS	
30	LXRb_TRANS	
31	PPARa_TRANS	
32	PPARd_TRANS	
33	PPARg_TRANS	
34	PPRE_CIS	
35	SREBP_CIS	
36	Ahr_CIS	xenobiotic metabolic process
37	CAR_TRANS	
38	PBREM_CIS	
39	PXR_TRANS	
40	PXRE_CIS	

Number	Endpoint	Go Process
41	AP_1_CIS	response to stress
42	HIF1a_CIS	
43	HSE_CIS	
44	MRE_CIS	
45	NRF1_CIS	
46	NRF2_ARE_CIS	
47	Oct_MLP_CIS	
48	p53_CIS	
49	Xbp1_CIS	
50	CRE_CIS	biosynthetic process
51	ERRa_TRANS	
52	ERRg_TRANS	
53	GR_TRANS	
54	GRE_CIS	
55	DR5_CIS	cell differentiation
56	RARa_TRANS	
57	RARb_TRANS	
58	RARg_TRANS	
59	RXRa_TRANS	
60	RXRb_TRANS	
61	NURR1_TRANS	
62	RORb_TRANS	
63	RORg_TRANS	
64	RORE_CIS	
65	Sox_CIS	
66	AP_2_CIS	anatomical structure development
67	BRE_CIS	
68	C_EBP_CIS	
69	FoxA2_CIS	
70	FoxO_CIS	
71	GATA_CIS	
72	GLI_CIS	
73	HNF4a_TRANS	
74	HNF6_CIS	
75	Myb_CIS	
76	Myc_CIS	
77	NFI_CIS	
78	Sp1_CIS	
79	STAT3_CIS	
80	TCF_b_cat_CIS	
81	TGFb_CIS	

There are differences in assay sensitivity by mode and receptor, based on expression and design differences.

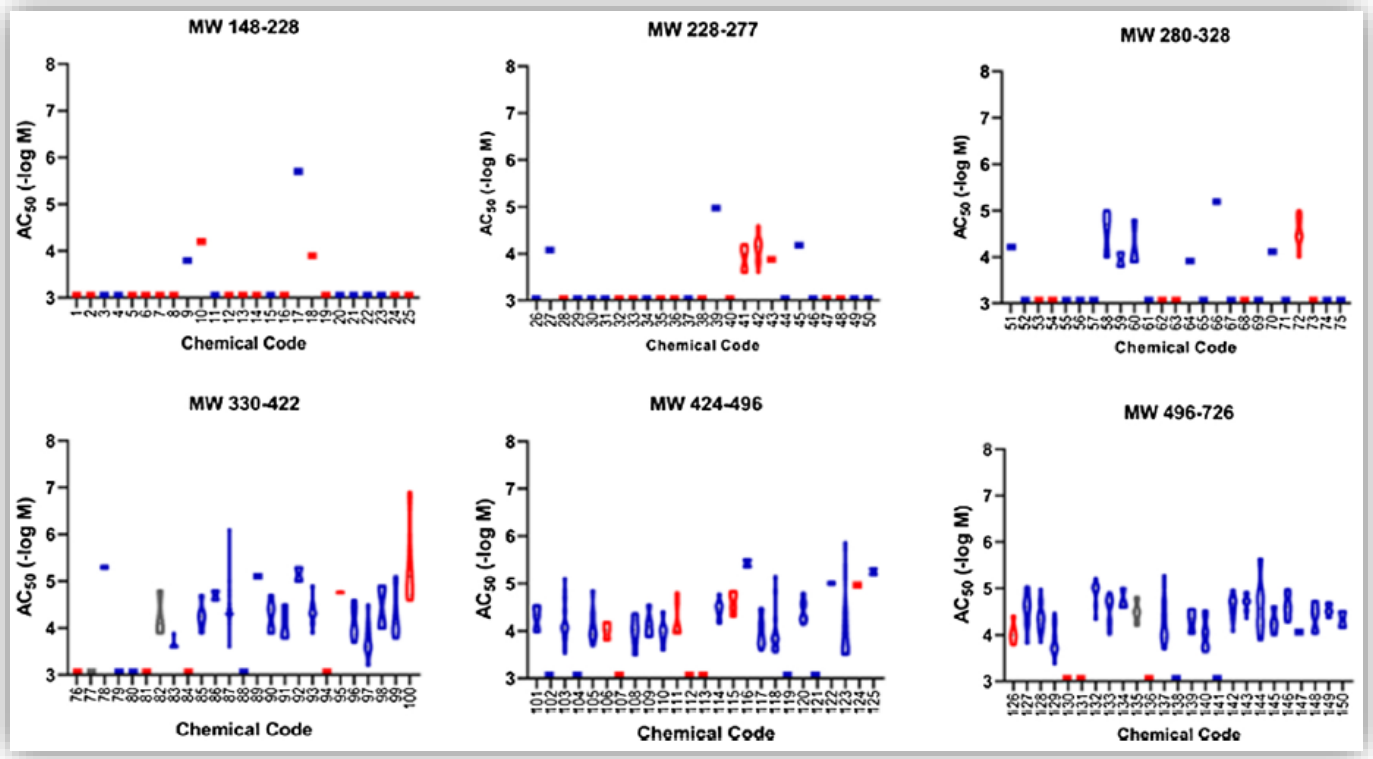
Table 1
Nuclear receptors included in FACTORIAL-TRANS assay.

#	Abbreviation	Receptor Name	Nomenclature	Reference Agonist (Fold-Increase)	*cis*-Factorial Assay (Fold-Increase)	Receptor Expression in HepG2[1]
1	FXR	Farnesoid X receptor	NR1H4	Lithocholic acid (3.5)	IR1 (1.9)	Moderate
2	AR	Androgen receptor	NR3C4	Testosterone propionate (44.1)	NA	Very low
3	RARγ	Retinoic acid receptor-γ	NR1B3	All-trans retinoic acid (3.9)	DR5 (20.2)	Moderate (RAR subfamily)[2]
4	GAL4	Yeast GAL4, negative control	GAL4	NA	NA	NA
5	RXRα	Retinoid X receptor-α	NR2B1	Bexarotene (18.5)	DR5 (8.3)	Moderate (RXR subfamily)[2]
6	GR	Glucocorticoid receptor	NR3C1	Betamethasone (29.1)	GRE (4.6)	Moderate
7	RARβ	Retinoic acid receptor-β	NR1B2	All-trans retinoic acid (1.6)	DR5 (20.2)	Moderate (RAR subfamily)[2]
8	RARα	Retinoic acid receptor-α	NR1B1	All-trans retinoic acid (5.5)	DR5 (20.2)	Moderate (RAR subfamily)[2]
9	PPARγ	Peroxisome proliferator-activated receptor-γ	NR1C2	Rosiglitazone maleate (44.8)	PPRE (3.8)	High
10	ERRγ	Estrogen-related receptor-γ	NR3B3	4-Nonylphenol, branched (2.7)	NA	NA
11	RORβ	RAR-related orphan receptor-β	NR1F1	SSR69071 (7.8)	RORE (5.9)	NA
12	ERα	Estrogen receptor-α	NR3A1	17β-Estradiol (22.6)	ERE (19.1)	Very low; full-length human ERα co-expressed in FACTORIAL-CIS
13	LXRα	Liver X receptor-α	NR1H3	Lynestrenol (13.9)	DR4 (2.3)	High (LXR subfamily)[2]
14	ERRα	Estrogen-related receptor-α	NR3B1	4-Nonylphenol, branched (2.7)	NA	NA
15	PXR	Pregnane X receptor	NR1I2	Rifampicin (3.8)	PXRE (9.1)	Moderate; full-length human PXR co-expressed in FACTORIAL-CIS
16	TRα	Thyroid hormone receptor-α	NR1A1	3,5,3′-Triiodothyronine (33.0)	NA	High
17	LXRβ	Liver X receptor-β	NR1H2	Lynestrenol (8.7)	DR4 (2.3)	High (LXR subfamily)[2]
18	CAR	Constitutive androstane receptor	NR1I3	p,p'-DDT (3.5)	PBREM (1.0)	Very low
19	PPARα	Peroxisome proliferator-activated receptor-α	NR1C1	Pirinixic acid (14.1)	PPRE (2.4)	Moderate
20	RORγ	RAR-related orphan receptor-γ	NR1F3	SSR69071 (14.2)	RORE (5.9)	NA
21	RXRβ	Retinoid X receptor-β	NR2B2	Bexarotene (15.2)	DR5 (8.3)	Moderate (RXR subfamily)[2]
22	HNF4α	Hepatocyte nuclear factor-4-α	NR2A1	NA	NA	High
23	NURR1	Nuclear receptor related 1	NR4A2	Bexarotene (24.6)	NA	NA
24	VDR	Vitamin D receptor	NR1I1	Ergocalciferol (32.6)	VDRE (1.2)	Very low
25	PPARδ	Peroxisome proliferator-activated receptor-δ	NR1C3	12-Hydroxyoctadecanoic acid (9.3)	PPRE (2.9)	NA

- Low- to negligible-expression in HepG2 cells of ERa and PXR was overcome by cotransfection of full-length receptors in the TRANS assay
- CAR and VDR have very low sensitivity to ligands due to reliance only on endogenous receptor expression in the host cell.

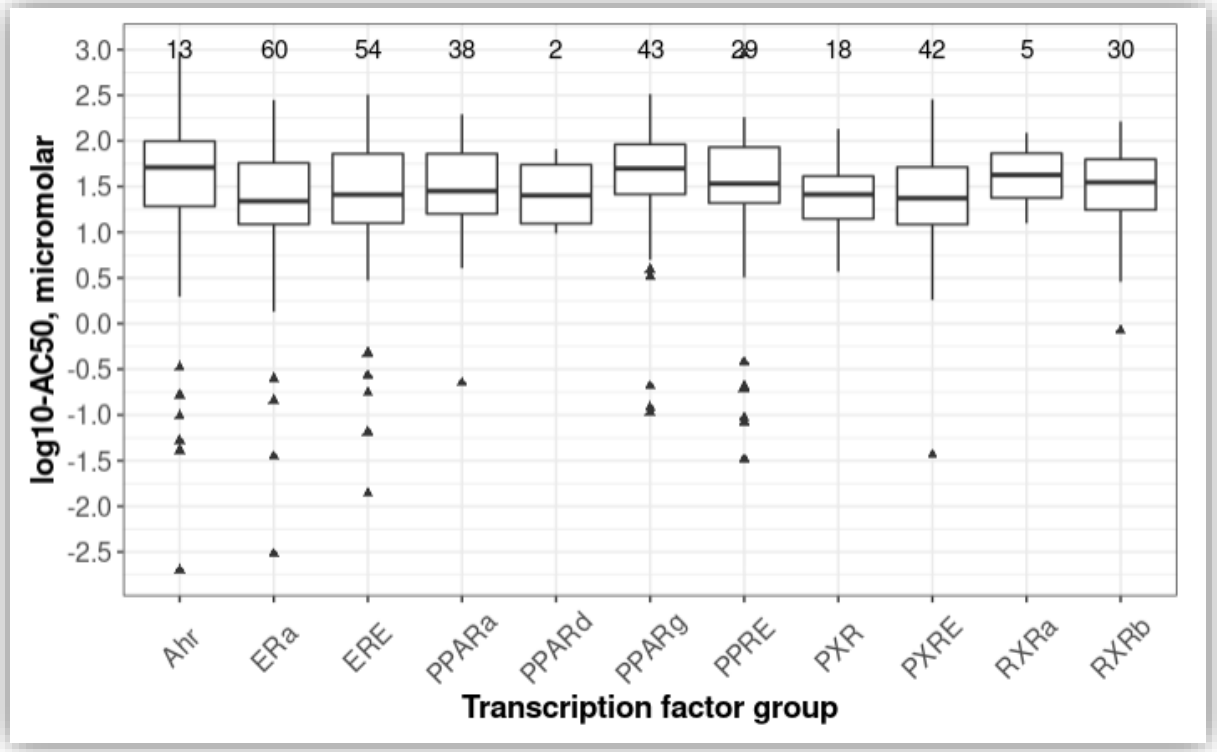
As with other assay platforms screened, lower MW often corresponded to more limited bioactivity, but there may be more than one reason.

- PFAS with molecular weight less than 330 g/mol appeared less likely to be active in the Attagene assays and more likely to "fail" analytical QC (defined as parent structure not detected).
- Activity was not detected for 76 distinct structures, of which 55 % failed analytical QC.
- 67% of the "failed" samples had predicted vapor pressures in excess of 100 mmHg, suggesting that chemical volatilisation may have played a role in limited bioactivity of some of these samples.
- The specific acid form of PFAS may also be important, as the free acid form of the chemical known as "GenX" (perfluoro-2-methyl-3-oxahexanoic acid (DTXSID70880215) did not have a high vapor pressure (was unlikely to have volatilised), but the ammonium salt form of this chemical (DTXSID40108559) showed activity as a PPARa agonist when solubilised in water (rather than DMSO).

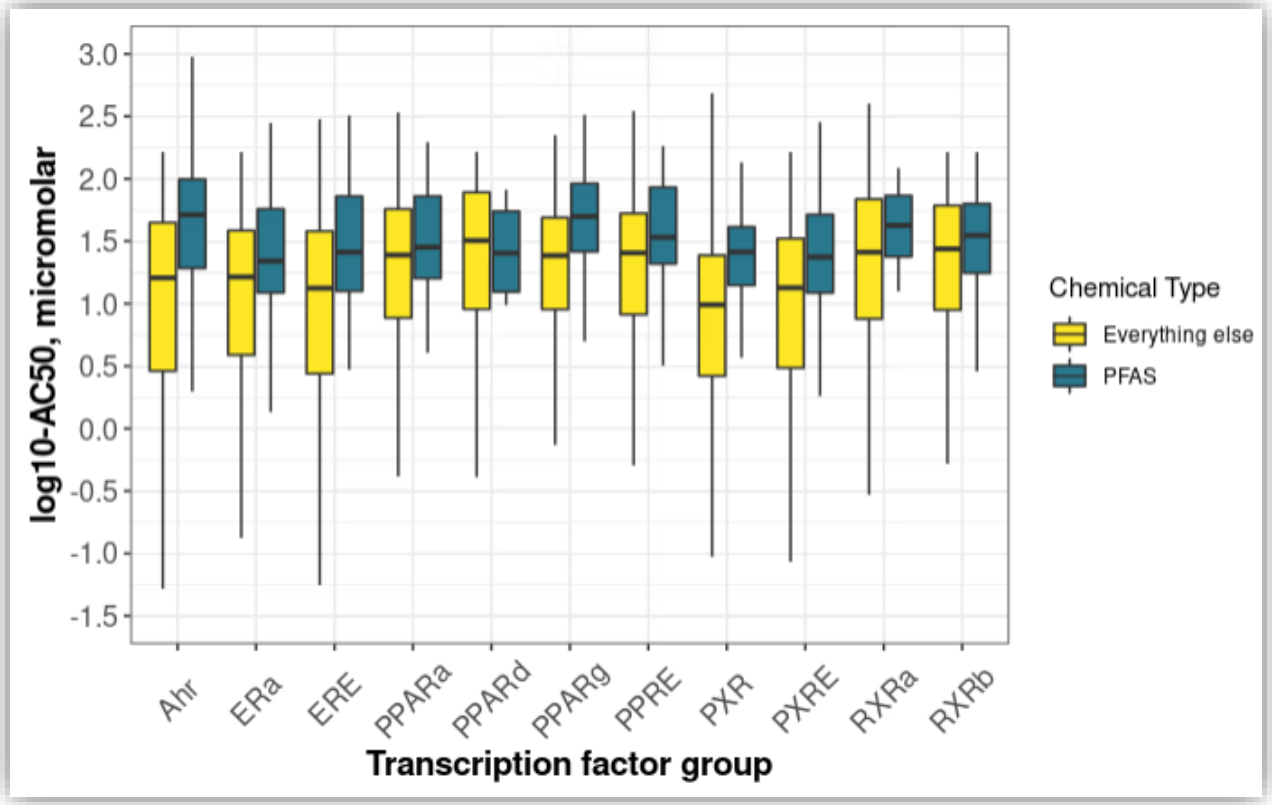


Houck et al. 2020, Fig1B.

Potency for the PFAS that were positive at key transcription factor targets tended to be somewhat left-shifted from the rest of the ToxCast library

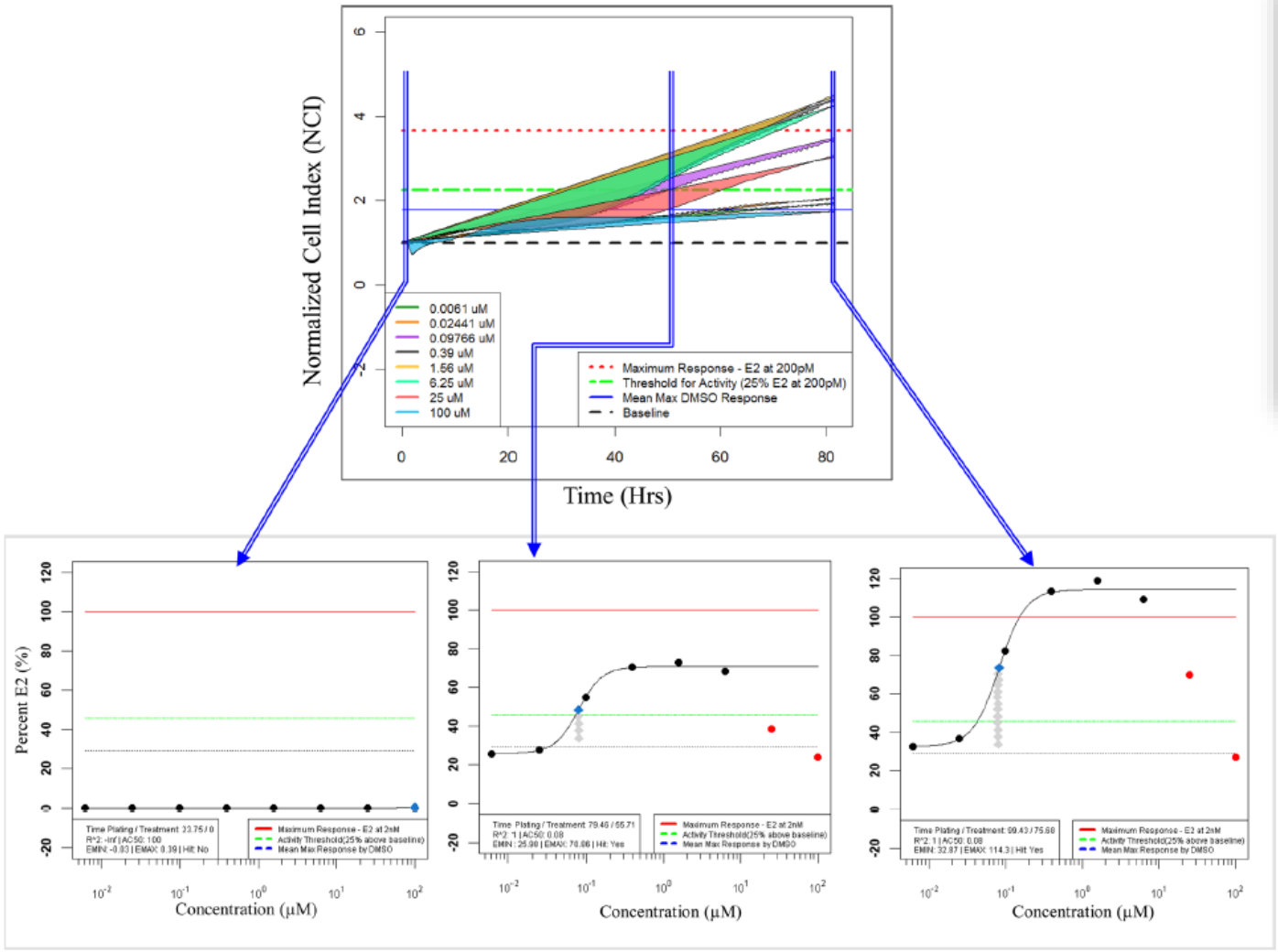


- The number of chemicals that simply hit one or more relevant assays for a particular transcription factor group can be examined in more depth for confirmation.



- Many PFAS were negative in the transcription factor activity screening
- Aryl hydrocarbon receptor (AhR), estrogen receptor alpha (ERa), PPAR alpha, delta, and gamma (PPARa,d,g), the pregnane X receptor (PXR), and RXR alpha and beta (RXRa,b) emerged as targets.

Estrogen receptor activity can be confirmed with orthogonal assays including ACEA: Real Time Cell Analysis Based on Electrical Impedance



Chemical Research in Toxicology

Article

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Real-Time Growth Kinetics Measuring Hormone Mimicry for ToxCast Chemicals in T-47D Human Ductal Carcinoma Cells

Daniel M. Rotroff,[†,‡] David J. Dix,[‡] Keith A. Houck,[‡] Robert J. Kavlock,[‡] Thomas B. Knudsen,[‡] Matthew T. Martin,[‡] David M. Reif,[‡] Ann M. Richard,[‡] Nisha S. Sipes,[‡] Yama A. Abassi,[§] Can Jin,[§] Melinda Stampfl,[§] and Richard S. Judson*[,‡]

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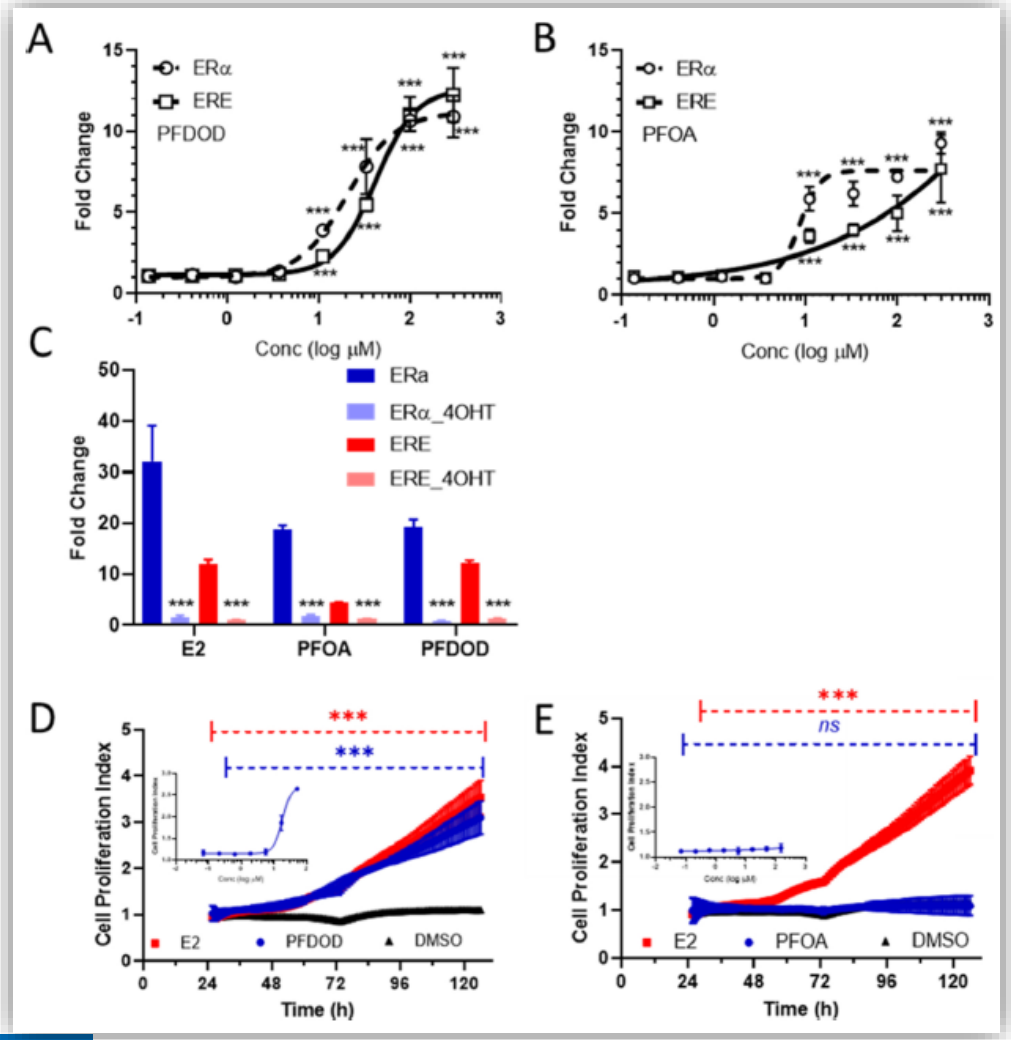
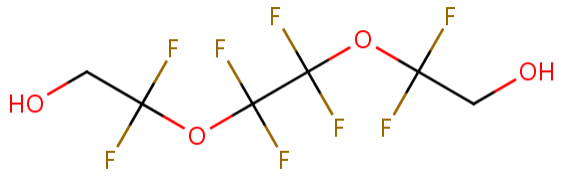
- Can measure cell proliferation or cytotoxicity depending on the direction
- Electrical impedance measured over 80 hr
- ACEA ER assay uses T-47D breast cancer cells

Confirmation of transcriptional responses with functional activity is an important strategy for ER bioactivity

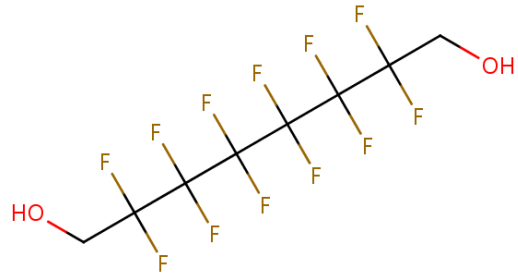


Houck et al. 2020, Fig5.

- 40-60 PFAS demonstrated some activity in the ATG ERa TRANS or ERE CIS assays; viewing these assays as orthogonal reduces the set to <10.
  - All of these were less potent than 17β-estradiol.
  - Acrylates and N-akyl perfluoroalkyl (linear) sulfonamide structural categories were significantly associated with ER activity.
- Adding in ACEA as another orthogonal assay to confirm specificity leads indicates few PFAS with transcription factor *and* functional ER-dependent cell proliferation



1H,1H,8H,8H-Perfluoro-3,6-dioxaoctane-1,8-diol

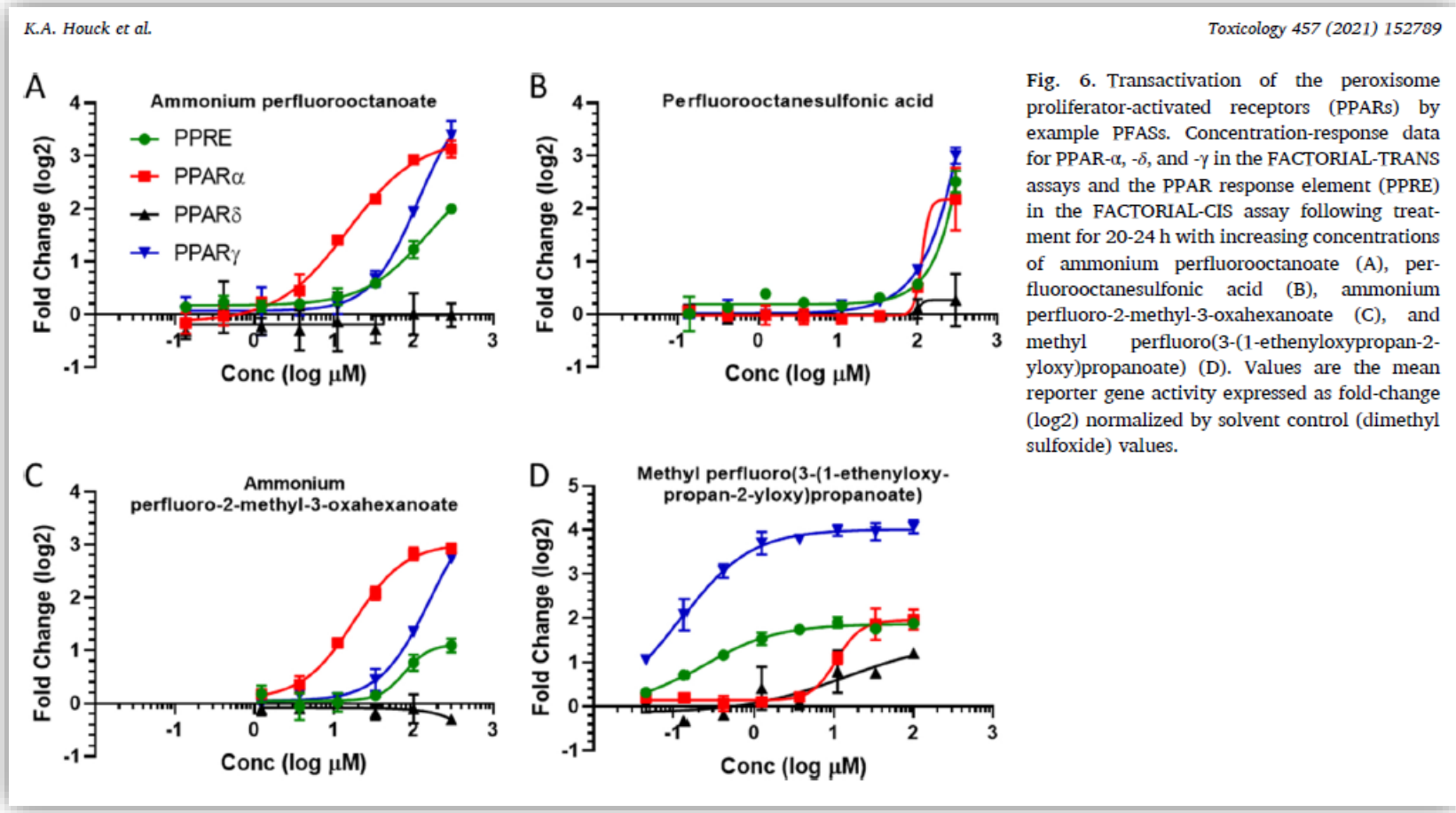


1H,1H,8H,8H-Perfluorooctane-1,8-diol

- PFOA activated ATG_ERa_TRANS and ERE_CIS but failed to produce functional ER-dependent cell proliferation in ACEA.

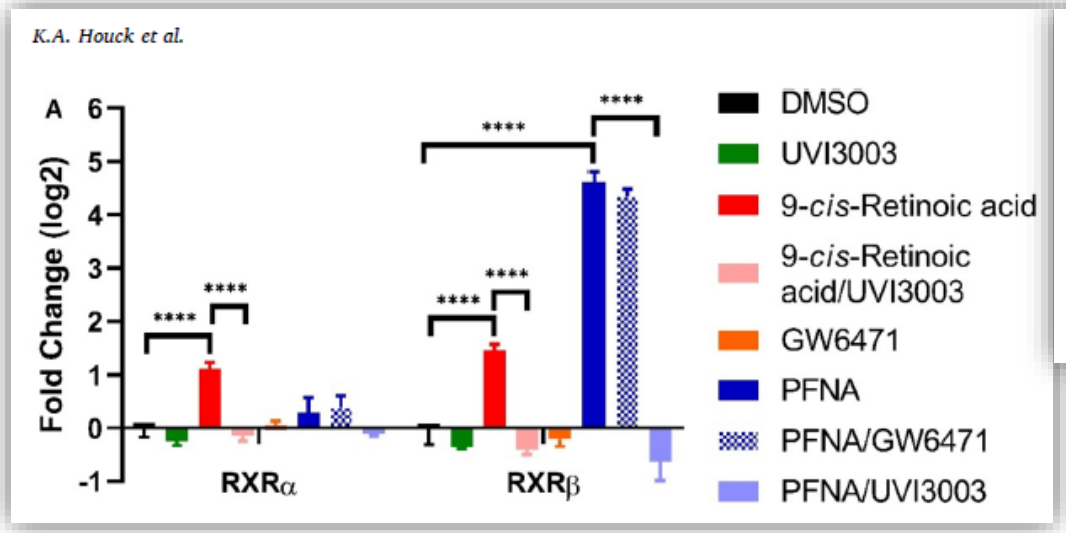
As expected PPAR activity was observed for a subset of PFAS.

- The TRANS assay contained endpoints for all three human PPARs (α,δ,γ) whereas the CIS assay contained a reporter gene controlled by a PPAR-response element that responds to all three PPARs endogenously expressed in the HepG2 host cells.
- Functional groups enriched within the actives were mostly carboxylates along with sulfonates, sulfonamides and a thenoylketone, which all have a negative ionic charge at physiological pH, consistent with known critical components for ligand-binding.
- Not much activity at PPARδ (smaller binding pocket?).



Houck et al. 2020, Fig6.

~17 PFAS activated RXRβ, with two of these active at RXRa



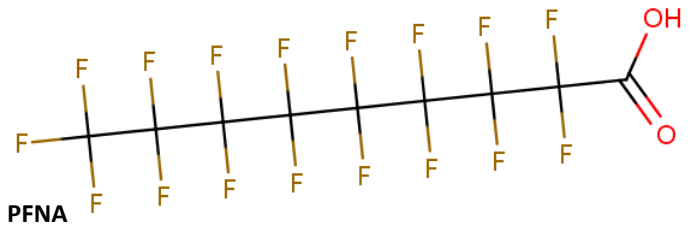
Toxicology 457 (2021) 152789

Fig. 8. PFAS activity for retinoid X receptors (RXR). A) Responses of RXRα and RXRβ to perfluorononanoic acid (PFNA) and effects of pharmacological agents UVI3003 (5 μM), a pan-RXR antagonist; 9-cis retinoic acid (0.02 μM), a pan-RXR agonist; and GW6471 (5 μM), a PPARα-selective antagonist; in the presence and absence of PFNA (66 μM). No significant activation of RXRα by PFNA was observed. Significance was established with an ordinary one-way ANOVA and Tukey's multiple comparisons test. (**** = P < .0001). B) Radioligand

Houck et al. 2020, Fig8A.

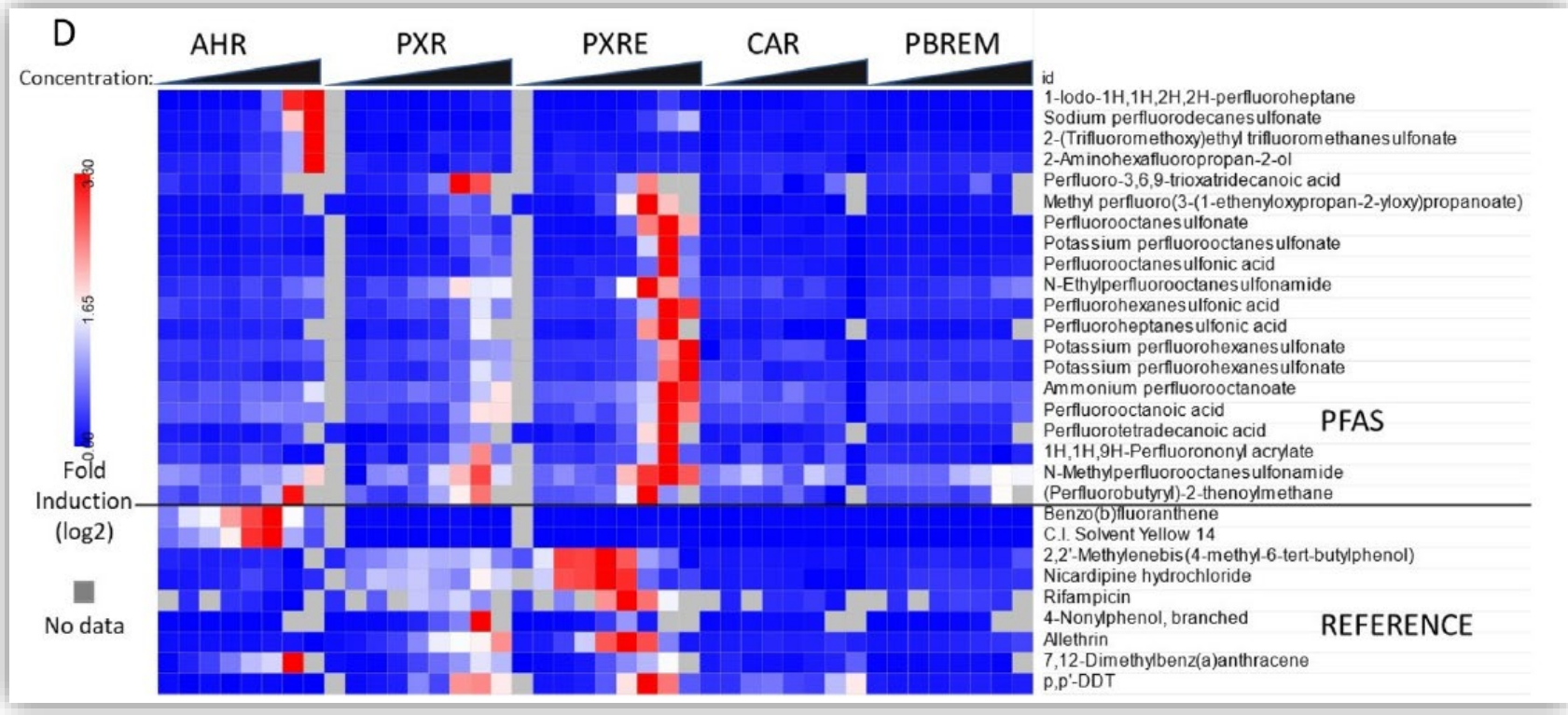
- Seventeen of the PFAS, mostly linear, fluorinated carboxylic acids, showed a novel finding of activation of RXRβ.
- Most also activated PPARa, PPARγ and NRF2, with varying levels of selectivity. Only two activated RXRa; however, NURR1 was activated, presumably through agonist effects on RXRβ .
- All are structurally related perfluorinated carboxylic acids and meet defined ligand structural requirements for RXR.

PFNA appears to work through RXR specifically: an RXR-selective antagonist, UVI3003 (DTXSID501024375), completely blocked PFNA activation of RXR, whereas the PPARα antagonist GW6471 was ineffective.



PFNA

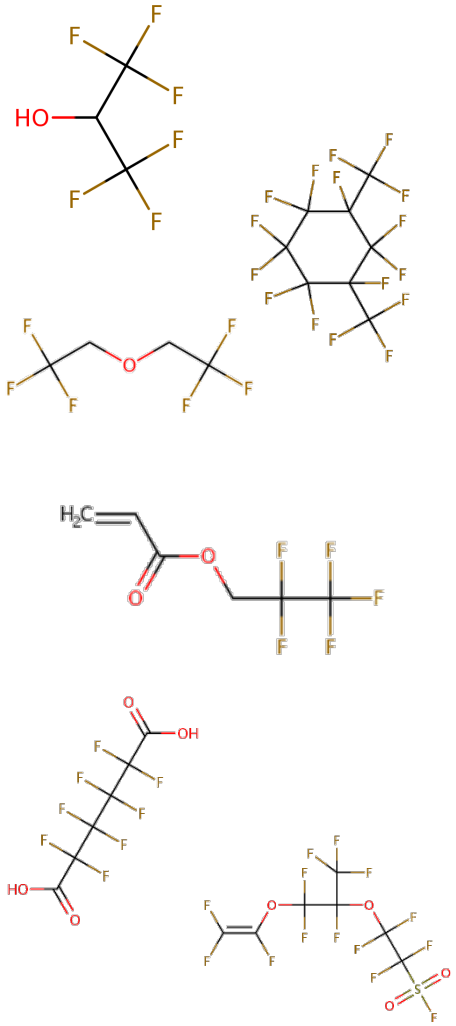
Xenobiotic nuclear receptor responses associated with hepatic metabolism may also be important targets to screen for PFAS bioactivity.



Houck et al. 2020, Fig3B.

- Many of the PFAS modulated the xenobiotic response, particularly PXR.
- Responses were generally modest with respect to potency and efficacy relative to prototypical PXR inducers.
- None of the PFAS were determined to be CAR activators, recognizing limitations in the FACTORIAL-CIS assay for CAR, likely due to negligible expression of CAR in HepG2 cells.
- Several PFAS structures activated the AhR, somewhat surprising in that all were linear fluoroalkyl molecules while the protypical activator is a polycyclic aromatic hydrocarbon. Except for sodium perfluorodecanesulfonate and 1-Iodo-1H,1H,2H,2H-perfluoroheptane, the responses were very weak with unknown *in vivo* relevance.

Take Home Messages…



- Chemical curation efforts are important to harmonise structure, naming, and identifiers across the PFAS space
- A chemical library of 430 PFAS was assembled for chemical screening, analytical method development, and other research needs
- A subset of 150 PFAS selected for *in vitro* toxicity and toxicokinetic testing to refine/support read across categories
- *In vitro* toxicity and toxicokinetic testing and the ongoing analysis demonstrate the diverse biological activities and toxicokinetic properties of PFAS
- More information at https://www.epa.gov/chemical-research/pfas-chemical-lists-and-tiered-testing-methods-descriptions

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