

## **REACT Program for Per- and Polyfluoroalkyl Substances**

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### **NTP Exploratory PFAS Library**

Per- and Polyfluoroalkyl Substances (PFAS) are industrial chemicals used for a variety of products including non-stick cookware, stain-resistant fabrics, food packaging, and firefighting foams. In January 2017, EPA's National Center for Environmental Assessment (NCEA) submitted a list of 30 PFAS to NTP for in-vitro screening. In response, NTP developed the Rapid Evaluation and Assessment of Chemical Toxicity (REACT) Program. Shortly before the NCEA nomination, the NTP Laboratory had started a collaborative screening project on 22 PFAS with the EPA's National Exposure Research Laboratory. NTPL combined the two chemical libraries (Table 1) into the NTP Exploratory PFAS Library. The PFAS chemicals are being screened using the assays listed in Table 2. As part of the effort to identify the biological activity that should be evaluated in these screening efforts, the DNTP Biomolecular Screening Branch has run a series of QSAR models on a set of 291 PFAS. NTP is presently designing in vitro assays that link to these model predictions. Because these efforts are exploratory to test specific hypotheses, some assays may not include all chemicals in the NTP Exploratory PFAS Library.

### **In Vivo and In Vitro Studies**

Several studies on GenX are underway. One study is examining the pharmacokinetics of GenX in rodents following single and repeat dosing. Since PFOA and PFOS are substrates for transporters, to better understand the role of transporters in the toxicity of PFAS, investigations are underway that examine the effects of PFOA and GenX on the activity of transporters along the blood brain barrier using both in vitro and in vivo models. Investigations are also underway to develop methods to evaluate the transporter mediated renal reuptake, which is believed to be responsible, in part, for the prolonged biological half-life of these chemicals. Another study is evaluating the effects of GenX and perfluorooctanoic acid (PFOA) on placental histopathology and gene expression in CD-1 mice.

### **EPA PFAS Screening Library**

In July of 2017, NTP and EPA's National Center for Computational Toxicology (NCCT) began a collaborative project on PFAS. As part of this collaboration NCCT is identifying a list of 75 PFAS chemicals of high priority for EPA and will provide aliquots of these chemicals, in a blinded fashion, to NTP for screening. This set of 75 has been selected from among a larger list of candidates because of interest in evaluating read-across extrapolations. NTP has made a commitment to share the findings with its agency partners and possibly identify other chemicals for screening. EPA is overseeing the QA/QC and solubility assessments of the 75 chemicals.

The NTP and NCCT collaboration also extends to screening this library. Because of the broad range of adverse effects induced by the prototype PFAS, PFOA and PFOS, the screening efforts need to evaluate a broad range of biological activity. To increase efficiency and reduce redundancy in these efforts the NTP and NCCT are working to determine which assays are most

relevant for screening PFAS and these two groups will share the screening efforts (see Table 3 for the assay list). At present, we are taking the approach that we will only use the EPA PFAS Screening Library for agreed upon assays. Thus, the reason for the discrimination between the NTP Exploratory PFAS Library and the EPA PFAS Screening Library. While this list does not limit what we do with the EPA PFAS Screening Library, we would like to have concurrence from EPA to add more assays.

Table 1. NTP Exploratory PFAS Library

CAS Registry No.	Name	Alternate Name
307-55-1	Perfluorododecanoic acid	PFDoA
2058-94-8	Perfluoroundecanoic acid	PFUnA
335-76-2	Perfluorodecanoic acid	PFDA
375-95-1	Perfluorononanoic acid	PFNA
375-85-9	Perfluoroheptanoic acid	PFHpA
307-24-4	Perfluorohexanoic acid	PFHxA
2706-90-3	Perfluoropentanoic acid	PFPeA
375-22-4	Perfluorobutyric acid	PFBA
335-77-3	Perfluorodecanesulfonate	PFDS
375-92-8	Perfluoroheptanesulfonate	PFHpS
355-46-4	Perfluorohexanesulfonate	PFHxS
2706-91-4	Perfluoropentansulfonate	PFPeS
375-73-5	Perfluorobutanesulfonate	PFBS
754-91-6	Perfluorooctanesulfonamide	PFOSA
39108-34-4	Fluorotelomer sulfonate 8:2	FtS 8:2
27619-97-2	Fluorotelomer sulfonate 6:2	FtS 6:2
2991-50-6	N-ethyl-N-((heptadecafluorooctyl)sulfonyl)glycine	NEtFOSAA
2355-31-9	N-(Heptadecafluorooctylsulfonyl)-N-methylglycine	NMeFOSAA
678-39-7	Fluorotelomer alcohol 8:2	FtOH 8:2
647-42-7	Fluorotelomer alcohol 6:2	FtOH 6:2
62037-80-3	Ammonium perfluoro(2-methyl-3-oxahexanoate)	GenX
958445-44-8	Ammonium 4,8-dioxa-3H-perfluorononanoate	ADONA
57678-01-0	6:2 fluorotelomer phosphate monoester	6:2 monoPAP
57677-95-9	6:2 Fluorotelomer phosphate diester	6:2 diPAP
57678-03-2	8:2 fluorotelomer phosphate monoester	8:2 monoPAP
678-41-1	8:2 Fluorotelomer phosphate diester	8:2 diPAP
943913-15-3	6:2/8:2 Fluorotelomer phosphate diester	6:2/8:2 diPAP
914637-49-3	5:3 Polyfluorinated acid	5:3 acid
335-67-1	Perfluorooctanoic acid	PFOA
1763-23-1	Perfluorooctanesulfonate	PFOS
801212-89-9	4-(heptafluoroisopropoxy)hexafluorobutanoic acid	PFPE-1
377-73-1	2,2,3,3-tetrafluoro-3-(trifluoromethoxy)propionic acid	PFPE-2
151772-58-6	Nonafluoro-3,6-dioxaheptanoic acid	PFPE-3
151772-59-7	Perfluoro-3,6,9-trioxadecanoic acid	PFPE-4
137780-69-9	Perfluoro-3,6-dioxadecanoic acid	PFPE-5
330562-41-9	Perfluoro-3,6,9-trioxatridecanoic acid	PFPE-6
863090-89-5	Perfluoro(4-methoxybutanoic) acid	PFPE-7
72629-94-8	Perfluorotridecanoic acid	PFTriA

Table 2. NTP Exploratory PFAS Library Assays

Endpoint of Interest	Assay
<b>Hepatotoxicity</b>	Cell viability and mitochondrial membrane potential in 2D HepaRG cells. (DNTP/NTP Laboratory)
<b>Immunotoxicity</b>	PFAS induced cytokine expression in rat and human peripheral blood lymphocytes (Contract)
<b>Placental model</b>	JEG cell viability (DNTP/NTP Laboratory)
<b>Milk protein production</b>	Examine inhibition of milk protein production in MCF-7 cells using the mammary gland model (NTP Laboratory)
<b>Renal transport</b>	Renal proximal tubule permeability assay in rats and humans (Human assay done under contract to Solvo; the rat assay is under development in DNTP/NTP Laboratory).

Table 3. EPA PFAS Screening Library Assays

Endpoint of Interest	NTP	EPA
<b>Hepatotoxicity</b>	3D HepaRG transcriptomics	
<b>Developmental toxicity</b>		Zebrafish embryo assay
<b>Immunotoxicity</b>	Human and rat cytokine alterations in peripheral blood lymphocytes	
<b>Developmental neurotoxicity</b>		Multi-electrode Array assay in neonatal cortical cells
<b>Hepatic clearance</b>	Metabolic clearance in 50 donor-pooled hepatocyte suspensions	
<b>BioSeek</b>	Cytokine alterations in human vascular endothelial cells	
<b>Plasma protein binding</b>		Serum protein binding assay using human serum
<b>Enterohepatic recirculation</b>		Qualyst B-CLEAR hepatocyte transporter assay
<b>In vitro disposition</b>	In vitro disposition in 3D HepaRG cells	