Evaluation of Per- and Poly fluoroalkyl Substances (PFAS) *in vitro* toxicity testing for developmental neurotoxicity

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Conflict of Interest Statement

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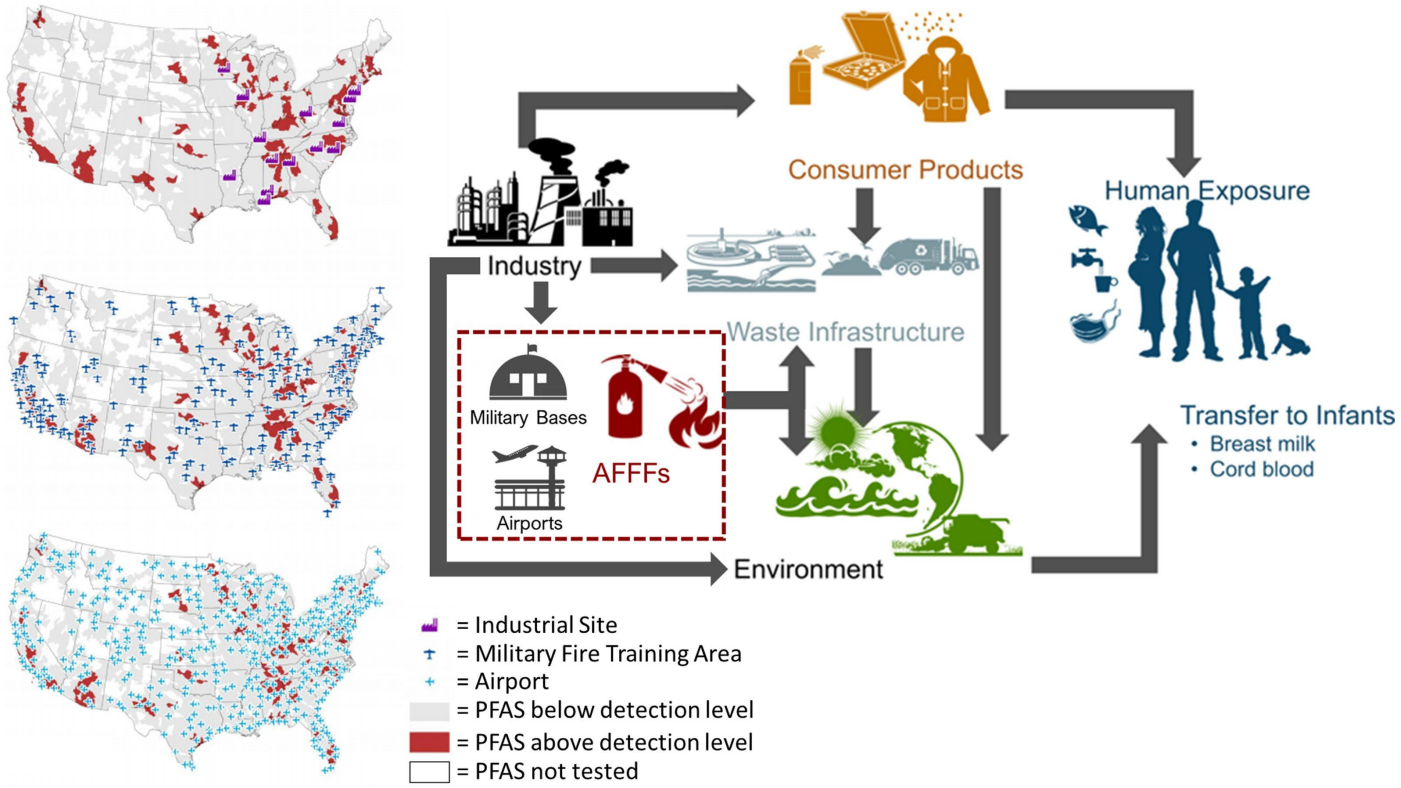
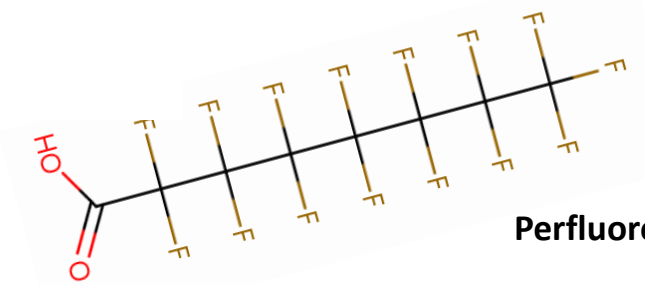
Per- and Poly fluoroalkyl Substances (PFAS)



Blake and Fenton, 2020 *Toxicology*

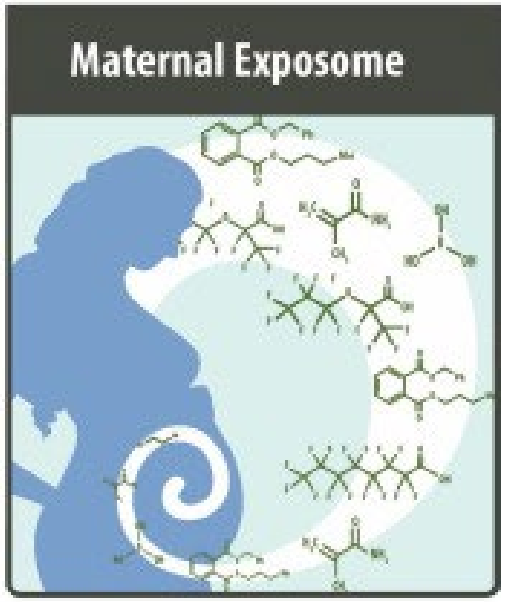
- **PFAS have widespread commercial and industrial applications**, such as water- and oil- repellents, surfactants, surface protectors, and fire-fighting foams.
- Core structure of PFAS: per- or poly- fluorinated carbon chains bonded to different functional groups (e.g. carboxylic or sulfonic acid).
- Long-chain PFAS have been identified as **highly persistent and bioaccumulative** and have been **detected in the environment, biota, and humans**.
- Only a small number of PFAS have been extensively evaluated for adverse human health potential, such as perfluorooctanoic acid (**PFOA**) and perfluorooctane sulfonic acid (**PFOS**), which are **currently under regulation internationally**.



Perfluorooctanoic acid (PFOA)

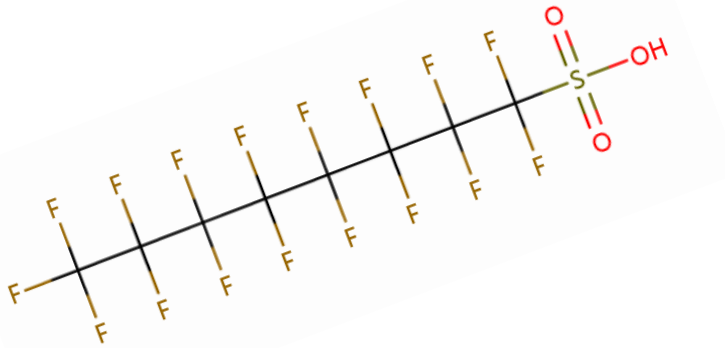
PFAS and developmental neurotoxicity (DNT)

- Little is known about the effects of PFAS exposure on the developing human nervous system.
- The **paucity of experimental data evaluating a large number of PFAS** is a discernable limitation in understanding human health effects of PFAS in the developing brain.
- Recent systematic reviews of available **human epidemiological studies** found that associations between perinatal PFAS exposure and adverse neurodevelopmental outcome are **inconsistent and inconclusive**.
- Some evidence of neurodevelopmental impairments in experimental **animal models exposed to PFOS and PFOA**, however findings are **conflicting**.
- Given the challenges of interpreting the DNT potential of PFAS from human and animal studies, **alternative approaches are needed** to evaluate the effects of PFAS exposure on adverse neurodevelopmental outcomes.



Rager et al., 2020 *Repro Tox*

This novel analysis demonstrates the power of using high-throughput screening and computational approaches to evaluate a large number of PFAS screened in a multi-dimensional battery of DNT new approach methods (NAMs).

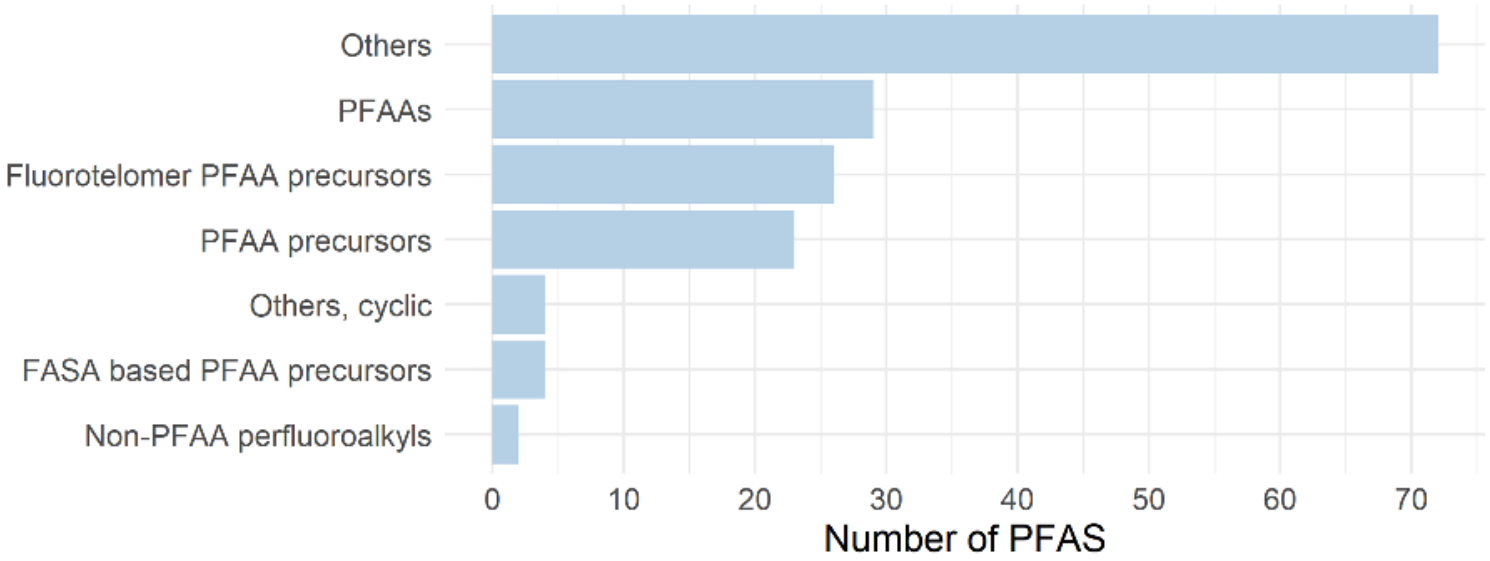


Perfluorooctanesulfonic acid (PFOS)

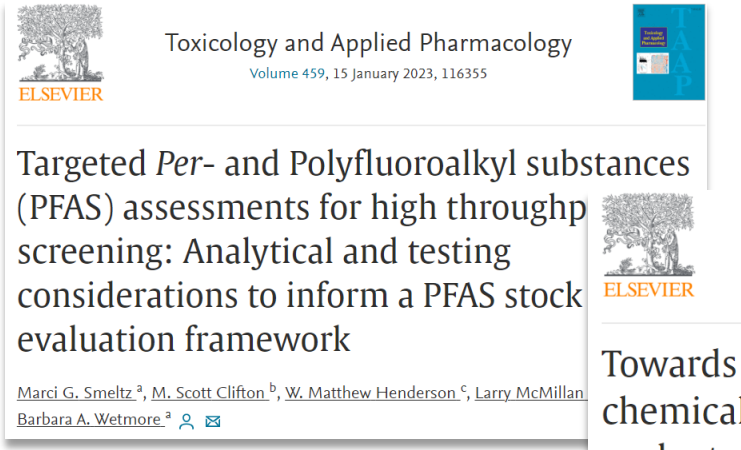
PFAS chemical selection

- 160 PFAS were screened in the DNT NAMs
- PFAS were prioritized and selected for testing based on factors such as structural category, solubility in dimethyl sulfoxide (DMSO), and structural diversity to support read-across.
- PFAS were selected prior to and after analytical quality control (QC) testing.

PFAS representing PFAS-Map OECD structural categories*



PFAA: perfluoalkyl acids



Complete list of PFAS:
https://comptox.epa.gov/dashboard/chemical-lists/EPAPFASINV

References:

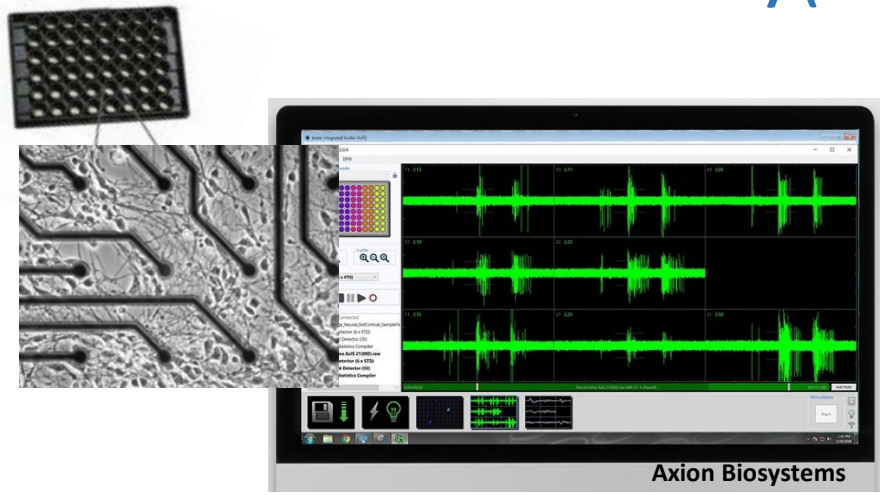
*Su and Rajan. (2021) A database framework for rapid screening of structure-function relationships in PFAS chemistry. *Scientific data.*

*OECD/UNEP, Reconciling Terminology of the Universe of Per- and Polyfluoroalkyl Substances: Recommendations and Practical Guidance. *Organisation for Economic Co-operation and Development*

PFAS screening approach in the DNT NAMs

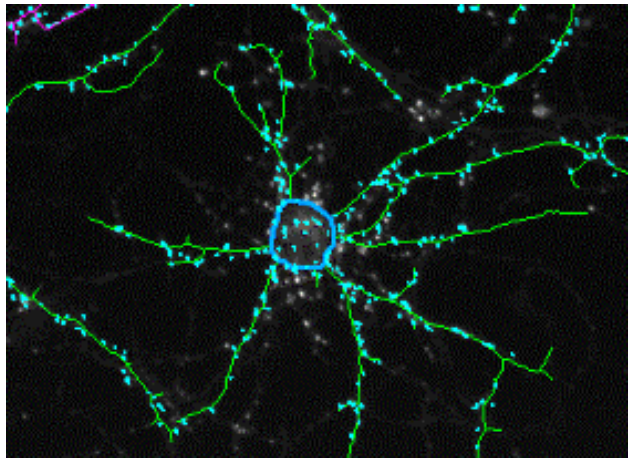
160 PFAS were screened in single-concentration or multi-concentration across four DNT NAMs (neural network formation assay, neurite outgrowth, proliferation, and apoptosis) using two assay technologies.

Microelectrode array (MEA) network formation assay (NFA)



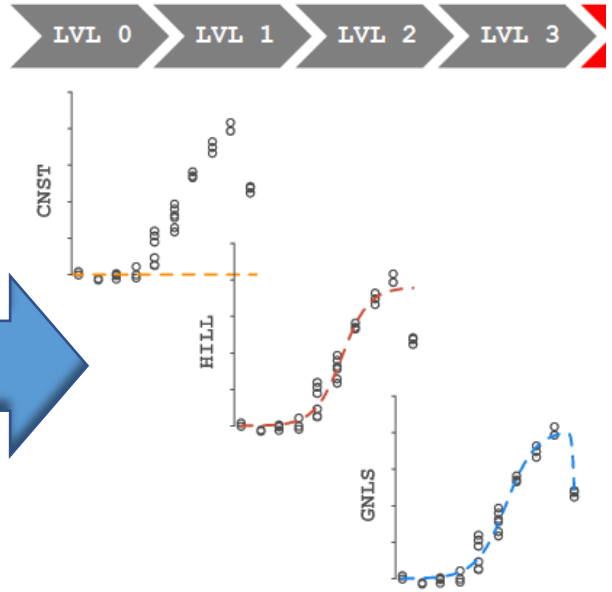
Activity type	Cell Culture
General activity	Primary rat cortical neurons Days in vitro (DIV): 5, 7, 9, 12
Network connectivity	
Bursting	
Oscillatory	
Cytotoxicity	

High-content imaging



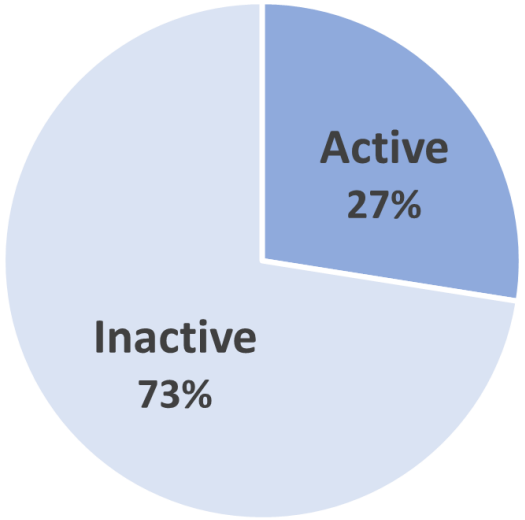
Assays	Cell culture
Neurite Outgrowth (NOG)	Primary rat neurons, human hN2 neural cells
Proliferation	Human hNP1 neuroprogenitors
Apoptosis	Human hNP1 neuroprogenitors

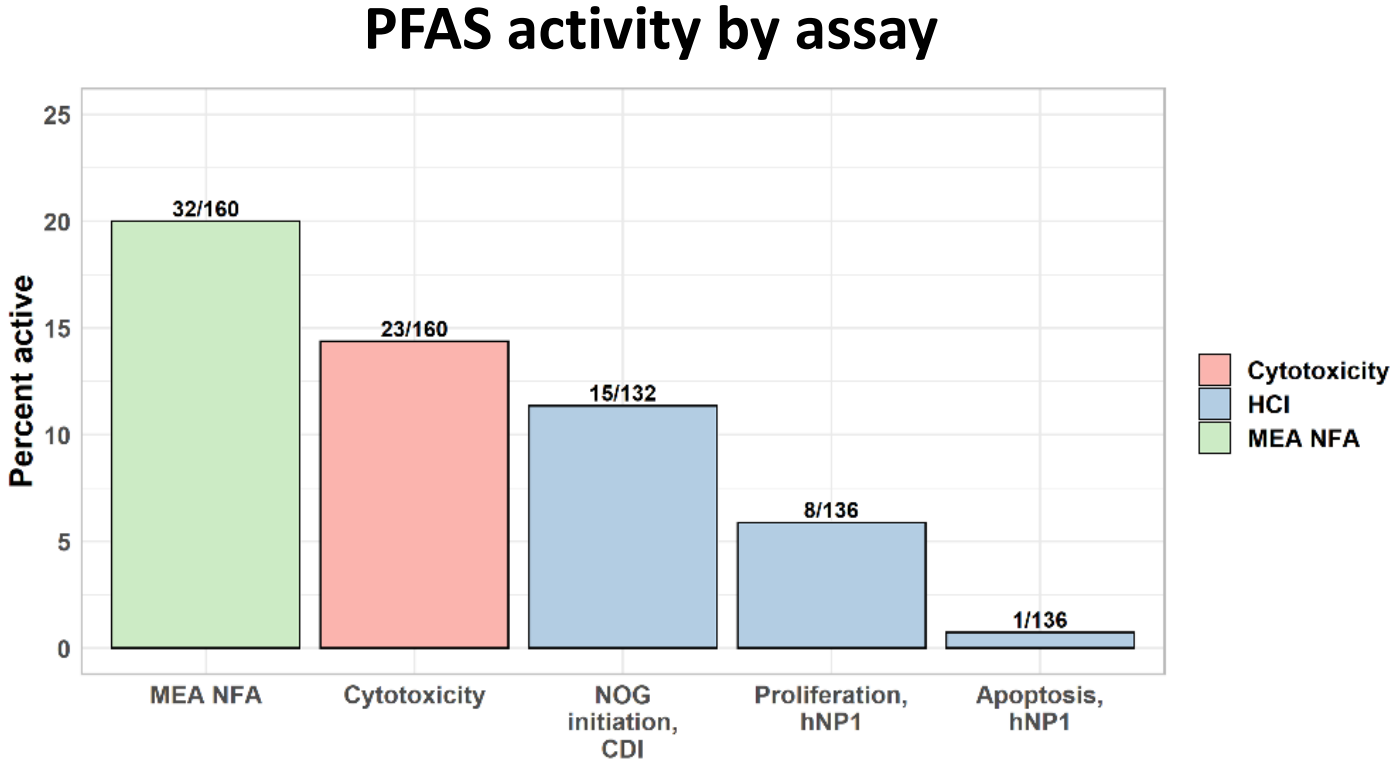
ToxCast Pipeline



The majority of PFAS were inactive in the DNT NAMs battery

Out of a set of 160 PFAS, 118 were inactive, leaving 42 active PFAS that decreased measures of neural network formation, neurite outgrowth, proliferation, or apoptosis.

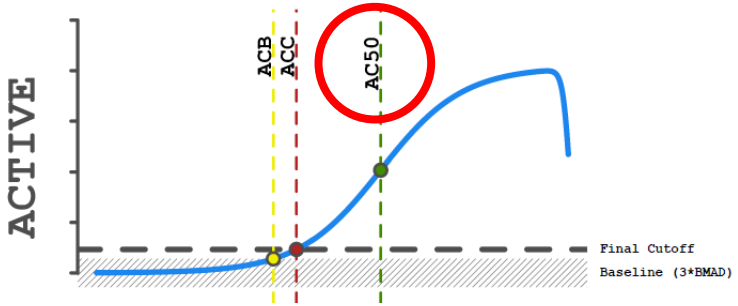


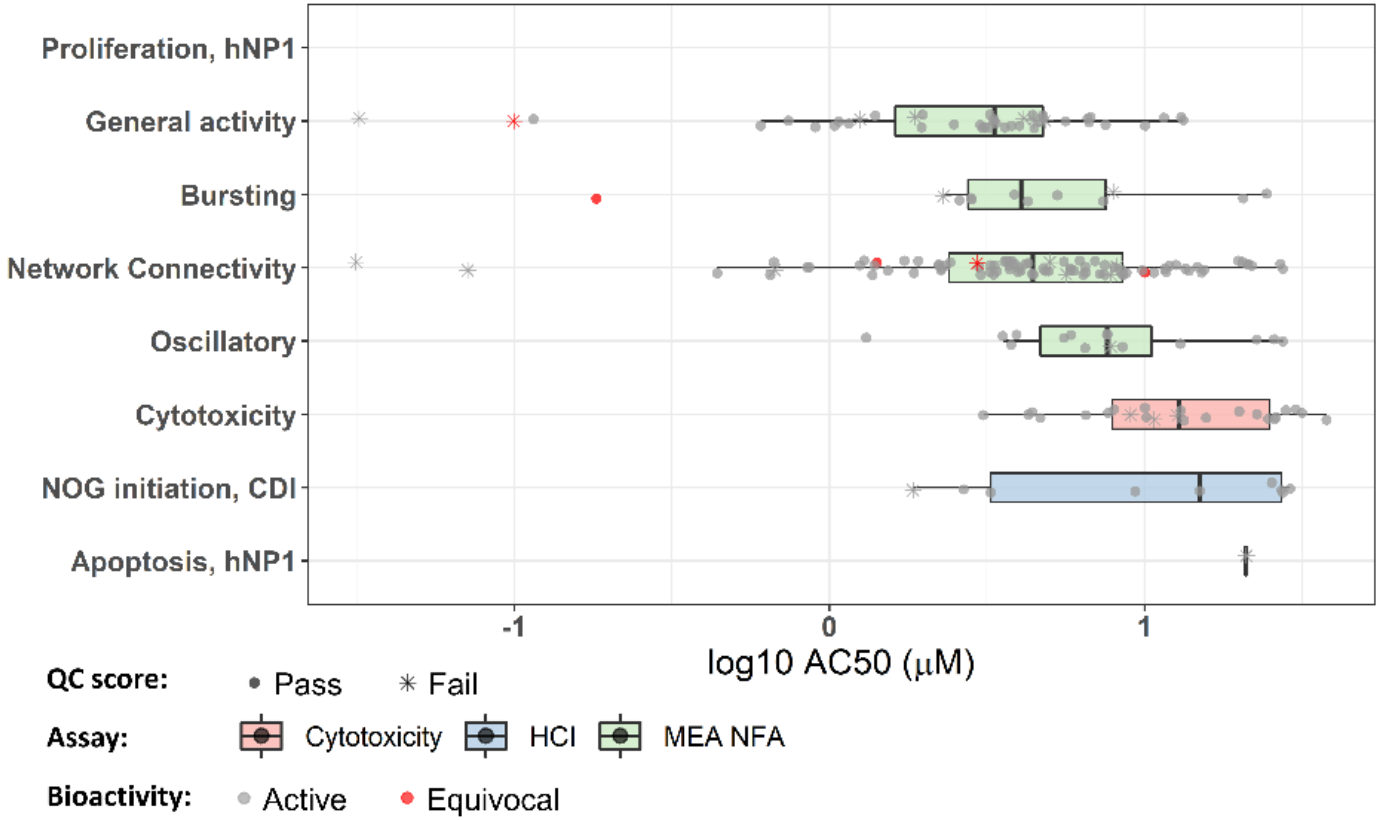


Carstens et al., 2023.

PFAS demonstrated the most potent activity in the MEA NFA compared to other DNT NAM assays

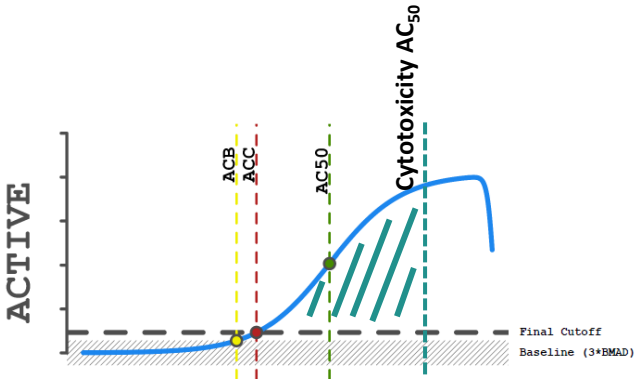
Potency: concentration at 50% maximal activity ($\mathbf{AC_{50}}$)



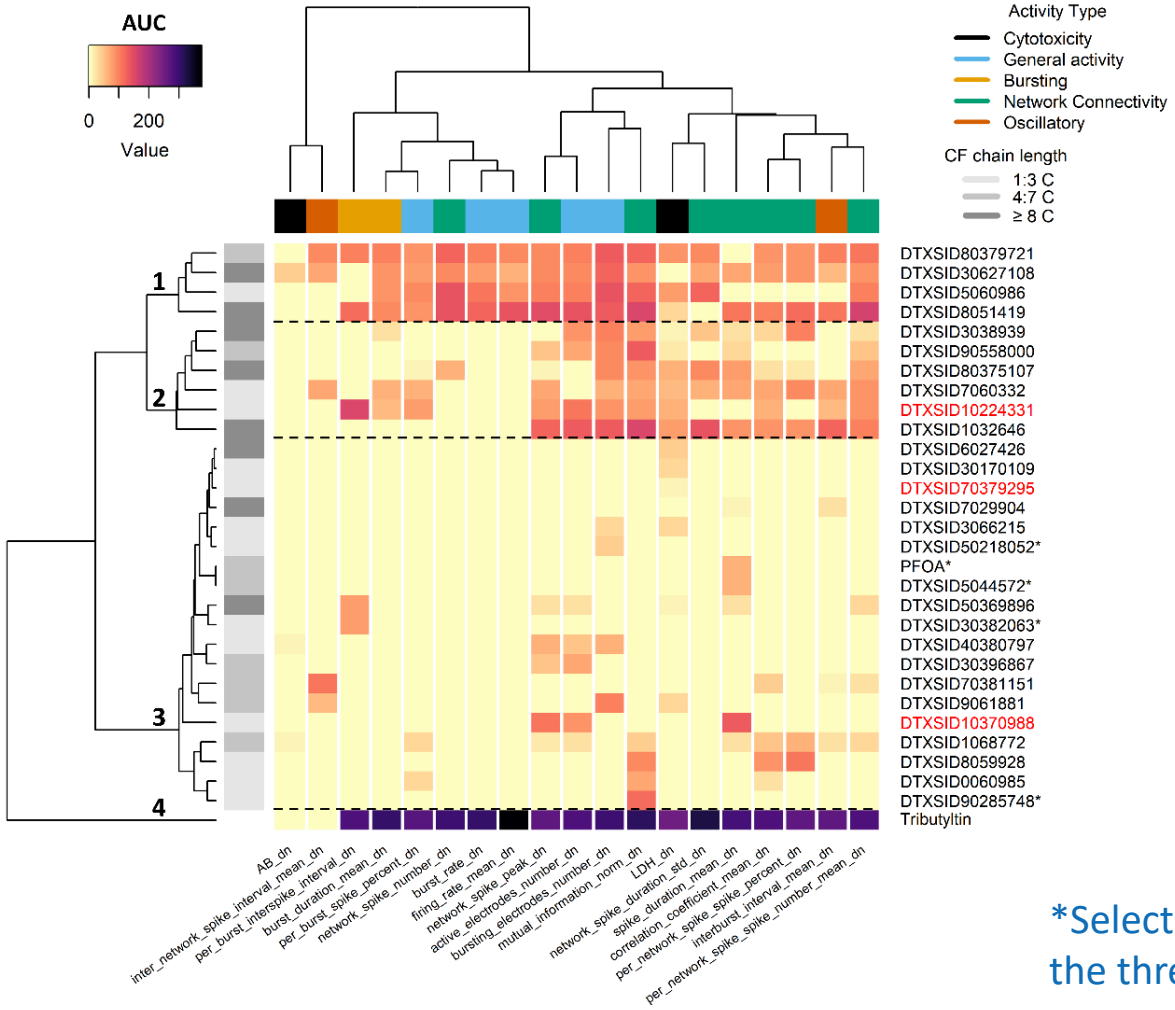


Carstens et al., 2023.

Out of 116 PFAS screened in multi-concentration MEA NFA, 24 PFAS demonstrate moderate or low selective activity*



Area under the curve (AUC)



Color key: AUC (yellow= no selective activity)

Rows: PFAS DTXSID's and tributyltin, an *in vivo* DNT reference positive. Red font indicates QC fails and * indicates equivocal activity.

Columns: MEA NFA endpoints

Row label bar (left): Perfluorinated carbon (CF) chain length.

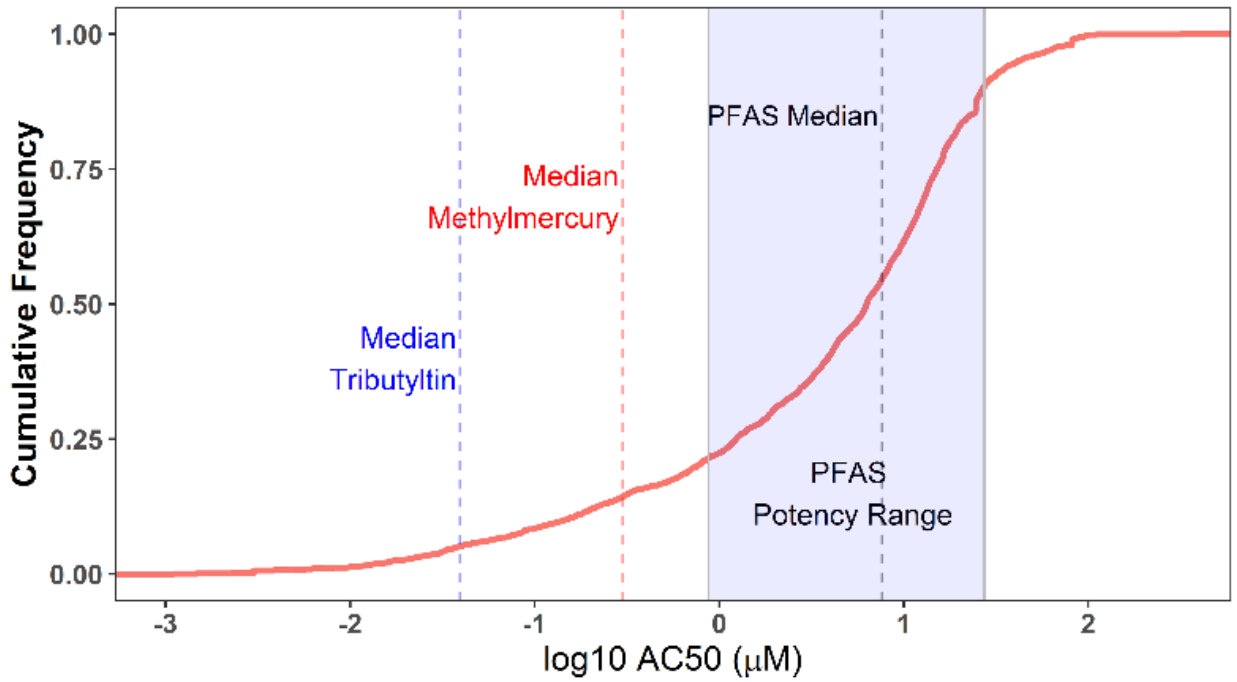
Column label bar (top): Types of neuronal activity

*Selective activity: activity below the threshold of cytotoxicity

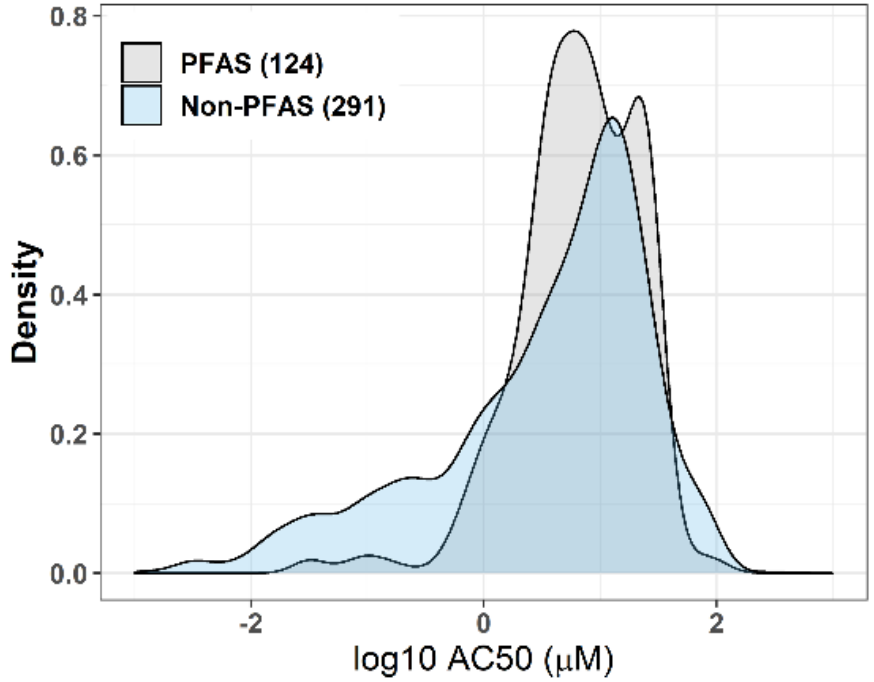
Carstens et al., 2023.

The median PFAS potency was near the median potency of all chemicals screened in the DNT NAMs and less potent than two neurodevelopmental toxicants

Cumulative density distribution of DNT NAMs potency (AC_{50})



Density Plot of DNT NAMs potency (AC_{50})

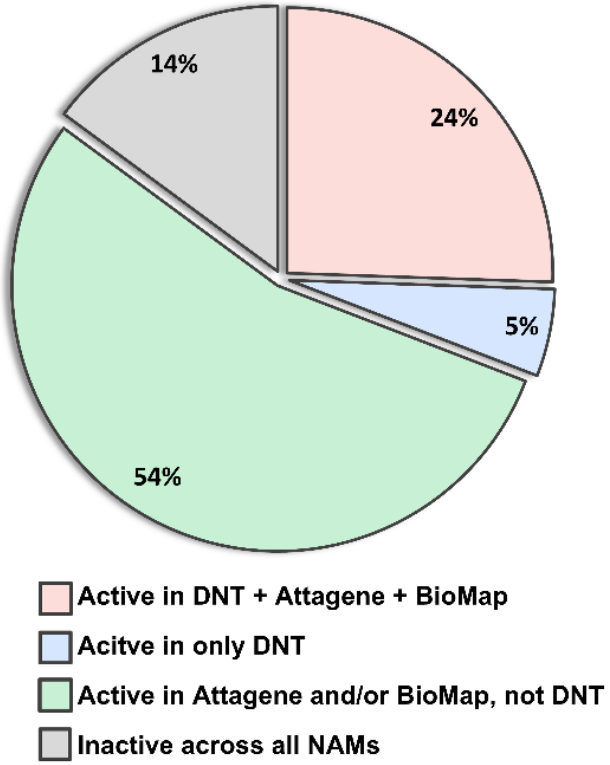


Carstens et al., 2023.

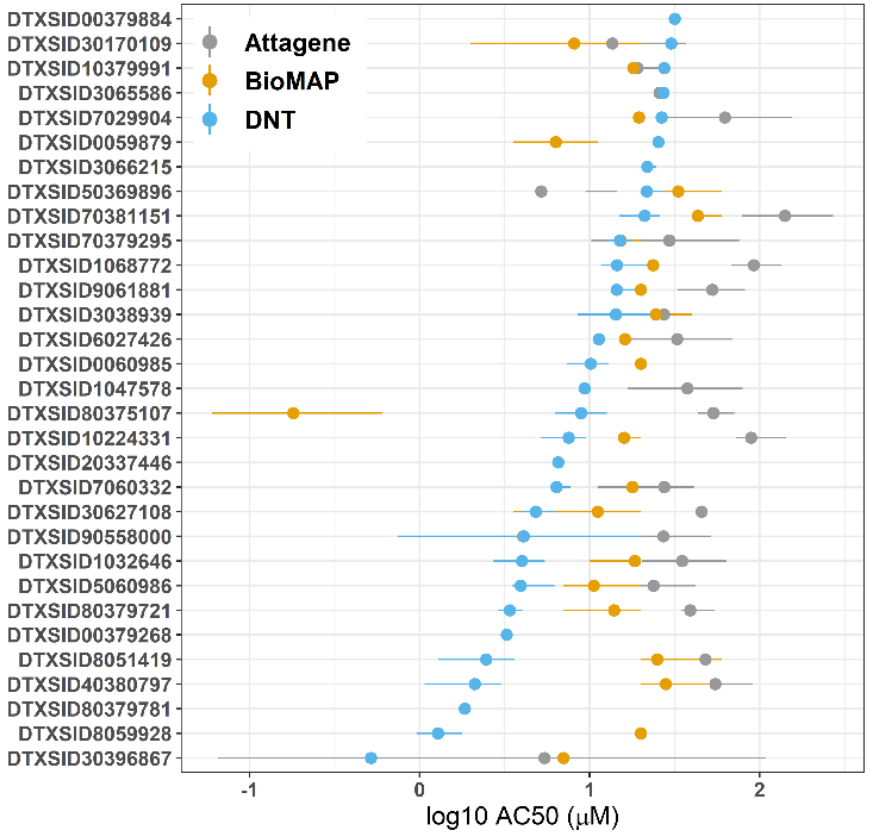
Active PFAS in the DNT NAMs were also active in other NAMs* in the ToxCast screening program

***BioMAP® Diversity PLUS panel:** phenotypic screening platform of primary human cells comprised of diverse tissue and organ systems (particularly vascular and immune biology)

***Attagene cis-Factorial and trans-Factorial assays:** transcriptional factor activity (cis-Factorial) or transfected nuclear receptor activity (trans-Factorial) in HepG2 cells



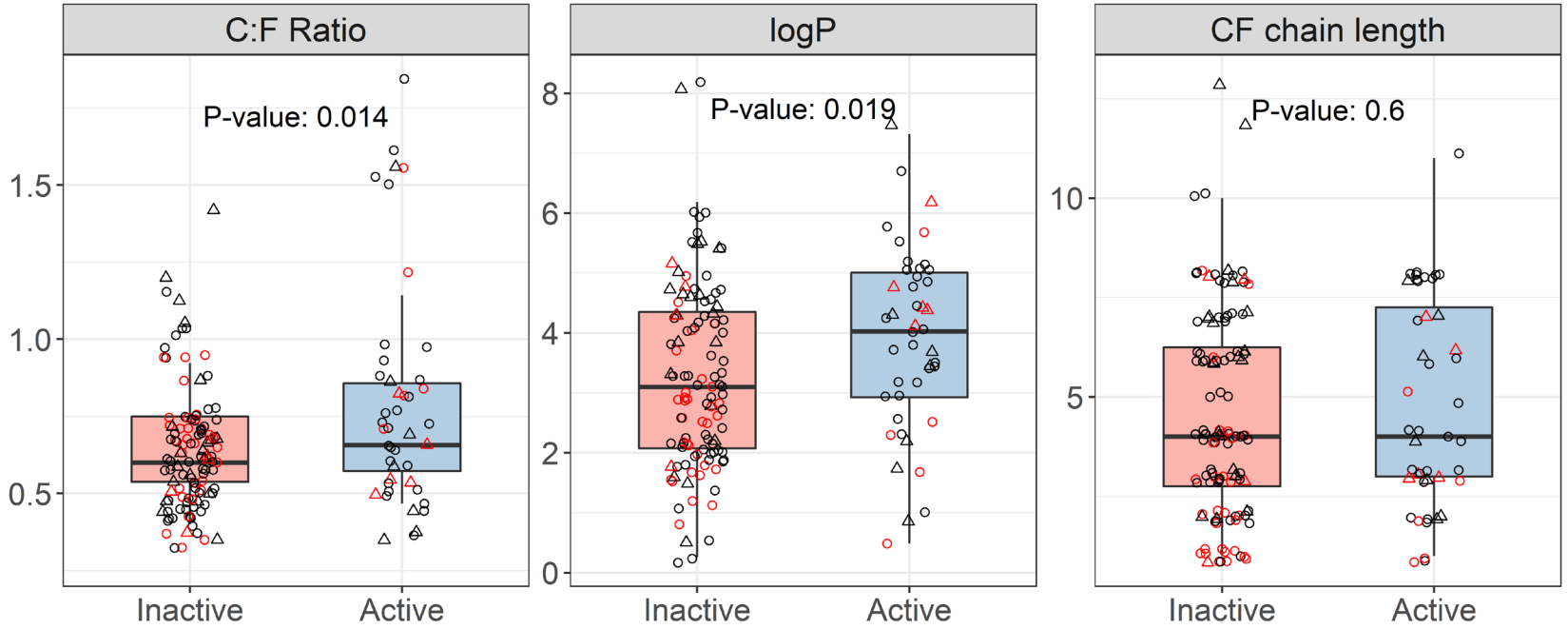
PFAS potency in the DNT NAMs versus BioMAP and Attagene assays



Carstens et al., 2023.

Physicochemical properties and structure feature descriptors

The **carbon: fluorine ratio** and **octanol: water partition coefficient (logP)** were increased in active PFAS in the DNT NAMs battery.



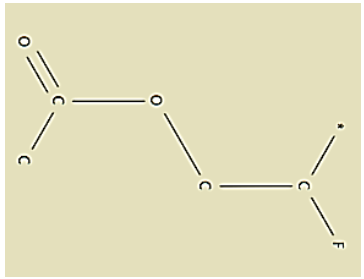
Carstens et al., 2023.

An initial set of 34 **PFAS-specific ToxPrints** categories were constructed (Patlewicz et. al, in prep) from combinations of the public set of 729 ChemoType ToxPrints (Yang et al., 2015)

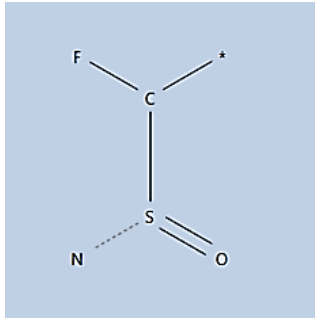
PFAS-specific ToxPrints

PFAS_perFoctyl

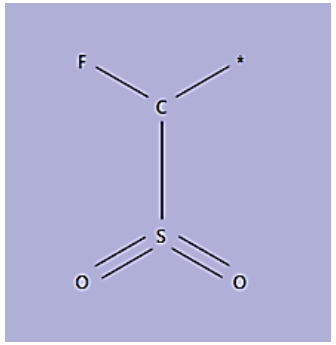
PFAS_acrylate



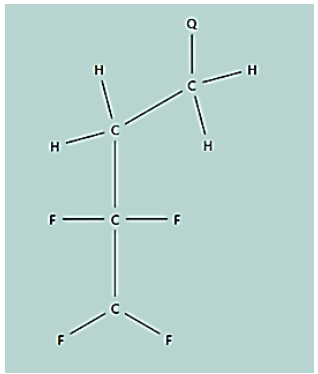
PFAS_sulfonamide



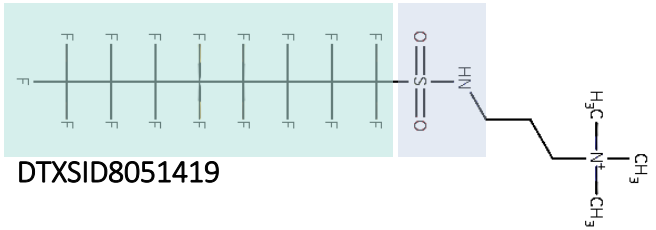
PFAS_sulfonyl



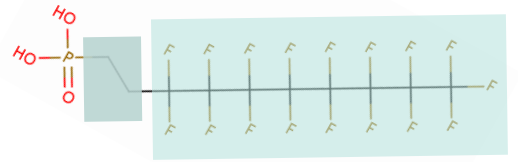
PFAS_oxydehydroxy



Top 4 most active PFAS in the DNT NAMs



DTXSID8051419



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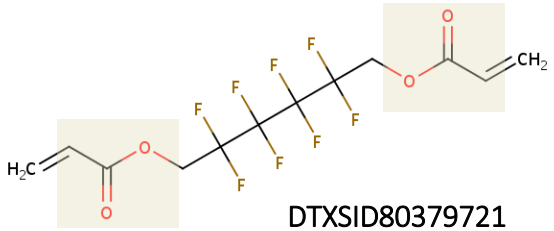

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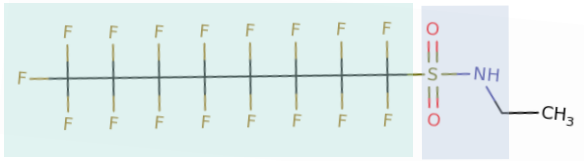
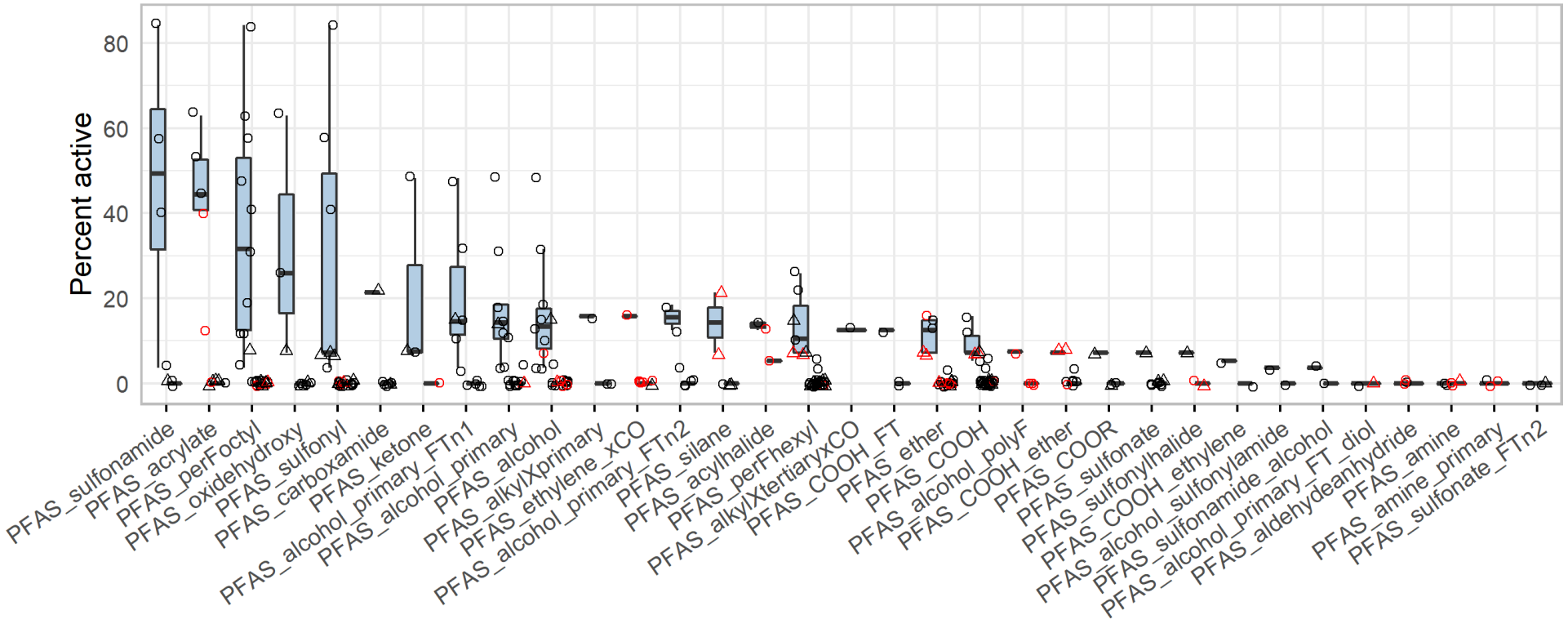


DTXSID1032646

https://comptox.epa.gov/dashboard/
https://chemotyper.org/

PFAS-specific ToxPrints reveal elevated DNT NAMs bioactivity in PFAS containing a subset of chemotypes

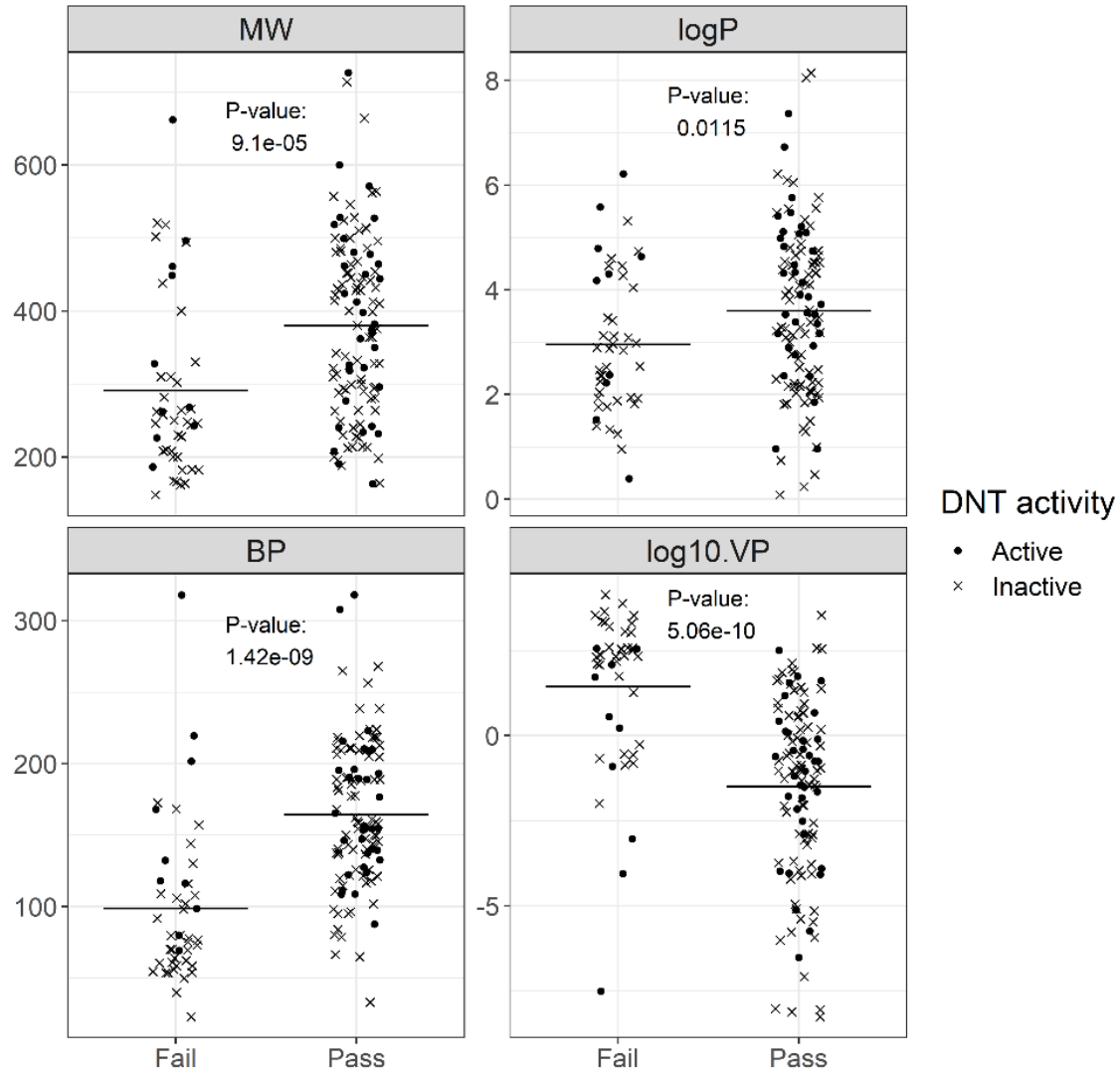


One ToxPrint containing a **carboxylic acid moiety** was enriched by significance in active PFAS compared to inactive PFAS in the DNT NAMs.

Carstens et al., 2023.

Analytical quality control (QC) for PFAS

- Analytical QC performed on PFAS stock solutions in DMSO indicated that 43/118 inactive PFAS failed QC testing, as such inactive PFAS may be explained by samples that were unstable or not amenable to screening in aqueous assay technologies.
- Of the 42 active PFAS, 10 failed QC testing; bioactivity may have resulted from uncharacterized metabolites and/or degradants.
- PFAS that failed QC typically demonstrated lower values for molecular weight, logP, and boiling point, as well as a higher vapor pressure, compared to PFAS that passed QC.



Carstens et al., 2023.

Conclusions

- A set of 160 PFAS representing distinct structural categories were largely inactive in the DNT NAMs and a small subset of PFAS demonstrated relatively low efficacy and high potency.
- We found that the majority of DNT NAMs-active PFAS were also active in other NAMs and that very few PFAS were *only* active in the DNT NAMs.
- Findings support recent studies that report an association between PFAS containing carboxylic acid or sulfonamide functional groups and elevated toxicity potential.
- Additional screening including PFAS representing overlapping and diverse functional groups and analysis of toxicokinetic parameters will be important for improving the interpretation of the DNT potential posed by these chemicals.

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Assay data:
Available in ToxCast invitrodb v3.5
https://doi.org/10.23645/epacomptox.6062623.v8
https://comptox.epa.gov/dashboard

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