Project Title and Scientific Abstract

Exploratory Analyses of Per- and Polyfluorinated Substances (PFAS) Exposures, Mechanisms of Immunotoxicity, and Adverse Outcome Pathway Development

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants found in over 99% of serum samples in the United States based on data from the National Health and Nutrition Examination Survey (NHANES). Exposure sources include drinking water, particularly in the vicinity of military or other sites using fire-fighting foams, consumer products, non-stick cookware, and take-out containers. Regulatory thresholds protective of public health focus on potential immunotoxicity as demonstrated from epidemiologic studies, but mechanisms are poorly understood, and most proposed regulatory values focus on individual PFAS despite there being nearly 9,000 known compounds. We propose several exploratory analyses that rely on the integration and synthesis of existing, publicly-available data and databases to explore issues around immunotoxicity and PFAS exposures to develop an adverse outcome pathway (AOP), based on NHANES biomonitoring data, human-cell line *in vitro* bioassay data from the U.S. Environmental Protection Agency's (US EPA) ToxCast computational toxicology program, US EPA's CompTox HTTK program, and available toxicological and epidemiologic data from the peer-reviewed literature. This is particularly relevant as we face a novel threat to the immune system through SARS-CoV-2/COVID 19 and the potential for other emerging threats. In addition, if it can be shown that *in vitro* assay data are associated with apical outcomes of regulatory interest, then this would represent the first instance of using *in vitro* data to develop regulatory thresholds protective of public health.

Overview and Background

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants found in over 99% of serum samples in the United States based on data from the National Health and Nutrition Examination Survey (NHANES) (Calafat et al. 2007; Kato et al. 2011). Exposure sources include drinking water, particularly in the vicinity of military or other sites using fire-fighting foams, consumer products, non-stick cookware, and take-out containers (Sunderland et al. 2019; Hu et al. 2018). Regulatory thresholds protective of public health focus on potential immunotoxicity as demonstrated from epidemiologic studies, but mechanisms are poorly understood, and most proposed regulatory values focus on individual PFAS (DeWitt et al. 2019; Cordner et al. 2019). There are nearly 9,000 known PFAS (http://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/) and that number grows daily as new substitute are proposed. Over 95% of the general population has demonstrated serum levels of the two most commonly measured PFAS: PFOA and PFOS (Calafat et al. 2007; Kato et al. 2011). PFASs are complex and can be categorized in different ways as shown in Figure 1. PFAS consist of perfluoroalkyl carboxylates (PFCAs) and sulfonates (PFSAs) with variable carbon chain length (C4 to C18), and either a carboxylic acid or sulfonate group. At neutral pH, these compounds have anionic end-groups. In addition, there are precursor compounds to PFAS that can metabolize to PFOS and PFOA once in the body. Currently, based on similar effects in animals, toxicokinetics and observed levels in human blood, a number of studies focus on the sum of four PFASs: PFOA, PFNA, PFHxS and PFOS as these four make up approximately half of what has been measured in food (EFSA 2020). For this analysis, we will start with what has been measured in serum from NHANES data (approximately 25 individual PFAS) as the most compelling measure of exposure from all sources in the US general population.

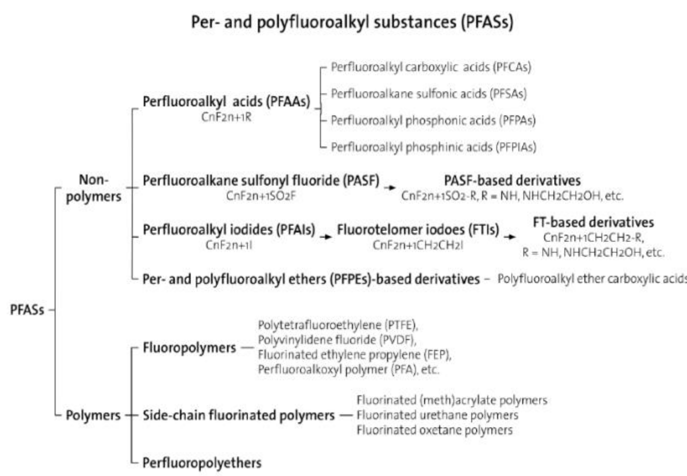

Figure 1: Classification of PFAS

Human epidemiologic data suggest that serum levels of PFOS and PFOA, in particular, are associated with reduced vaccine responses in longitudinal studies of children (DeWitt et al. 2019; Sunderland et al. 2019), and data are starting to be developed around mechanisms of immunotoxicity (Li et al. 2020). Based on these epidemiologic data, back-calculated ingestion doses and drinking water concentrations for individual PFAS