Prepared for

Airservices Australia

Subject

Evaluation of per- and poly- fluoroalkyl substances (PFASs) in Airservices Australia's Aviation Rescue Fire Fighting Service (ARFFS) Staff – 2018/2019

Project Team

Nilsson S, Aylward L, Kay M, Smurthwaite K, Toms LM, King L, Marrington S, Rotander A, Kirk M, Mueller J & Bräunig J

14 December 2020
UniQuest Project No: C02758-005

The main commercialisation company of

The University Of Queensland
AUSTRALIA

UNIQUEST

Acknowledgements

The project team would like to acknowledge the assistance, involvement and contribution of the following people: All participating ARFFS operational and retired staff and the staff at Sullivan and Nicolaides Pathology and Sonic Healthcare.
The Queensland Alliance for Environmental Health Sciences at the University of Queensland is co-funded by Queensland Health.

Suggested Citation:
Nilsson S,[1] Aylward L,[2] Kay M,[1] Smurthwaite K,[3] Toms LM,4 King L,[1] Marrington S,[1] Rotander A,[5] Kirk M,[3] Mueller J,[1] Bräunig J.[1] Evaluation of per- and poly- fluoroalkyl substances (PFASs) in Airservices Australia's Aviation Rescue Fire Fighting Service (ARFFS) Staff – 2018/2019. The University of Queensland, 2020.

1. Queensland Alliance for Environmental Health Sciences, The University of Queensland, Brisbane, QLD, Australia
2. Summit Toxicology, LLP, Falls Church, VA, USA
3. National Centre for Epidemiology and Population Health, Research School of Population Health, The Australian National University, Canberra, ACT, Australia
4. School of Public Health & Social Work, Queensland University of Technology, Brisbane, Australia
5. Man-Technology-Environment Research Centre, Örebro University, Örebro, Sweden

Direct Enquiries to:

Dr Jennifer Bräunig
Queensland Alliance for Environmental Health Sciences (QAEHS)
PACE Building
20 Cornwall St, Woolloongabba, Qld 4102
Email j.braunig@uq.edu.au
Web qaehs.centre.uq.edu.au
Centre phone +61 7 344 32443

Executive Summary

In 2018 a follow-up study to the 2013 Airservices Per– and Polyfluoroalkyl Substances (PFASs) Exposure Study was conducted. All Aviation Rescue Firefighting Services (ARFFS) staff as well as former staff, including retired and ex-staff, were invited to participate in the study to evaluate their PFAS serum concentration as well as measure some associated biochemical markers. The study was approved by the University of Queensland Human Research Ethics Committee under application number 2018001790.

The aims of the study were to

i) assess PFAS blood concentrations (integrative exposure) in Airservices current and former staff and evaluate links to 'work history';

ii) evaluate exposure trends including through re-recruitment of participants from the previous study and comparison of data with general population data;

iii) assess PFAS relevant biochemical markers and/or confounders associated with PFAS serum concentrations; and

iv) provide ongoing advice to Airservices to assess and minimise exposure risks to PFASs.

A total of 799 current and former Airservices staff took part in the study, of these 130 had previously participated in the 2013 Airservices PFAS Exposure study. Each participant provided a blood sample for PFAS and biochemical marker analysis and filled out a questionnaire about their work history, general health and lifestyle factors.

Six perfluoroalkyl acids (PFAAs), PFOA, PFNA, PFDA, PFHxS, PFHpS and PFOS, were detected in more than 90% of participants. Of these, average concentrations of PFHxS, PFHpS and PFOS, were elevated above the 95%ile of the general Australian population, while levels of PFNA and PFOA were similar to those found in the general population. The concentrations of PFOS, PFHxS and PFHpS were strongly correlated in serum of participants, indicating a common source of exposure to these chemicals.

Participants who started working prior to 2005, the year in which 3M LightWater aqueous film forming foam (AFFF) was substituted, showed average concentrations of PFHxS, PFHpS and PFOS higher than the general population, while those who started working for Airservices after 2005 had average concentrations similar to those of the general population. This suggests that substitution of 3M LightWater AFFF has been a successful measure to reduce occupational exposure in participants who started working after 2005.

An assessment of differences in PFAS serum concentrations by work stations did not show major differences in exposure between stations in firefighters employed prior to and post 2005. However, these analyses were limited by the fact that many Airservices staff have worked at more than one location throughout their career. Each station was represented by participants who had worked as firefighters for the particular station for four or more years (while not working at any other stations for

more than two years). Separate assessments were performed for participants who were employed before and after 2005.

An analysis by job position showed higher PFAS concentrations in Emergency Vehicle Technicians (EVTs) compared to firefighters (both in participants who started working before 2005 and after 2005). While this indicates potential higher exposure of EVTs to PFASs the evidence is not conclusive, as the group size of EVTs (n = 7) was small in comparison to the group of firefighters (n = 213) employed post 2005.

Participants who reported to be blood donors had lower average concentrations of PFOA, PFHxS, PFHpS and PFOS compared to those who did not report to be blood donors. Frequency of blood donation was also observed to be associated with lower concentrations of these chemicals.

Average concentrations of PFOA, PFHxS, PFHpS and PFOS declined by 55 – 65% between the two studies in 2013 and 2019. Using PFAS serum concentrations from the 130 participants who took part in both Airservices Exposure Studies it was possible to calculate average serum elimination half-lives for several PFASs. The average half-life was longest for PFHxS with 8.2 years, followed by PFHpS with 7.8 years and PFOS with 6.6 years. These results were in line with serum elimination half-lives reported in the scientific literature. No correlation was found between half-lives of serum elimination and their initial concentration. Among the individuals who took part in both studies the average decrease of PFOA concentrations was 58%, 43% for PFHxS, 45% for PFHpS and 49% for PFOS.

Multiple statistical approaches and assessments were used to evaluate the associations between biochemical markers, self-reported health issues and selected PFAA serum concentrations. This included assessment of overall linear relationships, and the risk of having out-of-range biomarker levels or any self-reported health issues with increasing PFAA concentration. The assessments were conducted by cross-sectional evaluation of potential associations between selected biochemical markers, self-reported health issues and selected PFAAs as a follow-up to the 2013 Airservices study. In addition, a subset of the participants from the 2013 Airservices study also participated in the current study, allowing for a longitudinal assessment of both changes in selected serum PFAAs and changes in biomarkers in those individuals.

Of all the assessed outcomes, some associations were found:

- For serum lipids, increasing levels of cholesterol were associated with increasing levels of PFOS, and increased LDL was associated with increasing levels of all four assessed PFAAs. No associations were found for HDL.
- Increasing levels of TSH, a biomarker for thyroid function, were found to be associated with increasing levels of serum PFOA. No associations with other PFAAs or for other thyroid hormone endpoints were found.
- Decreasing levels of ALT, a biomarker for liver damage, were found to be associated with increasing levels of PFOA.

- Biomarkers of kidney functions, urate and eGFR, were associated with PFOA concentrations. Urate levels were found to increase with increasing levels of PFOA, while a decreased risk of having abnormally low eGFR was observed in relation to increasing PFOA concentrations.
- Of the 12 categories of self-reported health conditions assessed (asthma, cancer (any, skin, prostate), cardiovascular disease, diabetes (Type 2), high blood pressure, kidney problems, liver problems, reproductive/fertility problems, serious arthritis and thyroid problems), a lower risk for self-reported serious arthritis and a higher risk of skin cancer was associated with increasing concentrations of serum PFOA. No review of medical records was performed to confirm the self-reported conditions.

Overall, the associations that were found were relatively small and did not result in an increased risk of out-of-range (potentially abnormal) values across the serum PFAA concentrations in this study.

In the longitudinal assessment conducted for the subset who participated in both the 2013 and 2019 study, no significant associations were found between changes over time in cholesterol, HDL, LDL or urate and the changes in PFAA concentration. However, the limited number of individuals in this assessment may have limited the chances of finding a temporal association.
A large number of statistical comparisons were conducted given the large number of outcomes and multiple exposure markers that were evaluated, and some associations may have been observed due to chance. In summary, this study provides some evidence of associations between levels of selected biochemical markers and PFAA exposure. In general, though, the associations that were observed were relatively small and further research is warranted to determine if such associations have any clinical significance.

Abbreviations

AFFF	Aqueous Film Forming Foam
ALT	Alanine aminotransferase
ARFF	Aviation Rescue and Fire Fighting
ARFFS	Aviation Rescue Fire Fighting Service
BMI	Body Mass Index
eGFR	Estimated glomerular filtration rate
HDL	High density lipoprotein
LDL	Low density lipoprotein
ng	Nanograms
PFAAs	Perfluoroalkyl acids
PFASs	Per- and Polyfuoroalkyl substances
PFHpA	Perfluoroheptanoic acid
PFOA	Perfluorooctanoic acid
PFNA	Perfluorononanoic acid
PFDA	Perfluorodecanoic acid
PFUnDA	Perfluoroundecanoic acid
PFBS	Perfluorobutanesulfonic acid
PFHxS	Perfluorohexanesulfonic acid
PFHpS	Perfluoroheptanesulfonic acid
PFOS	Perfluorooctanesulfonic acid
ppb	Parts Per Billion
RAAF	Royal Australian Air Force
SNP	Sullivan Nicolaides Pathology
T3	Triiodothyronine
T4	Thyroxine
TSH	Thyroid stimulating hormone
QAEHS	Queensland Alliance For Environmental Health Sciences
QC/QA	Quality Control and Quality Assurance

Contents

1. Introduction

Airservices Australia (“Airservices”) was established in 1995 as an Australian Government-owned corporation responsible for air traffic control and related aviation services for the Australian aviation industry; this includes the provision of aviation rescue and fire fighting (ARFF) services at major civilian airports around Australia. From the late 1970s until 2010, Airservices and its predecessors used various types of Aqueous Film Forming Foam (AFFF) for firefighting purposes. Historically, ARFF services staff were required to regularly train with these products to maintain their competency levels. The AFFF products that were used by Airservices have varied over time. Before 2010, these included 3M LightWater AFFF ('3M LightWater') and Ansul Ansulite 6% AFFF ('Ansulite'). Both of these products contain per- and poly- fluoroalkyl substances (PFASs), including perfluorooctanesulfonic acid (PFOS) and perfluorohexanesulfonic acid (PFHxS) as key ingredients in 3M LightWater and precursors to perfluorooctanoic acid (PFOA) as key ingredients in Ansulite. In 2010 Airservices switched to the use of Solberg RF6, a fluorine-free foam, at all locations with the exception of Darwin and Townsville (these latter locations completed the transition to Solberg RF6 in late 2019).

In 2013 Airservices commissioned The University of Queensland to conduct a study on the exposure of Airservices Aviation Rescue Fire Fighting Services staff (from here referred to as “Airservices staff”) to PFASs. The outcomes showed a positive correlation between the length of employment of staff as fire fighters and serum concentrations of some selected PFASs. Airservices commissioned a follow-up study which commenced in 2018, five years following the initial study. The aims of the study were as follows:

Aim 1: Assess PFAS blood concentrations (integrative exposure) in Airservices current and former staff and evaluate links to ‘work history’;

Aim 2: Evaluate exposure trends (which answers whether blood levels are consistently changing, and if so, how those trends compare to those observed in the general population);

Aim 3: Assess PFAS relevant biochemical markers and/or confounders associated with PFAS serum concentrations; and

Aim 4: Provide ongoing advice to Airservices to assess and minimise exposure risks to PFASs.

1.1. Background PFAS Information

Per- and poly- fluoroalkyl substances, or PFASs, is a collective term used for several classes of chemicals which have been heavily used in consumer products and industrial applications since the 1950s. Perfluorinated substances, such as perfluoroalkyl acids (PFAAs) have all hydrogen atoms on the carbon chain substituted by fluorine atoms, while polyfluorinated substances are only partially fluorinated and have some carbons bound to hydrogen (Figure 1)[1].

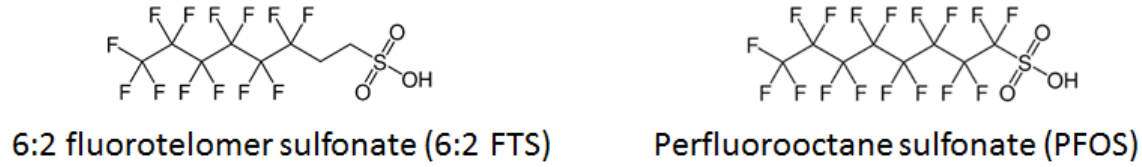

Figure 1: *Chemical structure of a polyfluorinated substance (left) and the related perfluorinated substance (right), both based on an 8 carbon chain length.*

The unique physico-chemical properties of PFASs led to their use in AFFF, where they are especially efficient at smothering hydrocarbon-fuel fires [2]. The repeated use of AFFF, especially during fire training exercises, has led not only to widespread environmental contamination with PFASs, but also to the exposure of firefighters with these chemicals [3]. These physio-chemical properties also affect how these chemicals are accumulated in the body. Many PFASs are biologically persistent and can be detected in the blood serum of individuals who are exposed. Figure 2 shows a simple timeline of the usage of PFASs in AFFF and points out some dates specific to Australia and Airservices (red).

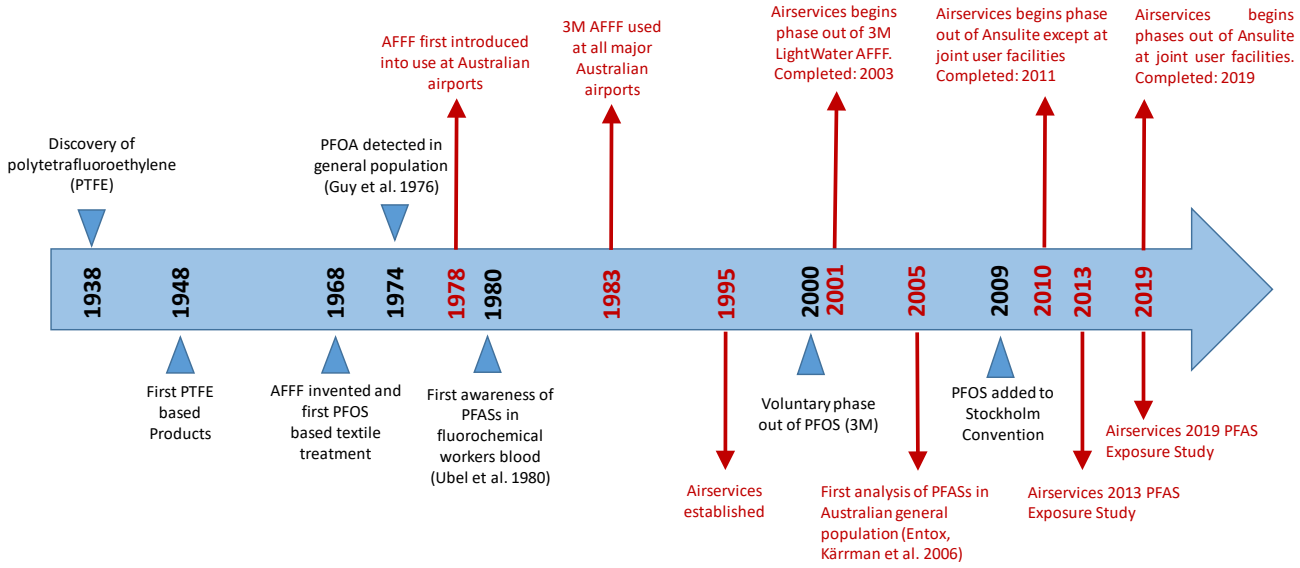

Figure 2: *Simple timeline of the use of PFASs with some Australia specific dates in red. [4, 5]*

The general population is exposed to PFASs through multiple different routes, including the use of consumer products, food and drinking water [6]. As a result, many PFASs are detected in serum samples taken from the general public, that is, people who have no occupational exposure to PFASs. Since 2002 the Queensland Alliance for Environmental Health Sciences (QAEHS) has conducted approximately biennial evaluations of the blood serum concentrations of PFASs in pooled samples of a general Australian population from South East Queensland [7-9]. Pooled samples mean that serum from 100 people of the same gender and age group is mixed into a single sample. The concentration measured in the pooled sample is an estimate of the average concentration in the sampled group.

These pooled sample results are the only data available to indicate exposure levels for the general Australian population. However, the data matches well with estimates of average concentrations in the general population in the USA [10], which was obtained based on measurements in individual samples. The pooled samples from South East Queensland have also allowed assessment of temporal trends of PFAS serum concentration in the Australian general population. The data suggest a decline in the levels of the most prominent PFASs (i.e. PFOA, PFOS, and PFHxS, all of which are PFAAs) in the Australian population from 2002/03 when compared to the most recent collection period for which data is available in 2016/17 (Figure 3, only PFOS shown). These datasets present important baseline values for the evaluation of PFASs in exposed populations and hence serve as a reference. The trends over time that are observed for many PFASs in the general population must be taken into account in the evaluation of the data for Airservices staff.

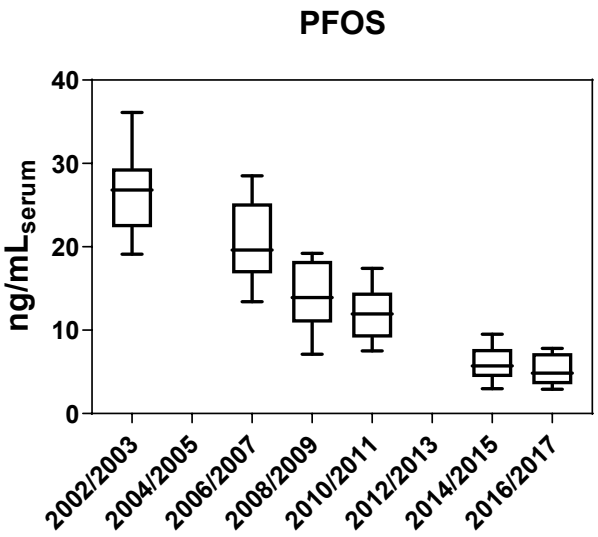

Figure 3: *Time-trend of PFOS concentrations in the general Australian population. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers minimum and maximum concentrations for PFOS in adults >16 years of age by collection year [7].*

1.2 Overview of 2013 Airservices Exposure Study

In 2013, all 731 ARFFS operational staff at Airservices were invited to participate in a study to evaluate their past exposure to AFFF by means of measuring the concentration of PFAAs in their blood serum. Of the 731 Airservices staff invited to participate in the study, 149 (20%) consented to take part. The three PFAAs found at the highest concentrations in Airservices staff, PFOS, PFHxS, and PFOA, were chosen as biomarkers for AFFF exposure. PFOS and PFHxS have been previously identified in different 3M LightWater AFFF formulations, while PFOA is a known breakdown product of PFAA precursors contained in Ansulite, as well as being found in 3M LightWater at low concentrations [11].

Participants were found to have concentrations of PFOA similar to those found in the general Australian population but elevated concentrations of PFOS and PFHxS. A probable explanation is that serum concentrations of PFOS and PFHxS were influenced by direct or indirect contact with some AFFF formulations. In addition, the concentrations of PFOS and PFHxS in serum of Airservices personnel are strongly correlated, which indicates that these two chemicals have come from the same source and most likely have the same exposure pathway. Geometric mean serum levels in Airservices staff were found to be approximately 20 times lower than reported levels in PFAS manufacturing workers from the U.S. who had high occupational exposure to these chemicals [12].

The concentrations of PFOS and PFHxS were found to be positively associated with length of employment working with AFFF 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 time period coincided with the phase-out of LightWater AFFF from Airservices training facilities starting in 2002 (completed in 2003) and suggested that the exposures to PFOS and PFHxS in AFFF had declined in recent years.

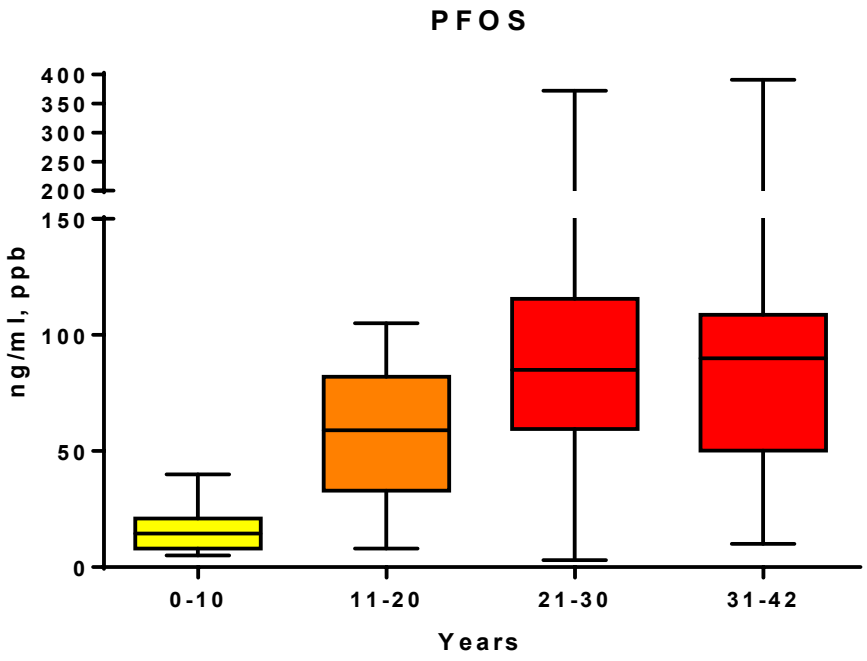

Figure 4. *PFOS concentrations (y-axis) in relation to years of employment in jobs with foam contact (x-axis), including jobs outside Airservices. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers min and max concentrations [3].*

There was no significant difference in PFAA blood serum levels between staff working at different ARFF stations across Australia. However, this conclusion should be accepted with caution for two reasons. First, there was a low participation rate for many of the stations. Second, many of the participants had been positioned at (i.e. rotated through) different stations during their Airservices employment.

Self-reporting of skin contact and frequency of contact with AFFF were used as an index of exposure. No relationship between PFOS levels and skin exposure were found. This index of exposure is limited as it relies on self-report and it only considers skin exposure to AFFF. Self-reporting of skin exposure does not capture other routes of potential exposure.

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

The results of the 2013 study were well received nationally and internationally and provided a platform for a follow-up study. Following on from the 2013 Airservices Exposure study, QAEHS was contracted to conduct a follow-up study 5 years later.

2. Methods

2.1. Study Objectives and Design

The objectives and study design of the 2018/2019 PFAS Exposure Study was developed over a series of workshops which included attendees from the University of Queensland, associated research organisations, and Airservices staff representing different areas of the company. Representatives of ARFFS included a Local Operations Manager, a Fire Commander, a Station Officer, a Leading Fire Fighter, an Emergency Vehicle Technician, as well as a representative from the United Firefighters Union.

To facilitate the development of a study design, a range of study design options were presented to the working group members and discussed in detail. Members of the working group were asked to discuss these options with their work peers to identify and justify a preferred study design that allowed achievement of study aims specific to each of the designs.

The working group reached a general consensus that any new study should combine a cross sectional approach adopting the study design from the 2013 Airservices Exposure Study with a focus on re-recruiting participants from the previous study for a longitudinal evaluation, but in addition being inclusive of all current, as well as former staff; i.e. retired and ex-Airservices staff.

Discussions at the two Technical Working Group Workshops identified some limitations of the 2013 study with regards to recruitment and implementation, the questionnaire, and logistics. All of these aspects were improved on in the new study design.

Together with the working group four study aims were developed:

Aim 1: Assess PFAS blood concentrations (integrative exposure) in Airservices current and former staff and evaluate links to ‘work history’;

Aim 2: Evaluate exposure trends (which answers whether blood levels are consistently changing, and if so, how those trends compare to those observed in the general population);

Aim 3: Assess PFAS relevant biochemical markers and/or confounders associated with PFAS serum concentrations; and

Aim 4: Provide ongoing advice to Airservices to assess and minimise exposure risks to PFASs.

The project was designed to measure both PFASs and a number of biochemical markers in as many personnel as were willing to participate, including in former staff. Furthermore, this 2018 study would seek to investigate temporal trends of PFAS body burden/exposure in Airservices staff by recruiting and enrolling staff who participated in the 2013 study.

A third workshop was held in February 2020 upon completion of data collection to present preliminary data and discuss analysis options.

2.2. Ethical Clearance

After the first two workshops the research team presented the study design to Airservices and it was approved formally in July 2018. A timeline of the study can be found in Figure 5. After the study was formally approved the Research Team sought ethical approval for study execution from the University of Queensland Human Research Ethics Committee, which was granted in October 2018 under clearance approval number 2018001790. Over the course of the project 4 amendments were made to the original ethics approval to (1) include additional questions in the participant questionnaire; (2) add total serum proteins to the biochemical parameters to be measured in participants' blood; (3) send out the results of the biochemical analysis to participants as soon as they were received by the research team; and (4) to send 10 randomly selected blood samples to two independent contract laboratories to measure PFAS concentrations independently as an additional quality control and quality assurance step.

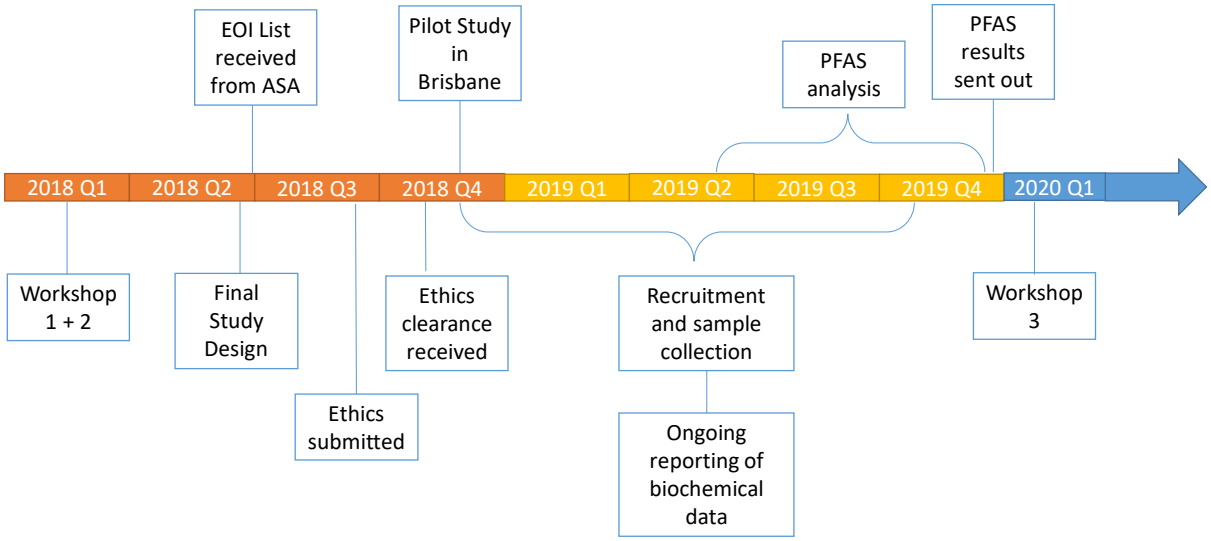

Figure 5: *Project timeline, January 2018 to April 2020.*

2.3. Participant Recruitment

Following study approval Airservices provided UQ with contact details of Airservices staff who had already expressed their interest in taking part in a second exposure study. This file was based on internal Airservices communications and included contact details of 667 individuals who had submitted an expression of interest (EOI). From this point onwards all participant recruitment was handled by UQ, without the direct involvement of Airservices. Airservices involvement in the following recruitment process was to send out memos to current and former staff and Union members outlining the study commencement and welcoming any new EOI's to register (see Table 1 for final EOI numbers) directly to the Research Team.

Table 1: *Final numbers of 'Expressions of Interest' to take part in the 2018 PFAS Exposure Study.*

Study EOI's	**No.**
Current Staff	625
Former Staff (Retired or ex-staff)	255
Total EOIs	**880**
Refusals (withdrew their participation during study)	**30** (20 current staff and 10 former staff)

A study database was created to assist in the recruitment process and was used to record participant contact information, contact attempts, any further information about their participation, track data collection, and track delivery of results to participants.

Recruitment and data collection were done progressively by location with a first pilot held in Brisbane (Nov-Dec 2018).

- Brisbane (Pilot)
- Sydney
- Canberra
- Melbourne (Tullamarine and Avalon)
- Perth and regional Western Australia
- Adelaide
- Hobart and Launceston
- Darwin
- Remaining regional areas throughout Australia

Throughout recruitment the study coordinators were in contact with the local operations managers to coordinate mobile phlebotomists for on-site collections at the larger stations. The study was further promoted by Airservices in Newsletters and Memos to all staff, but recruitment was handled directly by the Research Team. Each participant was contacted individually, as per the pilot protocol, and consent to participate in the study was recorded verbally. Current staff could choose if they wanted their blood sample collected by the mobile phlebotomist (if available at their work site) or at the closest collection clinic. Former staff were provided details of the closest collection clinic. The study coordinators called participants at two weekly/monthly intervals to follow up on outstanding questionnaires and blood samples.

Each participant was requested to complete a detailed questionnaire. The questionnaire was designed to capture demographic, lifestyle and work history information. Data from the questionnaire was used to integrate the results and investigate factors that could be associated with the PFAS and biochemical

concentrations. All participants were given the choice to fill in the questionnaire online or in paper format. The full questionnaire can be found in Appendix I.

2.4. Sample Analysis

Analysis of Biochemical Markers

Blood samples (3 SST tubes per participant) were collected by Sonic Healthcare/ Sullivan Nicolaides Pathology (SNP) either by mobile phlebotomist or at a collection clinic and all samples were sent to SNP in Brisbane, where biochemical analyses were performed on one of three collected SST tubes. Participants were not required to fast prior to blood collection. The specific biomarkers were chosen as the current scientific literature had reported these to be associated with PFAS exposure, or to be confounders that might influence PFAS concentrations. The biochemical markers determined are listed in Table 2. The two remaining SST tubes were transported to QAEHS, de-identified with participant codes and the blood serum was stored in the freezer prior to PFAS analysis. The results of the biochemical markers were reported back to QAEHS by SNP. QAEHS then sent out a letter to each of the participants within 2-3 weeks of the collection of the blood samples with the initial biochemistry results. These letters were all reviewed and signed by UQ medical doctor, Dr. Margaret Kay. Any abnormal biochemical results were flagged with advice provided to the participants that they arrange further follow up. Participants were encouraged to provide the results to their usual health provider so that the results could be part of their health record.

Table 2: *Biochemical marker and associated disease endpoints*

Biochemical endpoints	**Biochemical markers**
Lipid profile *Biomarkers for cardiovascular disease, metabolic effects*	Cholesterol, Triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL)
Thyroid function tests *Biomarkers for thyroid disease*	Thyroid Stimulating Hormone (TSH), Free Thyroxine (T4), Free Triiodothyronine (T3)
Liver function *Biomarkers for liver disease*	Alanine Aminotransferase (ALT)
Kidney function *Biomarkers for kidney disease*	Urate (Uric acid), creatinine, estimated Glomerular Filtration Rate (eGFR)
Serum proteins *Provide binding sites for PFASs in blood*	Globulin, Albumin, total protein

Analytical Methodology for PFAS Analysis in Serum

PFAS analysis was conducted at QAEHS laboratories. A full description of the analysis procedure can be found in Appendix III. Briefly, PFASs were extracted from serum with acetonitrile and analysed using high-performance liquid chromatography tandem mass spectrometry, and the quantification was based on isotope dilution methods.

Quality Control and Quality Assurance

Prior to commencing the analysis, a method validation was performed. Serum was extracted over a period of several months, and comprehensive quality control and quality assurance (QC/QA) was conducted to ensure precise and accurate results (Appendix II, Table A1). Serum samples were extracted in batches of 14 samples. Each batch also included intra and inter batch duplicates, pooled serum replicates and standard reference materials (NIST 1957) to establish variation and accuracy (Appendix III, Table A2). Blanks were also included to monitor any potential contamination. Stored serum samples from participants who participated in the previous study (n = 120) were extracted and analysed again in the same batch as the participants' most recently collected serum. In addition to the QC/QA that was performed during the analysis at QAEHS, ten randomly selected samples from 2018 were also sent to two additional laboratories to investigate variations in PFAS measurements between laboratories. The QAEHS lab also participated in two additional inter-laboratory comparison studies (AMAP 2020-01, GEQUAS, Appendix III, Table A3) concurrently to this study. The average variabilities of PFOA, PFHxS, PFHpS and PFOS in the inter-laboratory comparison was 16,%, 13%, 27% and 18% respectively, which is consistent with results of other published inter-laboratory comparisons for PFOA, PFHxS and PFOS [13], as well as other international inter-laboratory comparisons. Slightly higher variability of found for PFHpS, however, the concentration of several samples in the inter-laboratory comparison was very low and below or close to the limit of detection for all three laboratories, which can lead to greater variation. The variability of all QA/QC measurements for PFOA, PFHxS, PFHpS and PFOS in our lab, were lower than 8.1%. The reproducibility and accuracy of the QC/QA measurements were acceptable and are available in Appendix III, Table A1.

In conclusion, good reproducibility of duplicate and replicate analysis within our laboratory, acceptable reproducibility of all three inter-laboratory comparisons, and accurate measurements of the standard reference materials, provide confidence in the PFAS analysis results of this study.

Data Analysis

Reproducibility of QC/QA measurements were determined by calculating the coefficient of variance. To define the intervals of agreements between the reanalysed serum samples and the analysis in the 2013 study, Bland-Altman analysis was used. Average inter-laboratory coefficient of variation was 16% for all assessed PFAAs. The coefficient of variance was below 12% for all QC/QA measurements and

PFAAs within the QAEHS lab. No systematic pattern was found when comparing reanalysed serum samples to the reported measurements in 2013, indicating that the variation (coefficient of variance <8%), was random in these samples.

To determine if serum PFAS concentrations were elevated compared to the general Australian population, comparisons were made with the most recent PFAS data of the general population, obtained from pooled serum samples from South East Queensland [7]. Comparisons were always made between the same age groups. Individuals were considered as having elevated concentrations of PFASs if they fell above the estimated 95th percentile concentration in the general population (that is, the estimated level that 95% of the population is at or below). The estimated 95th percentile for the general population was derived for PFOA, PFHxS and PFOS by Toms et al. (2019) [7]. The 95th percentile for PFHpS was estimated by using the average of the mean:p95% ratio for PFOA, PFHxS and PFOS and applying this to the PFHpS data of the general Australian population derived in the 2017 round of pooled human biomonitoring samples from South East Queensland ([7], PFHpS data was unpublished).

For graphs and distribution calculations, values <MDL (Method Detection Limit) are not presented. In statistical analysis, values <MDL were included as MDL/√2. T-tests, ANCOVA and multiple linear regression assessments were performed to evaluate differences between groups, or the relationship between factors and outcomes, and the test that was used for each evaluation is presented in the results section. A more detailed description is also available in Appendix III for the assessment of the relationship between biochemical markers, self-reported health issues and PFAA concentrations. All statistical analysis were conducted using IBM SPSS (version 25, Chicago, IL) software. A p-value of <0.05 was considered statistically significant. Background information on basic statistics, applied in this report, is presented in Appendix II.

3. Results and Discussion

3.1. Participation Figures & Cohort Demographics

Of those who had expressed an interest to participate in the Exposure Study (n = 880), 91% participated and provided a blood sample (n = 799) and of these, 98% filled in a questionnaire (15 additional questionaries were filled in but no blood samples were provided, these participants were excluded from further analysis). Of the total participants 555 were currently employed by Airservices, while 244 were former staff (see Table 3).

Table 3: *Final participation figures, numbers of questionnaires and blood samples returned.*

Cohort Groups		**No. of samples provided**
Employment Status	Current Staff	555
	Former Staff	244
Overall totals for questionnaire & blood samples		**799 blood samples** (with 783 corresponding questionnaires)

The Airservices 2018/2019 cohort consisted of 799 participants, of which 97.5% were male and 2.5% female. The average age of the cohort was 52 years, ranging from 21-82 years. The majority of participants have held several different positions during their employment with Airservices, therefore, in the following, participants who held multiple positions are included in multiple job categories. Of all participants, 93% stated that they have worked as Firefighters, 42% as Officers, 18% as Senior Officers, 13% as Instructors and 5% as Emergency Vehicle Technicians (EVT).

For data analysis participants were grouped depending on when they started employment with Airservices. A total of 494 participants commenced employment prior to 2005 and therefore may have come into contact with 3M LightWater. A total of 140 participants started employment with Airservices between 2005 and 2010, while 135 started employment with Airservices after 2010. Further general information on demographics and work history is presented in Table A4 in Appendix IV.

3.2. PFAA Serum Concentration Data

Blood serum samples were analysed for a total of 40 different PFASs, however, many of these were not detected in the majority of the participants' sera and are therefore not discussed further. Nine PFASs, namely PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFBS, PFHxS, PFHpS and PFOS, were detected in more than 15% of the participants (Figure 6, Table A5 in Appendix IV). PFOS, PFOA and PFHxS were detected in all participants. PFNA, PFDA and PFHpS were detected in more than 90% of participants, while PFHpA, PFUnDA and PFBS were detected less frequently (respectively, 29%, 30% and 16%).

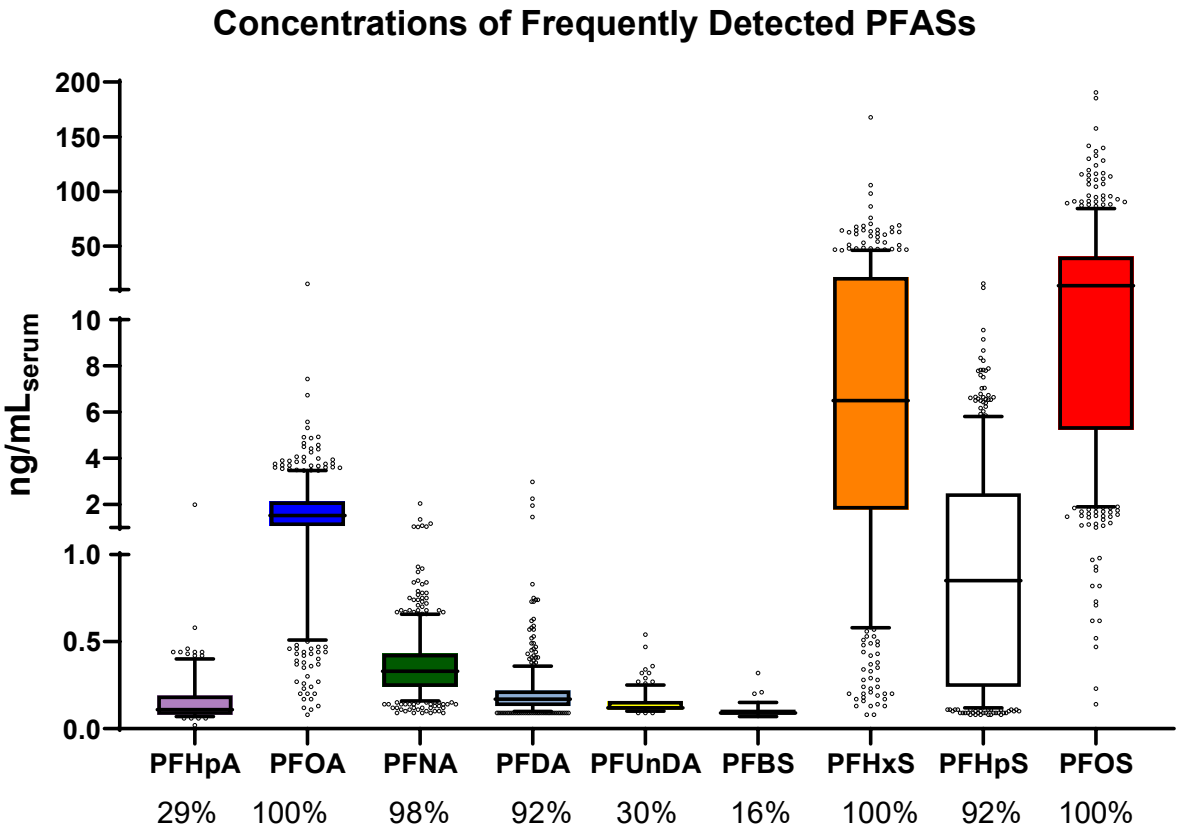

Figure 6: *Concentrations of PFASs in serum of 799 participants. Detection frequencies are reported below each compound. Only PFASs detected in more than 15% of the participants are presented. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers range to the 5th and 95th percentile concentrations. Individual dots represent bottom and top 5%. Note the broken y axis. For graphical explanation of the plot, see Appendix II, Figure A1.*

A strong correlation was found between PFOS and PFHxS, as well as PFOS and PFHpS and PFHpS and PFHxS (Appendix IV Figure A4). A correlation between these three chemicals suggests that they come from a common source and this has also been reported for the 2013 Airservices study. PFHpS was not quantified in the 2013 study, but could be quantified in the archived serum samples that were re-analysed. PFOS and PFHxS are two of the main components of 3M LightWater and are found at differing ratios to each other, depending on the production year of the formulation [11, 14]. PFHpS has also been found in different 3M LightWater formulations, as well as in groundwater at sites where AFFF were used [11]. LightWater was the firefighting foam used by Airservices until 2001/3, therefore it is likely that those participants with elevated levels of these three chemicals have been exposed to these chemicals directly or indirectly due to their occupation and specifically the use of AFFF containing these PFASs in their work.

3.3. Comparison with PFAA Levels Found in the General Australian Population

The pooled Australian data from South East Queensland's general population can be used to evaluate whether participants in the current study had PFAS concentrations above those expected in the general population, which may indicate exposure to PFASs through occupational sources. Since the data for the general populations stems from pooled samples, only an average PFAA concentration is available. However, the 95th percentile for PFOA, PFHxS and PFOS was estimated based on population variation from available datasets from the National Health and Nutrition Examination Survey (NHANES) in the United States [7, 15]. NHANES does not measure PFHpS concentrations in the general population, therefore the average of the population variation for PFOA, PFHxS and PFOS was applied to derive the 95th percentile for PFHpS. While this adds some uncertainty to the 95th percentile of PFHpS it still gives an indication of general population variation. The average age of the cohort was 52 years, therefore in the following, comparisons were made between the Airservices cohort and age group 46 – 60 of the general Australian population.

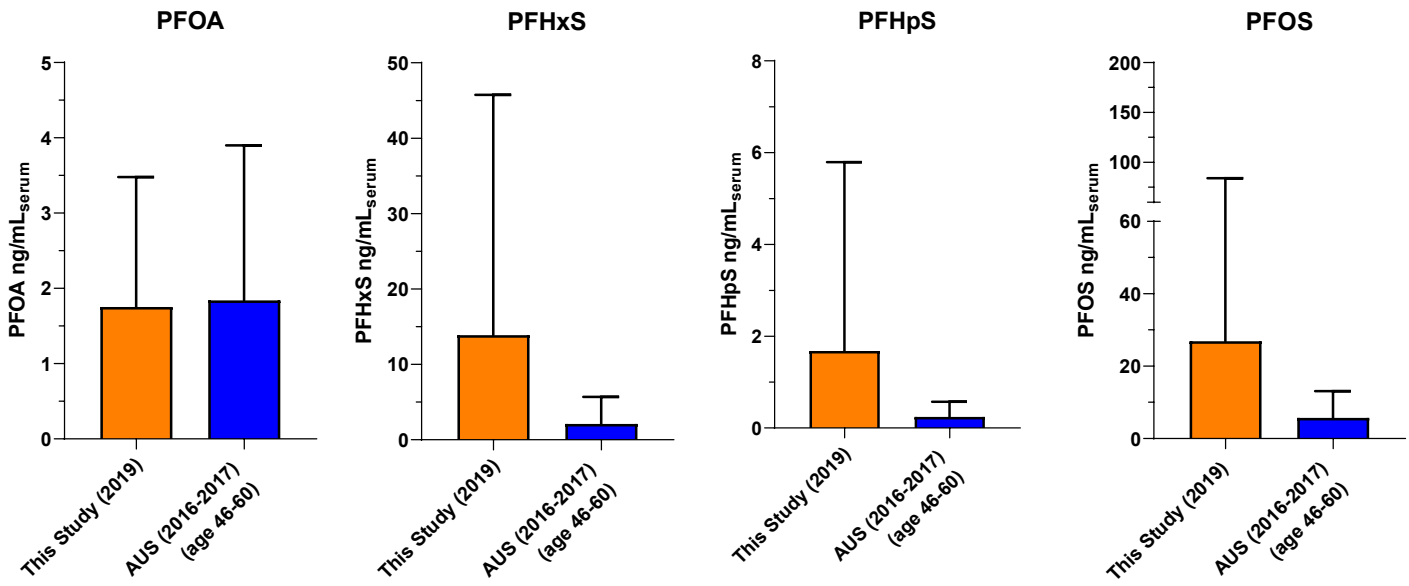

Figure 7: *Comparison of PFOA, PFHxS, PFHpS and PFOS concentrations in serum in study participants (orange) with those in the general Australian population (age 46-60) estimated from pooled samples from South East Queensland (blue) [7, 16]. This age group is used as comparison because the average age in this study falls within this age span (52 years). The bar presents the average concentration, and the error bar presents the estimated 95th percentile. For graphical explanation of the plot, see Appendix II Figure A1.*

A comparison of those PFASs that were detected in more than 90% of the cohort to the data of the general Australian population showed that average concentrations of three chemicals, PFHxS, PFHpS and PFOS, in Airservices participants were elevated above the average values of the general Australian population (Figure 7). The average concentrations of PFHxS, PFHpS and PFOS were 14 ng/mL, 1.7 ng/mL and 27 ng/mL respectively. In the pooled samples from the general Australian population (aged 46-60) the average concentrations of PFHxS were 2.1 ng/mL and of PFOS were of 5.7 ng/mL, estimated in 2016-2017 [7]. PFHpS

serum concentrations, although not published, were derived from the same pooled samples and the estimated average serum PFHpS concentration in 2016-2017 for age group 46-60 was 0.24 ng/mL (data unpublished). Concentrations of PFOA measured in this study showed values comparable to those measured in the general Australian population, indicating no increased exposure through occupational activities to this chemical. In the following sections of this report we have therefore concentrated on evaluation of the three chemicals that were found in elevated concentrations in the Airservices cohort, indicating potential occupational exposures, as well as PFOA, as it was detected in all participants and is one of the most frequently studied PFASs.

3.4. Analysis by Years of Employment

The cohort was categorised into three groups representing those participants who started working for Airservices before 2005, those who commenced working for Airservices between 2005 and 2010 (inclusive) and those who joined Airservices after 2010 (2011 onwards). These years were chosen according to the different firefighting foams being employed during those periods. Airservices staff could have been exposed to different PFASs from the 3M LightWater between approximately 1980 and 2005, and Ansulite between 2005 and 2010; since 2011 the PFAS-free Solberg RF6 has been used at all locations except Darwin and Townsville. These airports are operated jointly with the Royal Australian Air Force (RAAF) and the ARFF services agreement with Defence required the use of Ansulite to continue for a longer period of time; indeed, the transition to Solberg RF6 only occurred at these joint user facilities in 2019.

As is apparent in Figure 8, those participants who started working for Airservices prior to 2005 showed highest serum concentrations of PFOS, PFHxS and PFHpS. Participants who started working between 2005 and 2010 showed a higher average than those who started working only after 2010, however, neither of these two latter groups were elevated compared to the general Australian population, indicating limited occupational exposure to those chemicals in these subgroups.

Some individuals reported working for other employers where they may have come into contact with AFFF, such as the RAAF (n = 172). These participants have been coloured as red dots where they fall above the 95th percentile or below the 5th percentile. Figure A5 in Appendix IV shows the same figure, but excludes those participants who indicated they worked for other employers with potential AFFF exposure.

Length of employment at Airservices prior to 2005, i.e. during the time that 3M LightWater foam was used, influenced serum PFOS concentrations to some extent and is shown in Figure 9. In this case, length of employment is not tied to specific years, i.e. a person employed between 1-4 years may have been employed for this length of time at any time prior to 2005, not necessarily 2001-2005. Among those individuals employed at any time before 2005, there was no increase in PFOA concentration with increasing years of employment. However, increasing PFHxS, PFHpS and PFOS concentrations were associated with increasing years of employment (Figure 9, additional tables in Appendix IV Table A6).

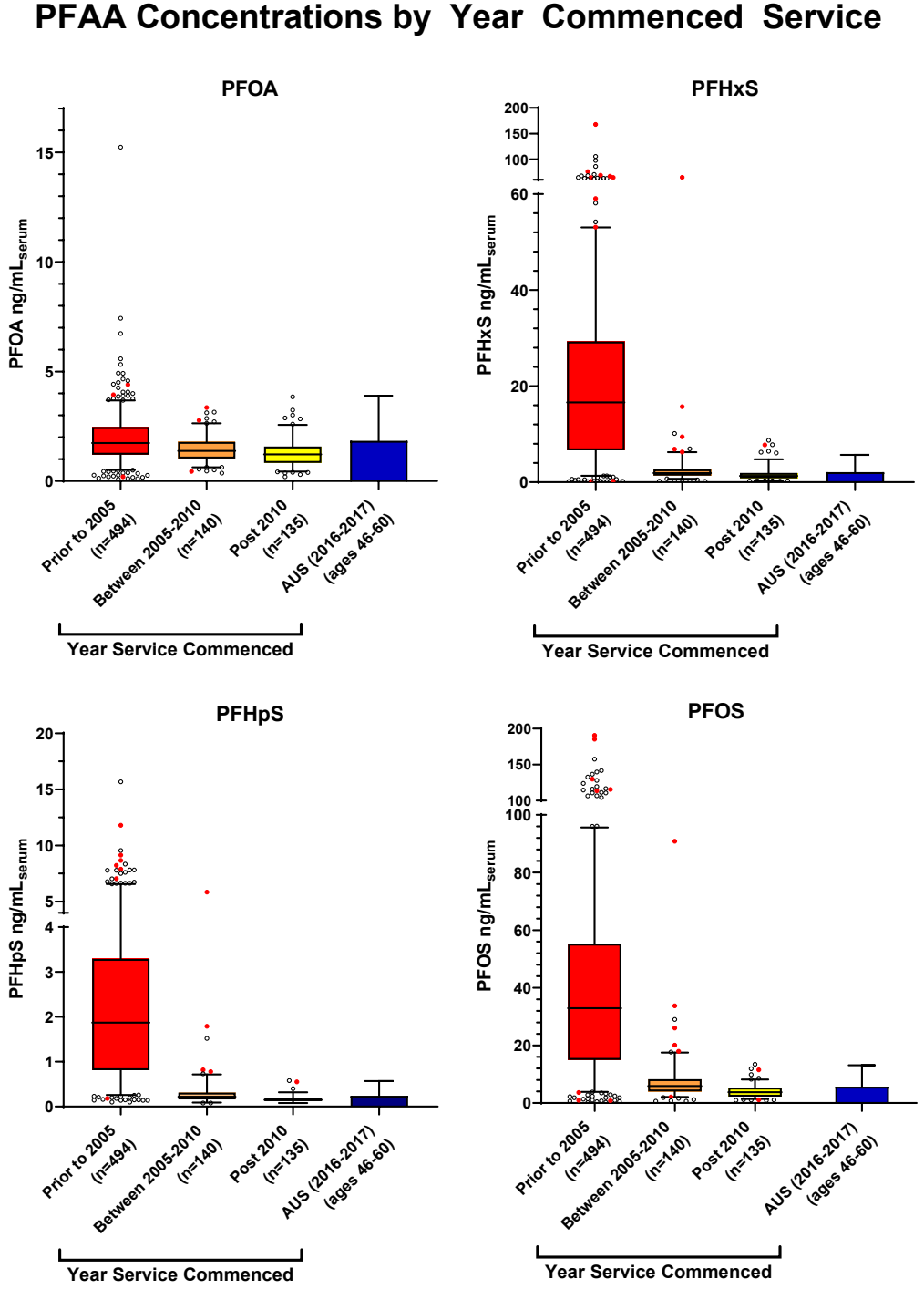

Figure 8: *PFAA concentrations grouped by category when employees commenced working for Airservices. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers range to the 5th and 95th percentile concentrations. Individual dots represent bottom and top 5%. For the general population, the bar presents the average concentration, and the error bar presents the estimated 95th percentile [7, 16]. For graphical explanation of the plot, see Appendix II. Red dots indicate individuals who reported working in other jobs that may have had AFFF exposure, and whose measurements fall above the 95th percentile or below the 5th percentile of the group.*

PFAA Concentrations by Number of Years Working with 3M-LightWater

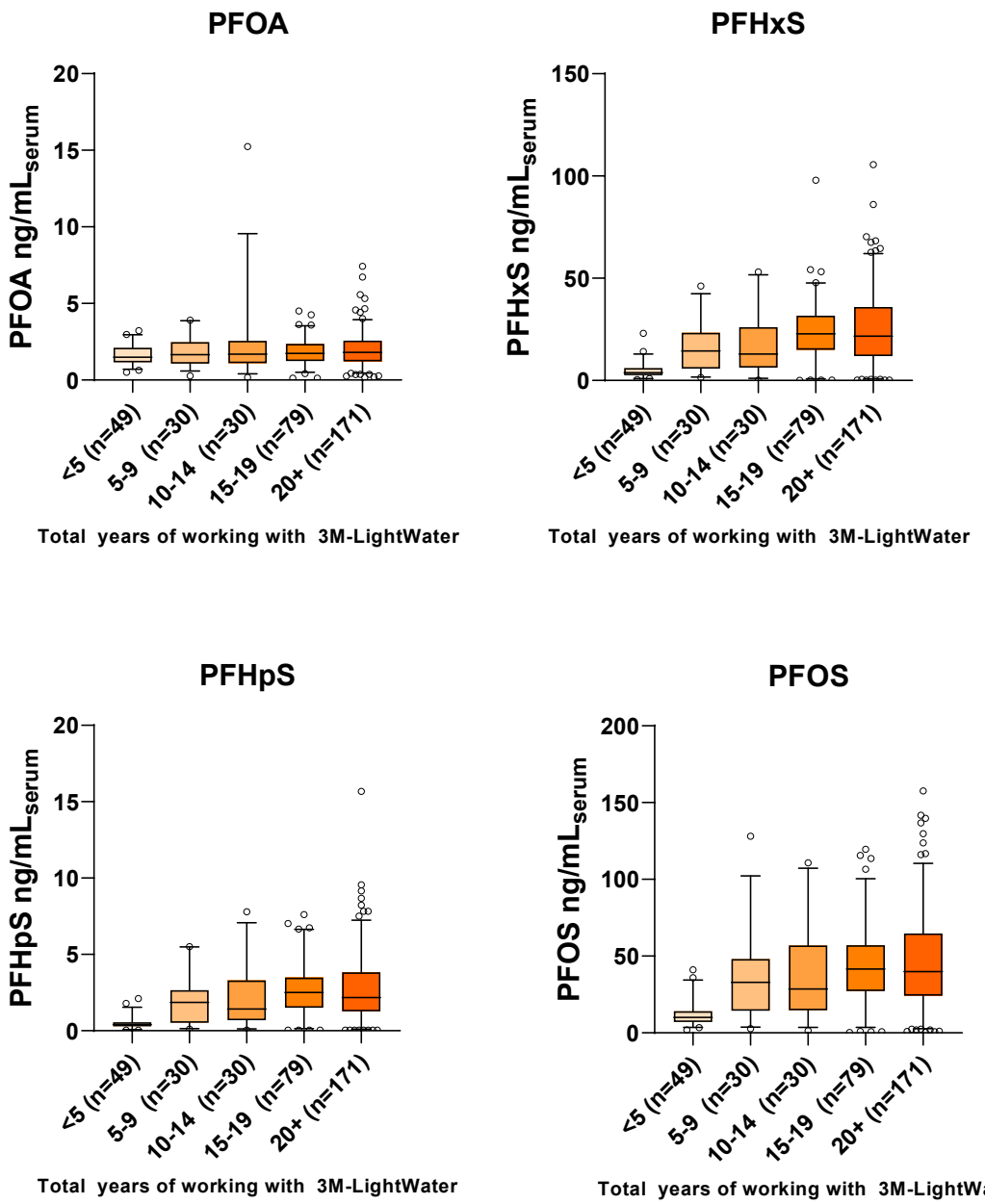

Figure 9: *Concentrations of PFASs in participants who started working for Airservices prior to 2005, depending on how many years in total they worked using 3M LightWater. Participants who have indicated that they have worked for other employers where they may have come into contact with AFFF, such as the RAAF, are not included in this graph. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers range to the 5th and 95th percentile concentrations. Individual dots represent bottom and top 5%. For graphical explanation of the plot, see Appendix II Figure A1*

3.5. Results by Work Station

Some limited analyses were undertaken to evaluate if PFAA serum concentrations vary by work location. However, direct comparisons between the stations should be made with caution as the Airservices cohort is transient, oftentimes working at different stations throughout their career. At least 20% of the cohort have worked at more than three stations throughout their career. Blood serum concentrations would represent the accumulated exposure from multiple work stations if the participant has worked at more than one station, and may therefore not show the true picture when assigning participant serum PFAS concentrations to only one station. Furthermore, low participation rates or stations with few employees further limit the analysis, as the confidentiality of participants cannot be safeguarded at low numbers. The cut-off for displaying station specific data was therefore set at a minimum of six participants or more.

For the analysis by station, which needs to be viewed with caution due to the above-mentioned constraints, the cohort was divided into two groups, those employed prior to 2005 and those employed post-2005. In each group only those participants were included who had worked as a firefighter during their employment at Airservices. Representatives from each station included participants who had worked at the particular station for four or more years, while not having worked at other stations for more than two years during their career. This further constraint was applied to ensure the data reflected those participants that had worked for a longer period of time at one particular station, while at the same time maintaining sufficiently high numbers in each group to safeguard confidentiality. Only firefighters were included to make sure each station would be represented with participants with the same potential exposure. Participants who indicated having AFFF exposure while working for other employees (i.e. RAAF) were excluded from this analysis. Participants were also excluded if they did not provide full information about the years they were employed.

Mean PFAS concentrations for each station, are presented in Appendix IV, Table A7, and the concentrations of PFOS for each station are presented in Figure 10. Comparisons show an overlap in the concentration ranges between the stations, indicating that there are no major differences in PFOS exposure between these stations. Although some stations have higher average levels than others, it is unknown, based on the information available, if the differences seen are significant. With the constraints applied and low participant numbers it is not possible to do a statistical evaluation of these results and they should be seen as a rough assessment only.

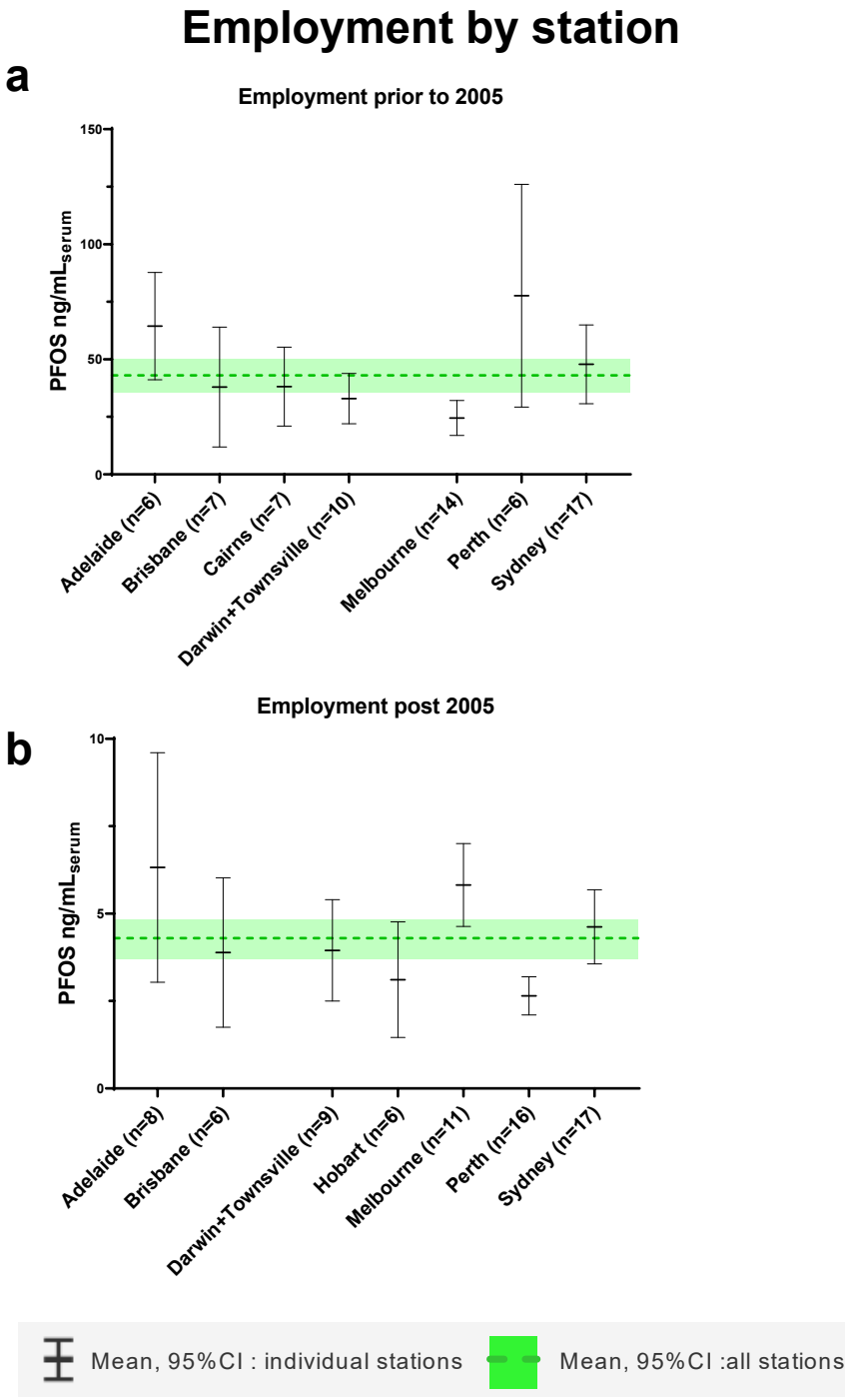

Figure 10: *PFOS serum concentration by Airservices working location for participants who worked as a firefighter at a certain station for a total of 4 or more years prior to 2005 (a) and years post 2005 (b). Representatives for each station only include participants who have not worked at other stations for more than 2 years. Participants who worked in other jobs with potential AFFF exposure, or in positions other than firefighters are excluded from this analysis. The lines indicate mean concentrations, and the whiskers represent the 95% Confidence Interval (95% CI). The scattered green line represents the overall mean and 95% CI of all stations.*

3.6. Results by Job Position

The level of exposure to PFASs may have varied depending on job position. Two main job categories, Emergency Vehicle Technicians (EVTs) and Firefighters, were explored for potential exposure differences. EVTs work on maintenance and repair of the fire engines, in the past often coming into direct contact with AFFF concentrate which may have led to high exposures, especially when limited personal protective equipment (PPE) was worn and skin of hands and arms were exposed. Of the EVTs that were in contact with AFFF most days, and who provided information regarding the use of PPE, an average of 60% stated that they did not wear any PPE (Table A4 in Appendix IV). In contrast to EVTs, the main exposure route for Firefighters is direct contact with AFFF (diluted) during firefighting events and training. Of the firefighters that provided PPE information, an average of 26% stated that no PPE was worn when in contact with AFFF most days (Table A4 in Appendix IV).

Participants who worked as EVTs had a higher average serum concentration of PFHxS, PFHpS and PFOS, compared to those who worked as firefighters before 2005, while 3M LightWater was in use (Figure 11a). For both EVTs and firefighters, the PFOS concentration was associated with years of employment (Table A8 and Figure A6 in Appendix IV). After 2005, participants would not have worked with 3M LightWater, however, exposure could still have been present due to contaminated infrastructure and materials. The average serum concentration of these PFASs was also greater in EVTs who were employed with Airservices after 2005, compared to participants who worked as firefighters (Figure 11 b). While this may indicate slightly higher ongoing exposure through job related activities for EVTs, it must be kept in mind that the number of participants in the EVT group was very small (n = 7) and therefore results may be skewed. Additionally, the average age of the seven EVTs were 10 years greater compared to the average age of the 213 firefighters. This age difference can in part explain the greater average concentration of PFASs in the EVTs, as serum PFAS concentrations have been reported to have a positive relationship with age in the general population [7].

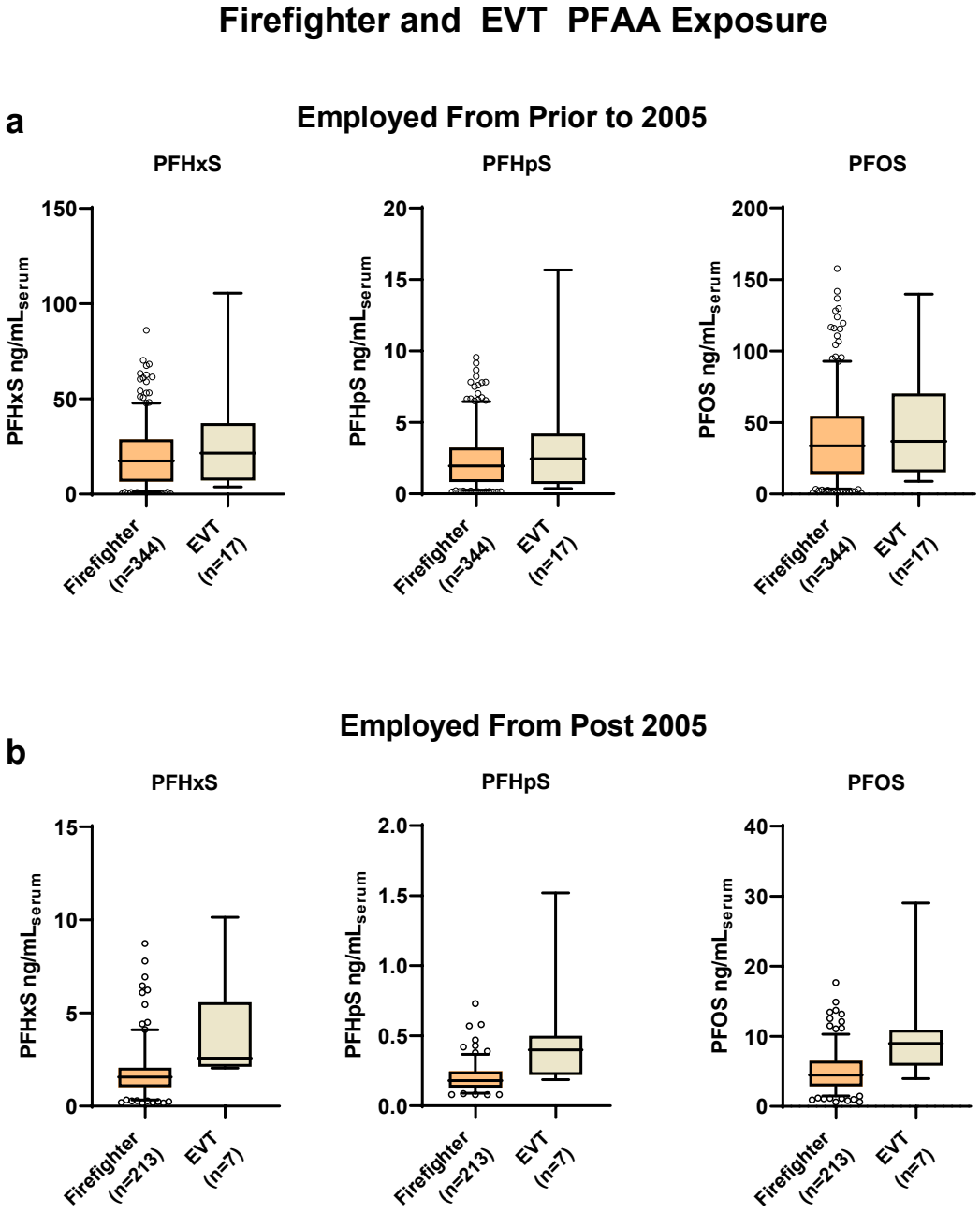

Figure 11. PFAS serum concentration in participants who have worked as EVTs and Firefighters prior to 2005(a) and post 2005(b). The figure only includes participants who indicated that they have not worked for other employers where they could have been in contact with AFFF. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers range to the 5th and 95th percentile concentrations. Individual dots represent bottom and top 5%. For graphical explanation of the plot, see Appendix II, Figure A1.

3.7. Additional PFAA Concentration Trends

The data of the cohort was further analysed to be able to evaluate any potential trends. When grouping the cohort into age groups and comparing these to the general population, it can be observed that only the older age groups have elevated PFHxS, PFHpS and PFOS concentrations compared to the general population (Figure 12), but this was not observed for PFOA. This can reflect the usage of AFFF containing PFASs in the older age groups or it can reflect the longer period of time to accumulate PFASs in older age groups. Multiple linear regression, assessing the relationship between PFAS concentration with age and years of employment is presented in Table A8 in Appendix IV (the relationship is also shown between Age and PFOS concentrations in participants who started working prior to 2005 in Figure A7). For participants who started working before 2005, age is a significant predictor of PFOA serum concentrations. However, age was not a significant predictor for PFHxS, PFHpS and PFOS serum concentrations, when taking the number of years working, as well as number of years since retirement into consideration (Appendix IV Table A6). Years of working with 3M LightWater was significantly positively associated with PFHxS, PFHpS and PFOS serum concentration (also shown in figure 9), while the number of years since retirement were negatively associated with the serum concentrations. This indicated that the higher PFOS/PFHxS/PFHpS concentrations measured in the older age groups were in fact associated with them working with the 3M LightWater product, and not with their age. In participants who commenced service after 2005, where average PFAA concentrations were not elevated, age was again a significant predictor for PFHxS, PFHpS and PFOS serum concentrations which has also been observed in the general population.

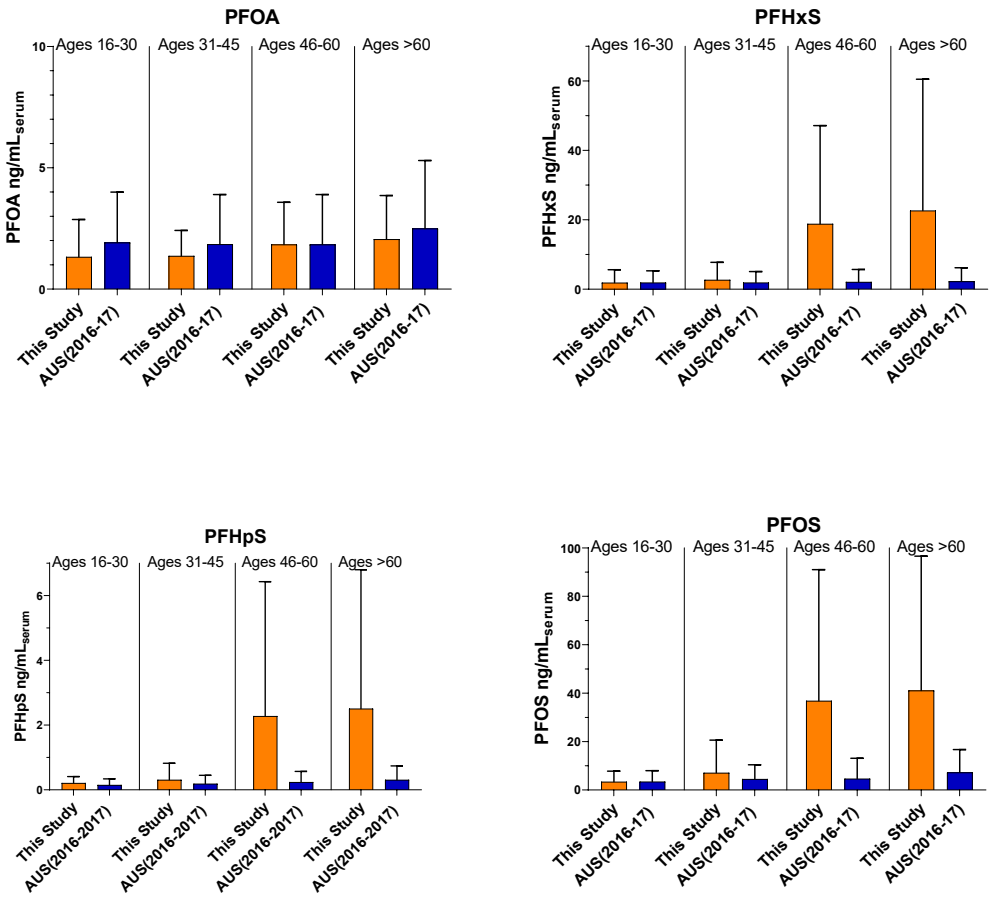

Figure 12: *PFOA, PFHxS, PFHpS and PFOS concentrations by age group (orange), and comparison to general Australian population (blue) [7, 16]. The bars present the average concentration, and the error bars present the estimated 95th percentile.*

The association between PFAS concentration and blood donation was also evaluated. Participants who reported that they were blood donors had a lower average concentration of PFOA, PFHxS, PFHpS and PFOS compared to participants who were not blood donors (Figure 13-14; Statistical test outcomes presented in Appendix IV; Multiple linear regression TableA8, t-test; Table A9). Increased frequency of reported blood donation can also be observed to be associated with lower PFAS concentration (Figure 13-14; Statistical outcomes presented in Appendix IV; ANCOVA, Table A10). In the 2013 study, participants who were blood donors were also found to have a lower PFAS concentrations compared to participants who were not blood donors and blood donation has also been reported in the literature to reduce concentrations of PFASs [17].

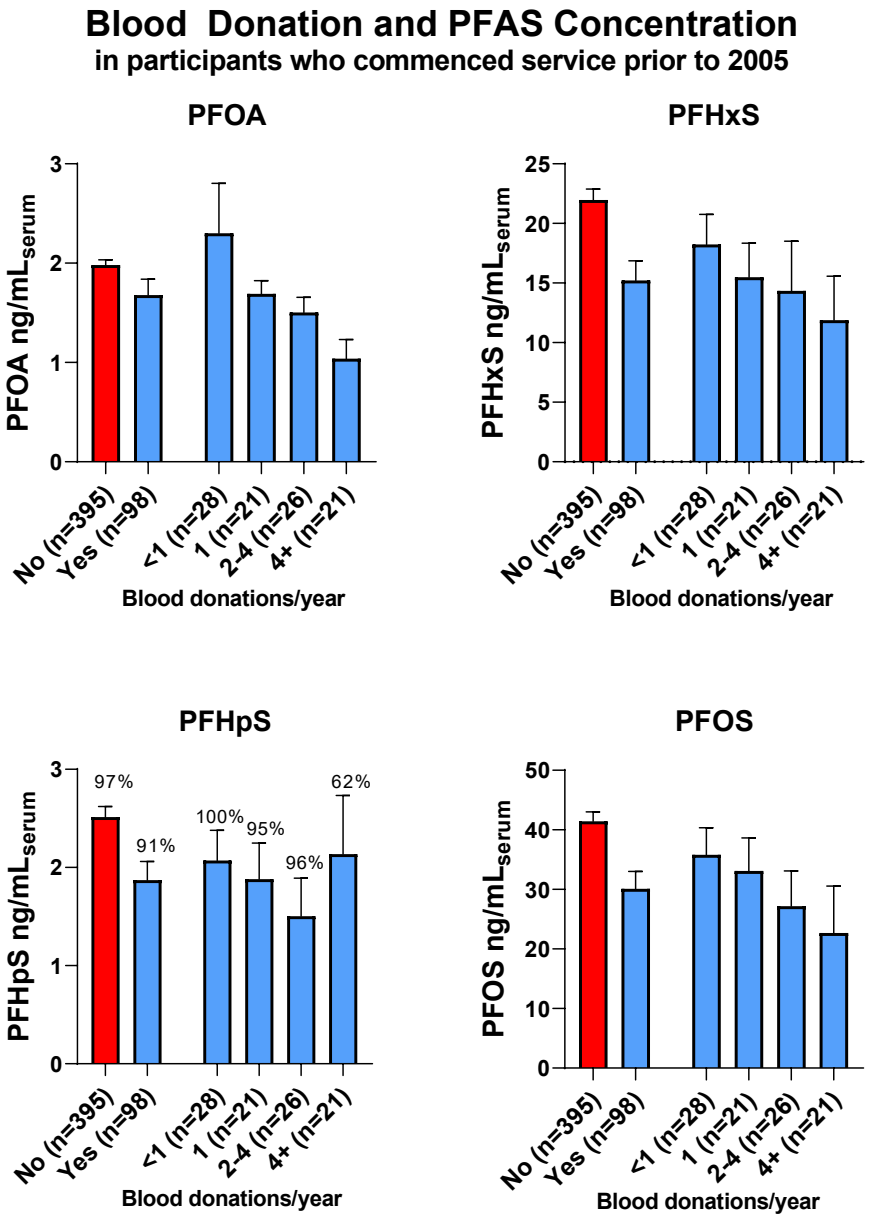

Figure 13: *Serum PFAS concentrations by blood donation ("No" or "Yes), and number of blood donations per year, in participants (n=492) who started working for Airservices before 2005, and therefore worked with 3M AFFF. The bars show the mean concentration, and the whiskers represent the standard error of the mean (SEM). The percentage above bars in the PFHpS graph represents the detection frequency for each group. PFOA, PFHxS and PFOS were detected in all participants. The means of blood donation "yes" and "no" were significantly different for all PFASs.*

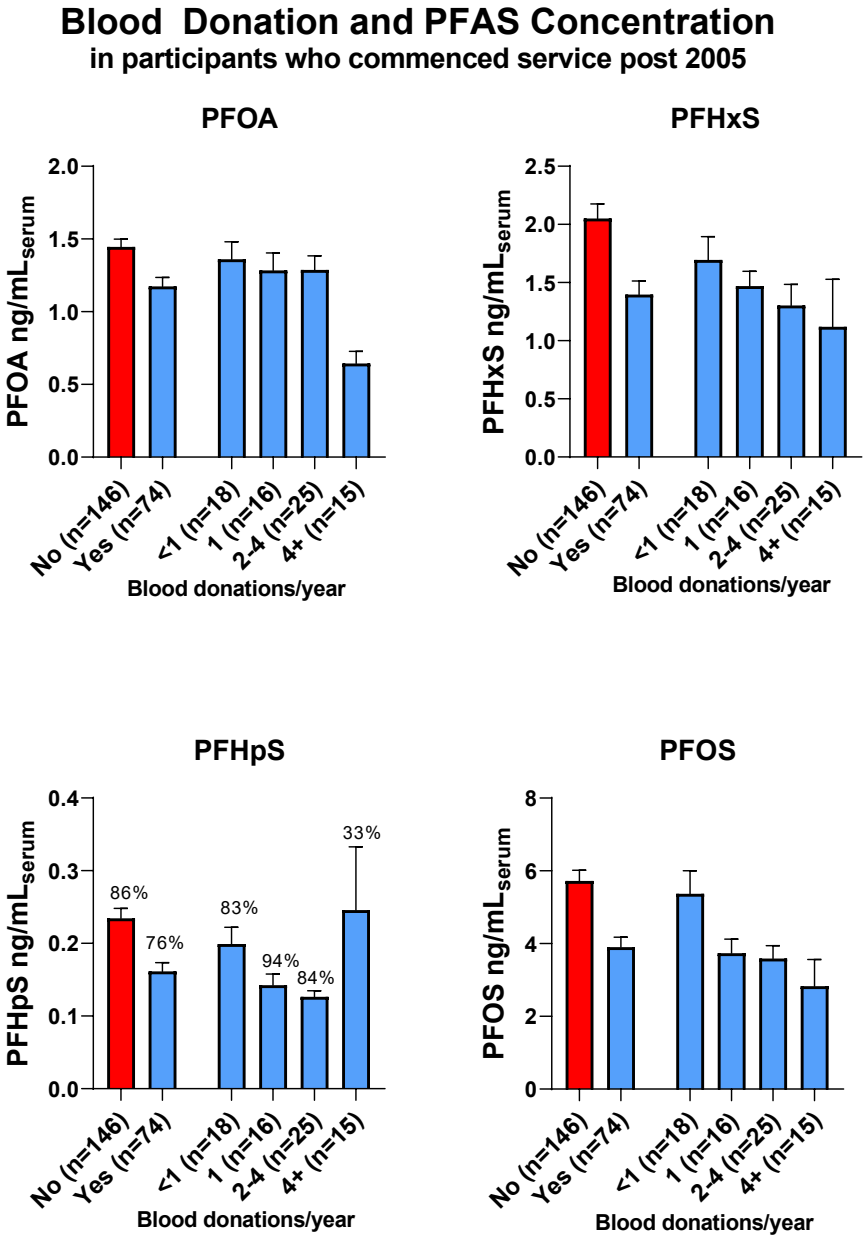

Figure 14: *Serum PFAS concentrations by blood donation ("No" or "Yes"), and number of blood donations per year, in 220 participants who have been employed by Airservices post 2005. The bars show the mean concentration, and the whiskers represent the standard error of the mean (SEM). The percentage above bars in the PFHpS graph represents the detection frequency for each group. PFOA, PFHxS and PFOS were detected in all participants. The means of blood donation "yes" and "no" were significantly different for all PFASs.*

3.8. Temporal PFAA Concentration Trends

Comparison to 2013 Study Cohort

Average serum concentrations of PFOA, PFOS, PFHxS and PFHpS measured in the 2019 study are 55-65% lower than levels measured in the 2013 study (Figure 15). Note that this comparison is of the overall averages in the two surveys, which include different individuals (with some overlap). Temporal trends of these PFAAs can also be observed in serum of the general Australian population. Average concentrations, estimated from pooled serum samples, in the age group 45-60, which is comparable to the average age of the Airservices cohort, are available for both 2011 and 2017. Comparing these two years, the average concentration of PFOS was unchanged, while PFOA, PFHxS and PFHpS decreased 37%, 16% and 29%, respectively, between these two measurements [7, 16]. In adults overall (over the age of 16) the decrease in the general Australian population between the two years was 53%, 37%, 27% and 56% for PFOA, PFHxS, PFHpS and PFOS, respectively. Similar to the comparison between the two Airservices studies, these averages include different individuals. The average decrease in the general population is lower compared to the average decrease in the Airservices study for all four assessed PFAAs, possibly explained by the low continuous exposure in the general population. The decline of serum PFAAs in the Airservices cohort is faster, as one of the main exposure routes has been eliminated.

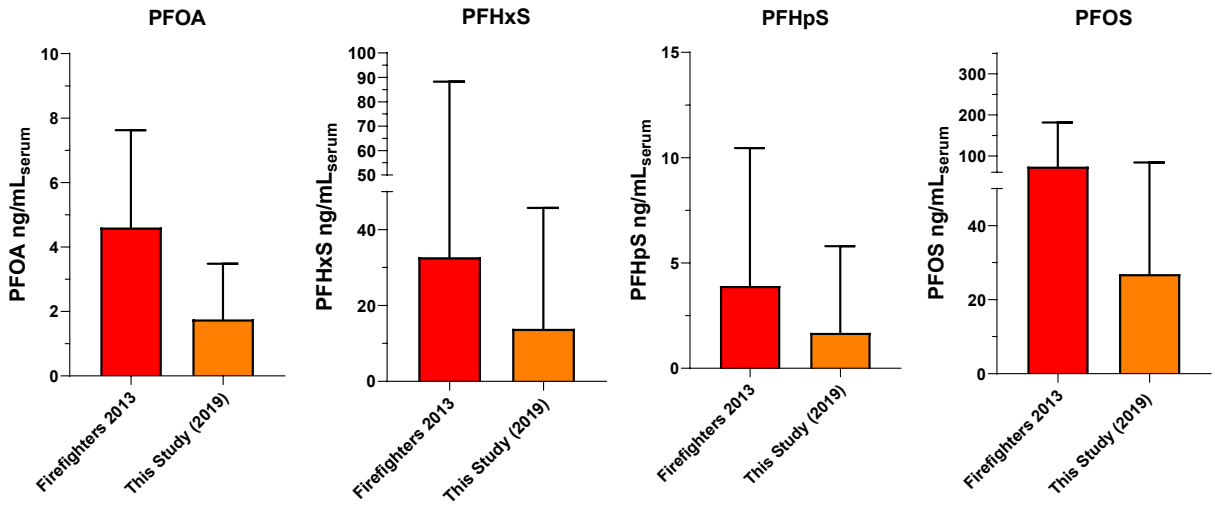

Figure 15: *Concentrations of PFOA, PFHxS, PFHpS and PFOS in the 2013 Airservices cohort (red, n =153 (For PFHpS only reanalysed samples were available for concentration quantification (n=120)) and the 2019 Airservices cohort (orange, n = 799). The bars display the average and the whiskers represent upper 95%ile.*

Change in PFAS Serum Concentrations since 2013 in Individuals with Repeat Measures

A subgroup of the total 799 participants had also taken part in the 2013 Airservices Exposure study. This longitudinal subcohort (those participants with serum measurements in both studies) provide a unique opportunity to determine the change in serum concentration over time. This allows the estimation of elimination half-lives of these chemicals. An elimination half-life is the time it takes for the concentration of a chemical in

serum to decrease by half. A total of 130 participants were re-recruited for the 2019 study from the 2013 study. Of these, 120 had indicated in 2013 that their serum sample may be stored at QAEHS for later research. These (n = 120) samples were re-analysed in 2019, together with the 2019 serum sample of those participants. The re-analysed sample concentrations were used, when available, for comparisons to minimise any differences in concentrations that could have been due to analytical differences.

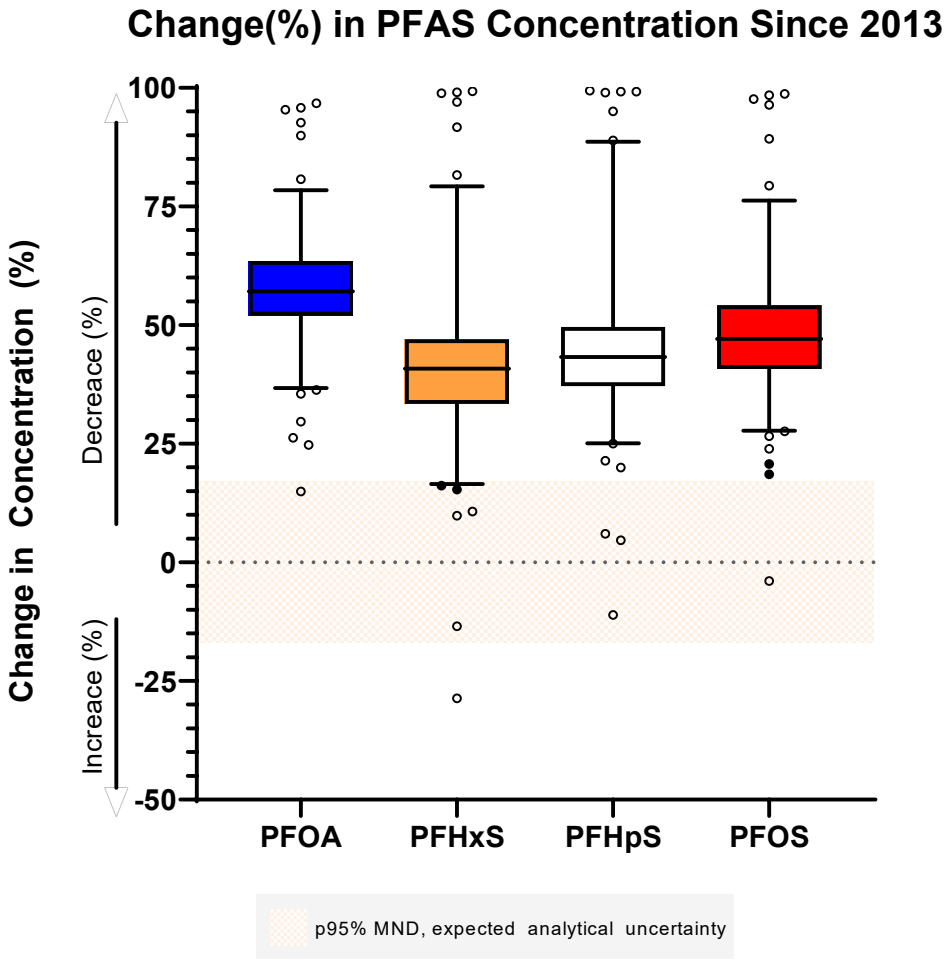

Figure 16: *Percent decrease in serum concentration of PFOA, PFHxS, PFHpS and PFOS between 2013 and 2019 of those study participants who took part in both studies (n=130). (120 participants had their archived serum re-analysed, and the reanalysed results were used for calculation of decrease. Only 10 participants did not have their serum re-analysed. The results reported in 2013 were used instead for these samples, hence a greater analytical variance can be present. Therefore when these results occur in the top or bottom 5% the dotpoints are filled black. The shaded area (95%ile of Mean Normalized Difference (MND) of repeated measurements) represents the % change that can be expected due to analytical uncertainty. Therefore, the dotpoints in the shaded area show no difference between the two years and should not necessarily be interpreted as indicating a change in concentration. Upper and lower ends of the box show 25% and 75%ile, respectively, the middle line shows the median value, whiskers represent upper 95%ile and lower 5th%ile.*

A comparison of serum concentrations between 2013 and 2019 of 130 participants showed an average decrease of 58% for PFOA, 42% for PFHxS, 45% for PFHpS and 49% for PFOS (Figure 16). A few participants had changes in measured concentration that were within the range of analytical uncertainty (shaded area in

the graph), indicating that any change that may have occurred was smaller than the analytical uncertainty. This could be expected when initial measured concentrations were already low relative to the analytical uncertainty bounds. Figure 17 shows the percent change in PFHxS as a function of 2013 sample concentration. Those participants who had low concentrations in 2013 were the ones who showed little or no change in concentration, or even an increase in concentration (PFHxS in one participant), between 2013 and 2019. It may be expected that for participants with very low concentrations, similar to those in the general population, the absolute amount of decrease or increase is within the range of the analytical uncertainty. From Figure 17 it can further be observed that the percentage decrease in PFHxS concentration was not concentration dependent. That is, individuals with higher 2013 PFHxS concentrations showed similar percentage decrease to those with lower PFHxS concentrations. This was also the case for PFOA, PFOS and PFHpS.

The individual decreases of PFAAs observed in this study can further be compared to decreases of PFAAs in individuals without elevated exposure. Unfortunately, no longitudinal data is available for a general Australian population, however, a longitudinal study conducted on a general Swedish population (Uppsala) assessed an overall 5 year decrease in PFOA, PFHxS and PFOS and found reductions of 41%, 20% and 38%, respectively, between 2006/2009 and 2011/2014, in 579 individuals (aged 75-80) [18]. In contrast to the comparison made in the previous section, where the average decline in the general Australian population was used, a longitudinal analysis of individual participants provides a more accurate estimation of the temporal trends of PFAAs in serum, as it removes errors associated with randomised participant selection. The average decrease in PFAAs was greater in 130 individuals in the Airservices cohort where longitudinal data was available for PFOA and PFOS compared to the general Swedish community, which may indicate an ongoing low background level of exposure in the general Swedish population, while in the Airservices cohort the major exposure source has been removed. Although the percentage decrease of PFHxS was much higher in the Airservices cohort compared to the general Swedish population, it was mentioned that a contamination of the drinking water source had occurred during the time of the Swedish study which would have been a source of higher and ongoing PFHxS exposure and therefore negating the use of this population as a background comparison for PFHxS decline.

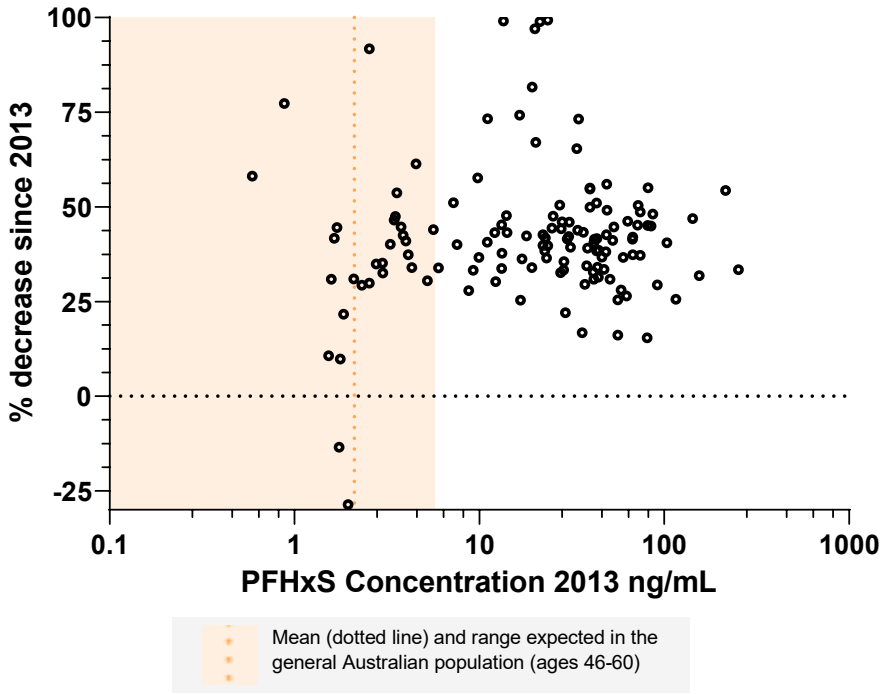

Figure 17: *Percentage decrease in concentration since 2013 plotted against the initial concentration in 2013. The shaded area represents the concentration that is expected in the general Australian population (up to the 95th percentile estimated from 2016/2017 [7], where the orange dotted line represents the average).*

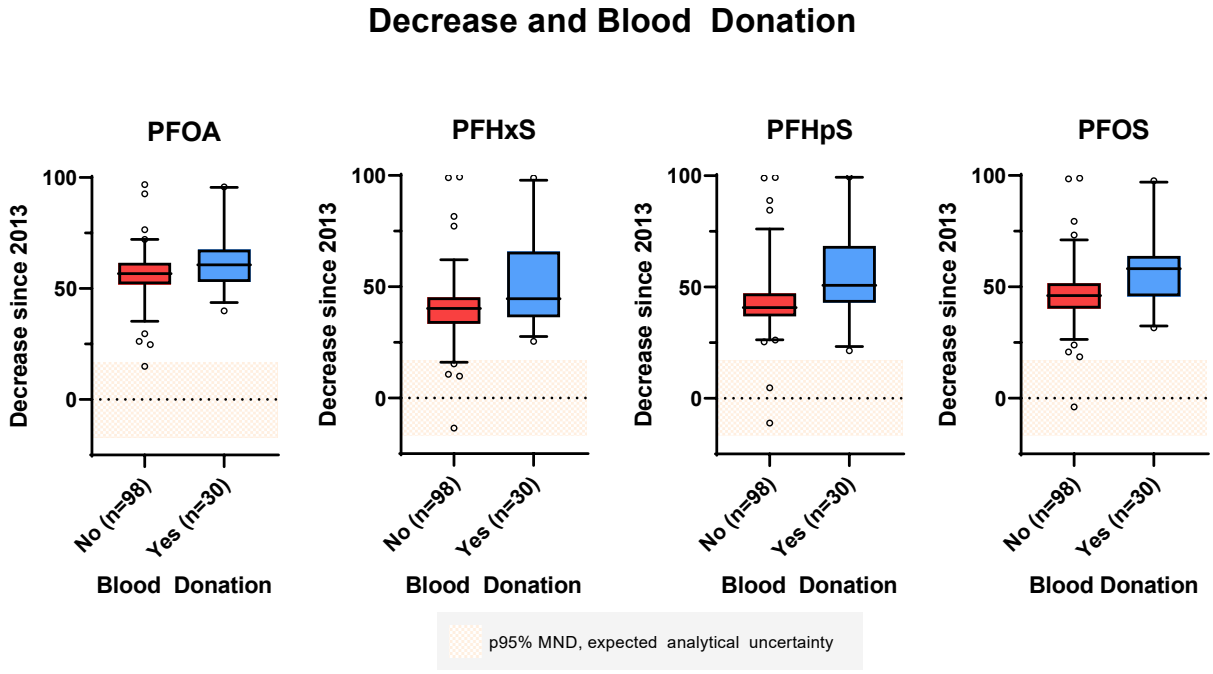

Figure 18: *Percent decrease in serum concentration of PFOA, PFHxS, PFHpS and PFOS between 2013 and 2019 of those study participants who were not blood donors (Red), and blood donors (Blue). The shaded area represents the % change that can be expected due to analytical uncertainty. Therefore, the dot points in the shaded area show no difference between the two years and signify a no change in concentration. Upper and lower end of the box show 25% and 75%ile, respectively, the middle line shows the median value, whiskers represent upper 95%ile and lower 5th%ile.*

In Figure 13 and 14 it was shown that study participants who donated blood had lower average PFAS serum concentrations compared to those who did not donate blood. Therefore, it was further assessed if those participants with two available serum time points (2013 and 2019) showed a greater average decrease of PFOA, PFHxS, PFHpS and PFOS. Participants who were blood donors (n=30) were found to have a greater average decrease in serum concentrations compared to participants who were not blood donors (Figure 18). Blood donation has been discussed as an effective way to reduce PFAS serum concentrations, however, it is not recommended as a treatment.

Elimination Half-lives in Longitudinal Cohort

An elimination half-life is the time it takes for a chemical to decrease in concentration by half. Elimination half-lives can be calculated where exposure to a chemical has ceased (or ongoing exposure is negligible or low compared to earlier exposure levels). In the Airservices cohort occupational exposure to PFHxS, PFHpS and PFOS decreased when the 3M LightWater foam was replaced in 2005. With two time points of PFAS serum concentrations this allows the calculation of half-lives for participants who had clearly elevated levels of these chemicals (above the 95%ile of the general Australian population), assuming ongoing background exposure was low. The longest average elimination half-life was observed for PFHxS with 8.2 years (Figure 19). This is in line with other studies [19-21] where PFHxS was seen to have a longer serum elimination half-live in comparison to PFOS and PFOA (Table 4). The average serum elimination half-live for PFHpS was 7.8 years, while it was 6.6 years for PFOS. No serum elimination half-live was determined in this way for PFOA, as most participants showed PFOA serum concentrations in line with the Australian general population. However, serum elimination half-lives were derived for PFOA using data from all participants who had either shown no-change between the two years or a decrease in concentration. The average half-life calculated for PFOA was 5.2 years, indicating the fastest elimination of the four PFAAs investigated. When calculating the half-lives for PFHxS, PFHpS and PFOS in the same way using the entire study cohort (omitting those participants who had shown an increase in concentration between the two years), slightly longer half-lives were derived with 8.7 years for PFHxS, 8.6 years for PFHpS and 6.7 years for PFOS. These half-lives are longer and show a wider range between participants, as they are influenced by ongoing background exposure to these chemicals. The majority of studies investigating the elimination half-lives of PFASs have assessed individuals with elevated PFAA concentrations, after end of exposure (Table 4). The elimination half-lives of PFAAs in the general population have also been assessed, although the majority of these studies have evaluated half-lives from cross sectional studies on populations over time, i.e. on independent populations (e.g. [16, 22, 23]. In these studies half-lives reported for PFOA are between 5.3 - 5.9 years for PFHxS 4.6 years, for PFHpS 5.3 years and for PFOS between 2.3 - 4.5 years. [16, 22],

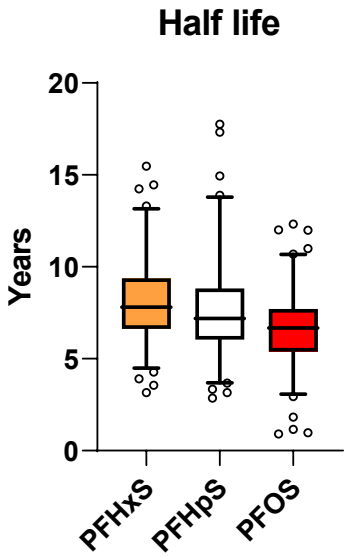

Figure 19: *Average half-lives for PFHxS (n=84), PFHpS (n=83) and PFOS (n=109). Half-life was only calculated for participants who had a change of PFAS concentration that is greater than the percent difference that can be expected from analytical variance, and in participants who have elevated concentrations compared to the general population. Upper and lower end of the box show 25% and 75%ile, respectively, the middle line shows the median value, whiskers represent upper 95%ile and lower 5th%ile.*

Table 4: *Average half-lives of serum elimination of PFOA, PFHxS, PFHpS and PFOS in various exposed cohorts.*

		Half-lives (Years) and range/confidence interval			
Reference		**PFOA**	**PFHxS**	**PFHpS**	**PFOS**
This study Airservices staff (n=130)	Participants with elevated PFAS levels*		8.2 (95% CI 7.7-8.7) (n=84)	7.8 (95% CI 7.2-8.4) (n=83)	6.6 (95% CI 5.2-7.0) (n=109)
	All Participants¥	5.2 (95% CI 4.8-5.9) (n=130)	8.7 (95% CI 7.8-9.6) (n=128)	8.6 (95% CI 5.8-10.4) (n=119)	6.7 (95% CI 6.2-7.2) (n=129)
Xu et al 2020 [24] Airport employees exposed to contaminated drinking water (n=26)		1.77 (95% CI 1.43, 2.31)	2.86 (95% CI 2.1, 4.47)	1.46 (95% CI 0.83, 6.25)	2.91 (95% CI 1.71, 9.63)
Li et al. 2019 [25] Residentially exposed community (n=108)		3 (95% CI 2.8-3.2)	4.7 (95% CI 4.3-5.2)	4.7 (95% CI 4.3-5.3)	2.9 (95% CI 2.7-3.1)
Li et al. 2017 [19] Residentially exposed community (n=20 male)		2.8 (95% CI 2.4- 3.4) (men)	7.4 (95% CI 6.0- 9.7) (men)		4.6 (95% CI 3.7- 6.1) (men)
Worley et al. 2017 [21] Residentially exposed community (n=45)		3.9 (range 3.5–4.1)	15.5 (range 13.4–17.6)		3.3 (range 3.0–3.6)
Olsen et al. 2007 [20] Fluorochemical production workers (n=6)		3.8 (range 3.1-4.4)	8.5 (range 6.4-10.6)		5.4 (range 3.9-6.9)

**Half-life was calculated for participants who had elevated levels of PFASs (>95th Percentile of the general population) and had a decrease since 2013. Only a few participants had elevated levels of PFOA, therefore half-life was not calculated for this compound.*

¥ Half-life was calculated for all participants that had a decrease or no change in concentration since 2013 (this half-life may be influenced by ongoing background exposure)

3.9 Assessment of Biomarkers and Self-reported Health Issues

This study provides an extensive dataset for a cross-sectional evaluation of potential associations between selected biochemical markers, self-reported health issues and selected PFAAs as a follow-up to the 2013 Airservices study. In addition, a subset of the participants in the 2013 Airservices study also participated in the current study, allowing for a longitudinal assessment of both changes in selected serum PFAAs and changes in biomarkers in those individuals. A detailed description, on the methodology and results is presented in Appendix V.

Below is an overview of the methods used in this assessment; refer to Appendix V for detail.

Method

Accounting for several factors that could potentially influence any of the measured biomarkers (see Table 2) or self-reported health issues, multiple statistical approaches and assessments were used to evaluate the associations between the outcomes and selected PFAA serum concentrations. Multiple linear regression was used to assess an overall linear relationship between the variation in biomarker levels and PFAA concentrations. In this assessment, PFAA concentration data were employed separately both as a continuous as well as a categorical variable (the latter as PFAA quartiles as presented in Appendix V Table A22, and where both across and between quartiles was investigated). Where significant associations were found, the relationship was further investigated by assessing the odds of having out-of-range biomarker levels (Appendix V Table A23), using logistic regression. Multiple linear regression was also used to assess the relationship between the change in biomarkers and change in PFAA concentration for participants with longitudinal data. In addition to measured biochemical markers, the participants provided self-reported health information vie the questionnaire. Logistic regression was used to investigate the relationship between having specific health issues and PFAA concentrations. An overview of all relationships assessed are presented in Table 5.

***Table 5**: Assessed associations between biochemical markers, self-reported health outcomes and PFAA concentrations (highlighted green). The numbers represent what models were used for assessment: 1) Multiple Linear Regression 2) Logistic Regression.*

Assessed Outcomes	Cross-Sectional				Longitudinal			
	PFOA	***PFHxS***	***PFHpS***	***PFOS***	***PFOA***	***PFHxS***	***PFHpS***	***PFOS***
Serum lipids								
Cholesterol	1	1	1	1, 2	1	1	1	1
HDL	1	1	1	1	1	1	1	1
LDL	1, 2	1, 2	1, 2	1, 2	1	1	1	1
Thyroid function markers								
TSH	1, 2	1	1	1				
T3	1	1	1	1				
T4	1	1	1	1				
Kidney function markers								
Urate	1, 2	1	1	1	1	1	1	1
eGFR	2	2	2	2				
Liver function markers								
ALT	1, 2	1	1	1				
Self-reported health conditions								
Asthma	2	2	2	2				
Cardiovascular disease	2	2	2	2				
Cancer (any)	2	2	2	2				
Cancer (Skin)	2	2	2	2				
Cancer (Prostate)	2	2	2	2				
Diabetes (Type 2)	2	2	2	2				
High blood pressure	2	2	2	2				
Kidney Problems	2	2	2	2				
Liver problems	2	2	2	2				
Reproductive/fertility problems	2	2	2	2				
Serious Arthritis	2	2	2	2				
Thyroid problems	2	2	2	2				

Blank: Not assessed

Results

An overview of the outcomes of the assessed associations between measured biochemical markers and selected PFAA concentrations are presented in Table 6 and Figure 20 (More detailed tables and figures are presented in Table A11-A21, and Figure A8-A9 in Appendix V).

Biochemical markers

Serum lipids (Total Cholesterol, HDL, LDL)

Measures of total cholesterol, HDL and LDL are used as biomarkers for cardiovascular disease. Total cholesterol (referred to as just “cholesterol” in this report), is the total amount of cholesterol in your blood, including HDL and LDL. HDL are lipids that facilitate the removal of cholesterol from the human body, and are often considered as “good cholesterol”. In contrast to HDL, LDL facilitate the transport of cholesterol to the tissues of the human body. LDL is often considered as “bad cholesterol” as high concentrations of LDL are linked with the development of cardiovascular disease outcomes. Several studies have reported positive

association between cholesterol levels an PFAA exposure, while associations between both HDL and LDL with PFAAs have been inconsistent [26].

In total, 646 participants were included for the analysis of an association between serum lipids and PFAA concentrations. In these participants, the average level of cholesterol was 5.48 (SD 0.99) mmol/L, the average HDL level was 1.35 (SD 0.34) mmol/L, and the average LDL level was 3.33 (SD 0.89) mmol/L.

The B-coefficients of the multivariate regression analysis is presented as "change in biomarker" in Appendix V, table A11. This is a measurement of the change in the outcome (biomarker), in relationship with one natural logarithm unit increase of PFAAs. The association between serum lipids and ln-transformed concentrations of PFAAs showed that increasing levels of cholesterol were associated with increasing PFAA levels, but this association only reached significance for PFOS. Increasing levels of LDL were positively associated with increasing ln- PFAA, with significant associations with PFOA, PFHxS and PFOS. HDL levels were not significantly associated with any of the assessed PFAAs.
In addition to the use of ln-transformed PFAA concentrations, associations between the changes of serum lipids with increasing quartiles of PFAAs were also assessed. Quartiles were assessed with quartile 1 (Q1) as a reference (lowest PFAA concentrations). Each quartile is a representation of a change in PFAA concentration from Q1. Additionally, a trend across all quartiles is assessed. Significantly associated relationships are presented graphically in Appendix V, Figure A8. The adjusted change in LDL between quartile 1 (Q1) and quartile 4 (Q4) of PFAAs were statistically significant for PFOS and PFHpS, where the trend associated with increasing PFOS-quartiles additionally was associated with increasing LDL. None of the other lipids demonstrated significant changes across quartiles that were associated with any of the PFAA. The associations between cholesterol, LDL and PFAAs were additionally assessed for any associations between increasing quartiles and the presence of out-of-range measurements (>5.5 mmol/L cholesterol >4 mmol/L LDL) using logistic regression. The OR of having out-of-range-values is presented in table A12, and no significant associations were found.

Liver function marker (ALT)

In this study, ALT was used as a biomarker for liver-function. ALT is a liver-enzyme, and when levels of this enzyme increase in the blood, it can indicate that the liver is damaged or diseased. Several studies have reported positive association between PFAA concentrations and ALT, but the results have not been consistent [26].
For the assessment of the association between ALT levels and PFAA concentrations, 781 participants were included. The average ALT level in these participants was 30.2 (SD 18) international units (IU)/L. There was an inverse association between ALT and ln-transformed concentrations of PFOA, indicating decreasing levels of ALT as serum PFOA increased, however there were no significant differences across quartiles. The associations between ALT and PFOA quartiles were additionally assessed for any associations between increasing quartiles and the presence of out-of-range measurements (M: >40 IU/L, F: >30 IU/L) using logistic regression and no significant associations were found. No statistically significant association between ALT and any of the other assessed PFAAs was found. The outcomes of the models are Table A13 and A14, in Appendix V)

Thyroid function markers (TSH, T3, T4)

The measurement of the blood concentrations of TSH, T3 and T4 are used as biological markers of thyroid function. TSH controls the thyroid hormone levels, and high and low concentrations of TSH can indicate an underactive or overactive thyroid gland, respectively. T4 and T3 hormones are produced by the thyroid gland and testing the levels of these hormones can also be used to assess thyroid function. Previous research does not show any clear or consistent evidence of an association between any of these hormones and PFAA concentrations [26].

In total, 759 participants were included in the analysis of the association between these markers and PFAA concentrations. The average concentrations of TSH, T3 and T4 were 1.5 (SD 1.4) mU/mL (milli-international units per litre), 4.5 (SD 0.5) pmol/L (picomoles/ litre), 12.9 (SD 1.4) pmol/L respectively. Associations between thyroid function markers and PFAA concentrations are presented in Appendix V; table A15. A significantly positive association was found between TSH and the trend of increasing quartiles of PFOA. However, TSH concentrations in PFOA Q4 were not significantly different from Q1. Nor was there an increased risk of any out of range values with increasing PFOA quartiles (Appendix V; Table A16). No significant associations were found between any of the other thyroid markers and PFAA concentrations.

Kidney function markers (Uric acid, eGFR)

Urate and eGFR were used as biomarkers for kidney function. Urate, the anion of uric acid, is the end product of purine metabolism. If the kidney function is reduced, urate can accumulate in the blood, and high measured levels of urate in the serum can therefore indicate poor kidney function. eGFR is an estimate of how much blood is filtered in the kidneys each minute, a low rate indicates that the kidneys are not working as well as they should. Previous research has shown limited evidence for an inverse association between eGFR and PFOS and PFOA, and no clear or consistent evidence of an association between uric acid and PFAA concentrations [26].

In total, 735 participants were included in the statistical analysis of associations between kidney function markers and PFAA concentrations. Of all participants, 2.4% were categorized as having low eGFR (<60 mL/min/1.73m2). The outcomes of the statistical analysis are presented in Appendix V; Table A17 and A18. The results from multiple regression analysis show that, after adjustment for potential confounders, urate was positively associated with both ln-transformed PFOA as well as with increasing PFOA quartiles (association is presented graphically in Appendix V; Figure A9). No significant associations were found for PFHxS, PFHpS or PFOS. The association between urate measurements and PFOA were further assessed through analysing the association between odds of out-of-range urate measurements (defined as>0.5 mmol/L for males and >0.4 mmol/L for females) with increasing quartiles through logistic regression. No significant association was found (Appendix V; Table A19). Logistic regression was also used to assess the odds of having low eGFR, and this assessment revealed that increasing PFOA concentrations were associated with a lower OR for eGFR. However, this association was not significant with increasing PFOA quartiles, or any with other PFAAs.

Self-reported health issues

Risk of high blood pressure, cardiovascular disease, kidney disease, liver problems, thyroid problems, diabetes, asthma, reproductive/fertility problems, arthritis and self-reported cancer outcomes, in relation to PFAA exposure, have all been of interest in previous studies but, to date, adequate or consistent evidence has not been provided [26]. The OR of having any of the self-reported health issues with increasing levels of PFAAs was assessed using logistic regression models (Appendix V; table A20).

All participants who answered the questionnaire were assessed and, of these, the following percentage stated that they had been diagnosed with: high blood pressure (22%); cardiovascular disease (7%); kidney disease (5%); liver problems (1.9%); thyroid problems (3%); diabetes type 1 (<1%); diabetes type 2(5%); asthma (12%); reproductive/fertility problems (3.4%); serious arthritis (6.5%); and, cancer (12%). Of those reporting having been diagnosed with cancer, skin cancer (52%) and prostate-cancer (23%) were the two most frequently reported. Because of the low number of participants with diabetes type 1 (<1%), this outcome was not assessed. Fully adjusted logistic regression shows that the OR for Serious Arthritis were <1 with each unit of increasing ln-PFOA levels, and the OR for Skin cancer was >1 for each unit of increasing ln-PFOA. The OR of being diagnosed with any of the other health issues was not significant.

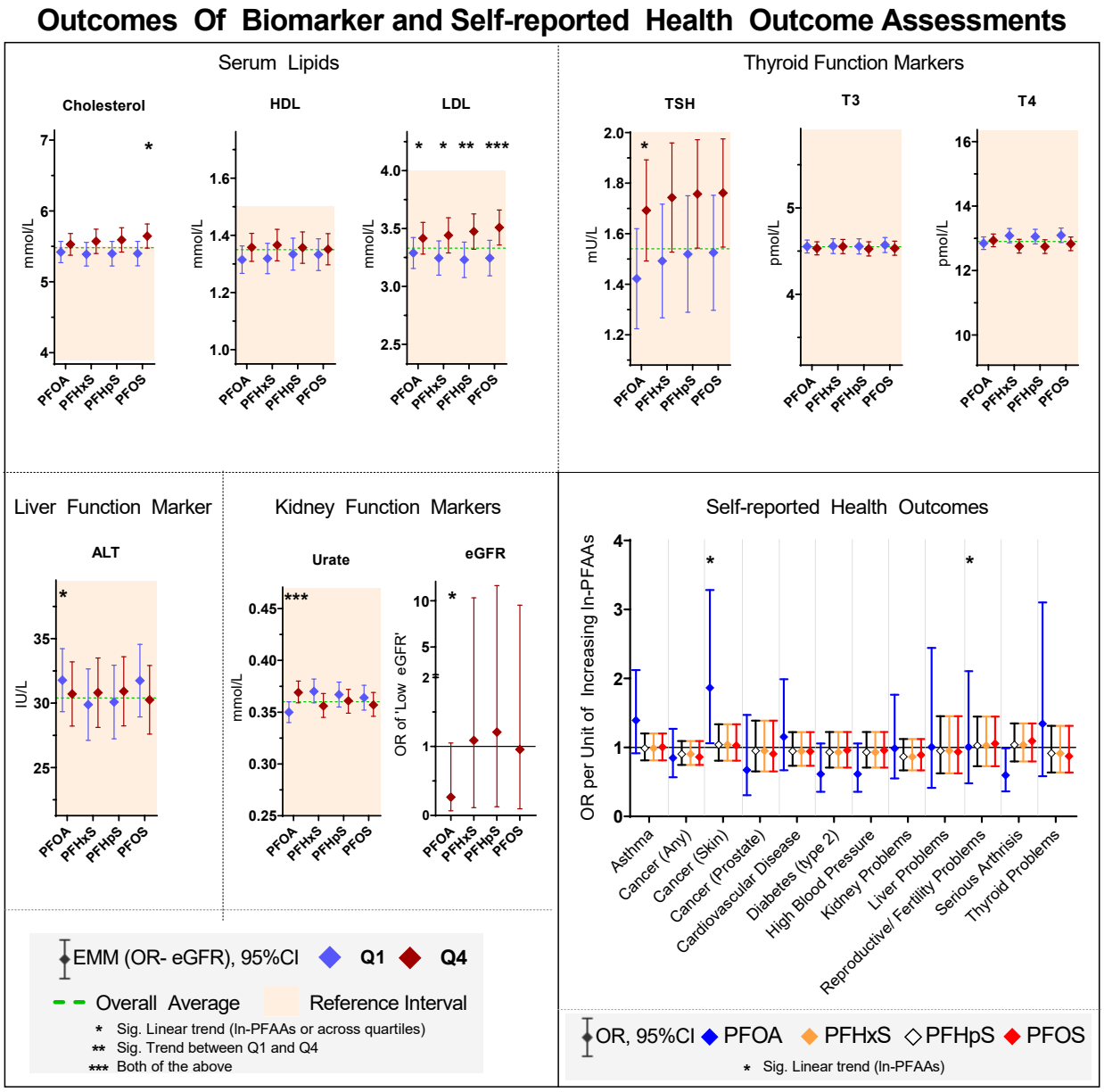

Figure 20. *Summary of the relationships between the biochemical markers, self-reported health issues and PFAA levels. For all biochemical markers apart from eGFR the symbols represent the co-variable -adjusted estimated marginal mean (EMM) of Quartile 1 (Q1; blue) and Quartile 4 (Q4; red). The dotted green line represents the overall average in all participants and the highlighted area represents the reference interval (RI) (where the reference differs between male, female and age, the RI for male, age 50 is displayed). For eGFR and self-reported health outcome graphs, the symbol represent the Odds Ratio (OR). In all graphs, the error bars represent the 95% confidence interval of the mean. *represent a significant linear trend (across ln-transformed concentrations or quartiles, or both) ** represents a significant trend between Q1 and Q4, while *** represent significant trends of all assessments. See Appendix V for concentration ranges of PFAA quartiles (Table A22) and for a graphical explanation of the plot (Figure A1).*

Assessment of biomarkers in the longitudinal data set

Assessments of cross-sectional design, such as presented above, assess the relationships at a specific point in time. However, this design prohibits any definite conclusions of causal relationships as exposure and outcome are simultaneously assessed and there are limitations to causal interference. To overcome these limitations, longitudinal data is useful to assess these association. Longitudinal data (i.e. two PFAA serum and biomarker measurements for the same person) is available for the 130 participants who participated in both the 2014 and this current study, and therefore, this data was used to assess the associations between change in biomarkers and change in PFOA, PFHxS and PFOS concentrations, as these compounds were quantified in all participants in both the 2013 and 2019 studies. PFHpS was not quantified in the 2013 study, and therefore, only participants who had their stored blood from 2013 re-analysed could be used in the assessment of PFHpS relationships (n=120). The change in biomarkers and PFAAs were defined by the ratio change, x(2019)/x(2013); i.e. the measurement in 2019 divided by the measurement in 2013. Where 2013 samples were stored and reanalysed for PFAAs, the reanalysed measurement was used for the 2013 measurement. The average ratio change for PFAA was 0.42, 0.58, 0.55 and 0.51 for PFOA, PFHxS, PFHpS and PFOS respectively, which represent a 58%, 42%, 45% and 49% decrease in respective PFAA concentration since 2013. The biomarkers assessed as outcomes were serum lipids (cholesterol, HDL and LDL) and the kidney function biomarker urate. The percentage change in biomarkers, in relation to the percentage change in PFOS concentrations are presented in Appendix V; Figure A10.

Serum lipids

In total, 99 participants were included in the statistical analysis of the association between change in cholesterol and LDL and change in PFOA, PFHxS and PFOS concentrations, while LDL measurements were available for 97 of these participants. Data on PFHpS was limited to 92 participants. Average cholesterol, HDL, and LDL concentrations in the participants increased, between 2014 and 2019, by 7, 3, and 10%, respectively (representing 2019/2013 ratio 1.07, 1.03, 1.10). None of the changes in the lipid biomarkers were significantly associated with the changes in any of the PFAA concentrations between 2013 and 2019 (Table A21; Figure A8).

Urate

In total, 124 participants were included in the statistical analysis of the association between change in urate and change in PFOA, PFHxS and PFOS concentrations while data was limited to 114 participants for PFHpS. The average ratio change in urate was 1.01, representing a 1% increase since 2013. No significant associations were found between changes in urate levels from 2013 to 2019 and changes in the serum concentrations of any of the PFAAs over the same time period (Table A21).

***Table 6**: Assessed associations between biochemical markers, self-reported health outcomes and PFAA concentrations*

Assessed Outcomes	Cross-Sectional				Longitudinal			
	PFOA	*PFHxS*	*PFHpS*	*PFOS*	*PFOA*	*PFHxS*	*PFHpS*	*PFOS*
Serum lipids								
Cholesterol	-	-	-	↑*	-	-	-	-
HDL	-	-	-	-	-	-	-	-
LDL	↑*	↑*	↑*	↑*	-	-	-	-
Thyroid function markers								
TSH	↑*	-	-	-				
T3	-	-	-	-				
T4	-	-	-	-				
Kidney function markers								
Urate	↑*	-	-	-	-	-	-	-
eGFR	*	-	-	-				
Liver function markers								
ALT	↓*	-	-	-				
Self-reported health conditions								
Asthma	-	-	-	-				
Cardiovascular disease	-	-	-	-				
Cancer (any)	-	-	-	-				
Cancer (Skin)	↑	-	-	-				
Cancer (Prostate)	-	-	-	-				
Diabetes (Type 2)	-	-	-	-				
High blood pressure	-	-	-	-				
Kidney Problems	-	-	-	-				
Liver problems	-	-	-	-				
Reproductive/fertility problems	-	-	-	-				
Serious Arthritis	↓	-	-	-				
Thyroid problems	-	-	-	-				

↑; (Red); Positive association

↓; (Blue); Negative association

**: Increasing PFAS concentrations do not increase the risk of out-of-range values*

-; (Green); Assessed but no association found,

Blank: Not assessed

Discussion and Limitations of the Assessment of Biochemical and Health-issues

Comparing these results to those of other exposure studies for PFAAs helps to contextualise these observations. The findings of an association between cholesterol, LDL and PFAAs in this study are consistent with previous studies. Positive associations between cholesterol and LDL and PFAAs (especially PFOA and PFOS) have previously been reported in several studies across different populations and age groups, in general population studies as well as populations with elevated exposure (including populations with residential PFAA exposure and occupational PFAA exposure) [27-29]. Longitudinal studies have also reported a reduction in the serum lipids as PFAA concentrations decrease after exposure ceases [30].

Some studies investigating kidney function biomarkers have shown positive relationships between serum uric acid and PFAA exposure and an inverse association between eGFR and PFAA exposure. In this study, PFOA levels were not considered elevated in the participants, however, a positive relationship between urate and

PFOA was found. A positive relationship has been reported in the general population in previous studies (e.g. [31]), but also in in populations with elevated exposure, such as PFOA production workers [32]. Previous studies have demonstrated a reverse relationship between eGFR and PFASs [26], whereas this study demonstrated a reduced risk of having an abnormally low eGFR (it is possible that our results could reflect a chance finding).

In other studies, the associations between other biomarkers (including markers for thyroid function and liver function) and PFAAs have not been consistent. In this current study, the associations between these biomarkers and PFOA were not consistent among the different measures of PFOA assessments (i.e. the association was not significant among both continuous and categorical PFOA scales). When considering the inconsistent findings of this study's results and those of previous studies, it is likely that any relationship is weak, or it is possible that the observed associations are simply chance findings.

The underlying mechanism by how PFAA concentrations may affect the assessed biomarkers is not yet clear and further research is needed. Previous literature has provided some evidence supporting a causal relationship between serum cholesterol and PFAA concentrations [30]. For the other biomarkers, a reverse causation is possible. For example, PFAAs have been found to be excreted mainly by the kidneys [33]. Therefore, decreased kidney function with a decreased filtration rate could potentially result in slower elimination and consequently increase serum PFAAs over time [34].

Using multiple different analytical approaches, our study provides an extensive assessment of this data set. However, this approach, with multiple statistical comparisons (>100), presents an increased risk of chance findings (type 1 error). Other limitations in this study include the small number of individuals (n=130) in the longitudinal arm, which decreases the statistical power. This longitudinal assessment only includes data gathered at two time-points, so only an assumed linear association can be explored. However, if the response of biomarkers lags behind changes in the PFAA concentration, it is likely that a non-linear association (if any) exists. Additional limitations for this study include the possibility of confounders, such as unreported health issues that could alter the biomarkers, or socioeconomic status (which can impact a number of biomarker outcomes). The use of self-reported data provides some understanding of the findings, but the accuracy of such data can be limited. Relationships between self-reported health issues and PFAA concentrations should be interpreted with caution. Similarly, the number of participants defined with health issues was small which also limits the statistical examination for associations. Additionally, it may take several years before a diagnosable health issues develops in response to PFAA exposure, making any association more difficult to identify.

4. Conclusion

The Airservices 2018/2019 Exposure study enrolled a total of 799 participants. Each participant filled out a questionnaire about work history and lifestyle. Serum samples were collected and analysed for health biomarkers as well as a suite of PFASs. With reference to the study aims, the findings of this are:

Aim 1: Assess PFAS blood concentrations (integrative exposure) in Airservices current and former staff and evaluate links to 'work history'.

The average levels of PFOA found in Airservices staff and ex staff that participated in this study were comparable to the general Australian population, while the levels of PFHxS, PFHpS and PFOS were elevated. These elevated concentrations were found in participants who had been employed by Airservices prior to 2005, when 3M LightWater was still in use. The group of participants who commenced service after this foam was phased out (post 2005) did not have higher average serum concentrations of PFHxS, PFHpS and PFOS compared to the general Australian population.

Aim 2: Evaluate exposure trends (which answers whether blood levels are consistently changing, and if so, how those trends compare to those observed in the general population).

Compared to the results from the 2013 study, it can be observed that the average PFAS concentrations of PFOA, PFHxS and PFOS among all participants (as well as PFHpS which was reanalysed in 120 of the participants from the 2013 study) were 55-65% lower, recognizing that the two groups overlap but are not the same individuals overall. Among staff who participated in both the 2013 survey and the current survey, the temporal trends of PFAS concentration could additionally be observed on an individual level. Among these individuals, an average decrease of 58% for PFOA, 42% for PFHxS, 45% for PFHpS and 49% for PFOS was observed. Compared to the general Australian population (pooled and cross-sectional data), as well as a general population from Sweden (PFOA and PFOS), the recoded average decreases of the Airservices cohort were higher, indicating faster clearance of the assessed PFAAs.

Aim 3: Assess PFAS relevant biochemical markers and/or confounders associated with PFAS serum concentrations.

Cholesterol and LDL were both positively associated with all PFAAs. There was a statistically significant relationship between LDL and all PFAAs, and between cholesterol and PFOS. The positive associations were further supported by longitudinal data. Lower increases in cholesterol and LDL levels were associated with a greater decrease in PFAAs, although not statistically significant. However, greater levels of PFAAs were *not* associated with a greater risk of out-of-range values or with a greater risk of hypertension or other cardiovascular disease. Although levels of PFOA in the Airservices cohort were similar to background levels in Australia, increasing levels of PFOA were positively associated with urate levels in the cross-sectional analysis. This relationship was not observed in longitudinal data. Increasing PFOA levels (continuous) were associated with a lower risk of low eGFR, and with lower levels of ALT. However, significant associations were not confirmed for increasing PFOA quartiles. TSH levels were positively associated with PFOA quartiles, but this trend was not significant when assessing continuous PFOA concentrations. Nor was there an increased risk of having out-of-range TSH values with increasing PFOA levels. No other statistically significant relationships were found between any measured biomarkers and PFAA concentrations.

There are potential limitations connected with any self-reported health data and the relationship with PFAA concentrations needs to be interpreted with caution. For example, issues such as unconfirmed diagnoses, or misunderstandings of health issues, can impact the reported results.
In summary, this study documents associations between some biochemical markers and PFAA exposure. However, the effects found are marginal and no associations with clinically significant health issues were identified.

Aim 4: Provide ongoing advice to Airservices to assess and minimise exposure risks to PFASs.
Decreasing average concentrations in serum of the Airservices cohort, as well as individual decreases in participants who participated in both studies, indicate that work health and safety practises that have been implemented by Airservices are working in reducing exposure. In particular, substitution of AFFF undertaken in 2005 and ultimate replacement in 2010 was clearly effective at reducing or eliminating elevated exposures. The study provides convincing evidence that the replacement of PFAS-based foams and health and safety controls to minimise PFAS exposure have been effective and there is minimal ongoing occupational PFAS exposure for Airservices staff.

5. Summary of Findings

The key findings of this study are as follows:

- Higher concentrations of PFHxS, PFHpS and PFOS were found in the serum of Airservices staff and ex staff members compared to the general Australian.
- Concentrations of PFOA were comparable with the levels in the general Australian population.
- Study participants who commenced service starting from 2005 had PFAS concentrations similar to those of the general population. This suggests that the exposure to the evaluated PFAAs have declined in recent years.
- PFAS concentrations were lower in participants who were regular blood donors.
- Elimination half-lives of PFHxS, PFHpS and PFOS were estimated to be 8.2 years, 7.8 years and 6.6 years, respectively
- The following associations were found in the assessed biochemical markers: Increasing levels of LDL were associated with increasing levels of all four assessed PFAAs, and cholesterol was positively associated with serum PFOS levels; the biomarkers of liver function, ALT, and thyroid function, TSH, had significant positive linear relationships with ln-PFOA concentration and PFOA quartiles respectively; and, biomarkers of kidney functions, urate and eGFR, were associated with PFOA concentrations, with urate positively associated with serum PFOA concentrations while a decreased risk of having abnormally low eGFR was observed in relation to increasing PFOA concentrations. The positive associations that were found were in general relatively small and did not result in an increased risk of levels above the clinical reference range. Lower risk of eGFR may have been a chance finding due to increased risk of Type 1 error possible in this analysis.
- No significant associations were found between temporal changes in cholesterol, HDL, LDL or urate and the changes in PFAA concentration in a subset of 130 individuals that participated in both the 2013 and 2019 study. Although, slight trends can be observed that supporting the findings of a

positive association between cholesterol, LDL and PFAA, the low number of individuals assessed limits the possibility of detecting any associations.

- Of the twelve categories of self-reported health conditions assessed, there were two significant relationships found: a lower risk (decreased OR) for self-reported Serious Arthritis was associated with increasing concentrations of serum PFOA; and, a higher risk (increased OR) for self-reported skin cancer was associated with increasing concentrations of serum PFOA. Limitations in the assessment of associations between self-reported health issues and PFAA concentrations must be considered.

6. References

1. Buck, R.C., J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury, and S.P. van Leeuwen, *Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins.* Integrated environmental assessment and management, 2011. **7**(4): p. 513-541.

2. Moody, C.A. and J.A. Field, *Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams.* Environmental science & technology, 2000. **34**(18): p. 3864-3870.

3. Rotander, A., L.-M.L. Toms, L. Aylward, M. Kay, and J.F. Mueller, *Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF).* Environment International, 2015. **82**: p. 28-34.

4. Kärrman, A., J.F. Mueller, B. van Bavel, F. Harden, L.-M.L. Toms, and G. Lindström, *Levels of 12 perfluorinated chemicals in pooled Australian serum, collected 2002–2003, in relation to age, gender, and region.* Environmental Science & Technology, 2006. **40**(12): p. 3742-3748.

5. Ubel, F., S. Sorenson, and D. Roach, *Health status of plant workers exposed to fluorochemicals-a preliminary report.* American Industrial Hygiene Association Journal, 1980. **41**(8): p. 584-589.

6. Sunderland, E.M., X.C. Hu, C. Dassuncao, A.K. Tokranov, C.C. Wagner, and J.G. Allen, *A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects.* Journal of Exposure Science & Environmental Epidemiology, 2019. **29**(2): p. 131-147.

7. Toms, L.M.L., J. Bräunig, S. Vijayasarathy, S. Phillips, P. Hobson, L.L. Aylward, M.D. Kirk, and J.F. Mueller, *Per- and polyfluoroalkyl substances (PFAS) in Australia: Current levels and estimated population reference values for selected compounds.* International Journal of Hygiene and Environmental Health, 2019.

8. Toms, L.M.L., J. Thompson, A. Rotander, P. Hobson, A.M. Calafat, K. Kato, X. Ye, S. Broomhall, F. Harden, and J.F. Mueller, *Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations in an Australian population from 2002 to 2011.* Environment International, 2014. **71**: p. 74-80.

9. Toms, L.-M.L., A.M. Calafat, K. Kato, J. Thompson, F. Harden, P. Hobson, A. Sjödin, and J.F. Mueller, *Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia.* Environmental Science & Technology, 2009. **43**(11): p. 4194-4199.

10. CDC, *Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey (NHANES) Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2013 - 2014. .* 2015-2016.

11. Backe, W.J., T.C. Day, and J.A. Field, *Zwitterionic, cationic, and anionic fluorinatedchemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS.* Environmental Science & Technology, 2013. **47**(10): p. 5226-5234.

12. Olsen, G.W., J.M. Burris, M.M. Burlew, and J.H. Mandel, *Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations.* Journal of occupational and environmental medicine, 2003. **45**(3): p. 260-270.

13. Barzen-Hanson, K.A., S.C. Roberts, S. Choyke, K. Oetjen, A. McAlees, N. Riddell, R. McCrindle, P.L. Ferguson, C.P. Higgins, and J.A. Field, *Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater.* Environmental Science & Technology, 2017. **51**(4): p. 2047-2057.

14. Aylward, L.L., E. Green, M. Porta, L.-M. Toms, E. Den Hond, C. Schulz, M. Gasull, J. Pumarega, A. Conrad, M. Kolossa-Gehring, G. Schoeters, and J.F. Mueller, *Population variation in biomonitoring data for persistent organic pollutants (POPs): An examination of multiple population-based datasets for application to Australian pooled biomonitoring data.* Environment International, 2014. **68**: p. 127-138.

15. Eriksson, U., J.F. Mueller, L.-M.L. Toms, P. Hobson, and A. Kärrman, *Temporal trends of PFSAs, PFCAs and selected precursors in Australian serum from 2002 to 2013.* Environmental Pollution, 2017.

16. Lorber, M., G.E. Eaglesham, P. Hobson, L.M.L. Toms, J.F. Mueller, and J.S. Thompson, *The effect of ongoing blood loss on human serum concentrations of perfluorinated acids.* Chemosphere, 2015. **118**: p. 170-177.

17. Stubleski, J., S. Salihovic, L. Lind, P.M. Lind, B. van Bavel, and A. Kärrman, *Changes in serum levels of perfluoroalkyl substances during a 10-year follow-up period in a large population-based cohort.* Environment International, 2016. **95**: p. 86-92.

18. Li, Y., T. Fletcher, D. Mucs, K. Scott, C.H. Lindh, P. Tallving, and K. Jakobsson, *Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water.* Occupational and Environmental Medicine, 2018. **75**(1): p. 46-51.

19. Olsen, G.W., J.M. Burris, D.J. Ehresman, J.W. Froehlich, A.M. Seacat, J.L. Butenhoff, and L.R. Zobel, *Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers.* Environmental Health Perspectives, 2007. **115**(9): p. 1298-1305.

20. Worley, R.R., S.M. Moore, B.C. Tierney, X. Ye, A.M. Calafat, S. Campbell, M.B. Woudneh, and J. Fisher, *Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community.* Environment international, 2017. **106**: p. 135-143.

21. Gomis, M.I., R. Vestergren, M. MacLeod, J.F. Mueller, and I.T. Cousins, *Historical human exposure to perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using population-based pharmacokinetic modelling.* Environment International, 2017. **108**(Supplement C): p. 92-102.

22. Olsen, G.W., C.C. Lange, M.E. Ellefson, D.C. Mair, T.R. Church, C.L. Goldberg, R.M. Herron, Z. Medhdizadehkashi, J.B. Nobiletti, J.A. Rios, W.K. Reagen, and L.R. Zobel, *Temporal Trends of Perfluoroalkyl Concentrations in American Red Cross Adult Blood Donors, 2000–2010.* Environmental Science & Technology, 2012. **46**(11): p. 6330-6338.

23. Xu, Y., T. Fletcher, D. Pineda, C.H. Lindh, C. Nilsson, A. Glynn, C. Vogs, K. Norström, K. Lilja, and K. Jakobsson, *Serum Half-Lives for Short-and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam.* Environmental Health Perspectives, 2020. **128**(7): p. 077004.

24. Li, Y., Y. Xu, K. Scott, C. Lindh, K. Jakobsson, and T. Fletcher, *Half-lives of PFOA, PFPeS, PFHxS, PFHpS and PFOS after end of exposure to contaminated drinking water.* Environmental Epidemiology, 2019. **3**.

25. Kirk, M., K. Smurthwaite, J. Bräunig, S. Trevenar, C. D'Este, R. Lucas, A. Lal, R. Korda, A. Clements, and J. Mueller, *The PFAS Health Study: Systematic Literature Review.* Canberra: The Australian National University, 2018.

26. Eriksen, K.T., O. Raaschou-Nielsen, J.K. McLaughlin, L. Lipworth, A. Tjønneland, K. Overvad, and M. Sørensen, *Association between Plasma PFOA and PFOS Levels and Total Cholesterol in a Middle-Aged Danish Population.* PLOS ONE, 2013. **8**(2): p. e56969.

27. Frisbee, S.J., A. Shankar, S.S. Knox, K. Steenland, D.A. Savitz, T. Fletcher, and A.M. Ducatman, *Perfluorooctanoic Acid, Perfluorooctanesulfonate, and Serum Lipids in Children and Adolescents: Results From the C8 Health Project.* Archives of Pediatrics & Adolescent Medicine, 2010. **164**(9): p. 860-869.

28. Sakr, C.J., K.H. Kreckmann, J.W. Green, P.J. Gillies, J.L. Reynolds, and R.C. Leonard, *Cross-Sectional Study of Lipids and Liver Enzymes Related to a Serum Biomarker of Exposure (ammonium perfluorooctanoate or APFO) as Part of a General Health Survey in a Cohort of Occupationally Exposed Workers.* 2007. **49**(10): p. 1086-1096.

29. Fitz-Simon, N., T. Fletcher, M. Luster, K. Steenland, A. Calafat, K. Kato, and e. al., *ERRATUM: Reductions in Serum Lipids with a 4-year Decline in Serum Perflourooctanoic Acid and Perfluorooctanesulfonic Acid.* Epidemiology, 2013. **24**(6): p. 941-941.

30. Shankar, A., J. Xiao, and A. Ducatman, *Perfluoroalkyl chemicals and elevated serum uric acid in US adults.* Clinical epidemiology, 2011. **3**: p. 251-258.

31. Costa, G., S. Sartori, and D. Consonni, *Thirty years of medical surveillance in perfluooctanoic acid production workers.* Journal of occupational and environmental medicine, 2009. **51**(3): p. 364-372.

32. Han, X., D.L. Nabb, M.H. Russell, G.L. Kennedy, and R.W. Rickard, *Renal Elimination of Perfluorocarboxylates (PFCAs).* Chemical Research in Toxicology, 2012. **25**(1): p. 35-46.

33. Kataria, A., H. Trachtman, L. Malaga-Dieguez, and L. Trasande, *Association between perfluoroalkyl acids and kidney function in a cross-sectional study of adolescents.* Environmental Health, 2015. **14**(1): p. 89.

7. Appendices

Appendix I. Questionnaire

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

Participant Code | | | | |

Evaluation of per- and poly- fluoroalkyl substances (PFASs) in Airservices Australia's Aviation Rescue Fire Fighting Services (ARFFS) staff – 2018 Exposure Study

Questionnaire for Participants

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

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.

If you need help or have any questions or comments?

Please ring us on *toll free number* **1800 370 760**

OR Mobile 0436 325 850

OR email us on *ASAstudy@uq.edu.au*

There are no right or wrong answers or trick questions in this survey. Your responses are simply to help better understand the data gathered in this study.

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.

Should you wish to withdraw at any stage from the study, or to withdraw any unprocessed data you have supplied, you are free to do so without prejudice.

YOUR DETAILS

1. Please state your date of birth

DD	MM	YYYY

2. What is your gender?

Male	1
Female	2
Gender not specified	3

3. What is your country of birth?

If you were NOT born in Australia – what year did you come to live in Australia?

YYYY

LIFESTYLE INFORMATION

We are now going to ask you a few questions about your lifestyle, your health, your diet and your work. This information is important for the interpretation of the chemical results obtained in this study.

4. Which of the following best describes your smoking status now?

I have never smoked	1	***(go to question 9)***
I used to smoke	2	
I now smoke occasionally	3	
I now smoke regularly	4	

5. If you used to smoke, how long ago did you quit smoking?

I quit within the last 6 months	1
I quit 6 – 12 months ago	2
I quit more than one year ago	3
I still smoke	4

6. In the LAST WEEK how many cigarettes did you usually smoke PER DAY?

Did not smoke at all	1
1 – 9 per day	2
10 – 19 per day	3
20 – 29 per day	4
30 – 49 per day	5
50 or more per day	6

7. Estimated number of years smoking?

		Number of years

8. How many times have you tried to quit smoking?

(Write '0' if never tried)

		Number of times

The next questions are about your use of alcoholic beverages during this past year. Some items below ask questions about how many **'standard drinks'** you have had.

When referring to 'a standard drink':

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 often do you have a drink containing alcohol?

Never	1	***(go to question 12)***

Monthly or less	2
Two to four times per month	3
Two to three times per week	4
Four or more times per week	5

10. How many standard drinks containing alcohol do you have on a typical day when you are drinking?

1 or 2 drinks	1
3 or 4 drinks	2
5 or 6 drinks	3
7, 8 or 9 drinks	4
10 or more drinks	5

11. How often do you have 5 or more standard drinks on one occasion?

Never	1
Less than monthly	2
Monthly	3
Weekly	4
Daily or almost daily	5

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

(Examples of moderate-strenuous exercise is fast walking, playing tennis, dancing, biking, swimming, going to the gym)

Never	1
Once a week	2
Two to three times a week	3
Four to five times a week	4
More than five times a week	5

13. Which diet best describes your normal diet?

Mixed Diet – meat and vegetables	1
Vegetarian	2
Vegan (no animal products)	3

14. On average, how often do you eat fish or other seafood, including tinned fish (e.g. tinned tuna)?

Never	1
Less than once a week	2
Once a week	3
Two to three times a week	4
Four to five times a week	5

Daily	6

15. On average, how often do you consume <u>milk and milk products</u>, including cheese?

Never	1
Less than once a week	2
Once a week	3
Two to three times a week	4
Four to five times a week	5
Daily	6

16. On average, how often do you consume <u>meat</u>?

Never	1
Less than once a week	2
Once a week	3
Two to three times a week	4
Four to five times a week	5
Daily	6

17. Are you a blood donor?

No	1	***(go to question 20)***
Yes	2	

18. If yes, how frequently do you donate?

Less than once a year	1
Once a year	2
2 to 4 times per year	3
More than 4 times per year	4

19. When was the approximate <u>date</u> (month & year) of your last blood donation?

MM	YYYY

YOUR HEALTH

20. **The next questions are about health problems you might have had at any time in your life. Has a doctor ever diagnosed you as having any of the following:** *Please answer '**Yes/No' to each condition***			**No**	**Yes**	**If 'YES' What was your age when first diagnosed?**	**If 'YES' please state type of problem/s *(if necessary)***
a. Diabetes (high blood sugar) type 1			1	2		
b. Diabetes (high blood sugar) type 2			1	2		
c. High blood pressure *(Hypertension)*			1	2		
d. Cardiovascular disease (*e.g. Heart Attack, Stroke, Angina)*			1	2		
e. Kidney problems *(incl. kidney stones)*			1	2		
f. Liver problems			1	2		
g. Asthma			1	2		
h. Reproductive or fertility problems			1	2		
i. Thyroid problems			1	2		
j. Serious Arthritis *(incl. Rheumatoid arthritis)*			1	2		
k. Chronic back or neck problems			1	2		
l. Cancer *please specify:*_____			1	2		
m. Any other chronic pain *please specify:*_____			1	2		
n. Other major physical illness *please specify:*_____			1	2		

21. Are you currently taking any of the following <u>medications</u>? **If, YES, please provide the <u>details</u> of the medication** *Please answer '**Yes/No' to each condition***	**No**	**Yes**	**If 'YES'** **What is the name of the medicine/s?**
1. Medication for **High Cholesterol**	1	2	
2. Medication for **Gout**	1	2	
3. Medication for **Diabetes**	1	2	
4. Medication for **Epilepsy**	1	2	
5. **Fluid tablets**	1	2	
6. **Antibiotics** *(incl. treatment for TB)*	1	2	
7. Medication for **Thyroid problems**	1	2	
8. **Chemotherapy** *(incl. methotrexate)*	1	2	

22. How tall are you without shoes? _____cm

Please note: heights are recorded on your driver's licence

23. How much do you weigh (in very little or no clothing and without shoes**)?** _____Kgs

OCCUPATIONAL HISTORY with ARFFS

We are now going to ask you some questions regarding your job assignments/employment as a fire fighter in roles currently or previously held at Airservices Australia, any of its predecessor organisations, or other agencies prior to or since your employment with Airservices (or its predecessors).

Instructions for Q24:

i). If you have held any of the below positions during your employment at Airservices Australia, please fill out the required information in the table below (i.e. duration, timeframe, employer, where/location).

ii). THEN complete the additional questions for each job/position

- Read '**Additional Question 1'** (see over page) **and write the number that corresponds to your answer in the table column** (e.g. write '3' if your answer is 'once a week').
- Then read '**Additional Question 2'** (see over page) **and again write the number that corresponds to your answer in the table column** (e.g. write '2' if your answer is 'yes – most of the time'). Then complete '**Additional Question 2a'** (write PPE you wore – **NB:** if you had more than one job please list PPE separately for each job).
- Then answer '**Addition Question 3'** **again by writing the number that corresponds to your answer in the table column.**

24. Have you ever held the following positions during your employment at Airservices Australia (or its predecessors)? If, YES, please write when and for what organisation?

Role	Duration	Timeframe	Employer	Where/location	Additional Question 1 *Please write corresponding number 1-5 for your answer*	Additional Question 2 *Please write corresponding number 1-4 for your answer*	Additional Question 3 *Please write corresponding number 1-5 for your answer*
e.g. Firefighter	***5 years***	***2005-2010***	***Airservices***	***Hobart, Cairns***	***3***	***4***	***3***
	2 years	***2011-2012***	***RAAF***	***RAAF Amberley***	***2***	***1***	***3***
Senior Officer							
Officer							
Firefighter							

Role	**Duration**	**Timeframe**	**Employer**	**Where/location**	**Additional Question 1** *Please write corresponding number 1-5 for your answer*	**Additional Question 2** *Please write corresponding number 1-4 for your answer*	**Additional Question 3** *Please write corresponding number 1-5 for your answer*
Instructor							
Emergency vehicle technician (EVT)							

Additional Question 1. **How frequently did you have contact with <u>aqueous film forming foams</u>** (incl. Ansul Ansulite and 3M Lightwater) **when employed?**

NOTE**: do NOT include exposure to **Solberg foam (i.e. RF6 or Training Foam)

Never	1
Less than once a month	2
Once a week	3
Twice a week	4
Most days	5

Additional Question 2. **Were you wearing any Personal Protective Equipment (PPE) when you were in contact with <u>aqueous film forming foams</u>** (incl. Ansul Ansulite and 3M Lightwater) **when employed in this position?**

NOTE**: do NOT include exposure to **Solberg foam (i.e. RF6 or Training Foam)

Yes - always	1
Yes – most of the time	2
Yes – only sometimes	3
No – none	4

Additional Question 2a. **Can you please list the types of PPE you were wearing while in contact with aqueous film forming foams during each job?**

Job Title:

Job Title:

Job Title:

Additional Question 3. **How much skin was routinely exposed to aqueous film forming foams during this job?**

NOTE**: do NOT include exposure to **Solberg foam (i.e. RF6 or Training Foam)

None	1
Mostly just hands	2
Hands and arms	3
Hands, arms, and trunk	4
Whole body skin exposure	5

23. Have you had any <u>other jobs at Airservices</u> (or its predecessors) in which you routinely handled or used aqueous film forming foams?

No 1 ***(go to question 25)***

Yes 2

24. If 'YES', please provided details below for each job:

Role and location within Airservices	Years Position Held	Describe foam use and contact (frequency and amount of skin exposure)

25. Have you had any <u>other</u> jobs (<u>NOT</u> at Airservices or its predecessors) in which you were in <u>contact with PFAS</u> or similar chemicals?

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

No 1 ***(go to question 27)***

Yes 2

26. If 'YES', please provided details below for each job:

Organisation/Location	Years Position Held	Describe foam use and contact (frequency and amount of skin exposure)

27. Are you or have you attended training at other ASA fire stations or fire training ground sites?

No 1 ***(go to question 29)***

Yes 2

28. If 'YES', please specify location and duration :

Location	Duration of training

29. Have you attended any emergency response where <u>aqueous film forming foams</u> (incl. Ansul Ansulite and 3M Lightwater) **were used?**

No 1 ***(go to question 31)***

Yes 2

30. If 'YES', please specify location and year of incident :

Location	Year of Incident

31. Have you ever lived <u>within 5km</u> of a <u>Defence base</u>?

No 1 ***(go to question 33)***

Yes 2

32. If 'YES', please specify location and years of residence:

Location	Years of Residence

33. Have you ever lived <u>within 5km</u> of an <u>airport</u>?

No 1 ***(go to question 35)***

Yes 2

34. If 'YES', please specify location and years of residence :

Location	Years of Residence

35. Can you think of any other ways you may have been exposed to <u>aqueous film forming foams</u>?

Hooray you're finished !!!!!
*Once again, **<u>Thank You</u>** for taking the time to complete this questionnaire.*

If you would like to make any comments, please write them below:

Appendix II. Background Information of Statistics Used in This Report

Terms

Descriptive Statistics

Central tendency

Mean; The mean is a value to describe central tendency. It is calculated by adding all individual values and dividing the sum by the number of values.

Median; The median describes the central tendency of the data set. It is the middle value of all sets of data arranged in the order lowest to highest (or vice versa). In the population, half of the population has values below the median while the other half has values above the median.

Distribution

Distribution in our samples

Standard Deviation (SD); To describe the scatter of the population, the SD is a useful measurement of the variability. The SD describes the variability around the mean. In a normal distribution, 68.3% of all measurements will fall between ±1 standard deviation, while 95,4% of all measurements will fall between ±2 standard deviations around the mean. In this report, the SD is often presented in tables in addition to mean values.

Coefficient of variation (CV); CV, also known as the relative standard deviation (RSD) describes the SD as a percentage in relation the mean of the sample population. In this report, we present our QAQC results in the form of CV of repeated measurements. A low CV represents a low variability in our measurements.

Quartiles and percentiles; The spread around the median is often described by percentiles and quartiles. These are determined by arranging all sets of data in the order lowest to highest. To determine the **quartiles**, this data set is grouped into four groups, where the first quartile (Q1) contains 1/4 of all values that have the lowest observations (i.e. the 25% of the lowest data points). The second quartile (Q2) contains the data points between the lowest 1/4 and median value, and so on. **Percentiles** is a measurement at which the percentage of the total values are at, or below this measurement. In this report, the 5^{th} and 95^{th} percentiles are presented in several graphs and tables, these values indicate that of all measurements, 5% and 95% falls below the 5^{th} and 95^{th} percentiles, respectively.

Distribution between our sample and the true population

Standard error of the mean (SEM); The standard error of the mean (SEM), is a value describing how far the estimated mean in our study population varies from the true population mean, if repeated samples are taken from the population. Generally, the greater number of samples included in a study, the lower the SEM (i.e. the closer our estimated mean is to the true mean of the population). In this report, the SEM is displayed in graphs presenting PFAS concentrations by blood donations (Figure 13-14).

Confidence interval (CI) The confidence intervals define the precision of the estimated mean in our study population in relation to the true mean of the population. Often, a 95% CI is used. With 95% CI, the confidence limits are two extremes of a measurement in which 95% of all observations would lie. Consequently, if the same experiment was repeated 100 times, 95 of these experiments would return a mean value within these two extreme values.

Statistical Analysis

P-value; In significance testing. The p-value is the probability of obtaining the observed results, under the assumption that there is no difference. The p-value is expressed as decimals, but we can also discuss it as converted percentage. For example, a p-value of 0.01 is 1% i.e. p=0.01 means that there is a 1% chance that the observed results happened by random chance. In our assessments, we consider a result to be significant when the p-value is <0.05, i.e. there is less than 5% chance that our results are random. In this report, we present p-values in the tables where all statistical outcomes are presented (see a few examples in Figure A1-A2).

Positive and reverse (negative) associations; In this report, we describe some associations as positive or reverse (negative). This is not the same as good and bad but reflects the direction of a relationship. A positive association between two variables indicates that when one of the variables increases, the other variable also increases. A reverse (negative) association indicates that when one variable increases, the other variable decreases. The associations are also interchangeably described as “relationships” and “correlation” in this report (e.g. a ‘positive relationship’ between two variables is the same as a ‘positive association’ between these variables. These variables can also be described as ‘positively correlated’).

Outcome, predictors, Co-variables (covariate) and adjusting; When assessing the association between two variables; an outcome and a predictor, there may be other variables that can be important to take into consideration; co-variables, also commonly called covariates. Examples of a co-variable include confounders, which are variables that can influence both the outcome and the predictor, and therefore, may be the true reason why we see an association. For example, when we assess the relationship between total number of years working, and serum PFAS concentration, PFAS

concentration is considered to be the outcome, while the number of years working is the predicting variable. An example of a confounding variable in this case is 'age'. Several monitoring studies have shown that PFAS concentrations tend to increase by age. Also, people that are older, may also have worked for longer. When assessing the association that we are interested in (as in the example above; the association between PFAS concentration and number of years working), we want to 'get rid' of the confounding effects (in the above example, the influence of age). When the confounding effects are controlled, we often refer to it as "**adjusted**" for confounders.

Statistical power; *The statistical power* is the likelihood that the study will detect an effect/a difference when there is an effect/a difference there to be detected. In this report, we mention "decrease in statistical power" as a limitation in our longitudinal analysis. By this we mean, that we have a lower chance of detecting a true relationship when we have a smaller sample size (only 130 participants were available for the longitudinal assessment).

Graphs Used in the Report

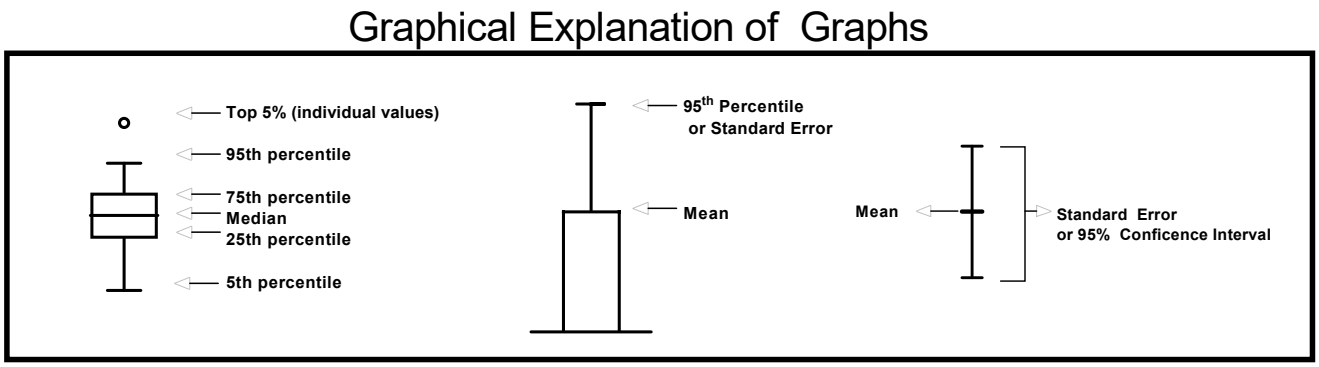

Figure A1. *Graphical explanation of the graphs.*

Statistical models used in this report

The outputs of the statistical models are shown in graphs or tables. Examples on how to interpret statistical outputs shown in the tables in this report are shown in Figure A2-A3. Some very basic background information of the statistical models is explained further below.

Correlation analysis

The relationship between two continuous variables can be assessed by a correlation analysis. The correlation analysis tests a linear relationship between two variables, in terms of how strong the relationship is, and in what direction the relationships goes. However, this analysis does not imply causality, only how the two variables co-vary. The outcome of this analysis is presented as the ***correlation coefficient ('r')*** which is a value between -1 and +1. If the variables that are assessed are not correlated, the correlation coefficient is close to zero. If the association is strongly correlated, the coefficient is closer to -1 or + 1. The stronger the correlation, the closer to -1 or +1 r is. The – or + represent negative or positive correlation respectively. It is worth to mention that a weak correlation can be statistically significant if the number of measurements/observations are large. In this report, we present a correlation analysis in Figure A2, where we assess the relationship between the different PFASs. In these graphs, an additional coefficient is presented, the **coefficient of determination**, **or 'r squared (r^2)'**. Where 'r' explains the strength of the relationship, the r^2 explains to what extent the variance of one of the variables explains the variance of the other variable. r^2 coefficient is described below in relation to regression analysis.

T-test

A t-test tells you the significance of the difference between two groups by comparing the means of these two groups. Therefore, a t-test can be used to determine if two groups are different from each other. In this report, the outcomes of t-tests are presented in tables, where the t-scores, and p-values are presented. The t-score is the ratio of the difference between the two groups, and the difference within both groups. The greater the t-value, the more different the two groups are. How significant this t-value is, is shown by the p-value. An example of how to interpret the outputs from a t-test is shown in Figure A2.

ANCOVA

In this report, ANCOVA analysis are used for several statistical assessments. ANCOVA (analysis of co-variance) models are used when assessing if there is a difference between groups, when more than two groups are compared. ANCOVA only provides information on the significance between the groups that are compared. To assess which groups are different, a so called post-hoc test is used. In this report, 'Bonferroni' or 'Tukey's' are used as post-hoc tests. The outcomes of the ANCOVA analysis in this report is either presented in tables or graphically (biochemical outcomes). When the outcomes are presented graphically, it is presented as **'estimated marginal means (EMM)'**. The EMM is the

predicted adjusted means of each group, i.e. the means predicted when all other co-variables are held constant. Where ANCOVA analysis is presented in tables, the adjusted beta **coefficient** (parameter estimates) is presented as 'change'. The coefficient represents the change in the outcome per one-unit change of the predictor, assuming all other predictors (co-variables) are held constant. The p-value is also presented in relation to this coefficient to show the significance. An example of how to interpret the outputs from an ANCOVA is shown in Figure A2.

Linear regression

In this report, we use linear regression to assess an overall linear association between two variables. A linear regression analysis is an approach to model the relationship between an outcome and one or several predictors. In this report, we use multiple linear regression models, which mean that we take several predictors into consideration. This is done to be able to adjust for co-variables. The association between the outcome and predictor is estimated through a "**Beta coefficient**". This Beta coefficient is interpreted as "for every one-unit increase in the predictor, the outcome changes by 'the B-coefficient' ". The Adjusted B-coefficients (95% CI of the B-coefficient) and the p-values for the B-coefficients are presented as the outcomes of our linear regression models. The adjusted B-coefficient, can be interpreted as 'for every one unit increase in the predictor, the outcome changes by 'the B-coefficient' when all other co-variables are held constant". For some models, the **coefficient of determination**, **or 'r squared (r^2)** is also presented to provide information on how well the predicting variables can explain the outcome. r^2 values range from 0-1(but can be converted to percentage for interpretation). The higher the r^2, the more the predicting variable can explain the assessed outcome. Example of how to interpret the outputs from linear regression analysis is shown in Figure A2 and Figure A3. Figure A2 show an example of a table showing the assessment of the relationship between PFAS concentrations and different factors (e.g. age, gender, how many years employment etc. Figure A3 is an example of a table showing the assessment of the relationship between biomarkers and PFAS concentration.

Logistic Regression

Linear regression (assessed above) can be used when the outcome is continuous. However, in some assessments, the outcome is dichotomous, i.e. categorical with only two outcomes. For example, when we assess the association between diagnosis of 'cardiovascular disease' and PFAS concentration, the outcome 'cardiovascular disease' can either be 'diagnosed' or 'not diagnosed'. In this case, logistic regression is used instead of linear regression. In this report, we use multiple logistic regression to adjust for co-variables. The outcome of a logistic regression is in this report presented as an '**odds ratio (OR)**'. The OR can be interpreted as 'for every one-unit increase in the predictor, the odds/risk of having the outcome become 'the OR'. If OR <1, the risk/odds of having the outcome is lower, while an OR>1 mean that there is an increased risk of having the outcome. Example of how to interpret the outputs from logistic regression analysis is shown in Figure A3.

In addition to the assessment between the risk of having a self-diagnosable health issue, we also apply logistic regression to assess the relationship between our measured biomarker and PFAS concentration. The levels of most biomarkers are on continuous scale. To be able to use logistic regression analysis we categorise the biomarker measurements as ‘out-of-range’ or ‘normal’ to be able to assess the risk of having ‘out-of-range’ levels with increasing PFAS concentrations. This is done in addition to our linear regression model as it provides some additional information on the relationships. For example, we could find a significant positive association in our linear regression analysis, this mean that with increasing PFAS concentrations, the levels of biomarkers also increase. However, to assess if the levels of biomarkers increase to levels that are considered ‘out of range’, we use logistic regression. This is further discussed in the biochemical section (Appendix V*)*

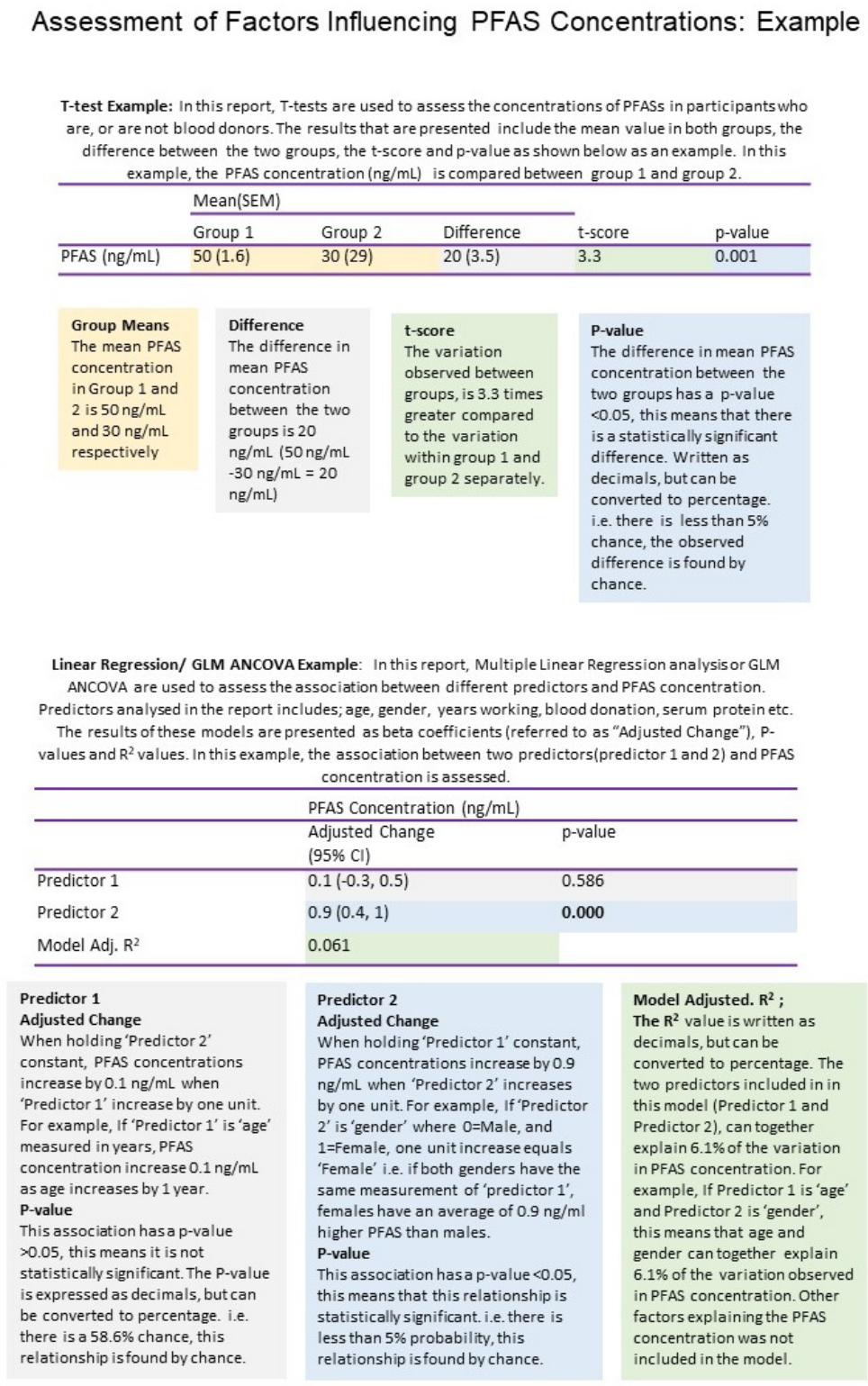

***Figure A2**. Example on how to interpret statistical outputs from t-tests, linear regression and GLM ANCOVA.*

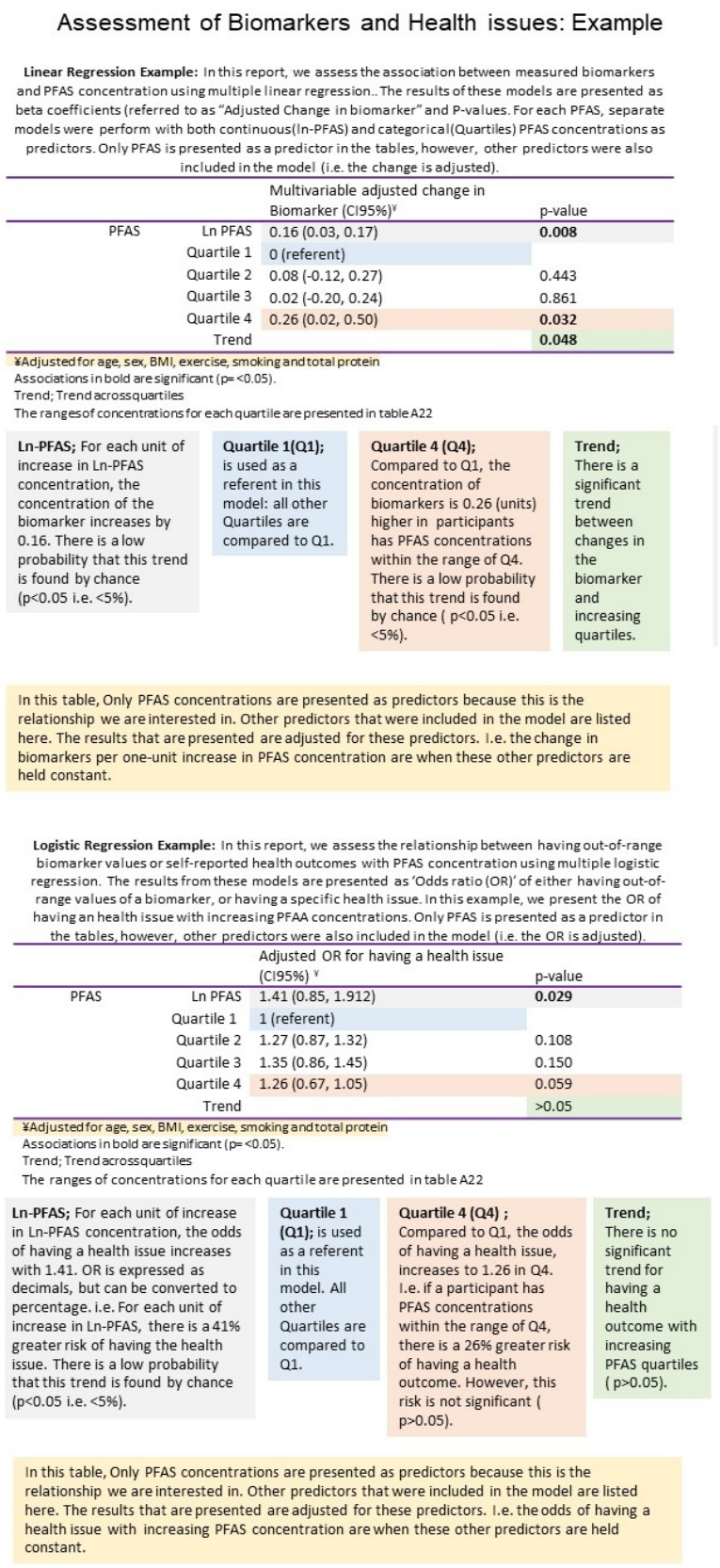

Figure A3. *Examples on how to interpret statistical outputs from linear and logistic regression where biochemical markers and health outcomes are assessed.*

Appendix III. Analysis and Quality Control and Assurance

Analytical Methodology for PFAA analysis

The analytical methodology was done according to previously published protocols [3, 7], with minor modifications to allow for extraction of a larger sample volume. Briefly, an aliquot of 1mL serum was transferred to a 15 mL falcon tube followed by addition of 10 µL labelled internal standard mix. Acetonitrile was used to precipitate the proteins and the extraction was facilitated by ultrasonication (30min) and vortex mixing. After centrifugation (4750 rpm, 30 min), the supernatant was transferred to fresh 15 mL falcon tubes and blown down to 1 mL using a gentle stream of nitrogen. The supernatant was filtrated into an LC vial through a 2 µm RC Membrane filter (Phenomenex) and blown down to 200 µL using nitrogen, after which 300 µL MilliQ and 10 µL recovery standard mix were added. PFAAs were analysed by HPLC-MS/MS using a high-performance liquid chromatograph (HPLC, Nexera, Shimadzu Corp., Kyoto Japan), coupled to a tandem mass spectrometer (SCIEX Triple Quad 6500+ ,Concord, Ontario, Canada) equipped with an electrospray ionisation and using scheduled multiple reaction monitoring mode (sMRM). A volume of 5 µL was injected and separation was achieved using a Kinetex 2.6 µm EVO C18, 100Å, 100 x 2.1 mm column (Phenomenex, Lane Cove) held at 50 °C, with a flow rate of 0.45 mL min^{-1} and a gradient elution using mobile phases 1% (Phase A) and 90% (Phase B) methanol, respectively, with 8mM ammonium acetate. Quantification was performed using the internal standard method with non-extracted standards. In this report, reported concentrations are for linear isomers of all PFASs, with the exception of PFOS, where the total concentration of both linear and branched isomers is presented. Method detection limits (MDLs) were determined using EPA guidelines (40 CFR 136 Appendix B), and set as 3.14 times the standard deviation of seven spiked replicates (Calf serum spiked at 0.5ng/mL and human pooled serum spiked at 1ng/mL). The MDL for all analyzed PFASs ranged from 0.04-0.1 ng/mL.

Quality Control and Quality Assurance (QC/QA) Outcomes

The average recoveries (standard deviation in brackets) for $^{13}C_4$-PFOA, $^{18}O_2$-PFHxS, $^{13}C_4$-PFOS were 93% (8%), 88% (8%) and 78% (9%), respectively (an acceptable recovery rate is generally set to be between 50% and 120%). The recovery of native spiked pooled samples (n=58) were 101% (7%) for PFOA, 103 (7%) for PFHxS, 95% (6%) for PFHpS and 106% (8%) for PFOS.

Reproducibility was calculated as the coefficient of variance (CV%) of each of the replicate and duplicate measurements and shown in Table A1. The accuracy of the analysis, estimated by analysing a reference sample (NIST 1957), was found to be within the acceptable range (Table A2). QAEHS performed well in the inter-laboratory studies which were performed concurrently to the Airservices study (AMAP 2020-01, GEQUAS). Z-scores for the AMAP study are shown in Table A3.

Table A1: *Coefficient of Variance (%) for the different repeated measurements. Only frequently detected PFASs (detected in >15% of participants) are shown.*

	Intra Batch Duplicate (n=58)*	Inter Batch Duplicate (n=57) *	Pooled Serum (n=58)	Spiked Pooled Serum (n=58)	Reanalysis of Stored Serum (n=120) *¥	Inter Laboratory Samples (n=10, 3 labs)
PFHpA	6.0	8.3	23	9.9		
PFOA	2.7	3.7	5.2	6.4	6.1	15
PFNA	4.2	4.8	8.4	8.6		8.2
PFDA	3.9	4.4	7.1	6.8		
PFUnDA	5.3	4.5	3.0	7.2		
PFBS	3.2	1.3		5.3		
PFHxS	2.6	3.4	6.8	7.1	6.9	13
PFHpS	3.1	3.9	12	8.1		27
PFOS	2.6	3.7	4.8	5.2	7.8	18

**Average Coefficient of Variance (CV) of all paired CVs is presented.*
¥ Reanalysed stored serum samples were compared to the results reported in the 2013 Airservices study. *Bland-Altman analysis, which assesses the intervals of agreements between the two values used in 2019 vs 2013, shows there is no major systematic pattern but rather indicates that the variance is random.*

Table A2: *Average (and standard deviation in brackets) PFAA concentrations (ng/mL) of NIST reference serum sample (NIST1957) analysed in this study (n=58), and reference values.*

	This Study	Reference
PFHpA	0.29(0.08)	0.31(0.05) [1]
PFOA	4.3(0.34)	5.0(0.44) [1]
PFNA	0.73(0.09)	0.88(0.08) [1]
PFDA	0.25(0.02)	0.39(0.12) [1]
PFUnDA	0.12(0.01)	0.17(0.04) [1]
PFHxS	3.35(0.21)	4(0.83) [1]
PFOS	18(1.11)	18(95%CI 4.2) [2]

CI= Confidence Interval

1) *NIST, National institute of standards and technology. U.S. Department of Commerce*
2) *Riddell et al., 2009 [35]*

Table A3: *Results (z-score) of inter-laboratory comparison studies AMAP 2020-01 (samples n=3) and G-EQUAS 64 (samples n=2).*

	PFHpA	PFHxA	PFOA	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
AMAP 2020-01	0.63-1.45	0.91-1.14	0.13-0.77	0.11-0.64	0.37-0.42	0.27-1.23	0.62-0.81	0.29-0.49	-0.05- -0.45

Appendix IV. Additional Information, Tables and Graphs

Table A4: *Demographics of the Airservices Cohort.*

General Demographics		***n (%)***		***Employment at Airservices***		***n (%)***	
Gender	Male	779 (97.5)		Year Commenced Service	<2005	494 (61.8)	
	Female	20 (2.5)			2005-2010	140 (17.5)	
					>2010	135 (16.9)	
Age	Average Age	51.53			No information	30 (3.8)	
	Min	21.70					
	Max	82.37		Have Held Position As:			Did not wear any PPE*
				Officer	Yes	332 (41.6)	17%
BMI*	<18.5	0 (0)			No	447 (55.9)	
	18-<25	149 (18.6)			No information	20 (2.5)	
	25-<30	424 (53.1)					
	≥30	205 (25.7)		Senior Officer	Yes	143 (17.9)	19%
	No information	21 (2.6)			No	635 (79.5)	
					No information	21 (2.6)	
Lifestyle							
Smoking Status	Never smoked	473 (59.2)		Fire Fighter	Yes	744 (93.1)	26%
	Used to smoke	274 (34.3)			No	35 (4.4)	
	Smoke occasionally	25 (3.1)			No information	20 (2.5)	
	Smoke regularly	10 (1.3)					
	No information	17 (2.1)		Instructor	Yes	102 (12.8)	10%
					No	676 (84.6)	
Alcohol Consumption	Never	43 (5.4)			No information	21 (2.6)	
	Monthly or less	140 (17.5)					
	2-4/ month	211 (26.4)		Emergency Vehicle Technician (EVT)	Yes	43 (5.4)	60%
	2-3/week	265 (33.2)			No	735 (92)	
	≥4/week	123 (15.4)			No information	21 (2.6)	
	No information	17 (2.1)					
				Other	Yes	21 (2.6)	
Exercise	Never	28 (3.5)			No	759 (95)	
	Once a week	77 (9.6)			No information	19 (2.4)	
	2-3/week	285 (35.7)					
	4-5/ week	261 (32.7)		Other PFAS exposure possibilities			
	>5/ week	132 (16.5)		Other jobs	Yes	173 (21.7)	
	No information	16 (2)		*(With potential* contact *with PFAS)*	No	607 (76)	
					No information	19 (2.4)	
Diet	Mixed Diet¥	760 (95.1)					
	Vegetarian	20 (2.5)		*L*ived within 5km of a*:*			
	Vegan	3 (0.4)		*Defence base*	Yes	184 (23)	
	No information	16 (2)			No	588 (73.6)	
					No information	27 (3.4)	
Blood Donor	No	598 (74.8)		*Airport*	Yes	286 (35.8)	
	Yes	184 (23)			No	490(61.3)	
	<1/ year	52 (6.5)			No information	23(2.9)	
	1/ year	40 (5)					
	2-4/year	52 (6.5)					
	>4 / year	38 (4.8)					
	No information	17 (2.1)					

Percentage may not add to 100% because of rounding
¥ Mixed Diet; Vegetables and meat
** Calculated for only participants who stated they were in contact with AFFF most days (In reference; the Questionnaire (Appendix I) Question 24; First row; Additional question 1:"5"; Additional question 2 "4".*

Table A5: *Detection frequency (%) and concentrations (ng/ml) of PFASs in the serum of Airservices staff and former members. Only PFASs detected in more than 15% of all participants are presented.*

		PFHpA	***PFOA***	***PFNA***	***PFDA***	***PFUnDA***	***PFBS***	***PFHxS***	***PFHpS***	***PFOS***
All Participants (n=799)	Detection Frequency (%)	29.04	100.0	98.12	92.24	29.66	16.02	100.0	91.49	100.0
	Average	0.16	1.7	0.36	0.2	0.14	0.1	14	1.7	27
	SD	0.16	1	0.17	0.18	0.06	0.03	17	2	30
	Median	0.11	1.5	0.33	0.17	0.12	0.09	6.5	0.85	14
	95th Percentile	0.4	3.4	0.65	0.36	0.24	0.14	46	5.8	84
	Range	<MDL-2.0	0.08-15	<MDL-2.1	<MDL-3	<MDL-0.54	<MDL-0.32	0.08-168	<MDL-16	0.14-191
Ages 16-30 (n=28)	Detection Frequency (%)	10.7	100.0	100.0	100.0	28.6	14.3	100.0	46.4	100.0
	Average	0.08	1.4	0.29	0.19	0.15	0.11	1.9	0.21	3.4
	SD	0.01	0.74	0.16	0.11	0.07	0.03	2.1	0.13	2.1
	Median	0.08	1.3	0.27	0.16	0.13	0.1	0.97	0.18	2.4
	95th Percentile	0.09	2.9	0.54	0.36	0.27	0.14	5.6	0.41	7.8
	Range	<MDL-0.09	0.39-3.2	0.09-0.92	0.09-0.63	<MDL-0.32	<MDL-0.15	0.35-9.4	<MDL-0.55	1.1-8.8
Ages 31-45 (n=263)	Detection Frequency (%)	20.5	100	98.1	94.3	29.3	20.2	100	88.2	100
	Average	0.15	1.4	0.32	0.2	0.14	0.1	2.7	0.31	7.1
	SD	0.27	0.59	0.14	0.17	0.06	0.04	3.3	0.38	7.1
	Median	0.09	1.3	0.3	0.17	0.12	0.09	1.8	0.2	5.1
	95th Percentile	0.22	2.4	0.56	0.42	0.21	0.15	7.7	0.82	21
	Range	<MDL-2	0.2-3.4	<MDL-1	<MDL-2.3	<MDL-0.47	<MDL-0.32	0.17-33	<MDL-3.5	0.62-57
Ages 46-60 (n=308)	Detection Frequency (%)	27.6	100	97.7	89.0	32.5	13.6	100	95.1	100
	Average	0.17	1.8	0.37	0.21	0.14	0.1	19	2.3	37
	SD	0.1	0.95	0.16	0.17	0.05	0.03	19	2.1	31
	95th Percentile	0.39	3.6	0.67	0.36	0.24	0.14	47	6.4	91
	Range	<MDL-0.46	0.08-5.58	<MDL-1.36	<MDL-1.96	<MDL-0.34	<MDL-0.2	0.08-168	0.09-16	0.14-185
Ages >60 (n=200)	Detection Frequency (%)	45	100	99	94	26	15	100	97	100
	Average	0.17	2.1	0.39	0.19	0.14	0.09	23	2.5	41
	SD	0.12	1.4	0.21	0.22	0.07	0.02	17	2	31
	Median	0.11	1.8	0.35	0.16	0.12	0.08	19	2	36
	95th Percentile	0.42	3.9	0.68	0.33	0.24	0.12	61	6.8	97
	Range	<MDL-2.0	0.17-15	<MDL-2.1	<MDL-3	<MDL-0.54	<MDL-0.13	0.08-76	<MDL-9.6	0.47-191

Only Values above MDL have been included for calculation of the distribution parameters.

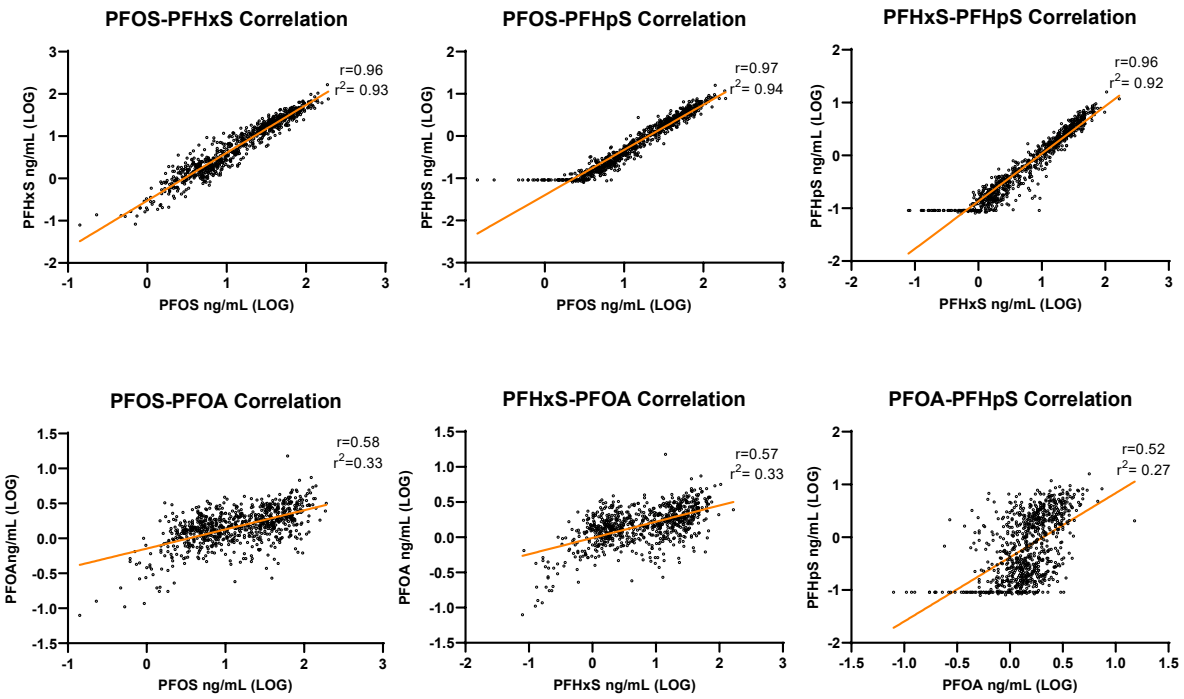

Figure A4. *Correlations between PFOA, PFHxS, PFHpS and PFOS concentrations in serum of the 799 participants. Values<MDL are included as MDL/Sqrt (2). r=correlation coefficient. r^2 coefficient of determination.*

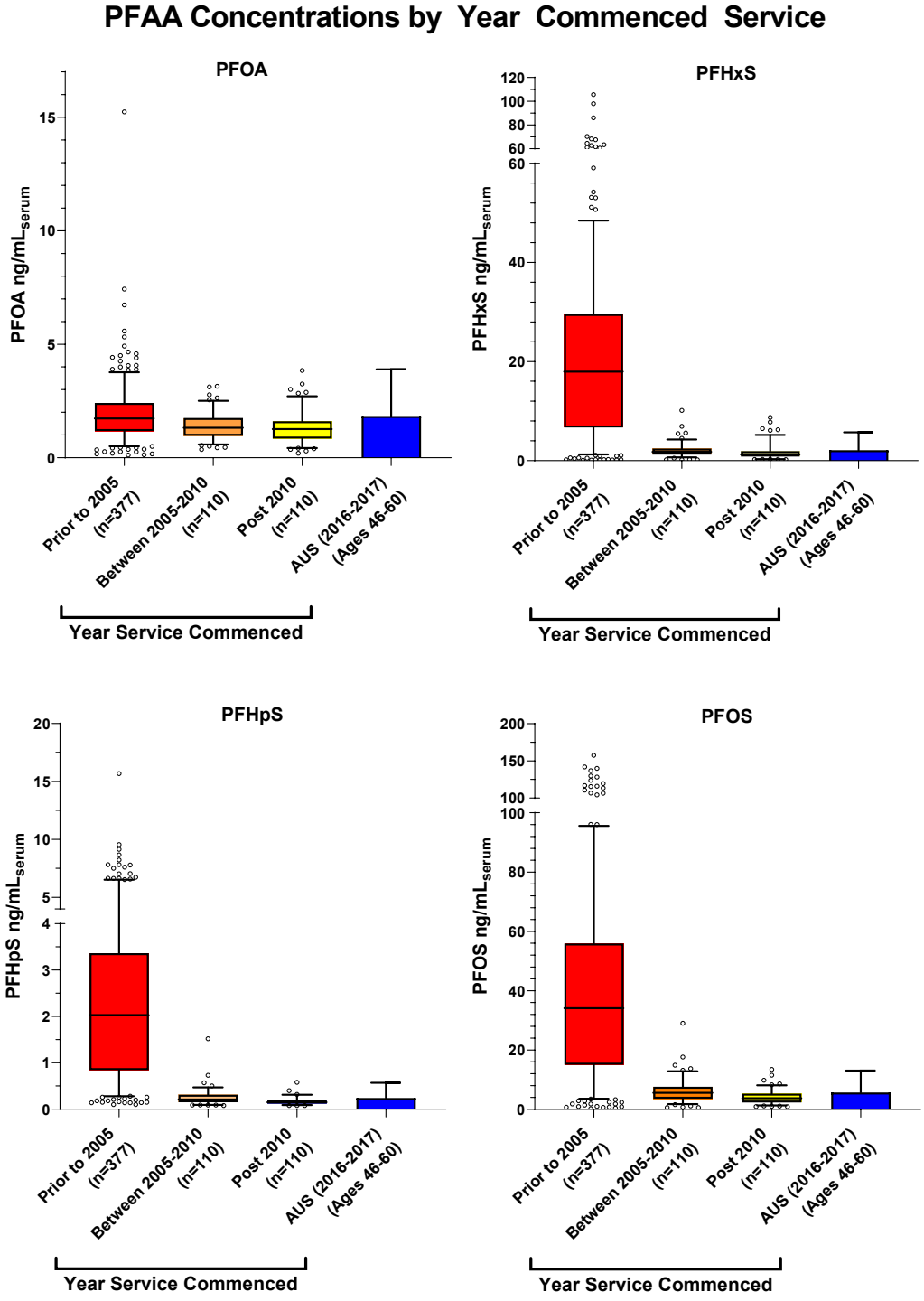

Figure A5. *PFAS concentration grouped by year when employees commenced service for Airservices, excluding participants who indicated having worked other employers where they may have come into contact with AFFF, such as the RAAF. The lines in the boxes indicate median concentrations, the outside of the boxes the 25th and 75th percentiles, and the whiskers range to the 5th and 95th percentile concentrations. Individual dots represent bottom and top 5%. For the general population, the bar presents the average concentration, and the error bar presents the estimated 95th percentile [7, 16].*

Table A6. *Results from GLM ANCOVA analysis assessing years working, and years since retirement.*

	Ln PFOA				Ln PFHxS				Ln PFHpS				Ln PFOS			
	Commenced Service: <2005		>2005		Commenced Service: <2005		>2005		Commenced Service: <2005		>2005		Commenced Service: <2005		>2005	
	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value
Age	0.024 (0.01, 0.038)	**0.001**	0.011 (-0.001, 0.023)	0.067	0.014 (-0.013, 0.04)	0.316	0.016 (0, 0.032)	**0.045**	0.017 (-0.009, 0.042)	0.198	0.041 (0.027, 0.056)	**0.000**	0.011 (-0.011, 0.034)	0.321	0.032 (0.018, 0.045)	**0.000**
Gender	-0.169 (-1.022, 0.685)	0.698	-0.166 (-0.476, 0.145)	0.295	-0.975 (-2.632, 0.681)	0.248	-0.588 (-1.009, -0.167)	**0.006**	-0.889 (-2.474, 0.696)	0.271	-0.792 (-1.166, -0.418)	**0.000**	-0.784 (-2.189, 0.62)	0.273	-0.539 (-0.887, -0.191)	**0.003**
Years Working*	-0.011 (-0.022, 0)	**0.045**	0.013 (-0.01, 0.035)	0.276	0.039 (0.018, 0.061)	**0.000**	0.036 (0.005, 0.066)	**0.024**	0.037 (0.016, 0.057)	**0.001**	0.051 (0.023, 0.078)	**0.000**	0.029 (0.011, 0.047)	**0.002**	0.024 (-0.001, 0.05)	0.060
Years Since Retirement	-0.014 (-0.024, -0.004)	**0.005**	0.069 (-0.054, 0.191)	0.269	-0.028 (-0.047, -0.009)	**0.004**	0.047 (-0.119, 0.213)	0.581	-0.036 (-0.054, -0.018)	**0.000**	0.08 (-0.068, 0.227)	0.288	-0.03 (-0.046, -0.014)	**0.000**	0.022 (-0.115, 0.159)	0.752
Model Adj. R2	0.026		0.034		0.116		0.100		0.130		0.342		0.103		0.206	

Additionally Adjusted	Ln PFOA				Ln PFHxS				Ln PFHpS				Ln PFOS			
	Commenced Service: <2005		>2005		Commenced Service: <2005		>2005		Commenced Service: <2005		>2005		Commenced Service: <2005		>2005	
	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value
Age	0.026 (0.013, 0.04)	**0.000**	**0.01 (-0.002, 0.022)**	0.103	0.017 (-0.01, 0.043)	0.213	0.015 (-0.001, 0.031)	0.067	0.019 (-0.006, 0.044)	0.134	0.04 (0.026, 0.054)	**0.000**	0.014 (-0.008, 0.036)	0.216	0.03 (0.017, 0.043)	**0.000**
Gender	-0.139 (-0.978, 0.7)	0.745	-0.139 (-0.45, 0.171)	0.378	-0.869 (-2.49, 0.753)	0.293	-0.528 (-0.943, -0.114)	**0.013**	-0.785 (-2.34, 0.769)	0.321	-0.734 (-1.1, -0.367)	**0.000**	-0.701 (-2.079, 0.677)	0.318	-0.491 (-0.834, -0.149)	**0.005**
Years Working*	-0.013 (-0.024, -0.002)	0.023	0.011 (-0.012, 0.034)	0.337	0.036 (0.015, 0.057)	**0.001**	0.032 (0.002, 0.062)	0.039	0.034 (0.014, 0.054)	**0.001**	0.047 (0.02, 0.074)	**0.001**	0.027 (0.009, 0.045)	**0.004**	0.022 (-0.003, 0.047)	0.090
Years Since Retirement	-0.015 (-0.025, -0.005)	**0.002**	0.085 (-0.037, 0.208)	0.171	-0.03 (-0.049, -0.011)	**0.002**	0.074 (-0.089, 0.238)	0.371	-0.038 (-0.056, -0.02)	**0.000**	0.107 (-0.038, 0.251)	0.146	-0.031 (-0.047, -0.015)	**0.000**	0.047 (-0.088, 0.182)	0.496
Total Protein	0.022 (0.007, 0.036)	**0.003**	0.006 (-0.011, 0.023)	0.453	0.033 (0.005, 0.061)	**0.022**	0.017 (-0.006, 0.039)	0.147	0.028 (0.001, 0.054)	**0.042**	0.016 (-0.004, 0.036)	0.107	0.027 (0.004, 0.051)	**0.023**	0.013 (-0.006, 0.032)	0.177
Blood Donor	-0.183 (-0.339, -0.028)	**0.021**	-0.167 (-0.312, -0.022)	**0.024**	-0.537 (-0.838, -0.235)	**0.001**	-0.297 (-0.49, -0.103)	**0.003**	-0.512 (-0.801, -0.224)	**0.001**	-0.289 (-0.46, -0.118)	**0.001**	-0.423 (-0.679, -0.167)	**0.001**	-0.258 (-0.418, -0.098)	**0.002**
Model Adj. R2	0.060		0.052		0.154		0.142		0.165		0.380		0.138		0.246	

Gender; Male (0) vs. Female (1)
Blood Donor; "no" (0) vs. "yes" (1)
**For commencement prior to 2005, "Years Working" do only include the number of years prior to 2005, even though some participants may have worked longer.*

Table A7. *Number of participants (n) and estimated (adjusted) mean¥ (95% Confidence interval of the mean) blood serum levels of PFOA, PFHxS, PFHpS and PFOS (ng/ml serum), for each fire station.*

	Employed before 2005×					Employed after 2005×				
		Mean(95% CI)					Mean(95% CI)			
Station	N	PFOA	PFHxS	PFHpS	PFOS	N	PFOA	PFHxS	PFHpS	PFOS
Adelaide	6	2.0 (0.62, 3.3)	36 (18, 54)	3.5 (1.7, 5.3)	64 (41, 88)	8	0.93 (0.47, 1.4)	3.1 (0.3, 5.8)	0.18 (0.1, 0.26)	6.3 (3, 9.6)
Brisbane	7	1.6 (1.2, 1.9)	18 (8.2, 28)	2.2 (0.7, 3.6)	38 (12, 64)	6	1.4 (0.73, 2)	1.5 (0.75, 2.3)	0.16 (0.06, 0.26)	3.9 (1.7, 6)
Cairns	7	2.5 (1.6, 3.4)	19 (10, 28)	2.7 (1.0, 4.4)	38 (21, 55)	<6				
Darwin	10	1.8 (1.2, 2.5)	14 (8.0, 21)	1.7 (0.93, 2.6)	33 (22, 44)	7	1.4 (1.08, 1.7)	1.5 (0.93, 2.1)	0.11 (0.05, 0.17)	3.6 (1.8, 5.5)
Hobart	<6					6	0.87 (0.16, 1.6)	0.63 (0.3, 1)	0.10 (0.03, 0.17)	3.1 (1.5, 4.8)
Melbourne	14	1.6 (1.1, 2)	13 (8.7, 17)	1.3 (0.86, 1.7)	25 (17, 32)	11	1.2 (0.72, 1.7)	1.7 (1.38, 2)	0.23 (0.18, 0.27)	5.8 (4.6, 7)
Perth	6	2.0 (0.99, 2.9)	38 (19, 58)	4.3 (1.8, 6.8)	78 (29, 126)	16	1.2 (0.98, 1.4)	1.3 (0.9, 1.8)	0.11 (0.07, 0.14)	2.6 (2.1, 3.2)
Sydney	17	2.3 (198, 2.6)	26 (17, 35)	3.2 (2.0, 4.4)	48 (31, 65)	20	1.3 (1.06, 1.6)	2.1 (1.52, 2.7)	0.18 (0.14, 0.22)	4.6 (3.6, 5.7)
All Stations	91	1.9 (1.8, 2.1)	23 (19, 26)	2.6 (2.2, 3.1)	45 (38, 51)	102	1.3 (1.15, 1.4)	1.7 (1.46, 2)	0.16 (0.14, 0.18)	4.5 (4.1, 5)

Stations with less than 6 participants are not included for confidentiality reasons.
×Employed for more than 4 years at the specific station as a firefighter, and less than 2 years at any other station.
¥ All stations include stations presented in this table, as well as other stations where <6 participants were representatives.

Table A8. *Results from Linear Regression analysis assessing years working for Firefighter and EVTs working prior to 2005*

	PFOS			
	Firefighters		EVTs	
	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value
Age	0.106 (-0.276, 0.488)	0.586	-1.232 (-3.351, 0.887)	0.233
Years Working*	**0.849 (0.433, 1.266)**	**0.000**	**2.992 (1.519, 4.465)**	**0.001**
Model Adj. R2	0.061		0.522	

**Ages working prior to 2005*

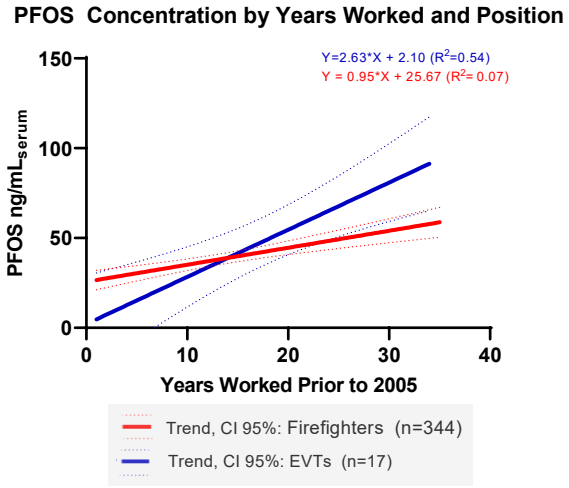

Figure A6: *The association (un-adjusted) between PFOS concentration (ng/ml) and years of working as EVTs and Firefighters in participants employed prior to 2005.*

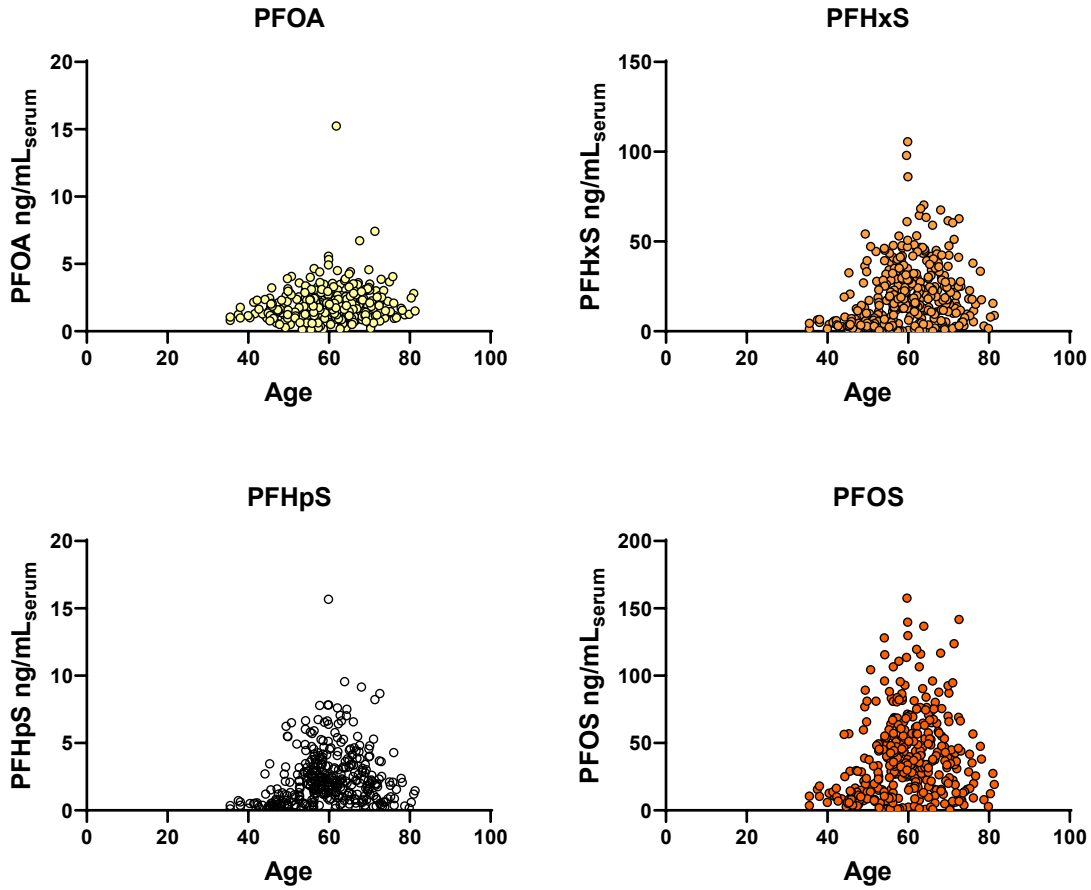

Figure A7: *Concentration of PFAAs (ng/ml) by age (years) of participants who started working for Airservices before 2005.*

Table A9. *Mean PFAA (ng/mL) concentrations grouped by blood donation (Yes, No), and outcomes of t-test in participants that commenced service prior and after 2005.*

	Commenced prior to 2005					Commenced after 2005				
	Mean(SEM)					Mean(SEM)				
	No Blood Donor (n=395)	Blood Donor (n=98)	Difference	t-score	p-value	No Blood Donor (n=146)	Blood Donor (n=74)	Difference	t-score	p-value
PFOA	2.0 (0.05)	1.7 (1.6)	0.31 (0.13)	2.324	**0.021**	1.5 (0.05)	1.2 (0.53)	0.27 (0.086)	3.147	**0.002**
PFHxS	22 (0.93)	15 (16.32	6.74 (2.0)	3.319	**0.001**	2.1 (0.12)	1.4 (0.12)	0.66 (0.194)	3.370	**0.001**
PFHpS	2.4 (0.11)	1.7 (1.79)	0.75 (0.23)	3.262	**0.001**	0.21 (0.01)	0.13 (0.09)	0.08 (0.017)	4.559	**0.000**
PFOS	41 (1.56)	30 (28.81)	11 (3.5)	3.285	**0.001**	5.7 (0.29)	3.9 (0.27)	1.8 (0.398)	4.583	**0.000**

Table A10: *Results from GLM ANCOVA analysis assessing blood donation*

	Ln PFOA				Ln PFHxS				Ln PFHpS				Ln PFOS			
	Commenced Service: <2005		>2005		Commenced Service: <2005		>2005		Commenced Service: <2005		>2005		Commenced Service: <2005		>2005	
Predictors	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value	Adjusted Change (95% CI)	p-value
Age	0.008 (0.002, 0.013)	**0.009**	0.011 (0.001, 0.02)	**0.025**	0.03 (0.019, 0.041)	**0.000**	0.019 (0.006, 0.031)	**0.005**	0.025 (0.014, 0.035)	**0.000**	0.049 (0.037, 0.061)	**0.000**	0.019 (0.01, 0.029)	**0.000**	0.032 (0.021, 0.043)	**0.000**
Gender	-0.212 (-1.061, 0.636)	0.623	-0.117 (-0.351, 0.117)	0.326	-1.214 (-2.845, 0.417)	**0.144**	-0.627 (-0.953, -0.301)	**0.000**	-1.138 (-2.736, 0.46)	**0.162**	-0.821 (-1.123, -0.518)	**0.000**	-0.96 (-2.379, 0.459)	0.184	-0.54 (-0.811, -0.268)	**0.000**
Donations/ year 4+	-0.87 (-1.137, -0.603)	**0.000**	-0.783 (-1.02, -0.545)	**0.000**	-1.678 (-2.191, -1.165)	**0.000**	-0.938 (-1.269, -0.607)	**0.000**	-1.481 (-1.983, -0.978)	**0.000**	-0.766 (-1.073, -0.46)	**0.000**	-1.307 (-1.753, -0.86)	**0.000**	-0.806 (-1.081, -0.531)	**0.000**
2-4	-0.223 (-0.464, 0.018)	0.069	-0.061 (-0.252, 0.129)	0.524	-0.618 (-1.08, -0.156)	**0.009**	-0.38 (-0.645, -0.115)	**0.005**	-0.707 (-1.16, -0.254)	**0.002**	-0.417 (-0.662, -0.172)	**0.001**	-0.475 (-0.877, -0.073)	**0.021**	-0.334 (-0.555, -0.114)	**0.003**
1	-0.044 (-0.312, 0.224)	0.747	-0.047 (-0.279, 0.185)	0.691	-0.201 (-0.716, 0.313)	0.443	-0.138 (-0.462, 0.185)	0.401	-0.295 (-0.8, 0.209)	0.251	-0.122 (-0.421, 0.178)	0.424	-0.118 (-0.566, 0.33)	0.605	-0.23 (-0.499, 0.039)	0.093
<1	0.08 (-0.155, 0.314)	0.505	-0.035 (-0.254, 0.183)	0.750	-0.004 (-0.455, 0.447)	0.986	-0.114 (-0.417, 0.19)	0.462	0.003 (-0.439, 0.444)	0.990	-0.141 (-0.422, 0.14)	0.324	0.027 (-0.365, 0.419)	0.893	-0.01 (-0.263, 0.243)	0.939
No Blood Donor	0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)		0 (Referent)	
Model Adj. R2	0.091		0.171		0.139		0.215		0.117		0.385		0.100		0.309	

Gender; Male (0) vs. Female (1)

Appendix V. Assessment of Biochemical Markers and Self-reported Health Issues

Methods

Assessment of the cross-sectional data set

In the cross-sectional dataset, the association of biochemical markers and self-reported health issues with PFAA serum concentrations was assessed.

Biochemical markers

*B*iochemical markers assessed included: serum lipids (Total Cholesterol, LDL, HDL), liver function marker (ALT), thyroid function markers (TSH, T3, T4) as well as the kidney function markers (Uric acid, *eGFR*). eGFR was assessed on a categorical scale and defined as 'low' (<60 mL/min/1.73m^2) or 'normal' (>60 mL/min/1.73m^2) [36]. All other biochemical markers were assessed on continuous scales.

Multiple linear regression was used to assess for the presence of a linear association between all biochemical markers of continuous scale and exposure (PFAA serum concentration). To assess the associations between eGFR (dichotomous) and PFAA exposure, logistic regression analysis was used to determine the odds-ratio (OR) of 'low eGFR' for increasing PFAA serum concentrations. Separate regression models were performed with each single PFAA exposure variable. These assessment methods were chosen as they make it possible to assess the relationships between the dependent outcomes and PFAA concentration, while also considering other independent variables which could be potential confounders. Linear regression is only used when the predicted outcome is continuous, while logistic regression is used when the predicted outcome is categorical. These assessment methods are also commonly used for evaluation of the relationship between biomarkers and PFAA concentrations.

PFAAs included in the statistical analysis were PFOA, PFHxS, PFHpS and PFOS, as these were the dominant PFAAs detected in both frequency (>90%) and concentration. The PFAA concentrations were analysed as both continuous and categorical variables in separate models in the linear regression models. For analysis as a continuous variable, PFAA concentrations were log-transformed to improve skewed distributions. For analysis as a categorical variable, PFAAs were grouped into quartiles (Q) of increasing exposure, where a trend was tested across increasing quartiles (ranges presented in Table A22), as well as between quartiles where quartile 1 (Q1) was used as the referent.

A number of potential confounders were considered, including: age, gender, body mass index (BMI), exercise, drinking habits, smoking habits, diet/seafood consumption, serum cholesterol levels and serum protein levels (albumin, globulin and total protein (albumin+ globulin)) (categorical; gender (male, female), BMI (< 25, 25-30, >30), exercise (<1/week, 1/week, 3+/week), drinking habits (<1/month, 1-2/month, 1-2/week, 3+/week), smoking habits (never, used to, current smoker), diet (vegetarian/vegan, mixed diet), seafood consumption (<1/week, 1/week, 2+ per week), where the group that is underlined was used as a referent. All covariates, apart from cholesterol and serum protein, measured in blood serum, were self-reported as part of the

questionnaire. The listed potential confounders have all been suggested to be associated with at least one of the outcomes assessed in this study. Associations between total PFAAs (Sum of PFOA, PFHxS, PFHpS and PFOS) and these potential confounders were assessed through univariate simple linear regression models. To avoid over-adjustment, only potential confounders which were statistically associated with total serum PFAA were included as variables in the final multivariable adjusted analysis.

All final models were adjusted for age, gender, exercise, smoking habits, and total serum protein (albumin+ globulin), as these were all associated with PFAA concentration. BMI was included as a potential confounder in assessments of all outcomes apart from thyroid function markers. Thyroid function may have an influence on BMI, rather than BMI influencing thyroid function [37], and therefore this is not included as a confounder in this analysis to avoid bias [38]. Cholesterol was additionally included as a covariate in assessments of urate outcomes, as it may be a potential confounder (or a causal intermediate) for this outcome [39]. Additionally, seafood consumption has also been suggested to be a potential confounder for urate outcomes, and therefore, we additionally adjusted for this co-variable in a sensitivity analysis and the magnitude change of β-coefficients between the two assessments were compared- including and excluding seafood consumption.
Participants were excluded from the serum lipid models (19%), kidney function markers models (6%) , thyroid function marker models (5%) and liver function marker models (2%), if they did not answer the questionaries or stated that they that they took cholesterol lowering medications, gout medications/fluid tablets, thyroid medication or stated that they had hepatitis, respectively.
General linear model analysis of covariance (GLM ANCOVA) was performed to estimate predicted outcomes (estimated marginal mean; EMM) of the biochemical markers. In this model, the outcome was defined as the dependent variable, PFAA quartiles were defined as fixed factors, and all other potential confounders were defined as co-variables. In this way, the EEM is adjusted for all potential confounders. These EEMs were used to present the adjusted data graphically, where significant differences were found between quartiles in the primary multiple linear regression models. Additionally, in associations where significant differences were found between PFAA quartiles, logistic regression was performed to predict the odds ratio (OR) of having out-of-range values of the outcomes, based on reference-values by Sullivan Nicolaides Pathology (SNP).

Self-reported health issues

Self-reported health conditions, reported by participants in the questionnaire, included: diabetes type 1 and 2, asthma, hypertension, cardiovascular disease, kidney problems, liver problems, thyroid problems, reproductive/fertility problems and cancer. Logistic regression analysis was performed to assess the odds of having any of these conditions with increasing PFAA concentration (both continuous and increasing quartiles). The same co-variables that were used in the models described above were also used in this analysis. All participants who filled in the questionnaire for each health outcome were included in the analysis.

Assessment of biomarkers in the longitudinal data set

The 130 participants that took part in both the study in 2013 and the current 2019 Airservices study were further included in a longitudinal assessment on the relationship between biomarkers and PFAA concentrations. This relationship was examined by linear regression of the ratio change in each biomarker measurement in relation to the ratio change in PFAA concentration.

The biomarkers that were measured in both studies were the serum lipids (cholesterol, HDL and LDL) as well as the kidney function biomarker urate. Albumin, globulin and total serum protein were also measured in both studies and the change in total serum protein was used as a co-variable in the linear regression. Additionally, the regression was adjusted by gender and the continuous co-variables age at baseline (the blood collection in 2013), time between the two blood collections, and change in BMI between the two blood collections, as these are possible confounders of the relationship between change in both biomarkers and PFAAs. Changes in other measured variables were not associated with ratio change in any PFAAs, and therefore were not included as co-variables in order to not over-adjust the models. These included change in exercise behaviour (defined as number of moderate exercise/week in 2018 compared to 2013; same, less, more), drinking behaviour (defined as number of drinks/week in 2018 compared to 2013; same, less, more) and smoking habit (no change, stopped since 2013, started since 2013).

Participants were omitted from the models assessing serum lipids (24%) and urate (5%) if the participants did not answer the questionnaire or if they stated that they took cholesterol lowering medication or gout medication, respectively.

Result Tables

Some very basic background information of the statistical models used, and how to interpret the statistical outputs shown in the tables are covered in Appendix II.

Biochemical markers

Serum lipids (Total Cholesterol, HDL, LDL)

Table A11. *Change (Linear regression coefficients (B-coefficients) of serum lipids; Cholesterol (mmol/L), HDL (mmol/L) and LDL (mmol/L) with increases in PFAA concentrations (ln-transformed and increasing quartiles).*

PFAA		**Multivariable adjusted change in cholesterol (CI95%)¥**	**p-value**	**Multivariable adjusted change in HDL (CI95%)¥**	**p-value**	**Multivariable adjusted change in LDL (CI95%)¥**	**p-value**
PFOA	**Ln PFOA**	0.125 (-0.011, 0.262)	0.071	0.021 (-0.023, 0.065)	0.343	**0.146 (0.025, 0.267)**	**0.018**
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	0.007 (-0.203, 0.216)	0.951	0.05 (-0.017, 0.117)	0.143	-0.008 (-0.193, 0.178)	0.934
	Quartile 3	0.144 (-0.068, 0.356)	0.183	0.061 (-0.007, 0.129)	0.078	0.066 (-0.123, 0.255)	0.493
	Quartile 4	0.109 (-0.112, 0.329)	0.333	0.043 (-0.028, 0.113)	0.234	0.127 (-0.069, 0.323)	0.204
	Trend		>0.05		>0.05		>0.05
PFHxS	**Ln PFHxS**	0.048 (-0.02, 0.116)	0.168	0.015 (-0.007, 0.036)	0.190	**0.061 (0, 0.121)**	**0.049**
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	0.15 (-0.061, 0.36)	0.164	0.056 (-0.011, 0.123)	0.104	0.097 (-0.09, 0.284)	0.310
	Quartile 3	0.048 (-0.195, 0.29)	0.701	0.034 (-0.044, 0.112)	0.390	0.064 (-0.153, 0.28)	0.564
	Quartile 4	0.182 (-0.084, 0.447)	0.179	0.047 (-0.038, 0.132)	0.278	0.196 (-0.041, 0.432)	0.104
	Trend		>0.05		>0.05		>0.05
PFHpS	**Ln PFHpS**	0.046 (-0.026, 0.118)	0.214	0.002 (-0.021, 0.025)	0.861	0.059 (-0.005, 0.123)	0.070
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	0.15 (-0.065, 0.366)	0.171	0.032 (-0.037, 0.101)	0.360	0.15 (-0.041, 0.341)	0.124
	Quartile 3	0.004 (-0.249, 0.256)	0.976	0.022 (-0.059, 0.103)	0.592	0.026 (-0.198, 0.25)	0.820
	Quartile 4	0.194 (-0.08, 0.468)	0.165	0.023 (-0.065, 0.111)	0.603	**0.244 (0.001, 0.488)**	**0.049**
	Trend		>0.05		>0.05		>0.05
PFOS	**Ln PFOS**	**0.082 (0.003, 0.161)**	**0.041**	0.011 (-0.015, 0.036)	0.411	**0.095 (0.025, 0.165)**	**0.008**
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	0.031 (-0.184, 0.247)	0.775	0.016 (-0.053, 0.086)	0.641	0.075 (-0.116, 0.266)	0.443
	Quartile 3	0.068 (-0.182, 0.319)	0.593	0.048 (-0.033, 0.128)	0.243	0.02 (-0.203, 0.243)	0.861
	Quartile 4	0.247 (-0.025, 0.518)	0.075	0.019 (-0.068, 0.106)	0.674	**0.263 (0.022, 0.504)**	**0.032**
	Trend		>0.05		>0.05		**0.048**

¥Adjusted for age, sex, BMI, exercise, smoking and total protein
Associations in bold are significant (p= <0.05).
Trend; Trend across quartiles
The ranges of concentrations for each quartile are presented in table A22

Table A12. *Odds Ratio (OR) of out-of-range serum cholesterol (>5.5 mmol/L) and serum LDL (>4 mmol/L) with increases in PFAA quartiles. Only associations that were statistically significant in simple linear regression analysis (presented in table XX) are presented.*

PFAA		Multivariable adjusted OR of out-of-range cholesterol (CI95%)¥	p-value	Multivariable adjusted OR of out-of-range LDL (CI95%)¥	p-value
PFOA	**Quartile 1**			1 (referent)	
	Quartile 2			1.343 (0.727, 2.483)	0.347
	Quartile 3			1.397 (0.755, 2.584)	0.286
	Quartile 4			1.637 (0.88, 3.046)	0.120
PFHxS	**Quartile 1**			1 (referent)	
	Quartile 2			1.022 (0.552, 1.891)	0.945
	Quartile 3			1.312 (0.667, 2.582)	0.431
	Quartile 4			1.557 (0.756, 3.204)	0.230
PFHpS	**Quartile 1**			1 (referent)	
	Quartile 2			1.071 (0.575, 1.995)	0.829
	Quartile 3			1.074 (0.522, 2.208)	0.846
	Quartile 4			1.636 (0.775, 3.456)	0.197
PFOS	**Quartile 1**	1 (referent)		1 (referent)	
	Quartile 2	0.956 (0.598, 1.529)	0.851	1.158 (0.616, 2.177)	0.649
	Quartile 3	1.104 (0.639, 1.908)	0.723	1.291 (0.629, 2.651)	0.486
	Quartile 4	1.388 (0.768, 2.51)	0.278	2.039 (0.962, 4.321)	0.063

¥Adjusted for age, sex, BMI, exercise, smoking and total protein
The ranges of concentrations for each quartile are presented in table A22

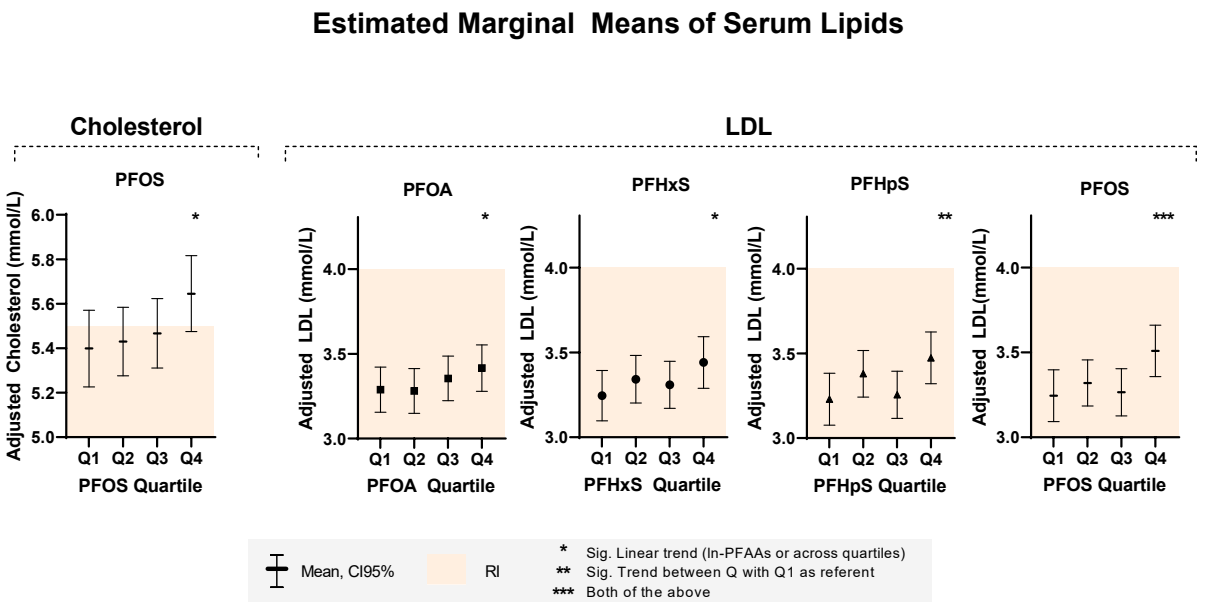

Figure A8. *The co-variable -adjusted estimated marginal means (EMM) of Cholesterol and LDL measurement (mmol/L) across PFAA quartiles (quartile ranges are presented in table 22). Only relationships that were significant (see Table A11) are presented. The symbols represent the mean and the error bars represents the 95% confidence intervals of the mean. * represent a significant (sig.) different EEM compared to Q1. ** represent a sig. linear trend (across ln-transformed concentrations or quartiles, or both). The highlighted area represents the reference interval (RI) (table A23). For graphical explanation of the plot, see Figure A1.*

Liver function marker (ALT)

Table A13: Changes (Linear regression coefficients (B-coefficients) of Liver-function biomarker ALT (IU/L) with increases in PFAA concentrations (ln-transformed and increasing quartiles).

PFAA		**Multivariable adjusted change in ALT (CI95%)¥**	**p-value**
PFOA	**Ln PFOA**	**-2.211 (-4.317, -0.106)**	**0.040**
	Quartile 1	0 (referent)	
	Quartile 2	-1.984 (-5.389, 1.422)	0.253
	Quartile 3	-2.568 (-6.036, 0.901)	0.147
	Quartile 4	-1.079 (-4.645, 2.488)	0.553
	Trend		>0.05
PFHxS	**Ln PFHxS**	0.073 (-0.984, 1.13)	0.892
	Quartile 1	0 (referent)	
	Quartile 2	0.513 (-2.995, 4.021)	0.774
	Quartile 3	0.326 (-3.817, 4.47)	0.877
	Quartile 4	0.816 (-3.469, 5.102)	0.709
	Trend		>0.05
PFHpS	**Ln PFHpS**	0.309 (-0.819, 1.437)	0.591
	Quartile 1	0 (referent)	
	Quartile 2	-0.15 (-3.746, 3.446)	0.935
	Quartile 3	0.272 (-3.972, 4.517)	0.900
	Quartile 4	0.699 (-3.66, 5.059)	0.753
	Trend		>0.05
PFOS	**Ln PFOS**	-0.553 (-1.779, 0.674)	0.377
	Quartile 1	0 (referent)	
	Quartile 2	-1.868 (-5.449, 1.713)	0.306
	Quartile 3	-2.279 (-6.43, 1.871)	0.281
	Quartile 4	-1.625 (-5.907, 2.657)	0.456
	Trend		>0.05

¥Adjusted for age, sex, BMI, exercise, smoking and total protein
Associations in bold are significant (p= <0.05).
Trend; Trend across quartiles
The ranges of concentrations for each quartile are presented in Table A22

Table A14. Odds Ratio (OR) of out-of-range liver-function biomarker ALT (M: >40 IU/L, F: >30 IU/L) with increases in PFOA quartiles. Only associations that were statistically significant in simple linear regression analysis (presented in table A13) are presented.

PFAA		**Multivariable adjusted OR of out-of-range ALT levels (CI95%)¥**	**p-value**
PFOA	**Quartile 1**	1 (referent)	
	Quartile 2	0.972 (0.572, 1.651)	0.915
	Quartile 3	0.895 (0.521, 1.537)	0.688
	Quartile 4	0.883 (0.499, 1.562)	0.669

¥Adjusted for age, sex, BMI, exercise, smoking and total protein
Associations in bold are significant (p= <0.05).
The ranges of concentrations for each quartile are presented in table A22

Thyroid function markers (TSH, T3, T4)

Table A15. Associations (Linear regression coefficients (B-coefficients) of thyroid-function biomarker TSH (mIU/mL), T3 (pmol/L) and T4 (pmol/L) with increases in PFAA concentrations (ln-transformed and increasing quartiles).

PFAA		Multivariable adjusted change in TSH(CI95%)¥	p-value	Multivariable adjusted change in T3(CI95%)¥	p-value	Multivariable adjusted change in T4 (CI95%)¥	p-value
PFOA	**Ln PFOA**	0.166 (-0.006, 0.338)	0.058	-0.02 (-0.085, 0.045)	0.547	0.061 (-0.108, 0.231)	0.479
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	0.046 (-0.231, 0.323)	0.746	-0.028 (-0.132, 0.077)	0.605	0.183 (-0.09, 0.456)	0.188
	Quartile 3	0.163 (-0.118, 0.443)	0.255	-0.001 (-0.107, 0.105)	0.988	0.086 (-0.191, 0.362)	0.543
	Quartile 4	0.267 (-0.022, 0.556)	0.071	-0.029 (-0.138, 0.08)	0.601	0.092 (-0.193, 0.377)	0.527
	Trend		**0.047**		>0.05		>0.05
PFHxS	**Ln PFHxS**	0.051 (-0.034, 0.136)	0.242	0.003 (-0.029, 0.035)	0.863	-0.039 (-0.123, 0.045)	0.363
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	-0.079 (-0.362, 0.205)	0.586	-0.066 (-0.173, 0.041)	0.227	-0.074 (-0.353, 0.205)	0.602
	Quartile 3	0.015 (-0.318, 0.348)	0.928	0.019 (-0.107, 0.145)	0.770	-0.159 (-0.488, 0.169)	0.340
	Quartile 4	0.246 (-0.099, 0.59)	0.162	-0.013 (-0.143, 0.118)	0.850	-0.339 (-0.678, 0.001)	0.051
	Trend		>0.05		>0.05		>0.05
PFHpS	**Ln PFHpS**	0.058 (-0.032, 0.149)	0.207	-0.007 (-0.041, 0.027)	0.688	-0.062 (-0.151, 0.027)	0.172
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	-0.162 (-0.452, 0.127)	0.271	-0.056 (-0.165, 0.053)	0.314	-0.011 (-0.296, 0.275)	0.942
	Quartile 3	0.011 (-0.328, 0.349)	0.950	0.042 (-0.086, 0.169)	0.523	-0.132 (-0.465, 0.202)	0.439
	Quartile 4	0.225 (-0.124, 0.575)	0.206	-0.035 (-0.167, 0.097)	0.604	-0.319 (-0.664, 0.026)	0.070
	Trend		>0.05		>0.05		>0.05
PFOS	**Ln PFOS**	0.055 (-0.043, 0.154)	0.271	0.002 (-0.035, 0.04)	0.901	-0.043 (-0.141, 0.054)	0.382
	Quartile 1	0 (referent)		0 (referent)		0 (referent)	
	Quartile 2	-0.145 (-0.435, 0.144)	0.325	-0.07 (-0.179, 0.04)	0.212	-0.141 (-0.427, 0.145)	0.334
	Quartile 3	-0.025 (-0.357, 0.307)	0.881	0 (-0.126, 0.126)	0.999	-0.209 (-0.538, 0.119)	0.210
	Quartile 4	0.224 (-0.122, 0.57)	0.203	-0.043 (-0.174, 0.088)	0.518	-0.273 (-0.615, 0.069)	0.117
	Trend		>0.05		>0.05		>0.05

¥Adjusted for age, sex, exercise, smoking and total protein
Associations in bold are significant (p= <0.05).
Trend; Trend across quartiles
The ranges of concentrations for each quartile are presented in table A22

Table A16. Odds Ratio (OR) of out-of-range thyroid-function biomarker TSH (>5 mIU/mL, >70yrs, >4 mIU/mL, 50-70yr, >3,5 mIU/mL 18-50 yrs) with increases in PFOA quartiles. Only associations that were statistically significant in simple linear regression analysis (presented in table A15) are presented

PFAA		Multivariable adjusted OR of out-of-range TSH levels (CI95%)¥	p-value
PFOA	**Quartile 1**	1 (referent)	
	Quartile 2	1.092 (0.263, 4.534)	0.904
	Quartile 3	1.003 (0.211, 4.773)	0.997
	Quartile 4	2.011 (0.474, 8.525)	0.343

¥Adjusted for age, sex, exercise, smoking and total protein
Associations in bold are significant (p= <0.05).
The ranges of concentrations for each quartile are presented in table A22

Kidney function markers (Uric acid, eGFR)

Table A17. Changes (Linear regression coefficients (B-coefficients) of kidney-function biomarker urate mmol/L with increases in PFAA concentrations (ln-transformed and increasing quartiles).

PFAA		Multivariable adjusted change in Urate (mmol/L) (CI95%)¥	p-value
PFOA	**Ln PFOA**	**0.013 (0.004, 0.022)**	**0.006**
	Quartile 1	0 (referent)	
	Quartile 2	0.008 (-0.006, 0.023)	0.246
	Quartile 3	**0.021 (0.007, 0.036)**	**0.004**
	Quartile 4	**0.02 (0.005, 0.034)**	**0.010**
	Trend		**0.003**
PFHxS	**Ln PFHxS**	-0.002 (-0.007, 0.002)	0.290
	Quartile 1	0 (referent)	
	Quartile 2	-0.01 (-0.025, 0.005)	0.180
	Quartile 3	-0.009 (-0.026, 0.008)	0.313
	Quartile 4	-0.014 (-0.032, 0.004)	0.130
	Trend		>0.05
PFHpS	**Ln PFHpS**	-0.001 (-0.006, 0.003)	0.544
	Quartile 1	0 (referent)	
	Quartile 2	-0.003 (-0.018, 0.012)	0.697
	Quartile 3	-0.01 (-0.028, 0.008)	0.268
	Quartile 4	-0.006 (-0.025, 0.013)	0.534
	Trend		>0.05
PFOS	**Ln PFOS**	-0.003 (-0.008, 0.002)	0.222
	Quartile 1	0 (referent)	
	Quartile 2	0.003 (-0.012, 0.018)	0.716
	Quartile 3	-0.003 (-0.02, 0.014)	0.745
	Quartile 4	-0.006 (-0.025, 0.012)	0.490
	Trend		>0.05

¥Adjusted for age, sex, BMI, exercise, smoking, cholesterol and total protein. Sensitive analysis with including seafood consumption did not change beta coefficient >10%.
Associations in bold are significant (p= <0.05).
Trend; Trend across quartiles
The ranges of concentrations for each quartile are presented in Table A22

Table A18. Odds ratio (OR) of having low eGFR(<60 mL/min/1.73m) , with increases in PFAA concentrations (ln-transformed and increasing quartiles).

PFAA		Adjusted OR for low eGFR (CI95%) ¥	p-value
PFOA	**Ln PFOA**	**0.41 (0.185, 0.912)**	**0.029**
	Quartile 1	1 (referent)	
	Quartile 2	0.276 (0.057, 1.325)	0.108
	Quartile 3	0.355 (0.086, 1.457)	0.150
	Quartile 4	0.265 (0.067, 1.05)	0.059
	Trend		>0.05
PFHxS	**Ln PFHxS**	1.113 (0.708, 1.748)	0.643
	Quartile 1	1 (referent)	
	Quartile 2	0.788 (0.059, 10.45)	0.857
	Quartile 3	1.9 (0.205, 17.642)	0.572
	Quartile 4	1.088 (0.115, 10.322)	0.942
	Trend		>0.05
PFHpS	**Ln PFHpS**	1.126 (0.708, 1.793)	0.616
	Quartile 1	1 (referent)	
	Quartile 2	0.343 (0.018, 6.463)	0.475
	Quartile 3	2.011 (0.218, 18.578)	0.538
	Quartile 4	1.208 (0.125, 11.638)	0.870
	Trend		>0.05
PFOS	**Ln PFOS**	1.106 (0.66, 1.851)	0.702
	Quartile 1	1 (referent)	
	Quartile 2	0.868 (0.074, 10.13)	0.910
	Quartile 3	1.862 (0.2, 17.347)	0.585
	Quartile 4	0.957 (0.096, 9.519)	0.970
	Trend		>0.05

¥Adjusted for age, sex, BMI, exercise, smoking and total protein
Low eGFR is considered <60 mL/min/1.73m
Associations in bold are significant (p= <0.05).
Trend; Trend across quartiles
The ranges of concentrations for each quartile are presented in Table A22

Table A19. Odds Ratio (OR) of out-of-range urate levels (>0.5 mmol/L(M), >0.4 mmol/L(F)) with increases in PFAA quartiles. Only associations that were statistically significant in simple linear regression analysis (presented in table A17) are presented.

PFAA		Multivariable adjusted OR of out-of-range urate levels (CI95%)¥	p-value
PFOA	**Quartile 1**	1 (referent)	
	Quartile 2	1.443 (0.443, 4.705)	0.543
	Quartile 3	1.781 (0.581, 5.457)	0.312
	Quartile 4	1.636 (0.508, 5.265)	0.409

¥Adjusted for age, sex, BMI, exercise, smoking, cholesterol and total protein
The ranges of concentrations for each quartile are presented in table A22

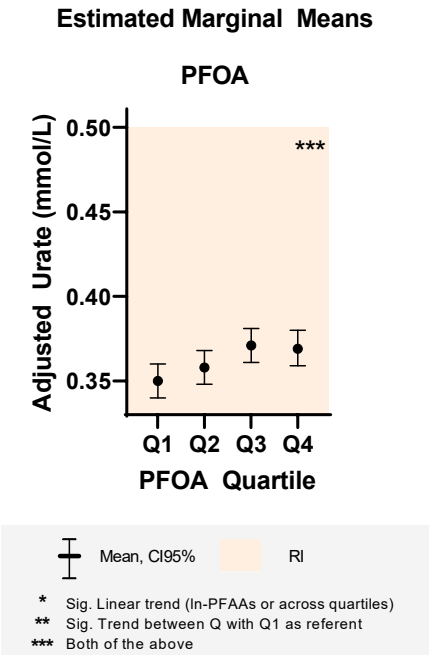

Figure A9. *The co-variable -adjusted estimated marginal means of urate measurements (mmol/L) across PFOA quartiles (quartile ranges are presented in Table A22). The symbols represent the mean and the error bars represents the 95% confidence intervals of the mean. The highlighted area represents the reference interval (RI) for male (female, RI <0.4 mmol/L). For graphical explanation of the plot, see Appendix III, Figure A1.*

Self-reported health issues

Table A20. Odds ratio (OR) of self-reported health issue for increasing ln-transformed PFAA concentrations.

	Multivariable adjusted OR (CI95%)¥ for self-reported health issues;											
Ln- PFAA	**Asthma**	**Cancer (any)**	**Cancer (Prostate)**	**Cancer (Skin)**	**Cardiovascular disease**	**Diabetes Type 2**	**High Blood Pressure**	**Kidney disease**	**Liver problems**	**Reproductive /fertility problems**	**Serious Arthritis**	**Thyroid problems**
PFOA	1.393 (0.915 - 2.121)	0.848 (0.567, 1.269)	0.672 (0.307, 1.471)	**1.864 (1.060, 3.281)***	1.153 (0.669, 1.988)	0.614 (0.357, 1.057)	0.811 (0.584, 1.127)	0.986 (0.551, 1.764)	1.005 (0.414, 2.442)	1.005 (0.480, 2.105)	**0.597 (0.362, 0.987)***	1.344 (0.582, 3.101)
PFHxS	0.988 (0.812, 1.202)	0.903 (0.746, 1.092)	0.951 (0.652, 1.388)	1.038 (0.807, 1.335)	0.946 (0.733, 1.222)	0.93 (0.707, 1.223)	*0.89 (0.759, 1.044)*	0.865 (0.667, 1.121)	0.953 (0.624, 1.453)	1.026 (0.727, 1.447)	1.037 (0.797, 1.348)	0.914 (0.636, 1.313)
PFHpS	1.005 (0.815, 1.24)	0.862 (0.702, 1.058)	0.906 (0.607, 1.352)	1.028 (0.791, 1.337)	0.941 (0.711, 1.247)	0.959 (0.709, 1.296)	*0.922 (0.777, 1.093)*	0.89 (0.666, 1.191)	0.935 (0.594, 1.472)	1.055 (0.735, 1.513)	1.093 (0.828, 1.442)	0.872 (0.592, 1.284)
PFOS	1.028 (0.816, 1.296)	0.849 (0.681, 1.058)	0.92 (0.593, 1.425)	1.022 (0.768, 1.36)	0.901 (0.669, 1.215)	0.928 (0.673, 1.28)	*0.888 (0.738, 1.068)*	0.868 (0.638, 1.181)	0.986 (0.599, 1.625)	0.999 (0.675, 1.478)	1.051 (0.775, 1.425)	0.835 (0.551, 1.264)

¥Adjusted for age, sex, BMI, exercise, smoking and total protein
** Associations in bold are significant (p= <0.05).*

Assessment of biomarkers in the longitudinal data set

Table A21. Changes (Linear regression coefficients (B-coefficients)) of the 2019/2014 ratio of cholesterol, HDL, LDL and urate with increasing 2019/2014 ratio of PFAAs.

	Multivariable adjusted change in 2019/2014 ratio							
Ratio PFAA (2019/2014)	**Ratio Cholesterol (2019/2014) (CI95%)¥**	**p-value**	**Ratio HDL (2019/2014) (CI95%)¥**	**p-value**	**Ratio LDL (2019/2014) (CI95%)¥**	**p-value**	**Ratio Urate (2019/2014) (CI95%)¥**	**p-value**
PFOA	0.014 (-0.211, 0.239)	0.903	0.011 (-0.287, 0.309)	0.942	-0.15 (-0.527, 0.228)	0.433	-0.212 (-0.511, 0.087)	0.163
PFHxS	0.055 (-0.107, 0.218)	0.500	-0.08 (-0.296, 0.135)	0.461	-0.028 (-0.304, 0.248)	0.840	-0.095 (-0.303, 0.113)	0.366
PFHpS	0.1 (-0.049, 0.248)	0.185	-0.022 (-0.232, 0.188)	0.835	0.075 (-0.185, 0.334)	0.568	0.006 (-0.199, 0.211)	0.957
PFOS	0.174 (-0.004, 0.352)	0.056	0.016 (-0.225, 0.257)	0.894	0.118 (-0.19, 0.425)	0.448	-0.104 (-0.339, 0.13)	0.379

¥Adjusted for sex, age at baseline, time between blood collection, change in BMI and change in total protein

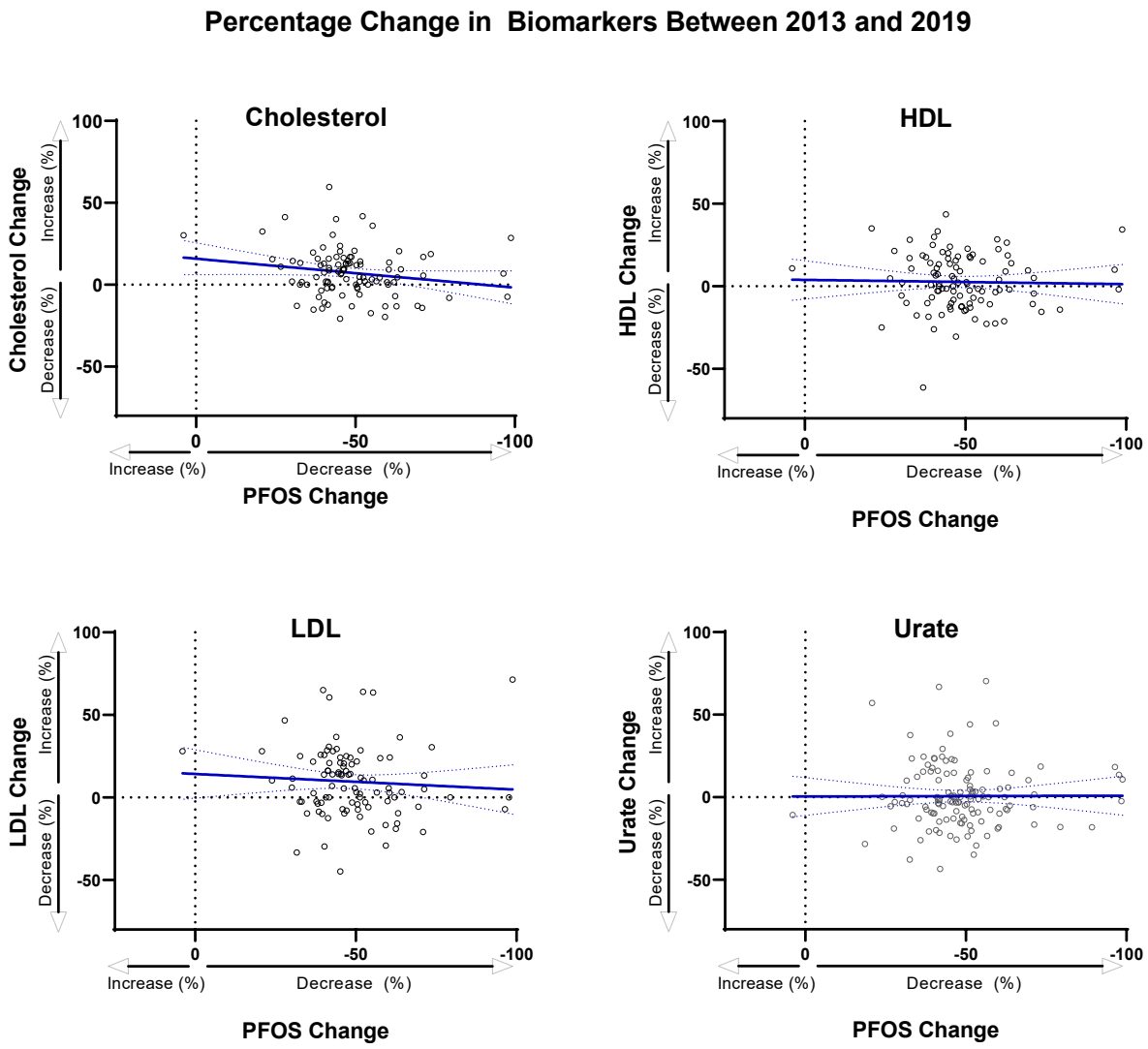

Figure A10. *Percentage change in biomarkers (cholesterol, HDL, LDL and Urate) and percentage change in PFOS concentrations between 2013 and 2019. Note the reverse x-axis. The blue line represents the unadjusted relationship generated from linear regression, where the dotted blue line represents the confidence interval on the association. None of the slopes of the associations were significantly different from 1, indicating no significant relationships between the changes in these biomarkers and the observed changes in PFOS concentrations.*

Additional Tables for Reference

Table A22. The PFAA concentration range (ng/mL) for each quartile in the assessment of each group of biochemical markers. For assessments of each group of biochemical markers, quartiles of PFAA concentrations were created. As different participants were excluded from each assessment, the ranges of the quartiles differ.

PFAAs (ng/mL)		Serum Lipids	Liver function	Thyroid function	Kidney function
PFOA	**Quartile 1**	<1.1	1.1	1.1	1.1
	Quartile 2	1.1-1.5	1.1-1.5	1.1-1.5	1.1-1.5
	Quartile 3	1.5-2.1	1.5-2.1	1.5-2.1	1.5-2.1
	Quartile 4	>2.1	>2.1	>2.1	>2.1
PFHxS	**Quartile 1**	<1.6	<1.8	<1.8	<1.8
	Quartile 2	1.6-4.2	1.8-6.5	1.8-6.5	1.8-6.0
	Quartile 3	4.2-18	6.5-22	6.5-22	6.0-21
	Quartile 4	>18	>22	>22	>21
PFHpS	**Quartile 1**	<0.17	<0.19	<0.19	<0.18
	Quartile 2	0.17-0.41	0.19-0.62	0.19-0.62	0.18-0.57
	Quartile 3	0.41-2.0	0.62-2.3	0.62-2.3	0.57-2.2
	Quartile 4	>2.0	>2.3	>2.3	>2.2
PFOS	**Quartile 1**	<4.8	<5.3	<5.3	<5.1
	Quartile 2	4.8-11	5.3-14	5.3-14	5.1-13
	Quartile 3	11-36	14-40	14-41	13-40
	Quartile 4	>36	>40	>41	>40

Table A23. Levels of biomarkers defined as "out-of-range" for logistic linear regression.

Biomarkers		Out-of-range values
Serum Lipids	**Cholesterol**	>5.5 mmol/L
	HDL	Not assessed
	LDL	>4mmol/L
Liver function	**ALT**	Male; >40 IU/L Female; >30 IU/L
Thyroid function	**TSH**	70yrs; >5 mIU/mL, 50-70 yrs; >4 mIU/mL, 18-50 yrs; >3.5 mIU/mL
	T3	Not assessed
	T4	Not assessed
Kidney function	**Urate**	Male: >0.5 mmol/L Female:>0.4 mmol/L
	eGFR	<60 mL/min/1.73m

IU; international units

Appendix References

1. Buck, R.C., J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury, and S.P. van Leeuwen, *Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins.* Integrated environmental assessment and management, 2011. **7**(4): p. 513-541.

2. Moody, C.A. and J.A. Field, *Perfluorinated surfactants and the environmental implications of their use in fire-fighting foams.* Environmental science & technology, 2000. **34**(18): p. 3864-3870.

3. Rotander, A., L.-M.L. Toms, L. Aylward, M. Kay, and J.F. Mueller, *Elevated levels of PFOS and PFHxS in firefighters exposed to aqueous film forming foam (AFFF).* Environment International, 2015. **82**: p. 28-34.

4. Kärrman, A., J.F. Mueller, B. van Bavel, F. Harden, L.-M.L. Toms, and G. Lindström, *Levels of 12 perfluorinated chemicals in pooled Australian serum, collected 2002–2003, in relation to age, gender, and region.* Environmental Science & Technology, 2006. **40**(12): p. 3742-3748.

5. Ubel, F., S. Sorenson, and D. Roach, *Health status of plant workers exposed to fluorochemicals-a preliminary report.* American Industrial Hygiene Association Journal, 1980. **41**(8): p. 584-589.

6. Sunderland, E.M., X.C. Hu, C. Dassuncao, A.K. Tokranov, C.C. Wagner, and J.G. Allen, *A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects.* Journal of Exposure Science & Environmental Epidemiology, 2019. **29**(2): p. 131-147.

7. Toms, L.M.L., J. Bräunig, S. Vijayasarathy, S. Phillips, P. Hobson, L.L. Aylward, M.D. Kirk, and J.F. Mueller, *Per- and polyfluoroalkyl substances (PFAS) in Australia: Current levels and estimated population reference values for selected compounds.* International Journal of Hygiene and Environmental Health, 2019.

8. Toms, L.M.L., J. Thompson, A. Rotander, P. Hobson, A.M. Calafat, K. Kato, X. Ye, S. Broomhall, F. Harden, and J.F. Mueller, *Decline in perfluorooctane sulfonate and perfluorooctanoate serum concentrations in an Australian population from 2002 to 2011.* Environment International, 2014. **71**: p. 74-80.

9. Toms, L.-M.L., A.M. Calafat, K. Kato, J. Thompson, F. Harden, P. Hobson, A. Sjödin, and J.F. Mueller, *Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia.* Environmental Science & Technology, 2009. **43**(11): p. 4194-4199.

10. CDC, *Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey (NHANES) Data. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2013 - 2014.* . 2015-2016.

11. Backe, W.J., T.C. Day, and J.A. Field, *Zwitterionic, cationic, and anionic fluorinatedchemicals in aqueous film forming foam formulations and groundwater from U.S. military bases by nonaqueous large-volume injection HPLC-MS/MS.* Environmental Science & Technology, 2013. **47**(10): p. 5226-5234.

12. Olsen, G.W., J.M. Burris, M.M. Burlew, and J.H. Mandel, *Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations.* Journal of occupational and environmental medicine, 2003. **45**(3): p. 260-270.

13. Lindström, G., A. Kärrman, and B. van Bavel, *Accuracy and precision in the determination of perfluorinated chemicals in human blood verified by interlaboratory comparisons.* Journal of Chromatography A, 2009. **1216**(3): p. 394-400.

14. Barzen-Hanson, K.A., S.C. Roberts, S. Choyke, K. Oetjen, A. McAlees, N. Riddell, R. McCrindle, P.L. Ferguson, C.P. Higgins, and J.A. Field, *Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater.* Environmental Science & Technology, 2017. **51**(4): p. 2047-2057.

15. Aylward, L.L., E. Green, M. Porta, L.-M. Toms, E. Den Hond, C. Schulz, M. Gasull, J. Pumarega, A. Conrad, M. Kolossa-Gehring, G. Schoeters, and J.F. Mueller, *Population variation in biomonitoring data for persistent organic pollutants (POPs): An examination of multiple population-based datasets for application to Australian pooled biomonitoring data.* Environment International, 2014. **68**: p. 127-138.

16. Eriksson, U., J.F. Mueller, L.-M.L. Toms, P. Hobson, and A. Kärrman, *Temporal trends of PFSAs, PFCAs and selected precursors in Australian serum from 2002 to 2013.* Environmental Pollution, 2017.

17. Lorber, M., G.E. Eaglesham, P. Hobson, L.M.L. Toms, J.F. Mueller, and J.S. Thompson, *The effect of ongoing blood loss on human serum concentrations of perfluorinated acids.* Chemosphere, 2015. **118**: p. 170-177.

18. Stubleski, J., S. Salihovic, L. Lind, P.M. Lind, B. van Bavel, and A. Kärrman, *Changes in serum levels of perfluoroalkyl substances during a 10-year follow-up period in a large population-based cohort.* Environment International, 2016. **95**: p. 86-92.

19. Li, Y., T. Fletcher, D. Mucs, K. Scott, C.H. Lindh, P. Tallving, and K. Jakobsson, *Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water.* Occupational and Environmental Medicine, 2018. **75**(1): p. 46-51.

20. Olsen, G.W., J.M. Burris, D.J. Ehresman, J.W. Froehlich, A.M. Seacat, J.L. Butenhoff, and L.R. Zobel, *Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers.* Environmental Health Perspectives, 2007. **115**(9): p. 1298-1305.

21. Worley, R.R., S.M. Moore, B.C. Tierney, X. Ye, A.M. Calafat, S. Campbell, M.B. Woudneh, and J. Fisher, *Per- and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community.* Environment International, 2017. **106**: p. 135-143.

22. Gomis, M.I., R. Vestergren, M. MacLeod, J.F. Mueller, and I.T. Cousins, *Historical human exposure to perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using population-based pharmacokinetic modelling.* Environment International, 2017. **108**(Supplement C): p. 92-102.

23. Olsen, G.W., C.C. Lange, M.E. Ellefson, D.C. Mair, T.R. Church, C.L. Goldberg, R.M. Herron, Z. Medhdizadehkashi, J.B. Nobiletti, J.A. Rios, W.K. Reagen, and L.R. Zobel, *Temporal Trends of*

Perfluoroalkyl Concentrations in American Red Cross Adult Blood Donors, 2000–2010. Environmental Science & Technology, 2012. **46**(11): p. 6330-6338.

24. Xu, Y., T. Fletcher, D. Pineda, C.H. Lindh, C. Nilsson, A. Glynn, C. Vogs, K. Norström, K. Lilja, and K. Jakobsson, *Serum Half-Lives for Short-and Long-Chain Perfluoroalkyl Acids after Ceasing Exposure from Drinking Water Contaminated by Firefighting Foam.* Environmental Health Perspectives, 2020. **128**(7): p. 077004.

25. Li, Y., Y. Xu, K. Scott, C. Lindh, K. Jakobsson, and T. Fletcher, *Half-lives of PFOA, PFPeS, PFHxS, PFHpS and PFOS after end of exposure to contaminated drinking water.* Environmental Epidemiology, 2019. **3**.

26. Kirk, M., K. Smurthwaite, J. Bräunig, S. Trevenar, C. D'Este, R. Lucas, A. Lal, R. Korda, A. Clements, and J. Mueller, *The PFAS Health Study: Systematic Literature Review.* Canberra: The Australian National University, 2018.

27. Eriksen, K.T., O. Raaschou-Nielsen, J.K. McLaughlin, L. Lipworth, A. Tjønneland, K. Overvad, and M. Sørensen, *Association between Plasma PFOA and PFOS Levels and Total Cholesterol in a Middle-Aged Danish Population.* PLOS ONE, 2013. **8**(2): p. e56969.

28. Frisbee, S.J., A. Shankar, S.S. Knox, K. Steenland, D.A. Savitz, T. Fletcher, and A.M. Ducatman, *Perfluorooctanoic Acid, Perfluorooctanesulfonate, and Serum Lipids in Children and Adolescents: Results From the C8 Health Project.* Archives of Pediatrics & Adolescent Medicine, 2010. **164**(9): p. 860-869.

29. Sakr, C.J., K.H. Kreckmann, J.W. Green, P.J. Gillies, J.L. Reynolds, and R.C. Leonard, *Cross-Sectional Study of Lipids and Liver Enzymes Related to a Serum Biomarker of Exposure (ammonium perfluorooctanoate or APFO) as Part of a General Health Survey in a Cohort of Occupationally Exposed Workers.* 2007. **49**(10): p. 1086-1096.

30. Fitz-Simon, N., T. Fletcher, M. Luster, K. Steenland, A. Calafat, K. Kato, and e. al., *ERRATUM: Reductions in Serum Lipids with a 4-year Decline in Serum Perflourooctanoic Acid and Perfluorooctanesulfonic Acid.* Epidemiology, 2013. **24**(6): p. 941-941.

31. Shankar, A., J. Xiao, and A. Ducatman, *Perfluoroalkyl chemicals and elevated serum uric acid in US adults.* Clinical epidemiology, 2011. **3**: p. 251-258.

32. Costa, G., S. Sartori, and D. Consonni, *Thirty years of medical surveillance in perfluooctanoic acid production workers.* Journal of occupational and environmental medicine, 2009. **51**(3): p. 364-372.

33. Han, X., D.L. Nabb, M.H. Russell, G.L. Kennedy, and R.W. Rickard, *Renal Elimination of Perfluorocarboxylates (PFCAs).* Chemical Research in Toxicology, 2012. **25**(1): p. 35-46.

34. Kataria, A., H. Trachtman, L. Malaga-Dieguez, and L. Trasande, *Association between perfluoroalkyl acids and kidney function in a cross-sectional study of adolescents.* Environmental Health, 2015. **14**(1): p. 89.

35. Riddell, N., G. Arsenault, J.P. Benskin, B. Chittim, J.W. Martin, A. McAlees, and R. McCrindle, *Branched perfluorooctane sulfonate isomer quantification and characterization in blood serum samples by HPLC/ESI-MS(/MS).* Environmental Science & Technology, 2009. **43**(20): p. 7902-7908.

36. SNP. Sullivann Nicolaides Pathology (2020). Estimated Glomerular Filtration Rate (eGFR). Available from: http://protocols.sonichealthcare.com/shared/IP061.pdf. 2020 [cited 2020 08 21].

37. Pearce, E.N., *Thyroid hormone and obesity.* Current Opinion in Endocrinology & Diabetes and Obesity, 2012. **19**(5): p. 408-13.

38. Cole, S.R., R.W. Platt, E.F. Schisterman, H. Chu, D. Westreich, D. Richardson, and C. Poole, *Illustrating bias due to conditioning on a collider.* International Journal of Epidemiology, 2010. **39**(2): p. 417-20.

39. Tonelli, M., C. Isles, T. Craven, A. Tonkin, M.A. Pfeffer, J. Shepherd, F.M. Sacks, C. Furberg, S.M. Cobbe, J. Simes, M. West, C. Packard, and G.C. Curhan, *Effect of Pravastatin on Rate of Kidney Function Loss in People With or at Risk for Coronary Disease.* Circulation, 2005. **112**(2): p. 171-178.

THE UNIVERSITY OF QUEENSLAND
AUSTRALIA

CREATE CHANGE

Contact details

Dr Jennifer Bräunig

T +61 7 **3346 1899**

E **j.braunig**@uq.edu.au

W qaehs.centre.uq.edu.au

CRICOS Provider Number 00025B