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

Kelly E. Carstens[1], Freudenrich TM[1], Wallace K[1], Choo S[2], Carpenter A[2], Richard, AM[1], Patlewicz, G[1], Wetmore BA[1], Paul Friedman K[1], Shafer TJ[1]

[1]Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, [2]ORISE Postdoctoral Research Participant

P480
SOT 2023

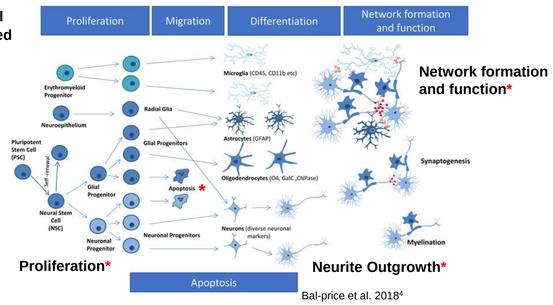
Kelly E. Carstens | Carstens.kelly@epa.gov | 919-541-3834

www.epa.gov

Introduction

- Thousands of PFAS exist in commerce however only a small number have been evaluated for adverse human health potential. Epidemiology and animal studies report conflicting evidence that PFOS or PFOA exposure may be associated with neurodevelopmental impairment.
- To provide developmental neurotoxicity (DNT) hazard information, *in vitro* singe concentration (sc) and multi-concentration (mc) screening data was generated from a battery of DNT new approach methods (NAMs) for a set of 160 PFAS[1].
- The DNT NAMs battery was comprised of the microelectrode array neuronal network formation assay (MEA NFA in rat cortical cells) and high-content imaging (HCI) assays to evaluate proliferation (human hNP1 cells), apoptosis (human hNP1 cells), and neurite outgrowth (NOG in human iCell Gluta cells). Data were curve-fit using the ToxCast Pipeline (tcpl)[2].
- Analytical quality control (QC) testing of solubilized stock samples was conducted with mass spectrometry. Chemicals were given a pass/ fail grade depending on the detection of the parent compound[3].

***Neurodevelopmental processes represented by assays in the EPA DNT NAMs battery**

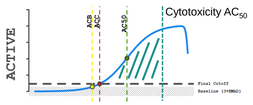


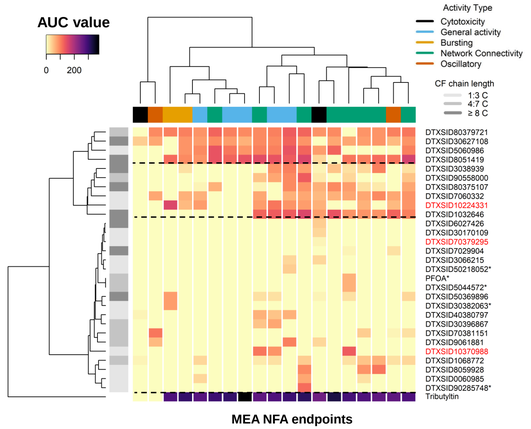
Network formation and function*

Bal-price et al. 2018[4]

24 PFAS demonstrate moderate or low selective activity.

Selective activity: area under the curve (AUC) below the threshold of the cytotoxicity AC_{50}.





Color key: AUC (yellow= no selective activity)

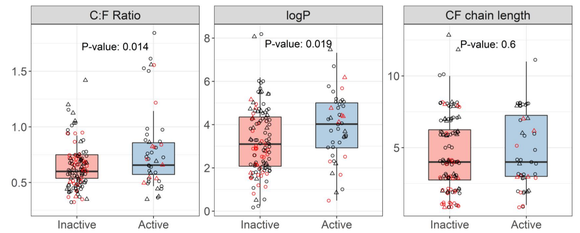
Rows: PFAS DTXSID's and tributyltin, an *in vivo* DNT reference positive. Red font indicates QC fails and * indicates equivocal activity.

Columns: MEA NFA endpoints

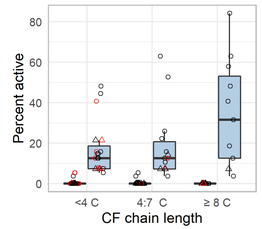
Row label bar (left): Perfluorinated carbon (CF) chain length.

Column label bar (top): Types of neuronal activity

Active PFAS have increased carbon: fluorine ratio and logP.



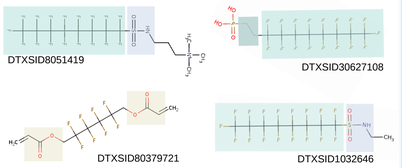
Perfluorinated carbon (CF) chain length ≥ 8 C may demonstrate elevated DNT NAM activity.



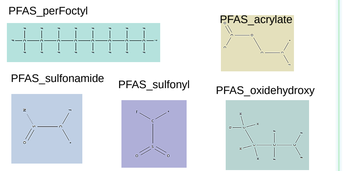
PFAS-specific ToxPrints reveal elevated DNT NAMs bioactivity in PFAS containing a subset of chemotypes.

A preliminary set of 34 PFAS-specific ToxPrints were constructed from combinations of the public set of 729 ToxPrints. A more expansive set of PFAS-specific ToxPrints is publicly available[6].

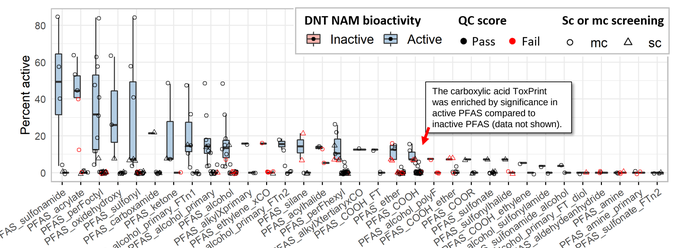
Top 4 most active PFAS in the DNT NAMs



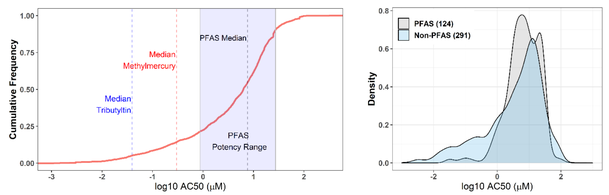
PFAS-specific ToxPrints



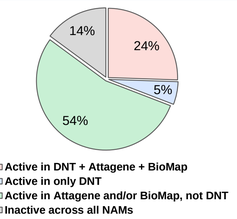
Bioactivity of PFAS chemicals containing each PFAS-specific ToxPrint (x-axis).



Active PFAS are less potent than two known neurodevelopmental toxicants.

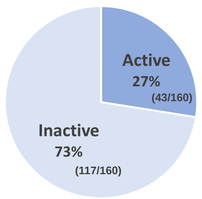


DNT NAMs-active PFAS were also active in other NAMs[7] .

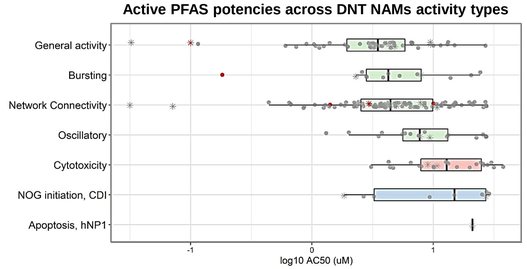


The majority of PFAS were inactive in the DNT NAMs battery.

PFAS Bioactivity



Active PFAS potencies across DNT NAMs activity types



❖ Endpoints measuring decreased 'general activity' in the MEA NFA were the most sensitive.

❖ Endpoints measuring apoptosis and proliferation (no actives) were the least sensitive.

Assay: Cytotoxicity HCI MEA NFA
Analytical QC score: Pass Fail
Bioactivity*: Active Equivocal

Bioactivity results were determined by hit-call thresholding in tcpl. An active response was defined as activity exceeding ± 3 baseline median absolute deviation. An equivocal was defined as low confidence in concentration response modeling[5].

Summary and Future Directions

- A subset of 160 PFAS, representing distinct PFAS-Map OECD structural categories, were largely inactive in the DNT NAMs and a subset of PFAS demonstrated relatively high potency and low efficacy.
- The majority of DNT NAMs-active PFAS were also active in other NAMs, suggesting may not demonstrate neural specific effects.
- Analytical QC testing indicated that 35/116 inactive samples and 10/44 active samples did not pass QC, as such careful interpretation is required. Some negatives may have been due to loss of the parent PFAS and some actives may have resulted from a mixture of parent and/or degradants of PFAS.
- PFAS containing ≥8 perfluorinated carbons and/or functional groups such as sulfonamides, carboxylic acid, and acrylates may be associated with elevated DNT potential.

Conclusion: This analysis demonstrates the power of using NAMs and computational approaches to evaluate trends in DNT bioactivity and PFAS chemical and structure feature descriptors. The current findings will help EPA prioritize which PFAS characteristics are of the highest concern for DNT potential.

Future Direction: Additional screening including PFAS chemicals containing ToxPrints that are currently underrepresented will be important for improving the interpretation of the DNT potential posed by specific structure feature descriptors.

References:
1: Patlewicz et al. (2019). A Chemical Category-Based Prioritization Approach for Selecting 75 Per- and Polyfluoroalkyl Substances (PFAS) for Tiered Toxicity and Toxicokinetic Testing. *Environ. Health Perspect.*
2: Filer et al. (2017). Tcpl: the ToxCast pipline for high-throughput screening data. *BioInformatics.*
3: Smeltz et al. (2022) Targeted Per- and Polyfluoroalkyl substances (PFAS) assessments for high throughput screening: Analytical and testing considerations to inform a PFAS stock quality evaluation framework. *Toxicol. Appl. Pharmacol.*
4: Bal-Price et al. (2018). Recommendation on test readiness criteria for new approach methods in toxicology: Exemplified for developmental neurotoxicity. *Altex.*
5: Carstens et al. (2023). Evaluation of Per- and Poly fluoroalkyl Substances (PFAS) *in vitro* toxicity testing for developmental neurotoxicity. *Chem. Res. Toxicol.*
6: Richard et al. (2023). A New CSRML Structure-Based Fingerprint Method for Profiling and Categorizing Per- and Polyfluoroalkyl Substances (PFAS). *Chem. Res. Toxicol.*
7: Houck et al. (2022) Evaluation of 147 perfluoroalkyl substances for immunotoxic and other (patho)physiological activities through phenotypic screening of human primary cells. *Altex.*