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

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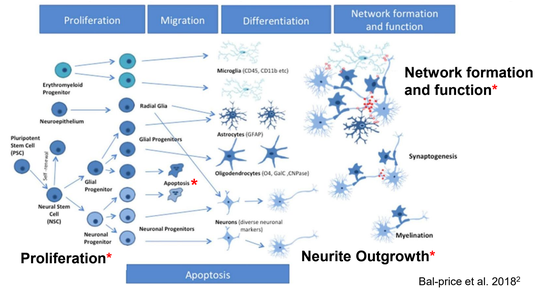
Kelly E. Carstens | Carstens.kelly@epa.gov | 919-541-3834

www.epa.gov

Introduction

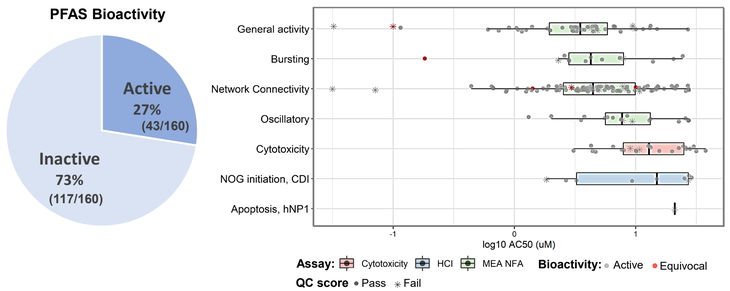
- Thousands of PFAS exist in commerce however only a small number have been evaluated for adverse human health potential. Epidemiology and animal studies report conflicting evidence that PFOS or PFOA exposure may be associated with neurodevelopmental impairment.
- To provide developmental neurotoxicity (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 (Patlewicz et al. poster PTh-33 for PFAS selection).
- 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 FCDI GlutaNeurons). Data were curve-fit using the ToxCast Pipeline (tcpl)[1].
- Analytical quality control (QC) testing indicated that 35/116 inactive samples and 10/44 active samples did not pass QC (the parent compound failed to be detected).

Neurodevelopmental processes represented in the EPA DNT NAMs battery*



Bal-price et al. 2018[2]

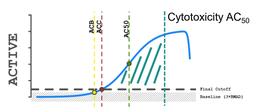
The majority of PFAS were inactive in the DNT NAMs battery.



- Endpoints measuring decreased 'general activity' in the MEA NFA were the most sensitive.
  - Endpoints measuring apoptosis and proliferation (no actives) were the least sensitive.

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

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



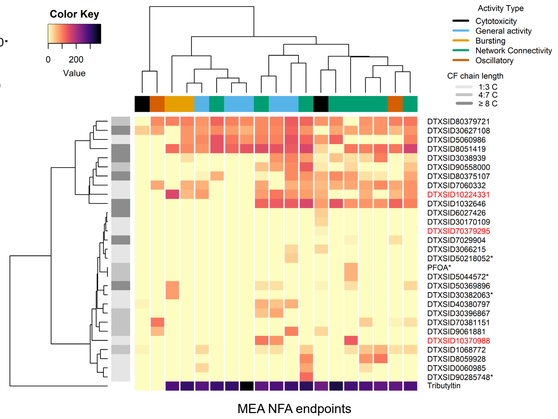
Color key: AUC (yellow= no selective activity)

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

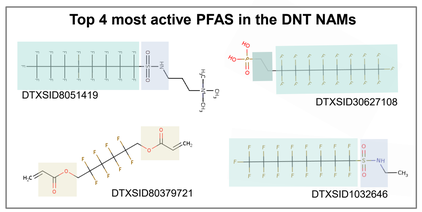
Columns: MEA NFA endpoints

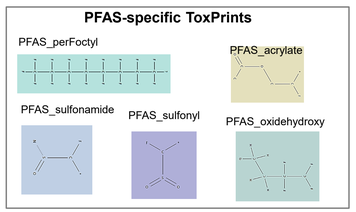
Row label bar (left): Perfluorinated carbon (CF) chain length.

Column label bar (top): Types of neuronal activity

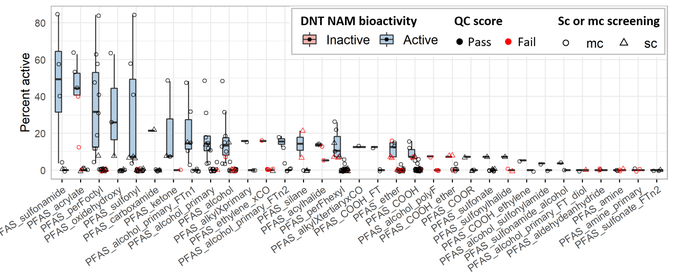


PFAS-specific ToxPrints reveal elevated DNT NAMs bioactivity in PFAS containing a subset of chemotypes.



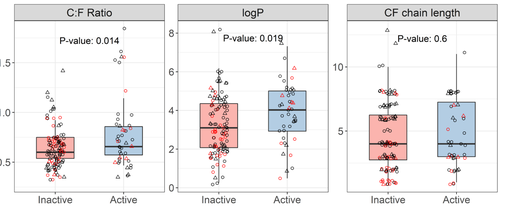


A preliminary set of 34 PFAS-specific ToxPrints[3] were constructed from combinations of the public set of 729 ToxPrints. A more expansive set of PFAS-specific ToxPrints is currently under development and will be made publicly available[4].

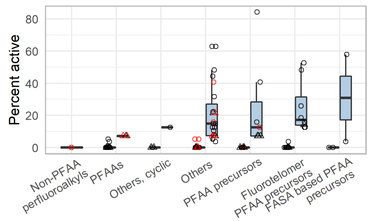


This poster does not reflect US EPA policy.

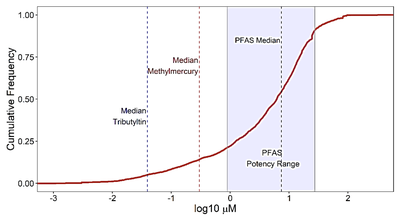
The carbon: fluorine ratio and logP were increased in active PFAS.



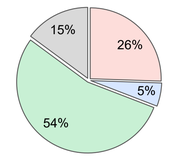
PFAS-Map OECD structure categories[5] did not reveal any clear trends in DNT NAMs bioactivity.



Active PFAS are less potent than two neurodevelopmental toxicants.

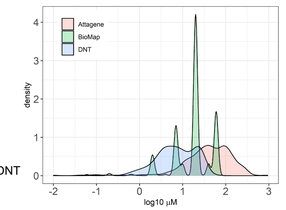


DNT NAMs-active PFAS were also active in other NAMs[6] .



- Active in DNT + Attagene + BioMap
- Acitve in only DNT
- Active in Attagene and/or BioMap, not DNT
- Inactive across all NAMs

DNT NAM potencies were decreased relative to other NAMs.



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 and the DNT NAM potencies were decreased relative to other NAMs, which may be explained by longer exposure durations or repeated dosing in the DNT NAMs.
- PFAS containing ≥8 perfluorinated carbons and/or functional groups such as sulfonamides or 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 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: Filer et al., (2017). Tcpl: the ToxCast pipline for high-throughput screening data. *BioInformatics*.
2: Bal-Price et al., (2018). Recommendation on test readiness criteria for new approach methods in toxicology: Exemplified for developmental neurotoxicity. *Altex*.
3: Patlewicz et al. (2022). In Preparation.
4: Richard et al. (2022). In Preparation.
5: Su and Rajan. A database framework for rapid screening of structure-function relationships in PFAS chemistry. *Scientific data*.
6: Houck et al., (2022) Evaluation of 147 perfluoroalkyl substances for immunotoxic and other (patho)physiological activities through phenotypic screening of human primary cells. *Altex*.

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