TITLE: **The EFFECTS of PFOA and PFHXS on HUMAN DEVELOPING NEURONS: AN *in vitro* STUDY.**

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INTRODUCTION: Per-fluoroalkyl substances (PFAS) are a large group of man-made chemicals that are commonly used to make Teflon cookware, water repellent clothing, stain resistant fabrics and carpets, cosmetics, fire-fighting foams, and products that are highly hydro/lipophobic (i.e., grease, oil, and water resistant. These chemicals end up in drinking water. The most common PFAS are perfluorooctanoic sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). PFAS substances are non-biodegradable and persistent in the environment and in human and animal bodies; they will stay in the body and accumulate over time in the blood. The ubiquitous nature of PFAS presents serious environmental risks. PFAS also contain endocrine disruptive properties that may cause damage to the embryo and/or fetus in utero. Studies have shown severe adverse effects in animals and humans such as immune system impairment, damage to liver, kidney and thyroid, prostate cancer, arthritis, and developmental delays. We are currently examining the effects of two perfluoroalkyl (PFAS) compounds: perfluorooctanoic acid (PFOA) and perfluoro hexanoic acid (PFHxS. To the best of our knowledge, there have been no detailed analyses of how exposures to PFOA and PFHxS effect human developing neurons. The purpose of this research is to examine how these compounds affect the developing human fetal brain neurons and their potential contributions to the occurrence of neurological diseases or disorders.

METHODS: To determine the potential adverse effects of PFAS, we utilized four fetal neuronal cell lines that represents early fetal brain neurons (i.e. female CRL-2266 and male CRL-2267 at 8 weeks gestation); (i.e. CRL-2149 female and male CRL-2142 at 24 weeks gestation). CRL 2266 and CRL 2267 can be partially differentiated via 48-hour retinoic acid (1uM) exposure. These neuroblastoma cell lines (NBC's) were exposed to PFOA and PFHXS under three different concentrations: low (0.2 uM/mL), medium (2.0 uM/mL), and high (20 uM/mL). After 48 hour incubations in humidified 5% CO2, the cells were fixed and stained with the hematoxylin and eosin (H&E). The H&E staining allowed us to observe and analyze the effects of PFHXS and PFOA for neuromodifications at morphological levels. Each concentration of cells was compared to the control, in which NBC's were not exposed to PFOA and PFHXS. We were able to carry out a morphological analysis in which central chromatolysis, axonal length, axon degeneration, and syncytia formation were examined.

RESULTS: The morphologic analyses showed significant changes in the NBC'S for both male and female origins beginning in the low concentration. There was an increased amount of chromatolysis and axon degenerations. As compared to the control, the number of cell clusters, which is an indication of cell growth, began to dwindle as the concentrations increase from low to high. There was an indication of syncytia formation and axonal elongation in both medium and

high concentrations for male and female fetal brain neurons when the NBC'S were exposed to PFOA and PFHXS.

Real Time PCR Analyses (2266 & 2267): Male and female cell lines were evaluated for the differential expressions of oxytocin receptor (OXYR) and arginine-vasopressin receptor (AVPR). Female cell line exposed to PFOA & PFHxS showed upregulation of AVPR; exposure to PFHXS upregulated OXYR; female cell lines, when exposed to PFOA, displayed both up and downregulation of OXYR. Male cell line, when exposed to PFOA & PFHXS showed upregulation of AVPR. Male cells, when exposed to PFOA and PFHxS showed significant upregulation of OXYR.

Real Time PCR Analyses (2142 & 2149): Male and female cell lines were evaluated for the differential expressions of oxytocin receptor (OXYR) and arginine-vasopressin receptor (AVPR). Female cell line exposed to PFOA and PFHxS showed downregulation of AVPR, whereas, exposure to PFOA upregulated OXYR and downregulation of OXYR in cells exposed to OXYR. Male cell line exposed to PFOA exhibited dichotomous response. Therefore, cell exposed to PFOA showed upregulation of AVPR but downregulation of AVPR in PFHxS. However, male cells exposed to PFOA and PFHxS showed significant downregulations of OXYR.

CONCLUSION: We concluded that exposures to PFOA & PFHXS at low concentrations induce significant neuro modifications in male and female fetal brain neurons. There is a possibility that PFOA & PFHXS may play a role in neurological disorders, causing damage to fetal brain cells in utero, during the early stages of gestation.

REFERENCES:

1. Trier, X., Granby, K., Christensen, J., 2011. Polyfluorinated surfactants (PFS) in paper and board coatings for food packaging. Environ. Sci. Pollut. Res. 18, 1108–1120.

2. Wang, S., Huang, J., Yang, Y., Hui, Y., 2013. First report of a Chinese PFOS alternative overlooked for 30 years: its toxicity, persistence, and presence in the environment. Environ. Sci. Technol. 47, 10163–10170.

3. Jin Heon Lee, Chae Kwan Lee, Chun-Hui Suh, Hee-Sook Kang, Choon-Pyo Hong, Suk-Nam Choi. Serum concentrations of per- and poly-fluoroalkyl substances and factors associated with exposure in the general adult population in South Korea. International Journal of Hygiene and Environmental Health. 220 (2017) 1046–1054.

4. Sealey LA, BW Hughes, A. Steinemann, JP Pestaner, DG Pace and O Bagasra. Role of Environmental Factors in Autism Development and Male Bias: Neuromodifying Effects of Fragrance. Environmental Research. 2015; Sept 23:142;731-738.

5. Sealey L.A., Hughes B.W., Sriskanda A.N., Guest J.R., Gibson A.D., Johnson-Williams L., Pace D.G., and Bagasra O. Environmental Factors in the Development of Autism Spectrum Disorders. Environment