Neurotoxicity of Perfluoroalkyl Substances (PFAS) Mixtures in Firefighting Materials

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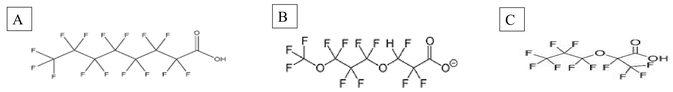
INTRODUCTION

- Perfluoroalkyl substances (PFAS) are synthetic compounds that are composed of fluorinated carbon chains.
- These compounds are used in wide range of applications due to oil, water, stain resistant properties such as clothing, carpets, fabrics for furniture, adhesives, paper packaging for food, and heat-resistant/non-stick cookware.
- The carbon-fluoride bond is very stable, thus PFAS are bioaccumulative in organisms and persistent in the environment.
- PFAS exposure is linked with many adverse health effects such as liver diseases, thyroid hormone disruption, decrease immune response and increase cholesterol levels[1,2].
- PFAS is detected in the blood of the general population but firefighters and first responders are at higher risk of exposure to PFAS[3,4].
- Aqueous Film Firefighting Foam (AFFF) contains PFAS mixtures due to their effectiveness in extinguishing fires especially fuel fire. Also, the turnout gear contains PFAS as a water repellent agent[5].
- Firefighters can be exposed to PFAS through inhaling combustion products of PFAS-containing consumer products such as carpets and furniture during firefighting or drinking PFAS-contaminated water.
- The concerns of PFAS toxicity led to voluntarily phasing out of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by their manufacturer.
- New AFFF formulation contain shorter chain PFAS such as GenX (6 carbon) and ADONA (7 carbon). These chemicals have very limited toxicological data.
- Many animal studies support potential neurological effects associated with PFAS exposure, focusing on PFOS and PFOA [6,7].

OBJECTIVES

- Assessing the developmental neurotoxicity (DNT) of GenX or ADONA using zebrafish (*Danio rerio*) as an animal model.
- Assessing effect of GenX and ADONA on the development of dopaminergic pathway.
- Assessing DNT of GenX /PFOA mixture and ADONA/PFOA mixture.



Chemical Structure of (A) PFOA, (B) ADONA, (C) GenX

RELEVANCE To NORA

This study will identify potential adverse effects of GenX, ADONA and PFOA exposures. This aim is in line with the goals of the national occupational research agenda (NORA) public safety sector. This sector aims to reduce public safety workers' hazardous exposure and to set standards for their protective clothing and equipment standard.

ZEBRAFISH AS AN ANIMAL MODEL

Why use Zebrafish:

- Epidemiological studies weren't conclusive in terms of linking PFAS exposure to neurobehavior and neurological diseases[1].
- The concern about GenX and ADONA is relatively recent, so there is a big need to characterize the risk of those chemicals using animal models.

Advantages of Zebrafish:

- The zebrafish has a sequenced genome and 80% homology of genes associated with human disease[8].
- The zebrafish is a well-established model to study DNT and neurobehavior[9].

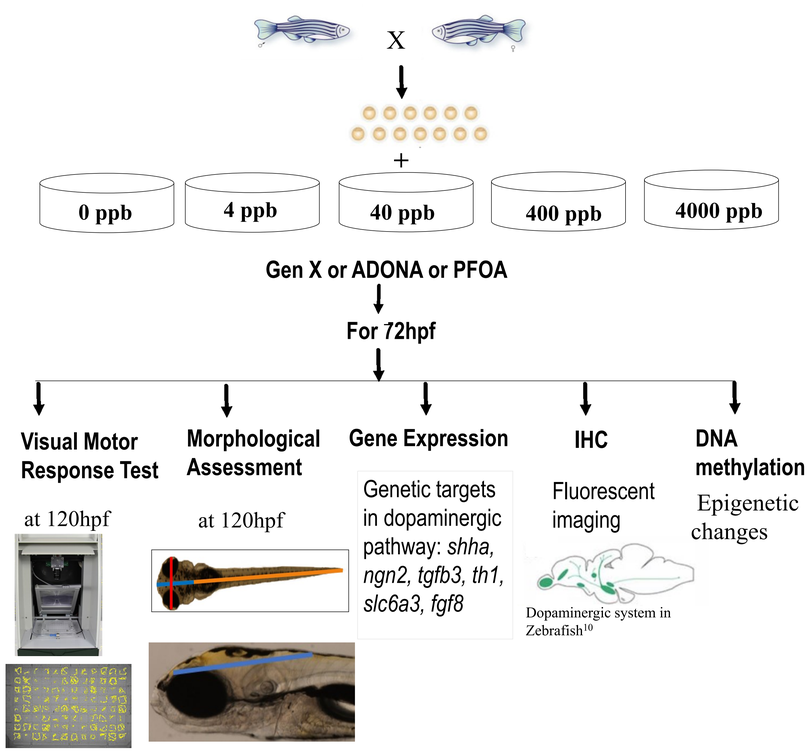
EXPERIMENTAL DESIGN

Breeding:

- Adult fish are bred in spawning tanks
- Embryos will be collected immediately after fertilization
- 50 embryos/ petri dish

DNT assessment of single chemical exposures:

1- Locomotor Activity Assessment
2- Morphological Assessment
3- Gene Expression
4- Immunohistochemistry (IHC)
5- loci-specific DNA methylation analysis



EXPERIMENTAL DESIGN (CONTINUED)

DNT assessment of binary chemical exposures:

Same set of experiments using GenX and PFOA or ADONA and PFOA mixtures. Concentrations of each chemical will be determined based on the results of single chemical exposures.

EXPECTED RESULTS

We are expecting that:

- GenX or ADONA exposure will result in a decrease in locomotor activity compared to PFOA.
- Gene expression of genes in the dopaminergic pathway will be affected by GenX and ADONA.
- Gene expression alterations are associated with changes in DNA methylation.
- Number of dopaminergic neurons will decrease in GenX and ADONA exposures.
- GenX/PFOA or ADONA/PFOA exposures will results in synergistic effect compared to single chemical exposure.
- **Research to practice potential :** results of this study will emphasize importance of cleaning turnout gear, and proper handling of AFFF waste. It also may influence changes of the AFFF formulation and standards for turnout gear.

FUTURE DIRECTIONS

- Long term effect of GenX and ADONA exposure as well as GenX/PFOA and ADONA/PFOA will be assessed by assessing the same endpoints on adult fish.
- The findings of this project will be used as preliminary data for a future R01 proposal (targeting Oct. 2020 submission).

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