Evaluation of perfluoroalkyl acids (PFAAs) in Airservices Australia's Aviation Rescue and Fire Fighting (ARFF) staff

FINAL REPORT

A consultancy funded by Airservices Australia

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Executive Summary

In 2013, all 731 Aviation Rescue and Fire Fighting (ARFF) operational staff at Airservices Australia were invited to participate in a study to evaluate their past exposure to aqueous film forming foam (AFFF) by means of measuring the concentration of fluorosurfactants in their blood serum. The three fluorosurfactants found at highest levels in ARFF staff, perfluorooctanesulfonic acid (PFOS), perfluorohexanesulfonic acid (PFHxS), and perfluorooctanoic acid (PFOA), were chosen as biomarkers for AFFF exposure. PFOS, PFOA, and PFHxS belong to the group perfluoroalkyl acids (PFAAs) and have been previously identified in different AFFF formulations. Despite many studies in people who have had higher exposure than most ARFF workers, there have been no health issues directly attributable to high levels of these chemicals. The study was conducted as part of a contribution to scientific understanding and this report presents the results of the analysis of PFAAs in serum of 150 employees who consented to participate in this study.

Participants were found to have levels of PFOA similar to those found in the general Australian population but higher levels of PFOS and PFHxS. The most likely explanation is that PFOS and PFHxS levels are influenced by direct or indirect contact with some AFFF formulations. In addition, the concentrations of PFOS and PFHxS in serum from ARFF personnel are strongly correlated, which indicates that these two chemicals have come from the same source. Serum levels in ARFF staff were found to be approximately 20 times lower compared to reported levels in manufacturing workers from the U.S. who have high occupational exposure to these chemicals.

The concentrations of PFOS and PFHxS were found to be positively associated with years of jobs with AFFF contact. Study participants who had worked ten years or less had levels of PFOS that were similar to or only slightly above those of the general population. This coincides with the phase out of Light Water AFFF from ARFF training facilities in 2002, and suggests that the exposures to PFOS and PFHxS in AFFF have declined in recent years. Blood donation was found to be linked to low PFAA levels.

There was no significant difference in PFAA blood serum levels across ARFF stations. Direct comparisons made between stations should be made with caution due to the low participation rate for the majority of the stations. In addition, the fact that many of the participants have been positioned at different stations during their ARFF employment, may not give a “true” picture. Self-reporting of skin contact and frequency of contact were used as an index of exposure. Using this index, there was no relationship between PFOS levels and skin exposure. This index of exposure is limited as it relies on self-report and it only considers skin exposure to AFFF, and does not capture other routes of potential exposure.

Possible associations between serum PFAA concentrations and five biochemical outcomes were assessed. The outcomes were serum cholesterol, triglycerides, high-density lipoproteins, low density lipoproteins, and uric acid. No statistical associations between any of these endpoints and serum PFAA concentrations were observed.

Glossary/Abbreviations

AFFF	Aqueous Film Forming Foam
ANOVA	Analysis Of Variance
ARFF	Aviation Rescue and Fire Fighting
BMI	Body Mass Index
Entox	National Research Centre for Environmental Toxicology
ng	Nanograms
PFAAs	Perfluoroalkyl acids
PFOA	Perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFHxS	Perfluorohexanesulfonic acid
ppb	Parts Per Billion
RSD	Relative Standard Deviation
SNP	Sullivan Nicolaides Pathology
QA/QC	Quality Assurance Quality Control

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1. Introduction

1.1. Background

Airservices Australia contracted Entox in 2012 requesting that the baseline exposure levels of ARFF staff to fluorosurfactants from past usage of aqueous film forming foam (AFFF) be determined. The target analytes selected were perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA), 6:2 fluorotelomer sulfonate (6:2 FTS), and associated fluorosurfactants, which have been previously identified in different Aqueous film forming foam (AFFF) formulations. The three fluorosurfactants found at highest levels in ARFF staff, PFOS, perfluorohexanesulfonic acid (PFHxS), and PFOA, were chosen as biomarkers for AFFF exposure. This study commenced after consultation with Airservices' Board of Directors, Executive General Managers, and staff associations.

PFOS and PFOA are persistent chemicals that remain present in people who have been exposed to them previously. Although there are no definitive health issues known to be associated with these chemicals, previous studies have suggested an association between some of the PFAAs and serum cholesterol and uric acid concentrations. It was therefore decided that it would be appropriate to measure concentrations of these chemicals in blood serum to evaluate whether elevated exposure to these chemicals has occurred in ARFF staff, and examine factors that were associated with higher or lower concentrations, including self-reported exposure through foam use and other factors. Thus, measurements of serum lipids and uric acid were also made on the collected blood samples, and potential associations between these biochemical markers and the measured PFAA levels were assessed.

Between the early 1980s and 2003, 3M™ Light Water™ was used by ARFF at fire training grounds around Australia. In accordance with Airservices' environmental policy, ARFF transitioned to Ansulite® in 2003 after 3M announced that it was withdrawing Light Water from manufacturing because PFOS, one of the main foam components, had been found to be persistent and accumulative in the environment. When testing of a number of batches of Ansulite revealed that PFOA was present at above trace levels in all samples tested, and that PFOS was present at above trace levels in some of the batches, the use of Ansulite for ongoing competency training ceased in January 2010. Since 2010, Solberg, a fluorosurfactant-free firefighting foam, has been used in operational responses only at all locations with the exception of the Defence/civilian joint user facilities at Townsville and Darwin which continue to use AFFF at Defence's request.

1.2. Fluorinated surfactants

Fluorinated surfactants belong to a broad group of surfactants, in which at least one hydrogen atom along the carbon backbone has been replaced with fluorine, and have been commercially available since the 1950s. The first available were the PFAAs PFOS and PFOA, which are perfluorinated surfactants meaning that all hydrogens on the eight-carbon long chain have been replaced with fluorine (Buck et al. 2012). Another PFAA that has been produced in high volumes is PFHxS, which is a member of the same chemical class as PFOS. PFAAs have been produced via either electrochemical fluorination (ECF) or telomerization processes and used in a wide variety of industrial applications and commercial products (for example electronic surface coatings, treatment of paper, and firefighting foams) (Buck et al. 2012). In the ECF process a significant amount of cleaved, branched and cyclic compounds are formed, which yields a complex mixture of different perfluoroalkyl chain lengths and branched structures. The telomerization process on the other hand exclusively yields an even number of fluorinated carbon chains (Kissa 2001). The strength of the carbon-fluorine bond contributes to the physicochemical properties of PFAAs, such as strong chemical and thermal stability, making them both industrially attractive and environmentally persistent. The combination of the hydrophobic fluorocarbon chain and the hydrophilic head group gives PFAAs repellency towards both water and oils (Figure 1) (Moody and Field 2000). Since PFAAs do not break down readily after being released into the environment, they are present in living organisms all over the world, including in people in the general community (Giesy and Kannan 2001; Kannan et al. 2004; Tao et al. 2006; Calafat et al. 2007; Kärrman et al. 2007).

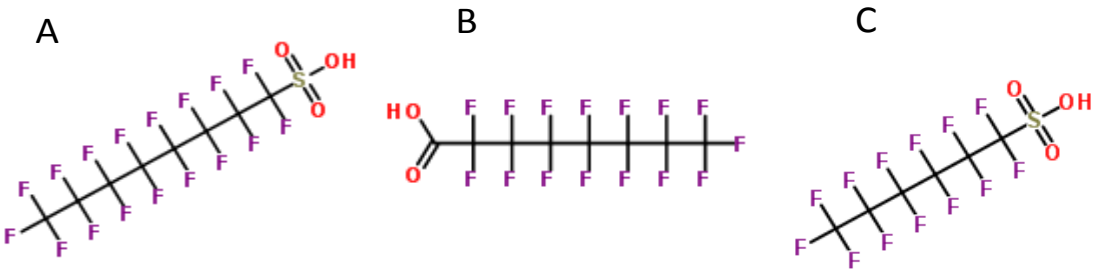

Figure 1. Chemical structure of A) PFOS, B) PFOA and C) perfluorohexanesulfonic acid (PFHxS).

Human exposure to PFAAs may occur through both direct and indirect exposure. Direct exposure implies that the PFAAs are present in the exposure source, and indirect exposure that a precursor compound undergo environmental break-down processes or metabolize in the body to PFAAs. An example of PFAA precursor compounds are the fluorotelomer alcohols (FTOHs), and perfluorooctane sulfonamidoethanols (FOSEs),

which are major raw material for surfactant and surface protection products (Buck et al. 2011). Degradation pathways of FTOHs and FOSEs that ultimately may result in the formation of PFAAs are through reaction with oxygen in the atmosphere (Young and Mabury, 2010), as well as degradation by bacteria in environmental compartments (Rhoads et al. 2008, Dinglasan et al. 2004). FOSE and FTOH have been confirmed to metabolize to PFOS (Xu et al. 2004) and PFOA (Martin et al. 2005), respectively, in liver cells of rats. For FTOH there is also strong evidence that the same can occur in the human body (Nilsson et al. 2013).

Potential pathways through which the general community can be exposed to PFAAs include:

- Ingestion of household dust (Kubwabo et al. 2005) or of foods contaminated during preparation, processing, or via contact with packaging materials (Begley et al. 2005; Tittlemier et al. 2006; Tittlemier et al. 2007; Ericson et al. 2008; Fromme et al. 2008).
- Contact with PFAA containing consumer products such as carpets and apparel (Trudel et al. 2008).
- Inhalation of indoor air (Shoeib et al. 2004).
- Exposure to PFOA through drinking water accidentally contaminated with this compound has been reported (Emmett et al. 2006; Holzer et al. 2008).

Definitive health risks associated with PFAA exposure in humans have not been shown. In animals, health effects in relation to PFOS and PFOA exposure include reproductive and developmental toxicity (negative effects on the reproductive ability of an organism and the development of its offspring) and carcinogenicity (the ability or tendency to induce cancer or increase its incidence) (Kudo and Kawashima 2003; Kennedy et al. 2004; Lau et al. 2007; Andersen et al. 2008). Grice et al. (2007) found no association between occupational PFOS exposure and several cancers, common human health conditions and birth weight. Similarly, Monroy et al. (2008) found no association between PFAA concentrations and gestation length, birth weight and gender.

1.3. Aqueous Film Forming Foam (AFFF)

Aqueous film forming foams (AFFFs) are complex mixtures containing fluorocarbon- and hydrocarbon- based surfactants used to extinguish fires involving highly flammable liquids. The fire-fighting efficiency of these foams is due to the unique physicochemical characteristics of fluorinated surfactants allowing for the formation and spreading of an aqueous film formed on top of lighter hydrocarbon fuels (Buck et al., 2012). The use of PFAAs in AFFF formulations has been linked to environmental contamination related to handling, storage and usage (de Solla et al., 2012). Substantially elevated levels of PFOS have been reported in water and biological samples, such as molluscs, turtles, and

wild mink, downstream from airports with a history of firefighting training activities (de Solla et al., 2012, Kärrman et al., 2011, Persson et al., 2013). In Cologne, Germany, elevated levels of PFOS and PFHxS were found in individuals who drank water from private wells contaminated with AFFF from a nearby airport (Weiss et al., 2012). Similarly, in Uppsala, Sweden, AFFF contaminated drinking water has been suggested as one plausible factor behind increasing exposure to PFHxS in Uppsala residents (Glynn et al. 2012). As some AFFF to a large extent constitutes proprietary fluorinated surfactants several studies in recent years have focused on identifying these unknown fluorinated compounds (D'Agostino and Mabury, 2014, Place and Field, 2012, Weiner et al. 2013). This is of particular interest since a significant portion of the total organofluorine in environmental and biological samples is in the form of unknown fluorinated chemicals, and it has been suggested that a portion of this unknown organofluorine likely originates in proprietary fluorinated surfactants that may enter the environment through use of AFFF (D'Agostino and Mabury, 2014). The above mentioned studies have identified a large number of novel and infrequently reported fluorinated surfactants in different AFFF formulations. Weiner et al. (2013) identified one surfactant in AFFF as a PFAA precursor in a biodegradation experiment and more research has been called for to examine more of these novel surfactants for their potential as PFAA precursors.

ARFF has historically used two fluorosurfactant based AFFF foams; Light Water™ produced by the 3M™ company and Ansulite® produced by Ansul Incorporated. Light Water, a PFOS-based AFFF formulation produced by ECF, was replaced by Airservices with Ansulite at around the same time (2002) the production of PFOS was voluntarily discontinued by 3M (3M 2000 a,b). Ansulite concentrate is telomere based and supposed to be free of both PFOS and PFOA. However, a chemical characterization requested by Airservices found PFOS and PFOA in Ansulite concentrate stored at Cairns airport, and PFOA in Ansulite concentrate stored at Brisbane Airport (AECOM Australia Pty Ltd, 2010). Consequently, in 2010 (approx.), Ansulite was replaced with Solberg, which is a fluorosurfactant-free firefighting foam and training with foam ceased in 2010.

2. Objectives and project design

This is an investigative study with the objective of determining whether ARFF employees were occupationally exposed to PFAAs through contact with AFFF foam formulations.

These objectives were met through:

- Assessing the concentration of PFOS, PFHxS and PFOA in blood sera of ARFF firefighters.
- Investigating potential correlations between PFAA levels and different parameters targeted by a questionnaire, such as number of working years.
- Comparing findings with other Australian and international data, including data from groups with high occupational exposure.
- Investigating potential correlations between levels of PFAAs and levels of blood lipids and uric acid.

2.1. Questionnaire

A questionnaire was designed to capture information about basic demographic factors (age, gender), dietary patterns including alcohol consumption, lifestyle factors such as exercise patterns, current medications, health conditions, work history, self-described skin contact rates with foam, and other factors (Appendix A).

2.2. Ethics

The ethics approval for this study was granted by The University of Queensland Medical Research Ethics Committee on 22 November 2012, and three amendments were approved on 28 February 2013, 29 October 2013, and 18 February 2014. The project was allocated Clearance Number 2012001216.

2.3. Outline of study plan

To achieve the objectives of the study we carried out the work in 5 stages:

Stage 1 – Recruitment

Entox had the task of recruiting ARFF employees to participate in the study. Recruitment packs were sent out to 22 ARFF Airport fire stations (731 packs in total). The Fire Station Manager at each station had previously been briefed to facilitate the distribution of the packs to all operational staff. The packs included two information documents about the study, one information document about AFFF foam, one document providing answer to frequently asked questions about PFOS and PFOA and related health effects, one consent form, one questionnaire, and one laboratory request form from Sullivan Nicolaides Pathology, (SNP) (Appendix A). Airservices supported the recruitment process by advertising the study in their fortnightly newsletter. Each questionnaire and consent form was coded and the key for these codes were stored separately to ensure confidentiality was maintained at all times. Laboratory identification was enabled by a unique code and all samples were de-identified when the analyses were done.

Staff who wished to participate completed the consent form and questionnaire and arranged to have their blood taken at a convenient time.

Stage 2 – Sample Collection and storage

Approximately 30 ml of blood was collected at a SNP collection centre or a SNP partner collection centre. Western Diagnostics Pathology in Perth was contracted to collect blood samples in Alice Springs and Broome. All samples were couriered to SNP (Taringa laboratory) where the serum was analyzed for blood chemicals. The blood samples were stored at -20 degrees Celsius and shipped on dry ice. The remaining serum was then sent to Entox for PFAA analysis.

Stage 3 – Analysis and quality control

The samples were prepared and extracted in a clean lab facility at Entox. All the tubes and vials used were marked with ID numbers. Validated analytical methods and a state-of-the-art mass spectrometer were used for analysis of PFAAs. All samples were de-identified when analysed.

Stage 4 - Gather, Collate And Interpret Data

The first page of the questionnaire and the consent form including personal details were kept securely and separately from the questionnaires. The electronic documents containing personal details about the participants were securely stored and only accessible by the project manager. Chemical results were transferred into an electronic database (Excel) that allowed basic statistical analysis of the data.

The measured serum concentrations were compared with:

- Australian data - serum collected in Queensland in 2006/07.
- International data – US health survey from 1999-00.
- Manufacturing workers with high occupational exposure - 3M Decatur plant in Alabama, USA.

Statistical analyses of the measured PFAA levels were conducted to examine:

- Factors potentially associated with serum PFAA levels, and
- Potential relationships between serum lipid and uric acid concentrations and the measured PFAA concentrations.

Stage 5 – Report Preparation

In Stage 5, a detailed report was prepared to present the research findings and provide informed understanding of the results of this project. This report includes:

- Tables and figures
- Sample storage
- Blood quantities tested

- Interpretation of data
- Comparison of results with current data on PFAA levels in Australia and the US.
- Comparison of results with data on PFAA levels in a highly exposed occupational group.

3. Sample Analysis

3.1. Analysis of biochemical markers

A number of blood chemicals were analyzed at SNP, Taringa, Brisbane, Queensland, and a selection of those were studied in relation to serum levels of PFAAs. The specific biomedical markers were chosen because previous studies had reported/suggested that these may be altered by exposure to PFAAs. In total we examined total serum cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) levels, triglycerides, and uric acid.

3.2. Analytical Methodology for PFAA analysis

The analytical methodology and quality assurance/quality control (QA/QC) are described in detail in Appendix B. In short, PFAAs were extracted from serum with acetonitrile followed by analysis using high-performance liquid chromatography-tandem mass spectrometry, and the quantification was based on isotope dilution methods.

3.3. Statistical analysis

An analysis of variance (ANOVA) was conducted to assess the major factors that influence blood levels of PFOS, PFHxS, and PFOA (Table B2, Appendix B). Factors investigated included age, sex, serum protein levels, current smoking, blood donor (yes versus no), years of employment on jobs with foam contact (ARFF and other jobs with foam contact), and current ARFF station.

Potential associations between measured serum lipids and uric acid levels were assessed to examine whether the concentration of these markers or the risk of out-of-range levels of these markers were influenced by serum PFAA concentrations. Additional factors potentially influencing these markers were also considered, including age, sex, body mass index (BMI), exercise intensity, alcohol consumption, current smoking status, and serum protein levels.

4. Results

The focus of this report is on the evaluation of the three most prevalent PFAAs (PFOS, PFHxS and PFOA) in serum of ARFF firefighters, which have been associated with AFFF. Although another fluorosurfactant, 6:2 FTS, has been identified as a major constituent in some AFFF formulations and high levels have been reported in AFFF contaminated groundwater (Schultz et al. 2004), this fluorosurfactant was not detected in the serum of ARFF staff. A total of 150 participants were recruited from 18 different airport fire stations. This is a 21% participation rate based on the 731 recruitment packs that were sent out in March 2013. The number of participants recruited at each airport ranged from 2-23 and the participation rate ranged from 11%-53%. (Table B3, Appendix B). The largest number of participants was from Melbourne (23 people) and the highest participation rate was from Rockhampton (53%).

4.1. Levels of PFAAs in ARFF firefighters

Blood serum concentrations of PFOS, PFHxS and PFOA are summarized for the different stations in Table B3, Appendix B. Serum concentrations of PFOS ranged between 3 ng/ml and 391 ng/ml serum. The highest average concentrations were found in ARFF staff currently working in Rockhampton, Karratha, Adelaide, Sydney and Coolangatta. A good agreement between the median value and the average value in Table B3 indicates symmetry in the data. On the other hand, an increasing difference between the median and the average is caused by an increasing skewness in the data, which can be illustrated with Avalon where a couple of high observations give a relatively high average compared to the median. Direct comparisons between stations should be made with caution since the low participation rate for the majority of the stations, as well as the fact that many of the participants have been positioned at different stations during their ARFF employment, may not give a completely "true" picture. Data is only presented for the stations with six participants or more to safeguard participant confidentiality.

A strong correlation was found between PFOS and PFHxS concentrations (R^2=0.93), which suggests that these two chemicals have a common source of exposure (Figure 2). PFHxS has along side PFOS been found as one of the components in Light Water and a strong correlation supports the theory that serum levels of PFOS and PFHxS in ARFF staff have been influenced by direct or indirect contact with AFFF.

A significantly weaker correlation (R^2=0.43) was found between PFOS and PFOA, which suggests that the exposure scenario of these two chemicals varies more compared to PFOS and PFHxS (Figure 3). It is probable that AFFF is a less important source of PFOA exposure in this case. This is further supported by the finding that the levels of PFOA were less elevated compared to PFOS and PFHxS, which will be discussed further.

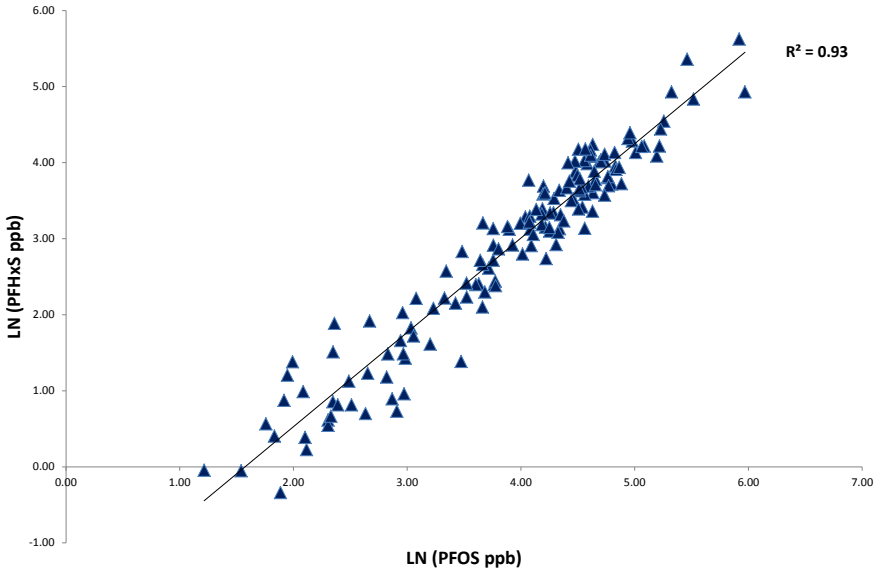

Figure 2. Correlation between PFOS and PFHxS concentrations in serum of 150 ARFF firefighters. R^2=correlation coefficient.

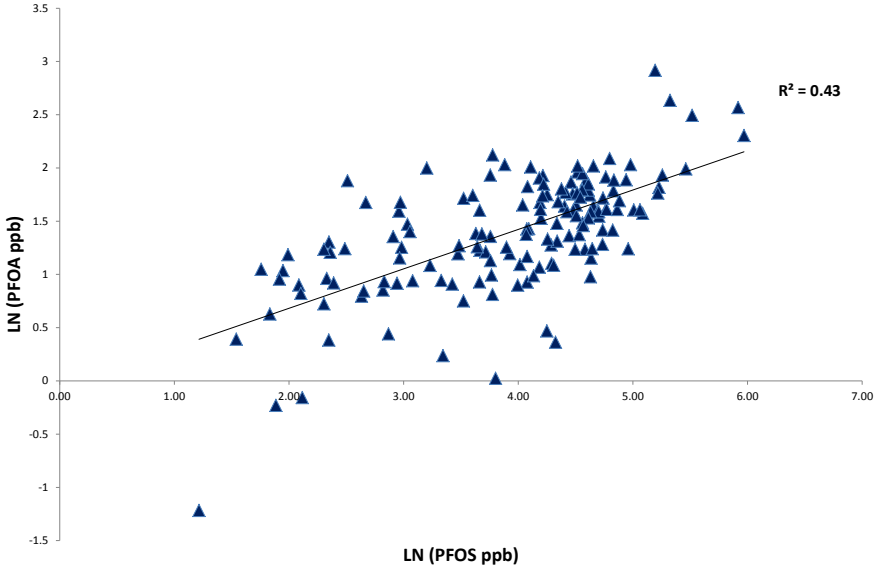

Figure 3. Correlation between PFOS and PFOA concentrations in serum of 150 ARFF firefighters. R^2=correlation coefficient.

4.2. Evaluation of factors influencing blood levels of PFAAs

The results of the ANOVA are presented in Table B2, Appendix B. Levels of PFOS, PFHxS and PFOA were negatively associated with sex (lower levels in females than in males). These relationships are consistent with previous data indicating that women have lower levels of PFAAs than men (Ericson et al. 2007, Kato et al. 2011, Toms et al. 2009). However, the results for female participants should be interpreted with some caution, since there were very few female participants in the study.

Levels of all three PFAAs were negatively associated with blood donation (lower levels in persons who report blood donation) (Figure C1, Appendix C). This finding is consistent with previous literature that indicates that PFAAs bind to protein components in the blood. As a result, removal of blood (through blood donation or disease treatment for hemochromatosis) acts as an elimination pathway and results in lower blood concentrations of these compounds (Thompson et al. 2010).

The concentrations of all three biomarkers were significantly (PFOS and PFHxS) or borderline significantly (PFOA) positively associated with years of jobs with foam contact (Figure 4). Study participants who had worked ten years or less had levels of PFOS that were similar to or only slightly above those of the general population. This coincides with the phase out of Light Water AFFF from ARFF training facilities in 2002. After 20 years of exposure PFOS levels seemed to level off (Figure 4). The concentrations were also independently associated with age. However, as expected, there was a strong and significant interaction between age and years of foam exposure that modifies the independent relationships of the two factors with blood levels of the compounds. There was no statistically significant difference in PFAA blood levels across ARFF stations.

Direct exposure (to the PFAAs themselves) and indirect exposure (to a precursor compound that metabolizes in the body to a PFAA) to AFFF may have occurred through different routes and may have varied with many different factors, both in space and time. Two plausible exposure routes are skin contact with foam and inhalation of aerosolized foam present in air during foam training activities. Skin contact may have occurred with the actual foam as well as, for example, with protective gear impregnated with foam. Quantifying and documenting the exposure experienced by employees is always difficult. In this study we relied on retrospective self-reporting by participants of the direct exposure of the skin to these foams. Other exposure routes, such as inhalation of aerosolized foam during training, ingestion of foam or contact with safety equipment, were not possible to capture.

To examine if there was any association between skin exposure and levels of PFOS, an index of skin exposure was calculated from the participants' self-reporting of exposure. The formula for this skin exposure index was: (years of exposure x frequency) + (years of exposure x skin contact). Analysis demonstrated no relationship between this index of skin exposure and PFOS. This finding needs to be interpreted cautiously because the index only considered direct skin exposure to AFFF and was a relatively crude measure of skin exposure as it relied solely on self-reporting.

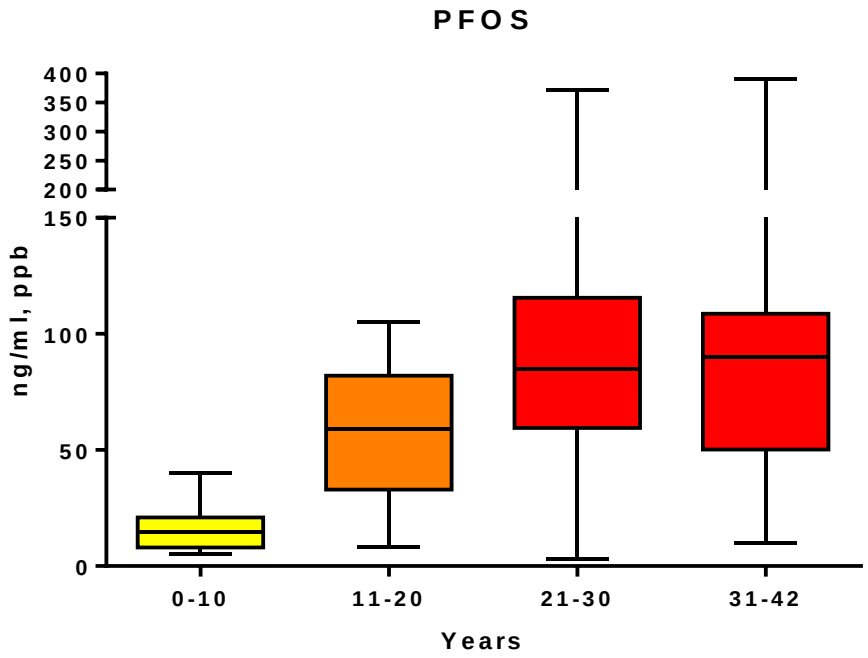

Figure 4. PFOS concentrations (y-axis) in relation to number of years of jobs with foam contact (x-axis), including jobs outside ARFF. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers min and max concentrations.

In addition to skin contact, elevated air levels at the training grounds during AFFF usage is a potential exposure route. Such exposure is likely to be influenced by training routines, weather conditions and breathing apparatus usage. In one occupational exposure study, high levels of a PFOA precursor compound (8:2 FTOH) was identified in air and was associated with significantly elevated levels of PFOA in whole blood of professional ski-wax technicians applying flouro-based ski waxes indoors and under heat (Nilsson et al. 2013). Drinking water may be contaminated with PFAAs if it is sourced from local groundwater. However, concentrations of PFAAs in community water in Australia are typically very low and do not contribute significantly to exposure (Thompson et al. 2011). Where community water is used at fire stations, drinking water is not envisaged to be a relevant source of contamination.

None of the measured chemicals were associated with current smoking behaviour; however, very few of the participants (<5%) currently smoke.

4.3. Comparison with PFAA levels in the general population

The results of PFAAs in blood from the ARFF employees is compared with PFAA levels in the general population in Australia and overseas from two large study groups (Figure 5, 6 and 7). Data on PFAA concentrations in the Australian population are available from biomonitoring data conducted in Australia every 2 years. These

biomonitoring data were obtained by collecting de-identified blood serum based on gender and age. The samples were pooled with approximately 30 samples per pool. For this comparison the average concentration of PFAAs available from 84 pools (n=2420) of both males and females was used. The samples, collected in 2006/2007, were obtained from Sullivan Nicolaides Pathology and analysed at the Center for Disease Control (CDC), Atlanta, Georgia, USA. Since the samples were pooled and only average PFAA concentrations could be calculated. The 95th percentile was then estimated based on population variation from available biomonitoring datasets from the U.S., Canada, Germany, and the Catalonian region of Spain (Aylward et al., in prep).

In ARFF staff the serum levels of PFOS were significantly higher compared to the levels found in the general population in Australia and the US (Figure 5). The median level was 66 ng/ml in ARFF staff compared to 15 ng/ml and 30 ng/ml in the general population in Australia and the US, respectively (Toms et al., 2009; Kato et al., 2011).

The serum levels of PFHxS in ARFF staff were also significantly higher than general population levels in Australia and the US (Figure 6). The median level was 25 ng/ml in ARFF staff compared to 3 ng/ml and 2 ng/ml in the general population in Australia and the US, respectively.

In Figures 5 and 6, PFOS and PFHxS have relatively high whiskers extending above the bars. This indicates that the ARFF data is skewed (i.e. a number of individuals are outliers with serum concentrations substantially higher than the median). This is not the case in Figure 7 in which the PFOA levels in the serum of ARFF staff are comparable to the levels in the Australian and the US general population (Figure 7). As mentioned previously this suggests that AFFF has not been a major source of PFOA exposure.

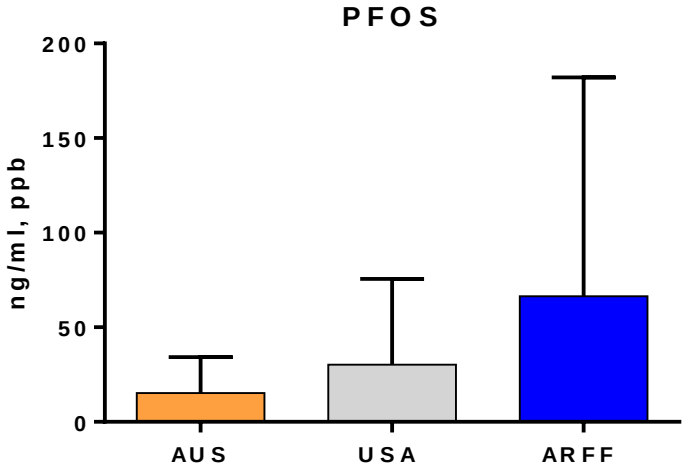

Figure 5. Serum concentrations of PFOS (ng/ml) in 84 pooled samples from Queensland, Australia (AUS), from 2006/2007 (n=2420), and individual samples from a US health survey (USA) from 1999-2000 (n=1562), and in 150 ARFF staff members. The whiskers indicate the 95th percentile and the columns indicate median concentrations for ARFF and USA, and average concentrations for AUS.

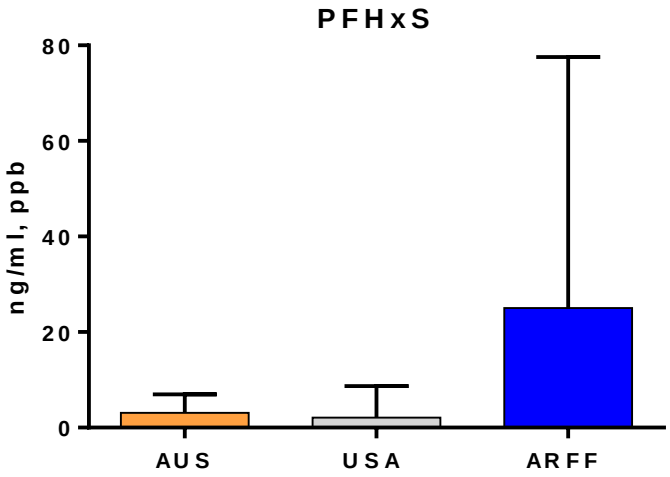

Figure 6. Serum concentrations of PFHxS (ng/ml) in 84 pooled samples from Queensland, Australia (AUS), from 2006/2007 (n=2420), and individual samples from a US health survey (USA) from 1999-2000 (n=1562), and in 150 ARFF staff members. The whiskers indicate the 95th percentile and the columns indicate median concentrations for ARFF and USA, and average concentrations for AUS.

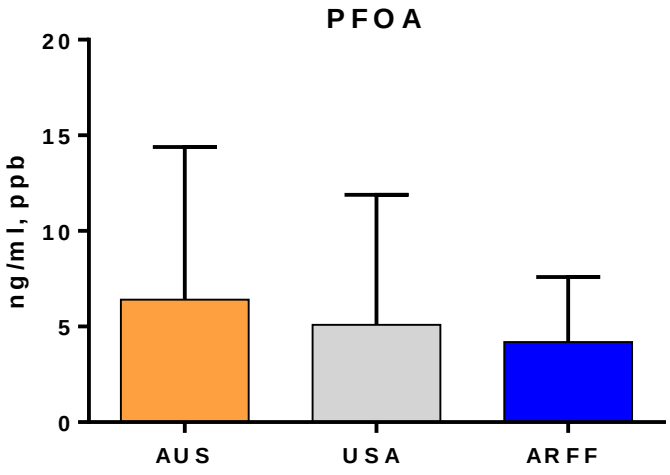

Figure 7. Serum concentrations of PFOA (ng/ml) in 84 pooled samples from Queensland, Australia (AUS), from 2006/2007 (n=2420), and individual samples from a US health survey (USA) from 1999-2000 (n=1562), and in 150 ARFF staff members. The whiskers indicate the 95th percentile and the columns indicate median concentrations for ARFF and USA, and average concentrations for AUS.

4.4. Comparison with PFAA levels in a highly occupationally exposed group

The concentrations of PFOS found in the serum of the ARFF employees were compared to data from 263 highly exposed fluorosurfactant manufacturing workers from the 3M Decatur plant in Alabama, USA (78 % males, 18% females). The geometric mean serum level of PFOS in ARFF staff was approximately 20 times lower than in the 3M workers (Figure 8). Comparing maximum levels, the highest level in an ARFF firefighter was 391 ng/ml compared to 10060 ng/ml in a Decatur employee (Olsen et al. 2003).

In this study, serum from Decatur workers was collected at a time when exposure was ongoing (i.e. they were working at the plant with fluorochemical manufacturing at the time of blood collection). Hence if assuming that Light Water has been an important source for PFOS (and PFHxS) exposure to ARFF staff a direct comparison between these two groups is complicated by the fact that an important part of the PFOS exposure would have ceased around 2002 when Light Water was replaced with Ansulite. This assumption is supported by the result that the highest PFOS levels were, without exception, found in firefighters or EVTs who were working when Light Water was still in use. Previous studies have calculated the half-life of serum elimination as 5 years for PFOS and 8 years for PFHxS (Olsen et al. 2007). This means that the PFOS and PFHxS levels may remain high in individuals who had been working with AirServices when Light Water was in use, even though Light Water is no longer in use.

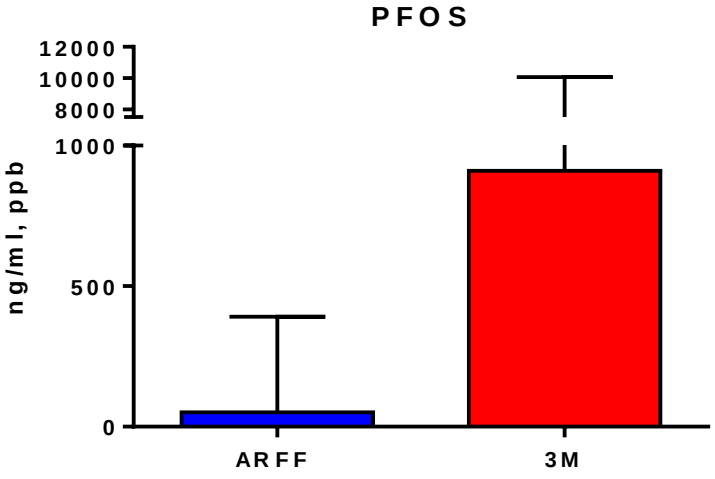

Figure 8. Geometric mean serum concentrations of PFOS (ng/ml) in 150 ARFF staff and in highly exposed fluorosurfactant manufacturing workers (sera extracted and analyzed in 2000) from the 3M plant in Decatur, Alabama, USA (n=263). The whiskers indicate maximum concentrations. Notice the different scales on the Y-axis.

4.5. Biochemical outcome measures and associations

We examined five biochemical outcome measures for possible associations with exposure to the three PFAAs covered by this study. Previous studies have found relationships between some PFAA compounds and serum lipid and serum uric acid concentration. In particular, in populations exposed to PFAAs, cholesterol (Steenland et al., 2009; Nelson et al., 2010; Lin et al., 2009), low-density lipoprotein (LDL) levels, and triglycerides (Steenland et al., 2009) have been positively associated with blood PFOA or PFOS concentrations. However, Nelson et al. (2010) found the opposite for PFHxS. In addition, serum uric acid levels have also been positively associated with PFAA concentrations (Steenland et al 2010). As a result, we examined cholesterol and other lipids (triglycerides, HDL, and LDL) and serum uric acid to evaluate whether associations between these biochemical markers and PFAA blood levels were apparent in the ARFF population. These biochemical markers are known to be associated with health issues in clinical medicine, although there has been no definitive evidence that changes related to the PFAAs have health consequences.

We examined potential relationships between these five biochemical outcomes and blood PFAA concentrations as well as covariates (age, sex, Body mass index (BMI), current smoking behavior, current exercise intensity, alcohol consumption, and serum protein concentrations) in an ANOVA. Based on the initial examination of relationships between the outcome variables and PFAA concentrations and covariates, we developed final regression models predicting changes in the measured levels of these outcomes (Table D1, Appendix D). The models for cholesterol, LDL, HDL, and triglycerides were limited to participants who did not report taking cholesterol-lowering medication. The model for uric acid omitted one participant who reported taking medication to treat gout. Only individuals (in this case 93% of the participants) with reported height and weight measures (to allow calculation of BMI) were included in the models.

Before beginning the regression exercise, we examined whether serum PFOA, PFOS, or PFHxS concentrations were associated with key covariates. No significant associations between these compounds and BMI or total serum protein were observed.

Cholesterol

Increased cholesterol levels are associated with increased cardiovascular disease risk. Total serum cholesterol was not associated with any of the covariates except total serum protein levels (albumin plus globulin). No association with PFOA, PFOS, or PFHxS was observed.

HDL

Higher HDL is desirable because HDL appears to provide a protective effect on cardiovascular disease risk. HDL levels were higher in females than males. HDL levels were lower in persons with higher BMI (Figure D3A, Appendix D) and lower in current smokers than in non-smokers. No association with PFOA, PFOS, or PFHxS was observed.

LDL
Increased LDL levels are associated with increased cardiovascular disease risk. LDL was positively associated with total serum protein levels. No association with PFOA, PFOS, or PFHxS was observed.

Triglycerides
Increased triglyceride levels are associated with increased cardiovascular disease risk. Serum triglycerides were positively associated with BMI (Figure D3B, Appendix D), with being a current smoker, and with total serum protein. A slight negative association (in the protective direction) was observed with increasing PFOA concentrations. Because PFAA compounds have been reported to associate with proteins in blood (Butenhoff et al. 2012), we tested whether there was a significant statistical interaction between PFOA and serum proteins. In models with an interaction term added, neither the associations individually with PFOA and protein, nor the interaction term coefficient, were significant. These results are difficult to interpret statistically, but the finding of decreased triglyceride levels associated with increasing PFOA concentrations should be viewed cautiously. Figure D1, Appendix D, shows the unadjusted association between triglycerides and PFOA.

Uric Acid
Elevated serum uric acid is associated with increased risk of cardiovascular disease. The most important predictor of uric acid was sex: uric acid was lower in females than in males (Figure D2, Appendix D). Uric acid was positively associated with BMI and serum protein concentrations (Figure D3C, Appendix D). No association with PFOA, PFOS, or PFHxS was observed.

Using logistic regression, we examined odds ratios of the likelihood of having an out-of-range value for each of the five outcome variables as a function of PFAA concentration, taking into account the covariates identified in the ANOVA. The risk of having an adverse out of range value (below the normal range for HDL, above the normal range for the remaining outcomes) was not statistically related to the serum concentrations of any of the PFAAs.

Serum protein
None of the PFAA concentrations were significantly associated with serum total protein levels. Because PFAAs bind to serum proteins (D'eon et al. 2010), a positive relationship between blood levels and serum protein levels would be expected. However, it is possible that the influence of other factors in this population was much greater than the influence of serum protein levels, making any association difficult to detect.

Overall, the results of these analyses do not provide any evidence of a measurable relationship between serum PFAA concentrations and the biochemical outcomes examined here. The possible exception is a slight tendency towards lower serum triglyceride levels with increasing PFOA levels. However, PFOA levels were not elevated in the ARFF staff compared to the general population data and a lower triglyceride level is not considered unhealthy. This association is very slight, suggesting no clinically meaningful relationship is present.

5. Summary of Findings

This study sought to determine concentrations of PFAAs in ARFF employees potentially exposed to AFFF during their work. The findings of this study are as follows:

- Higher levels of PFOS and PFHxS were found in the serum of ARFF staff compared to the general Australian and US population.
- The PFOA levels were comparable with the levels in the general Australian and US population.
- The PFOS levels were much lower (approximately 20 times lower) compared to a highly exposed occupational group of fluorosurfactant manufacturing workers.
- A positive correlation was found between PFOS and PFHxS and years employed in jobs with foam contact.
- Study participants who had worked ten years or less had levels of PFOS that were similar to or only slightly above those of the general population. This suggests that the exposure to PFOS and PFHxS in AFFF have declined in recent years.
- Skin exposure itself was not associated with PFOS levels - indicating that other exposure routes may have contributed to these levels or that self-reporting of exposure routes was not robust enough to provide an adequate correlation.
- PFAA levels were lower in participants who were regular blood donors.
- No relationship was found between serum PFAA concentrations and any of the five biochemical outcome measures examined, with the exception of a slight relationship in the non-adverse direction between PFOA levels and decreasing serum triglycerides.
- Despite many studies in people who have had higher exposure than most ARFF workers, there have been no health issues directly attributable to high levels of PFOS, PFHxS and PFOA.

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Appendix A

PARTICIPANT INFORMATION LEAFLET

A study to evaluate Perfluorinated compounds in the Blood serum of Airservices Australia's Aviation Rescue and Fire Fighting (ARFF) staff

THE UNIVERSITY OF QUEENSLAND

Investigators: Prof Jochen Mueller and his team at the National Research Centre for Environmental Toxicology (ENTOX) at The University of Queensland.

General Information

Perfluorinated compounds (PFCs) including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are chemicals that are commonly detected in the blood of many people in the general community in Australia, the US, Europe, and many other countries. This is an investigative study to assess whether exposure to these chemicals has occurred through contact with various aqueous film forming fire-fighting foam formulations. It is important to understand that there is no information to suggest that even very high exposure to these chemicals poses any serious health risk.

We would like to invite you to take part in a research project to be conducted by Prof Jochen Mueller from ENTOX in conjunction with Airservices. The purpose of this study is to find out the levels of these environmental pollutants in people who work within ARFF at Airservices and to try to determine whether there has been exposure in the workplace.

A **blood sample** (about 50mls) will be obtained by a blood collector from SNP laboratories. The blood will then be analysed for PFCs. The study team will also measure other common chemicals that are normally found in the body e.g. uric acid and blood fats. The purpose of this study is to help us to identify the sources of these perfluorinated compounds in the community.

Information about these chemicals has previously been provided to Airservices and to employees. A copy of the document "**Frequently Asked Questions**" was distributed to ARFF employees to provide further details about these chemicals. A copy of this document is attached for your reference.

If you agree to take part in this study:

You will be asked to complete a **questionnaire** and to provide a **blood sample**.

How will you benefit from this study?

The study will use individual blood serum samples from participants. These samples will be analysed to ascertain the levels of PFCs. You will be able to obtain the study results once the study is completed. These results will be presented with information to help you understand them. There will be **no immediate personal benefit** to you from this study. The level of PFCs in any person's blood is the result of a lifetime accumulation from many

different exposures. The main benefit of this study is to provide long term information that will assist us in understanding whether occupational exposure of ARFF staff has occurred.

Will the information you give be confidential?
Your information will be treated with the utmost confidentiality. All information will be coded when it is collected for this study and will be stored in a computer using these codes. You will not be personally identified for the purposes of this study. Only the study staff at ENTOX will know that you have participated. Although the results of the study may be published in scientific literature, your identity will not be revealed and will remain confidential. All personal information from the consent form will be kept secure and separate from the other material including your completed questionnaire.

Airservices will not be informed about who has participated nor will they receive any individual test results. Furthermore, it will not be possible for the results of this study to be used by Airservices to identify individual employees for any reason (favourably or unfavourably). However, the summarised results will inform Airservices about the levels of exposure of the PFC and related chemicals that was present in the blood of the employee group. These data will be contextualised using Australian and international studies.

Do you need to take part in this study?
Your participation is voluntary. You do not need to take part in the study unless you want to do so.

Possible risks
There are limited risks associated with this study. There may be some discomfort and/or bruising following the blood test but this is expected to be temporary. Specially trained blood collectors will take your blood.

What if I change my mind?
If you wish to withdraw your consent to participate at any time then we will destroy any results already obtained in the study. You can notify the study team at Uniquest on 07-3274 9060 if you wish to withdraw your consent.

Will I be told the results of the study?
A general report that **summarises** the research findings will be sent to **all** participants, on completion of the study. Airservices will also receive the general report. These reports will **not** identify any specific employee, so your personal results will remain **confidential**. It is intended that these results will assist Airservices to find out whether occupational exposure to these chemicals has occurred. Study staff will be able to provide further explanation of results for all participants as required.

In addition to the general report, participants will also be able to request a summary of their **personal blood results** for PFCs. The consent form provides you with the opportunity to request this information. This report will include your PFC chemical level. This level will be compared to the range of results found in ARFF employees and to the range of results found in the general Australian community and overseas populations. Your personal result may be 'High' or 'Low'. It is important to understand that, currently, there

is **no known serious health risk** related to high exposure to these chemicals. There is **no treatment** for high levels of these chemicals.

Your personal report will also include **your results for other blood chemicals** (such as uric acid and blood fats) and the normal range for these chemicals. If any of these results are abnormal, the letter you receive will include a recommendation for you to attend your usual medical service to discuss these results and arrange further review. Abnormal findings are common in the general community, even when the person is not aware of these. The study staff **will not** be able to provide a clinical interpretation of these results because interpretation of these results requires a full medical history and examination. Your usual medical service will be able to interpret this information for you. It will be **your responsibility** to seek any further medical follow up if you wish to do so and there may be costs associated with this. It will be **your responsibility to fund** all costs related to any medical or clinical follow up after you receive these results.

What will happen to my stored blood sample at the end of the study?
When the study is completed, it is planned that your stored blood sample will be routinely disposed of in accordance with the University's guidelines.

HOWEVER, on the consent form the researchers will ask you if you could **also agree** to have **your blood sample stored for future research** on PFCs.

If you **do not** wish your blood to be stored for future testing, then do not sign this second part of the consent. If you **change your mind**, you can withdraw your consent to participate at any time and request that your sample be destroyed. Your results will remain confidential at all times. You will not be provided with any results from future testing of this blood sample.

Contacts
This study has been cleared by one of the human research ethics committees of the University of Queensland in accordance with the National Health and Medical Research Council's guidelines. You are of course, free to discuss your participation in this study with project staff (Prof Jochen Mueller contactable on 07-30009197, or the Secretary at the National Research Centre for Environmental Toxicology contactable on 07-32749003). If you would like to speak to an officer of the University not involved in the study, you may contact the **Ethics Officer** on 07-3365 3924.

Human health FAQ sheet

This FAQ sheet has been prepared to assist ARFF staff in understanding the information contained in the independent experts' human health reports. These FAQs were prepared following the first presentation by Professor Brian Priestly and Professor Jochen Mueller on 9 February 2011 and will be updated as required. For further information, please consider the experts' reports and presentations, or speak to your Station Commander or Hub Manager.

What is PFOS/PFOA and where is it found?
PFOS and the related chemical PFOA are types of perfluorocarbons that are used as, and referred to, as fluorosurfactants. Fluorosurfactants are a type of man-made organic compound that alter the surface tension of a liquid. They are found in everyday products such as fabric softener, water and stain repelling agents and non-stick cookware. Fluorosurfactants are also used in aqueous film forming foams (**AFFFs**).

Have I been exposed to PFOS/PFOA?
Yes. Fluorosurfactants are found in the blood of the general population all over the world. The sources of exposure in the general population are not fully understood, but it is known that exposure to trace levels can occur through the food supply and in some cases, from the presence of these chemicals in drinking water supplies. In the case of fire fighters, it is possible that small amounts of PFOS could be absorbed through the skin when handling the concentrated or diluted AFFFs, but there is currently no direct evidence of the extent to which this could occur.

What health effects in humans, if any, have been linked to exposure to PFOS/PFOA?
Possible health effects in humans from exposure to PFOS and PFOA are under study in workers exposed occupationally (ie manufacturing workers), in residents in communities with elevated exposures and in larger studies of the general population in the USA. The results of studies so far are not conclusive. There are some indications of alterations in serum lipids and other biochemical indicators in association with measured blood levels of PFOS, PFOA and other perfluorocarbons, but overall the studies available to date do not clearly establish a relationship to adverse health effects, even in heavily exposed manufacturing workers (up to 1,000 fold higher than the general population). The potential health effects studied to date in humans, and the inconsistencies and qualification of any conclusions that can be drawn from these studies, are set out in more detail in the experts' independent reports.

Is Airservices going to implement a form of health testing for ARFF staff? If so, what would the testing involve?
Yes. Airservices is currently consulting with ARFF staff, the UFU and CPSU to discuss the particular form and details of the health testing. The testing would likely involve having a pathology laboratory collect one blood sample from each participant in the study. Privacy and ethical considerations will be fully considered and properly addressed.

What would the test results tell me?
The blood test results should show whether PFOS exposure in fire fighters is similar to, or higher than, PFOS exposure in the general population; or the more unlikely outcome that PFOS blood levels are more like heavily exposed manufacturing workers. It is most unlikely that a health-testing result in an individual could be linked to any adverse health effect in that individual that is attributable to PFOS exposure.

Do you expect that ARFF staff will have high levels of PFOS in their blood? If so, what would this mean for me?
Blood levels of PFOS and related chemicals have never been measured in fire fighters, so it is difficult to predict what the levels might be. The blood levels will be related to the amount of exposure that has occurred and over how long that exposure occurred, since PFOS and related compounds are slowly eliminated from the body. Fire fighters have had opportunities for exposure to PFOS and related compounds because of the presence of these chemicals in AFFFs. Because of this, it is possible that blood levels in some ARFF staff may be higher than those in the general population, but the levels will probably be lower than those measured in workers using and manufacturing these chemicals directly on a day-to-day basis. Studies in such workers have not demonstrated any clear health effects associated with exposure.

The experts' reports talk about both human and animal studies. Should I be concerned about the results of the animal studies?
Studies of chemical effects in animals are conducted at very high doses in order to give scientists an idea of what effects might occur and to provide a basis for estimating safe human doses. For example, adverse effects are observed in animal studies of PFOA only at exposures that lead to blood levels 1,000 to 10,000 times higher than the levels observed in people in the general population. While such studies help scientists to identify toxic responses to a chemical, such information can not readily be used to evaluate potential effects in humans who are exposed at much lower concentrations. Animal studies have also been used to establish conservative PFOS and PFOA health-based exposure standards for humans, but in the case of both perfluorocarbons, the marked differences in the way animals and humans clear them from the body complicates the extrapolations. While everyone in the community has been exposed to PFOS and PFOA, mainly through food sources, estimates of PFOS exposures in the general community are substantially less than intakes considered to represent a safe exposure level based on animal studies.

Why are PFOS and related chemicals being phased out of use?
The manufacture and use of PFOS is being discontinued through international agreements and voluntary actions by manufacturers primarily because of its persistence in the environment, rather than because of any established health effects. Fluorosurfactants break down very slowly in the environment under naturally occurring conditions. They are resistant to biological and most chemical degradations. Because of this, they tend to accumulate in the food chain and in human tissue. The international scientific community has identified this characteristic as undesirable because of the potential for unforeseen effects resulting from accumulating levels and the difficulty in removing these chemicals from the environment once they are released.

INFORMATION ON FIRE FIGHTING FOAM

Airservices uses fire fighting foam at its aviation fire and rescue stations at airports around the country. This foam acts as a thermal and evaporation barrier to inhibit and extinguish a fire in the event of an emergency.

Since the 1980's we have used various types of foams at most airports around Australia. This foam is used for emergency response and training purposes in order to extinguish and prevent fuel fires.

Internationally and locally, a number of studies are underway into the use of foam to fight major fires and the long-term effects of this on the environment.

The foams previously used by Airservices, and which are still used in Australia and internationally by other fire fighting authorities, contain materials which are persistent in the environment, known as fluorosurfactants.

These are extremely common and used in many other products, including water and stain repellents, lubricants (in the automotive and airline industries), anti-reflective coatings, fabrics, textiles, paper and leather as well as in wax, polishes, paints, varnishes, cleaning products and non-stick applications such as Teflon.

In an effort to be more environmentally responsible, Airservices has transitioned to a more environmentally friendly fire fighting and fluorosurfactant-free foam that enables us to continue to maintain our ability to protect the travelling public from the threat of aviation fires.

The new fire fighting foam

Airservices has been at the forefront in monitoring international trends and studies in relation to the use of these materials. This resulted in the transition to a new fire fighting foam, called Solberg RF6 in 2010.

This product has similar fire suppressing properties to deal with aviation fires but has been designed by the manufacturer to be more environmentally friendly. Airservices also independently conducted its own testing of the new product.

International standards

Airservices is closely monitoring national and world-wide development of policies, procedures and research related to fluorosurfactants. There are no internationally agreed thresholds for exposure. Australia does not currently have any guidelines around exposure to these materials.

Scientific research

Studies going back 40 years have not established any conclusive health risk in relation to these materials. There is no definite link, established either scientifically or medically, between specific or general health problems and long term exposure to the materials found in fire fighting foam.

Additionally, Airservices has undertaken independent health studies of potential health risks for our aviation fire fighters. These indicated that there are no specific health concerns likely to be associated with exposure to fluorosurfactants.

Research also suggests that the far greater exposure to these chemicals is in the home, through products such as stain and water repellants (e.g. ScotchGard) and non-stick applications (e.g. Teflon and "greaseproof paper"). It is well accepted that almost everyone is likely to have some level of these chemicals in their systems due to the significant range of products containing fluorosurfactants.

For more information
p 1300 301 120 (within Australia)
t 02 6268 4233 or +61 2 6268 4233 (outside Australia)
e info@airservicesaustralia.com
www.airservicesaustralia.com

12-091JUNE Corporate Communication

Please place in envelope and send back to ENTOX after completion.

THE UNIVERSITY OF QUEENSLAND

PARTICIPANT CONSENT FORM

A study to evaluate perfluorinated compounds in the blood serum of Airservices Australia's Aviation Rescue and Fire Fighting (ARFF) staff

Investigators: Prof Jochen Mueller and his team at the National Research Centre for Environmental Toxicology (ENTOX) at The University of Queensland.

Part A – Consent to participate.
Part B – Request for and consent to receive a report of the individual results gathered in this study.
Part C – Consent to storage of the blood sample for future research.

Participant Identification: (please print)
Full Name: _____

Address: _____

_____

Telephone: _____

Part A – Consent to participate

I, ……..., agree to participate in this research project. (name)

(Please tick each box to acknowledge each statement is correct.)

- ☐ I have been given clear information about this study and I understand this information.
- ☐ I have been informed of any risks to my health or well-being.
- ☐ I have been given the opportunity to have a member of my family or a friend present while the study was explained to me.
- ☐ I have been assured that no personal information from my questionnaire or my personal results will be provided to my employer or published, so my identity will be kept confidential.
- ☐ I am aware that this study has been cleared by one of the human ethics committees of the University of Queensland.
- ☐ I am aware that I may request further information about the project as it proceeds.
- ☐ I understand that I may withdraw my consent at any time.
- ☐ I give permission for the study team to contact me to clarify questionnaire responses if necessary.

Signed:…… Date:……

Name ……

Witness…….

Consent form (perfluorinated compounds and Airservices staff)(cont'd)
Part B – Request for and consent to receive a report of the individual results gather in this study.

I, ……..., request a copy of my results.
(name)

(Please tick each box to acknowledge each statement is correct.)

☐ I request that the researchers send me a copy of my personal chemical results and I understand that I am responsible for safeguarding these results.

☐ The above address is the correct address to which these results are to be sent.

☐ I will notify the researchers if I change my address or if I wish this information to be sent to a different address in the future.

☐ I understand that this personal report may indicate a high or low result of the environmental chemicals being measured and that this level is my current lifetime exposure.

☐ I understand that if the uric acid or blood fat results are abnormal then the researchers will alert me to this fact but will **not** be able to provide a clinical interpretation of these results.

☐ I understand that it is my responsibility to seek further medical follow up with my usual medical service and that any costs associated with any medical or clinical follow up will be my responsibility to fund.

Signed:…… Date:…….

Name ……

Witness……

Part C – Consent to for storage of the blood sample for future research.

I, ……..., agree that my blood can be stored for future research. (name)

(Please tick each box to acknowledge each statement is correct.)

☐ I consent to allow the researchers to store any unused blood from my blood sample for future work on environmental pollutants.

☐ I understand that I will not receive any results related to any future testing.

Signed:…… Date:…….

Name ……

Witness……..

Please place in envelope and send back to ENTOX!

Please place in envelope and send back to ENTOX after completion.

THE UNIVERSITY OF QUEENSLAND

A study to evaluate perfluorinated compounds in the blood serum of Airservices Australia's Aviation Rescue and Fire Fighting (ARFF) staff

Questionnaire for Participants

Please complete the following questionnaire providing as much detail as possible.

Your answers will be kept strictly confidential.

This page with your details will be stored separately from your answers.

Only the researchers will have access to these details and these will be kept securely as approved by one of The University of Queensland's Ethics Committees.

No information gathered from you during this project will be provided directly to your employer.

Airservices will receive a report containing combined information. This information will not identify you as an individual at any time.

Please print all answers.

Name:……

Residential Address:……..…

……

……

……

Email Address:……

Best Telephone Contact:……

Office Use	
Code	

Office Use	
Code	

Where boxes are provided for answers, please tick the box that best fits the answer. We appreciate your time in completing this questionnaire which will provide us with the information we need to know about you, your lifestyle, your health, your diet and your work. This information is important for the interpretation of the chemical results obtained in this study.

Please print your answers.

Personal Information

1. What is your date of birth? …….

2. Gender: Male ☐ Female ☐

3. What is your country of birth? ……

   In you were not born in Australia –
   What year did you come to live in Australia? .....

Lifestyle Information

4. Have you ever smoked? Yes ☐ No ☐

   If No – go to Question 8

5. How many **years** have you smoked for in total?
   ……

6. How **many cigarettes** did you (or do you) smoke per day?
   ……..

7. Do you still smoke? Yes ☐ No ☐

   If No – In what year (approximately) did you have your last cigarette?
   ……

8. How many **standard drinks** do you have in one week (on average)
   ……

   (1 **standard drink** = 1 pot (QLD) or middy (NSW) full strength beer,
   1 can (375ml) of mid-strength beer, 100ml (small glass) of wine, 1 nip of spirits)

9. How many **times each week** do you do moderate-strenuous **exercise** (on average)?

(Examples of moderate-strenuous exercise is fast walking, tennis, dancing, biking)

- ☐ Less than twice a week
- ☐ Three-four times a week
- ☐ Five of more times per week

10. Which diet best describes your **normal diet**?

- ☐ Mixed Diet – meat and vegetables
- ☐ Vegetarian but with dairy products and eggs
- ☐ Strictly Vegetarian (no milk and no eggs)

11. On average, how often do you eat **fish** or other seafood?

- ☐ Never
- ☐ Less than once a week
- ☐ Once a week
- ☐ Twice a week
- ☐ More than twice a week

12. On average how often do you consume **milk and milk products**, including cheese?

- ☐ Never
- ☐ Less than once a week
- ☐ Once a week
- ☐ Twice a week
- ☐ More than twice a week

13. On average, how often do you consume **meat**?

- ☐ Never
- ☐ Less than once a week
- ☐ Once a week
- ☐ Twice a week
- ☐ More than twice a week

14. Are you a **blood donor**? Yes ☐ No ☐

If yes, how frequently do you donate blood?

- ☐ Less than once a year
- ☐ Once a year
- ☐ 2 to 4 times per year
- ☐ >4 times per year

If yes, when was the approximate **date** of your last blood donation? ……..

Health Information

15. Do you currently have any **chronic health problems**? Please **tick** Yes or No for each condition. If **Yes**, Please provide the **details** requested.

Category	Y	N	**Describe** (date of diagnosis, type of problem and severity)
Diabetes			
Heart disease			
High blood pressure			
Kidney problems (including kidney stones)			
Liver problems			
Asthma			
Reproductive or fertility problems			
Thyroid problems			
Serious Arthritis (e.g. Rheumatoid arthritis)			
Cancer			
Other health conditions?			List:

16. Are you currently taking any **medications** for the following conditions? Please **tick** Yes of No for each medication. If **Yes**, Please provide the **details** requested.

Medication	Y	N	If yes – what is the **name** of the medicine/s
For high cholesterol			
For gout			
For diabetes			
For epilepsy			
Fluid tablets			
Antibiotics (including treatment for TB)			
For thyroid problems			

Chemotherapy (including methotrexate)			

Occupational History

17. Starting with your **current** assignment and working backwards to your first job with ARFF,

Please list **where** you worked and the (approximate) dates that you worked in that job.

Location	Dates

18. In the table below, Please **Tick** the **Job assignments** that you have held **at Air Services**.

Senior Officer – means a senior Fire Commander or Superintendant in an operational or non-operational role

Officer – means a Sub Station Officer, Station Officer or Fire Commander

Fire Fighter – means a trained fire fighter below the rank of Sub Station Officer

Instructor – means an Instructor at the Learning Academy, or a Check and Standards Officer

EVT – means an Emergency Vehicle Technician or Maintenance Officer who works with ARFF emergency vehicles

For each of these jobs, please fill in the information requested: list years and location.

For Example:

✔	Senior Officer	1993-1995 1997-1998	Townsville Cairns

Tick	Job assignment	Years	Location
	a. Senior Officer		
	b. Officer		
	c. Firefighter		
	d. Instructor		
	e. Emergency vehicle technician (EVT)		

19. For **each** of the jobs that you ticked in Question 18 above ONLY, please provide the following details about your **contact** with aqueous film forming foams (including Ansulite AFFF and 3M Lightwater) by **ticking** the relevant boxes. Note: Do not include exposure to Solberg foam in this response.

a. SENIOR OFFICER

How frequently did you have contact with aqueous film forming foams? **Tick One Box ONLY**	
	Never
	Less than once a month
	Once a week
	Twice a week
	Most days

How much skin was routinely exposed to aqueous film forming foam during this job? **Tick One Box ONLY**	
	Mostly just hands
	Hands and arms
	Hands, arms, and trunk
	Whole body skin exposure

19 (continued)

b. OFFICER

How frequently did you have contact with aqueous film forming foam? **Tick One Box ONLY**	
	Never
	Less than once a month
	Once a week
	Twice a week
	Most days

How much skin was routinely exposed to aqueous film forming foam during this job? **Tick One Box ONLY**	
	Mostly just hands
	Hands and arms
	Hands, arms, and trunk
	Whole body skin exposure

19 (continued)

c. FIRE FIGHTER

How frequently did you have contact with aqueous film forming foam? **Tick One Box ONLY**	
	Never
	Less than once a month
	Once a week
	Twice a week
	Most days

How much skin was routinely exposed to aqueous film forming foam during this job? **Tick One Box ONLY**	
	Mostly just hands
	Hands and arms
	Hands, arms, and trunk
	Whole body skin exposure

19 (continued)

d. INSTRUCTOR

How frequently did you have contact with aqueous film forming foam? **Tick One Box ONLY**	
	Never
	Less than once a month
	Once a week
	Twice a week
	Most days

How much skin was routinely exposed to aqueous film forming foam during this job? **Tick One Box ONLY**	
	Mostly just hands
	Hands and arms
	Hands, arms, and trunk
	Whole body skin exposure

19 (continued)

e. EMERGENCY VEHICLE TECHNICIAN (EVT)

How frequently did you have contact with aqueous film forming foam? **Tick One Box ONLY**	
	Never
	Less than once a month
	Once a week
	Twice a week
	Most days

How much skin was routinely exposed to aqueous film forming foam during this job? **Tick One Box ONLY**	
	Mostly just hands
	Hands and arms
	Hands, arms, and trunk
	Whole body skin exposure

20. Have you had any **<u>other</u> jobs at Airservices**
in which you routinely handled or used aqueous film forming foams?

Yes ☐ No ☐

If Yes, please provide the **details** requested below:

Role and location within Airservices	Dates	Describe foam use and contact (frequency, amount of skin exposure)

21. Have you ever had any **<u>other</u> jobs** (**<u>NOT</u>** at Airservices)
in which you were in **contact with PFCs** or similar chemicals?

(e.g. Firefighter (voluntary, military), facility producing/processing PFCs or similar chemicals, carpet cleaning, retreating carpets or rugs, or professional carpet installation)

Yes ☐ No ☐

If Yes, please provide the **details** requested below:

Organisation/Location	Dates	Describe foam use and contact (frequency, amount of skin exposure)

<u>THANK YOU</u> <u>FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE</u>.

Please place in envelope and send back to ENTOX!

Sullivan Nicolaides PATHOLOGY

Clinical Trials

Laboratory Request Form

PATIENT IDENTIFICATION	SEX	DATE OF BIRTH

ADDRESS

Airservice Australia Project, ENTOX, 39 Kessels Rd, Coopers Plains, Qld 4108

TESTS REQUESTED

E/LFT

HDL/LDL

Attention Collection Staff

Please collect 4 x 8.5ml SST tubes. Transport all tubes to SNP Taringa Laboratory.

Please call Courtney Butler (Clinical Trials Manager – Taringa – 07 33778782) if you have any queries.

Date of Collection: ☐☐ / ☐☐☐ / ☐☐☐☐ (DD / MMM / YYYY)

Time of Collection: ☐☐ : ☐☐ (24hr clock)

...../...../.....

MB2
CHDL

COLL

SULLIVAN NICOLAIDES PTY LTD ACN 078 202 196 APA

134 Whitmore Street Taringa 4068

COPY REPORTS TO

SNP Clinical Trials Manager

REFERRER

M 1 2 4 6 7

ENTOX – Dr Jochen Mueller

M12467
CXB34

ACCOUNT NAME

Airservice Australia Project

COLL INIT	LOC CODE
U	BR400
COLL CODE	PAY CAT
D	AIRAP

SST Tube	EDTA Tube	LiH Tube	CIT Tube	PPT Tube
ACD Tube	Vacu Tube	Rand Urine	Jar Other	Histo
Pap Slide	ThP ThinPrep	Chlam Swab	Transc Red swab	Plain Black swab
Unspun	Frozen	Card	Other	

Attention Collection Staff

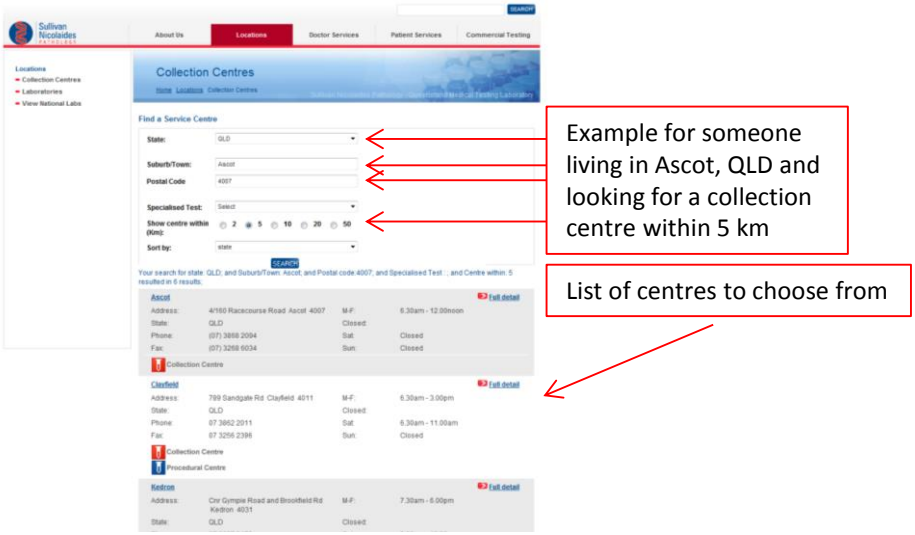
1. Collect **4 x 8.5ml SST Vacutainer**. Gently invert x 5.
2. Centrifuge SSTs.
3. Transport all specimens to SNP Taringa.

Attention SRA/Sendaways Staff

1. Send **3 x 8.5ml SST Vacutainers** to the attention of Clinical Trials Department.

Effective Date: 16 January 2013 Issue 1 ::ODMA\MeridioIS\GTARNT60#73918#5

The easiest way of finding the blood collection centre closest to you is to type in your details (state/suburb/postal code) on one of the websites listed below the figure, which illustrates an example.



Brisbane/Cairns/Coolangatta/Darwin/Hamilton Island/Mackay/Rocky/Sunshine Coast/Townsville
SNP – Sullivan Nicolaides Pathology
http://www.snp.com.au/locations/Collection-Centres.aspx

Sydney
DHM - Douglass Hanly Moir Pathology
http://www.dhm.com.au/our-locations/collection-centres.aspx

Melbourne & Avalon Airport
Melbourne Pathology
http://www.mps.com.au/locations/collection-centres.aspx

Adelaide
Clinpath Laboratories
http://www.clinpath.com.au/locations/Collection-Centres.aspx

Perth
Clinipath Pathology
http://www.clinipathpathology.com.au/locations/Collection-Centres.aspx

Canberra
Capital Pathology
http://www.capitalpath.com.au/locations/collection-centres.aspx

Tasmania - Launceston
Launceston Pathology
http://www.launcestonpath.com.au/locations/Collection-Centres.aspx

Tasmania - Hobart
Hobart Pathology
http://www.hobartpath.com.au/locations/Collection-Centres.aspx

Appendix B

Analytical Methodology for PFAA analysis

An aliquot of 200 µl serum was transferred to a 2 ml Eppendorf tube followed by addition of the ^{13}C-labeled internal standards. Acetonitrile was used to precipitate the proteins and the extraction was facilitated by ultrasonication and vortex mixing. After centrifugation, the supernatant was filtrated into a LC vial through a 2 µm GHP membrane (Pall, East Hills, NY, USA) and reduced to 200 µl using nitrogen, after which 300 µl 5 mM ammonium acetate in water and the performance standards $^{13}C_8$-PFOS,$^{13}C_8$-PFOA were added. PFAAs were determined by HPLC-MS/MS using an AB/Sciex API5500Q mass spectrometer (AB/Sciex, Concord, Ontario, Canada) equipped with an electrospray (TurboV) interface coupled to a Shimadzu Nexera HPLC system (Shimadzu Corp., Kyoto, Japan). Separation was achieved using a 4 micron 50x2.0mm Phenomenex C18 Gemini column (Phenomenex, Torrance, CA) run at 45 ^{o}C, and a flow rate of 0.3 mL min-1.

Quality Assurance and Quality Control (QA/QC)

Quantification was performed using the internal standard method with non-extracted standards dissolved in 30% methanol in aqueous 5 mM ammonium acetate. The recoveries for $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS, $^{13}C_4$-PFOA, ranged between 70% to 100% (an acceptable recovery rate is generally set to be between 50% and 120%). Ultra pure water was used as a procedural blank and was prepared for each batch of 10-20 samples and extracted in the same way as the real samples. Reproducibility was calculated as the relative standard deviation (RSD) of seven individual analyses of a QA/QC pooled serum sample on different days and was found to be below 10% for PFOS, PFHxS and PFOA. The accuracy of the analysis, estimated by analysing a reference sample, was found to be within the acceptable range (Table 2).

Table B1. Accuracy of serum PFAA analysis (ng/mL) by analysing a NIST reference serum sample (SRM 1957).

	PFOS	PFHxS	PFOA
This study	10.3	2.8	3.6
Reference value[1]	10.6[2]	-	5.0
MTM[3]	9.7	2.9	3.4

[1)] NIST, National institute of standards and technology. U.S. Department of Commerce

[2)] Riddell et al., 2009

[3)] Man-Technology-Environment Research Centre, Örebro University, Sweden. The MTM PFAA method has been evaluated in an interlaboratory study on fish muscle with satisfactory z-scores (z<2)(van Leeuwen et al., 2009).

Statistical analysis

The regression was conducted on the log10-transformed blood concentrations of the three analytes. Because age and total years of jobs with foam contact are highly correlated, an interaction term for these two variables was included in the regression.

The final model for prediction of blood concentrations of the three analytes is of the form:

$$Log10PFOA = \beta0 + \beta1(sex) + \beta2\ (F) + \beta3(Age) + \beta4\ (Donor) + \beta5(Age * F)$$

Where

sex is 1 for females and 0 for males, F is years of foam exposure, Age is age in years, Donor is 1 for blood donor vs. 0 for non-donors, and β values represent the regression coefficients in Table ANOVA.

Table B2. Results from ANOVA analysis.

	PFOS		PFHxS		PFOA	
	Beta (SE)	p value	Beta (SE)	p value	Beta (SE)	p value
Female vs. male	-0.348 (0.14)	0.014	-0.424 (0.182)	0.021	-0.228 (0.098)	0.021
Foam exposure (yrs)	0.077 (0.014)	<0.001	0.088 (0.019)	<0.001	0.018 (0.01)	0.084
Age (yrs)	0.037 (0.005)	<0.001	0.046 (0.006)	<0.001	0.012 (0.003)	0.001
Blood donor (yes vs. no)	-0.239 (0.056)	<0.001	-0.319 (0.073)	<0.001	-0.105 (0.04)	0.009
Interaction term (age x yrs of foam exposure)	-0.001 (0.0003)	<0.001	-0.002 (0.0003)	<0.001	-0.0003 (0.0002)	0.06
Adj. R2 for model:	0.498		0.497		0.162	

Table B3. Number of participants (n) for each airport fire station, percentage participation (%) based on 731 recruitment packs that were sent out to 21 airport fire stations, and blood serum levels of PFOS, PFHxS and PFOA (ng/ml serum, ppb) in ARFF firefighters. M=median, A=arithmetic mean, min=lowest concentration, max=highest concentration.

			PFOS				PFHxS				PFOA			
Station	n	%	M	A	min	max	M	A	min	max	M	A	min	max
Adelaide	11	27	99	86	21	145	52	45	6	72	5	5	3	8
Alice Springs[2]	2	17	-	-	-	-	-	-	-	-	-	-	-	-
Avalon	7	32	11	39	10	142	2	19	2	81	3	3	1	6
Brisbane	10	13	62	67	8	118	25	27	1	56	4	4	1	7
Broome[1]	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Cairns	7	17	59	61	25	102	23	20	8	34	4	4	3	6
Canberra	7	28	62	49	14	94	23	19	3	36	5	4	2	6
Coolangatta	6	19	71	86	12	184	40	40	2	75	6	5	4	7
Darwin	9	21	40	61	7	150	10	24	1	62	4	4	1	6
Hamilton Island[1]	0	0	-	-	-	-	-	-	-	-	-	-	-	-
Hobart	8	36	49	73	31	192	18	27	4	94	4	5	2	8
Karratha	6	40	109	117	49	186	59	56	23	85	4	5	4	6
Launceston[2]	3	15	-	-	-	-	-	-	-	-	-	-	-	-
Mackay	6	38	51	61	18	132	26	25	2	44	5	5	3	8
Melbourne	23	25	59	52	3	105	27	36	23	56	3	4	0.3	8
Perth	8	11	50	64	6	180	21	25	1	60	4	6	1	18
Rockhampton	10	53	110	149	7	391	45	73	1	277	5	6	1	13
Sydney	17	19	96	78	6	205	38	38	2	138	5	5	2	14
Sunshine coast	6	33	58	63	17	114	20	27	4	65	4	5	3	8
Townsville[2]	4	11	-	-	-	-	-	-	-	-	-	-	-	-
Yulara[1]	0	0	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	**150**	**21**	**66**	**74**	**3**	**391**	**25**	**33**	**1**	**277**	**4**	**5**	**0.3**	**18**

[1] No data available due to 0 participants

[2] Stations with less than 6 participants are only included in the total for confidentiality reasons.

Appendix C

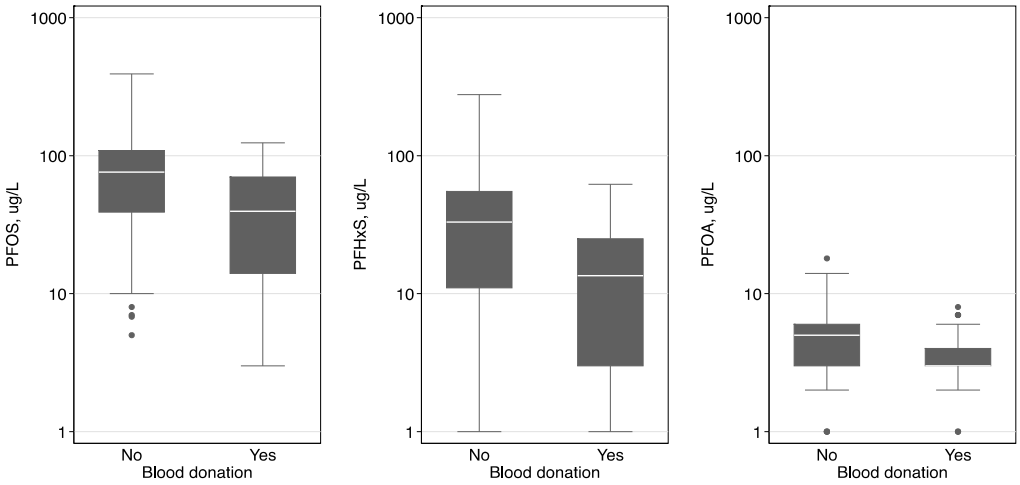


Figure C1. Blood concentrations of PFOS, PFHxS and PFOA by blood donation.

Appendix D

Table D1. Models based on participants with measured BMI and not taking cholesterol-lowering medications (for cholesterol, HDL, LDL, and triglycerides models) or gout medication (uric acid model).

			Beta (SE) *p value*		
Parameter	Cholesterol (n=135)	HDL (n=122)	LDL (n=134)	Triglycerides (n=121)	Uric acid (n=138)
Age (yrs)	*NS*	*NS*	*NS*	*NS*	*NS*
Sex (female vs. male)	*NS*	0.30 (0.14) *p<0.03*	*NS*	*NS*	-0.08 (0.03) *p<0.01*
BMI (kg/m^2)	*NS*	-0.04 (0.007) *p<0.001*	*NS*	0.07 (0.02) *p<0.001*	0.004 (0.0015) *p<0.01*
Current smoker (Y vs. N)	*NS*	-0.25 (0.15) *p<0.1*	*NS*	*NS*	*NS*
Total serum protein (albumin+globulin)	0.07 (0.02) *p<0.001*	*NS*	0.06 (0.02) *p<0.003*	*NS*	0.003 (0.001) *p<0.05*
log10 PFOA	*NS*	*NS*	*NS*	-0.71 (0.36) *p<0.1*	*NS*
log10 PFOS	*NS*	*NS*	*NS*	*NS*	*NS*
log10 PFHxS	*NS*	*NS*	*NS*	*NS*	*NS*
Model Adj. R^2:	0.08	0.26	0.06	0.09	0.15

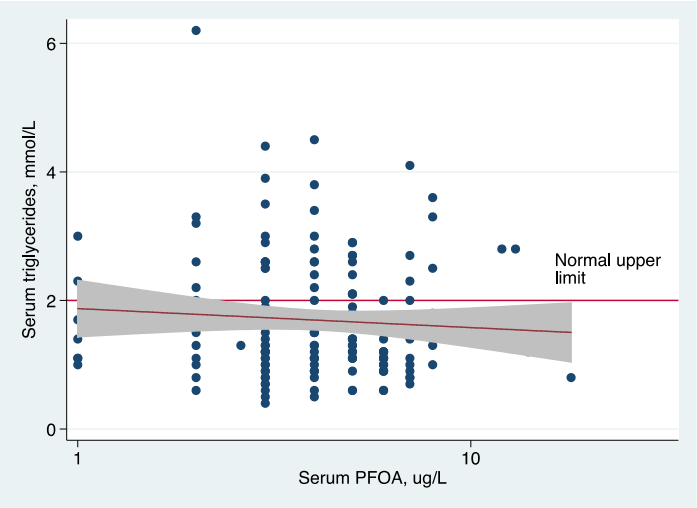


Figure D1. Serum triglycerides as a function of serum PFOA

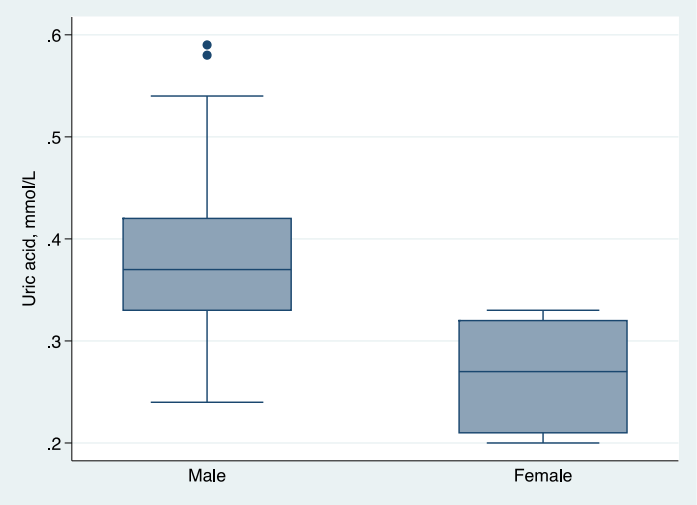


Figure D2. Uric acid by sex.

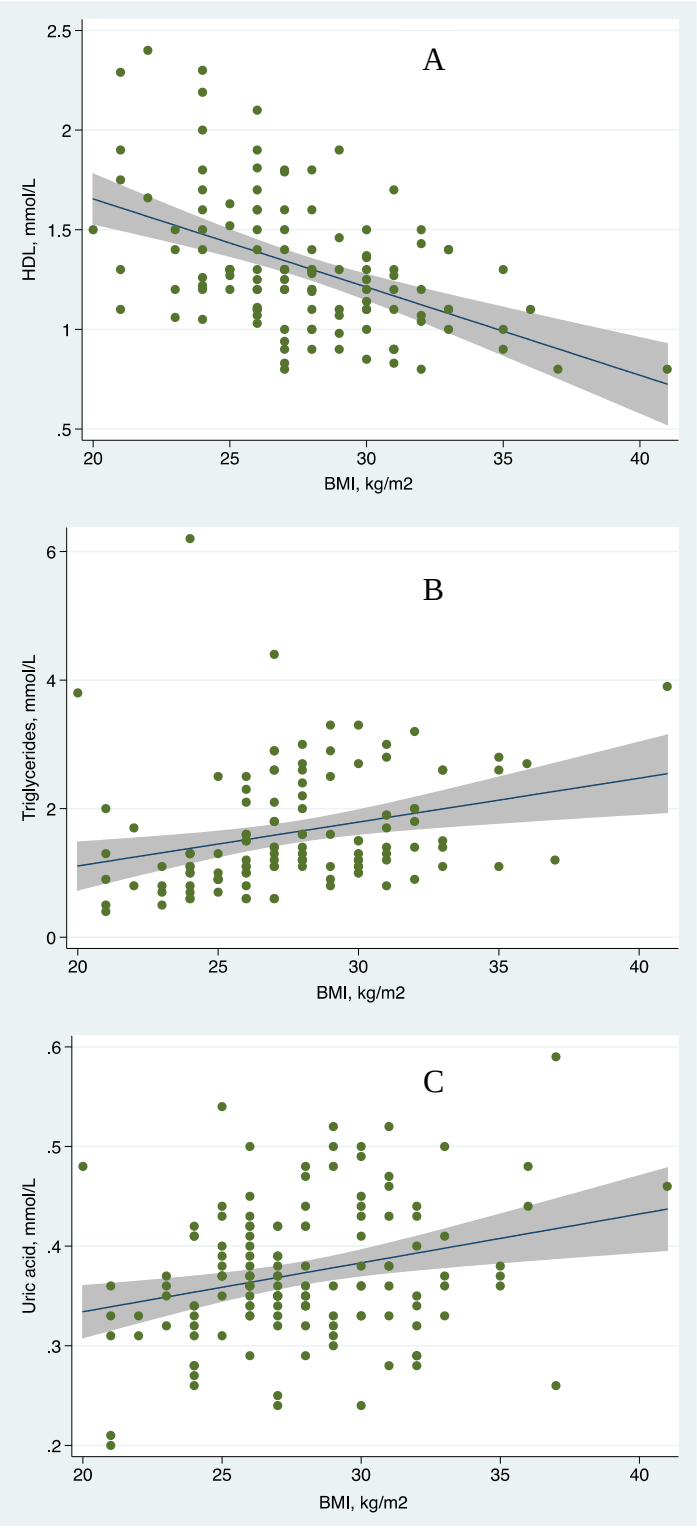


Figure D3. Unadjusted relationships between HDL (A) and triglycerides (B) and BMI in individuals not taking cholesterol-lowering medication, and between uric acid (C) and BMI in individuals not taking medication to treat gout.